ORIGINAL ARTICLE

Sustained Coinfections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Cystic Fibrosis

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Abstract

Rationale: *Staphylococcus aureus* and *Pseudomonas aeruginosa* often infect the airways in cystic fibrosis (CF). Because registry studies show higher prevalence of *P. aeruginosa* versus *S. aureus* in older patients with CF, a common assumption is that *P. aeruginosa* replaces *S. aureus* over time. *In vitro*, *P. aeruginosa* can outgrow and kill *S. aureus*. However, it is unknown how rapidly *P. aeruginosa* replaces *S. aureus* in patients with CF.

Methods: We studied a longitudinal cohort of children and adults with CF who had quantitative sputum cultures. We determined the abundance of *P. aeruginosa* and *S. aureus* in cfu/ml. We determined the duration and persistence of infections and measured longitudinal changes in culture positivity and abundance for each organism.

Measurements and Main Results: Between 2004 and 2017, 134 patients had ⩾10 quantitative cultures, with median observation time of 10.15 years. One hundred twenty-four patients had at least one positive culture for *P. aeruginosa*, and 123 had at least one positive culture for *S. aureus*. Both species had median abundance of $>10^6$ cfu/ml. Culture abundance was stable over time for both organisms. There was an increase in the prevalence of *S. aureus*/*P. aeruginosa* coinfection but no decrease in *S. aureus* prevalence within individuals over time.

Conclusions: *S. aureus* and *P. aeruginosa* are abundant in CF sputum cultures. Contrary to common assumption, we found no pattern of replacement of *S. aureus* by *P. aeruginosa*. Many patients with CF have durable long-term coinfection with these organisms. New strategies are needed to prevent and treat these infections.

Keywords: *Staphylococcus aureus*; *Pseudomonas aeruginosa* sputum; cystic fibrosis; methicillin-resistant *Staphylococcus aureus*

At a Glance Commentary

Scientific Knowledge on the Subject: *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections are common in cystic fibrosis. *S. aureus* is more prevalent in children and adolescents, whereas *P. aeruginosa* is more prevalent in adults.

What This Study Adds to the Field: Patients with cystic fibrosis have durable, highly abundant respiratory coinfections with *S. aureus* and *P. aeruginosa*. Contrary to common assumption, *P. aeruginosa* replacement of *S. aureus* within individuals is not routinely observed in longitudinal follow-up.

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This article has a related editorial.

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Respiratory infections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* increase the risk of disease progression in cystic fibrosis (CF) (1–3). These organisms represent the two most prevalent CF respiratory pathogens (4). Registry studies indicate that *S. aureus* is most prevalent in younger patients, whereas *P. aeruginosa* is the most prevalent above age 24 (5). These cross-sectional data support a common, yet unproven, assumption that *P. aeruginosa* replaces *S. aureus* over time (6–12). Although this phenomenon of *P. aeruginosa* outcompeting *S. aureus* can be demonstrated *in vitro* (9, 12, 13), conclusive evidence of pathogen replacement in people with CF would require a longitudinal cohort study. It is possible that some differences in pathogen prevalence between older and younger patients might be explained by factors unrelated to direct bacterial competition. For instance, different birth cohorts were exposed to different treatments in early life. Older patients were born before eradication therapy for *P. aeruginosa* was available but were treated for *S. aureus* (14). Patients born in the era of inhaled antibiotic therapy now can suppress *P. aeruginosa* (15–17) but may not eliminate *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA) (18).

To measure how quickly *P. aeruginosa* replaces *S. aureus* in people with CF, we examined microbiology results from a longitudinal cohort of patients with CF. Because there could be doubt about the pathologic significance of *S. aureus* on oropharyngeal culture swab owing to its presence in healthy subjects (7, 19), we focused on quantitative cultures of sputum or BAL. With quantitation, long-term trends in the microbial population structure may become apparent. For instance, in a patient with both *S. aureus* and *P. aeruginosa*, one organism may begin to dominate.

Using this cohort, we determined the sequence of lower respiratory infections, the duration of infections, and the effect of incident infections on the presence and abundance of baseline pathogen. We hypothesized that *P. aeruginosa* would dominate and ultimately replace *S. aureus* in quantitative cultures from individuals with CF within one decade of follow-up.

Methods

Ethics Statement

The institutional review board of the University of Iowa approved this study (# 201905718). A waiver of informed consent was granted owing to the study being minimal in risk.

Study Design

This is a longitudinal, retrospective, single-center cohort study of adult and pediatric patients with CF who had chronic sputum production. The primary aim was to determine whether *S. aureus* prevalence would decrease over time in individuals with CF, coincident with pathogen replacement by *P. aeruginosa*. In examining the prevalence of respiratory microorganisms by age cohort in the Cystic Fibrosis Foundation (CFF) annual report (5), we noted an approximately 10% decrease in *S. aureus* prevalence with each decade. We estimated that 130 individuals, observed for a period of 10 years, would be required to detect this 10% reduction in *S. aureus* prevalence with $\alpha = 0.05$ and power = 0.80 using a 1-sided McNemar's exact test. Secondary endpoints included quantitative changes in sputum density of *S. aureus* and *P. aeruginosa*.

Inclusion and Exclusion Criteria

We included patients with CF who received care at the University of Iowa between 2004 and 2017. All subjects had diagnostic sweat chloride measurements or two *CFTR* mutations. For longitudinal analysis, we required each subject have at least 10 quantitative respiratory cultures of the lower respiratory tract (e.g., sputum or BAL). We excluded patients with CFTR-related metabolic syndrome. We excluded cultures that did not result in quantitation and cultures obtained after lung transplantation. In addition to microbiology data, we obtained electronic prescription data to determine whether infections were treated. Electronic prescriptions were available after 2009.

Definitions of MRSA and Methicillin-Sensitive *S. aureus*

We defined MRSA if it was explicitly named in the report, if there was phenotypic resistance to oxacillin or cefoxitin, or if there was molecular detection of PBP2a. We defined methicillin-sensitive *S. aureus* (MSSA) if it was declared on the report or if the *S. aureus* failed to meet above definitions of MRSA. A minority of *S. aureus* isolates could not be classified.

Definitions of Prevalence

Pathogen prevalence was the number of subjects with a culture positive for a given organism divided by the number of subjects cultured during the same calendar year. To determine microbial prevalence by age cohorts defined in the CFF annual report (5), we calculated the age of each subject as of the last day of the calendar year. We combined age <2 and ages 2–6 into a single group owing to few observations. Within each age category, we divided the number of subject-years that *S. aureus* or *P. aeruginosa* were present on quantitative culture by the number of subject-years a quantitative culture was obtained.

Duration and Persistence of Infections

The duration of infection was the time difference (in years) between the initial and final positive culture. The persistence of an organism was the percentage of quantitative cultures that were positive after the initial positive culture. The persistence was not calculated if an organism was only identified on the final culture for an individual. Similarly, we determined the time difference between the first and last cultures that were simultaneously positive for both *S. aureus* and *P. aeruginosa* (referred to as double-positive cultures). The persistence of coinfections was the percentage of cultures that were double-positive in between the initial and final double-positive cultures. For individuals with a single double-positive culture, the duration of coinfection was zero. If there were no intervening cultures between the first and last double-positive culture, the persistence of coinfection was not calculated.

Sequence of Appearance and Disappearance of Bacterial Species

For individuals with infections by multiple organisms, we calculated the time difference between the debut cultures of MSSA, MRSA, *Haemophilus influenzae*, and *P. aeruginosa*. To determine whether the appearance of a competitor species was temporally associated with disappearance of a preexisting species, we calculated the time difference between the last appearance of each organism versus the debut of a potential competitor.

Table 1. Longitudinal Cohort of Subjects with CF Analyzed from January 1, 2004, to December 31, 2017

	Patients without Quantitative Cultures (*Total = 66*)	Patients with 1–9 Quantitative Cultures (*Total = 137*)	Patients with ⩾10 Quantitative Cultures (*Total = 134*)
Sex and genotypes, *n* (%)			
Sex, F	31 (47.0)	68 (49.6)	61 (45.5)
F508del/F508del	32 (48.5)	65 (47.4)	79 (59.0)
F508del/other	26 (39.4)	57 (41.6)	47 (35.1)
Age, yr, median (IQR)			
Birth year	2007 (1992–2013)	1999 (1986–2007)	1986 (1976–1997)
At first culture*	3.40 (0.30–19.10)	8.84 (0.31–23.72)	19.34 (8.08–28.48)
At first quantitative culture	—	11.21 (2.23–24.33)	19.66 (8.69–28.48)
Follow-up duration, median (IQR)			
Years between first and last cultures*	1.25 (0.52–3.68)	5.48 (2.66–9.72)	11.98 (7.94–13.57)
Years between first and last quantitative cultures	—	2.08 (0.00–4.47)	10.15 (6.73–11.84)
Number of cultures*	7 (3–14)	14 (5–28)	35 (26–41)
Number of quantitative cultures	—	2 (1–5)	25 (17–33)
Final clinical status, *n* (%)			
Lung transplant	0 (0)	7 (5.1)	24 (17.9)
Death without transplant	1 (1.5)	7 (5.1)	14 (10.4)
Alive, no transplant, and cultured after January 1, 2016	53 (80.3)	92 (67.2)	92 (68.7)
Other (e.g., transfer of care)	12 (18.2)	31 (22.6)	4 (3.0)
Culture results, median (IQR)*			
Any *S. aureus*	50 (75.8)	120 (87.6)	126 (94.0)
MSSA	46 (69.7)	100 (73.0)	111 (82.8)
MRSA	5 (7.6)	64 (46.7)	79 (59.0)
P. aeruginosa	34 (51.5)	101 (73.7)	128 (95.5)
Either *S. aureus* or *P. aeruginosa*	59 (89.4)	133 (97.1)	134 (100)
Both *S. aureus* and *P. aeruginosa*	25 (37.9)	88 (64.2)	120 (89.6)
Simultaneous *S. aureus* and *P. aeruginosa*	21 (31.8)	71 (51.8)	114 (85.1)

Definition of abbreviations: CF = cystic fibrosis; IQR = interquartile range; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-sensitive *S. aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*.
*Includes nonquantitative CF respiratory culture studies such as oropharyngeal swab in addition to quantitative cultures. All cultures obtained after lung transplant were excluded.

Changes in Abundance of Baseline Infections following Incident Infection with Competing Species

In patients who were infected by both *S. aureus* and *P. aeruginosa*, we determined which infection was established first by identifying the calendar years that each organism debuted on quantitative culture. We monitored the abundance of each organism from 3 years before to 5 years after the appearance of the opposite species. Subjects whose first *S. aureus* and *P. aeruginosa* occurred in the same year were analyzed separately. To control for the potential overrepresentation of subjects having more follow-up cultures owing to more severe disease, we calculated the mean $\log_{10}$ cfu/ml of the established organisms on an annual basis. Because organism abundance for both species was not normally distributed, we used Spearman's correlation to test for a relationship between culture density and time following detection of the opposite species.

Longitudinal Changes in Annual Culture Status

We determined positivity for *S. aureus* and *P. aeruginosa* for each year a subject had at least one quantitative culture. Owing to a decrease in the number of quantitative cultures in 2017 because of changes in center practice, we compared organism prevalence between each subject's initial year of observation and the final year up to the year 2016 using exact McNemar's test.

Statistics and Data Analysis

We used RStudio version 1.2.5001 (RStudio, Inc.) and SAS version 9.4 (SAS Institute) for data analysis and statistical tests. For nonnormal distributions, we used nonparametric statistical tests. We reported central tendency and variation with median and interquartile range. Box plots were made using R default settings.

Data Availability

Deidentified microbiologic data and source codes corresponding to results presented in this article will be made available upon publication and for up to 3 years after final publication. Written requests for data should be made by e-mail to the corresponding author. Data will be transferred contingent upon completion of a data transfer agreement.

Results

Subjects and Sources of Quantitative Cultures

To measure replacement of *S. aureus* by *P. aeruginosa*, we identified subjects with

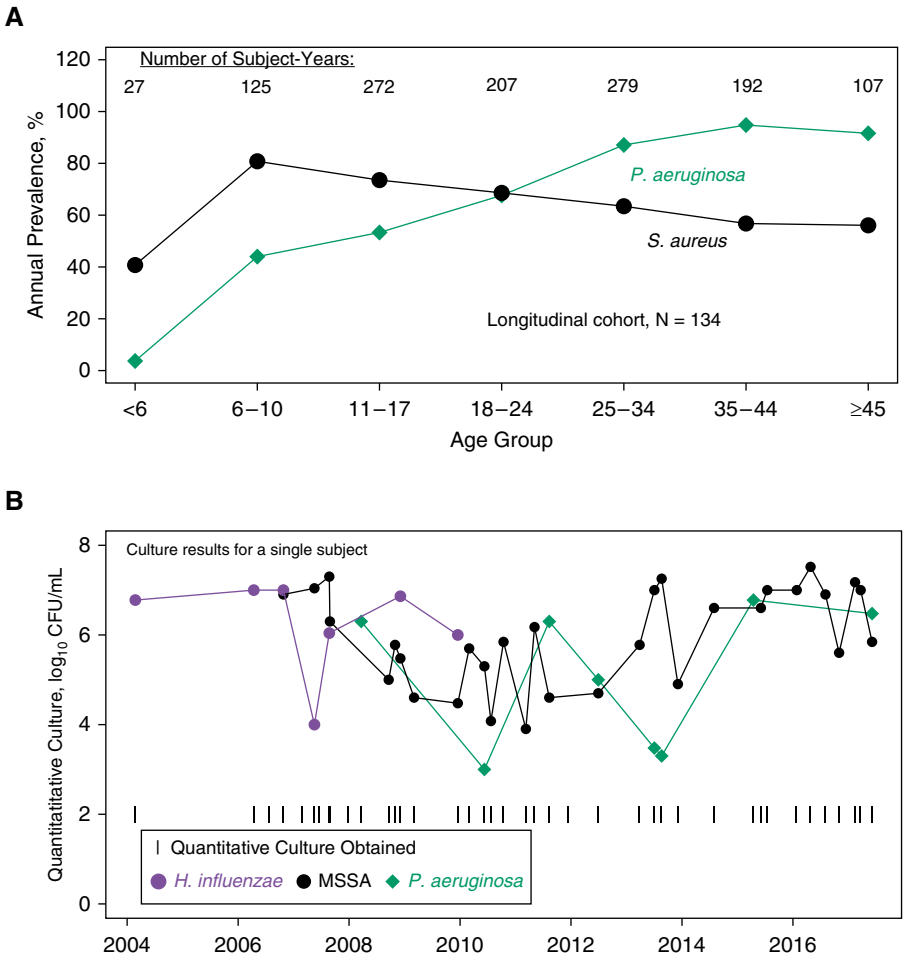

Figure 1. (*A*) Average annual prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* between 2004 and 2017, stratified by age cohorts (in years) defined in the Cystic Fibrosis Foundation Annual Report. Data are from subjects who had at least 10 quantitative cultures ($N = 134$). Consistent with registry-wide data from the same time interval, *P. aeruginosa* prevalence is greater than *S. aureus* prevalence among adult patients. (*B*) Quantitative culture results for a representative single subject. *H. influenzae* was present early. MSSA and *P. aeruginosa* were subsequently identified and remained stable for nearly 10 years. *H. influenzae* = *Haemophilus influenzae*; MSSA = methicillin-sensitive *Staphylococcus aureus*.

CF who had quantitative respiratory cultures. Between January 1, 2004, and December 31, 2017, our center cared for 337 patients with CF. The standard practice included quantitative sputum cultures (*see* online supplement 1 and 2). One hundred thirty-four patients had at least 10 quantitative cultures of either sputum or BAL. These patients were similar to the remainder of the center in terms of sex and genotype but were older and had more associated complications (Table 1). Patients with 10 quantitative cultures routinely produced sputum, had lower FEV_1, and had higher *P. aeruginosa* prevalence (*see* online supplement 3–5).

For patients with 10 quantitative cultures, their median starting age was 19.66 years, near the age cohort at which *P. aeruginosa* surpasses *S. aureus* in prevalence (5). The median follow-up was 10.15 years and 25 quantitative cultures. We used this longitudinal group to determine the duration and sequence of infections and to ascertain how quickly *S. aureus* is replaced by *P. aeruginosa*.

Prevalence and Abundance of *S. aureus* and *P. aeruginosa* in Quantitative Respiratory Cultures

Most patients with quantitative respiratory cultures had at least one culture positive for *S. aureus* or *P. aeruginosa* (Table 1). Consistent with the CFF registry (5), *S. aureus* was more prevalent in younger patients and *P. aeruginosa* was more prevalent in older patients (Figure 1A). Although this suggested that *P. aeruginosa* might replace *S. aureus* over time, when we examined individual patients longitudinally (Figure 1B), we observed that some individuals had sustained and abundant loads of both *S. aureus* and *P. aeruginosa*, often for several years.

To systematically compare the abundance of common CF pathogens, we determined their culture density in cfu/ml (Table 2). As expected, bacteria were significantly more abundant than fungi. Most bacteria, including *P. aeruginosa* and *S. aureus*, had median culture density of more than 10^6 cfu/ml.

Simultaneous Abundance of *S. aureus* and *P. aeruginosa*

We considered the possibility that *P. aeruginosa* would dominate *S. aureus* when both were present. One hundred seven patients had simultaneous growth of *S. aureus* and *P. aeruginosa*. From these patients, we identified 1,195 double-positive cultures. The median density of *P. aeruginosa* (in $\log_{10}$ cfu/ml) was 6.52 and *S. aureus* was 6.42 (Figure 2A). Although the difference was statistically significant by Wilcoxon signed-rank test, we did not consider the absolute difference in bacterial abundance to be biologically significant. To determine whether some of the cultures display dominance of one pathogen, we compared the abundance of *P. aeruginosa* and *S. aureus* by scatter plot (Figure 2B). We found that some double-positive cultures had dominance of either *S. aureus* or *P. aeruginosa*. However, most cultures had high density of both species, and few cultures had low abundance of both pathogens. Our findings were similar when we analyzed MRSA and MSSA cultures separately (*see* online supplement 6).

Duration and Persistence of Infections by Bacterial Pathogen

A hallmark of CF pathogens is their ability to establish chronic bacterial infections of the airways. Using our longitudinal cohort, we measured the chronicity of bacterial infections by two methods: duration and persistence. We defined duration as the time from initial to final positive culture. Because the duration of infection does not represent

Table 2. Microorganism Abundance by Species

Organism	$\log_{10}$ cfu/ml [*Median (IQR)*]	Number of Studies* (*Total = 3,522*)
P. aeruginosa	6.7 (5.9–7.0)	2,267
S. aureus†	6.5 (5.3–7.0)	2,046
MRSA	6.6 (5.7–7.0)	1,084
MSSA	6.3 (5.0–7.0)	972
H. influenzae	6.3 (5.5–7.0)	209
Other bacteria‡	6.5 (5.8–7.0)	1,803
Fungus	3.5 (3.0–4.0)	99

Definition of abbreviations: CF = cystic fibrosis; *H. influenzae* = *Haemophilus influenzae*; IQR = interquartile range; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-sensitive *S. aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*.
*Subjects with CF who had at least one quantitative culture positive for the indicated species and quantified in cfu/ml. All subjects had at least 10 quantitative culture studies between January 1, 2004, and December 31, 2017.
†Some *S. aureus* cultures could not be classified as either MRSA or MSSA. Occasionally, MRSA and MSSA were present on the same culture.
‡Sum of all bacteria other than *P. aeruginosa*, *S. aureus*, and *H. influenzae*.

how frequently the pathogen was cultured and could be biased by how late the organism appeared during the observation period, we defined persistence as the percentage of cultures that were positive following an initial positive culture for that organism.

We compared the duration and persistence of the benchmark pathogen *P. aeruginosa* with MRSA, MSSA, and *H. influenzae* (Figure 3). *P. aeruginosa* infections had the longest duration (median 7.6 yr) and the greatest persistence (median of 92.9%). By contrast, *H. influenzae* infections were brief (median 1.4 yr) and had low persistence. *S. aureus* was between these two extremes, with a median duration of approximately 5 years. The persistence of *S. aureus* infections was associated with methicillin resistance; 28.6% of subsequent cultures were positive for MSSA after incident infection, whereas 76.2% of subsequent cultures were positive for MRSA.

Sequence of Bacterial Appearance and Disappearance

H. influenzae and *S. aureus* are often considered early CF pathogens and *P. aeruginosa* a later pathogen. This sequence could occur by multiple mechanisms. Early *S. aureus* infections could be replaced by *P. aeruginosa*, or chronic *P. aeruginosa* infections may represent a barrier to

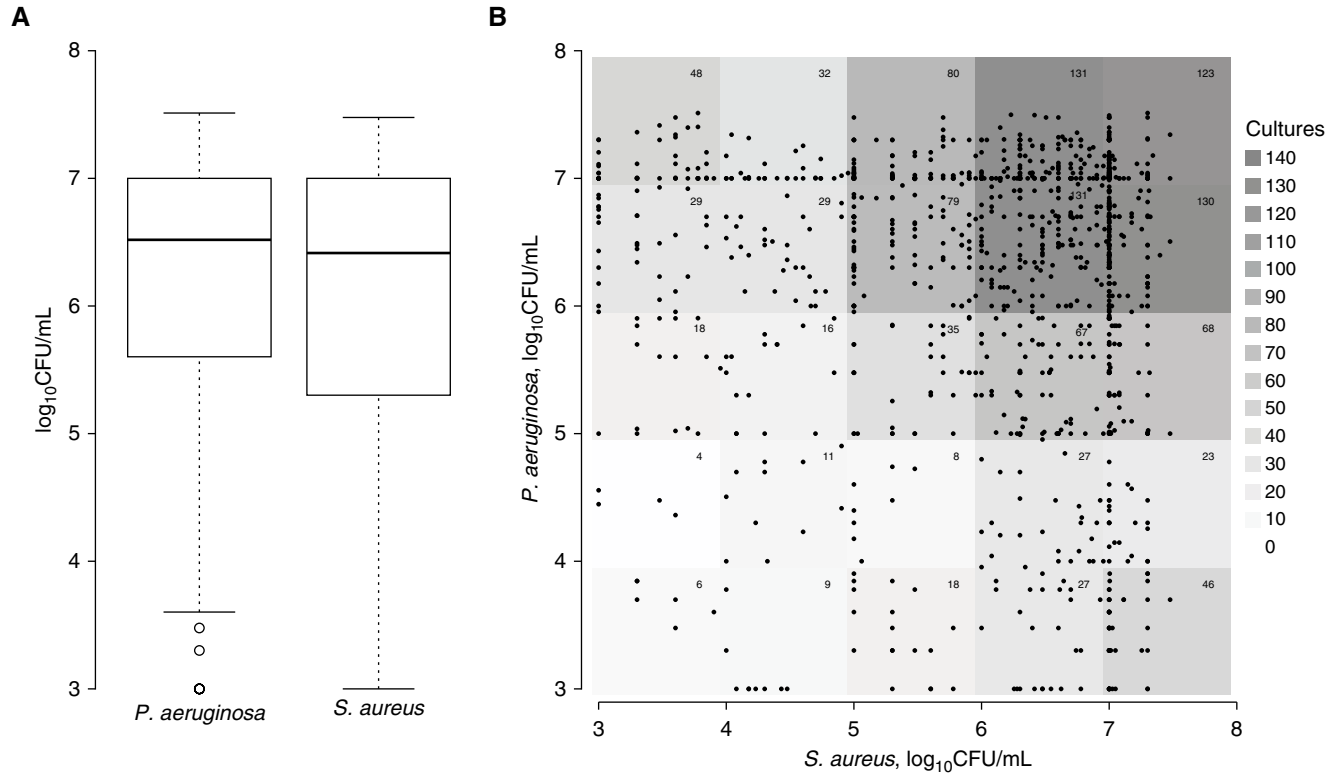

Figure 2. (*A*) Abundance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in patients with simultaneous positive cultures. *P. aeruginosa* was slightly more abundant than *S. aureus* (medians, 6.52 vs. 6.42; $P = 0.02$ by Wilcoxon signed-rank test), but the difference may be biologically insignificant. (*B*) Scatter diagram displaying abundance of *S. aureus* and *P. aeruginosa* in double-positive cultures. Because many points are superimposed, the number of cultures within a 1-log range of titer are indicated at the top right corner of each log unit and graphically depicted by the background shading. Some double-positive cultures had dominant *P. aeruginosa*, whereas others had dominant *S. aureus*. Few had low abundance of both organisms. There was weak negative correlation between *S. aureus* and *P. aeruginosa* abundance (Spearman rho = −0.067, $P = 0.02$). $n = 107$ patients, 1,195 double-positive cultures.

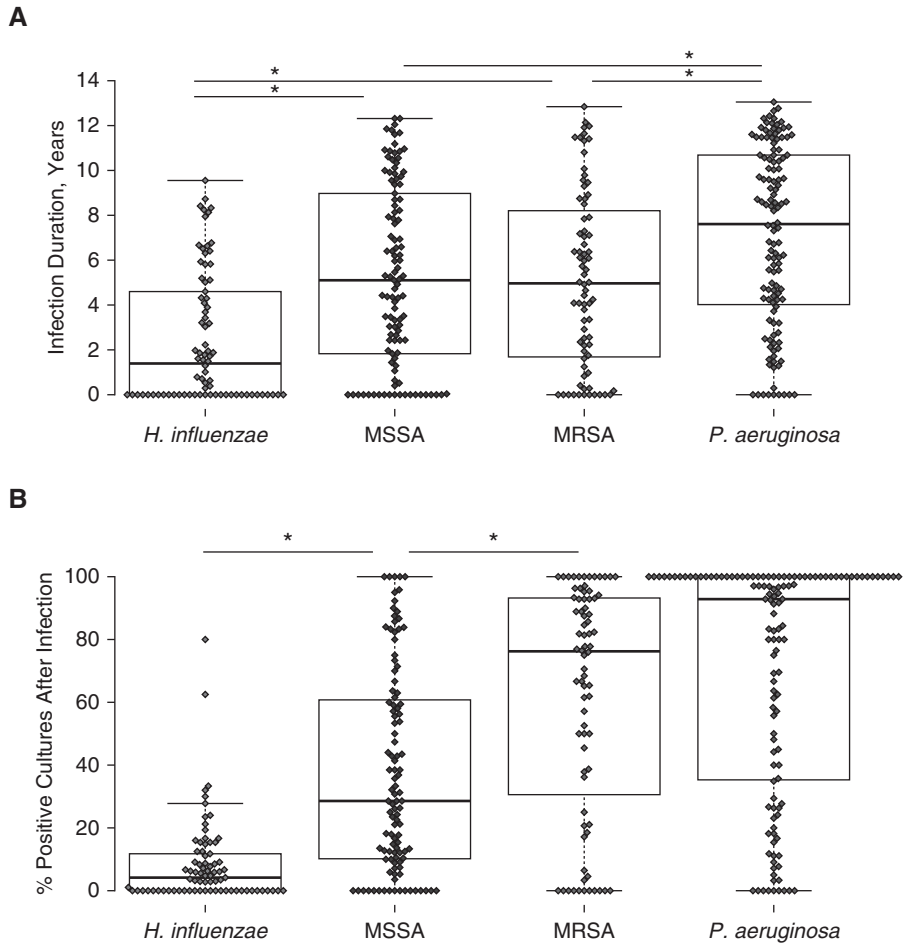

Figure 3. (*A*) Duration of infections within individual patients between January 1, 2004, and December 31, 2017. Dots represent the time (in years) from the first positive culture to the last positive culture for the organisms listed at bottom. $N = 134$ subjects; 78 *Haemophilus influenzae*, 109 methicillin-sensitive *Staphylococcus aureus* (MSSA), 76 methicillin-resistant *Staphylococcus aureus* (MRSA), 124 *Pseudomonas aeruginosa*. (*B*) Persistence of bacterial species after initial positive infection. Dots represent percentage of cultures that are positive for the organism listed after the first positive culture. Dots are not displayed if the subject's only positive culture for an organism was the final culture. $N = 134$ subjects; 77 *H. influenzae*, 107 MSSA, 76 MRSA, 124 *P. aeruginosa*. $^*P < 0.05$ by Wilcoxon signed-rank test.

acquisition of new *S. aureus* infections. Because of this, we hypothesized that *P. aeruginosa* would generally appear later than *S. aureus*. We calculated the sequence in which four bacterial species appeared, including *H. influenzae*, MSSA, MRSA, and *P. aeruginosa*. We measured the time of the first documented infection with each pathogen following the appearance of a reference "early" pathogen (*see* online supplement 7). In our cohort, the sequence of pathogen appearance varied by individual. Some patients had *P. aeruginosa* for several years before they cultured MSSA, whereas others had MSSA for years before *P. aeruginosa*. MRSA, an emerging pathogen of the early 2000s, appeared later than all other pathogens examined.

We predicted that pathogen replacement events would be temporally linked to the appearance of a competitor species. To identify these replacement events, we measured the time each organism last appeared relative to the debut of a potential competitor (*see* online supplement 8). The only example of this temporal association was the disappearance of *H. influenzae* approximately 1 year after the appearance of MRSA. However, because *H. influenzae* displays the weakest persistence, this temporal relationship could be coincidental. *S. aureus*, often assumed to be replaced by *P. aeruginosa*, was identified at least 5 years after the first culture of *P. aeruginosa*. These data either could be consistent with long-term coinfections with *S. aureus* and *P. aeruginosa* or might indicate that *P. aeruginosa* infections do not prevent *S. aureus* acquisition or reinfection.

Duration of *S. aureus*/*P. aeruginosa* Coinfections

We measured the duration of *S. aureus*/*P. aeruginosa* coinfections. One hundred seven patients from the longitudinal cohort had at least one double-positive culture. When we examined MSSA and MRSA infections separately, the median duration of coinfection was between 3 and 4 years (Figure 4). Because MRSA appeared later in time, this could underestimate the duration of MRSA infections. Because 43 subjects had both MRSA and MSSA, the median duration of coinfection with *P. aeruginosa* and either phenotype of S. *aureus* was 5.3 years. MRSA was more likely to appear consistently in quantitative respiratory cultures than MSSA (Figure 4B).

Changes in Abundance of Baseline Pathogens following Incident Infection with a Potential Competing Species

We hypothesized that *S. aureus* abundance would decrease following appearance of *P. aeruginosa*. We examined 42 subjects with baseline *S. aureus* who developed *P. aeruginosa* infection in a later calendar year. To control for the potential bias of oversampling from patients with more frequent follow up, we calculated an annualized average of culture density. We measured *S. aureus* abundance for up to 3 years before and 5 years after *P. aeruginosa* infection (Figures 5A and 5B). From Year 0 to Year 5, the average *S. aureus* titer was stable. We performed similar analysis for *P. aeruginosa*. Twenty-nine patients had baseline *P. aeruginosa* and developed *S. aureus* infection in a later calendar year. Among these patients, the *P. aeruginosa* culture density was stable following *S. aureus* infection. Among patients who first had *S. aureus* and *P. aeruginosa* in the same year, both species were stable in the following years (*see* online supplement 9). Titers tended to be higher for MRSA than MSSA in patients coinfected with *P. aeruginosa* (*see* online supplement 10).

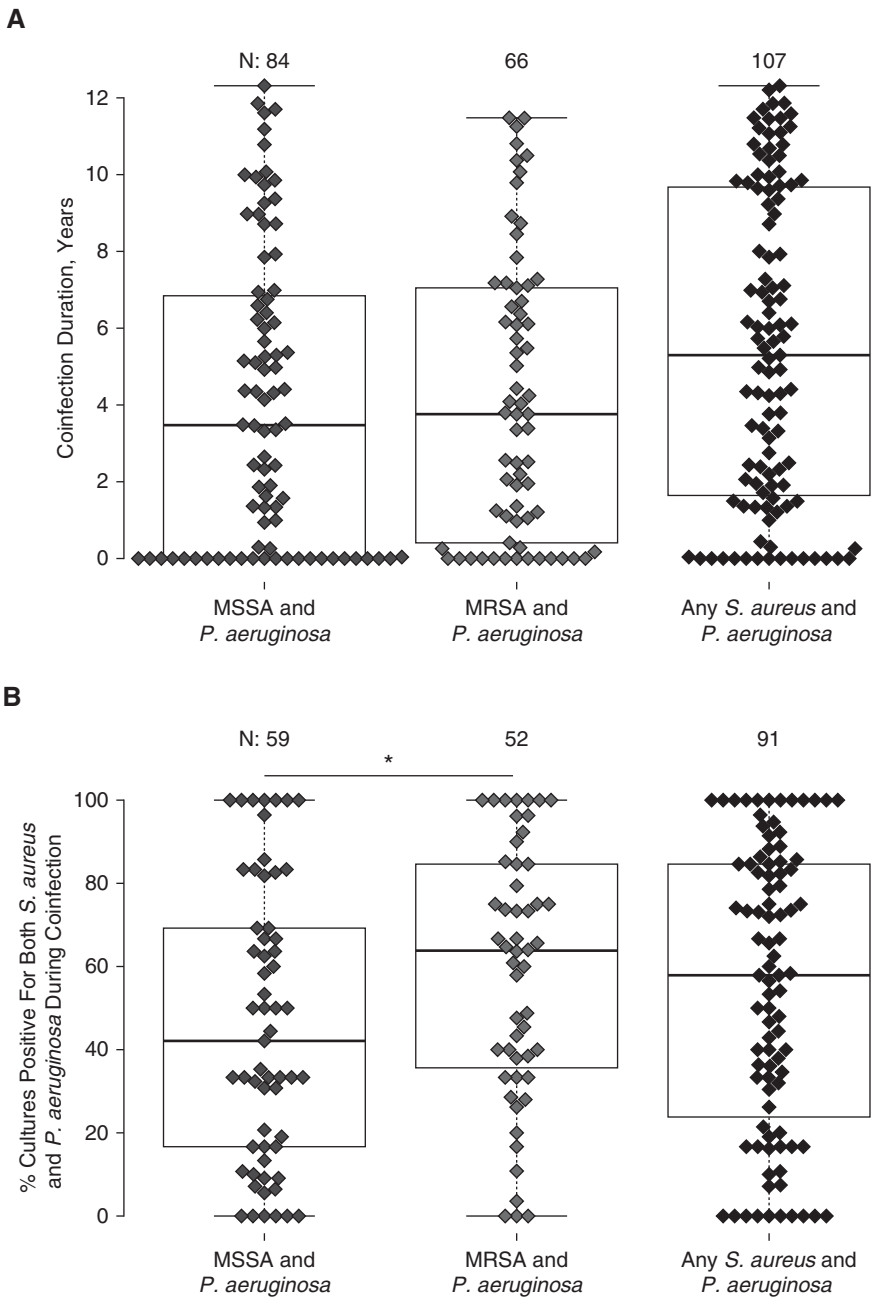

Figure 4. (*A*) Duration of positivity for *Staphylococcus aureus* and *Pseudomonas aeruginosa* between January 1, 2004, and December 31, 2017. Dots represent time from initial to final cultures that were simultaneously positive for *Pseudomonas aeruginosa* and methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), or either phenotype of *S. aureus*. The number of individuals per group is shown at top. Forty-three individuals had both MSSA and MRSA during the study. (*B*) Percentage of cultures that were double-positive for *S. aureus* and *P. aeruginosa* among subjects with ⩾2 double-positive cultures. Dots represent the percentage of cultures between the first and last double-positive culture that were simultaneously positive for *P. aeruginosa* and the subtype of *S. aureus* listed at bottom. The number of individuals per group is given at the top. Initial and final cultures were excluded from this statistic, as they are double-positive by definition. Subjects having only two double-positive cultures obtained consecutively were excluded. MRSA had greater persistence compared with MSSA. *$P < 0.05$ by Wilcoxon signed-rank test.

Trends in Positivity for *S. aureus* and *P. aeruginosa* within Individuals

Because *P. aeruginosa* is thought to replace *S. aureus* in the CF airway, we followed individual patients over time to see if their annual culture positivity for *P. aeruginosa* increased and *S. aureus* decreased. There was inadequate coverage in 2017 because of a shift in clinical practice, with discontinuation of quantitative cultures (*see* online supplement 2 and 11). Therefore, we compared the initial culture year to the last culture year through 2016 for 134 individuals with longitudinal culture results, Figure 5C. In this longitudinal analysis, *S. aureus* showed a trend toward increasing (from 60.4% to 68.7%). There was a statistically significant increase in *P. aeruginosa* (from 58.2% to 73.9%). Coinfections with *S. aureus* and *P. aeruginosa* were common and rose substantially over time, from 30.6% to 50.7% (Table 3). In summary, the net change in infection state for the cohort was in the direction of coinfection, rather than replacement of *S. aureus* by *P. aeruginosa* (Figure 5D). Although we observed some reversions from coinfected status back to *S. aureus* or *P. aeruginosa* only, these were relatively less common (*see* online supplement 12). Replacement of *S. aureus* by *P. aeruginosa* occurred in two individuals, whereas *S. aureus* replaced *P. aeruginosa* in one individual. The annual infection state for each member of the cohort is depicted graphically in Figure 5E. Both pathogens were persistently identified in individuals with CF.

Treatment of *S. aureus* and *P. aeruginosa*

A potential explanation for sustained growth of *S. aureus* in people with CF would be a medical decision to forgo antistaphylococcal treatment. Therefore, we obtained electronic prescription records following implementation of Epic in 2009. We found widespread prescription of antibiotics directed against both *S. aureus* and *P. aeruginosa* (*see* online supplement 13 and 14). Thus, both organisms demonstrate sustained persistence despite intensive antimicrobial therapy.

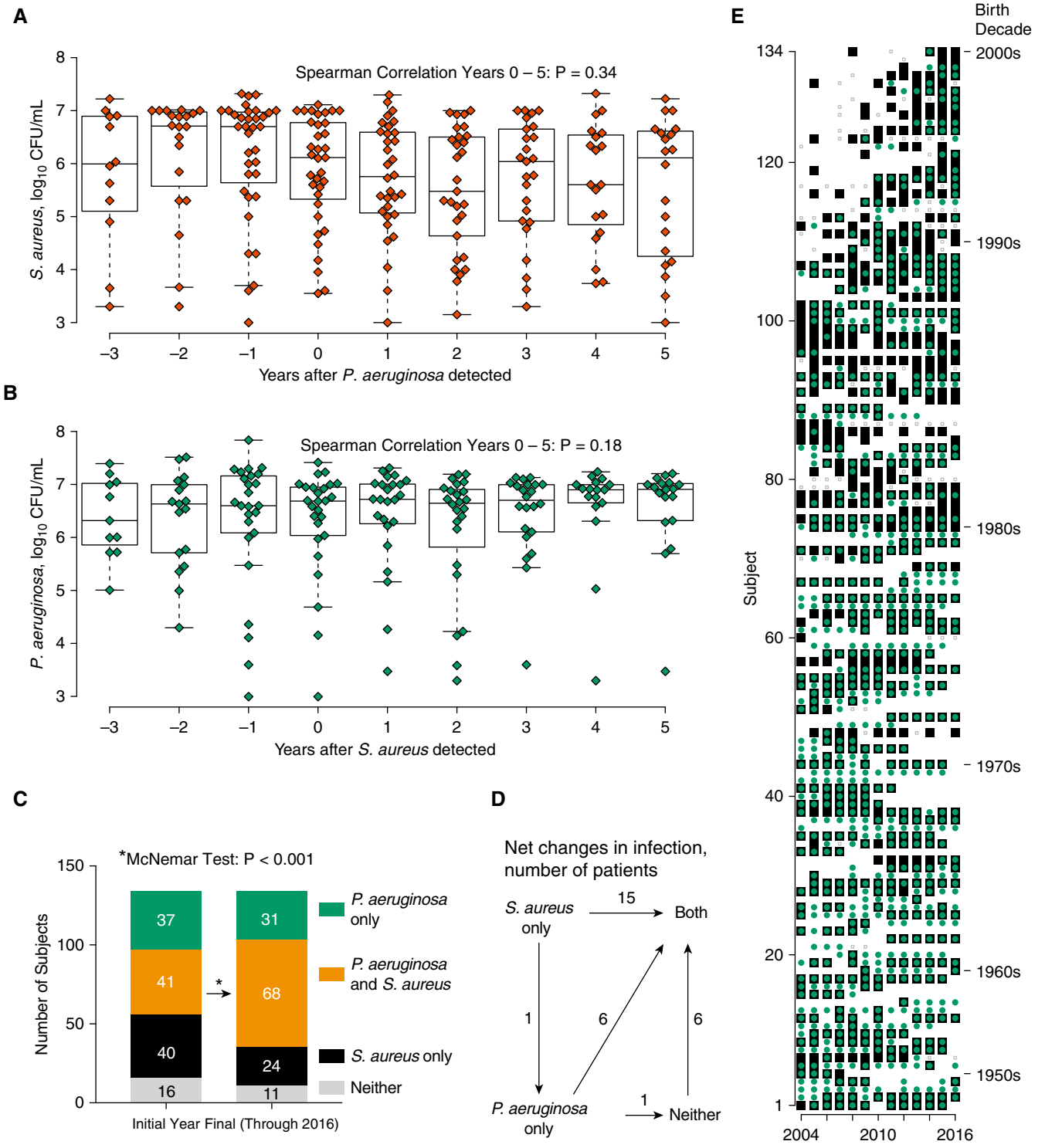

Figure 5. (*A*) Changes in *Staphylococcus aureus* abundance following incident *Pseudomonas aeruginosa* infection. Data show annualized mean culture density of *S. aureus* (on $\log_{10}$ scale) for 42 patients who had *S. aureus* before *P. aeruginosa*. For years 0–5, Spearman rho = −0.075, $P = 0.34$. (*B*) Changes in *P. aeruginosa* abundance following incident *S. aureus* infection. Data represent mean density of *P. aeruginosa* for 29 patients who had *P. aeruginosa* before *S. aureus*. For Years 0–5, Spearman rho = 0.12, $P = 0.18$. (*C*) Annual prevalence of *S. aureus*, *P. aeruginosa*, and dual infection between each subject's initial and final year up to 2016. A statistically significant increase in dual infection was detected. Neither organism decreased in prevalence when examined individually. (*D*) Data represent net changes in infection status for individuals between their first and final year up to 2016.

Table 3. Longitudinal Culture Positivity for *S. aureus* and *P. aeruginosa*

Organism	Initial Year [*n* (%)]	Final through 2016 [*n* (%)]	OR (95% CI)*	*P* Value†
S. aureus	81 (60.4)	92 (68.7)	1.5 (0.87–2.57)	0.17
P. aeruginosa	78 (58.2)	99 (73.9)	4.5 (1.86–10.9)	0.0003
S. aureus and *P. aeruginosa*	41 (30.6)	68 (50.7)	2.6 (1.48–4.52)	0.0007

Definition of abbreviations: CI = confidence interval; OR = odds ratio; *P. aeruginosa* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*.
*Odds ratio using Mantel-Haenszel method and 95% CI.
†*P* value calculated by Exact McNemar's test, based on *N* = 134 individuals.

Discussion

In this longitudinal CF cohort study, we report trends in quantitative microbial burden with extensive follow-up. We found that both *S. aureus* and *P. aeruginosa* are abundant and persistent in the CF airway. Compared with *H. influenzae*, *S. aureus* has similar abundance on quantitative cultures but persists longer within individuals. Although *S. aureus* is commonly considered an early pathogen relative to *P. aeruginosa*, the sequence of infections is variable. Baseline infections with either species persist for years following the appearance of the opposite species, and both organisms are abundant in coinfected patients. Contrary to common assumption, we rarely observed replacement of *S. aureus* by *P. aeruginosa* within individuals, despite an average follow-up time of 10 years. We found a trend toward accumulation of both species over time, despite apparent attempts to treat both pathogens.

Comparison with Previous Studies

There is growing recognition of the prevalence of *S. aureus* and *P. aeruginosa* coinfection in CF (4). Molecular analysis of these bacteria shows that the same pulsed-field types can remain for years (20, 21). Our data show that these organisms are also stable in terms of quantitative abundance. The abundance of either *S. aureus* or *P. aeruginosa* predicts low microbial diversity and neutrophilic inflammation in early CF lung disease (22). Coinfection with these organisms may be consequential for patients (23–29). Adults with *S. aureus* and *P. aeruginosa* experience worse outcomes than those with *S. aureus* alone (27). MRSA is recognized as a risk factor for poorer CF outcomes (3, 30, 31). Compared with MSSA, MRSA is associated with worse outcomes in combination with *P. aeruginosa* (25). Our data show that MRSA infections were more persistent than MSSA, suggesting a potential mechanism for this difference.

The recent development of CFTR modulator therapies raises hope that these drugs could correct the underlying host defense defects in CF, thereby preventing or clearing chronic pulmonary infections. However, epidemiologic studies show that chronic bacterial infections persist in patients with CF despite CFTR modulator therapy (32–35). This indicates a continued need to determine mechanisms of bacterial persistence.

Advantages of This Study

Our study addresses unanswered questions about coinfections with *S. aureus* and *P. aeruginosa*. It was not clear from previous literature which of these two species dominates in coinfections, how long coinfections last, how often these organisms are isolated from the same cultures, and whether one organism is eventually replaced. Our study focused on sputum and BAL because *S. aureus* can be found in the oropharynx of healthy children without CF (19). Although sputum is expectorated through the mouth, multiple studies show that sputum has greater sensitivity and specificity for predicting endobronchial pathogens versus oropharyngeal swabs (36–38). Thus, sputum is considered by some to be a "credible surrogate" for BAL (39). Moreover, quantitation allows us to determine the abundance of both organisms over time.

Limitations

Our findings should be interpreted in the context of the high local MRSA prevalence, which may impair *S. aureus* eradication. Future studies involving larger cohorts with lower MRSA could reveal *S. aureus* attrition. Our focus on quantitative cultures limits this study to patients with chronic sputum production. Thus, we cannot describe potential interactions between *S. aureus* and *P. aeruginosa* in younger patients or the sequence of infections that occur in the first years after birth. The dynamic range of our quantitative cultures may not capture the most or least abundant infections. However, unlike molecular metagenomic techniques, quantitative cultures are relatively easier to interpret. We did not correlate coinfection with clinical outcomes such as FEV_1, as these comparisons have been made by others. We also did not calculate the changes in pathogen abundance following treatment with antibiotics

Figure 5. (*Continued*). Transition from *S. aureus* to *P. aeruginosa* was rare and was less common in this cohort than the transition from either organism alone to dual infection. (*E*) Longitudinal trends in *S. aureus* and *P. aeruginosa* positivity for the cystic fibrosis cohort. Each row is a subject, displayed in order by year of birth. The youngest individual within a birth decade is indicated at the right. The *x*-axis shows years in which a subject had a quantitative culture. Symbols represent positivity for *S. aureus* (black squares), *P. aeruginosa* (green circles), dual infection (green circle inside a black square), or absence of both (gray dots).

or CFTR modulators. Finally, our database did not include information about infections with small colony variant *S. aureus*, which are associated with antibiotic resistance and poorer outcomes (26).

Conclusions

S. aureus and *P. aeruginosa* establish stable, long-term coinfections in people with CF. Both organisms are abundant in sputum and BAL. New strategies are needed to prevent and treat infections by these CF pathogens. ■

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