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ORIGINAL ARTICLE

Viability of *Pseudomonas aeruginosa* in cough aerosols generated by persons with cystic fibrosis

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ABSTRACT

Background Person-to-person transmission of respiratory pathogens, including *Pseudomonas aeruginosa*, is a challenge facing many cystic fibrosis (CF) centres. Viable *P aeruginosa* are contained in aerosols produced during coughing, raising the possibility of airborne transmission.

Methods Using purpose-built equipment, we measured viable *P aeruginosa* in cough aerosols at 1, 2 and 4 m from the subject (distance) and after allowing aerosols to age for 5, 15 and 45 min in a slowly rotating drum to minimise gravitational settling and inertial impaction (duration). Aerosol particles were captured and sized employing an Anderson Impactor and cultured using conventional microbiology. Sputum was also cultured and lung function and respiratory muscle strength measured.

Results Nineteen patients with CF, mean age 25.8 (SD 9.2) years, chronically infected with *P aeruginosa*, and 10 healthy controls, 26.5 (8.7) years, participated. Viable *P aeruginosa* were detected in cough aerosols from all patients with CF, but not from controls; travelling 4 m in 17/18 (94%) and persisting for 45 min in 14/18 (78%) of the CF group. Marked inter-subject heterogeneity of *P aeruginosa* aerosol colony counts was seen and correlated strongly (r=0.73–0.90) with sputum bacterial loads. Modelling decay of viable *P aeruginosa* in a clinic room suggested that at the recommended ventilation rate of two air changes per hour almost 50 min were required for 90% to be removed after an infected patient left the room.

Conclusions Viable *P aeruginosa* in cough aerosols travel further and last longer than recognised previously, providing additional evidence of airborne transmission between patients with CF.

Key messages

What is the key question?

- Several studies have suggested that airborne transmission of *Pseudomonas aeruginosa* among patients with cystic fibrosis (CF) is feasible; however, it is unclear how far cough generated aerosols travel or how long they remain viable in the airborne phase.

What is the bottom line?

- Using two validated cough aerosol sampling systems to measure the viability of *P aeruginosa* in droplet nuclei we demonstrate that patients with CF produce cough aerosols containing viable organisms that are capable of travelling up to 4 m and persisting in the air for up to 45 min.

Why read on?

- Data presented here challenge current CF infection control practices of separating patients by 1–2 m to prevent cross-infection by respiratory bacteria, suggesting a re-examination of current infection control practices within the CF community is needed.

INTRODUCTION

Communicable respiratory infections are an important cause of mortality and morbidity and result in healthcare costs worth billions of dollars.[1 2] The person-to-person transmission of respiratory pathogens may occur by three key routes: *contact transmission*, where infectious respiratory secretions spread by direct contact or indirectly through a contaminated intermediate object or person; *droplet transmission*, where large infectious droplets travel directly over short distances from the respiratory tract to deposit onto mucosal surfaces (eg, nasal and conjunctival mucous membranes); and *airborne transmission*, involving dissemination of small droplet nuclei within the respirable size range that remain infectious over time and distance and are inhaled by susceptible individuals.[3] Airborne transmission may pose a significant risk for hospital-acquired respiratory infection,[4] with evidence that it is an important mode of acquisition for many respiratory infections, including *Mycobacterium tuberculosis* (TB) and *Bordetella pertussis*.[5 6]

Cystic fibrosis (CF) is the most common life-limiting autosomal-recessive disorder in Caucasians with most affected persons dying in their third-to-fourth decade from respiratory failure complicating chronic pulmonary infections.[7] *Pseudomonas aeruginosa* is the most common pathogen in CF lung disease, and once infection is established there is an accelerated decline in lung function, quality of life and survival.[8 9] While many patients with CF acquire *P aeruginosa* sporadically from the environment, molecular-typing studies show shared strains are common in some

CF centres, suggesting person-to-person transmission may also be occurring.[10 11] In Australia, *P aeruginosa* strains AUST-01 and AUST-02 are responsible for 40% of chronic infections[11] and are associated with worse clinical outcomes and increased treatment requirements.[11 12] The mechanisms of cross-infection in CF are unknown. However, despite CF centres implementing contact and droplet transmission control measures, *P aeruginosa* cross-infection continues[11 13 14] and is only interrupted by implementing strict patient segregation policies.[15 16]

It seems that a plausible mechanism of transmission between patients is the airborne route. Indeed, patients with CF infected with *P aeruginosa* produce droplet nuclei containing viable bacteria.[17] Furthermore, studies using air sampling in CF clinical settings have demonstrated viable *P aeruginosa* after physiotherapy and in corridors outside inpatient cubicle rooms housing patients with shared strain *P aeruginosa* infection.[18 19] Others have also suggested that airborne transmission is feasible, with shared and mucoid *P aeruginosa* strains having a survival advantage.[20 21] However, methods used in previous studies were unable to demonstrate how long viable bacteria were present in aerosols or how far they could travel from the original source. We therefore sought to enhance understanding of *P aeruginosa* aerosol production in CF in order to (i) determine the duration and distance of viable cough aerosols, (ii) identify the factors influencing pathogen viability and to (iii) understand the effects of room ventilation on controlling the duration of viable airborne *P aeruginosa*.

METHODS

Subjects

Patients with CF (n=19) aged >12 years old and chronically infected with *P aeruginosa* were recruited from the Royal Children's Hospital (RCH) and The Prince Charles Hospital (TPCH), Brisbane, Australia. Exclusion criteria included recent pneumothorax, haemoptysis and pregnancy. Healthy, non-smoking controls (n=10) were recruited from hospital staff and their families. Further details are provided in the online supplementary methods. The RCH (HREC/11/QRCH/44), The University of Queensland (2012000615) and the Queensland University of Technology Human Research Ethics Committees (1100000618) approved the study and participants (or their guardians) provided their written, informed consent.

Clinical measurements

Subjects attended two study days separated by a mean (SD) 7.5 (4.7) days (range 2–21). A sputum sample was collected from each patient with CF on the day of testing. Cough manoeuvres were supervised by a healthcare professional and emergency equipment was available.

Forced expiratory volume in one-second (FEV_1) and forced vital capacity (FVC) were measured on all subjects according to ATS/ERS guidelines.[22] Respiratory muscle strength was measured by maximum inspiratory (MIP) and maximum expiratory (MEP) mouth pressures using a Micro Medical Respiratory Pressure Meter at TPCH. Weight, height, age, sex and clinical status (exacerbation or clinically stable) were recorded.

Determining the distance viable cough aerosols travel

A 'Distance Rig' (see online supplementary methods) permitted measurement of cough aerosols in a particle-free environment at distances up to 4 m from the subject (see online supplementary figure S1).[23] An Andersen Impactor (28.3 L/min) captured and measured viable cough aerosol particles,[17] and a Lasair II-110 optical particle counter (OPC) measured real-time particle concentrations and verified the absence of room air aerosol contamination in the Distance Rig. Andersen Impactor and OPC samples were collected through a common circular isokinetic inlet.

Subjects sat with their head in the Rig breathing high-efficiency particulate air (HEPA)-filtered air for 2 min to purge residual room air from their lungs before coughing as frequently and strongly as was comfortable for 5 min. The Andersen Impactor sampled continuously during the voluntary cough phase. Cough numbers were recorded manually. Cough aerosols were collected at 1, 2 and 4 m (order randomised) from each subject, who performed duplicate cough manoeuvres at each distance.

Determining duration of aerosol viability

A Duration Rig (see online supplementary methods) comprised a 0.4 m^3 airtight stainless steel cylinder (illustrated in online supplementary figure S3) to assess the duration of aerosol particle viability in the airborne phase. The cylinder was rotated at 1.7 rpm so that gravitational settling and inertial impaction of particles was minimised.[24] The system was flushed with HEPA-filtered air before sample collection so that cough aerosols were not contaminated by room air aerosols.

Subjects were seated and fitted with a snorkel-style mouthpiece (CareFusion Australia) connected to 10 cm of flexible tubing. A nose clip was attached and subjects breathed filtered air for 2 min, and then coughed as much as was comfortable for 2 min, with cough numbers recorded manually. The Rig was isolated, and the resultant cough aerosols were aged for 5, 15 or 45 min. Suspended particles were collected with an Andersen Impactor at the conclusion of the ageing period. Measurements after 5 and 15 min ageing were performed in duplicate while, for logistic reasons, only one measurement was performed after 45 min ageing.

Microbiology

Cough aerosol cultures were performed with chocolate bacitracin (300 mg/mL) agar using previously described incubation conditions and colony-forming units (CFU) count protocols.[17] Quantitative (sputum) and qualitative cultures were performed on respiratory samples using standard techniques. After presumptive screening, *P aeruginosa* isolates were confirmed by duplex real-time (RT) PCR assays.[11] *P aeruginosa* isolates were genotyped using enterobacterial repetitive intergenic consensus-PCR and clusters analysed alongside a previously characterised panel of Australian shared *P aeruginosa* strains.[11]

Statistical analysis and modelling of airborne *P aeruginosa*

Continuous variables are reported as mean and SD, and binary variables as frequency and percentage. Analyses were performed using χ^2 test, one-way and mixed-effects analysis of variance with subjects as the random factor, followed by the least significant difference procedure. Repeatability was estimated by intraclass correlation using a two-way mixed effects analysis of variance. CFU data were transformed as log(X+1), and their means and 95% CI were back transformed to the original units for ease of interpretation. We also performed a sensitivity analysis to account for the potential 'stacking' of organisms in the cascade impactor (see online supplementary methods). *P aeruginosa* decay over time was assessed using the Duration Rig data (5, 15 and 45 min). The half-life of airborne *P aeruginosa* was estimated from the slope of the decay curve. A model (see online supplementary methods) estimated the relative effects of ventilation and airborne biological decay on *P aeruginosa*

removal in different healthcare settings, including the study site.[20 25] Ventilation rates were expressed in the standard units of air changes per hour (ACH), which describe how many times a volume of air equal to the volume of a room enters each hour.

RESULTS

Subjects were well matched for age, sex and height, but those with CF had lower weight, body mass index and lung function (table 1). All subjects completed the cough manoeuvres safely. Four patients with CF were studied during a pulmonary exacerbation. Within the CF group there was no significant change in spirometry between study days (intraclass correlation coefficient (95% CI) 0.79 (0.48 to 0.92)). Similarly, total CFUs for *P aeruginosa* in cough aerosols, cough counts and log *P aeruginosa* sputum concentration within subjects were highly repeatable (see online supplementary table S1).

Table 1 Demographic, clinical and microbiological characteristics of the study subjects at time of initial cough aerosol experiment, according to type of subject*

Characteristic	Subjects with cystic fibrosis (N=19)	Healthy control subjects (N=10)	p Value
Age—years	25.8±9.2	26.5±8.7	0.85
(range)	14.4–43.4	16.1–41.5	
Male sex—no. (%)	10 (52.6)	4 (40.0)	0.52
Weight (kg)	63.0±11.7	74.5±16.1	0.04
Height (cm)	169.6±6.7	173.3±1.9	0.30
Body mass index	21.8±3.1	24.6±2.9	0.03
FEV_1			
Value (L)	2.03±0.70	3.80±0.80	<0.001
Percent of predicted value	53.9±19.0	96.1±11.5	<0.001
FVC			
Value (L)	3.23±0.69	4.68±0.98	<0.001
Percent of predicted value	72.9±15.9	100.2±9.2	<0.001
MIP†			
Value (cmH_2O)	105.78±27.38	95.12±19.13	0.38
Percent of predicted value	116.7±30.6	108.4±18.1	0.53
MEP†			
Value (cmH_2O)	126.23±31.67	130.15±28.91	0.79
Percent of predicted value	108.5±24.0	112.7±23.3	0.72
Number of coughs per minute	26.9±18.5	37.8±23.5	0.18
Infection – no. (%)			
Pseudomonas aeruginosa	19 (100.0)	–	–
Mucoid *P aeruginosa*	15 (78.9)	–	–
Non-mucoid *P aeruginosa*	18 (94.7)	–	–
Staphylococcus aureus	5 (26.3)	–	–
Stenotrophomonas maltophilia	2 (10.5)	–	–
Trichosporon/Arxula spp.	3 (15.8)	–	–
Aspergillus spp.	3 (15.8)	–	–
Scedosporium spp.	2 (10.5)	–	–
Candida spp.	1 (5.7)	–	–
Sputum *P aeruginosa* concentration—colony-forming units per mL‡	1.37×10^8 (2.16×10^7–1.06×10^9)	–	–

*Values are means±SD unless otherwise indicated.
†Maximal static inspiratory and expiratory mouth pressures (MIPS and MEPS) were calculated for only 12/19 test subjects and 7/10 control subjects.
‡Data are presented as median values (IQR). Only 18/19 test subjects were able to expectorate sputum on the initial experimental day.

Eighteen patients with CF produced sputum and one provided a cough swab (table 1), with *P aeruginosa* isolated from all and 15/19 (79%) yielding at least one mucoid isolate. Sputum *P aeruginosa* concentrations ranged from 4.0×10^6 to 4.2×10^9 CFU/mL. Genotypic analysis revealed 12 strains shared commonly within the Australian CF community[11]; AUST-01 (1), AUST-02 (7), AUST-06 (2), AUST-07 (1) and AUST-19 (1), and seven unique strains. Five patients harboured two different strains. Other organisms cultured are detailed in table 1.

P aeruginosa was cultured in cough aerosols from all patients with CF, but not from any controls. Viable *P aeruginosa* was isolated at 4 m from 17/18 (94%) and at all distances from 16/18 (89%) of the CF group (figure 1). Aerosolised *P aeruginosa* remained viable for 45 min from 14/18 (78%) subjects in the duration experiments (figure 2). Most viable *P aeruginosa* isolates were cultured from particles <3.3 µm (table 2) and the strains cultured from cough aerosols were genotypically indistinguishable from those isolated in respiratory samples from the same patient. *Stenotrophomonas maltophilia* and *Arxula adeninivorans* were also cultured in cough aerosols and sputum from two patients. No other respiratory pathogens cultured in sputum were isolated from cough aerosols. No *P aeruginosa* or other microbes identified in cough aerosols were isolated from surface swabs or blank aerosol samples taken during the studies (see online supplementary methods).

Marked intersubject heterogeneity was seen in the total numbers of viable *P aeruginosa* cultured from cough aerosols (figure 1; see online supplementary table S2). Measured *P aeruginosa* CFUs decreased with the distance the aerosol travelled and with the ageing time; CFUs were significantly higher at 1 m than 4 m, and at 5 min than 45 min (table 2). CFUs remained significantly higher for both distance and duration when corrected for the effects of stacking (see online supplementary table S3). In both distance and duration studies, strong correlations were seen between total, small and large fraction cough aerosols (r=0.90–1.0). A positive correlation was found between sputum and aerosol *P aeruginosa* CFUs in both distance (r=0.73–0.78) and duration (r=0.85–0.90; see online supplementary table S4) studies, although there was no significant correlation found between clinical or demographic variables and cough aerosols produced in either study. Correlations for these variables were unchanged when measured CFUs were corrected for stacking (see online supplementary table S5)

The influences of biological decay and room ventilation on *P aeruginosa* in cough aerosols were modelled (see online supplementary methods). Environmental conditions in the Duration Rig were similar to those in the air-conditioned clinical setting. The number of viable *P aeruginosa* in cough aerosol followed an exponential decay with a half-life of 50 min (95% CI 30 to 151 min). Room ventilation removed additional viable *P aeruginosa* and became the dominant mechanism of removal at ventilation rates above the biological decay rate of the cough aerosol (ie, approximately 0.85 ACH). Figure 3 shows the time taken to achieve 10, 50, 90, 99 and 99.9% reductions in airborne *P aeruginosa* following the departure of a source patient, including the combined effects of biological decay and room ventilation. At 2 ACH, 90% of viable airborne *P aeruginosa* are removed in approximately 49 min (95% CI 41 to 61 min), decreasing as ventilation rate increases to approximately 24 min (95% CI 22 to 26 min) at 5 ACH and to 13 min (95% CI 12 to 13 min) at 10 ACH.

DISCUSSION

Cross-infection between patients with CF can adversely affect health outcomes, but how this occurs and the transmission

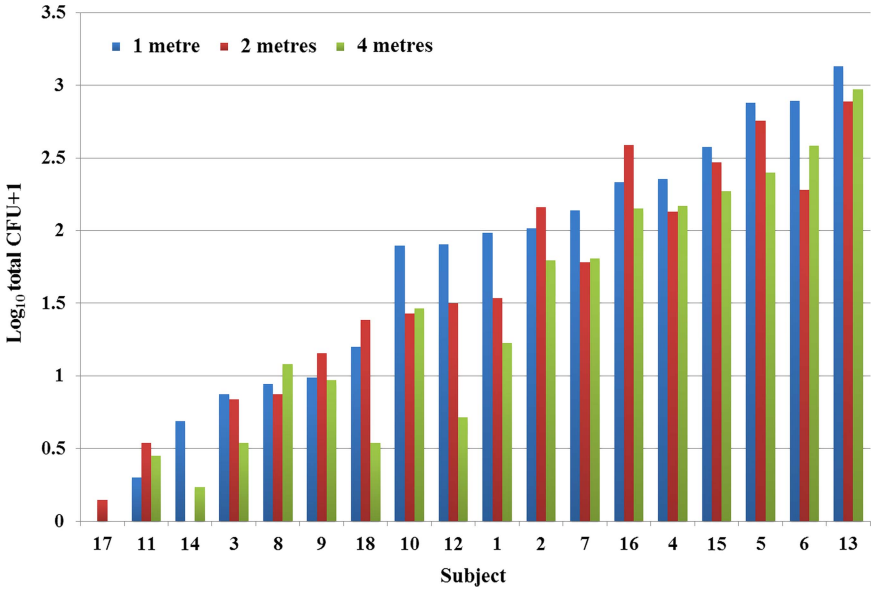

Figure 1 Log_{10} combined total colony-forming unit counts of *Pseudomonas aeruginosa* isolated at 1, 2 and 4 m from the Distance Rig cough aerosol cultures of subjects with cystic fibrosis.

pathways involved remain uncertain. We have shown that patients with CF produce aerosols containing viable *P aeruginosa* during coughing. Particles within the respirable range persist over distance and time, providing a potential vehicle for airborne transmission between patients. Marked intersubject heterogeneity of aerosol production was observed, suggesting that some patients may be 'super-spreaders' of *P aeruginosa*. Finally, sputum *P aeruginosa* concentration was a strong predictor of viable cough aerosol concentrations.

Evidence of highly prevalent shared *P aeruginosa* strains within CF populations suggests person-to-person transmission.[10 11 26] Using air sampling equipment both in the clinical setting, viable *P aeruginosa* have been demonstrated close to patients with shared strain *P aeruginosa* infection.[18 19] The clinical significance of these findings was unclear and led us to study the size distribution of cough aerosols, and we have previously demonstrated *P aeruginosa* can be present in aerosols generated by coughing.[17] However, we were unable to determine how far these aerosols travelled or the duration they remained viable in the airborne phase. An *in vitro* aerosol production system has demonstrated that artificially produced *P aeruginosa* aerosols can survive up to 45 min and that survival was greater with mucoid *P aeruginosa*.[20 21] The present study confirms aerosols produced during coughing by infected patients contain viable *P aeruginosa* that travel up to 4 m and stay suspended for at least 45 min. These data add to existing evidence that airborne transmission is likely to play a role in patients with CF acquiring *P aeruginosa*.

Our study demonstrates that droplet nuclei, generated by coughing, and containing viable *P aeruginosa*, are small enough to stay airborne for extended periods and over long distances. They are also well within the size range to deposit within the tracheobronchial tree following inhalation.[27] Such droplets can be efficiently disseminated to locations far from their origin by mechanical room air ventilation systems, which often recirculate up to 80–90% of air used for heating or cooling. The role of room ventilation as a potentiator of airborne transmission of microbes such as *M tuberculosis*, measles, SARS-coronavirus and varicella zoster is well documented.[4]

Published guidelines for ventilation in hospitals suggest that 2 ACH of fresh (ie, non-recirculated) air are required for clinic rooms and general wards, 3 ACH are recommended for procedure rooms and 12 ACH are needed for isolation rooms.[28 29] We have previously published results of measuring ACH in various

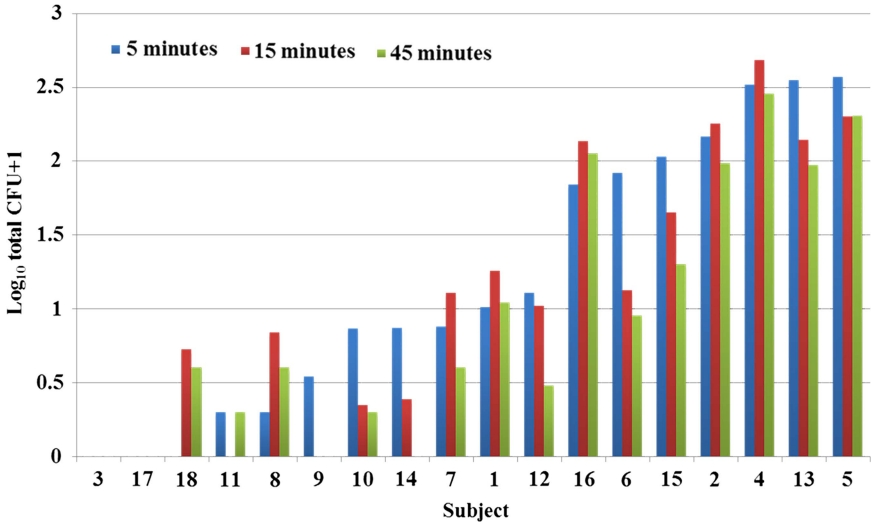

Figure 2 Log_{10} combined total colony-forming unit counts of *Pseudomonas aeruginosa* isolated after 5, 15 and 45 min from the Duration Rig cough aerosol cultures of subjects with cystic fibrosis.

Table 2 Colony-forming unit (CFU) counts of *Pseudomonas aeruginosa* isolated from the cough aerosol cultures of subjects with cystic fibrosis (CFUs in total aerosol, and in large and small particle fractions are shown)*

Distance	1 m	2 m	4 m	ANOVA p value†
Total‡	52.6 (40.9–67.6)[a]	37.3 (28.9–48.0)[a]	24.8 (19.2–32.0)[b]	0.001
Large fraction§	27.4 (21.4–34.9)[a]	19.9 (15.5–25.5)[a]	12.6 (9.7–16.2)[b]	<0.001
Small fraction¶	27.6 (21.2–35.9)[a]	19.6 (15.0–25.5)[ab]	14.3 (10.9–18.7)[b]	0.005
Duration	**5 min**	**15 min**	**45 min**	
Total‡	14.6 (11.0–19.2)[a]	11.9 (8.9–15.7)[a]	7.7 (5.4–11.0)[b]	0.046
Large fraction§	4.2 (3.1–5.5)[a]	3.9 (2.9–5.2)[a]	2.3 (1.5–3.4)[a]	0.074
Small fraction¶	11.9 (9.1–15.6)[a]	9.0 (6.7–11.8)[ab]	6.2 (4.3–8.8)[b]	0.031

*Values are means (95% CI). Within sets and rows, a different superscript letter ([a] or [b]) denotes a significant difference ($p<0.05$) between other distances or durations.
†ANOVA for trend across distance or duration.
‡Total CFU counts represent the *P aeruginosa* CFUs isolated from all six Andersen Impactor stages (aerosol particles sizes 0.65 to >7.0 μm).
§Large particle fraction CFU counts represent the *P aeruginosa* CFUs isolated from stages 1, 2 and 3 (aerosol particle sizes >7, 4.7–7.0 and 3.3–4.7 μm, respectively) of the Andersen Impactor.
¶Small particle fraction CFU counts represent the *P aeruginosa* CFUs isolated from stages 4, 5 and 6 (aerosol particle sizes 2.1–3.3, 1.1–2.1 and 0.65–1.1 μm, respectively) of the Andersen Impactor.

clinical locations within TPCH and found that these meet or exceed recommended levels in most cases, with an extreme example of almost 24 ACH in an isolation room in the emergency department.[25] However, the data from the present study suggest that a clinic room with the recommended two ACH may require almost an hour after a patient harbouring *P aeruginosa* leaves the room before 90% of the viable bacteria are removed from the air (see online supplementary methods). These data have substantial implications for CF infection control practices. Our modelling suggests that increasing room ventilation provides some benefit; however, the time required to remove 90% of potentially infectious aerosols still exceeds 10 min despite substantial increases in ACH (figure 3). A new paradigm may be needed, such as employing within CF centres advanced ventilation and ultraviolet germicidal irradiation (UVGI) techniques to achieve infection control, as is done for TB clinics.[30 31]

This study has several important limitations. The infectious inoculum required to establish *P aeruginosa* infection in CF is unknown and may vary between patients.[20] Thus, it is impossible to estimate to what extent viable aerosols of *P aeruginosa* need to be removed to eliminate the risk of infection in susceptible patients. In many CF clinical settings, contact and droplet infection control measures have been implemented, but reduced rates of shared *P aeruginosa* strain infections have been reported only in centres employing strict cohort segregation based upon molecular typing surveillance.[15] It therefore remains uncertain whether these simpler measures are sufficient, and if preventing airborne transmission should also be considered when strict segregation of large patient cohorts attending major CF centres is not always feasible.[14]

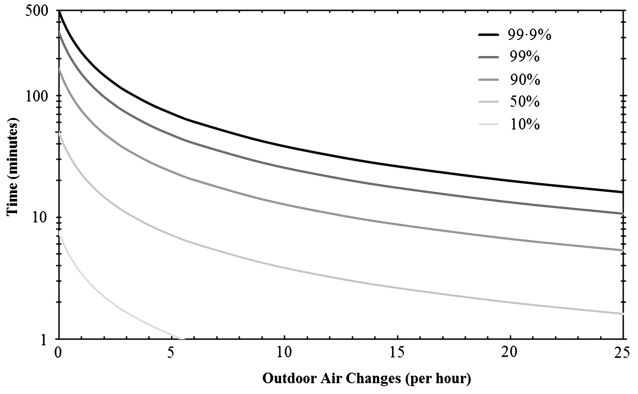

Figure 3 Time taken to achieve 10, 50, 90, 99 and 99.9% reductions in airborne *Pseudomonas aeruginosa* due to the combined effects of biological decay and room ventilation.

Our study included a small number of older patients with chronic *P aeruginosa* infection and moderately severe airflow obstruction. Thus, we cannot comment on viable aerosol production during coughing by younger or healthier patients or in those with transient *P aeruginosa* infections. In addition, we used culture media that selected for aerobic gram-negative bacteria and we cannot provide data on other microbes, including *Staphylococcus aureus*, non-tuberculous mycobacteria or respiratory viruses.[14 32 33] Future studies using other selective media to determine the viability of other microorganisms in cough aerosols are now required. Due to bactericidal effects, it is also possible that the bacitracin used in the culture media reduced the recovery of some *P aeruginosa* organisms. Finally, modelling data assume that the room air is perfectly mixed, which is rarely the case. As such, our estimates of the time taken to remove viable *P aeruginosa* from a clinic room may be overestimates or underestimates of real-world scenarios (see online supplementary methods).

The present study uses bacterial infection in patients with CF to demonstrate the potential for aerosol transmission in healthcare settings and adds to studies suggesting similar mechanisms for *M tuberculosis* and for *B pertussis* in non-human primate models.[5 6] Our results provide further evidence, which challenges the dogma that CF bacterial pathogens within droplet nuclei have limited survival in air. Indeed, airborne transmission may be a more important mechanism of cross-infection within the CF community than previously considered to be the case. Furthermore, our data add to the controversy generated by the current USA CF Foundation guidelines that only one person with CF attend a CF Foundation-sponsored or CF Centre-sponsored event[34]; recommendations strongly opposed by some adults with CF.[35]

In summary, this study indicates that when patients with CF harbouring *P aeruginosa* cough, they produce an aerosol containing viable organisms capable of travelling 4 m and persisting in the air for 45 min. These data challenge current CF infection control guidelines that remain in place in many parts of the world, including the separation of patients by 1–2 m to prevent cross-infection by respiratory bacteria.[13 36] Consequently, further studies on transmission are needed and infection control

practices recommended currently for the CF community may need to be re-evaluated.

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Contributors LDK, GRJ, TJK, LM and SCB contributed equally to the work. LDK, GRJ, KG, PDS, CEW, LM and SCB conceived the study and led the funding application. All authors contributed to the experimental design and management of the study. LDK and GRJ supervised by LM led the development and verification of the sampling Rigs and collection of the cough aerosol samples. JC, MW, CEW and SCB contributed to subject recruitment and collection of clinical data and samples. TJK, JAK and KAR led the microbiological sampling, culture, identification and genotyping. PKO led the statistical analyses. Manuscript was written by LDK, TJK, PDS and SCB, with input from coauthors. SCB had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests None.

Patient consent Obtained.

Ethics approval Royal Children's Hospital Human Research Ethics Committee, The University of Queensland Medical Research Ethics Committee, Queensland University of Technology Human Research Ethics Committee.

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ONLINE SUPPLEMENTARY MATERIAL

VIABILITY OF *PSEUDOMONAS AERUGINOSA* IN COUGH AEROSOLS GENERATED BY PERSONS WITH CYSTIC FIBROSIS.

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METHODS

Recruitment

Subjects with CF were recruited from the two CF centres by personal invitation from the Investigators (SCB, CEW) or the Study Coordinators (MEW, JC). All CF patients were >12-years of age, had chronic *Pseudomonas aeruginosa* infection and at least one positive sputum culture in the prior 12-months. The majority of patients were studied during clinical stability, however, four patients were studied close to the completion of intravenous antibiotics for logistic reasons (e.g. travelling long distance, avoid missing school attendance). Healthy control participants were recruited from contact with staff at both healthcare facilities including relatives of the staff for the younger controls. Nineteen CF patients with CF and 10 healthy controls participated. Two patients and two controls performed only one of the experiment days.

Distance of cough aerosols: 'Distance Rig'

The Distance Rig consisted of an expandable wind tunnel that allowed high-efficiency particulate air (HEPA)-filtered air to be introduced upstream of the subject;[1] ensuring unidirectional air flow of particle-free air and permitting measurement of cough aerosols at distances of 0 to 4-metres from the subject without interference from other particle sources (Figure E1). The air velocity was maintained at 0.1-metres/sec, typical of a mechanically-ventilated indoor environment, such as a hospital.[2-4] The Rig was positively pressurized to prevent room air contamination.

Viable aerosol and total particle sampling methods

A six-stage Andersen Impactor (Thermo Scientific, Franklin, MA) captured and sized viable cough aerosols in the six stages between 0.6 and >7 μm.[5] A vacuum pump was used to

draw 28.3 L/min of air through the Impactor. The pump flow was checked daily using a rotameter, and all Andersen Impactor O-rings were inspected for wear. Exhaust air from the pump was HEPA-filtered.

A Lasair II-110 (Particle Measuring Systems, Boulder, CO) optical particle counter (OPC) measured real-time particle concentration in six channels between 0.1 and >5 µm using a sample flow of 28.3 L/min, permitting detection of very low concentrations. The OPC verified that there were no room air particles present before each test, subjects' lungs were free of residual room air, and no contamination of aerosols occurred during testing. It also confirmed that the total particle concentration in the Rig was <0.01 particles per cm^3 (p/cc) prior to the subject coughing, which was approximately 10^4 times lower than the room air concentration and similar to an ISO 4 cleanroom.

The Andersen Impactor and OPC samples were collected through a common 12.5 cm circular, sharp-edged isokinetic inlet. It was set parallel to the airflow and provided 100% particle aspiration efficiency at the tunnel air velocity of 0.1 metres/sec.[6] Smoke visualization tests confirmed sample extraction by the inlet was uniform. One metre of conductive tubing transported samples from the inlet to the Andersen Impactor and OPC. The tubing residence time was 1-sec for Andersen samples and 0.06-sec for OPC samples. Sample losses due to gravitational settling and inertial impaction were from 0 to 8% and 0 to 1%, respectively, and diffusion losses were negligible.[6]

Air velocity was monitored continuously using a 9535 hot-wire anemometer (TSI Inc., St. Paul, MN). Temperature and water vapour concentration were measured simultaneously

upstream of the participant and at the sampling inlet by HC2-CO4 probes and a HygroLog NT data logger (Rotronic AG, Bassersdorf).

Duration of cough aerosols: 'Duration Rig'

A 0.4 metre^3 airtight stainless steel cylinder was used for collecting and aging cough aerosols to assess viability in the airborne phase (Figure E2). A variable-speed drive was used to set the rotation rate (1.7 rpm) of the Rig, which minimized gravitational settling and inertial impaction of particles.[7, 8] The system was flushed with HEPA-filtered air before sample collection so that cough aerosols were prevented from being contaminated by room air aerosols.

Viable aerosol and total particle sampling methods

The Andersen Impactor and OPC described previously were used during sampling. We estimate over 95% of cough aerosol particles in the size range of the Andersen Impactor remained airborne during the aging period.[9]

An 8-cm circular inlet was positioned 30-cm inside the Rig along the rotation axis. This provided 100% aspiration efficiency of particles in the Andersen and OPC size ranges.[6] Samples were transported via straight conductive tubing, with residence times of 0.8 sec for Andersen samples and 0.09 sec for OPC samples. Sample losses across the Andersen Impactor size range due to gravitational settling and inertial impaction were from 0 to 6% and 0 to 1%, respectively. Temperature and water vapour concentration were measured simultaneously inside and outside of the Duration Rig by the same probes described above.

Before each test, the Duration Rig was flushed with HEPA-filtered air using a 3M Air-Mate respirator (St. Paul, MN). Once the OPC confirmed a particle concentration <0.01 p/cc, a 5-minute blank sample was taken by the Andersen Impactor. The Air-Mate provided filtered air to replace that extracted by the pump. The OPC confirmed the Rig was free of contamination, and the Rig was then isolated by entry and exit valves.

Cleaning and quality assurance

The Distance and Duration Rigs were disinfected thoroughly at the end of each study day using 0.15% (w/v) benzalkonium chloride (Glitz^{TM}, Pascoe's Pty Ltd, Australia), followed by 70% (v/v) ethanol. HEPA-filtered air dried both devices. Non-disposable items, collection tubes, inlet valves and other rigging devices were sterilized using standard hospital procedures. Andersen Impactors were decontaminated with 70% (v/v) ethanol and air dried. Surface swabs were collected into Amies Agar Gel Transport Medium (COPAN Diagnostics Inc., CA, USA) and blank aerosol samples were collected at the start and end of each day. For Duration studies, additional blank aerosol samples were collected between each experiment.

Quality assurance surface swabs were enriched in LB broth (Sigma-Aldrich Pty Ltd, New South Wales, Australia) for 24-hours, and then subcultured onto chocolate bacitracin and colistin nalidixic acid agar (Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) at 35^0C for 72 hours, and Sabouraud agar (Thermo Fisher Scientific Australia Pty Ltd) at 28^0C for 72 hours. Blank aerosol samples were incubated aerobically at 35^0C for 72-hours.

Modelling of airborne *Pseudomonas aeruginosa*

To estimate the time taken to remove airborne *P. aeruginosa* following the departure of a source patient, we used a simple model based on the airborne biological inactivation rate we measured experimentally combined with a range of room ventilation rates.[10] This approach assumed that these were the two major mechanisms by which airborne *P. aeruginosa* was removed; biological inactivation (i.e. 'die-off') and dilution with room ventilation air.[10] Our modelling focussed solely on the risk posed by airborne cough aerosol droplet nuclei containing *P. aeruginosa*, as distinct from contact or droplet transmission, as this is the transmission mode affected by these removal processes.

The role of airborne biological decay and room air ventilation was considered to be negligible in determining the fate of particles in the size range collected by the first (i.e. 'input') stage of the viable sampler. This input stage collects particles >7μm and such droplet nuclei were not considered to fall within the airborne size range (Dp <5μm) for the purposes of this study. The decision to exclude the larger droplet nuclei is further supported by the expectation that droplet nuclei form initially as much larger droplets before drying to their equilibrium size and collection by the viable sampler. That initial size is approximately twice the diameter of the droplet residue collected by the viable sampler.[11] The smaller surface area to mass ratios of these larger particles results in the droplets' movement and fate being dominated by momentum acquired within the cough exhalation jet and the influence of gravity immediately after the cough. Because of these influences the larger droplets and droplet residues tend to impact on surfaces during, or soon after, the cough event so that they do not remain airborne long enough to have their fate altered significantly by the ventilation and room air currents.[12-14] Hence, deposition of particles is not incorporated into airborne models. While we observed small numbers of viable particles on the input stage of the viable sampler

following extended storage, this was mostly due to the counteraction of the gravitational settling process provided by the Duration Rig.[9] Thus, under 'real world' conditions, particles in the size range of the Andersen Impactor input stage would deposit due to gravitational settling very shortly after their release, in contrast to those in the airborne range, which remain suspended for extended periods.[14] We therefore considered the particles collected on the input stage to not be relevant to our modelling.

Empirically, the effect of ventilation on the concentration of airborne pathogens, or indeed any particle, over time follows a first-order exponential decay.[11, 15] Likewise, the biological inactivation of a pathogen in response to environmental challenges is represented in the same manner.[11] Therefore, the concentration of *P. aeruginosa* at a given point in time can be calculated using equation 1:

$$\log_e(N_t) = \log_e(N_0) - (k_{bio} + k_{vent}) \times t \qquad (1)^1$$

Where:

N_t $= P.aeruginosa$ concentration at time t (CFU L^{-1})

N_0 $=$ $P.aeruginosa$ concentration at time 0 (CFU L^{-1})

k_{bio} $=$ biological inactivation rate of $P.aeruginosa$ (h^{-1})

k_{vent} $=$ removal of $P.aeruginosa$ due to room ventilation rate (ACH^{-1})

t $=$ time (h)

A practical example of when to employ such models is when seeking to adhere to the US Centers for Disease Control and Prevention recommendation of allowing sufficient time for ≥99% removal of airborne contaminants following the departure of a patient with suspected or confirmed tuberculosis from a room before another patient enters.[16]

Similarly, the model can help determine the time required to achieve a specified reduction in airborne *P. aeruginosa* in a CF clinic setting by taking into account the known room ventilation rate. Figure 3 in the paper shows that it would take approximately 50-min to achieve this removal, due to combined effect of ventilation and biological inactivation, in a clinic room ventilated at the guideline rate of two ACH.[17-19] Figure 3 also shows the time taken to remove specified amounts of *P. aeruginosa* at the ventilation guidelines prescribed for other clinical settings.[17]

In addition to assuming that ventilation and biological decay are the major mechanisms which remove airborne *P. aeruginosa* from room air, we made two other key assumptions that underpin equation 1 and its use that affect the accuracy of our predictions. Firstly, and most importantly, the pathogen is assumed to be to be perfectly mixed with the room air, which is almost never the case in practice.[17] There are regions where the concentration can be higher or lower than that assumed under perfect mixing. For example, this could occur due to proximity of the pathogen source or a room ventilation inlet, respectively. However, this assumption is characteristic of all the classic airborne transmission models.[17] If a conservative approach to infection control is required, then it is appropriate to err towards a greater amount of pathogen removal, such as 90%, to account for this when determining how long to wait before the next person enters a room.

Secondly, as the majority of ventilation air is usually recirculated it is prudent to base estimates of room clearance on only the outdoor air component of ventilation air, which is the method we have used to present the estimates in this paper.[20] For example, if 25% of air supplied to a room is ‘fresh’ outdoor air and the total ventilation rate is 8 ACH, then the outdoor air exchange rate is 2 ACH and this is the appropriate figure to use when modelling

or reading off Figure 3 in the paper. Likewise, ventilation guidelines for healthcare settings often specify a minimum total ventilation rate and the proportion that must be outdoor air (e.g. 6 ACH total with at least 2 ACH outdoor). In assessing a room ventilated at these guideline values, the ventilation rate would be set to 2 ACH, rather than 6. This approach considers all recirculated ventilation air as contaminated and incapable of diluting the concentration of a pathogen. While this may not be the case in practice, particularly where recirculated air is filtered, it allows for conservative exposure risks to be determined. This method has its basis in the enduring work of Riley and colleagues in revisiting the earlier work of Wells, and developing what is now known as the Wells-Riley equation for estimating the probability of airborne pathogen transmission indoors.[21, 22] As the infectious inoculum of *P. aeruginosa* is unknown, it is appropriate to employ this conservative approach to infection control.

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Table S1. Repeatability of log transformed combined total colony-forming units (CFU) of *Pseudomonas aeruginosa*, total number of coughs, FEV1 actual and log_{10} sputum *P. aeruginosa* in subjects with cystic fibrosis.

	Experiment	Repeatability	95% CI
Combined total	Distance	0.92	0.86 - 0.97
	Duration	0.89	0.79 - 0.96
Total number of coughs	Distance	0.95	0.90 - 0.98
	Duration	0.95	0.91 - 0.98
FEV_1 actual		0.79	0.48 - 0.92
log_{10} sputum *P. aeruginosa*		0.71	0.35 - 0.89

Table S2. Comparison of between subjects, within subjects, duplicates and ratios for log transformed small, large and combined total particle fraction colony-forming units (CFU) of *Pseudomonas aeruginosa* isolated from the cough aerosol cultures of subjects with cystic fibrosis and the number of coughs per minute in distance and duration experiments.

Characteristic	Between subjects	Within subjects	Duplicates	Between/Within Ratio	Between/Duplicates Ratio
Distance					
Small	21.5	0.6	0.2	38.1	99.4
Large	21.8	0.5	0.2	44.7	113.9
Combined total	24.6	0.5	0.3	46.5	112.8
Number of coughs	1245.6	20.6	6.6	60.6	189.9
Duration					
Small	16.8	0. 6	0.3	32.9	56.6
Large	9.8	0.5	0.2	22.3	47.1
Combined total	17.8	0.6	0.3	31.9	69.4
Number of coughs	1523.3	14.8	20.5	100.6	74.3

Table S3. Colony-forming unit (CFU) counts of *Pseudomonas aeruginosa*, corrected for stacking, isolated from the cough aerosol cultures of subjects with cystic fibrosis. CFUs in total aerosol, and in large and small particle fractions are shown*.

Distance	1 metre	2 metres	4 metres	ANOVA P-value†
Total‡	59.3 (45.6 – 77.0)[a]	39.4 (30.2-51.3)[b]	26.3 (20.1-34.3)[c]	0.001
Large fraction§	30.2 (23.4-38.9)[a]	21.0 (16.2-27.1)[b]	13.2 (10.1-17.2)[c]	<0.001
Small fraction‖	31.7 (24.0-41.7)[a]	20.8 (15.6-27.5)[b]	15.3 (11.5-20.3)[b]	0.003
Duration	5 minutes	15 minutes	45 minutes	
Total‡	15.2 (11.4-20.1)[a]	12.3 (9.2-16.4)[a]	7.9 (5.5-11.3)[b]	0.043
Large fraction§	4.3 (3.2-5.7)[a]	4.0 (2.9-5.3)[a]	2.3 (1.5-3.4)[a]	0.073
Small fraction‖	12.5 (9.5-16.5)[a]	9.3 (7.0-12.3)[ab]	6.4 (4.4-9.1)[b]	0.029

* Values are means (95% CI). Within sets and rows a different superscript letter ([a] or [b] or [c]) denotes a significant difference (P<0.05) between other distances or durations.

† ANOVA for trend across distance or duration

‡ Total CFU counts represent the *P. aeruginosa* CFUs isolated from all six Andersen Impactor Stages (aerosol particles sizes 0.65 to >7.0 µm).

§ Large particle fraction CFU counts represent the *P. aeruginosa* CFUs isolated from Stages 1, 2 and 3 (aerosol particle sizes >7, 4.7-7.0 and 3.3-4.7 µm, respectively) of the Andersen Impactor.

‖ Small particle fraction CFU counts represent the *P. aeruginosa* CFUs isolated from Stages 4, 5 and 6 (aerosol particle sizes 2.1-3.3, 1.1-2.1 and 0.65-1.1 µm, respectively) of the Andersen Impactor.

Table S4. Correlation between clinical characteristics, number of coughs performed, sputum *Pseudomonas aeruginosa* concentration and log transformed combined total colony-forming units of *P. aeruginosa* isolated from the cough aerosol cultures of subjects with cystic fibrosis.

Characteristic	Pearson correlation coefficient					
	1 metre	2 metres	4 metres	5 minutes	15 minutes	45 minutes
Age	-0.05	-0.2	-0.25	0.02	-0.02	-0.11
Body-mass index	-0.37	-0.46	-0.36	-0.16	-0.19	-0.24
FEV_1 Value - litres	0.08	0.22	0.21	-0.12	0.08	0.06
FEV_1 Percent of predicted value	0.09	0.25	0.22	0.02	0.24	0.23
FVC Value - litres	0.05	0.13	0.16	-0.22	-0.07	-0.11
FVC Percent of predicted value	0.07	0.2	0.2	0	0.18	0.17
MIP Value - cmH2O	-0.1	-0.14	-0.16	-0.29	-0.33	-0.36
MIP Percent of predicted value	-0.31	-0.26	-0.28	-0.34	-0.35	-0.27
MEP Value - cmH2O	0.27	0.29	0.22	0.12	0.14	0.05
MEP Percent of predicted value	-0.15	-0.02	-0.09	-0.22	-0.12	-0.06
Number of coughs performed	-0.05	0.04	-0.09	0.23	0.26	0.26
Sputum *P. aeruginosa* concentration	0.73*	0.73*	0.78†	0.90†	0.85†	0.85†

* Correlations achieving statistical significance of 0.01.

† Correlations achieving statistical significance of <0.01.

Table S5. Correlation between clinical characteristics, number of coughs performed, sputum *Pseudomonas aeruginosa* concentration and log transformed combined total colony-forming units of *P. aeruginosa* corrected for stacking isolated from the cough aerosol cultures of subjects with cystic fibrosis.

Characteristic	Pearson correlation coefficient					
	1 metre	2 metres	4 metres	5 minutes	15 minutes	45 minutes
Age	-0.06	-0.20	-0.26	0.02	-0.02	-0.10
Body-mass index	-0.37	-0.46	-0.36	-0.16	-0.19	-0.23
FEV_1 Value - litres	0.08	0.22	0.21	-0.12	0.07	0.06
FEV_1 Percent of predicted value	0.10	0.25	0.22	0.03	0.23	0.22
FVC Value - litres	0.05	0.13	0.16	-0.22	-0.08	-0.12
FVC Percent of predicted value	0.08	0.20	0.21	0.00	0.17	0.16
MIP Value - cmH2O	-0.10	-0.14	-0.16	-0.29	-0.33	-0.36
MIP Percent of predicted value	-0.28	-0.26	-0.26	-0.33	-0.35	-0.27
MEP Value - cmH2O	0.24	0.27	0.20	0.12	0.14	0.05
MEP Percent of predicted value	-0.13	-0.01	-0.08	-0.21	-0.12	-0.06
Number of coughs performed	-0.05	0.04	-0.09	0.24	0.26	0.26
Sputum *P. aeruginosa* concentration	0.71*	0.73*	0.77†	0.90†	0.85†	0.85†

* Correlations achieving statistical significance of 0.01.

† Correlations achieving statistical significance of <0.01.

Figure S1. Schematic diagram of the Distance Rig.

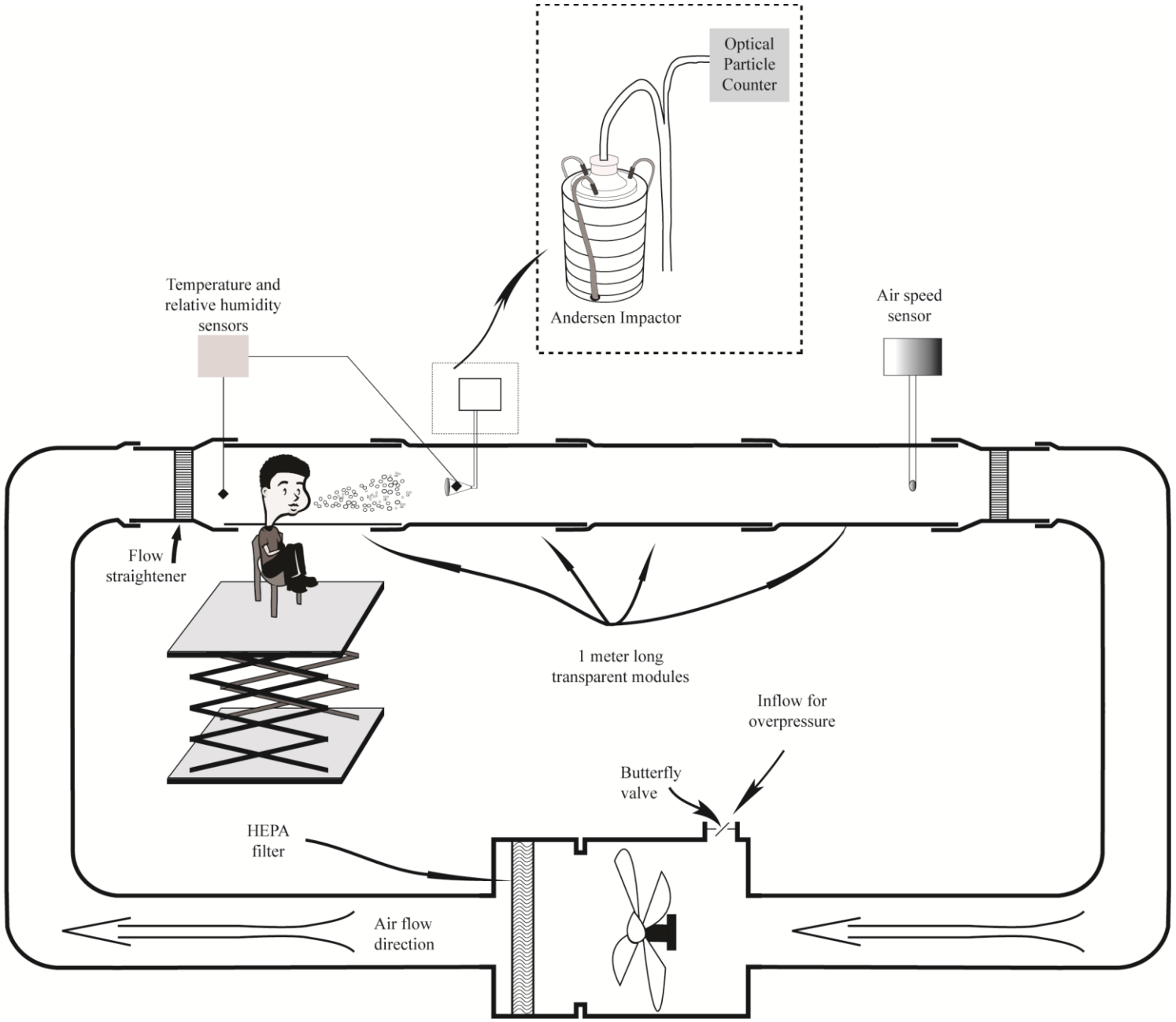

Figure S2. Schematic diagram of the Duration Rig.