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

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42 R.E.S, G.R.J and N.J conducted the cough studies and acquired the data. R.E.S, K.A.R and
43 L.J.S performed microbiological analysis. P.O'R and E.B led the data analysis. M.E.W and
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58 **At a Glance Commentary.**

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

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

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

72

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

75

76

77 **ABSTRACT**

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

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

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

89 **Measurements and Main Results:** During uncovered coughing (reference maneuver), 19/25
90 (76%) participants produced aerosols containing *P. aeruginosa*, with a positive correlation
91 found between sputum *P. aeruginosa* concentration (CFU/mL) and aerosol *P. aeruginosa*
92 CFUs. There was a reduction in aerosol *P. aeruginosa* load during coughing with surgical
93 mask, coughing with N95 mask and cough etiquette compared with uncovered coughing
94 ($p<0.001$). A similar reduction in total CFUs was observed for both masks during coughing,
95 yet participants rated surgical masks more comfortable ($p=0.013$). Cough etiquette provided
96 approximately half the reduction of viable aerosols of the mask interventions during
97 voluntary cough. Talking was a low viable aerosol producing activity.

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

101 Word count = 248

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

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117 **INTRODUCTION**

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

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

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

152

153 **METHODS**

154 *See* online supplement for additional details.

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

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

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

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

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

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

192 **Microbiology:** Standardized qualitative and quantitative sputum cultures were performed, as
193 previously described (20). *P. aeruginosa* identification was confirmed by oxidase testing,
194 42°C growth and MALDI-TOF mass spectrometry. *P. aeruginosa* genotyping was
195 undertaken using the Sequenom iPLEX20SNP assay, as previously described (29).

196 **Statistical analysis:** A sample size calculation based on our earlier work (20) suggested 21
197 patients were required to demonstrate a 40 percentage point reduction in the presence or
198 absence of *P. aeruginosa* with 80% power with a two-tailed $p=0.05$. Therefore, 25 patients
199 with CF and chronic *P. aeruginosa* were enrolled, allowing for 10-15% dropout to achieve a
200 sample size of 21. SPSS version 22 was used for statistical analysis. Categorical variable
201 associations were examined using Fisher's Exact test. Continuous variables describing
202 participant characteristics were examined using a Student t-test. Clinical and demographic
203 variables were compared with the log transformed total *P. aeruginosa* aerosol colony forming
204 units (CFUs) in the uncovered cough maneuver using a Pearson correlation test. The paired t-
205 test based on log transformed CFU was used to compare each intervention maneuver with the
206 reference maneuver (uncovered coughing). Results were stratified by the level of aerosol
207 production as follows: participants were classed as high, low or nil viable aerosol producers
208 during the uncovered cough maneuver using an arbitrary pre-defined total CFU of ≥ 10 or
209 < 10 , respectively, accumulated across the six-stages of the Andersen Impactor (Figure 1).

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

212

213 **RESULTS**

214 **Participant overview:** Twenty-five (15 male) adult participants with a mean (SD) age of
215 31.3 (7.8) years, FEV₁ 50.7 (17.4) % predicted and BMI 22.1 (2.8) kg/m² were recruited
216 (Table 1). Twenty-two (88%) participants were on maintenance azithromycin. Eleven (44%)
217 participants were established on chronic inhaled antibiotic therapy (either continuous or
218 cycling alternate month) and the remaining 14 (56%) participants had been prescribed inhaled
219 antibiotic therapy (less frequently than alternate months) in the 12 months prior to the study.
220 Eleven participants (44%) had received intravenous antibiotics in the week prior to their
221 study involvement. The mean (SD) age, FEV₁ % predicted and BMI of patients attending
222 TPCH ACFC in 2015 was 30.7 (9.9) years, 66.7 (24.1) % predicted and 23.0 (4.2) kg/m²,
223 respectively. In the preceding calendar year, of 282 patients reviewed at TPCH ACFC, 68.4%
224 had chronic *P. aeruginosa* infection.

225 **Sputum microbiology:** *P. aeruginosa* was cultured from the 24 sputum samples provided at
226 a mean concentration of 6.3 x 10⁷ CFU/mL (95% CI, 2.6 x 10⁷ – 15.0 x 10⁷; Table 1).
227 Genotyping identified 12 different *P. aeruginosa* strains, including five common Australian
228 shared strains (11); AUST-01 (n=6), AUST-02 (n=9), AUST-06 (n=6), AUST-07 (n=3),
229 AUST-13 (n=1), five other minor shared strains, and two unique strains. Eight participants
230 harbored more than one *P. aeruginosa* strain; two different strains were detected in seven
231 participants, while one participant was infected with three major Australian shared strains
232 (AUST-01, AUST-02 and AUST-06). Other CF pathogens identified in the sputum on the
233 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 *maltophilia* 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 ($p=0.21$).

256 **Talk maneuvers.** No aerosol CFUs were recovered from either talk maneuvers for 23/24
257 (96%) participants and a single aerosol *P. aeruginosa* CFU was cultured from the remaining
258 two participants (one masked and one unmasked study; Table 2).

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

264 High viable aerosol production (total CFUs ≥ 10) was observed in 14/19 (74%) participants
265 who cultured at least one CFU in the uncovered cough maneuver. In these participants a
266 reduction in aerosol *P. aeruginosa* concentration (log CFU) was demonstrated with each
267 strategy designed to interrupt aerosol dispersal: surgical mask (-94%); N95 mask (-94%);
268 cough etiquette (-53%). The surgical mask ($p < 0.001$) and the N95 mask ($p < 0.001$) were both
269 effective in reducing infectious airborne dispersal compared to uncovered coughing, with
270 cough etiquette providing less reduction in mean *P. aeruginosa* CFUs than both masks (Table
271 3).

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

280 0.02) grams and 0.02 (0.00-0.04) grams, respectively; $p=0.23$). Cough numbers for each
281 maneuver were similar and there was no difference in the number of coughs during the N95
282 mask ($p=0.15$) and cough etiquette ($p=0.52$) tests compared to the uncovered coughing
283 intervention (Table S1), indicating that fatigue or participant motivation were unlikely to
284 have impacted the accuracy of the maneuvers.

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

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

290

291 **DISCUSSION**

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

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

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

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

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

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

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

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

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

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

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

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

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

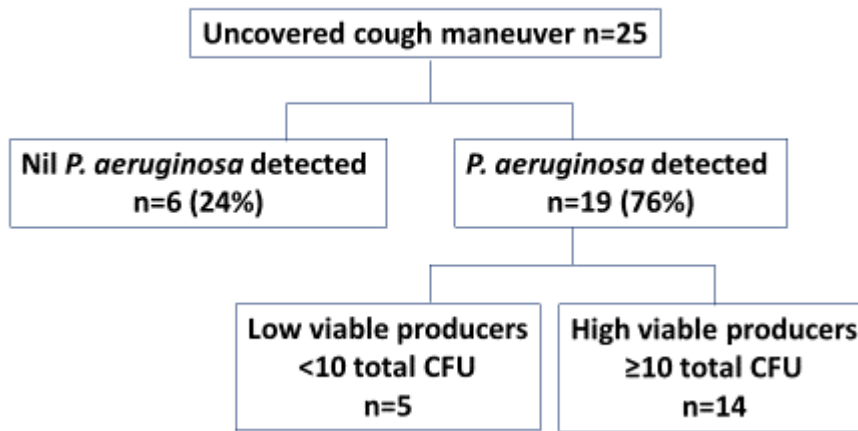
422 mask application by the wearer and a longer duration of mask wear in a CF cohort impacts
423 wearer tolerability and mask effectiveness.

424

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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,
440 confidence interval

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

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

451

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

452

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

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

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

456

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

460

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

467

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

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

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