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1 **Microbial Interactions in the Cystic Fibrosis Airway**

2

3 **Running Title: Microbial Interactions in CF**

4

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25 Abstract

26 Interactions in the airway ecology of cystic fibrosis may alter organism persistence and clinical outcomes.
27 Better understanding of such interactions could guide clinical decisions. We fitted logistic regression models
28 using generalized estimating equations to longitudinal two-year patient cohorts in the Cystic Fibrosis
29 Foundation Patient Registry, 2003-2011 to study associations between airway organisms present in each
30 calendar year and their presence in the subsequent year. Models were adjusted for clinical characteristics and
31 multiple observations per patient and tested for sensitivity to cystic fibrosis-specific treatments. The study
32 included 28,042 patients aged six and older from 257 accredited US Care Centers and Affiliates with sputum
33 cultures for at least two consecutive years for methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant
34 *S aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*,
35 *Achromobacter xylosoxidans*, and *Candida* and *Aspergillus* species. We analyzed 99.8% of 538,458 sputum
36 cultures from the patients during the study period. Methicillin-sensitive *S aureus* was negatively associated with
37 subsequent *P aeruginosa*. *P aeruginosa* was negatively associated with subsequent *B cepacia* complex, *A*
38 *xylosoxidans*, and *S maltophilia*. *B cepacia* complex was negatively associated with future presence of all
39 studied bacteria and *Aspergillus* species. *P aeruginosa*, *B cepacia* complex and *S maltophilia* were each
40 reciprocally and positively associated with *Aspergillus* species. Independent of patient characteristics, studied
41 organisms interact and alter outcomes of treatment decisions, sometimes in unexpected ways. By inhibiting *P*
42 *aeruginosa*, methicillin-sensitive *S aureus* may delay lung disease progression. *P aeruginosa* and *B cepacia*
43 complex may inhibit other organisms by decreasing airway biodiversity, potentially worsening lung disease.

44

45 Introduction

46 In the United States, Cystic Fibrosis (CF) affects roughly 30,000 people, reducing life expectancy by more than
47 50% (1). Progressive pulmonary disease, marked by recurrent exacerbations, bacterial infection and declining
48 lung function, drives morbidity and mortality (2–5). Studies of the CF lung reveal a diverse microbiology with
49 methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeruginosa* being the two most

50 commonly isolated organisms from the airway (1, 2). Opportunistic organisms including *Burkholderia cepacia*
51 complex, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, non-tuberculous *Mycobacteria* and
52 fungal organisms commonly colonize and infect patients with CF.

53 The presence of different organisms alters long term outcomes of patients with CF. MSSA appears to
54 enhance survival while *B cepacia* complex may presage catastrophic decline in health (6, 7). Acquisition of
55 MRSA and *P aeruginosa* are associated with accelerated lung disease (8–12). Published cross-sectional data
56 from the CF Foundation Patient Registry (CFFPR) show that dominant airway infections differ with age (1).
57 MSSA most commonly infects pediatric patients, while *P aeruginosa* infection increases with age and
58 commonly dominates the bacterial community in adult patients (13). Without clear understanding of the
59 underlying microbial interactions, efforts to prevent, treat or eradicate specific organisms such as *P aeruginosa*
60 may produce unexpected and undesirable outcomes. A double-blinded randomized controlled study in 2002
61 showed that prophylactic treatment of MSSA with cephalexin in infants and young children with CF led to
62 earlier colonization with *P aeruginosa* (14). Prophylaxis with ciprofloxacin had no effects on preventing
63 infection with *P aeruginosa* (15). Similarly, eradication therapy for *P aeruginosa* increased infection with *S*
64 *maltophilia* (15).

65 While antibiotic therapy may play a role, existing infections themselves appear to alter the rest of the
66 microbiota (7, 16–19) and may thus alter clinical disease course. *In vitro* and non-human animal models show
67 evidence of interspecies interaction between *P aeruginosa* and other pathogens including MSSA and *B*
68 *cenoecepacia*. Mathematical models of disease progression explore potential airway interactions between *P*
69 *aeruginosa*, MSSA and *Burkholderia* species; results from these models are consistent with observational data
70 in CF (1) and illustrate the potential impact of managing these organisms (7).

71 In this study, we focus on eight common CF airway pathogens and show how the presence of each in a
72 given study year is associated with observed infections in the following year. By improving our understanding
73 of interactions between organisms, we seek to enhance understanding of the underlying mechanisms of

74 changing airway microbial ecology which may help us anticipate the impacts of changing practice on clinical
75 outcomes.

76

77 **Methods**

78 *Study Design and Data*

79 We analyzed data from the CFFPR, 2003–2011, which contains prospectively collected patient data from 257
80 US CF Foundation accredited care centers and affiliated programs. Data were gathered according to a defined
81 protocol after obtaining written consent from adult patients or parental consent with assent from minors. The
82 data include patient demographics, clinical measurements of CF disease, treatment information, number of
83 clinic visits, and culture results from routine quarterly and acute illness samples. These data were monitored to
84 confirm fidelity to medical charts (20).

85 We obtained approval from the University of Utah Investigational Review Board (IRB) for the
86 performance of this study with a waiver for informed consent and approval from the Data Use Committee of the
87 US CF Foundation for access to and use of the CF Foundation Patient Registry. We participate in data collection
88 after written informed consent for the CF Foundation Patient Registry with separate approval from the IRB.

89

90 *Study population and definitions*

91 Our study cohort included all CFFPR subjects who had at least two consecutive years of data between 2003 and
92 2011. Since 2003, the CFFPR records each culture result separately for every patient; previously, the CFFPR
93 reported only a single annualized result per year thus guiding our study period selection. Patients younger than
94 six years were excluded because they cannot reliably perform pulmonary function testing and usually do not
95 produce sputum. We used sputum culture results to determine presence of infection. The CFFPR records
96 organisms as present or absent for each culture and records the number of cultures obtained each given year.
97 Presence of infection with a particular organism within a given calendar year was defined as at least one
98 positive culture for that organism within that year. We focused on eight common infections, for which the

99 CFFPR contains sufficient data for analysis: MSSA, methicillin resistant *S aureus* (MRSA), *B cepacia* complex,
100 *P aeruginosa*, *S maltophilia*, *A xylosoxidans*, *Candida* species and *Aspergillus* species. We identified and
101 adjusted for the following patient characteristics as potential confounders in statistical models: age, age at
102 diagnosis of CF, sex, CF-related diabetes, pancreatic sufficiency, weight-for-age *z*-score, percent predicted
103 forced expiratory volume in one second (FEV₁%), and acute pulmonary exacerbations (APE). Most of these
104 characteristics were previously found to predict 5-year survival (6). We defined patients as diabetic in a given
105 year if the condition was present at any time during that year. We defined patients as pancreatic sufficient in a
106 given year if they were noted to be sufficient for all encounters and did not use pancreatic enzymes during that
107 year. For sensitivity analyses to determine the effect of adjustment of associations for different interventions, we
108 used treatment data on oral azithromycin, inhaled aztreonam, tobramycin, recombinant human DNase (DNase),
109 and hypertonic saline, days per year of therapy with home intravenous (IV) antibiotics, hospitalization days for
110 pulmonary exacerbation treatment, and lung transplantation.

111

112 *Statistical Analysis*

113 We fitted cross-sectional univariable logistic regression models (21) with presence of each organism in a given
114 year as the outcome and each other organism studied as an individual explanatory variable. Cross-sectional
115 multivariable models were then fitted with presence of each organism as the outcome and with all other
116 organisms as explanatory variables with and without additional adjustment for clinical characteristics. These
117 models were fitted separately in each calendar year, 2003-2011. We fitted similar multivariable logistic models
118 relating organisms in each year *t* to presence of each organism in year *t+1*, where *t*=2003-2010, with and
119 without additional adjustment for clinical characteristics. Finally, we fitted a single combined model across all
120 observation years for each patient using generalized estimating equations with an independence working
121 correlation matrix. This model uses multiple observations per individual across the study years. Using the
122 combined model increases the power of our analysis and reduces the size of confidence intervals (22, 23). It
123 makes the assumption that the associations between organisms from one year to the next are the same across the

124 calendar years. See the Online Supplement for detailed methods regarding the individual steps to fitting the
125 combined model. Sensitivity analyses examined the impact of adjusting for treatments used. All analyses were
126 performed using the R statistical system (24).

127

128 **Results**

129 *Participants*

130 We found 28,042 patients aged six years or older in the CFFPR from 257 care centers and affiliated programs
131 accredited by the US CF Foundation between January 1, 2003 and December 31, 2011 with at least two
132 consecutive years of culture data (Table 1). These patients had a total of 538,458 sets of sputum culture results
133 of which 537,396 (99.8%) were included in our analysis (Figure 1). Culture results were excluded only for lack
134 of same-patient cultures in contiguous years. From 2003 to 2011, the median cross-sectional age of patients
135 increased from 16.6 to 19.1 (Table 1). CF-related diabetes prevalence nearly doubled over this time period from
136 9.62% to 17.5%. A minority of patients were pancreatic sufficient; this increased between 2003 and 2011 from
137 6.53% to 11.2%. There were marginal changes in the median FEV₁% and mean number of APE during the
138 study period. The changes in pediatric and adult groups were similar (Tables S1 and S2).

139

140 *Infection Prevalence*

141 There were changes in percentages of patients with positive cultures for the eight most common infections
142 recorded in the CFFPR between 2003 and 2011 (Table 1, Figure 1, and Tables S1 and S2). Percentages of
143 patients infected by MRSA and *Candida* species more than doubled, and percentages with *S maltophilia*, *A*
144 *xylosoxidans*, and *Aspergillus* species also increased. Between 2003 and 2011, there were statistically
145 significant small decreases in percentages of patients infected by MSSA (50.2% to 49.2%) and *P aeruginosa*
146 (63.8% to 57.3%).

147

148 *Cross Sectional Associations between Airway Infections*

149 Figure 2 shows estimated odds ratios from multivariable logistic regression models fitted in each year 2003-
150 2011 with each organism as an outcome and the set of all other organisms as explanatory variables, with and
151 without adjustment for clinical variables and number of cultures. Estimates were remarkably consistent across
152 calendar years. *P aeruginosa* infection was negatively associated with MSSA, *B cepacia* complex, *S maltophilia*
153 and *A xylosoxidans* and was positively associated with *Aspergillus* species infections for every study year with
154 or without adjustment for the presence of other organisms (Figure 2A, D, E, F and H, respectively). *P*
155 *aeruginosa* and *B cepacia* complex were negatively associated in every study year with or without adjustment
156 for other organisms, and additional adjustments for clinical characteristics intensified these associations (Figure
157 2B and D). After adjustment for other organisms and with or without adjustment for clinical characteristics, *P*
158 *aeruginosa* infection was negatively associated with MRSA for each year from 2004 through 2011 (Figure 2C).
159 *A xylosoxidans* infections were associated with *B cepacia* complex infections every study year with large
160 negative coefficients suggesting that co-infections are uncommon (Figure 2D). In contrast, *S maltophilia* was
161 associated with *Aspergillus* species infections every year with large positive coefficients suggesting that co-
162 infections are common (Figure 2H). Odds ratios from logistic regression models examining univariable,
163 unadjusted associations between organisms are similar to the full adjusted results (Figure S1).

164

165 *Associations between Present and Future Airway Infections*

166 Figure 3 shows the estimated odds ratios from multivariable models for the associations of microbial infection
167 status for each of the years 2004 through 2011 (years $t+1$) with infections in the previous year where $t=2003$ -
168 2010, by year (21). Associations were similar for every two year period examined, with some variation in
169 degree of statistical significance for individual relationships.

170 For each organism, the predictor most strongly associated with its presence in year $t+1$ was presence of
171 the same organism in year t (Figure S2). MSSA, *B cepacia* complex, *S maltophilia* and *A xylosoxidans* in year t
172 were all negatively associated with *P aeruginosa* in each year $t+1$ (Figure 3B). *Candida* or *Aspergillus* species

173 were not typically associated with subsequent *P aeruginosa* (Figure 3B). Other organisms besides MSSA and *B*
174 *cepacia* complex in each year t were infrequently associated with MRSA in each year $t+1$. MSSA in three years
175 t (2005, 2008, and 2010) and *B cepacia* complex infections in two years t (2005 and 2007) had potentially
176 significant negative statistical and clinical associations with MRSA (Figure 3C). Adjustment for clinical
177 variables did not substantially change the estimates or their statistical significance (Figure 3).

178 When using all observations simultaneously in the combined model, the relationships between an
179 organism seen in year t with a different organism in year $t+1$ (Figure 4, Tables S3 and S4) were similar to all
180 relationships seen in multivariable logistic regression models for each of the two-year models reported above
181 but with much narrower confidence intervals (Figure 3). Adjustment for clinical characteristics produced
182 similar results (Figure 4, Tables S3 and S4). These models again showed that organism presence in year t was
183 most strongly associated with the same organisms in year $t+1$ (Figure S3). Sensitivity analyses in which we
184 additionally adjusted, one at a time, for clinical treatments given in year t showed that associations were not
185 substantially altered.

186

187 *Missingness of Data*

188 Our study population included patients who had two consecutive years of culture data at least once between
189 2003 and 2011 (Table 1). We analyzed cohorts and patients for missing data. The proportion of patients
190 excluded for any two-year cohort ranged from 4.6% to 7.5% (Table S5). There were small but statistically
191 significant differences between study patients and patients with missing data. Excluded patients tended to be
192 older, with higher prevalence of diabetes and slightly decreased lung function, however, they also appeared in
193 other ways to be healthier, with better nutritional status, fewer APE and higher rates of pancreatic sufficiency
194 (Tables S6, S7 and S8).

195

196 **Discussion**

197 Our study of microbiology in the human airway reveals microbial interactions that may alter therapeutic
198 responses in individuals with CF. Current organisms change the odds of finding other microbes concurrently
199 and in the future. Among studied organisms, the likelihood of retention is highest for *P aeruginosa*, MRSA, and
200 especially *B cepacia* complex, the most clinically pathogenic organisms. In contrast, MSSA, the only organism
201 associated with increased 5-year survival in CF (6), is the least likely to persist from one year to the next. The
202 presence of *P aeruginosa*, MRSA and *B cepacia* complex all reduce the likelihood of culturing MSSA in future
203 years thus likely reducing patient survival.

204 Prior studies of small groups of patients using molecular analysis methods show that a patient is more
205 likely to retain current infections than lose them (25, 26). The chronicity of specific infections (27), their
206 interactions with human host defenses and their clinical outcomes are previously studied (28). Microbial
207 species-to-species interactions among pathogens similar to those found in the CF airway have been studied in
208 various *in vitro* and non-human *in vivo* model systems (17, 18, 29–37), and the clinical effects of the
209 combination of *P aeruginosa* and MSSA have been explored (38), but interspecies microbial interactions in the
210 CF airway are minimally explored, and recent calls to expand this knowledge base remain outstanding (16, 39).

211 Our study answers this call quantitatively by precisely showing that current infections alter the odds of
212 finding other organisms in subsequent years. Our study uses an approximately thousand-fold or larger group of
213 patients than prior studies examining organism persistence alone, thus providing the additional power necessary
214 to explore interspecies interactions with confidence. We demonstrate these associations in extensive univariable
215 and multivariable models (adjusted for other infections and clinical variables), and we demonstrate a lack of
216 sensitivity to varying clinical status and all common treatments. The stability of the results despite variability in
217 clinical states and prescribing of common treatments underscores the need to understand potential effects of
218 persistent microbial interactions to avoid undesirable clinical outcomes.

219 The strongest association between organisms was the negative one between MSSA and *P aeruginosa*
220 between years t and $t+1$ in both directions (Figures 2, 3 and 4). All associations were independent of the

221 presence of other organisms (Figure 4 and Table S3) and the severity of clinical characteristics (Figure 4 and
222 Table S4), and they were insensitive to the use of multiple CF-specific therapies including chronic and acute
223 antibiotic treatments. MSSA may limit acquisition and reduce persistence of *P aeruginosa* infection in some
224 patients, and *P aeruginosa* may supplant MSSA infection in others (1). These observations are consistent with
225 previous findings that elimination of MSSA leads to more rapid infection with *P aeruginosa* (14).

226 The two most harmful bacterial pathogens in CF, *P aeruginosa* and *B cepacia* complex, are also the
227 organisms in our study that were associated with the greatest number of other studied infections (Figure 4). The
228 presence of either *P aeruginosa* or *B cepacia* complex was associated with lower odds of future MSSA, *S*
229 *maltophilia*, and *A xylosoxidans*. *B cepacia* complex was additionally associated with lower odds for concurrent
230 (Figure 2C, turquoise cluster) and subsequent MRSA (Figure 4C). By limiting acquisition or persistence of
231 other infections, *P aeruginosa* and *B cepacia* complex may decrease microbial diversity. Loss of diversity in CF
232 airway ecology is linked with worsening lung disease, an observation consistent with the increased
233 pathogenicity of *P aeruginosa* and *B cepacia* complex in CF (13, 19).

234 Our study shows that *S maltophilia* and *A xylosoxidans* each had less influence on each other and the
235 other six organisms in our study than did *P aeruginosa* or *B cepacia* complex (Figures 2, 3 and 4). The
236 decreased impact on diversity may help explain, for example, why *S maltophilia* seems less pathogenic in CF
237 (40). Our study supports previous findings that intermittent infection with *S maltophilia* does not substantially
238 affect progression of lung disease or survival (41–43).

239 *Aspergillus* and *Candida* species are the most commonly cultured fungal infections in the CF airway
240 (44). The extent and nature of interaction between fungal and bacterial infections in the CF airway is unclear.
241 Previous *in vitro* and non-human *in vivo* model based research demonstrated inhibition of biofilm formation in
242 both *Candida* and *Aspergillus* by *P aeruginosa* (29, 30, 37). Our study based on clinical observation suggests
243 that *P aeruginosa* and *S maltophilia* infections are associated with higher rather than lower odds of concurrent
244 and subsequent *Aspergillus* infection (Figures 2H, S1, 3H and 4H). MRSA and *S maltophilia* were associated
245 with slightly higher odds of subsequent *Candida* infection (Figure 4G). Only *B cepacia* complex was associated

246 with lower odds for a future fungal infection, and only for *Aspergillus* species (Figure 4G). The discrepancies
247 between our observations and those from non-human models of *P aeruginosa* and *Aspergillus* interactions (29,
248 30, 37) may merely reflect differences between model system and human airway conditions but may indicate
249 the presence of important differences in microbial virulence (45). Evidence of interspecies interactions may help
250 explain why approximately a third of efforts to eradicate pathogens from the CF airway fail (46, 47): perhaps a
251 non-targeted concurrent infection promotes persistence of a target organism. The clinical impacts of these
252 associations remain uncertain but their potential for altering disease course and outcomes invites further study.

253 Our findings suggest that microbial interaction exists in the airways of patients with CF regardless of
254 treatments and events that may modify the presence of microbes. Potential interaction mechanisms may be
255 considered. First, microbes produce antimicrobial agents (31). The strength of associations between MSSA, *P*
256 *aeruginosa* and *B cepacia* complex (Figure 4A, B and D) are consistent with prior findings that these organisms
257 produce novel antimicrobials (48–52). Second, organisms may compete for airway resources such as iron (32,
258 53). Third, organisms may interact with human host defenses or with each other to modify interactions with yet
259 other organisms (35, 54). Finally, our confirmation of the consistency and strength of microbial interactions
260 regardless of mechanism suggests additional areas for investigation of clinical impacts. Expanded knowledge of
261 microbial interactions may explain unexpected outcomes of antimicrobial therapy (14, 15), better delineate the
262 pathogenesis of pulmonary exacerbations in CF that punctuate and accelerate the course of disease (55), and
263 improve predictions of long term outcomes critical to the well-being of patients (6, 7, 56).

264

265 *Limitations*

266 First, there may be unrecorded treatment decisions that affect airway ecology. However prior studies show that
267 short-course antibiotic therapy only transiently affects a CF patient's individual microbiota (26, 57). Moreover,
268 our analysis showed that adjustment for various treatments did not materially alter results. Second, we were
269 limited to studying the eight organisms for which there are sufficient culture data present in the CFFPR. This
270 excluded direct study of many CF airway organisms that are infrequently present, under reported, not collected

271 during the study period (such as non-tuberculous *Mycobacteria*) or only identifiable by non-culture methods
272 (58). The use of culture data is subject to variable rates, by organism, of false positive or negative results;
273 however, similar difficulties affect recovery of organisms by non-culture methods (58). Furthermore, results
274 from conventional aerobic sputum cultures are the data that drive clinical decisions in treating patients with CF,
275 are correlated with results from culture independent methods for identifying the common aerobic CF infections
276 analyzed in our study, especially *P aeruginosa* (26, 58), and provide the basis of prior reports showing
277 associations between organisms and survival outcomes (6, 9). Lastly, there are potential biases from
278 missingness of data that prevented inclusion of some patients in the analysis. However, the proportion of
279 patients with missingness was quite low (Tables S6, S7 and S8). The patients in the CFFPR during the study
280 period who never had sufficient data for inclusion accounted for 0.2% to 0.9% of all CFFPR patients during
281 each year of the study period (Tables S7 and S8). There may also be data in the CFFPR that were partially
282 available for adjustment of our models but which we excluded from the analysis for various reasons. For
283 example, we did not use genotype data because it was unavailable for a large proportion of the patients studied
284 and because it is less successful as a predictor of long term clinical outcomes compared to clinical phenotype
285 variables such as we previously used to predict 5-year survival outcomes (6).

286

287 *Conclusions*

288 This study helps clinicians understand how current microbiology may play a role in shaping the overall
289 subsequent microbiology of the CF airway. Mechanistic studies are needed to understand specifically how
290 MSSA may limit infection with *P aeruginosa* and how *P aeruginosa* and *B cepacia* complex may limit co-
291 infecting organisms. Such understanding has the potential to influence strategic decisions in CF clinical care.
292 While a bacterial pathogen found in an otherwise healthy host is often met with an attempt at eradication, this
293 strategy in CF may be defeated by interspecies interactions that promote persistence of multiple species and
294 have unintended consequences even when seemingly successful. Eliminating specific infections within the
295 diverse CF airway ecology may disrupt a delicate balance and accelerate the time to infection of more

296 pathogenic organisms. Determining the potential influence of each organism on the CF microbiome may help
297 clinicians understand the extended impact of modifying a patient's airway ecology and ultimately improve
298 patient survival.

299

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307 Some of the data and results were reported by Dr. Granchelli in preliminary form at the 37th European
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310 analysis. Drs. Granchelli, Adler and Liou conceived the initial idea for this paper. All authors participated in the
311 design and execution of the analysis and interpretation of the data. Dr. Liou obtained funding, acquired the raw
312 data, oversaw security and integrity of the data and with the assistance of Ms. Judy Jensen obtained necessary
313 permissions from the University of Utah IRB and the CFF Patient Registry Comparative Effectiveness Research
314 Committee to proceed with the study. Drs. Granchelli and Liou drafted the manuscript but all authors
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337

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355 novel antibiotic and reports being a member of the Clinical Research Review Committee at the US CFF and
356 reviewing grant applications and being a member of the Editorial Board at *CHEST* and reviewing papers for
357 various journals regarding CF and statistical analyses in Medicine.

358

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360 **Figure Legends**

361 **Figure 1. Prevalence of positive cultures for studied infections, 2003-2011.** Eight curves show the changing
362 prevalence of the organisms studied for the patients in the CFFPR for each of the years 2003 through 2011 able
363 to produce samples for microbiologic cultures. The figure is similar to prior figures showing the data in
364 somewhat different ways (59).

365 **Figure 2. Adjusted cross-sectional associations between airway infections.** Forest plots show the adjusted
366 odds ratios (circles) and 99% confidence intervals (bars) of having a positive culture for each of the eight
367 studied organisms within each study year comparing presence versus absence of a positive culture for each of
368 the other seven organisms in the same year. The outcomes were (A) MSSA: methicillin sensitive
369 *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA: methicillin resistant *S aureus*, (D) BCC:
370 *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas maltophilia*, (F) AX: *Achromobacter xylosoxidans*,
371 (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Purple results are from models adjusted by the presence
372 of the remaining six other organisms. Turquoise results are from models additionally adjusted for clinical
373 characteristics: age, sex, late diagnosis of CF, best FEV₁% in each year, annual number of APE, pancreatic
374 sufficiency and diabetes status and weight-for-age z-score.

375 **Figure 3. Adjusted associations between airway infections in years 2003-2010 with other organisms in**
376 **subsequent years 2004-2011.** Forest plots show the odds ratio (circles) and 99% confidence intervals (bars) of
377 having a positive culture in years $t+1$ for each of the eight studied organisms when each of the other organisms
378 were present in respective years t where $t=2003-2010$. The outcomes from years $t+1$ were (A) MSSA:
379 methicillin sensitive *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA: methicillin resistant
380 *S aureus*, (D) BCC: *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas maltophilia*, (F) AX:
381 *Achromobacter xylosoxidans*, (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Red results are from
382 models adjusted by the presence of the remaining six organisms. Green results are from models additionally
383 adjusted for clinical characteristics in years t : age, sex, late diagnosis of CF, best FEV₁% in each year, annual
384 number of APE, pancreatic sufficiency and diabetes status and weight-for-age z-score.

385 **Figure 4. Adjusted associations between airway infections in year t with other organisms in year $t+1$.** Each
386 forest plot shows the odds ratios (circles) and 99% confidence intervals (bars) from the combined model
387 utilizing the entire 2003-2011 CFFPR data set for each of the eight studied organisms in year $t+1$ when each of
388 the other organisms were present in respective years t where $t=2003-2010$. The outcomes from years $t+1$ were
389 (A) MSSA: methicillin sensitive *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA:
390 methicillin resistant *S aureus*, (D) BCC: *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas*
391 *maltophilia*, (F) AX: *Achromobacter xylosoxidans*, (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Red
392 results are from models adjusted by the presence of the remaining six organisms. Green results are from models
393 additionally adjusted for clinical characteristics for years t : age, sex, late diagnosis of CF, best FEV₁% in each
394 year, annual number of APE, pancreatic sufficiency and diabetes status and weight-for-age z -score.

1 **Table 1. Selected Characteristics of Study Patients**

Patient Characteristics	Year									
	2003	2004	2005	2006	2007	2008	2009	2010	2011	
Number Enrolled	15626	16428	16933	17483	18305	18786	19384	19818	20534	
Number of cultures ^a	42995 (0.996)	47516 (0.999)	51826 (1.000)	55922 (0.999)	61442 (1.000)	65319 (1.000)	68312 (1.000)	70294 (0.999)	74409 (1.000)	
Cultures per Patient ^b	2 (1 - 4)	2 (1 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 5)	
Age ^b	16.6 (11.4 - 24.0)	17.0 (11.7 - 24.5)	17.2 (11.8 - 24.8)	17.6 (12.0 - 25.3)	17.8 (12.1 - 25.9)	18.1 (12.2 - 26.4)	18.4 (12.4 - 27.0)	18.7 (12.5 - 27.4)	19.1 (12.7 - 27.9)	
Female ^c	7446 (47.7)	7811 (47.5)	8050 (47.5)	8319 (47.6)	8764 (47.9)	9060 (48.2)	9393 (48.5)	9618 (48.5)	9949 (48.5)	
Pancreatic Sufficiency ^c	1018 (6.53)	1198 (7.30)	1189 (7.03)	1365 (7.81)	1457 (7.96)	1654 (8.80)	1830 (9.44)	2419 (12.2)	2300 (11.2)	
Diabetes ^c	1503 (9.62)	1744 (10.6)	1919 (11.3)	2410 (13.8)	2664 (14.6)	2845 (15.1)	3022 (15.6)	3270 (16.5)	3601 (17.5)	
FEV ₁ % ^b	81.0 (58.7 - 97.0)	81.5 (59.2 - 97.2)	82.0 (59.6 - 97.5)	82.6 (60.2 - 97.7)	83.0 (60.4 - 98.5)	83.2 (60.7 - 98.7)	83.5 (60.7 - 99.0)	83.9 (61.3 - 99.1)	83.7 (61.3 - 99.0)	
Acute Pulmonary Exacerbations ^b	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	
Weight-for-Age z-score ^b	-0.52 (-1.23 - 0.18)	-0.47 (-1.20 - 0.21)	-0.45 (-1.17 - 0.22)	-0.40 (-1.12 - 0.25)	-0.38 (-1.10 - 0.27)	-0.33 (-1.06 - 0.30)	-0.29 (-1.04 - 0.33)	-0.27 (-1.04 - 0.35)	-0.25 (-1.03 - 0.36)	
MSSA ^c	7852 (50.2)	8292 (50.5)	8497 (50.2)	8704 (49.8)	9140 (49.9)	9299 (49.5)	9574 (49.4)	9759 (49.2)	10104 (49.2)	
<i>P aeruginosa</i> ^c	9969 (63.8)	10469 (63.7)	10656 (62.9)	10729 (61.4)	11100 (60.6)	11123 (59.2)	11323 (58.4)	11482 (57.9)	11767 (57.3)	
MRSA ^c	2034 (13.0)	2642 (16.1)	3155 (18.6)	3588 (20.5)	4150 (22.7)	4576 (24.4)	5005 (25.8)	5514 (27.8)	5801 (28.3)	
<i>Burkholderia</i> Complex ^c	584 (3.74)	580 (3.53)	622 (3.67)	607 (3.47)	622 (3.40)	618 (3.29)	619 (3.19)	697 (3.52)	756 (3.68)	
<i>S maltophilia</i> ^c	1868 (12.0)	2064 (12.6)	2243 (13.2)	2320 (13.3)	2440 (13.3)	2457 (13.1)	2616 (13.5)	2881 (14.5)	3010 (14.7)	
<i>A xylooxidans</i> ^c	1028 (6.58)	1095 (6.67)	1166 (6.89)	1240 (7.09)	1236 (6.75)	1355 (7.21)	1343 (6.93)	1468 (7.41)	1501 (7.31)	
<i>Candida</i> species ^c	1295 (8.29)	1177 (7.16)	1365 (8.06)	1370 (7.84)	1391 (7.60)	1609 (8.56)	1661 (8.57)	3129 (15.8)	3559 (17.3)	
<i>Aspergillus</i> species ^c	2458 (15.7)	2677 (16.3)	2724 (16.1)	2913 (16.7)	2996 (16.4)	3190 (17.0)	3141 (16.2)	3409 (17.2)	3678 (17.9)	

^a Number (fraction of cultures obtained from study participants with usable data)^b Median (1st and 3rd quartiles)^c Number of Patients (percent of enrolled patients)

2

Figure 1

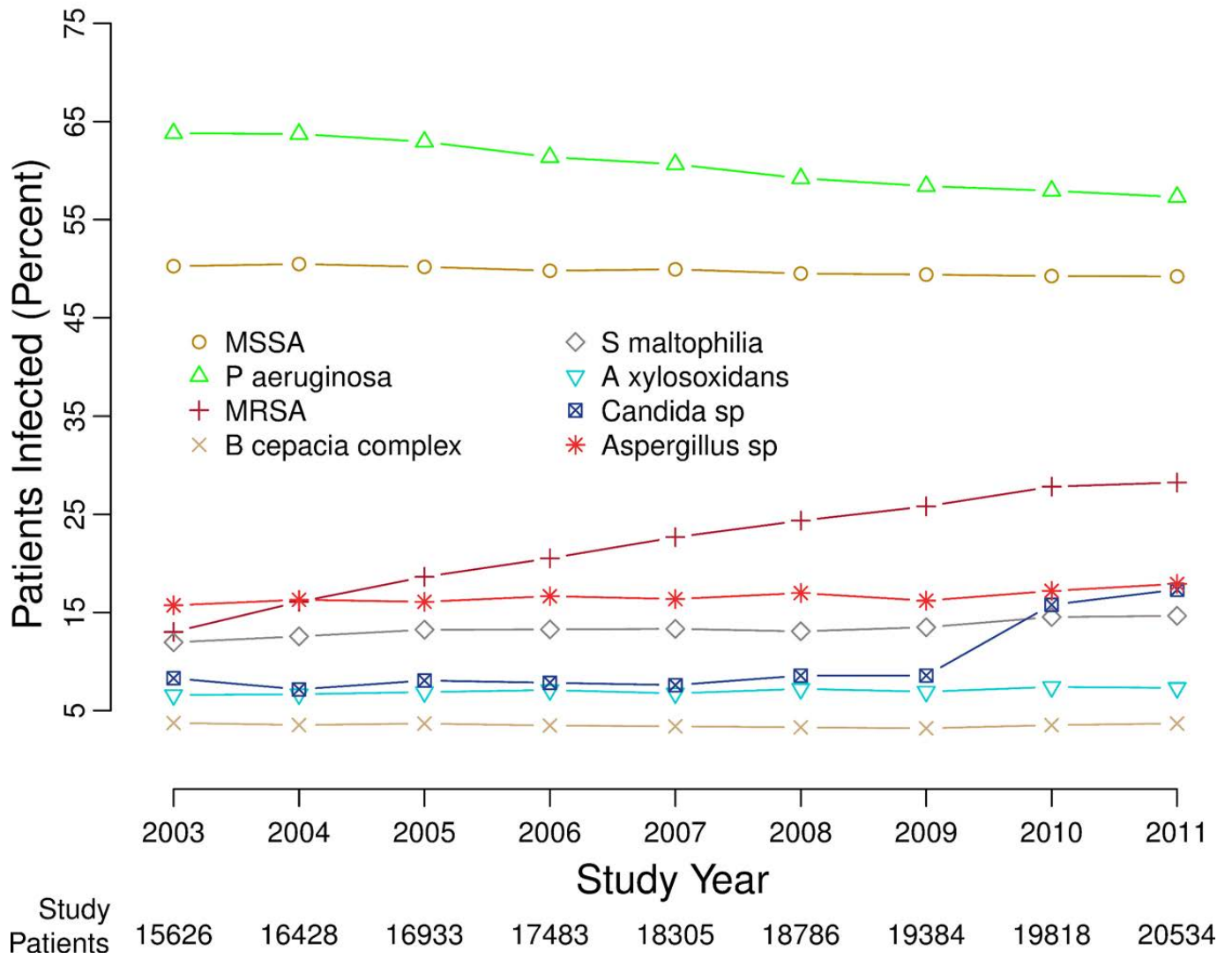


Figure 2

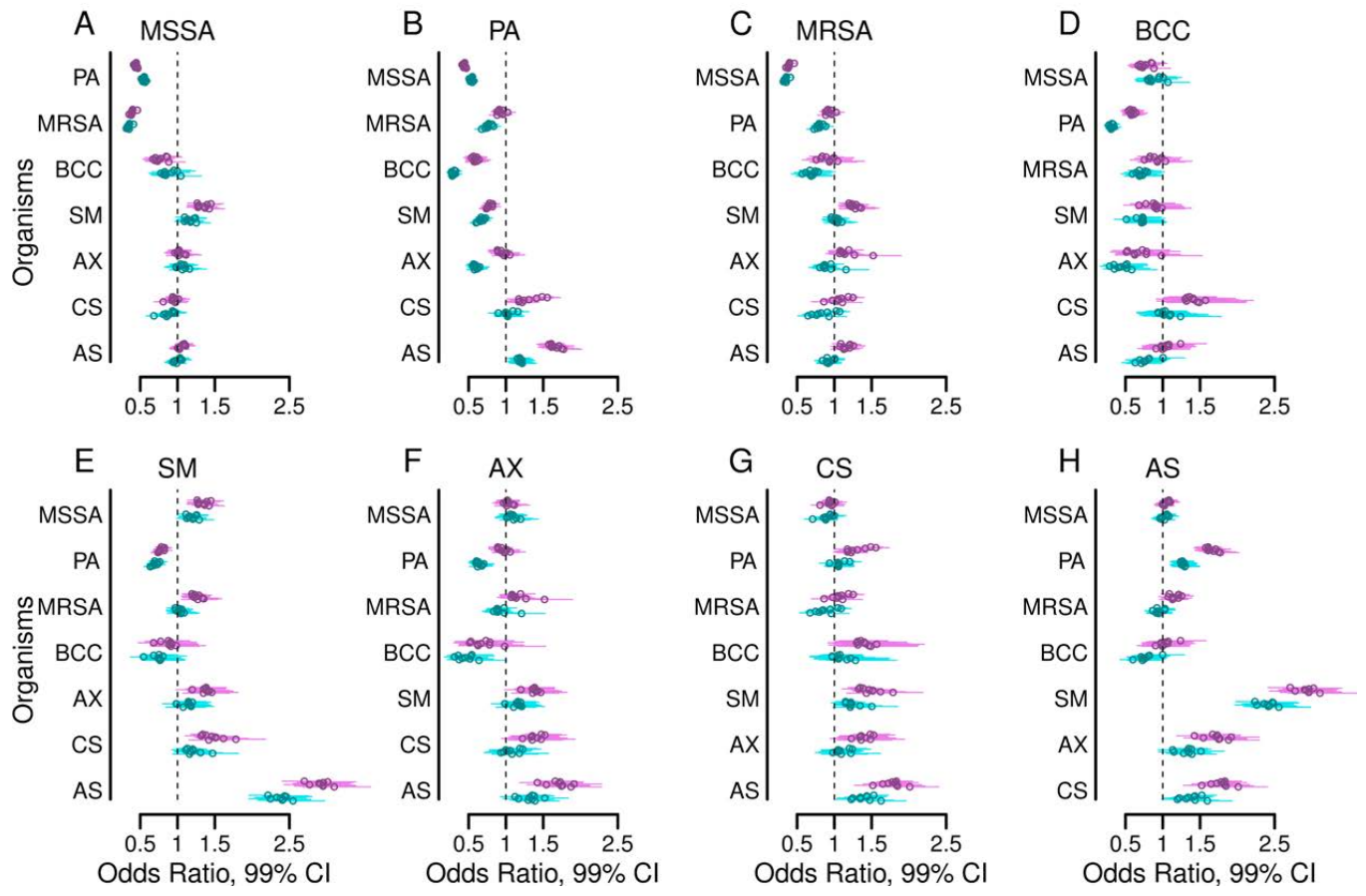


Figure 3

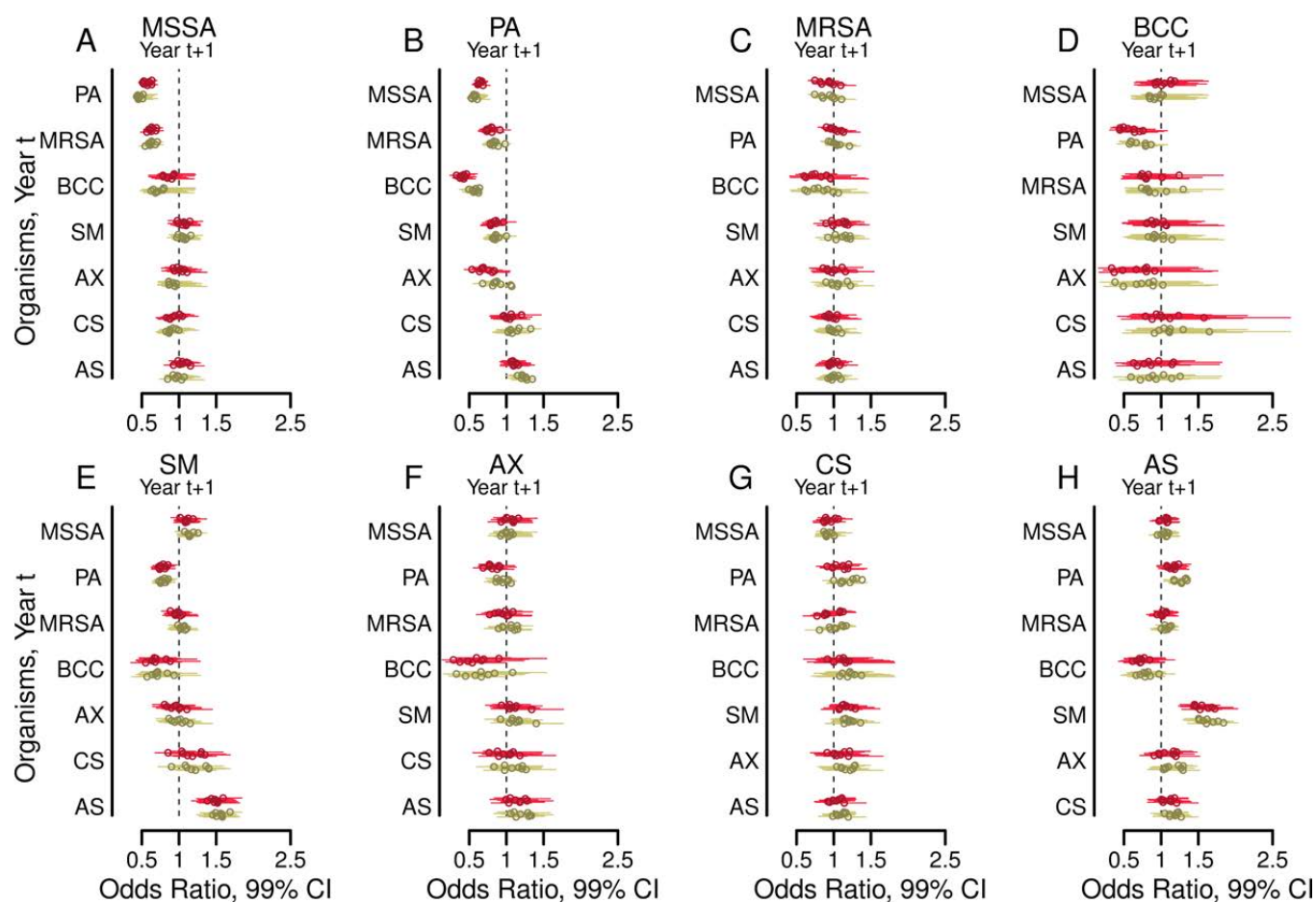


Figure 4

