Cough-generated aerosols of *Pseudomonas aeruginosa* and other Gram-negative bacteria from patients with cystic fibrosis

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**ABSTRACT**

**Background:** *Pseudomonas aeruginosa* is the most common bacterial pathogen in patients with cystic fibrosis (CF). Current infection control guidelines aim to prevent transmission via contact and respiratory droplet routes and do not consider the possibility of airborne transmission. It was hypothesised that subjects with CF produce viable respirable bacterial aerosols with coughing.

**Methods:** A cross-sectional study was undertaken of 15 children and 13 adults with CF, 26 chronically infected with *P aeruginosa*. A cough aerosol sampling system enabled fractioning of respiratory particles of different sizes and culture of viable Gram-negative non-fermentative bacteria. Cough aerosols were collected during 5 min of voluntary coughing and during a sputum induction procedure when tolerated. Standardised quantitative culture and genotyping techniques were used.

**Results:** *P aeruginosa* was isolated in cough aerosols of 25 subjects (89%), 22 of whom produced sputum samples. *P aeruginosa* from sputum and paired cough aerosols were indistinguishable by molecular typing. In four cases the same genotype was isolated from ambient room air. Approximately 70% of viable aerosols collected during voluntary coughing were of particles ≤3.3 μm aerodynamic diameter. *P aeruginosa*, Burkholderia cepacia complex and other bacteria may occur in airborne. To our knowledge, the particle size distribution of aerosols from patients with CF has not been reported. We hypothesised that, during voluntary coughing and during sputum induction, subjects with CF produce viable aerosols that are respirable. To test this hypothesis we modified a cough aerosol sampling system (CASS) recently developed to measure cough-generated aerosols from patients with *Mycobacterium tuberculosis*.14

Our primary aim was to determine the concentration and particle size distribution of cough aerosols containing cultivable *P aeruginosa* and other Gram-negative bacteria from children and adults with CF. We also sought to determine whether concentrations of cough aerosols detected were related to clinical parameters and clonality of *P aeruginosa* strains.

**METHODS**

**Subjects**

Subjects with CF were recruited from both the inpatient and outpatient services at the Royal
Cystic fibrosis

Children’s Hospital and The Prince Charles Hospital in Brisbane, Australia. Inclusion criteria were age >9 years, a confirmed diagnosis of CF and culture of P aeruginosa or B cepacia complex from sputum on at least one occasion within the previous 12 months. Exclusion criteria included known pregnancy, pneumothorax within the previous 6 months, history of cough syncope or vomiting associated with coughing. After the first subject experienced recurrence of mild haemoptysis during the cough study, we excluded those with haemoptysis in the previous 7 days. Subjects were excluded from hypertonic saline inhalation if there was a history of intolerance of hypertonic saline, presence of asthma symptoms or a forced expiratory volume in 1 s (FEV1) ≤40% predicted and no previous trials of hypertonic saline. Subjects were asked to withhold all nebulised therapy for 12 h prior to testing.

Cough aerosol sampling system (CASS)
The equipment used was a modification of that developed previously. In brief, a subject coughs through a mouthpiece connected to afferent tubing into a chamber whereupon a vacuum pump draws exhaled air and generated respiratory particles through one of two Anderson six-stage impactors. Each stage has 400 holes of decreasing diameter through which appropriately-sized aerosolised particles will penetrate and deposit on an agar plate. A “settle plate” of the same agar was placed inside the chamber to capture larger droplets. Larger particles (droplets) would be expected to deposit in the afferent limb tubing, the settle plate and the walls of the chamber. Additional details are provided in the online data supplement.

CASS protocol
The Andersen impactors were loaded with agar plates at room temperature. The tubing from the vacuum pump was attached to the port for the first six-stage impactor in the CASS. After the first session of coughing, the tubing was moved to the second sampler. All unused ports were occluded with plastic tape.

Subjects were instructed to cough into the CASS as frequently and as strongly as was comfortable for 5 min. At the onset of coughing the timer (set for 5 min) controlling the power to the vacuum pump was started. Cough strength was assessed as strong, moderate or weak and cough frequency was assessed quantitatively.

If hypertonic saline could be tolerated, the first sampling was done during voluntary coughing and the second 5-minute sample was collected during inhalation of 5 ml 4.5% saline delivered by a handheld ultrasonic nebuliser (Microneb Allersearch distributed by Becton Dickinson, North Ryde, Australia). Subjects were pretreated with albuterol metered dose inhaler (88 µg per puff), 4 puffs via spacer (Volumatic, Allen & Hanburys, UK). If hypertonic saline was not considered safe, sampling was done with the subject using tidal breathing for 5 min. Sputum samples were collected if produced.

Clinical parameters
Pulmonary function testing
Forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were obtained according to standard guidelines prior to the cough study. Respiratory muscle strength was assessed using maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) (Morgan Pmax) at the paediatric centre and using a Micro Medical Respiratory Pressure Meter (Micro Medical, Rochester, UK) at the adult centre.

Other
Age, gender, presence of current exacerbation of disease, height, weight and body mass index were recorded.

Room air sampling and air exchange
Using a centrifugal air sampler, two samples were obtained before each cough aerosol study: one during the subject’s performance of spirometry and one during the cough aerosol study. The indoor air temperature and relative humidity were measured with a thermo hygrometer (Rotronic HygroPalM 2, Rotronic Instrument Corp, Huntington, New York, USA) at the beginning of each study. Effective air exchange rates in the consultation rooms used for CASS testing and in the pulmonary function laboratory at the adult centre were determined using carbon dioxide as a tracer gas. Further details are provided in the online supplement.

Microbiology
CASS aerosol samples and chamber settle plate
Cultures were performed using chocolate bacitracin (500 µg/ml) agar in aerobic conditions at 35°C. After 48 h and 72 h incubation, a colony forming unit (CFU) count was performed on each plate including individual colonial P aeruginosa morphotypes and the combined total CFU count of P aeruginosa and other Gram-negative bacteria. Following presumptive screening (characteristic colonial appearance, presence of oxidase and growth at 42°C), the identity of each P aeruginosa isolate was confirmed by species-specific oprL gene PCR. Other non-fermenting Gram-negative bacteria detected throughout the study were identified using a combination of API 20NE (bioMerieux), amplified 16S rDNA restriction analysis (ARDRA) and recA-based PCR analysis.

Each Andersen sampler stage contains 400 holes and each CFU is regarded as the result of an infectious particle within a specific size range impacting on the agar. Colony counts exceeding 400 have been interpreted in two ways: an accepted “positive-hole” correction model taking into account the probability of multiple hits through each hole and a conservative model of a maximum count of 400 only. The total sum of P aeruginosa or B cepacia complex colonies counted (total count) in all the Andersen stages for 5 min of voluntary coughing and for 5 min hypertonic saline study or tidal breathing was calculated, as was the sum of the colonies from stages 4, 5 and 6 (<3.3 µm, termed “small aerosol fraction”).

Sputum samples, afferent limb cultures and air samples
Standard quantitative culture methods were used. For air samples and afferent limb cultures, only Gram-negative non-fermentative bacteria were assessed. Isolates were identified as above with molecular strain typing of P aeruginosa isolates. Further details are provided in the online data supplement.

Analysis of data
Counts for individual components and the totals for Andersen stages 1–6 (total) and for Andersen stages 4–6 (small fraction) were logarithmically transformed before analysis to correct for skewness. Means and 95% confidence limits (95% CI) were back transformed from log to linear scales for presentation. The paired differences between counts during voluntary coughing and each of the hypertonic and tidal breathing studies were analysed by paired t tests and mean differences were also back transformed from log to linear scales to calculate the ratios of counts during voluntary coughing and each of the hypertonic...
and tidal breathing studies. Correlation coefficients were estimated between logarithmically transformed total counts and clinical and demographic factors where available for all subjects. The Fisher exact test was used for the association between positive air samples and high total counts. All reported p values are two-sided. Linear regression was used to estimate the slope of the relationship between FEV₁ and total count. All analyses were performed with SPSS software Version 15.

RESULTS

CASS studies
Twenty-eight subjects (15 children, 13 adults) were consecutively recruited and completed 5 min of voluntary coughing. Twenty subjects were administered nebulised hypertonic saline and seven subjects had measurements during tidal breathing. One subject performed the voluntary cough only. Thirteen subjects were studied during a pulmonary exacerbation (table 1).

Sputum microbiology
In the 12 months before the study, 27 subjects had sputum that cultured positive for *P aeruginosa* and one subject had cultured *B cenocepacia* (table 2). Of the 27 patients with *P aeruginosa* infection, all adults (n = 12) and 14 children had chronic infection based on the Leeds criteria.22 One child had recently cleared a new infection with *P aeruginosa* following an eradication course of antibiotic therapy and cultured normal respiratory flora from a sputum sample collected on the day of testing. The patient with *B cenocepacia* had chronic infection based on the Leeds criteria (table 2).22 On the study day, 23 subjects provided expectorated sputum samples. Of these, one subject grew *Aspergillus* species and yeasts. Molecular strain typing analyses were performed with SPSS software Version 15.

Table 1  Demographic and baseline clinical factors of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Children (n = 15)</th>
<th>Adults (n = 13)</th>
<th>All (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range) age (years)</td>
<td>13.5 (9.9–16.6)</td>
<td>25.8 (18.8–48.8)</td>
<td>16.4 (9.9–48.8)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/7</td>
<td>9/4</td>
<td>17/11</td>
</tr>
<tr>
<td>Current exacerbation</td>
<td>7 (47%)</td>
<td>8 (46%)</td>
<td>13 (46%)</td>
</tr>
<tr>
<td>Mean (SD) BMI (kg/m²)</td>
<td>17.9 (2.5)</td>
<td>22.6 (3.7)</td>
<td>20.1 (3.9)</td>
</tr>
<tr>
<td>Mean (SD) Z score for weight</td>
<td>−0.6 (0.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) Z score for height</td>
<td>−0.5 (1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) FEV₁ (% predicted)</td>
<td>67.0 (22.5)</td>
<td>52.4 (19.6)</td>
<td>60.2 (22.1)</td>
</tr>
<tr>
<td>Mean (SD) FVC (% predicted)</td>
<td>77.0 (19.8)</td>
<td>70.7 (15.4)</td>
<td>74.1 (17.7)</td>
</tr>
<tr>
<td>Mean (SD) peak flow (l/s)</td>
<td>4.6 (2.2)</td>
<td>6.1 (1.9)</td>
<td>5.4 (2.2)</td>
</tr>
<tr>
<td>Mean (SD) MEP (cm H₂O)</td>
<td>82.8 (32.8)</td>
<td>101.0 (30.1)</td>
<td>91.1 (32.3)</td>
</tr>
<tr>
<td>Mean (SD) MEP (cm H₂O)</td>
<td>113.1 (42.9)</td>
<td>103.6 (29.5)</td>
<td>109.1 (37.4)</td>
</tr>
</tbody>
</table>

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MEP, maximum expiratory pressure; MIP, maximum inspiratory pressure.

Settle plate and air sampling microbiology
The chamber settle plate and afferent limb equipment was not changed between the two components of the study for individual subjects with quantitative culture, reflecting large droplet deposition for both components of the study combined. The mean total count for the settle plate was 6 CFU (95% CI 3 to 14). The mean total count for the afferent limb was 56 CFU/ml wash fluid (95% CI 10 to 303).

Mean (SE) air exchange rates ranged between 9.77 (0.06) and 19.40 (0.70) exchanges per hour in the testing rooms. A total of 101 air samples were collected before and during testing. Sixteen subjects cultured unique strains of *P aeruginosa* during testing of 14 patients. The unique strains isolated did not match any sputum or CASS isolates. Five air samples cultured AES2 strain during testing of four subjects with AES2 strain of *P aeruginosa*. For these four subjects, sputum, cough aerosol and air samples all cultivated the same strain. Three of the AES2 positive air samples were collected during pulmonary function testing and two during background testing in the CASS study rooms. Positive air samples were associated with a high concentration in cough aerosols. If only subjects with AES2 were considered, four out of five subjects with total cough aerosol counts...
>1000 CFU had positive air samples and no subjects out of the 10 with lower total CFU counts had positive air samples (p = 0.005). Temperature and humidity did not vary significantly between study sites or study days at each site (data not shown).

**DISCUSSION**

This is the first study to report the magnitude, variability and particle size distribution of culturable aerosols of Gram-negative bacteria produced by coughing in patients with CF. Although there is evidence of cultivable Gram-negative bacteria in the large droplets within the afferent tubing and settle plates in the cough chamber, a large proportion of cultivable particles were found to be in a size range that is likely to deposit in the lower respiratory tract. Genetically indistinguishable bacteria were identified in expectorated sputum and in the cough-generated aerosols, and in four experiments the same organisms were also isolated from the ambient room air. This supports the assertion that the sources of the bacteria are the patients rather than the hospital or nearby environment.

Aerosolisation of respiratory tract particles during coughing and sneezing and even during tidal breathing is a well-recognised phenomenon associated with the spread of many infections including measles, influenza and tuberculosis. The majority of respiratory pathogens have been thought to be spread by large droplets that settle within an approximate 1 m range of an individual, providing a low risk of airborne infection. Infection control practices for most CF centres reflect recently published infection control guidelines suggesting that patients should maintain a distance of at least 1 m to reduce the risk of cross-infection. The risk of acquisition of infection from respiratory aerosols is complex and probably relates to the pathogen type, concentration of the organism in the aerosol, the susceptibility of exposed individuals and the environment (air movement, relative humidity, temperature, etc). Limited studies have examined particle size distribution of respiratory aerosols and most have reported large droplet formation, predominantly particles with a diameter of >8 μm. More recently, Papineni and Rosenthal reported that 85% of particles were <1 μm and that coughing produced more aerosol particles than did breathing or talking. The first published study to use a CASS examined patients with tuberculosis and, like our study, found that most of the respiratory particles were <3.5 μm. Our study shows that patients with CF produce culturable aerosols in a wide range of particle sizes including both respiratory
CF centres in the UK, Europe and Australia has suggested cross-
identification of genetically related
bacteria. Infection may result from direct inhalation of aerosolised
spreaders (eg, in severe acute respiratory syndrome).32 33
Patients as “disseminators” (eg, in tuberculosis) or “super-
cough aerosols with a log normal distribution. Such a
result of stronger cough, may have an increased risk of
factors influencing the extent of isolation of Gram-negative
organisms in cough aerosols are likely to be complex, including
both host factors and bacterial factors such as enhanced survival
in air. Our data show that the concentration of bacteria in the
sputum and the forced expiratory flow rates were related to
cough aerosol concentration, with a trend for association with
higher peak flow and higher body mass index. These data
suggest that patients with milder lung disease, perhaps as a
result of stronger cough, may have an increased risk of
producing infectious aerosols. This warrants further investiga-
tion as the improvement in clinical outcomes in patients with
CF may potentially increase the risk of spread of clonal strains
of *P aeruginosa* and other Gram-negative bacteria.

The only air samples that cultured *P aeruginosa* which
matched clinical samples from sputum or CASS samples were
clonal AES2 strains. Given that patients who had positive air
samples also had high total aerosol counts, we were unable to
determine if the density of infection on its own—or whether, in
addition, the nature of the specific infection—contributed to
the positive air samples. The source of the *P aeruginosa* air
isolates that did not match any clinical samples is unknown and
environmental sampling of surfaces was not undertaken. It is
possible that environmental sources such as sinks may have
been involved as hand washing occurred during testing. The
measured air exchange rates in the study rooms provide an
important perspective as air sampling was performed for 12 min
on each occasion and, during this period, 2–4 complete air
exchanges occurred. Higher rates and density of positive air
samples may be anticipated in less well ventilated rooms.

Nebulised hypertonic saline is now recognised as improving
mucociliary clearance.34 We sought to determine if hypertonic
saline-induced cough further enhanced the production of
bacterial aerosols, but we found similar results to those seen
with voluntary coughing and much greater than those obtained
during tidal breathing. Treatments such as physiotherapy,
mucolytic agents and even nebulised antibiotics which can
induce coughing are likely to result in similar cough-induced
daerosols as with voluntary coughing. Although only seen in
three of seven patients tested during tidal breathing, the
presence of *P aeruginosa* in the cough aerosols and in the small
aerosol fraction from two patients warrants further study as
any reassessment of infection control recommendations to
incorporate the role of airborne transmission may not only
apply to coughing patients.

Figure 2 Particle size distribution of logarithmic corrected total cough
aerosol counts in colony forming units (CFU) with 95% confidence
intervals during voluntary coughing according to Andersen stage.

**Event limit of size range (micron)**
- 7.0
- 4.7
- 3.3
- 2.1
- 1.1
- 0.65

**Anatomical deposition site**
- Upper airway
- - bronchi
- - - alveoli

**Figure 3** Correlation of baseline forced expiratory volume in 1 s (FEV1) with 
logarithmic total corrected count from cough aerosols during voluntary coughing. CFU, colony forming unit; +, positive ambient air samples isolated.
select group of patients who did not undertake hypertonic saline-induced cough studies and further work is required to evaluate the extent to which tidal breathing is associated with the generation of potentially infective particles. Finally, while this study provides evidence that patients with CF and Gram-negative infection can produce potentially infectious cough aerosols, we cannot draw conclusions about transmission to susceptible individuals. A recent study examined the survival of *P. aeruginosa* in vitro and found bacterial survival, at least for a limited time period of <90 s, to be favoured by lower temperature and mucoid phenotype. While providing further evidence that airborne transmission is plausible, transmission by this route is yet to be proved beyond doubt.

In conclusion, this study shows that patients with CF infected with *P. aeruginosa* can produce respirable infectious cough aerosols in a wide range of concentrations of a log normal distribution. We also detected other non-fermenting Gram-negative bacteria including *B. cepacia* in the small aerosol fraction, suggesting that airborne transmission of such organisms is biologically plausible. Further studies of potential airborne transmission of bacterial pathogens in patients with CF are warranted to provide a scientific basis for infection control recommendations to prevent the spread of multidrug-resistant or clonal strains of *P. aeruginosa* and other Gram-negative bacteria in this patient population.

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**Competing interests:** None.

**Ethics approval:** The study was approved by the ethics committees of both CF centres and the University of Queensland and the Institutional Review Board of UMDNJ. Informed consent was obtained from all subjects and in addition from the parents or guardians of all young people under 18 years of age.

**Provenance and peer review:** Not commissioned; externally peer reviewed.

**REFERENCES**


