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Face masks and cough etiquette reduce the cough aerosol concentration of *Pseudomonas aeruginosa* in people with cystic fibrosis

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At a Glance Commentary.

Scientific Knowledge on the Subject: *Pseudomonas aeruginosa* is the dominant airways infection in people with cystic fibrosis (CF). People can harbor genetically indistinguishable strains of *P. aeruginosa*, which suggests that cross infection may be an important mode of transmission, although the mechanisms are not well understand. Droplet nuclei containing *P.*

aeruginosa produced during coughing can remain viable for extended periods, raising the possibility of airborne transmission. The CF Foundation recommends that people with CF wear a surgical mask in communal areas to reduce pathogen acquisition and transmission.

What This Study Adds to the Field: This comparative observational study demonstrated that surgical masks and the N95 masks are effective in reducing aerosols containing viable *P. aeruginosa* 2-metres from source during coughing in people with CF. Short-term use of face masks was well tolerated in people with CF lung disease, with the surgical mask rated more comfortable than the N95 mask. Cough etiquette reduced viable aerosols to a lesser extent than face masks.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

ABSTRACT

Rationale: People with cystic fibrosis (CF) generate *Pseudomonas aeruginosa* in droplet nuclei during coughing. The use of surgical masks has been recommended in healthcare settings to minimise pathogen transmission between CF patients.

Objective: To determine if face masks and cough etiquette reduce viable *P. aeruginosa* aerosolised during cough.

Methods: Twenty-five adults with CF and chronic *P. aeruginosa* infection were recruited. Participants performed six talking and coughing maneuvers, with or without face masks (surgical and N95) and hand covering the mouth when coughing (cough etiquette) in an aerosol-sampling device. An Andersen Impactor sampled the aerosol at 2-meters from each participant. Quantitative sputum and aerosol bacterial cultures were performed and participants rated the mask comfort levels during the cough maneuvers.

Measurements and Main Results: During uncovered coughing (reference maneuver), 19/25 (76%) participants produced aerosols containing *P. aeruginosa*, with a positive correlation found between sputum *P. aeruginosa* concentration (CFU/mL) and aerosol *P. aeruginosa* CFUs. There was a reduction in aerosol *P. aeruginosa* load during coughing with surgical mask, coughing with N95 mask and cough etiquette compared with uncovered coughing ($p<0.001$). A similar reduction in total CFUs was observed for both masks during coughing, yet participants rated surgical masks more comfortable ($p=0.013$). Cough etiquette provided approximately half the reduction of viable aerosols of the mask interventions during voluntary cough. Talking was a low viable aerosol producing activity.

Conclusions: Face masks reduce cough generated *P. aeruginosa* aerosols, with the surgical mask providing enhanced comfort. Cough etiquette was less effective at reducing viable aerosols.

Word count = 248

Key words: cystic fibrosis; infection control; surgical mask, N95 mask; cough etiquette

INTRODUCTION

Pseudomonas aeruginosa is the dominant pathogen in the airways of people with cystic fibrosis (CF), with a prevalence of up to 70% in adults (1-3). Chronic *P. aeruginosa* infection is associated with pulmonary function decline, increased exacerbations, poorer health-related quality of life and reduced survival (4-6). Studies have demonstrated that unrelated people with CF can harbor genetically indistinguishable strains, suggesting person-to-person spread of *P. aeruginosa* (7-11). Consequently, CF infection control guidelines published in 2003 sought to minimise potential contact and droplet transmission of pathogens and recommended cohort segregation according to microbiological status, single room inpatient accommodation and a separation distance of 1-meter between people with CF (12). Although such measures are thought to have contributed to a reduction in epidemic *P. aeruginosa* (13) and *Burkholderia cenocepacia* strain acquisition (14), cross infection with CF pathogens has continued (11, 15, 16).

Airborne transmission of *P. aeruginosa* was first suggested as a possible mode of cross-infection by studies that assessed environmental air contamination in CF clinical care settings (17) and cough aerosols from people with CF (18). Particle size diameter is used to categorize respiratory aerosols into droplets ($>5\mu\text{m}$) and droplet nuclei ($\leq 5\mu\text{m}$); with the latter a consequence of droplet evaporation and capable of airborne transmission (19). Our earlier work demonstrated that cough generated droplet nuclei containing *P. aeruginosa* in the respirable size range remained viable for up to 45-minutes and were detected up to 4-meters from the source (20). The updated CF infection control guidelines published in 2014 recommended a separation distance of 2-meters and included the specific recommendation for people with CF to wear a surgical mask in communal areas of healthcare facilities (21).

Face masks and cough etiquette (i.e. coughing into the hand or arm) are strategies that may interrupt aerosol dispersal. The primary role of the surgical mask is to prevent contamination of the environment by infectious droplets. The relatively low efficiency capture of aerosols, particularly during cough, and incomplete seal may allow particles to escape around the perimeter (22). The N95 mask provides inward protection from inhaled airborne pathogens, and it is reasonable to also expect limitation of aerosolised infectious material generated by the wearer. To date there is limited evidence of outward protection by surgical and N95 masks and the tolerability of these interventions has not been widely studied in patients with lung disease. Therefore, this study aimed to determine and compare the effectiveness and comfort of two commonly utilized face masks and cough etiquette on dispersal of aerosolised *P. aeruginosa* during talking and coughing in adults with CF. Some of the results of this study have been previously reported in the form of an abstract (23).

METHODS

See online supplement for additional details.

Participants: Participants (n=25) were recruited from The Prince Charles Hospital (TPCH) Adult Cystic Fibrosis Centre (ACFC), Brisbane, Australia in 2015. Eligible participants had a confirmed CF diagnosis, aged ≥ 18 years and chronic *P. aeruginosa* infection as determined by modified Leed's criteria (3). Exclusion criteria comprised recent haemoptysis ($>50\text{ml}$), pneumothorax, pregnancy and cough syncope. Written, informed participant consent was obtained and the study was approved by the local ethics committee.

Aerosol sampling system: Using a validated, closed wind-tunnel system, viable aerosols were collected at 2-meters from participants (24); the sampling distance was defined in

accordance with the current US CFF Infection Prevention and Control Guidelines recommending 2-meters between-person separation (21). Seated participants positioned their head within the tunnel with a weighted material cover rested over the shoulders and a slight positive tunnel pressure to prevent room air contamination (20).

Aerosol sampling protocol: Participants completed six maneuvers on a single day: 1) talking; 2) talking wearing a tied surgical mask (Kimberley-Clark TECNOL Fluidshield Fog-Free Surgical Mask, Georgia, USA); 3) uncovered coughing (reference maneuver); 4) coughing wearing a tied surgical mask; 5) coughing wearing an N95 mask (Kimberley-Clark N95 Particulate Filter Respirator, NSW, Australia); 6) coughing with their hand covering their mouth (cough etiquette). The testing order of the uncovered cough maneuver, coughing wearing a surgical mask and coughing wearing an N95 mask were randomized to minimize potential bias in aerosol production resulting from fatigue and airway clearance with repeated coughing (25). For logistical reasons, the cough etiquette test was the final maneuver performed and participants were asked to adopt their usual mouth covering technique (a glove was worn on the cough covering hand to minimize microbial dispersal from skin). Participants rested for ≥ 20 minutes between each maneuver. Face masks were sized and applied by a trained healthcare professional.

Within the tunnel each participant completed 2-minutes of tidal breathing in high-efficiency particulate air (HEPA) filtered air to washout residual room air, then 5-minutes of the respective maneuver (talk/cough) during which aerosols were continuously sampled through a six-stage Andersen Impactor (Thermo Scientific™), followed by 2-minutes of tidal breathing. Participants then completed a 5-point comfort rating score (26) and provided comments regarding mask wear during coughing. Masks were weighed before and immediately after each maneuver.

Clinical Measurements: Demographic and clinical measurements were recorded, including: age, gender, body mass index (BMI) and intravenous antibiotic use in the week prior. A sputum sample was collected on the study day. Spirometry (FEV₁ and FVC) was performed according to ATS/ERS standards (27) and the Global Lung Index (GLI) predicted scale (28) applied.

Microbiology: Standardized qualitative and quantitative sputum cultures were performed, as previously described (20). *P. aeruginosa* identification was confirmed by oxidase testing, 42°C growth and MALDI-TOF mass spectrometry. *P. aeruginosa* genotyping was undertaken using the Sequenom iPLEX20SNP assay, as previously described (29).

Statistical analysis: A sample size calculation based on our earlier work (20) suggested 21 patients were required to demonstrate a 40 percentage point reduction in the presence or absence of *P. aeruginosa* with 80% power with a two-tailed $p=0.05$. Therefore, 25 patients with CF and chronic *P. aeruginosa* were enrolled, allowing for 10-15% dropout to achieve a sample size of 21. SPSS version 22 was used for statistical analysis. Categorical variable associations were examined using Fisher's Exact test. Continuous variables describing participant characteristics were examined using a Student t-test. Clinical and demographic variables were compared with the log transformed total *P. aeruginosa* aerosol colony forming units (CFUs) in the uncovered cough maneuver using a Pearson correlation test. The paired t-test based on log transformed CFU was used to compare each intervention maneuver with the reference maneuver (uncovered coughing). Results were stratified by the level of aerosol production as follows: participants were classed as high, low or nil viable aerosol producers during the uncovered cough maneuver using an arbitrary pre-defined total CFU of ≥ 10 or <10 , respectively, accumulated across the six-stages of the Andersen Impactor (Figure 1).

The Mann-Whitney U test was used to examine change in mask weight. McNemar's test was used for matched pairs to compare comfort scores for the two mask types.

RESULTS

Participant overview: Twenty-five (15 male) adult participants with a mean (SD) age of 31.3 (7.8) years, FEV₁ 50.7 (17.4) % predicted and BMI 22.1 (2.8) kg/m² were recruited (Table 1). Twenty-two (88%) participants were on maintenance azithromycin. Eleven (44%) participants were established on chronic inhaled antibiotic therapy (either continuous or cycling alternate month) and the remaining 14 (56%) participants had been prescribed inhaled antibiotic therapy (less frequently than alternate months) in the 12 months prior to the study. Eleven participants (44%) had received intravenous antibiotics in the week prior to their study involvement. The mean (SD) age, FEV₁ % predicted and BMI of patients attending TPOCH ACFC in 2015 was 30.7 (9.9) years, 66.7 (24.1) % predicted and 23.0 (4.2) kg/m², respectively. In the preceding calendar year, of 282 patients reviewed at TPOCH ACFC, 68.4% had chronic *P. aeruginosa* infection.

Sputum microbiology: *P. aeruginosa* was cultured from the 24 sputum samples provided at a mean concentration of 6.3×10^7 CFU/mL (95% CI, $2.6 \times 10^7 - 15.0 \times 10^7$; Table 1). Genotyping identified 12 different *P. aeruginosa* strains, including five common Australian shared strains (11); AUST-01 (n=6), AUST-02 (n=9), AUST-06 (n=6), AUST-07 (n=3), AUST-13 (n=1), five other minor shared strains, and two unique strains. Eight participants harbored more than one *P. aeruginosa* strain; two different strains were detected in seven participants, while one participant was infected with three major Australian shared strains (AUST-01, AUST-02 and AUST-06). Other CF pathogens identified in the sputum on the testing day comprised: *Stenotrophomonas maltophilia* (n=3), *Aspergillus fumigatus* (n=3),

234 *Haemophilus influenzae* (n=1) and *Burkholderia cenocepacia* (n=1). Five participants had a
235 history of intermittent *Staphylococcus aureus* in the 12 months prior to the study but *S.*
236 *aureus* was not isolated from sputum of participants on the day of testing.

237 **Aerosol sampling:**

238 **Uncovered cough maneuver.** Of the 25 participants, 19 (76%) produced aerosols containing
239 viable *P. aeruginosa* in the uncovered cough maneuver at 2-meters (Table 2). The participant
240 unable to produce a sputum sample, was one of the six who did not culture *P. aeruginosa* on
241 the cough aerosol plates. Molecular typing revealed that for 16 individuals, the *P. aeruginosa*
242 genotype(s) detected in the individual's cough aerosols were genetically indistinguishable to
243 those isolated in their matched sputum sample. Three other participants (two with one
244 indistinguishable *P. aeruginosa* genotype in their matched aerosol/sputum combination; one
245 with two indistinguishable *P. aeruginosa* strains in their matched aerosol/sputum
246 combination) were each found to have one additional *P. aeruginosa* strain in their aerosol
247 cultures that was not detected in their sputum. The three participants who had isolated *S.*
248 *malophilia* in their sputum also generated aerosols that grew this organism (confirmed by
249 MALDI-TOF).

250 When the total *P. aeruginosa* CFUs of uncovered cough aerosols was associated with
251 demographic, clinical and microbiology parameters, a statistically significant correlation was
252 identified only between log transformed sputum *P. aeruginosa* counts and total aerosol load
253 ($r=0.55$, $p=0.01$). The mean (SD) percentage of culturable particles within the respirable size
254 range ($\leq 4.7\mu\text{m}$, collected on Andersen stages 3 to 6) were 71% (27) in the uncovered cough
255 maneuver and 86% (30) in the cough etiquette maneuver ($p=0.21$).

Talk maneuvers. No aerosol CFUs were recovered from either talk maneuvers for 23/24 (96%) participants and a single aerosol *P. aeruginosa* CFU was cultured from the remaining two participants (one masked and one unmasked study; Table 2).

Face masks and cough etiquette maneuvers. Of the 19 participants that produced culture positive aerosols during uncovered coughing, two (11%) produced *P. aeruginosa* positive aerosols wearing the surgical mask, and four (21%) grew *P. aeruginosa* in their aerosol cultures when wearing the N95 mask (Table 2). In contrast, 68% of these participants (n=13) grew *P. aeruginosa* in their aerosols using cough etiquette (Table 2).

High viable aerosol production (total CFUs ≥ 10) was observed in 14/19 (74%) participants who cultured at least one CFU in the uncovered cough maneuver. In these participants a reduction in aerosol *P. aeruginosa* concentration (log CFU) was demonstrated with each strategy designed to interrupt aerosol dispersal: surgical mask (-94%); N95 mask (-94%); cough etiquette (-53%). The surgical mask ($p < 0.001$) and the N95 mask ($p < 0.001$) were both effective in reducing infectious airborne dispersal compared to uncovered coughing, with cough etiquette providing less reduction in mean *P. aeruginosa* CFUs than both masks (Table 3).

Tolerability of the masks during cough maneuvers varied, with 13 (54%) participants providing a higher comfort rating to the surgical mask than the N95 mask, compared with two (8%) who provided a higher comfort rating to the N95 mask and nine (38%) who had no preference ($p = 0.013$) (Table 4). Key comments regarding the masks by the participants included: a perceived restriction to or reduction in ease of breathing (n=15, [N95=12]), sensation of heat (n=9, [N95=6]), sensation of dampness (n=3, all surgical) and rubbing/pressure from the mask during coughing (n=2, [N95=1]). There was a similar change in weight of the surgical mask and the N95 mask (median (interquartile range) of 0.01 (0.00-

0.02) grams and 0.02 (0.00-0.04) grams, respectively; $p=0.23$). Cough numbers for each maneuver were similar and there was no difference in the number of coughs during the N95 mask ($p=0.15$) and cough etiquette ($p=0.52$) tests compared to the uncovered coughing intervention (Table S1), indicating that fatigue or participant motivation were unlikely to have impacted the accuracy of the maneuvers.

Infection Control: *P. aeruginosa* or other CF pathogens were not cultured from blank aerosol tests or surface swabs of the tunnel.

Adverse events: Overall, the maneuvers were well tolerated; however, one participant (listed for lung transplant and FEV₁ 32.5% predicted) discontinued the N95 mask study due to claustrophobia and increased dyspnoea after mask application.

DISCUSSION

This study demonstrates that at 2-meters from source, both surgical and N95 masks are highly effective in reducing aerosols containing viable *P. aeruginosa* in the droplet nuclei size range during voluntary coughing in people with CF. Our study uses a system that mimics a hospital environment and has several strengths; it was designed to reflect real-world occurrences by using a model that allows determination of air contamination at the recommended separation distance between people with CF and by investigating the effect of cough etiquette and two commonly available masks in hospitals on aerosol dispersal. The cough maneuvers examined may also closely replicate aerosol production during airway clearance sessions and spirometry procedures. Furthermore, we provide much-needed information on the short-term tolerability of mask wearing by persons with lung disease, which is an often-overlooked dimension of infection control.

Cross-infection between people with CF has increasingly been reported over the past two decades, initially with *Burkholderia cepacia* complex (30, 31), *P. aeruginosa* (9-11) and more recently *Mycobacterium abscessus* (15, 32). Progressive changes to infection control policies have been implemented including cohort segregation and changes to practice for patients with CF in the clinic and inpatient facility. Airborne transmission of CF pathogens in aerosol droplet nuclei has been suggested and such evidence has contributed to the enhanced rigor of these policies (17, 18). Increased separation distance between people with CF and the wearing of surgical masks during hospital visits are now recommended (21). Evidence to support the effectiveness of the latter strategy has been limited. Two previous studies evaluated the effect of surgical mask wearing on bioaerosol spread within a CF cohort. Our finding of a strong protective effect with surgical masks during cough maneuvers corroborate with and strengthen those of Driessche *et al.* who demonstrated an 86% reduction in environmental detection of airborne *P. aeruginosa* concentration during mask wearing compared to the reference (coughing without a surgical mask) in a controlled laboratory model (33). In contrast, Zuckerman and colleagues found no difference in rates of air contamination of outpatient exam rooms between mask wearing and unmasked patients with CF, although the overall positive air sample yield was low, including in the control group without mask (0.7%) (34).

Three quarters of the participants that were studied produced aerosols containing viable *P. aeruginosa* at 2-meters during uncovered coughing. The only predictor of expired aerosol containing viable bacteria was the sputum load of *P. aeruginosa*, which agrees with the results of our earlier work (20), further suggesting burden of infection is a very important determinant of potential infectiousness. A previous study using artificially generated aerosols demonstrated that *P. aeruginosa* respiratory samples with a mucoid phenotype exhibited

improved survival, although this advantage did not extend to epidemic strains (35). The aerosol samples from the uncovered cough maneuvers in our study revealed both unique and shared Australian strains of *P. aeruginosa*, but the overall numbers were too small to make any conclusions regarding survival characteristics.

To date, very few studies have directly compared the performance of various respiratory hygiene strategies *in vivo* and to our knowledge, this study is the first that investigates the outward protective effects of various interventions in a CF population with chronic *P. aeruginosa* infection. We demonstrated that surgical and N95 masks both significantly reduce the potential for bioaerosol dispersal with >90% reduction in the mean total CFUs. Previous evidence suggested that impaction of microbe-laden droplets directly onto an obstruction such as a hand or surgical mask during coughing limits the formation of airborne droplet nuclei (36); our findings support this suggestion. Likewise, an earlier study also found that surgical and N95 masks were equally effective in interrupting aerosol transmission of influenza in nine patients (37). A different study considered the impact of cough etiquette strategies (including the hand, a tissue, an arm) on preventing cough aerosols in healthy volunteers (38). Similar to our findings, they reported that the expelled cough aerosol was not completely blocked by cough etiquette, therefore posing a potential risk for airborne transmission (38). Importantly, the majority of viable aerosol detected during uncovered coughing in the current study was in the respirable size range (<4.7 μ m) and this was not significantly different for the cough etiquette maneuver.

Our study also demonstrates that talking is a low infectious-aerosol producing activity, potentially indicating that the implementation of mask policies for people with CF within the hospital should especially target relevant high risk clinical settings. During times of known high aerosol producing activities (performing spirometry and airway clearance) in the clinical

setting when a mask is not be feasible, then other considerations such as high air exchange rates, negative pressure rooms and/or adequate washout time periods between patients in individual rooms is important.

Although short mask wear duration was overall well tolerated by people with CF, it was perceived as being less comfortable when compared with no mask for the participant, particularly the N95 mask where additional outward protection was not observed. This may be an important factor when considering adherence to correct wearing technique, especially over extended periods. In healthy subjects and healthcare professionals, the use of face masks has been associated with increased breathing resistance (39), headaches (40), and physical discomfort (41, 42), and tolerability lessens with increased duration of wear (43). Furthermore, the physiological effects and comfort of mask wear amongst people with respiratory conditions has not been studied extensively and may have adverse impact. One study that compared surgical and N95 mask efficacy in patients with confirmed influenza reported that one participant (from a cohort of 10) was unable to complete the short protocol due to respiratory distress (37). However, Dharmadhikari and colleagues investigated the clinical efficacy of surgical masks over extended periods in patients with multi-drug resistant tuberculosis and demonstrated a 56% reduction in transmission risk. Patients wore surgical masks for up to 12-hours duration, with permitted interruptions for meals and medication, although it was reported that patients with respiratory distress were not enrolled into the study and incentives were used to encourage adherence (44). The tolerability of mask wear in people with respiratory infection and lung disease is an area that requires further investigation.

High rates of mask interference by the wearer have been reported in people with CF in the outpatient setting (34). In our study, the participants were unable to touch or readjust the

masks during the testing period, as the arms were positioned outside of the tunnel. However, mask movement and slippage during the coughing maneuvers were observed for some participants. In fact, it was noted that three of the four participants who had detectable *P. aeruginosa* CFUs in the N95 mask cough maneuver experienced mask movement, which may have led to an ineffective facial seal and contributed to the release of viable aerosol.

The participants in this study comprised an adult cohort with moderate to severe lung disease. Based on earlier data demonstrating a strong correlation between *P. aeruginosa* sputum density and cough aerosol concentration (18, 20), sputum-producing participants were enrolled as a robust means of assessing mask efficacy within the clinical setting. Our results, nevertheless, highlight the need for additional studies to determine the role and effectiveness of masks in CF pediatric populations, or for those with preserved lung function and/or those who rarely expectorate sputum. Despite this, there are some important caveats to consider: i) one participant in the current study did not produce a sputum sample and importantly, was one of the six participants who did not culture *P. aeruginosa* in their cough aerosol. However, our earlier data demonstrates non-productive patients can produce viable aerosols (18, 20); ii) quantitative sputum microbiology is not routinely performed in most CF Centers serviced by clinical microbiology laboratories; iii) other clinical parameters do not predict viable aerosol production (18, 20) and iv) the infective inoculum of *P. aeruginosa* (or other CF pathogens) is not known and therefore we cannot estimate the extent of infection risk for an individual following single (or for that matter multiple) exposure episodes to cough aerosols. Taken together, we suggest that a universal mask wearing approach across both adult and pediatric CF Centers should be strongly considered to mitigate the risk of person-to-person spread of CF pathogens.

Limitations of our study include that the application and check of mask fit by a health care professional and the short mask wearing duration of less than 10-minutes may not reflect the typical application in a clinical setting; therefore the study may overestimate the protective effect of face masks. Furthermore, the cough etiquette technique adopted by participants differed widely, which may impact the results but does allow a more real-world situation. The culture media used in the Andersen Cascade Impactors and incubation conditions were selective for non-fastidious, aerobic gram-negative bacteria and did not allow the investigation of cough aerosol viability of other CF pathogens such as *H. influenzae*, *Staphylococcus aureus* and non-tuberculous mycobacteria. Whilst we focused specifically on *P. aeruginosa* in this study, our earlier work has demonstrated similar findings of viable aerosols of common CF pathogens (18, 20), thus it is likely that the effectiveness of masks would generalize to other bacteria in people with CF. Finally, compared to the clinically stable patients, we observed a greater proportion of participants receiving intravenous antibiotics for pulmonary exacerbations that were low viable aerosol producers. However, the current study was not powered to examine the impact of clinical status (clinical stability versus pulmonary exacerbation) and antimicrobial therapies on viable aerosol production. Further studies are required to address this clinically important question.

In conclusion, masks are a simple and relatively inexpensive method to effectively interrupt aerosol dispersal of *P. aeruginosa* in droplet nuclei generated during coughing in people with CF, with the surgical mask providing enhanced wearer comfort. These data support the USA CFF Infection Prevention and Control Guidelines for individuals with CF to wear a surgical mask to reduce environmental contamination and potential viable aerosol spread associated with coughing in communal areas of health facilities (21). Cough etiquette reduces viable bacterial aerosols, but not to the same extent as masks. Future studies will assess whether

422 mask application by the wearer and a longer duration of mask wear in a CF cohort impacts
423 wearer tolerability and mask effectiveness.

424

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428 nursing team at The Prince Charles Hospital.

429

430

431 **Figure 1.** Results of the uncovered cough (reference) maneuver

432

433

434 **Table 1.** Baseline demographic and clinical characteristics of the study participants

Participant Characteristics	Participants categorized according to viable <i>Pseudomonas aeruginosa</i> aerosol CFUs in the uncovered cough (reference) maneuver			
	All participants (n=25)	< 10 CFUs (n=11)	≥ 10 CFUs (n=14)	<i>p</i> value*
Age, years, mean (SD)	31.3 (7.8)	32.4 (7.0)	30.4 (8.5)	0.53
Sex, male, n (%)	15 (60.0)	6 (54.5)	9 (64.3)	0.70
BMI, mean (SD)	22.1 (2.8)	21.8 (3.1)	22.3 (2.7)	0.70
FEV ₁ % predicted, mean (SD)	50.7 (17.4)	52.6 (15.8)	49.2 (19.1)	0.64
IV antibiotics administered in previous 7 days, n (%)	11 (44.0)	7 (63.6)	4 (28.6)	0.12
<i>Pseudomonas aeruginosa</i> CFUs/mL x 10 ⁷ in sputum, mean (95% CI) † ‡	6.3 (2.6 - 15.0)	2.0 (0.4 - 10.0)	14 (5.9 - 35.0)	

435

436 *Pairwise comparison between participants with <10 (including nil) and ≥10 viable *P. aeruginosa* aerosol CFUs

437 †A sputum sample was provided by 24/25 participants

438 ‡Value is geometric mean

439 *Definition of abbreviations:* BMI, body mass index; FEV₁, forced expiratory volume in 1 sec; IV, intravenous; CFU, colony forming unit; CI, confidence interval

440

441 **Table 2.** Number of participants with detectable aerosol *P. aeruginosa* colony forming unit (CFU) counts across each study maneuver*

Maneuver	Participants with detectable <i>P. aeruginosa</i> CFUs, n (%)	Stratification of participants with detectable <i>P. aeruginosa</i> CFUs into high and low viable aerosol production	
		<10*	≥10*
Uncovered coughing (Reference)	19 / 25 (76.0)	5 / 5	14 / 14
Talking†	1 / 24 (4.2)	0 / 5	1 / 13
Talking wearing a surgical mask†	1 / 24 (4.2)	0 / 5	1 / 13
Coughing wearing a surgical mask	2 / 25 (8.0)	0 / 5	2 / 14
Coughing wearing an N95 mask‡	4 / 24 (16.7)	1 / 5	3 / 14
Cough etiquette	13 / 25 (52.0)	2 / 5	11 / 14

442

443 *Participants were stratified according to a pre-defined definition of high (≥ 10 CFU) and low (<10 CFU) viable aerosol production of detectable
444 *P. aeruginosa* CFU during the uncovered cough maneuver.

445 †One participant did not complete the maneuver (insufficient culture media available)

446 ‡One participant did not complete the maneuver (due to adverse event)

447 *Definition of abbreviations:* CFUs, colony forming units

Table 3. *Pseudomonas aeruginosa* total colony-forming unit (CFU) counts for each of the maneuvers compared to uncovered coughing (reference) for the high viable aerosol producers (n=14)

Maneuver	Log ₁₀ <i>P. aeruginosa</i> CFUs mean (95% CI)	<i>p</i> value
Uncovered coughing (reference)	1.66 (1.41-1.91)	-
Talking*	0.02 (0.00-0.07)	<0.001
Talking wearing a surgical mask*	0.02 (0.00-0.07)	<0.001
Coughing wearing a surgical mask	0.11 (0.00-0.32)	<0.001
Coughing wearing an N95 mask†	0.13 (0.00-0.30)	<0.001
Cough etiquette	0.90 (0.50-1.30)	<0.001

*One participant did not complete the maneuver (insufficient culture media available)

†One participant did not complete the maneuver (due to adverse event)

Definition of abbreviations: CI, confidence interval; CFUs, colony forming units

Table 4. A matched pairs comparison of the comfort levels during cough maneuvers while wearing the surgical mask and the N95 mask (n=24) *

		N95 mask			Total
		Poor	Sufficient	Good	
Surgical mask	Poor	3	1	0	4
	Sufficient	11	2	1	14
	Good	0	2	4	6
	Total	14	5	5	24

* Comfort level categories: a) very poor, b) poor, c) sufficient, d) good, e) very good. A low number of responses were obtained in the a) very poor and e) very good categories; therefore, for the analysis, the categories of a) very poor and b) poor were combined (“poor”), as were the categories of d) good and e) very good (“good”).

The boxed area represents participants (n=9) who reported no preference for the comfort of the surgical mask or the N95 mask. Numbers to the right of the boxed area represent participants (n=2) who provided a higher comfort rating to the N95 mask and those to the left represent participants (n=13) who provided a higher comfort rating to the surgical mask.

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