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Title:

Surgical masks reduce airborne spread of *Pseudomonas aeruginosa* in colonized cystic fibrosis patients

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Authors' contributions: KVD, PT, MAC and BSQ acquired the data (recruitment, cough studies and/or microbiology work-up). KVD and NH conducted and are responsible for the data analysis. KVD, MFC, RdG and BJM conceived and designed the study and helped obtaining funding. KVD, NH and JEAZ drafted the manuscript. All authors revised the manuscript prior to submission. DPS and JEAZ supervised the study.

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At a Glance Commentary: Our data provide evidence for the new Cystic Fibrosis Foundation guideline that advises people with CF to wear a surgical mask in healthcare settings, to reduce the risk of airborne pathogen spread. The availability of evidence for this new guideline could contribute to its uptake and should help other countries deciding on adoption of this guideline.

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

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Abstract

Rationale: There are increasing concerns about airborne transmission of pathogens between individuals with cystic fibrosis. The new United States cystic fibrosis infection control guidelines (Cystic Fibrosis Foundation) advise all people with cystic fibrosis to wear a surgical mask in healthcare settings. While it is generally accepted that masks reduce bacterial droplet spread, the effect on airborne transmission is much less clear.

Objectives: To determine if surgical masks are effective in reducing the airborne spread of *Pseudomonas aeruginosa* during coughing by individuals with cystic fibrosis.

Methods: Patients attending Vancouver's cystic fibrosis clinics in 2014 from whom *P. aeruginosa* was isolated from sputum in both of the preceding two years and who consented to study enrolment were invited to participate. We used a controlled human aerosol model to evaluate the effect of surgical masks on the viable *P. aeruginosa* cough aerosol load. Statistical comparisons were done using generalized linear mixed models.

Measurements and Main Results: Eleven patients, aged 16-77 years, participated. Viable *P. aeruginosa* was isolated during 12 sessions from 6/11 (55%) participants. In these patients, wearing a surgical mask reduced the airborne *P. aeruginosa* load by an average of 88% (95% CI 81-96%).

Conclusions: Wearing a surgical mask significantly reduced the airborne *P. aeruginosa* load in cystic fibrosis patients colonized with *P. aeruginosa*. Our data support the new Cystic Fibrosis Foundation recommendation for people with cystic fibrosis to wear a surgical mask in healthcare settings, to reduce the risk of aerosol pathogen spread.

Word count for the abstract: 242

Key words: Infection Control, Disease Transmission, Evidence-Based Medicine

Introduction

The recently updated Cystic Fibrosis (CF) Foundation infection control guidelines advise people with CF to wear a surgical mask in healthcare settings to reduce the risk of transmission or acquisition of CF pathogens (1). A study from Liverpool demonstrated a three-fold higher risk of death or lung transplantation in those infected with an epidemic *Pseudomonas aeruginosa* strain, compared with those infected with environmentally acquired bacteria, highlighting the concern about transmission of epidemic strains in clinical care settings (2). The new recommendation for CF patients to wear masks was supported by studies showing “infectious droplets in the air” of CF clinics. Cough aerosol particles smaller than 60 μm will evaporate to particles smaller than 5 μm before touching the ground, classically referred to as airborne droplet nuclei (3). Droplets are larger particles that do not stay in the air beyond 30 seconds. Both *Burkholderia cepacia* complex and *P. aeruginosa* have been isolated from patient room air samples (1, 4). It has been suggested that airborne dissemination may be the most important factor in patient-to-patient spread of epidemic strains of *P. aeruginosa* during CF center outbreaks, but this remains undetermined (4, 5). Also, for emerging mycobacterial infections in CF patients, there are concerns about airborne transmission (1).

Surgical masks were originally developed to prevent droplet contamination of operating fields. Very little is known about their ability to prevent droplet nuclei generation. Some investigators suggest that droplet nuclei cannot be formed as the originator droplets will be impacted in the mask (6). Others argue that small infectious particles originating from the lung, rather than impacting on an obstructing mask will follow airstreams flowing around the edges of relatively loose fitting surgical masks (7). Both arguments relate directly to Newton’s laws of motion: Newton’s first law states objects in motion will continue to move in the same direction unless

acted upon by an unbalanced force. Therefore, large particles will impact on an obstructing object (a mask) while small particles do not need much force ($F=ma$; Newton's second law) to change direction and might be able to follow airstreams around the mask's edges.

We aimed to determine if surgical masks were effective in reducing the airborne spread of *P. aeruginosa* by people with CF when coughing.

Methods

We recruited CF patients for whom our repository laboratory received a *P. aeruginosa* isolate both in 2012 and 2013. We used a modification (Figure E1 in the online data supplement) of our previously developed controlled human aerosol model (8) to evaluate the effect of surgical masks (Kimberly-Clark Type 1 procedure masks, Dallas, United States) on the viable *P. aeruginosa* cough aerosol load. Because we were primarily interested in airborne transmission, we only took delayed aerosol samples collected one minute after coughing. Statistical comparisons were done using generalized linear mixed models. (See online supplement for more detailed methods.) The Children's & Women's Health Centre Research Ethics Board approved the study and written consent was obtained from all subjects.

Results

Eleven participants aged 16 to 77 were evaluated in 22 cough sessions. Sixteen otherwise eligible people declined participation. Table E1 in the online data supplement lists clinical characteristics of study participants.

Aerosolized viable *P. aeruginosa* were recovered after 12 sessions from 6 participants (Figure 1). Among those producing infected aerosol particles (6/11; 55%), the airborne *Pseudomonas* load was reduced by 88% when wearing a surgical mask compared to no mask (95% CI 81-96%) ($p = 0.03$). The protective effect of surgical masks was 86% (95% CI 74-99%) when stratifying only for aerosol particles smaller than 4.7 μm ($p = 0.03$). There was no significant difference related to the session order (control vs. intervention; data not shown). A Pearson correlation coefficient between 0.98 and 1.00 demonstrated an excellent fit for the statistical models.

Discussion

The controlled human aerosol model used in our study produced relatively precise and repeatable results despite a small sample size. Among CF patients colonized with *P. aeruginosa* and producing infectious aerosol particles (55%), we demonstrated a greater than 80% reduction in infectious aerosol particle production when wearing a surgical mask while coughing.

Three previous studies examined the effect of surgical masks on the dispersal of airborne viable microorganisms from patients. Milton asked patients with influenza to cough into a cone and sampled their aerosols with slit impactors (9). The influenza copy number in the $\leq 5 \mu\text{m}$ fraction (the airborne droplet nuclei) was reduced by 64% (95% CI 33-81%) in mask sessions compared

to preceding control sessions. Not surprisingly, the copy number in the $> 5 \mu\text{m}$ fraction (the droplets) was reduced to a greater extent: 96% (95% CI 71-99%). Dharmadhikari monitored tuberculosis (TB) skin test conversion in guinea pigs housed in cages receiving exhaust air from a TB treatment facility in eMalahleni, South Africa (10). The study reported a 56% (95% CI: 33-71%) reduction in infectious aerosol production, as measured by fewer guinea pigs becoming infected, when TB patients wore surgical masks. Zuckerman reported 8% air contamination when sampling exam rooms with cascade impactors placed 3 feet from CF patients (11). In a follow-up study at 6 feet, few samples were positive, both with occupants with (2/149) or without (1/154) masks (12). The CF Foundation does not recommend the wearing of masks inside (well-ventilated) exam rooms (1).

Our study has several limitations. Firstly, aerosols from strong coughs may impact more effectively onto surgical masks ($F=ma$) and the strong artificial coughs produced during the study could have led to an overestimation of the protective effect. In real life patients would not be coughing as frequently and might cover their coughs (e.g. cough in their hand or elbow). Secondly, while we demonstrated that smoke particles below $10 \mu\text{m}$ were homogeneously mixed before sampling (Figure E2 in the online data supplement), this is not a guarantee that the same sized particles from real cough aerosols were equally well mixed. Thirdly, while our study demonstrated an important reduction in *P. aeruginosa*-containing airborne particles, we do not know the contagiousness of airborne *P. aeruginosa*. It would however seem reasonable to assume that masks are at least as effective in reducing droplet spread of *P. aeruginosa* as they are against airborne spread (9). Lastly, we only studied the outward protective effect of masks. The inward protection of surgical masks (to protect the wearer) has yet to be established (13).

Despite these study limitations, we demonstrated that surgical masks were effective in reducing the airborne load of *P. aeruginosa* when colonized individuals with CF cough. Our data provide evidence for the new CF Foundation guideline to wear surgical masks in healthcare settings.

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Figure 1: Effect of surgical masks on the airborne *P. aeruginosa* load originating from cough aerosols

The dashed lines represent 12 cough sessions from 6 different subjects. The solid line at the bottom of the graph represents 10 cough sessions from 5 different subjects in whom no viable *P. aeruginosa* from cough aerosols could be sampled in the control or in the mask cylinder.

Online Data Supplement

Full methods section

Patients with CF between 7 and 77 years old for whom our repository laboratory received a *P. aeruginosa* isolate in both of the preceding two years, were recruited in 2014 from BC Children's Hospital and St. Paul's Hospital, Vancouver, Canada. Exclusion criteria included: claustrophobia; pregnancy; hemoptysis in the preceding week; pneumothorax or pulmonary hemorrhage in the last 5 years; prior brain hemorrhage; prior lung transplantation; and because of infection control considerations a previous or current infection with *Burkholderia spp.*; methicillin resistant *Staphylococcus aureus* (MRSA); mycobacteria; *Pandora*; *Ralstonia* or multidrug resistant *Achromobacter species*.

The Children's & Women's Health Centre Research Ethics Board approved the study and participants provided written, informed consent. Participants received no remuneration.

To evaluate the effect of surgical masks on *P. aeruginosa* aerosol production we used a modification (Figure E1) of our previously developed controlled human aerosol model (E1). Participants were asked to cough in two cylindrical tanks from which the air was extracted through six-stage viable Andersen cascade impactors (Thermo Fisher Scientific, Waltham, United States) to measure concentration and particle size distribution of microorganisms in the cylinders' air (E2, 3). Participants moved back and forth between a cylinder where they wore a surgical mask (Kimberly-Clark Type 1 procedure masks, Dallas, United States) while coughing and a control cylinder where they coughed without a mask (42 coughs in total during one session; detailed protocol in Figure E1). This cough protocol was designed to allow for an improved distribution of the potentially more productive initial coughs between the two cylinders (E1). We

randomized patients to cough first into the control cylinder (no mask) or the intervention cylinder (participant wearing mask). This two-cylinder approach also limited the possibility of bias that could otherwise arise from studying intervention and control at a different time, with potential for differential impact of humidity, temperature, antibiotics exposure, and airway clearance techniques on *P. aeruginosa* aerosol generation. Air was dispersed within the cylinders for 30 seconds with two fans (Figure E1). This allowed for a homogenous mix of particles below 10 μm in diameter, which was validated by performing multiple (twenty-four) 2-point measurements of dipropylene/triethylene glycol smoke concentrations with a laser photometer (DustTrak, TSI, Shoreview, United States), demonstrated in Figure E2.

We did not include settle plates on the cylinder floor to capture large droplets (illustrated in reference E1) because settle plates remained negative for *P. aeruginosa* during two pilot experiments (data not shown) at the same time cylinder air samples were positive, and because the inclusion of settle plates would increase study protocol complexity for the participant (who would have to be asked to take the lids off before coughing and put them back on after coughing). Because we were primarily interested in airborne transmission (as opposed to droplet transmission), we also only took delayed aerosol samples (starting one minute after a subject's last cough). We excluded microorganisms collected from stage 1 in the Andersen impactor from the analysis because this stage captures particles above 7 μm , for which we did not demonstrate homogeneity (E4). We also performed a stratified analysis applying a more stringent definition of droplet nuclei (E5) by excluding stage 2, which captured particles between 4.7 and 7 μm .

Equipment decontamination between participants occurred as per Figure E1.

The Andersen impactors were loaded with Petri dishes containing LB agar (1.5% agar, 10 g l⁻¹ tryptone, 5 g l⁻¹ yeast extract and 10 g l⁻¹ sodium chloride). The plates were coded such that the

investigator performing the microbiology work-up was blinded to the intervention. Following aerobic incubation at 35-37°C for 2-4 days, colonies morphologically resembling *P. aeruginosa* were identified by MALDI-TOF mass spectrometry (BioTyper, Bruker Daltonics, East Milton, Canada) using the recommended settings. Numbers of *P. aeruginosa* colony forming units (CFU's) were adjusted according to published positive-hole correction tables (E6). Petri dishes overgrown by “rapid growers” impairing CFU counting were discarded together with the Petri dish from the same stage from the impactor from the cough cylinder used for the alternative condition.

Demographic and clinical characteristics were recorded: age, gender, presence of current exacerbation of disease, forced expiratory volume in 1 s (for stable patients FEV₁ result available closest to the study date and for patients with an exacerbation FEV₁ result from the study date), antibiotic prophylaxis regimen at the time of the study, and sputum culture results. Sputum culture results were graded as scant, light, moderate and heavy corresponding to growth of *P. aeruginosa* in respectively the first, second, third and fourth quadrant of a four quadrant-streaked commercially available blood agar plate.

We used generalized linear mixed models to measure the effect of masks on airborne *Pseudomonas* load. The effect was expressed as a relative difference with its 95% confidence interval. Matching within individuals was taken into account (as in a matched case-control study). We used a negative binomial response distribution to allow for over dispersion. Next, to an offset of the *P. aeruginosa* production for the controls, we assessed the impact of session (first or second session) and cough protocol sequences (for which participants were randomized as described above) by including these variables as independent effects. Model building based on

Wald-tests reduced complexity. We assessed goodness of fit by comparing predicted and observed outcomes using the Pearson correlation coefficient. Significance was set at $p < 0.05$. For more details related to those methods see Agresti (E7). All analyses were done with R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

References for the full methods section

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Figure E1: Design of the controlled human aerosol model

Description	Decontamination
1 Cough session protocol: Written on the cylinder wall in front of the participant. Participants are asked to move back and forth between the two cylinders during each cough session. Participants were asked to produce 1 cough in <i>cylinder one</i> , followed by 3 (1+2) coughs in <i>cylinder two</i> , followed by 11 (2+3+3+3) coughs back in <i>cylinder one</i> , followed by 9 (3+3+3) coughs in <i>cylinder two</i> , followed by 9 (3+3+3) coughs back in <i>cylinder one</i> , and finally 9 (3+3+3) coughs in <i>cylinder two</i> (a total of 21 coughs in each cylinder).	
2 Cough cylinders: Two 950-liter cylindrical volumes [radius 60 cm and height 84 cm; three times higher compared to the pilot model (E1)]. One control cylinder and one intervention (mask) cylinder. A height adjustable frame allows alignment of the cylinders' geometric centre with the subject's mouth.	
i Dome: electrostatic dissipative (reduces particle attraction) polycarbonate (SciCron Technologies)	UV, H ₂ O ₂ , EtOH
ii Seal between dome and floor: electrically conductive nickel-plated graphite in silicone (Parker)	UV, autoclave
iii Cylinder floor: a grounded electrically conductive carbon in UHMW polyethylene sheet (Quadrant)	UV, H ₂ O ₂ , EtOH
3 Fans: Two 92mm fans (GlacialTech) in each cylinder blow air forward in a diagonal plane during 30 seconds each time the participant has moved out of a cylinder and the kayak seal has been closed.	VHP
4 Kayak seal: To allow for increased particle containment. Is closed (photo c) with a sliding mechanism when there is no participant standing in the cylinder	autoclave
5 Exhaust hole: Covered by a metal plate to prevent droplets from being projected directly in the impactor during unobstructed (control) coughing	autoclave (plate), H ₂ O ₂ , EtOH
6 Impactor: Two six-stage viable Andersen cascade impactors (Thermo Scientific) to measure concentration and particle size distribution of microorganisms in the cylinder air. For the current study we took samples during 5 minutes. The impactors were loaded with culture dished containing LB medium.	autoclave
7 Valve: To timely initiate and stop aerosol sampling	autoclave
8 In-line HEPA filter (Advantec/Whatman) to prevent contamination of the flow regulator, vacuum tubing, and vacuum pumps	autoclave
9 Flow regulators (Cole-Parmer) to equalize the flow through the impactors at 28.3 liter/minute	n/a
UV: 95 Watt ultraviolet light for 5 minutes before disassembly and after reassembling (photo f); H ₂ O ₂ : surface wash with accelerated hydrogen peroxide; EtOH: surface spray with 70% ethanol; VHP: Vaporized Hydrogen Peroxide Sterilizer. The person pictured is a volunteer (not a study participant).	

Figure E2: Example of a 2-point smoke concentration sampling experiment to confirm particle homogeneity

Real-time measurement of the particle concentration in the 10 µm fraction with a laser photometer (DustTrak, TSI, Shoreview, United States). The experiment is carried out during air dispersion by the cylinder fans (Figure E1). The graph shows this results in comparable particle concentrations in both sampling points (b and d).

a: release of dipropylene/triethylene glycol smoke in the cylinder chamber with a smoke stick (Dragon Puffer): for the experiment shown the smoke was released near the cylinder centre

b: sampling of the particle concentration at the first sampling point: for the experiment shown this point was located at the exhaust hole (cylinder front)

c: when switching from the first to the second sampling point, the particle concentration in the tubing (connecting the second sampling point to the laser photometer) is being sampled for a couple of seconds, which explains the observed notch

d: sampling of the particle concentration at the second sampling point, for the experiment shown this point was located in the back of the cylinder (Southwest next to the cylinder wall).

The 1, 2.5 and 5 µm fractions also had a comparable concentration at the two sampling points (data not shown in this graph). In total, 24 experiments were carried out to demonstrate that 30 seconds of air dispersion with the cylinder fans is sufficient to obtain a homogenous mix of the measured particles; using different combinations of a smoke release point and sampling points.

Table E1: Demographic and clinical characteristics of study participants

	All subjects	negative cough aerosols for <i>P. aeruginosa</i>	positive cough aerosols for <i>P. aeruginosa</i>
Median (range) age (years)	39 (16 - 77)	32 (16 - 54)	45 (33 - 77)
Gender (M/F)	7/4	3/2	4/2
Clinical state:			
stable	10	4	6
exacerbation	1	1	0
Mean FEV₁ (% predicted)	61	68	55
Sputum culture for <i>P. aeruginosa</i>			
negative	1	1	0
positive:			
scant or light growth	2	1	1
moderate or heavy growth	5	2	3
not available from study day	3	1*	2 [†]
Antibiotic prophylaxis			
oral azithromycin	7	3	4
nebulized:			
tobramycin	4	3	1
colistin	2	1	1
aztreonam	4	2	2

FEV₁: forced expiratory volume in 1 s.

* One participant who was unable to expectorate sputum demonstrated no growth on culture from a cough swap. [†] Two participants for which there was no same day sputum sample available demonstrated heavy growth both on the most recent sample (respectively 5 and 2 months before) and on the first processed sample following the study date (respectively 1 and 6 weeks later).