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Importance of beta-lactam pharmacokinetics and pharmacodynamics on the recovery of microbial diversity in the airway of persons with cystic fibrosis

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ABSTRACT

Cystic fibrosis (CF) is a chronic lung disease characterized by acute pulmonary exacerbations (PEs) that are frequently treated with antibiotics. The impact of antibiotics on airway microbial diversity remains a critical knowledge gap. We sought to define the association between beta-lactam pharmacokinetic (PK) and pharmacodynamic target attainment on richness and alpha diversity. Twenty-seven children <18 years of age with CF participated in the prospective study. Airway samples were collected at hospital admission for PEx, end of antibiotic treatment (Tr), and >1 month in follow-up (FU). Metagenomic sequencing was performed to determine richness, alpha diversity, and the presence of antibiotic resistance genes. Free plasma beta-lactam levels were measured, and PK modeling was performed to determine time above the minimum inhibitory concentration ($fT > MIC$). 52% of study subjects had sufficient $fT > MIC$ for optimal bacterial killing. There were no significant differences in demographics or PEx characteristics, except for F508del homozygosity. No significant differences were noted in richness or alpha diversity at individual time points, and both groups experienced a decrease in richness and alpha diversity at Tr compared with PEx. However, alpha diversity remained decreased at FU compared with PEx in those with sufficient $fT > MIC$ but increased in those with insufficient $fT > MIC$ (Shannon -0.222 vs $+0.452$, $p=0.031$, and inverse Simpson -1.376 vs $+1.388$, $p=0.032$). Fluoroquinolone resistance was also more frequently detected in those with insufficient $fT > MIC$ (log2 fold change (log2FC) 2.29 , $p=0.025$). These findings suggest sufficient beta-lactam $fT > MIC$ is associated with suppressed recovery of alpha diversity following the antibiotic exposure period.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease that affects more than 30 000 people in the USA and 70 000 people worldwide.¹ Patients suffer from recurrent and chronic

Significance of this study

What is already known about this subject?

- Persons with cystic fibrosis (CF) have experience episodic pulmonary exacerbations (PEs) that are multifactorial and associated with decreased lung function and inflammation.
- Decreased microbial diversity in the CF airway is associated with decreased lung function.
- Antibiotic treatment for PEs influences microbial richness and alpha diversity in the CF airway.

What are the new findings?

- This study is the first validation study showing that beta-lactam antibiotic pharmacokinetic (PK)/pharmacodynamic (PD) target attainment during treatment is associated with suppressed recovery of microbial diversity following a PEx in children and adolescents with CF.
- Differences in community-level antibiotic resistance are also noted based on beta-lactam antibiotic PK/PD.

How might these results change the focus of research or clinical practice?

- Future research studies should assess the impact of beta-lactam antibiotic PK/PD on recovery of microbial diversity and lung function in adults with CF who have more advanced lung disease.

pulmonary infections that are strongly associated with morbidity and mortality.² Acute pulmonary exacerbations (PEs) are frequently treated with antibiotic therapy directed against pathogens associated with disease progression.³ In particular, *Pseudomonas aeruginosa* is often treated with an aminoglycoside plus beta-lactam combination such as tobramycin plus ceftazidime.^{3 4} Aminoglycoside antibiotics are routinely optimized for bacterial killing using

therapeutic drug monitoring to target a maximum concentration of 20–30 µg/mL for tobramycin and 80–120 µg/mL for amikacin.⁵ However, beta-lactams are not routinely optimized in a similar way, often due to their wide therapeutic range and lack of availability of drug assays. Additionally, persons with CF have augmented renal clearance, furthering their excretion of beta-lactam antibiotics compared with the general population.^{6,7} Pharmacokinetic (PK) modeling used to predict pharmacodynamic (PD) indices to optimize bacterial killing can provide valuable information to guide antibiotic therapy.^{8–10} Prior studies have found that the free drug concentration of beta-lactams over the time above the minimum inhibitory concentration ($fT > MIC$) was associated with improvements in lung function following treatment of a PEx.^{11,12} Additionally, suboptimal beta-lactam PK/PD may unintentionally promote bacterial resistance instead of pathogen killing,^{13,14} which in turn has been shown to be associated with decreased lung function.^{15–17}

Recent use of culture-independent next-generation sequencing has identified diverse communities of microbes in the CF airway, leading to an alteration in the traditional understanding of the role of infection in progressive lung disease.¹⁸ Decreased microbial diversity of the lungs, believed to be caused by the dominance of opportunistic and pathogenic bacterial taxa such as *P. aeruginosa* and the frequent use of antibiotics, is associated with declining lung function.^{19–21} Our prior work has suggested that sufficient beta-lactam PK/PD is associated with delayed recovery of baseline microbial diversity following treatment of a PEx.²² The objective of the current study was to test the hypothesis that beta-lactam antibiotic PK/PD is associated with changes in richness and alpha diversity following treatment of a PEx. Our secondary goal was to determine associations between antibiotic PK/PD, antibiotic resistance, and lung function.

MATERIALS AND METHODS

Study design

This was a prospective, observational cohort study of children with CF admitted to the hospital for treatment of PEx with intravenous beta-lactam antibiotics. The study period was from May 2018 to November 2019. Parental permission for those who were <18 years of age and assent were also obtained from subjects 7–17 years of age. Written informed consent was obtained from study subjects who were 18 years of age. Respiratory samples (including sputum and/or oropharyngeal (OP) swabs) were collected when patients were admitted for treatment with intravenous antibiotics (PEX) at the end of antibiotic treatment (Tr) course and again at their next follow-up (FU) clinic appointment. Blood samples for PK analyses of beta-lactam antibiotics were also collected during the hospitalization. Additional information regarding demographics (age, weight, height, race, ethnicity, and cystic fibrosis transmembrane conductance regulator (CFTR) mutation), PEX characteristics, medication use (CFTR modulators, oral maintenance antibiotics, inhaled maintenance antibiotics, insulin, PEX treatment antibiotics, and PEX steroid use), microbiology (concurrent PEX respiratory culture results), laboratory values (serum creatinine) and pulmonary function tests (PFTs) were also obtained. Baseline lung function was defined as the best PFT in the 6 months preceding the

PEX event. Baseline per cent predicted forced expiratory volume in one second (FEV_1) was used to determine the study subject's disease stage (early, $FEV_1 > 70\%$; intermediate, $FEV_1 40\%–70\%$; or advanced, $FEV_1 < 40\%$).²³ PEX was defined using modified Fuchs' criteria,²⁴ where patients were admitted to the hospital for intravenous antibiotic treatment and had at least one of the following signs or symptoms: increased cough, increased sputum production, hemoptysis, increased sinus discharge, sinus pain, increased dyspnea, malaise/fatigue, anorexia/weight loss, fever of $>38^\circ C$, change in exam of the chest, decrease in FEV_1 of $\geq 10\%$, or radiographical changes.

PK sampling design and plasma concentration determination

A sparse PK sampling design was used similar to the one we had previously published.¹¹ Briefly, samples were collected after achieving steady state (>24 hours after initiation of antibiotics) and were obtained at four time points: trough ($\times 2$), peak (1 hour after infusion), and mid-dose (3–4 hours after infusion). Samples were placed on ice and processed immediately to maintain concentration integrity, and then were frozen at $-80^\circ C$. Samples were shipped on dry ice to the Pharmacanalytical Laboratory at the University of Southern California. There the free drug plasma concentrations for ceftriaxone, ceftazidime, piperacillin, and meropenem were determined from each sample using multiplex liquid chromatography–mass spectroscopy.¹¹

PK/PD modeling

PK modeling of beta-lactam antibiotics was performed using Bayesian estimation in MW/Pharm V.3.80 (Mediware), as previously published.^{8,9,11} Briefly, study subject age, weight, height, serum creatinine (measured at hospital admission), antibiotic dose, and measured plasma concentrations were all incorporated into the model to develop the time–concentration curve. Each beta-lactam antibiotic was assessed for the free plasma concentration above the minimum inhibitory concentration (MIC) of the selected pathogens determine the $fT > MIC$. To be deemed sufficient, the $fT > MIC$ had to be 40% of the dosing interval for carbapenems, 50% of the dosing interval for penicillins, and 70% of the dosing interval for cephalosporins.¹⁰ For each study subject, their concurrent clinical respiratory culture results were used to identify the MIC. If more than one Gram-negative bacteria were identified, the highest MIC was used. The MIC_{50} used for *Staphylococcus aureus* isolates were based on published reports, as our laboratory does not report MICs for the beta-lactams used in this study.^{25,26} Persons with concurrent methicillin-resistant *Staphylococcus aureus* (MRSA) detected on respiratory culture also had to be on MRSA-directed therapy to not misclassify any subjects. Lastly, if the culture did not grow a pathogen for which to extrapolate an MIC (eg, only grew normal respiratory flora), antibiotic exposure was evaluated against the MIC_{50} for all *P. aeruginosa* isolates collected at our institution from persons with CF in 2016 ($n=125$). *P. aeruginosa* was selected as our standard because it is one of the more common pathogens in persons with CF, and as such, antibiotic regimens for treatment of PEX are often directed against this pathogen.³ Specifically, we used the following MICs for those persons

whose cultures grew normal respiratory flora: cefepime MIC₅₀ 8 µg/mL, ceftazidime MIC₅₀ 4 µg/mL, meropenem MIC₅₀ 4 µg/mL, and piperacillin/tazobactam MIC₅₀ 16 µg/mL.

Respiratory sample collection and processing

Sputum samples were collected in a sterile specimen cup, and OP swab specimens were collected using an Eswab with Amies media (Copan). Sputum and OP swab samples were stored at 4°C before sample processing. Sputum samples were homogenized by mixing 1:1 v/v with dithiothreitol (Fisher Healthcare) and sterile normal saline, vortexed, and heated in a 37°C heated bead bath for 15 min. Amies media was transferred directly into a sterile cryovial. All samples were pelleted through centrifugation (12 000g×10 min). Supernatants and pellets were then stored separately at –80°C.

Bacterial DNA extraction and metagenomic sequencing

The aforementioned frozen cell pellets were rapidly thawed and mixed with 1 mL of sterile phosphate-buffered saline, and then bacterial DNA was extracted using a QIAamp DNA Microbiome kit (Qiagen). DNA quantity and quality were measured using Qubit (ThermoFisher Scientific) and Bioanalyzer (Agilent), respectively. DNA was diluted to a concentration of 10–20 ng/µL, and 5 µL was used for library construction using Nextera XT Library Prep kit (Illumina). Libraries were sequenced on a NextSeq 500 (Illumina) at the GW Genomics Core. Twelve to fifteen libraries were sequenced per run using the Mid-Output 2×150 cycle kit.

BIOINFORMATIC ANALYSES

Sequences were examined for quality and trimmed using FastQC and FlexBar prior to downstream analyses.²⁷ Human sequences were filtered using KneadData.²⁸ Alignment of sequences for taxonomic profiling was performed using MetaPhlAn2.²⁹ AmrPlusPlus was used to align filtered and trimmed bacterial sequences against antibiotic resistance genes in Galaxy.³⁰ Taxonomic and antibiotic class resistance count tables were imported into Rstudio V3.6.1 for subsequent analyses using the packages DESeq2 V1.24.0, ggplot2 V3.2.0, phyloseq V1.28.0, and vegan V2.5–6.³¹ Explicit V2.10.5 was used to determine the number of observed bacterial species, Shannon Index, and the Inverse Simpson Index.³²

Sequencing quality and sensitivity analyses

The results of our sequenced respiratory samples, including quality control and sensitivity analyses, have been previously published.³³ Briefly, sequencing data of satisfactory quality was available from 71 of 81 study timepoints, and concurrently sequenced Zymo controls were concordant with their expected microbial community composition ($R^2=0.71$, $p=0.002$). The mean number of sequences per sample was 10.1 million (SD 4.2 million); the mean number of sequences aligned to our bacterial sequencing library was 7.9 million (SD 5.2 million); and the mean number of sequences aligned to our antibiotic resistance gene library was 16K (SD 34 000). Our mean Good coverage was 1 (SD 0). Of the 71 samples, 21 were from sputum and 50 were from OP swabs; the breakdown of these sample types based on time point and $fT>MIC$ is shown in online supplemental

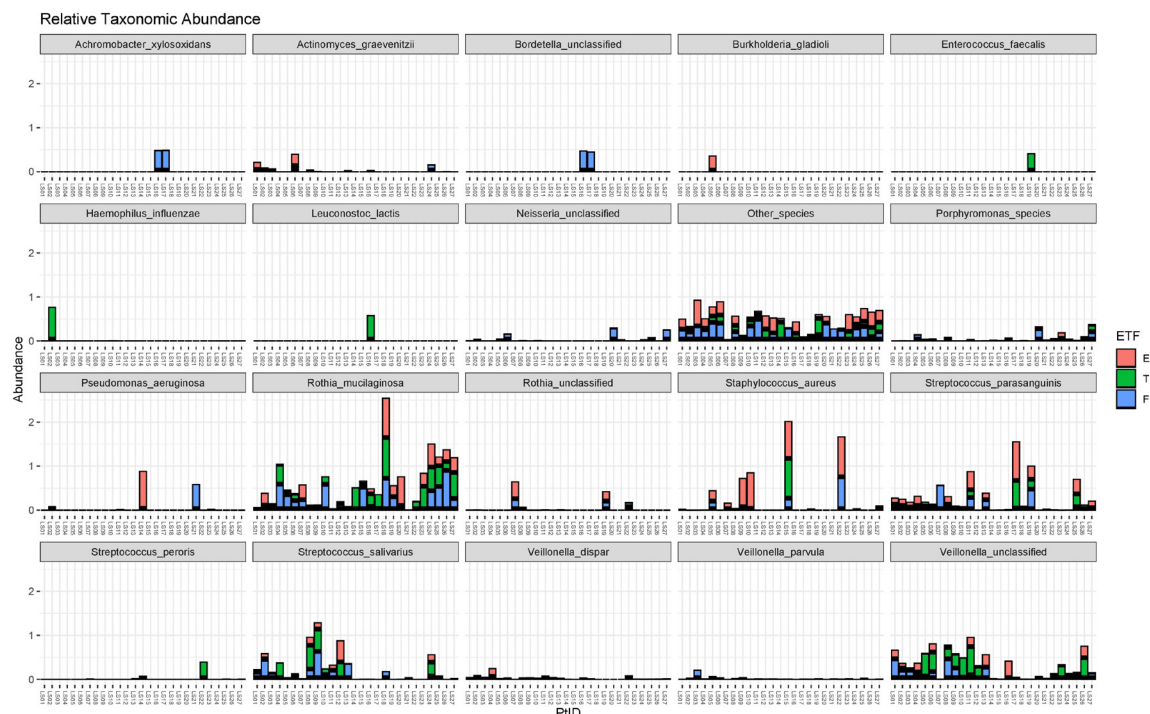


Figure 1 Relative bacterial taxonomic abundance. The 19 bacterial species that contributed to at least 20% of the total relative abundance of one or more samples compared to the other 176 bacterial species are shown. E, exacerbation sample; F, follow-up sample; PtID, participant ID; T, treatment sample.

Table 1 Study subject demographics and baseline clinical characteristics

	fT>MIC sufficient (n=14)	fT>MIC not sufficient (n=13)	P value
Age, * predicted mean years (SE)	9.7 (1.3)	10.2 (1.3)	0.788
Gender† (n, % female)	8 (57)	4 (31)	0.168
Race, ‡ n (%)			0.076
White	10 (71)	12 (92)	
Black	4 (29)	0 (0)	
Other	0 (0)	1 (8)	
Ethnicity, ‡ n (%)			0.345
Hispanic	4 (29)	6 (46)	
Not Hispanic	10 (71)	7 (54)	
CFTR genotype, ‡ n (%)			0.020
F508del homozygous	3 (21)	9 (69)	
F508del heterozygous	7 (50)	4 (31)	
Other	4 (29)	0 (0)	
Disease stage, ‡ n (%)			0.511
Early (FEV ₁ >70%)	12 (86)	11 (84)	
Intermediate (FEV ₁ 40%–70%)	0 (0)	1 (8)	
Advanced (FEV ₁ <40%)	0 (0)	0 (0)	
NA (age<6 years)	2 (14)	1 (8)	
BMI, * predicted mean (SE)	16.7 (1.2)	17.6 (1.3)	0.859
Maintenance azithromycin, ‡ n (% yes)	1 (7)	3 (23)	0.244
Maintenance inhaled antibiotic, ‡ n (% yes)	5 (36)	5 (38)	0.833
CFTR modulator, ‡ n (% yes)	2 (14)	5 (38)	0.152
Home insulin, ‡ n (% yes)	1 (7)	0 (0)	0.326
Baseline lung function, ‡ predicted mean (SE)	(n=12)	(n=12)	
% predicted FEV ₁	86.8 (5.68)	106.1 (5.68)	0.053
% predicted FVC	93.1 (5.79)	106.8 (5.79)	0.175
% predicted FEF25–75	72.6 (12.66)	118.8 (12.66)	0.037

*General linear model with Gaussian family and identity link.

† χ^2 .

‡General linear model with Gaussian family and identity link, controlling for demographic characteristics.

§Square transform.

BMI, Body Mass Index; CFTR, cystic fibrosis transmembrane conductance regulator; FEF25–75, forced expiratory flow 25–75; FEV₁, forced expiratory volume in one second; fT>MIC, time above the minimum inhibitory concentration; FVC, forced vital capacity.

table 1. A sensitivity analysis of three matched sputum and OP swab pairs from three study subjects found a significant correlation between their microbial communities (R^2 range 0.779–0.968, all $p<0.001$) and high similarity demonstrated by beta diversity measures (Morisita-Horn index range 0.861–0.908), suggesting that the mixed respiratory sample design in our study would allow for accurate interpretation of our findings.

Statistical analyses

Comparison of categorical and continuous variables was performed in STATA/IC V.15.1. χ^2 was used for comparisons of categorical variables between groups. Generalized

linear models were used for comparison of continuous variables between groups using a Gaussian variance function and an identity link function. Normality of continuous data was tested using Shapiro-Wilk test prior to analyses and was transformed if the variable was non-normal, and it did not contain negative values. In comparisons of richness and alpha diversity, analyses of differences of fT>MIC were performed controlling for sample type collected (sputum vs OP swab) and discordance of sample types across time points. In comparisons of pulmonary function, analyses of differences of fT>MIC were performed controlling for demographic characteristics (age, gender, race, CFTR genotype, and disease stage). Permutational analysis of variance was performed in Rstudio using the *adonis* function for the analysis and partitioning sums of squares using Bray-Curtis dissimilarities and controlling for repeated patient samples using the *strata* function.

RESULTS

Study subjects and baseline clinical parameters

Twenty-seven children and adolescents ≤ 18 years of age were recruited to participate. The median age of the total study cohort was 9.4 years (range 1.4–18.7 years). Fifty-six per cent of the study subjects ($n=15$) were male; race was reported as 81% ($n=22$) white and 15% ($n=4$) black; 37% ($n=10$) of the study subjects had Hispanic ethnicity. Forty-four per cent ($n=12$) were homozygous for an F508del mutation, while 41% ($n=11$) were heterozygous for an F508del mutation. At the time of study enrollment, 26% ($n=7$) of the subjects were receiving CFTR modulators; 37% ($n=10$) were on a maintenance inhaled antibiotic; 15% ($n=4$) were on maintenance azithromycin; and 4% ($n=1$) were receiving insulin. The mean best FEV₁ per cent predicted in the 6 months preceding their PEx was $96.4\% \pm 16.7\%$. Based on these results, 85% ($n=23$) of the study subjects were considered to be in an early disease stage (FEV₁>70%), while 4% ($n=1$) were at an intermediate disease stage (FEV₁ between 40% and 70%), and 11% ($n=3$) were too young for PFTs.²³

PEx characteristics

The most common symptoms at PEx onset were cough (85%, $n=23$), change in sputum quality (44%, $n=12$), and dyspnea (29%, $n=8$). The most common signs at PEx onset were decrease in FEV₁ by >10% (59%, $n=16$), change in exam of the chest (41%, $n=11$), and new infiltrate on chest X-ray (41%, $n=11$). The most common beta-lactam antibiotic received was ceftazidime (44%, $n=12$), followed by cefepime (19%, $n=5$) and piperacillin–tazobactam (19%, $n=5$). Nineteen per cent ($n=5$) received two beta-lactams for >72 hours during the treatment period. Sixty-three per cent ($n=17$) of the study subjects also received tobramycin, while 19% ($n=5$) also received vancomycin. Fifty-two per cent ($n=13$) were also given steroids as part of their treatment. The most common pathogens identified by respiratory culture were *P. aeruginosa* (33%, $n=9$), followed by methicillin-sensitive *S. aureus* (15%, $n=4$) and MRSA (11%, $n=3$). Forty-four per cent ($n=12$) of the study subjects had respiratory cultures obtained that only grew normal respiratory flora.

Microbiome characteristics

A total of 195 bacterial species were identified across all samples collected (n=71). Forty-four bacterial species contributed to at least 5% of the total relative abundance of one or more samples, while 34 species contributed to at least 10% of the total relative abundance of one or more samples. Nineteen species contributed to at least 20% of the total relative abundance of one or more samples; the relative abundance of these species in contrast to the other remaining species is shown in [figure 1](#). In contrast to clinical cultures obtained at PEx, the five bacteria with the highest overall relative abundance detected by sequencing in PEx samples were *Rothia mucilaginosa*, *Streptococcus parasanguinis*, *Veillonella* sp unclassified, *S. aureus*, and *S. salivarius*. Of the 26 PEx samples that were successfully sequenced, these bacterial taxa were detected in the following proportion of samples: *R. mucilaginosa* (92%), *S. parasanguinis* (73%), *Veillonella* sp. unclassified (88%), *S. aureus* (46%), and *S. salivarius* (65%) ([figure 1](#)).

Pharmacokinetics and $fT>MIC$

Fifty-two per cent (n=14) of the study subjects achieved an $fT>MIC$ that was deemed sufficient for optimal bacterial killing, based on at least one beta-lactam administered during the treatment period. No study subjects were reclassified based on lack of appropriate treatment for MRSA. When comparing baseline demographics, no significant differences in age, gender, race, ethnicity, disease stage, CFTR modulator use, or maintenance antibiotics were noted ([table 1](#)). Those who were $fT>MIC$ not sufficient were more likely to have a CFTR genotype of F508del homozygous (69%, n=9), while those who achieved $fT>MIC$ sufficiency were more likely to have a CFTR genotype of F508del heterozygous (50%, n=7). No significant differences were noted in PEx characteristics, including PFTs at PEx onset, bacteria or viruses identified from respiratory samples, antibiotics given, duration of antibiotic therapy, or receipt of steroids ([table 2](#)). While there were no significant differences in

Table 2 PEx characteristics and treatments received

	$fT>MIC$ sufficient (n=14)	$fT>MIC$ not sufficient (n=13)	P value
Pulmonary function at exacerbation, * predicted mean (SE)	(n=12 with PFTs)	(n=11 with PFTs)	
% predicted FEV ₁ †	76.7 (6.29)	87.2 (6.74)	0.296
% predicted FVC	82.2 (7.00)	93.7 (7.50)	0.368
% predicted FEF _{25–75}	77.9 (11.43)	67.3 (10.68)	0.588
Bacterial and viral pathogens			
MRSA positive, ‡ n (% yes)	1 (7)	2 (15)	0.496
MSSA positive, ‡ n (% yes)	3 (21)	1 (8)	0.315
<i>Pseudomonas aeruginosa</i> positive, ‡ n (% yes)	4 (29)	5 (38)	0.586
<i>Achromobacter xylosoxidans</i> positive, ‡ n (% yes)	1 (7)	0 (0)	0.326
<i>Stenotrophomonas maltophilia</i> positive, ‡ n (% yes)	1 (7)	0 (0)	0.326
<i>Haemophilus influenzae</i> positive, ‡ n (% yes)	1 (7)	0 (0)	0.290
No pathogen present, ‡ n, (% yes)	7 (50)	5 (38)	0.547
Respiratory pathogen panel ‡	(n=6 tested)	(n=5 tested)	
Rhino/enterovirus (n)	2	1	0.999
Negative (n)	4	4	
PEx treatments			
Primary beta-lactam antibiotic, ‡ n (%)			0.124
Ceftriaxone	2 (14)	0 (0)	
Ceftazidime	8 (57)	4 (31)	
Cefepime	1 (7)	4 (31)	
Piperacillin–tazobactam	1 (7)	4 (31)	
Meropenem	2 (14)	1 (8)	
Narrow spectrum, ‡ n (% yes)	11 (79)	8 (62)	0.333
Concurrent aminoglycosides, ‡ n (% yes)	8 (57)	9 (69)	0.516
Concurrent MRSA-directed therapy, ‡ n (% yes)	3 (21)	2 (15)	0.686
Total days of antibiotic therapy, * predicted mean (SE)	18.2 (1.57)	13.4 (1.66)	0.086
Steroids given, ‡ n (% yes)	5 (36)	8 (62)	0.180
$fT>MIC$, * mean (SE)	87.5 (6.4)	28.2 (6.8)	<0.001
Creatinine clearance, §* mean (SE)	148.1 (15.8)	224.9 (16.7)	0.002

*General linear model with Gaussian family and identity link, controlling for demographic characteristics.

†Square transform.

‡ χ^2 .

§Log transform.

FEF_{25–75}, forced expiratory flow 25–75; FEV₁, forced expiratory volume in one second; $fT>MIC$, time above the minimum inhibitory concentration; FVC, forced vital capacity; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PEx, pulmonary exacerbation; PFT, pulmonary function test.

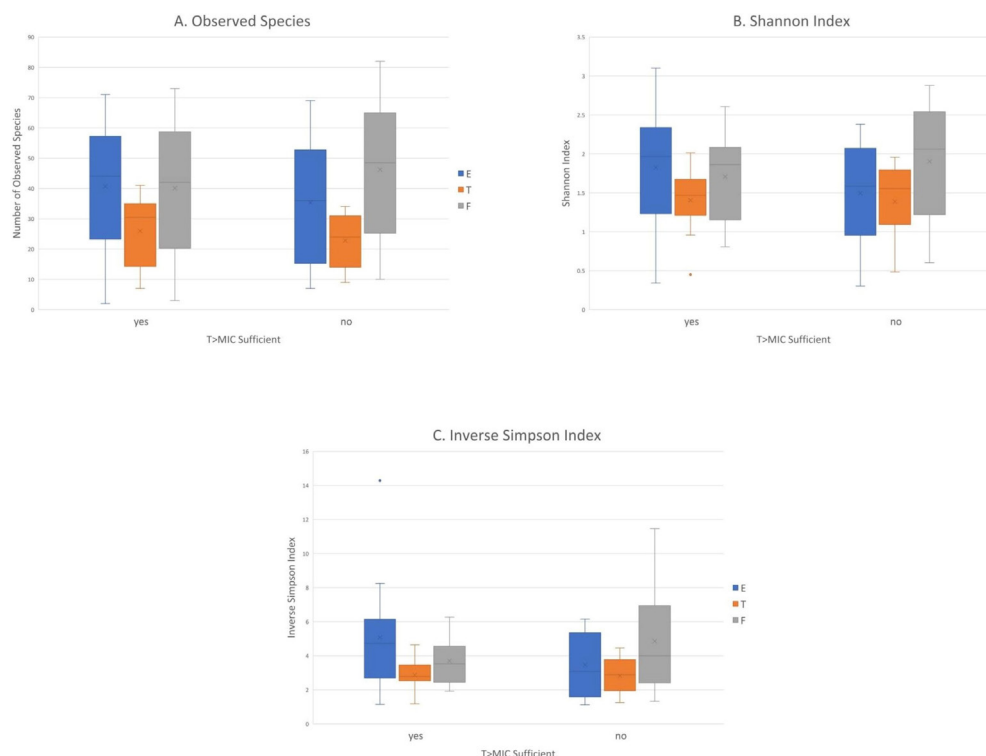


Figure 2 Box and whiskers plot of richness and alpha diversity. Observed indicates observed bacterial taxa; Shannon indicates Shannon diversity; 'no' indicates $fT > MIC$ not sufficient; 'yes' indicates $fT > MIC$ sufficient; 'x' corresponds to mean values. The center horizontal line corresponds to the median. Colored boxes represent the IQRs. Small circles represent individual values. E, exacerbation sample; F, follow-up sample; $fT > MIC$, time above the minimum inhibitory concentration; T, treatment sample.

beta-lactam used for treatment, ceftazidime was more commonly used in the $fT > MIC$ sufficient group (57%, $n=8$, vs 31%, $n=4$), and cefepime and piperacillin–tazobactam were more commonly used in the $fT > MIC$ insufficient group (both 31%, $n=4$, vs 7%, $n=1$). Additionally, while no significance was noted, antibiotic days were longer in the $fT > MIC$ sufficient group (18.2 vs 13.4). The mean $fT > MIC$ of the beta-lactam antibiotics used was significantly higher in the $fT > MIC$ sufficient group (87.5 vs 28.2, $p < 0.001$) (table 2 and online supplemental figure 1). Additionally, the mean creatinine clearance was significantly higher in the $fT > MIC$ insufficient group (148.1 vs 224.9, $p = 0.002$) (table 2).

Association between $fT > MIC$ and airway microbiome characteristics

No significant differences in richness or alpha diversity measured at each individual time point were noted between those who achieved sufficient $fT > MIC$ compared with those who did not (figure 2A–C and online supplemental table 2). When evaluating changes in richness and alpha diversity between time points, both groups had a decrease at Tr compared with PEx (figure 3A–C, online supplemental table 3). However, alpha diversity remained decreased at FU compared with PEx in those with sufficient $fT > MIC$ but increased in those with insufficient $fT > MIC$ (Shannon -0.222 vs $+0.452$, $p = 0.031$, and inverse Simpson -1.376 vs $+1.388$, $p = 0.032$). No significant differences were noted in intraperson beta diversity (figure 3D) or overall community composition (online supplemental figure 2)

between those who achieved sufficient $fT > MIC$ compared with those who did not. However, several bacterial taxa were over-represented or under-represented in those with sufficient $fT > MIC$ compared with those without, including *P. aeruginosa* (log2 fold change (log2FC) -7.9 , $p = 0.033$) and *Burkholderia gladioli* (log2FC -26.7 , $p < 0.001$), which were over-represented in the $fT > MIC$ insufficient group (online supplemental figure 3).

Association between $fT > MIC$ and antibiotic resistance

The relative abundance of antibiotic resistance genes identified by metagenomic sequencing is shown in figure 4. Respiratory samples from study subjects who achieved sufficient $fT > MIC$ were significantly more likely to have resistance to macrolides, lincosamides, and streptogramin A and B drugs (log2FC -0.96 , $p = 0.025$) compared with those with insufficient $fT > MIC$. Conversely, those with insufficient $fT > MIC$ were more likely to have fluoroquinolone resistance (log2FC 2.29, $p = 0.025$) compared with those with sufficient $fT > MIC$.

Association between $fT > MIC$ and recovery of pulmonary function

There were significant differences and trends toward significance in baseline pulmonary function with those who were $fT > MIC$ sufficient having a lower % predicted FEV₁ (86.8 vs 106.1, $p = 0.053$) and a lower % predicted FEF_{25–75} (72.6 vs 118.8, $p = 0.037$) than those who were $fT > MIC$ insufficient (table 1). These differences persisted across time

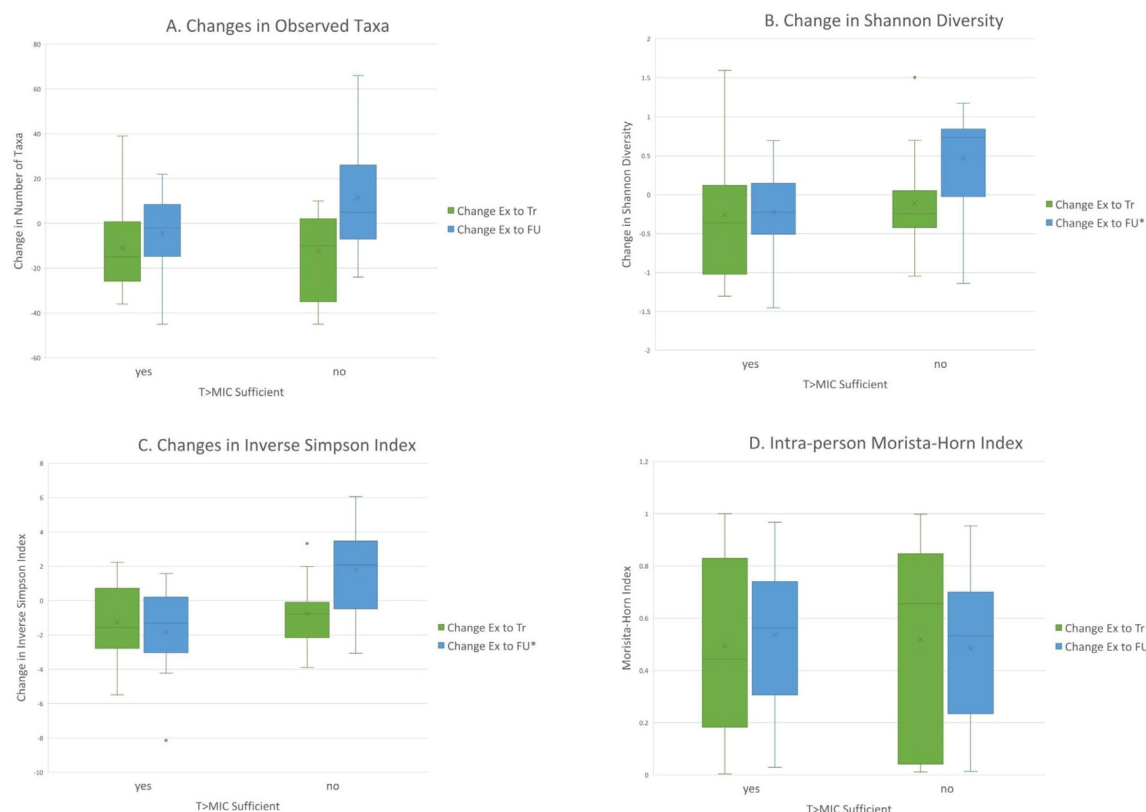


Figure 3 Box and whiskers plot of changes in richness, alpha diversity, and beta diversity across time points. (A) Changes in observed taxa. (B) Changes in Shannon Diversity. (C) Changes in Inverse Simpson Index. (D) Intraperson Morista-Horn Index. 'No' indicates $fT > MIC$ not sufficient; 'yes' indicates $fT > MIC$ sufficient; 'x' corresponds to mean values. The center horizontal line corresponds to the median. Colored boxes represent the IQRs. Small circles represent individual values. * $P < 0.05$. Ex, exacerbation; Tr, end of antibiotic treatment; $fT > MIC$, time above the minimum inhibitory concentration; FU, follow-up.

points, with those who were $fT > MIC$ sufficient consistently having lower pulmonary function results (table 2 and online supplemental table 4). However, when we normalized the results based on baseline lung function (eg, end of treatment FEV_1 /baseline FEV_1), no significant differences in per cent recovery of baseline pulmonary function were noted based on $fT > MIC$ (online supplemental figure 4 and table 4).

DISCUSSION

This study validates our previous findings that beta-lactam PK/PD target attainment is associated with recovery of alpha diversity following antibiotic treatment for a pulmonary exacerbation.²² Importantly, the differences between those who achieved sufficient beta-lactam $fT > MIC$ and those who did not were based on the change in alpha diversity between time points, not the individual time points themselves. This suggests that the recovery of alpha diversity that occurs following antibiotic exposure cannot be explained simply by the diversity of the microbial community prior to starting antibiotic treatment. However, as this study only captures antibiotic treatment around a single pulmonary exacerbation, we cannot draw conclusions about how repeated antibiotic exposures drive down microbial diversity over time. We were not able to show that sufficient beta-lactam $fT > MIC$ influenced recovery of pulmonary function in this cohort. Differences in antibiotic resistance were identified between the $fT > MIC$ sufficient

and not-sufficient groups, although in drug classes besides the beta-lactams.

It has been shown that decreased richness and microbial diversity in the airway of persons with CF are associated with lower pulmonary function.^{34–36} As antibiotics are frequently used to treat pulmonary exacerbation in persons with CF, it is logical that antibiotics would be an important contributor. However, the sustained effects of intermittent antibiotic treatment on microbial diversity remain unclear. Prior longitudinal studies showed that decreases in microbial diversity associated with progressive lung functional decline can be explained by antibiotic exposure.²¹ Studies of a single antibiotic treatment course have produced conflicting results. Some have found a limited impact of antibiotics on the relative abundance of particular pathogens or alpha diversity in general,³⁴ while others have found a distinct shift in both pathogen relative abundance and alpha diversity.³⁷ In a prior analysis of data from this cohort of study subjects, we found a significant decrease in richness at the end of antibiotic treatment compared to pulmonary exacerbation onset and FU.³⁸ Our findings in this study suggest that a sustained decrease in alpha diversity after antibiotic treatment is not related to the use of the antibiotic itself but to the beta-lactam antibiotic PK/PD. This is a factor that has not been frequently explored, and it does provide a plausible explanation for the discrepancies in the literature.

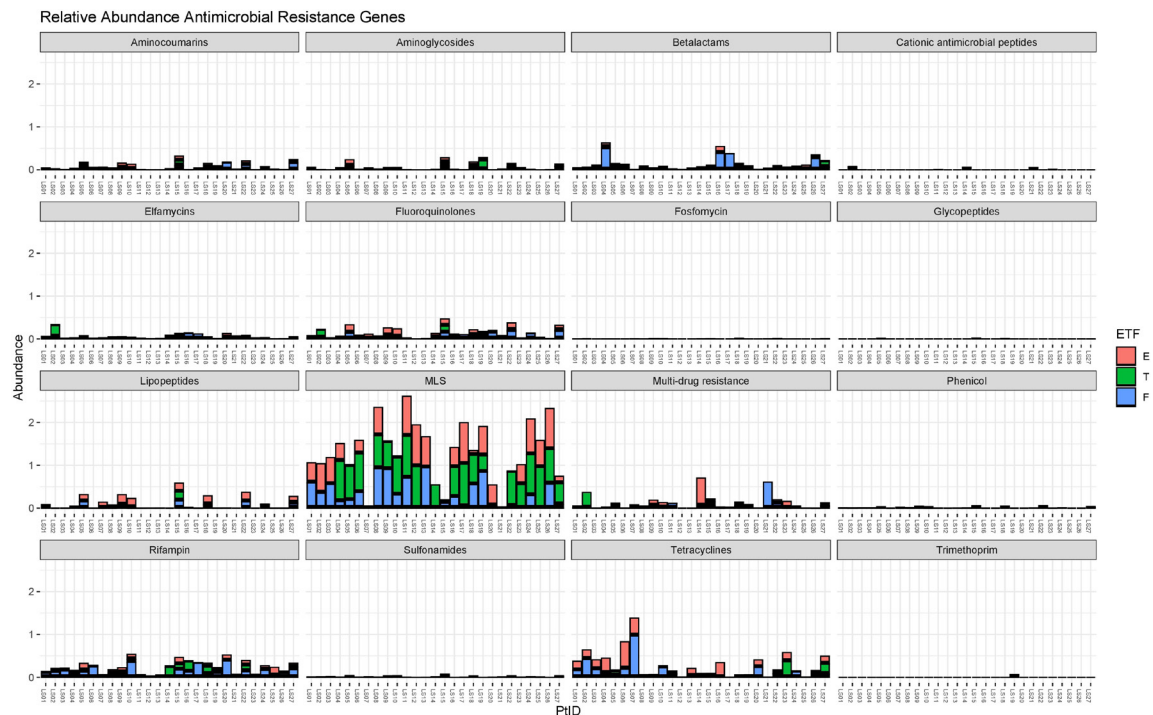


Figure 4 Relative abundance of antimicrobial resistance genes. E, exacerbation sample; F, follow-up sample; MLS, macrolides, lincosamides, and streptogramin A and B drugs; PtID, participant ID; T, treatment sample.

Our study also found that sufficient versus insufficient beta-lactam PK/PD was associated with significant differences in the relative abundance of many bacterial pathogens and 'normal' flora. It is possible that these differences are dependent on the beta-lactam MICs of the organism rather than the direct impact of the antibiotic exposure. For example, *B. gladioli* (which is often resistant to beta-lactam antibiotics³⁹) and *P. aeruginosa* (which often has high beta-lactam MICs⁴⁰) were over-represented in the beta-lactam $fT > MIC$ not sufficient group in our study.

In our study, we found fluoroquinolone resistance to be more commonly present in those with insufficient beta-lactam $fT > MIC$, whereas macrolide/lincosamide/streptogramin resistance was more commonly present in those with sufficient beta-lactam $fT > MIC$. However, despite having an excellent Good coverage for our taxonomic analysis, the number of sequences that could be aligned with antibiotic resistance genes was low (<0.2% of total sequencing reads). Prior studies have suggested a minimum sequencing depth of 50 million reads is necessary to adequately characterize antibiotic resistance by mechanism⁴¹; however, we reported our results by drug class to partially overcome this limitation. A higher sequencing depth may be necessary in order to be able to truly describe the role of antibiotic resistance within our samples.

While antibiotic PK/PD is important for understanding shifts in the microbial community, many clinicians are rightly more concerned with the impact on clinical outcomes. Our previous work and the work of others have shown that improved beta-lactam PK/PD is associated with improved recovery of pulmonary function.^{11 12} In this study cohort, we did not find beta-lactam $fT > MIC$ to be associated with recovery of pulmonary function following

pulmonary exacerbation. However, it is important to note that this relatively small study cohort was generally young with good baseline lung function, and that those with sufficient $fT > MIC$ were starting at a significantly lower baseline. These factors may have influenced our findings.

Our study has some limitations that prevent us from drawing more definitive conclusions. These include a relatively small number of subjects from a single center, their young age, relatively good baseline lung function, as well as the inability of some of them to produce sputum. As such, our findings may not be generalizable to adults with CF who have advanced disease stage ($FEV_1 < 40\%$). Additionally, the small number of study subjects means that we were likely underpowered to detect some significant differences. Particularly, the $fT > MIC$ sufficient group had lower baseline lung function which persisted across study time points, higher microbial community diversity at exacerbation onset, and longer duration of antibiotic treatment. A larger cohort would increase the power to detect if these features were significant factors. We also did not measure antibiotic concentrations directly within lung fluid but instead are imputing the significance of serum antibiotic concentrations on treatment of lung infection. Lastly, the interpretation of microbial community diversity in upper airway specimens as representative of diversity within the lungs remains a limitation in studies of children with CF.

In summary, beta-lactam $fT > MIC$ is associated with recovery of microbial diversity following antibiotic treatment for pulmonary exacerbation in persons with CF. Future studies should be conducted in an older population with more advanced lung disease (including those persons who can regularly produce sputum) to validate that these findings are generalizable across CF populations. Likewise,

a study in a larger cohort would provide more evidence to determine if beta-lactam PK/PD is an important factor in recovery of pulmonary function. Lastly, long-term FU studies are necessary to determine if this delayed recovery of microbial diversity is sustained and cumulative, or if recovery is gained after a longer period of time following antibiotic treatment. Further research is needed before these findings should influence changes in clinical practice.

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Contributors AH conceptualized and designed the study; performed the pharmacokinetic modeling, bioinformatic analysis, and data analysis; and wrote the original manuscript. AH, AB, HC, IS, and ACK were all involved in study subject recruitment. AH and AB performed the data collection. AB performed the sample processing and DNA extraction. KAC supervised the metagenomic sequencing. SL performed the antibiotic assays. AH, ETZ, RJF, and KAC were involved in the selection of analysis methods and interpretation of findings. All authors were involved in manuscript revision and approved the final version as written.

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REFERENCES

- MacKenzie T, Gifford AH, Sabadosa KA, *et al*. Longevity of patients with cystic fibrosis in 2000 to 2010 and beyond: survival analysis of the cystic fibrosis Foundation patient registry. *Ann Intern Med* 2014;161:233–41.
- Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. *N Engl J Med* 1996;335:179–88.
- Flume PA, Mogayzel PJ, Robinson KA, *et al*. Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. *Am J Respir Crit Care Med* 2009;180:802–8.
- Sanders DB, Solomon GM, Beckett VV, *et al*. Standardized treatment of pulmonary exacerbations (STOP) study: observations at the initiation of intravenous antibiotics for cystic fibrosis pulmonary exacerbations. *J Cyst Fibros* 2017;16:592–9.
- Zobell JT, Waters CD, Young DC, *et al*. Optimization of anti-pseudomonal antibiotics for cystic fibrosis pulmonary exacerbations: II. cephalosporins and penicillins. *Pediatr Pulmonol* 2013;48:107–22.
- Udy AA, Roberts JA, Lipman J. Implications of augmented renal clearance in critically ill patients. *Nat Rev Nephrol* 2011;7:539–43.
- Prandota J. Clinical pharmacology of antibiotics and other drugs in cystic fibrosis. *Drugs* 1988;35:542–78.
- Jelliffe RW, Schumitzky A, Bayard D, *et al*. Model-based, goal-oriented, individualised drug therapy. Linkage of population modelling, new 'multiple model' dosage design, bayesian feedback and individualised target goals. *Clin Pharmacokinet* 1998;34:57–77.
- Proost JH, Meijer DK. MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med* 1992;22:155–63.
- Downes KJ, Hahn A, Wiles J, *et al*. Dose optimisation of antibiotics in children: application of pharmacokinetics/pharmacodynamics in paediatrics. *Int J Antimicrob Agents* 2014;43:223–30.
- Hahn A, Jensen C, Fanous H, *et al*. Relationship of pulmonary outcomes, microbiology, and serum antibiotic concentrations in cystic fibrosis patients. *J Pediatr Pharmacol Ther* 2018;23:379–89.
- Kuti JL, Pettit RS, Neu N, *et al*. Meropenem time above the MIC exposure is predictive of response in cystic fibrosis children with acute pulmonary exacerbations. *Diagn Microbiol Infect Dis* 2018;91:294–7.
- Nair CG, Chao C, Ryall B, *et al*. Sub-lethal concentrations of antibiotics increase mutation frequency in the cystic fibrosis pathogen *Pseudomonas aeruginosa*. *Lett Appl Microbiol* 2013;56:149–54.
- Tam VH, Ledesma KR, Schilling AN, *et al*. In vivo dynamics of carbapenem-resistant *Pseudomonas aeruginosa* selection after suboptimal dosing. *Diagn Microbiol Infect Dis* 2009;64:427–33.
- Smith AL, Fiel SB, Mayer-Hamblett N, *et al*. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* 2003;123:1495–502.
- Waters VJ, Kidd TJ, Canton R, *et al*. Reconciling antimicrobial susceptibility testing and clinical response in antimicrobial treatment of chronic cystic fibrosis lung infections. *Clin Infect Dis* 2019;69:1812–6.
- Hahn A, Burrell A, Fanous H, *et al*. Antibiotic multidrug resistance in the cystic fibrosis airway microbiome is associated with decreased diversity. *Heliyon* 2018;4:e00795.
- Huang YJ, LiPuma JJ. The microbiome in cystic fibrosis. *Clin Chest Med* 2016;37:59–67.
- Coburn B, Wang PW, Diaz Caballero J, *et al*. Lung microbiota across age and disease stage in cystic fibrosis. *Sci Rep* 2015;5:10241.
- Zemanick ET, Wagner BD, Robertson CE, *et al*. Assessment of airway microbiota and inflammation in cystic fibrosis using multiple sampling methods. *Ann Am Thorac Soc* 2015;12:221–9.
- Zhao J, Schloss PD, Kalikin LM, *et al*. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A* 2012;109:5809–14.

- 22 Hahn A, Fanous H, Jensen C, *et al.* Changes in microbiome diversity following beta-lactam antibiotic treatment are associated with therapeutic versus subtherapeutic antibiotic exposure in cystic fibrosis. *Sci Rep* 2019;9:2534.
- 23 Konstan MW, Wagener JS, VanDevanter DR. Characterizing aggressiveness and predicting future progression of CF lung disease. *J Cyst Fibros* 2009;8 Suppl 1:S15–19.
- 24 Fuchs HJ, Borowitz DS, Christiansen DH, *et al.* Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N Engl J Med* 1994;331:637–42.
- 25 Washington JA, Jones RN, Gerlach EH, *et al.* Multicenter comparison of in vitro activities of FK-037, cefepime, ceftriaxone, ceftazidime, and cefuroxime. *Antimicrob Agents Chemother* 1993;37:1696–700.
- 26 Sweeney D, Shinabarger DL, Arhin FF, *et al.* Comparative in vitro activity of oritavancin and other agents against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 2017;87:121–8.
- 27 Roehr JT, Dieterich C, Reinert K. Flexbar 3.0 - SIMD and multicore parallelization. *Bioinformatics* 2017;33:2941–2.
- 28 McIver LJ, Abu-Ali G, Franzosa EA, *et al.* BioBakery: a meta'omic analysis environment. *Bioinformatics* 2018;34:1235–7.
- 29 Segata N, Waldron L, Ballarini A, *et al.* Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods* 2012;9:811–4.
- 30 Lakin SM, Dean C, Noyes NR, *et al.* MEGARes: an antimicrobial resistance database for high throughput sequencing. *Nucleic Acids Res* 2017;45:D574–80.
- 31 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550.
- 32 Robertson CE, Harris JK, Wagner BD, *et al.* Explicet: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data. *Bioinformatics* 2013;29:3100–1.
- 33 Felton E, Burrell A, Chaney H, *et al.* Inflammation in children with cystic fibrosis: contribution of bacterial production of long-chain fatty acids. *Pediatr Res* 2021. doi:10.1038/s41390-021-01419-4. [Epub ahead of print: 02 Mar 2021].
- 34 Fodor AA, Klem ER, Gilpin DF, *et al.* The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. *PLoS One* 2012;7:e45001.
- 35 Zemanick ET, Harris JK, Wagner BD, *et al.* Inflammation and airway microbiota during cystic fibrosis pulmonary exacerbations. *PLoS One* 2013;8:e62917.
- 36 Carmody LA, Caverly LJ, Foster BK, *et al.* Fluctuations in airway bacterial communities associated with clinical states and disease stages in cystic fibrosis. *PLoS One* 2018;13:e0194060.
- 37 Smith DJ, Badrick AC, Zakrzewski M, *et al.* Pyrosequencing reveals transient cystic fibrosis lung microbiome changes with intravenous antibiotics. *Eur Respir J* 2014;44:922–30.
- 38 Felton E, Burrell A, Chaney H. Bacterial functional profiling of the cystic fibrosis airway across clinical states. *TBD* 2021.
- 39 Segonds C, Clavel-Batut P, Thouverez M, *et al.* Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. *J Clin Microbiol* 2009;47:1510–6.
- 40 Kuti JL, Pettit RS, Neu N, *et al.* Microbiological activity of ceftolozane/tazobactam, ceftazidime, meropenem, and piperacillin/tazobactam against *Pseudomonas aeruginosa* isolated from children with cystic fibrosis. *Diagn Microbiol Infect Dis* 2015;83:53–5.
- 41 Zaheer R, Noyes N, Ortega Polo R, *et al.* Impact of sequencing depth on the characterization of the microbiome and resistome. *Sci Rep* 2018;8:5890.