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MAJOR ARTICLE

Impact of Antibiotics on the Lung Microbiome and Lung Function in Children With Cystic Fibrosis 1 Year After Hospitalization for an Initial Pulmonary Exacerbation

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Background. Cystic fibrosis (CF) is characterized by recurrent pulmonary exacerbations (PEx) and lung function decline. PEx are frequently treated with antibiotics. However, little is known about the effects of antibiotics on the airway microbiome of persons with CF over time. The purpose of this study was to evaluate changes in the microbiome and lung function in persons with CF over 1 year following an initial study pulmonary exacerbation (iPEx).

Methods. Twenty children aged ≤18 years with CF were enrolled in the study, which occurred prior to the routine administration of highly effective modulator therapy. Respiratory samples and spirometry were obtained at a minimum of quarterly visits and up to 1 year after an iPEx. Metagenomic sequencing was performed, and bacterial taxa were assigned using MetaPhlAn 2.0. Paired *t* test, analysis of variance, and generalized least squares regression were used to compare outcome variables.

Results. The mean age of study participants at the time of the iPEx was 10.6 years. There were 3 ± 1.6 PEx treated with antibiotics per person during the study period. Bacterial richness was similar at 1 year compared to iPEx (40.3 vs 39.3, $P = .852$), whereas the mean Shannon diversity index was significantly higher at 1 year (2.84 vs 1.62, $P < .001$). The number of PEx treated with antibiotics was not associated with changes in microbial diversity but was associated with changes in lung function.

Conclusions. In our 1-year prospective study, we found that microbial diversity increased despite decreases in lung function associated with repeated PEx events requiring antibiotic therapy.

Keywords. antibiotics; cystic fibrosis; microbiome; pulmonary exacerbations.

Cystic fibrosis (CF) is an autosomal recessive disease that affects 30 000 people in the United States and 70 000 around the world [1–3]. While it causes disease in multiple organ systems, lung inflammation is a major cause of morbidity and mortality in persons with CF [1, 4]. Pulmonary exacerbations (PEx) are frequently associated with a decline in lung function [5, 6]. Respiratory cultures during PEx typically grow *Pseudomonas aeruginosa* or *Staphylococcus aureus* in adults with CF, well-known pathogenic organisms found to be associated with poor lung function [4, 7–10]. However, colonization of the airways of children with CF by a set of organisms occurs early in life [4, 11]. Once the lung is colonized, there is a repeated cycle of infection, inflammation, and lung tissue damage that results in progressive loss of lung function and consequently progression of disease [4, 11].

The complex microbiome has previously been described in CF; recent advances in microbiome analysis, specifically culture-independent methods, have aided in the knowledge of the diversity of bacterial species present in the lungs of persons with CF [7, 11–13]. Traditionally, culture-based techniques have been used to detect key microbial species; in comparison, molecular methodologies have been shown to discover additional species as well as information regarding bacterial functional diversity [7, 10, 12–15]. A combination of both culture-based and non-culture-based methodologies may better define the ecology of the CF airway [10], especially in younger children without established CF pathogens identified in respiratory culture [16, 17].

PEx are often polymicrobial in nature and frequently treated with oral or intravenous (IV) antibiotics [5, 18–20]. Antibiotic exposure has been shown to affect bacterial community within the airways: it can decrease microbial diversity, influence the

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presence of dominant pathogenic bacteria (eg, *P aeruginosa*), and alter disease progression [21–24]. Little is published about the changes in lung microbiome seen longitudinally; however, studies published to date suggest that repeated PEx and treatment with antibiotics have a significant impact on diversity and lung function in adults with advanced disease [22]. The objective of this study was to describe the cumulative effect a treatment course with IV antibiotic therapy has on the diversity of the lung microbiome and lung function in children with CF 1 year after treatment for an initial study pulmonary exacerbation (iPEx). Specifically, we looked at evaluating changes in α-diversity, microbial richness, and spirometry over the 1-year collection period, considering antibiotic treatment courses for PEx between the iPEx and the 1-year follow-up.

METHODS

Study Participants' Clinical Data and Pulmonary Exacerbations

Twenty children ≤18 years of age were prospectively followed over the course of 1 year following hospitalization and treatment for an iPEx with IV antibiotics from May 2017 through December 2018 (Supplementary Figure 1). These children were enrolled in a prior study [25], with each child with CF hospitalized for PEx during the study period approached for participation. Those children who also enrolled in our biorepository study and who had 1 year of samples collected by December 2018 were included in the study presented here (20 of 27 original study participants). Baseline demographic information, including the type of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation, whether the study participants were on suppressive inhaled and/or oral antibiotics, and whether participants were on a CFTR modulator during the collection period, were documented. Additional information collected included respiratory culture results, pulmonary function testing results, and the number of PEx treated with antibiotics during the study period.

Patient Consent Statement

Written consent was obtained from the study participant (for those aged 18 years) or the parent (for those aged <18 years), with assent obtained for children aged 7–17 years. The design of the work has been approved by local ethical committees and the Institutional Review Board at Children's National Hospital (Pro8047, approved 29 August 2016 and Pro6781, approved 8 December 2015).

Respiratory Samples, DNA Sequencing, and Bioinformatic Approach

Respiratory samples (either sputum or oropharyngeal swab) were collected at the iPEx and then at a minimum of quarterly visits up to the 1-year follow-up sample (Supplementary Figure 1). The type of visit in which respiratory samples were collected was designated as well, sick, or hospitalization. Well visits included routine outpatient pulmonary visits, where no signs/symptoms of a PEx were present and lung function was around baseline. Sick visits were defined as an acute outpatient pulmonary clinic visit or emergency department visit where the participant had signs/symptoms of PEx and was subsequently managed with antimicrobial therapy, steroids, and/or watchful waiting. Hospitalization was defined as any admission to the hospital for IV antibiotics.

Respiratory samples were processed, bacterial DNA was extracted, and metagenomic sequencing was performed as previously described [15, 25]. In brief, the DNA extraction process included a bead-beating step to ensure adequate isolation of gram-positive bacteria. Bacterial taxa were assigned using MetaPhlAn 2.0 [26]. HUMANn2 was used to determine the bacterial gene and pathway expression of the respiratory DNA sequencing samples, including long-chain fatty acid (LCFA) pathways [27].

Lung Function

Lung function of the study participants was determined via spirometry performed at iPEx, end of treatment of the iPEx, minimum of quarterly collection visits, and at the 1-year follow-up visit, in conjunction with the collection of the respiratory samples. Specific spirometry parameters studied included percentage predicted forced expiratory volume in 1 second ($ppFEV_1$), percentage predicted forced vital capacity (ppFVC), and percentage predicted forced expiratory flow between 25%–75% of vital capacity ($ppFEF_{25–75}$). Baseline lung function was considered the best spirometric parameter in the 6 months preceding the iPEx [6]. Lung function was considered below baseline at iPEx if the spirometric parameter was decreased by >10%. Similarly, if lung function was within and/or greater than 10% of baseline at the 1 year follow-up, it was interpreted as returning to baseline [6].

Statistical Analysis

Taxonomic count tables obtained from sequencing of the respiratory samples were imported into Rstudio version 3.6.1 to analyze species richness and relative abundance, as well as α- and β-diversity and differential abundance using *vegan* version 2.5-6 [28], *phyloseq* version 1.28.0 [29], and *DESeq2* [30] packages. The α-diversity was measured using both the Shannon index ($q = 1$) and the inverse Simpson index ($q = 2$), whereas β-diversity was measured using the Morisita index of dispersion and Bray-Curtis [31]. Paired 2-tailed *t* test was used to compare the average changes in diversity measures and spirometry parameters observed at the 1-year follow-up compared to baseline and iPEx. A repeated-measures analysis of variance (ANOVA) was performed to evaluate significant differences in lung function measured longitudinally. A random-effects linear regression model using the generalized least squares estimator was performed in Stata/IC version 15.1 for associations between antibiotic courses for PEx events and changes in

richness and diversity, lung function, and LCFA pathways. The association between lung function, α-diversity, and relative bacterial abundance was also evaluated, with variables of interest for the model being identified using Spearman correlations. Those variables (ie, bacterial species) with an unadjusted *P* value of <.05 were included in the model. Last, we controlled for repeated participant samples, whether the study participant was receiving CFTR modulator therapy, type of sample (oropharyngeal swab vs sputum vs bronchoalveolar lavage), and timing of sample collection (well vs sick vs hospitalization) in our analysis when relevant.

RESULTS

Study Participants' Demographics and Clinical Data

The mean ± standard deviation age of study participants at the time of the iPEx was 10.6 ± 5.1 years, with an age range of 1–18 years at study enrollment (Table 1). Fifty-five percent of the participants were male; 20% were Black, 80% White, and 30% of Hispanic ethnicity. Thirty-five percent were homozygous and 50% heterozygous for F508del-CFTR mutation. Ninety-five percent of the study participants had a history of growth on respiratory cultures obtained prior to the study period and included well-established pathogens implicated in persons with CF: *P aeruginosa* (65%), methicillin-sensitive *S aureus* (40%), and methicillin-resistant *S aureus* (35%), among others (Table 1).

Table 1. Study Participants' Demographic and Baseline Clinical Data

Clinical Parameters	No. (%) (N = 20)
Sex	
Male	11 (55)
Female	9 (45)
Age at initial PEx, y, mean ± SD	10.6 ± 5.1
Race	
White	16 (80)
African American	4 (20)
Ethnicity	
Hispanic/Latino	6 (30)
Not Hispanic/Latino	14 (70)
Cystic fibrosis genotype	
F508del homozygous	7 (35)
F508del heterozygous	10 (50)
Other	3 (15)
Concomitant diagnosis of asthma	14 (70)
Prior organisms on respiratory culture	
Pseudomonas aeruginosa	13 (65)
Methicillin-sensitive *Staphylococcus aureus*	8 (40)
Methicillin-resistant *Staphylococcus aureus*	7 (35)
Stenotrophomonas maltophilia	7 (35)
Streptococcus spp[a]	7 (35)
Achromobacter xylosoxidans	5 (30)
Moraxella catarrhalis	3 (15)
Haemophilus influenzae	2 (10)
Unidentified gram-negative rod	2 (10)
Burkholderia cepacia	1 (5)
Elizabethkingae meningoseptica	1 (5)
Pseudomonas putida	1 (5)
Aspergillus fumigatus	1 (5)
CFTR modulator use	
At baseline/initial PEx	5 (25)
During 1-y study period[b]	4 (20)
Suppressive antibiotic therapy	
Inhaled antibiotics	9 (45)
Oral azithromycin	1 (5)
Inhaled ± oral antibiotics	9 (45)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; PEx, pulmonary exacerbation; SD, standard deviation.

[a]Includes *Streptococcus agalactiae* (n = 3), *Streptococcus pneumoniae* (n = 1), *Streptococcus pyogenes* (n = 1), and group C (n = 1) and group G (n = 1) *Streptococcus*.

[b]Discontinued due to drug side effects.

Encounter Descriptions and Pulmonary Exacerbations

All study participants were hospitalized for treatment of an initial PEx at study enrollment [25]. Following the initial 3 study visits (PEx onset, end of antibiotic treatment, and follow-up), there was a total of 214 additional documented encounters with an average of 10.7 ± 3.9 encounters per study participant during the 1-year follow-up period (Table 2). These encounters corresponded to a total of 60 PEx events treated with antibiotics, with a mean of 3 ± 1.6 PEx events per study participant (Table 2). The most commonly used IV antibiotics were tobramycin and ceftazidime, while the most commonly used oral antibiotic was amoxicillin-clavulanate (Table 2). Inhaled antibiotics were not used as part of treatment for PEx.

Respiratory Samples

A total of 106 respiratory samples were obtained during the study period, of which 98 (92%) were successfully sequenced (n = 3 not collected for sequencing; n = 5 failed sequencing). Forty-six percent of the samples were collected during sick visits, 35% during well visits, and 19.4% during hospitalization (Table 2). During the 1-year study period, 5 study participants had growth of new organisms. Eighteen of the 20 participants (90%) had samples successfully obtained and sequenced at the 1-year follow-up, of which 45% were a well visit, 30% were a sick visit, and 25% were a hospitalization. All collected respiratory samples were also cultured in our clinical microbiology laboratory following Cystic Fibrosis Foundation–recommended practices; the majority (70%) only had normal respiratory flora on culture at that visit.

Bacterial Richness and α-Diversity

Overall, bacterial richness was not significantly different at 1 year compared to iPEx (n = 18; 40.3 vs 39.3, *P* = .852) but was significantly increased compared to the end of antibiotic treatment (n = 14; 38.4 vs 23.8, *P* = .018) (Figure 1*A*). The mean Shannon diversity index was significantly higher at the 1-year

Table 2. Respiratory Sample Description and Associated Clinical Data

Collected Sample Information	No. (%)
Total No. of samples collected during the 1-y study period	106
No. of samples successfully sequenced during the 1-y study period	98 (92)
Proportion during well visits	34 (35)
Proportion during sick visits	45 (46)
Proportion during hospitalizations	19 (19)
Sample type	
Oropharyngeal swab	64 (65)
Sputum	30 (31)
Bronchoalveolar lavage	4 (4)
Longitudinal sample information	
Organisms on respiratory culture at initial PEx	
Pseudomonas aeruginosa	5 (25)
Methicillin-sensitive *Staphylococcus aureus*	3 (15)
Methicillin-resistant *Staphylococcus aureus*	3 (15)
Stenotrophomonas maltophilia	1 (5)
Achromobacter xylosoxidans	1 (5)
Moraxella catarrhalis	1 (5)
Haemophilus influenzae	1 (5)
Unidentified gram-negative rod	1 (5)
New growth of organisms during the 1-y study period	
Stenotrophomonas maltophilia	2 (10)
Pseudomonas aeruginosa	1 (5)
Moraxella catarrhalis	1 (5)
Aspergillus fumigatus	1 (5)
1-y follow-up sample information	
No. of samples successfully sequenced at 1-y follow-up	18 (90)
Type of visit at 1-y follow-up sample collection	
Well outpatient visit	9 (45)
Sick outpatient visit	6 (30)
Hospitalization	5 (25)
Organisms on respiratory culture at 1-y follow-up	
Pseudomonas aeruginosa	5 (25)
Achromobacter xylosoxidans	1 (5)
Only normal respiratory flora	14 (70)
Proportion on antibiotic therapy at 1-y follow-up	
Inhaled suppressive antibiotic therapy	9 (45)
Suppressive oral antibiotic therapy (includes QMWF azithromycin)	4 (15)
Treatment with oral antibiotic therapy	3 (15)
Treatment with IV antibiotic therapy	5 (25)
Any antibiotic therapy	14 (80)

Abbreviations: IV, intravenous; PEx, pulmonary exacerbation; QMWF, taken every Monday, Wednesday, and Friday.

follow-up compared to iPEx (n = 18; 2.84 vs 1.62, $P < .001$) and after completion of antibiotic treatment (n = 14; 2.71 vs 1.34, $P < .001$) (Figure 1*B*). There was no change in the inverse Simpson index between the 1-year follow-up and iPEx (n = 18; 5.12 vs 4.43, $P = .326$); however, it was significantly increased at the 1-year follow-up compared to end of antibiotic treatment (n = 14; 4.50 vs 2.82, $P = .009$) (Figure 1*C*).

As the type of visit at the follow-up sample could have influenced our findings, we also performed the paired *t* test by groups. There was no significant change in mean number of observed species at 1 year compared to iPEx, but there was a significant increase in bacterial richness at 1 year compared to completion of treatment of iPEx when follow-up samples were obtained during well (n = 6; 37 vs 18.5, $P = .043$) or sick (n = 4; 52.3 vs 23.8, $P = .006$) visits (Figure 1*A*). Shannon diversity was significantly increased at 1 year compared to iPEx in samples obtained during sick visits only (n = 6; 3.33 vs 1.88, $P = .001$) but approached significance for hospitalized visits (n = 5; 2.99 vs 1.80, $P = .056$) (Figure 1*B*). Shannon diversity was significantly increased in all 3 types of visits at 1 year compared to end of treatment (well: n = 6, 2.37 vs 1.10, $P = .045$; sick: n = 4, 3.29 vs 1.47, $P = .001$; hospitalized: n = 4, 2.63 vs 1.59, $P = .035$). There were no significant changes in inverse Simpson index with regard to type of visit comparing 1 year and iPEx, but approached significance for the change between 1 year and end of treatment for the sick visits (n = 4; 6.15 vs 3.08, $P = .055$) and hospitalization (n = 4; 4.59 vs 2.93, $P = .053$) (Figure 1*C*). The sample-level diversity data are shown in Supplementary Figure 2.

Relative Taxonomic Abundance

A total of 202 species were identified across all samples during the collection period, of which 149 species (74%) were seen at the 1-year follow-up. There was an average of 34.7 ± 17.6 bacterial species identified per sample (range, 2–67) during the collection period, and an average of 40.8 ± 16.3 species per sample seen at the 1-year follow-up (range, 11–67). The average number of species per sample identified at the iPEx was 37.4 ± 23.2 (range, 2–67). Supplementary Figure 3*B* shows the relative abundance of the top 20 bacterial species by sample and compares those present at iPEx (E) and at the 1-year follow-up (F). Five species had an average relative abundance of >1% and were present in >50% of the samples at the 1-year follow-up. *Rothia mucilaginosa* was commonly seen among all samples at the 1-year follow-up, with an average relative abundance of 21.3%. The remainder of the most relatively abundant species, in order of highest relative abundance to lowest, were *Streptococcus salivarius* (16.2%), *Veillonella* species (13.3%), *Streptococcus parasanguinis* (9.1%), and *Granulicatella* species (2.3%). While *S aureus* also had a high relative abundance across the cohort (9.6%), it was only present in less than half of the participant samples obtained at 1 year (n = 8 of 18). *Pseudomonas aeruginosa* had a relative abundance of <1% and was only present in 1 of the 1-year samples based on sequencing results, in contrast to having 5 participants with positive respiratory cultures (Table 2). Supplementary Figure 3*A* shows the relative abundance of the top 20 bacterial species per sample based on type of visit (well [W], sick [S], hospitalized [H]) and included all samples during the 1-year collection period. The most common species were similar to the 1-year data and included *Veillonella* species (20.1%), *R mucilaginosa* (15.7%), *S salivarius* (15.4%), *S parasanguinis* (9.2%), *S aureus* (8%), and *Granulicatella* species (2%). Additional data on

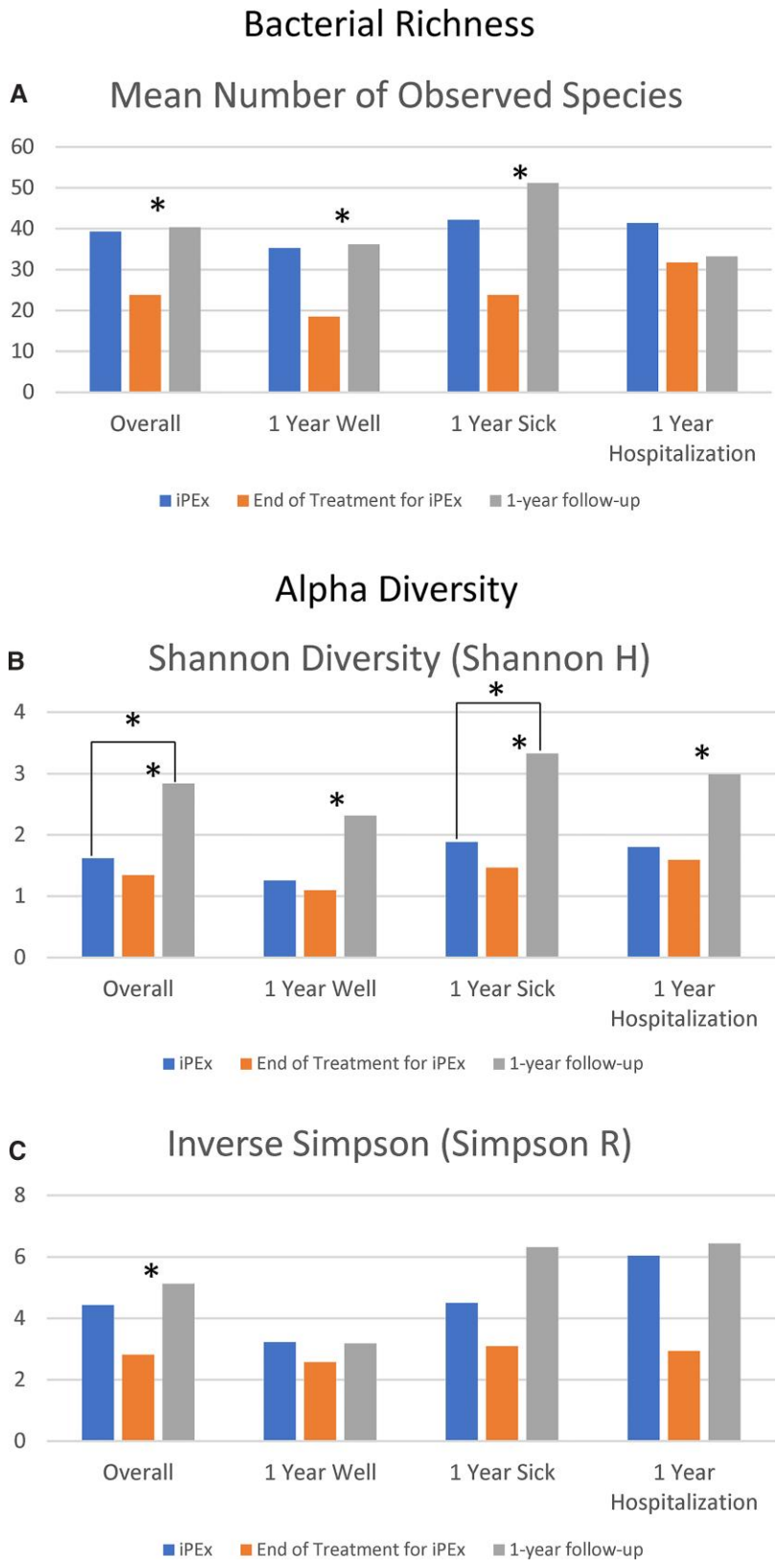

Figure 1. Changes in bacterial richness and α-diversity at 1 year compared to initial pulmonary exacerbation (iPEx) and after antibiotic treatment for iPEx. *A*, Bacterial richness, defined by mean number of observed species. *B*, Shannon diversity index. *C*, Inverse Simpson index. Blue indicates iPEx; orange, end of treatment for iPEx; gray, 1 year. Column set 1 represents overall change at 1 year, and sets 2–3 are divided by type of visit at 1 year (well, sick, hospitalization). $^*P < .05$. Associations measured using a paired *t* test between iPEx or end of treatment and follow-up.

β-diversity, differential bacterial species abundance, and the relationship between LCFAs and with PEx events are shown in the Supplementary Results (Supplementary Figures 4, 5, and 6, respectively).

Lung Function

All of the study participants were classified as early-stage CF, as their ppFEV$_1$ was ≥70% at baseline [32] with a mean baseline ppFEV$_1$ of 102% ± 0.16. Three of the 20 study participants did not have baseline spirometry data given that their age at study onset was <6 years; 2 of these participants had spirometry performed by the 1-year follow-up. ppFEV$_1$ was below baseline in 55% of the participants at the iPEx, and 90% had improvement after treatment of the iPEx. At the 1-year follow-up, only 55% had lung function at or better than baseline with the caveat that only 45% were presenting for a well visit. The average percentage change in ppFEV$_1$ from baseline was −9.01% ± 0.13 ($P = .010$; Figure 2*A*), whereas the average percentage increase in ppFEV$_1$ compared to iPEx was +12.8% ± 0.30 ($P = .065$; Figure 2*A*). The mean values of ppFEV$_1$, ppFVC, and ppFEF$_{25–75}$ at the 1-year follow-up were 90%, 97%, and 81%, respectively. While there was a significant decrease in all parameters at 1 year compared to baseline, the percentage change in ppFEV$_1$ and ppFVC remained within 10% (Figure 2*A*–*C*). There was significant recovery of ppFEF$_{25–75}$ at 1 year compared to iPEx (+26.5% ± 0.48; $P = .02$) but an overall significant decrease from baseline (−15.6% ± 0.24, $P = .005$) (Figure 2*C*). To better understand the impact on lung function longitudinally, we also evaluated the change at 3, 6, and 9 months (±1 month) (Supplementary Figure 7). There were no significant differences in lung function noted between these intervals. Using a repeated-measures ANOVA, we found the following based on the month the value was measured: ppFEV$_1$, $P = .829$; ppFVC, $P = .677$; and ppFEF$_{25–75}$, $P = .914$.

Impact of Repeated Antibiotic Courses on the Lung Microbiome and Lung Function

A wide range of both IV and oral antibiotics were administered during the study period (Table 3). Controlling for the type of visit (well, sick, hospitalized) of the 1-year follow-up sample, we found that the number of antibiotic courses prescribed was not associated with the change in richness from iPEx to 1-year follow-up ($P = .719$). Likewise, there was not an association with the change in the Shannon index ($P = .647$), the inverse Simpson index ($P = .557$), or the Morisita index of dispersion ($P = .822$). We also evaluated the association of the number of antibiotic courses prescribed for PEx and the percentage change in lung function from baseline (prior to iPEx) and the 1-year follow-up. We did not find a significant association for the change in ppFEV$_1$ ($P = .132$). We did find a significant association between the change in ppFVC when controlling for type of visit ($P < .001$). There was no significant association found for ppFEF$_{25–75}$ ($P = .701$). In all cases, the trendline showed that more antibiotic courses were associated with a greater percentage decrease in lung function (Supplementary Figure 8).

DISCUSSION

Our study evaluated for changes of the lung microbiome and associated changes in lung function in children with early-stage CF longitudinally over a year, following treatment for iPEx with IV antibiotics. These data were collected from a subset of children with CF for which more information about their iPEx has previously been published [25]. We found that species richness, defined as the number of observed species, and α-diversity, which considers both species richness and dominance/evenness of species, were significantly increased at the 1-year follow-up mark compared to completion of antimicrobial treatment for iPEx, irrespective of the clinical state (well, sick, or hospitalized). This occurred despite a significant decrease in species richness after completion of treatment for iPEx compared to number of observed species at the onset of the iPEx and despite the occurrence of additional PEx treated with both and/or either antibiotics or steroids as well as being on suppressive antibiotics during the study period. These findings were in concordance with previous studies showing that species diversity is affected by antibiotics but that lung microbial diversity can increase after removal of antibiotic pressure [7, 12].

As with previous studies, the most common pathogens isolated from culture-based methods were *P aeruginosa* and *S aureus*. *Staphylococcus aureus* is the most prevalent pathogen in children and adolescents with CF, yet 40% of adults remain colonized; *P aeruginosa* is the most common pathogen colonizing adults with CF [7]. The more common pathogenic bacteria are acquired in temporal succession in persons with CF, with *S aureus* being present early in life followed by infections caused by *P aeruginosa* and *Burkholderia cepacia* [7, 12]. The latter 2 pathogens subsequently persist as colonizers of the CF lung, often with relative abundances >50% using sequencing approaches similar to ours [4, 7, 11, 12]. At our center, *S aureus* is found at a lower rate than nationally reported data, likely due to regional variability [2, 33]. *Pseudomonas aeruginosa* is typically reported at rates similarly to the national population [2, 33]. However, despite the presence of *P aeruginosa* on 65% of respiratory cultures prior to the collection period, only 25% had *P aeruginosa* detected in culture at the 1-year follow-up and it was not among the top 20 species, contributing the most to overall relative abundance of our study cohort (Supplementary Results). As our study population encompassed children with early-stage disease, it likely explains why our sequencing findings were not consistent with those published in persons with moderate to more severe CF disease [7, 12]. It is interesting to note, however, the differences in culture results and the relative abundance information found by sequencing, which supports prior

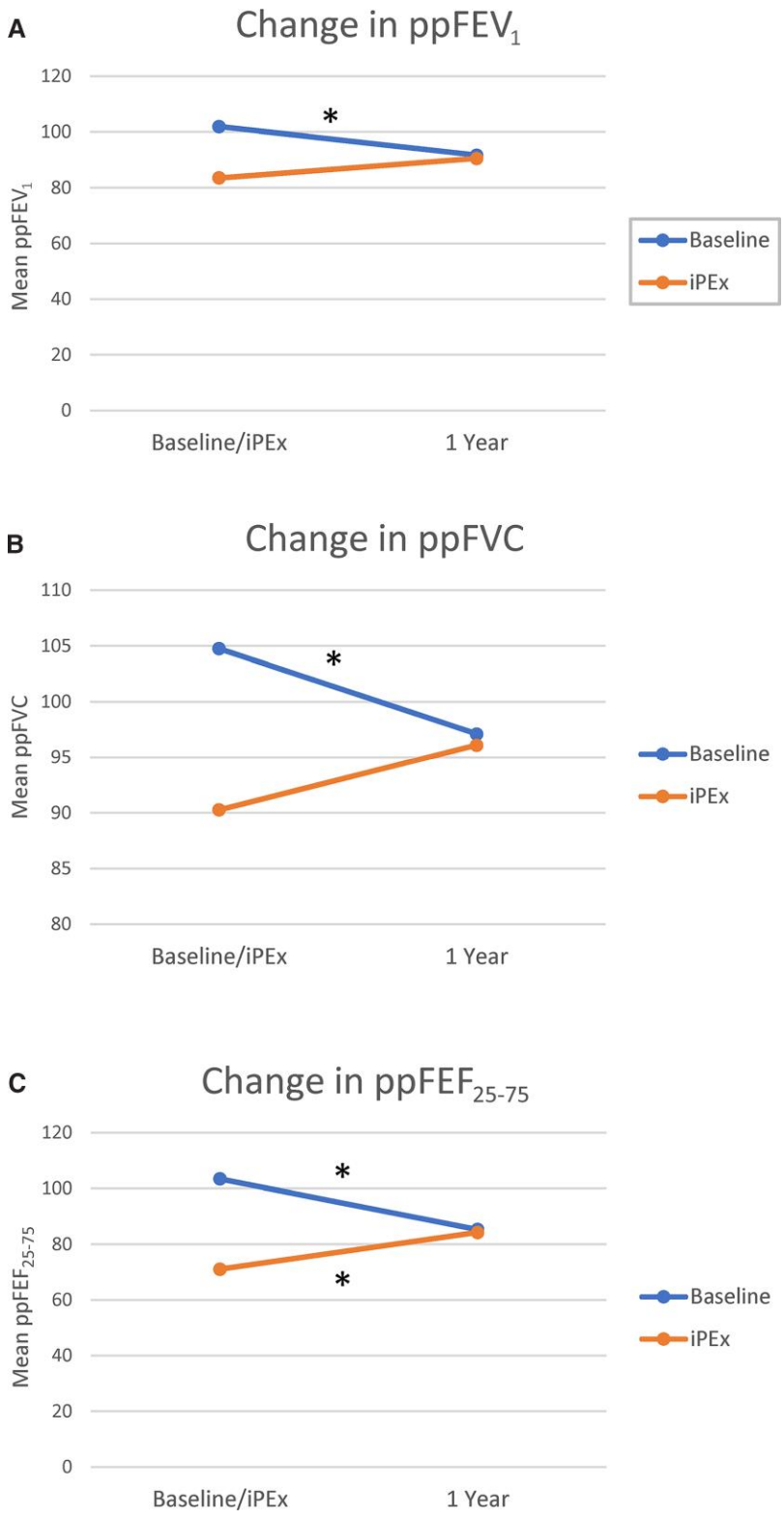

Figure 2. Changes in spirometry parameters at 1 year compared to baseline and initial pulmonary exacerbation (iPEx) to describe changes in lung function. *A*, Changes in percentage predicted forced expiratory volume in 1 second ($ppFEV_1$). *B*, Changes in percentage predicted forced vital capacity (ppFVC). *C*, Changes in percentage predicted forced expiratory flow between 25%–75% of vital capacity ($ppFEF_{25–75}$). Blue line indicates baseline to 1 year; orange, iPEx to 1 year. $^*P < .05$. Associations measured using a paired *t* test between baseline or iPEx and follow-up.

Table 3. Pulmonary Exacerbations During the Study Period

Treatment	No. (%)
Antibiotic treatment of initial PEx[a]	
Tobramycin	13 (65)
Ceftazidime	10 (50)
Meropenem	4 (20)
Piperacillin-tazobactam	4 (20)
Vancomycin	4 (20)
Cefepime	2 (10)
Ceftriaxone	2 (10)
Aztreonam	1 (5)
PO TMP-SMX	1 (5)
PO ciprofloxacin	1 (5)
PO linezolid	1 (5)
PO amoxicillin	1 (5)
Average No. of subsequent PEx, mean ± SD	3 ± 1.58
Antibiotic treatment of PEx	
PO antibiotics only	34 (57)
IV ± PO antibiotics	26 (43)
Antibiotics prescribed for PO only[a]	
Amoxicillin-clavulanate	16 (47)
Ciprofloxacin	10 (29)
TMP-SMX	10 (29)
Azithromycin	1 (2.9)
Levofloxacin	1 (2.9)
Antibiotics prescribed for IV ± PO[a]	
Tobramycin	12 (46)
Ceftazidime	8 (31)
Meropenem	7 (27)
PO TMP-SMX	7 (27)
Cefepime	6 (23)
Piperacillin-tazobactam	6 (23)
Vancomycin	6 (23)
Aztreonam	4 (15)
PO azithromycin	2 (8)
Ceftaroline	1 (4)
Ceftriaxone	1 (4)
PO levofloxacin	1 (4)
PO linezolid	1 (4)
PO rifampin	1 (4)
Courses of steroids prescribed (both with and without antibiotics), No.	
PO steroids	25
IV steroids	13

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: IV, intravenous; PEx, pulmonary exacerbation; PO, per oral; SD, standard deviation; TMP-SMX, trimethoprim-sulfamethoxazole.

[a]Multiple antibiotics were often prescribed for each individual PEx and thus totals do not add up to 100%.

suggestions that sequencing can assist traditional culture-based methodologies in understanding the full ecological environment in children with CF [10].

While *S aureus* had a high relative abundance with a mean of 13% at 1 year, it was present in only 8 of 18 samples, had a relative abundance of >1% in only 5 of the study participants, and was not differentially abundant at 1 year compared to iPEx or when comparing the different types of visits throughout the collection period. A previous study performed by Garcia-Nuñez et al showed that despite low relative abundance of *Staphylococcus* genus on genome sequencing from the bronchial microbiome in persons with CF with early-stage disease and not on chronic antibiotic therapy, a relative abundance of >0.26% at baseline suffered more PEx, more often due to *S aureus* [12]. Interestingly, samples with high *S aureus* relative abundance of >1% at 1 year were predominantly well visits (60%), with samples obtained during hospitalizations the second most common (40%). As supported by previous studies, the occurrence of pulmonary exacerbations is associated with changes in microbial composition, and the presence of dominant taxa are more likely only observed in more advanced disease [12].

The most relatively abundant species seen both across the study cohort and at the 1-year follow-up were anaerobic bacteria (*Rothia*, *Streptococcus*, *Veillonella*, and *Granulicatella* species), consistent with prior studies showing a predominance of anaerobic species in early disease [7]. As mentioned, a decline in anaerobic species is associated with an increase in relative abundance of more pathogenic species and may be associated with increasing the virulence of these species and/or increasing the ability of these species to colonize the lung, cause recurrent PEx, and aid in antibiotic resistance [4, 7, 11, 12]. These anaerobic species were present regardless of the type of visit in which the sample was obtained. In a model designed to more granularly assess the impact of microbial diversity and relative abundance of microbial species on lung function at any given time, we found 6 bacterial species of >200 identified that were significantly associated with lung function. All of these species would traditionally be considered nonpathogenic in persons with CF. Further studies are needed to see if these bacteria continue to be significant in additional patient cohorts.

The diagnosis of PEx in a person with CF is frequently associated with a decline in lung function, and management frequently focuses on the goal of a return to baseline and/or to a health status better than what preceded the PEx [6]. While the majority of our study participants had improvement in lung function at the 1-year follow-up compared to the iPEx, regardless of the type of visit, the comparison to baseline lung function was significantly decreased. Interestingly, this appeared to be driven by the initial exacerbation, as there were no significant differences in pulmonary function when assessed between months 3, 6, 9, and the 1-year follow-up. When evaluating for the number of antibiotic courses received (as indicative as to the frequency of moderate to severe PEx), we did find a significant association between antibiotic courses and the decline in ppFVC and a trend for the other parameters. Taken together, these findings support the notion that although antibiotic treatment for PEx often improves pulmonary function in the short term, lung function decline from PEx over time remains problematic in persons with CF [34].

There are several limitations to our current study. First, our participants had mild CF disease; further decline in microbial diversity and/or lung function, including the presence of an association between the 2 parameters, may be seen in more advanced and progressive disease. We also had a small sample size, which decreases the precision of our estimates; thus, our study may be underpowered to appreciate differences that truly exist. Correspondingly, we evaluated our study cohort over a relatively short collection period (1 year). Last, PEx was defined and dependent on the treating physician.

In conclusion, in our 1-year prospective evaluation of children with CF hospitalized for IV antibiotic treatment of an initial study PEx, we found that microbial diversity remained high despite decreases in lung function associated with repeated PEx events requiring antibiotic therapy. Furthermore, sequencing provided a broader assessment of the ecological environment of the airway compared to culture data alone, especially in regard to the abundance of traditional CF pathogens. Future longitudinal studies that encompass the progression from early lung disease to more moderate to severe disease could provide further insight on expected changes in lung microbiome and lung function in persons with CF and how they are influenced by antibiotic therapies.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. Z. I. and A. H., with support from A. C. K., conceptualized and designed the study. A. H., A. B., H. C., I. S., and A. C. K were all involved in study participant recruitment. Z. I., E. F., A. B., and A. H. performed data collection. A. B. performed sample processing and DNA extraction. K. A. C. supervised metagenomic sequencing. Z. I., E. F., and A. H. performed bioinformatic analysis and data analysis. A. H., R. J. F., E. T. Z., and K. A. C. were involved in selection of analysis methods and interpretation of findings. Z. I. wrote the original draft of the manuscript, with contributions by E. F. All authors were involved in manuscript revision and approved of the final version of the manuscript.

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Data availability. The sequence datasets supporting the conclusions of this article are available in the National Center for Biotechnology Information Sequence Read Archive repository under BioProjects PRJNA615628 and PRJNA846291.

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