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Microbial Interactions in the Cystic Fibrosis Airway

Running Title: Microbial Interactions in CF

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Abstract

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

Introduction

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

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

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

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

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

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

Methods

Study Design and Data

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

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

Study population and definitions

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

CFFPR contains sufficient data for analysis: MSSA, methicillin resistant *S aureus* (MRSA), *B cepacia* complex, *P aeruginosa*, *S maltophilia, A xylosoxidans, Candida* species and *Aspergillus* species. We identified and adjusted for the following patient characteristics as potential confounders in statistical models: age, age at diagnosis of CF, sex, CF-related diabetes, pancreatic sufficiency, weight-for-age *z*-score, percent predicted forced expiratory volume in one second (FEV_1%), and acute pulmonary exacerbations (APE). Most of these characteristics were previously found to predict 5-year survival (6). We defined patients as diabetic in a given year if the condition was present at any time during that year. We defined patients as pancreatic sufficient in a given year if they were noted to be sufficient for all encounters and did not use pancreatic enzymes during that year. For sensitivity analyses to determine the effect of adjustment of associations for different interventions, we used treatment data on oral azithromycin, inhaled aztreonam, tobramycin, recombinant human DNase (DNase), and hypertonic saline, days per year of therapy with home intravenous (IV) antibiotics, hospitalization days for pulmonary exacerbation treatment, and lung transplantation.

Statistical Analysis

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

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

Results

Participants

We found 28,042 patients aged six years or older in the CFFPR from 257 care centers and affiliated programs accredited by the US CF Foundation between January 1, 2003 and December 31, 2011 with at least two consecutive years of culture data (Table 1). These patients had a total of 538,458 sets of sputum culture results of which 537,396 (99.8%) were included in our analysis (Figure 1). Culture results were excluded only for lack of same-patient cultures in contiguous years. From 2003 to 2011, the median cross-sectional age of patients increased from 16.6 to 19.1 (Table 1). CF-related diabetes prevalence nearly doubled over this time period from 9.62% to 17.5%. A minority of patients were pancreatic sufficient; this increased between 2003 and 2011 from 6.53% to 11.2%. There were marginal changes in the median FEV_1% and mean number of APE during the study period. The changes in pediatric and adult groups were similar (Tables S1 and S2).

Infection Prevalence

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

Cross Sectional Associations between Airway Infections

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

Associations between Present and Future Airway Infections

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

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

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

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

Missingness of Data

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

Discussion

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

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

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

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

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

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

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

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

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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

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

Conclusions

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

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

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Some of the data and results were reported by Dr. Granchelli in preliminary form at the 37th European Cystic Fibrosis Society Meetings, June 11-14, 2013 in Gothenburg, Sweden. Drs. Adler and Liou had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Granchelli, Adler and Liou conceived the initial idea for this paper. All authors participated in the design and execution of the analysis and interpretation of the data. Dr. Liou obtained funding, acquired the raw data, oversaw security and integrity of the data and with the assistance of Ms. Judy Jensen obtained necessary permissions from the University of Utah IRB and the CFF Patient Registry Comparative Effectiveness Research Committee to proceed with the study. Drs. Granchelli and Liou drafted the manuscript but all authors participated in critical reviews and revisions of the work. Professor Sir David Cox throughout the study provided pivotal advice on many statistical aspects. Dr. Liou oversaw clinically oriented aspects of the work.

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Conflicts of Interest

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Figure Legends

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

Figure 2. Adjusted cross-sectional associations between airway infections. Forest plots show the adjusted odds ratios (circles) and 99% confidence intervals (bars) of having a positive culture for each of the eight studied organisms within each study year comparing presence versus absence of a positive culture for each of the other seven organisms in the same year. The outcomes were (A) MSSA: methicillin sensitive *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA: methicillin resistant *S aureus*, (D) BCC: *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas maltophilia,* (F) AX: *Achromobacter xylosoxidans*, (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Purple results are from models adjusted by the presence of the remaining six other organisms. Turquoise results are from models additionally adjusted for clinical characteristics: age, sex, late diagnosis of CF, best FEV_1% in each year, annual number of APE, pancreatic sufficiency and diabetes status and weight-for-age *z*-score.

Figure 3. Adjusted associations between airway infections in years 2003-2010 with other organisms in subsequent years 2004-2011. Forest plots show the odds ratio (circles) and 99% confidence intervals (bars) of having a positive culture in years *t+1* for each of the eight studied organisms when each of the other organisms were present in respective years *t* where *t*=2003-2010. The outcomes from years *t+1* were (A) MSSA: methicillin sensitive *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA: methicillin resistant *S aureus*, (D) BCC: *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas maltophilia,* (F) AX: *Achromobacter xylosoxidans*, (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Red results are from models adjusted by the presence of the remaining six organisms. Green results are from models additionally adjusted for clinical characteristics in years *t*: age, sex, late diagnosis of CF, best FEV_1% in each year, annual number of APE, pancreatic sufficiency and diabetes status and weight-for-age *z*-score.

Figure 4. Adjusted associations between airway infections in year *t* with other organisms in year *t+1*. Each forest plot shows the odds ratios (circles) and 99% confidence intervals (bars) from the combined model utilizing the entire 2003-2011 CFFPR data set for each of the eight studied organisms in year *t+1* when each of the other organisms were present in respective years *t* where *t*=2003-2010. The outcomes from years *t+1* were (A) MSSA: methicillin sensitive *Staphylococcus aureus*, (B) PA: *Pseudomonas aeruginosa*, (C) MRSA: methicillin resistant *S aureus*, (D) BCC: *Burkholderia cepacia* complex, (E) SM: *Stenotrophomonas maltophilia,* (F) AX: *Achromobacter xylosoxidans*, (G) CS: *Candida* species, (H) AS: *Aspergillus* species. Red results are from models adjusted by the presence of the remaining six organisms. Green results are from models additionally adjusted for clinical characteristics for years *t*: age, sex, late diagnosis of CF, best FEV_1% in each year, annual number of APE, pancreatic sufficiency and diabetes status and weight-for-age *z*-score.

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)
P aeruginosa[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)
Burkholderia 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)
S maltophilia[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)
A xylosoxidans[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)
Candida 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)
Aspergillus 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)

Figure 1

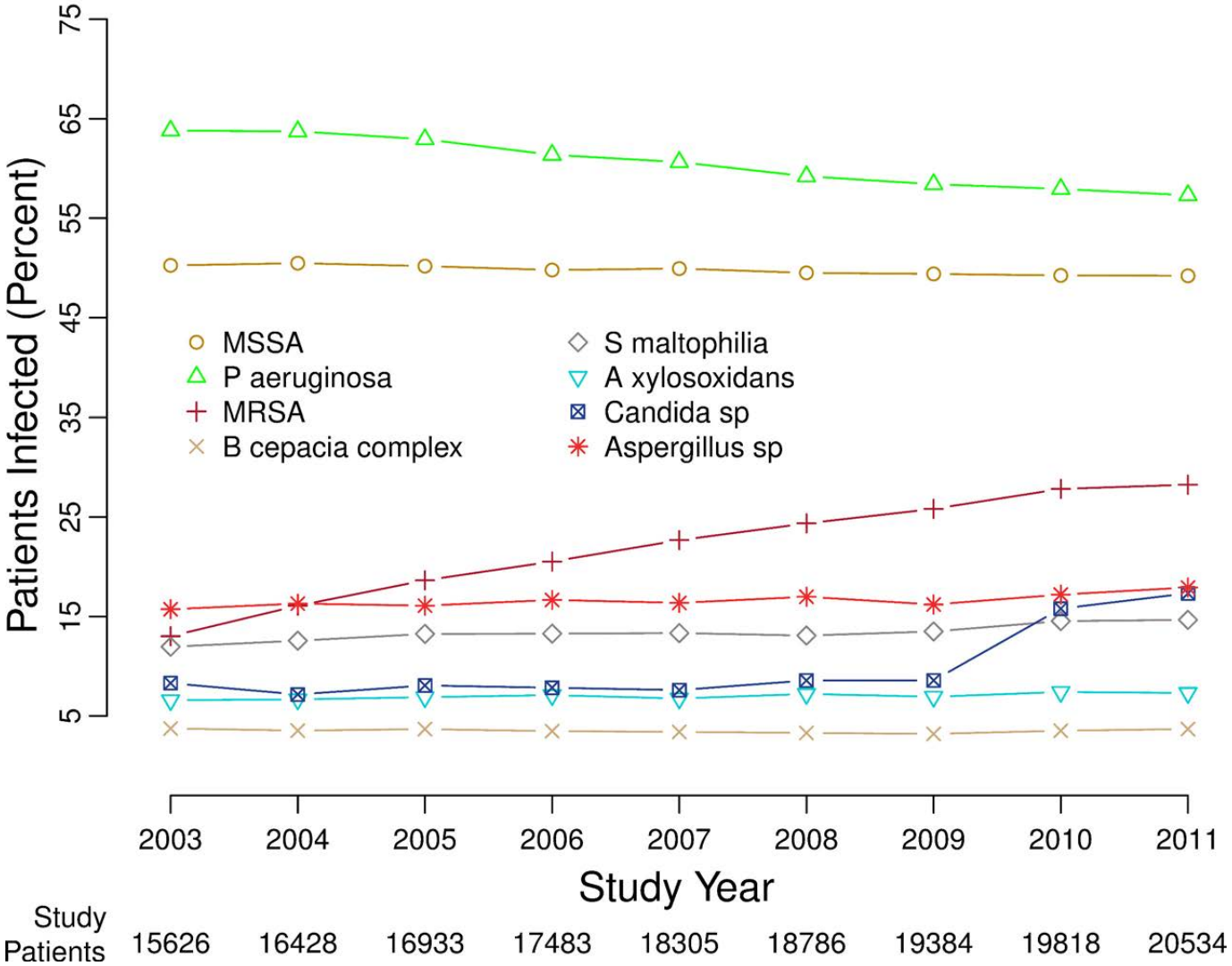

Figure 2

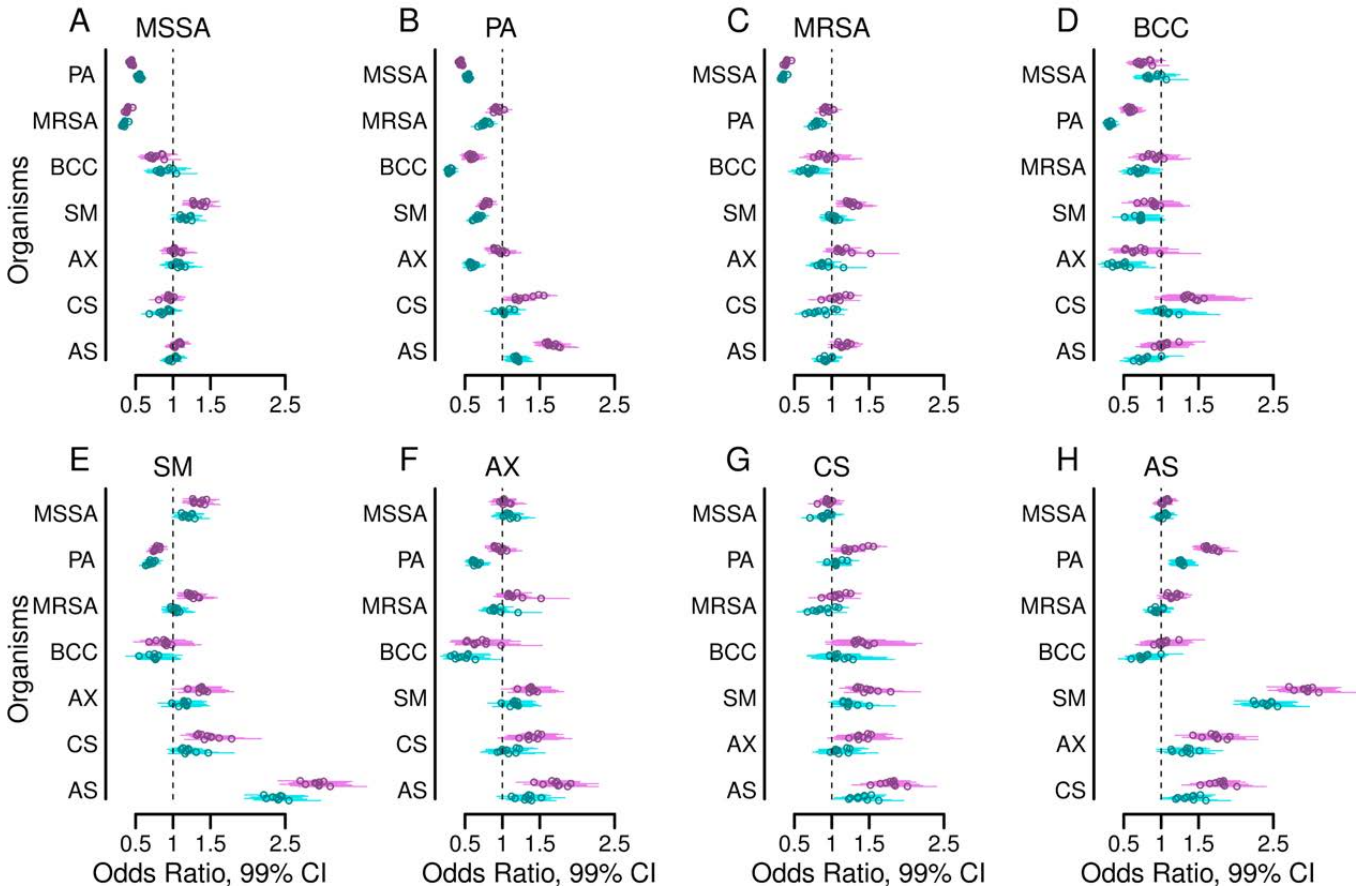

Figure 3

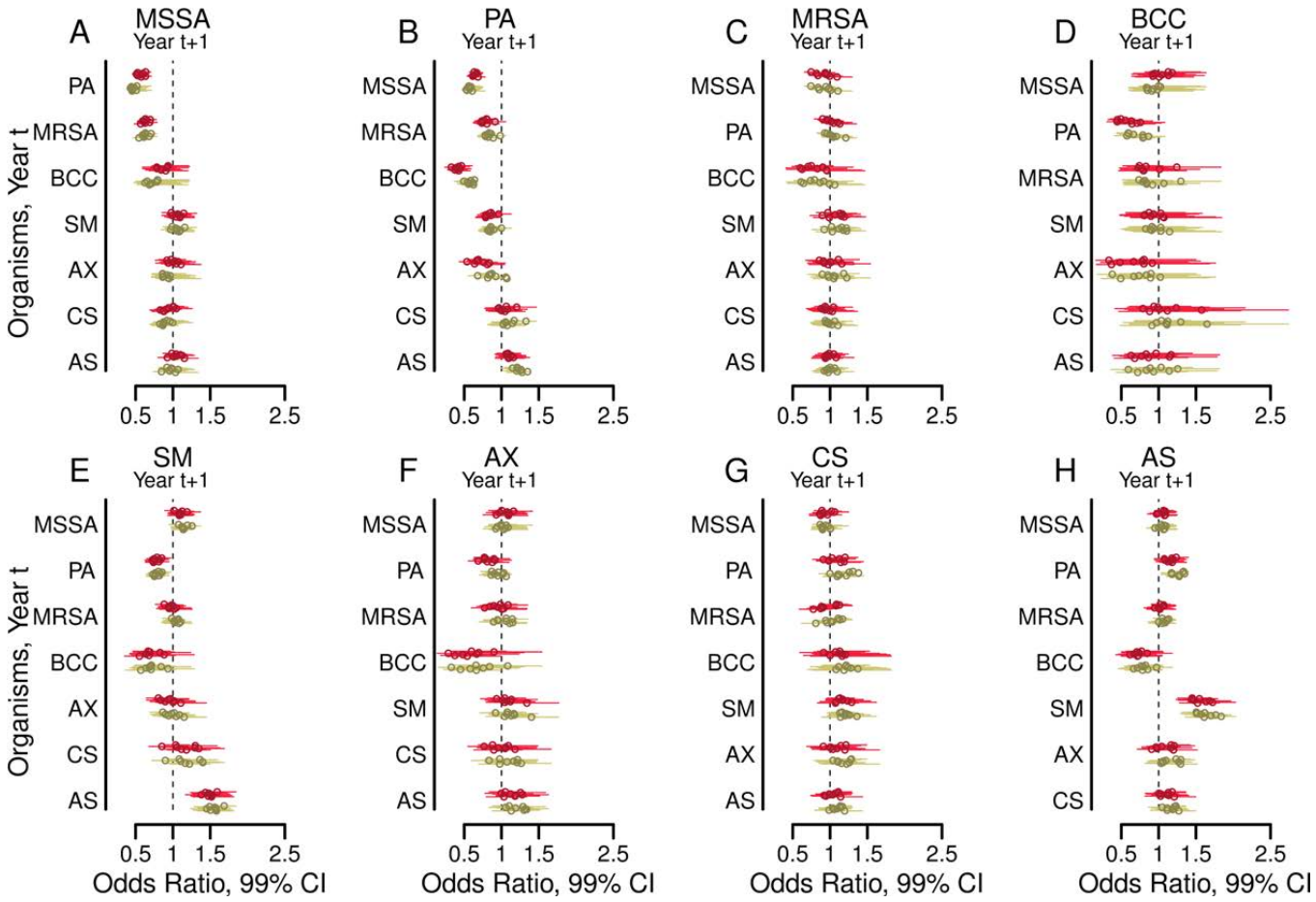

Figure 4