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Randomized Trial of Biofilm Testing to Select Antibiotics for Cystic Fibrosis Airway Infection*

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SUMMARY

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Contributions of authors. S.M.M., J.C.E., and J.L.B. wrote the manuscript, and S.McN., R.D.S., D.M.O., D.R., M.F.K., R.A., D.H., P.M.J., and R.L.G. edited it. S.M.M., J.C.E., and J.L.B. had full access to all of the data in the study and take full responsibility for the integrity of all of the data and the accuracy of the data analysis, including all adverse effects.

Rationale—In cystic fibrosis (CF), conventional antibiotic susceptibility results correlate poorly with clinical outcomes. We hypothesized that biofilm testing would more accurately reflect the susceptibilities of bacteria infecting CF airways.

Methods—A multi-center randomized pilot trial was conducted to assess the efficacy and safety of using biofilm susceptibility testing of *Pseudomonas aeruginosa* sputum isolates to guide antibiotic regimens for chronic airway infections in clinically stable adolescent and adult CF patients. Thirty-nine participants were randomized to biofilm or conventional treatment groups; 14-day courses of two antibiotics were selected according to an activity-based algorithm using the corresponding susceptibility results.

Results—Of the agents tested, meropenem was most active against biofilm-grown bacteria, and was included in regimens for about half of each study group. For nineteen of 39 randomized participants, randomization to the other study group would not have changed the antibiotic classes of the assigned regimen. Study groups were comparable at baseline, and had similar mean decreases in bacterial density, measured in log₁₀ colony forming units per gram of sputum (biofilm, -2.94 [SD 2.83], versus conventional, -3.27 [SD 3.09]), and mean increases in forced expiratory volume in one second, measured in liters (0.18 [SD 0.20] versus 0.12 [SD 0.22]).

Conclusions—In this pilot study, antibiotic regimens based on biofilm testing did not differ significantly from regimens based on conventional testing in terms of microbiological and clinical responses. The predictive value of biofilm testing may nonetheless warrant evaluation in an adequately powered clinical trial in younger CF patients or those experiencing acute pulmonary exacerbation.

Keywords

Pseudomonas aeruginosa; intravenous antibiotics; antibiotic resistance; antibiotic susceptibility testing; broth microdilution testing; inhibitory quotient; sputum bacterial density; lung function

INTRODUCTION

Individuals with cystic fibrosis (CF) have chronic endobronchial infections punctuated by recurrent acute exacerbations, with progressive lung damage and premature death. *Pseudomonas aeruginosa* is the single most important pathogen in CF. Approximately 55% of CF patients overall and more than 75% of patients >18 years are infected with this organism.^{1,2} Surveillance cultures of airway secretions yield a mean of ~2.5 morphologically distinct isolates of *P. aeruginosa* per patient.³ Intravenous and inhaled antibiotics are mainstays of CF therapy; multi-drug combinations improve efficacy and minimize emergence of antibiotic resistance.⁴ However, resistance remains an important clinical problem. For example, the prevalence of tobramycin-resistant *P. aeruginosa* has increased from 11.8% to 30.4% over the past decade.^{3,5}

Despite evidence that conventional susceptibility results do not reliably predict treatment responses in CF,^{6,7} current guidelines recommend that these be used to guide antibiotic selection.⁸ However, conventional susceptibility methods approved by the Clinical Laboratory Standards Institute (CLSI)⁹ do not account for the ability of *P. aeruginosa* to form biofilms within CF airways,¹⁰ or the marked differences in susceptibility of planktonic versus biofilm-grown *P. aeruginosa*.¹¹

P. aeruginosa biofilms display marked resistance to antibiotics commonly used to treat CF airway infections.^{12,13} Bacterial cells in biofilms are generally slowly dividing, thus use of log phase cells in conventional susceptibility testing may not reflect the susceptibility of *P.*

aeruginosa within CF airways. To evaluate the anti-biofilm activity of available antibiotics, clinically feasible biofilm susceptibility methods have been developed.^{11,14,15}

The specific aims of this pilot study were to assess the safety and measure the microbiological and pulmonary effects of using anti-biofilm activity to assign antibiotic regimens for chronic CF airway infection. These aims were achieved by performing a randomized controlled trial of antibiotic regimens assigned on the basis of biofilm susceptibility testing as compared to conventional testing. The hypothesis was that participants receiving antibiotics based on anti-biofilm activity would have a greater decrease in *P. aeruginosa* sputum density than those receiving treatment based on conventional activity.

MATERIALS AND METHODS

Participants were enrolled between February 2004 and October 2007 at seven US CF centers: Nationwide Children's Hospital (Columbus, Ohio), Children's Hospital of Pittsburgh (Pittsburgh, Pennsylvania), Washington University (St. Louis, Missouri), Baylor College of Medicine (Houston, Texas), the University of Iowa (Iowa City, Iowa), University Hospital (Cincinnati, Ohio), Seattle Children's Hospital (Seattle, Washington), and the University of Washington Medical Center (Seattle, Washington). The IRB at each site approved the protocol and amendments. Participants provided written informed consent (and assent when applicable). Inclusion and exclusion criteria were as described in Supplementary Material.

Sputum samples obtained at Visit 1 (Day -21 to -14) were cultured quantitatively.³ For each *P. aeruginosa* morphotype present at 10% of the most prevalent, antibiotic susceptibilities were determined by semi-automated broth microdilution³ (Sensititre System, AccuMed, Westlake, Ohio) and biofilm susceptibility testing.¹¹ Multiresistance was defined as resistance to all agents in at least two of three classes (aminoglycosides, fluoroquinolones, beta-lactams) by either conventional or biofilm testing, based on CLSI breakpoints.¹⁶

Conventional and biofilm regimens of two antibiotics were selected centrally using a published algorithm,¹⁷ with not more than one drug from each antibiotic class, as follows: aminoglycosides (amikacin, tobramycin); beta-lactams (ceftazidime, meropenem, piperacillin-tazobactam, ticarcillin-clavulanate); fluoroquinolones (ciprofloxacin); macrolides (azithromycin). For each bacterial morphotype, the algorithm calculated the conventional minimum inhibitory quotient (MIQ) and the biofilm minimum inhibitory quotient (BIQ) of each drug, defined as achievable serum concentration divided by conventional or biofilm minimum inhibitory concentration (MIC or BIC), respectively. For each subject's isolate set, MIQ and BIQ composite scores were used to rank antibiotics from most to least active. For each testing method, the two most active drugs from different antibiotic classes were selected. When the most active antibiotics had the same composite scores, a tiebreaker rule based on ease of administration, cost, and likelihood of adverse drug reaction was applied (hierarchy, most to least preferred: azithromycin, ciprofloxacin, ceftazidime, meropenem, ticarcillin-clavulanate, piperacillin-tazobactam, tobramycin, amikacin). To define drug allergies, the beta-lactams were subdivided into penicillin, cephalosporin, and carbapenem subclasses. If a participant had a history of allergic reaction, that class or subclass of antibiotics was excluded from potential assignment.

Participants were assigned to treatment groups using a block randomization procedure, stratified by study site. Participants and site personnel were informed of antibiotic assignments, but were not told the testing method (conventional or biofilm) on which this was based. The effectiveness of this blinding was not evaluated.

At Visit 2 (Day 0), spirometry was performed,¹⁸ and participants started on a 14-day course of their assigned antibiotic regimen; recommended dosing was as described in Supplementary Material. Participants assigned to regimens that included an IV antibiotic had the option of home IV therapy (OptionCare, Buffalo Grove, Illinois) or admission to a General Clinical Research Center; all such participants who initiated treatment received the majority of the 14-day course at home. Visit 3 (Day 7 +/- 2 days) was an interim visit or telephone call to monitor adverse events and review medication use. Visit 4 (Day 14 +/- 2 days) was timed to occur 12–48 hours after the last antibiotic dose, a time at which residual concentrations in airway secretions were deemed unlikely to affect bacterial growth. A sputum sample was collected for quantitative culture and spirometry was performed.

The primary outcome measure was microbiological response, change in *P. aeruginosa* sputum density, calculated as $\log_{10}$ of end-of-treatment density minus $\log_{10}$ of screening density, in CFU/g. The secondary outcome measure was pulmonary response, change in forced expiratory volume in one second (FEV₁), calculated as end-of-treatment FEV₁ minus the baseline FEV₁, in liters.

A CF Foundation Data Monitoring Committee conducted one interim review to assess study conduct and safety. A 10% rate of acute pulmonary exacerbation or need to change antibiotics in either treatment group was the pre-specified threshold for considering early study termination.

This pilot trial was designed to determine feasibility and estimate treatment effect and variability for planning a full-scale trial; owing to cost constraints, it was not powered to detect significant differences in microbiological or clinical efficacy between treatment groups. The target sample size was 20 randomized participants in each treatment group. Outcomes were analyzed using linear regression models that adjusted for baseline values, defined as sputum density measured at screening and FEV₁ measured at initiation of antibiotic treatment. Analyses were performed using STATA™ (versions 9.2 and 10.0, College Station, TX) and S-plus (version 6.2, Insightful Corp., Seattle); modified intent-to-treat comparisons included all randomized participants who received at least one dose of their assigned regimen.

RESULTS

Enrollment and randomization

The flow of participants through the protocol is shown in Figure 1. Forty randomizations resulted in 39 participants assigned to treatment. One individual was randomized but withdrawn prior to treatment and subsequently re-randomized and treated; data from the completed treatment course are included here. One participant from each group discontinued treatment due to adverse events. Study groups were comparable at baseline with regard to age, race, genotype, pancreatic status, baseline lung function, and *P. aeruginosa* density and morphotype number (Table 1). The proportion of males was higher in the biofilm group.

Susceptibility testing results

Susceptibility results were analyzed in terms of both inhibitory concentrations and inhibitory quotients; the latter represents a normalization of the former to enable direct comparison of activities for different antibiotics. The inhibitory quotient (defined under “Methods”) is the converse of the inhibitory concentration, in that a higher inhibitory quotient indicates higher activity of a given drug against the set of tested organisms. Biofilm and conventional testing of *P. aeruginosa* isolates at screening indicated that most agents were less active against biofilm-grown organisms (Table 2). Meropenem was most active against biofilms, while piperacillin/tazobactam and meropenem were most active against planktonic organisms. In

this set of isolates from CF adults, azithromycin showed poor activity by both methods, in contrast to the previous finding of good anti-biofilm activity against isolates from younger patients.¹¹ The largest fold-difference between biofilm and conventional testing was for piperacillin/tazobactam (16-fold more active against planktonic organisms). Lesser differences were seen for ceftazidime (8-fold) and meropenem (4-fold). Six participants (three in each arm) had at least one multiresistant isolate; all three with a conventionally multiresistant isolate also had biofilm multiresistance.

Antibiotic combinations

Participants were assigned to only 12 of 21 potential regimens (Table 3). The most frequent regimens included meropenem (52%) and ciprofloxacin (49%). Azithromycin-containing regimens were used for only 2 participants (5%), both in the biofilm group. No participant received ceftazidime and tobramycin, a combination commonly used in CF clinical practice; moreover, this combination would have been the biofilm regimen for only a single participant randomized to the conventional group, and would not have been the conventional regimen for any participant randomized to the biofilm group.

For each participant, the treatment regimens based on each method were categorized according to drug class or subclass, and cross-tabulated (not shown). The observed agreement between drug class combinations selected by BIC and MIC methods was 49%. Of the 19 participants who would have received the same drug class combinations had they been assigned to the other study group, six received meropenem/ciprofloxacin and five each received meropenem/aminoglycoside or anti-pseudomonal penicillin/ciprofloxacin.

Microbiological and clinical outcomes

Changes in sputum density (primary outcome) and FEV₁ (secondary outcome) were analyzed under a modified intent-to-treat principle, with inclusion of all participants who received at least one dose of their assigned regimen (Table 4). For all participants combined, the mean change in sputum density from baseline to end of treatment was -3.09 log₁₀ CFU/g (95% CI, -4.15 to -2.02). For biofilm group relative to conventional group, the estimated treatment effects were 0.28 log₁₀ CFU/g (95% CI, -1.98 to 2.54) for *P. aeruginosa* sputum density (i.e., the decrease in density was numerically greater in the conventional group) and 0.07 L (95% CI, -0.08 to 0.22) for FEV₁ (i.e., the increase in FEV₁ was numerically greater in the biofilm group), after adjustment for baseline values; these numerical differences were not statistically significant. Because the baseline sputum densities were somewhat imbalanced between groups (Table 1), the adjusted estimates of treatment effect shown in Table 4 were considered more conservative than unadjusted estimates. No within-participant relationship between changes in sputum density and FEV₁ was discerned (data not shown). Among the 31 participants with microbiological endpoint data (17 in biofilm group, 14 in conventional group), 23 had ≥ 1 log₁₀ CFU/g decrease in sputum density (13 in biofilm group, 10 in conventional group), and *P. aeruginosa* was not detected at the end of study in 7 of these (4 in biofilm group, 3 in conventional group). Lack of detectable *P. aeruginosa* at end of study did not correlate with initial sputum density (6.70 log₁₀ CFU/g, vs 7.67 for non-eradicators), mucoidy of isolates on initial culture, or antibiotic combination received (data not shown).

Among participants with microbiological endpoint data, a post-hoc analysis compared those infected with at least one multiresistant isolate (n=5) and those infected with only susceptible isolates (n=26), irrespective of study group assignment. The change in *P. aeruginosa* sputum density for these post-hoc groups was comparable: -3.4 log₁₀ CFU/g (SD 1.5) versus -3.0 log₁₀ CFU/g (SD 3.1), respectively.

A second post-hoc analysis compared those receiving two IV agents (i.e., beta-lactam plus aminoglycoside, n=15) and those receiving a single oral agent (i.e., ciprofloxacin or azithromycin) plus a single IV agent (n=18; 15 with microbiological endpoint data), irrespective of study group assignment. For the former, the change in sputum density was $-4.0 \log_{10}$ CFU/g (SD 2.5), whereas for the latter it was $-2.2 \log_{10}$ CFU/g (SD 3.2) (p=0.09). This trend toward a significant difference was not seen for lung function (data not shown).

Safety

Seventy treatment-emergent adverse events were reported (33 in biofilm group, 37 in conventional group). Thirteen participants in each group experienced 1 adverse event. No adverse event was judged serious. The most commonly reported adverse events were diarrhea (n=6), fatigue (n=6), headache (n=5), and oral candidiasis (n=4).

DISCUSSION

This pilot study of adults and adolescents with clinically stable CF airway infection confirmed the feasibility and safety of assigning systemic antibiotic regimens based on biofilm testing, and demonstrated an effect on *P. aeruginosa* sputum density and FEV₁ comparable to treatment based on conventional testing. Its failure to detect differences in outcome between the biofilm and conventional groups may be attributable to several factors, including small sample size (thus limiting statistical power), the clinical stability (i.e., lack of pulmonary exacerbations) of the participants, and the antibiotic susceptibilities of *P. aeruginosa* with which they were infected. Potentially compounding this last factor is the extreme variability of sputum antibiograms in individual CF patients with chronic *P. aeruginosa* infection, wherein morphologically indistinguishable isolates can exhibit markedly variable resistance phenotypes (on average, three antibiograms per morphotype).¹⁹

A previous retrospective study of *P. aeruginosa* from younger patients demonstrated marked differences between biofilm and conventional susceptibilities.¹¹ In the present study, differences between biofilm and conventional susceptibilities were less substantial, and for half of those randomized, being switched to the other study group would not have changed either of the assigned drug classes. We surmise that as patients age, repeated antibiotic exposure of their individual strains results in selection of subclones with diminished planktonic and biofilm susceptibility (or more consistently detectable resistance) across all drug classes. Thus, even an adequately powered study of older CF patients at clinical baseline might not detect a differential treatment effect of biofilm and conventional regimens. Nonetheless, this study provides data enabling the statistical power of IV antibiotic trials in this patient population (clinically stable adults with CF) to be calculated with precision for the first time.

In this study, FEV₁ % predicted improved 7.4% relative to baseline for all participants combined, similar to the effect of inhaled antibiotics.^{20,21} *P. aeruginosa* sputum density decreased $\sim 3 \log_{10}$ CFU/g in both study groups (which is greater than the microbiological effect of inhaled antibiotics),^{20,21} and also in a post-hoc group with multiresistant isolates. More surprisingly, for 7 of 39 participants (18%), *P. aeruginosa* was not detected at end of treatment. For CF clinicians who question the prevailing US approach of using IV antibiotics only during acute pulmonary exacerbations, these data suggest that elective IV therapy may be warranted in some clinically stable patients.

The microbiological response observed in both study groups and in those with multiresistant isolates may have reflected our approach to assigning antibiotics based on drug activity. We used a selection algorithm that prioritized drugs according to inhibitory quotient, which compares achievable serum concentration to *in vitro* susceptibility.¹⁷ Thus, we picked the

drugs for which the MIC or BIC of the isolates was the farthest below the corresponding achievable serum concentration. Meropenem was highly active against both planktonic and biofilm-grown bacteria, and was included in about half of the regimens in each study group. Although historically popular as a first-line regimen, the combination of ceftazidime and tobramycin was not assigned during the study. This may presage a clinical trend towards decreasing ceftazidime use and increasing meropenem use, owing to the greater activity of the latter against contemporary CF isolates.²²

Use of the pharmacodynamic concept of drug activity, which is seldom implemented in chronic CF airway infection, was intended to avoid bias associated with choosing drugs based on clinical judgment or arbitrary guidelines. Drug activity also accounts for potential sub-inhibitory or supra-inhibitory antibiotic effects that dichotomization of susceptibility results (i.e., interpretation as “susceptible” or “resistant”) may obscure. To implement this approach, clinical laboratories that perform broth microdilution or Etest would simply report the raw susceptibility data; a publicly available computer-based algorithm would then be used to calculate inhibitory quotients and assign drug regimens.¹⁷

In CF patients with acute exacerbation of non-multiresistant *P. aeruginosa* airway infection, randomized trials of single drugs or two-drug combinations have demonstrated decreases of 1 to 5 log₁₀ CFU/g,^{4,22-24} with particularly favorable effects observed for meropenem.²² In contrast, in multiresistant *P. aeruginosa* infection, both a retrospective study of clinically assigned regimens and a randomized controlled trial of regimens based on multiple combination bactericidal testing (MCBT) have shown only minor decreases in sputum bacterial density.^{25,26} Moreover, a post-hoc analysis of participants in the placebo arm of the inhaled tobramycin trial treated for acute exacerbation found no correlation between MIC results and changes in FEV₁; sputum bacterial density was not examined.⁶ Such studies have cast doubt on whether susceptibility testing can predict clinical outcomes, leading some to suggest that CF clinicians should forego susceptibility testing altogether.^{7,27}

To assume that growth of biofilms in a 96-well plate in the clinical laboratory can adequately represent the complex biochemical milieu and microbial ecology that shape CF airway infection would be naïve. The structure and physiology of biofilms in the CF airway probably reflect various factors (e.g., bacterial alginate, neutrophil DNA, respiratory mucins) that may be difficult to simulate in current testing systems.²⁸ However, post-hoc analysis of isolates from the MCBT trial suggests that biofilm testing might improve microbiological and clinical outcomes in CF airway infection.²⁹ That analysis compared outcomes according to whether biofilms formed by isolates cultured from adults with CF at onset of acute exacerbation were susceptible to the agents with which they had been treated. Participants with at least one biofilm-susceptible isolate (n=61) had significant decreases in sputum bacterial density (p=0.02) and length of stay (p=0.04), and trends toward less treatment failure and prolonged time to next exacerbation, compared to those with none (n=49). Conversely, post-hoc analysis of isolates from an inhaled tobramycin eradication trial performed in CF patients <6 yrs at clinical baseline with recent acquisition of *P. aeruginosa*³⁰ showed that initial isolates from children in whom eradication failed had higher tobramycin BICs than those in whom eradication occurred, despite similar initial tobramycin MICs in both groups, suggesting that biofilm testing may predict those at risk of treatment failure (Lucas Hoffman, Jessica Foster, S.M.M., J.C.E., J.L.B., and R.L.G., unpublished results). Taken together, these findings suggest that adequately powered trials are needed to evaluate whether biofilm susceptibility testing has predictive value for CF adults experiencing acute exacerbation of chronic *P. aeruginosa* airway infection, as well as for clinically stable younger patients with recent acquisition of *P. aeruginosa*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BIC	biofilm inhibitory concentration
BIC₅₀	50 th percentile of biofilm inhibitory concentration
BIC₉₀	90 th percentile of biofilm inhibitory concentration
BIQ	biofilm inhibitory quotient
BIQ₅₀	50 th percentile of biofilm inhibitory quotient
BIQ₉₀	90 th percentile of biofilm inhibitory quotient
CF	cystic fibrosis
CFTR	CF transmembrane conductance regulator gene
CFU	colony forming units
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
FEF₂₅₋₇₅	forced expiratory flow between 25% and 75% of FVC
FEV₁	forced expiratory volume in one second
FVC	forced vital capacity
IRB	Institutional Review Board

IV	intravenous
log₁₀	logarithm ₁₀
MCBT	multiple combination bactericidal testing
MIC	minimum inhibitory concentration
MIC₅₀	50 th percentile of minimum inhibitory concentration
MIC₉₀	90 th percentile of minimum inhibitory concentration
MIQ	minimum inhibitory quotient
MIQ₅₀	50 th percentile of minimum inhibitory quotient
MIQ₉₀	90 th percentile of minimum inhibitory quotient
SD	standard deviation

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Figure 1. Flow of participants through each stage of the protocol

*Repeat screenings were allowed. Most common reasons for screen failures included multi-resistant bacteria (n=11), insufficient bacterial density or lack of growth (n=5), inadequate biofilm growth (n=5), history of antibiotic allergy or intolerance (n=4), insufficient susceptibility data (n=3), and acute pulmonary exacerbation (n=3).

**The 39 randomized participants included 1 individual who was randomized twice: when first randomized, the individual was withdrawn prior to initiating treatment owing to decline in lung function; six months later the same individual was re-screened and randomized a second time, and treatment was initiated and completed. Treatment group and assigned

antibiotic regimen were the same for both randomizations (MIC group; meropenem/tobramycin). Data for the second randomization are summarized in the flow chart.

[†]Completion of treatment was defined as having received at least 10 days of the assigned antibiotic regimen.

Table 1

Baseline characteristics of randomized subjects, by study group.

	BIC group (N = 20)	MIC group (N = 19)	p-value ^a
Age, mean (SD)	32.0 (9.3)	28.2 (8.6)	0.19
Gender, n (%)			
Male	16 (80.0%)	9 (47.4%)	0.03
Female	4 (20.0%)	10 (52.6%)	
Race / ethnicity, n (%)			
Caucasian	20 (100%)	19 (100%)	
CFTR genotype, n (%)			
F508del homozygous	11 (55.0%)	9 (47.4%)	0.70
F508del heterozygous	7 (35.0%)	9 (47.4%)	
Other (non DF508)	1 (5.0%)	1 (5.3%)	
Missing	1 (5.0%)	0	
Pancreatic status, n (%)			
Sufficient	2 (10.0%)	3 (15.8%)	0.59
Insufficient	18 (90.0%)	16 (84.2%)	
Baseline sputum microbiology ^b			
<i>P. aeruginosa</i> density in log ₁₀ CFU/g of sputum, mean (SD)	7.20 (1.08)	7.69 (1.10)	0.17
<i>P. aeruginosa</i> morphotypes isolated from sputum specimen, mean (SD)	2.7 (0.9)	3.1 (1.0)	0.15
<i>P. aeruginosa</i> morphotypes tested, mean (SD)	1.7 (0.7)	1.9 (0.9)	0.37
Baseline lung function ^c			
FEV ₁ in L, mean (SD)	2.5 (1.1)	2.4 (1.3)	0.85
FEV ₁ % predicted, mean (SD)	62.2 (23.2)	64.0 (28.2)	0.83
FVC in L, mean (SD)	3.8 (1.1)	3.5 (1.5)	0.50
FEF _{25-75%} , mean (SD)	1.7 (1.5)	1.8 (1.7)	0.84

^a p-values obtained from t-tests with unequal variances (continuous variables) or from chi-squared tests (categorical variables)

^b Total density of *P. aeruginosa* was calculated by summing CFU/g for all *P. aeruginosa* morphotypes isolated, and then transforming the data to the logarithm₁₀ (log₁₀) value.

^c Baseline lung function data were available for 18 subjects in the BIC group and 17 subjects in the MIC group. FVC = forced vital capacity; FEF₂₅₋₇₅ = forced expiratory flow between 25% and 75% of FVC.

Table 2

Antibiotic susceptibilities of *P. aeruginosa* isolated at screening from subsequently randomized subjects.

	Achievable serum conc mg/L	Biofilm susceptibilities, mg/L N = 69 isolates ^a		Planktonic susceptibilities, mg/L N = 69 isolates ^b	
		BIC ₅₀ (BIQ ₅₀) ^b	BIC ₉₀ (BIQ ₉₀) ^b	MIC ₅₀ (MIQ ₅₀) ^b	MIC ₉₀ (MIQ ₉₀) ^b
Amikacin	16	32 (0.5)	128 (0.125)	16 (1)	128 (0.125)
Azithromycin	2	>16 (<0.125)	>16 (<0.125)	>16 (<0.125)	>16 (<0.125)
Ceftazidime	8	16 (0.5)	>64 (<0.125)	2 (4)	32 (0.25)
Ciprofloxacin	1	1 (1)	8 (0.125)	1 (1)	4 (0.25)
Meropenem	4	1 (4)	16 (0.25)	0.25 (16)	4 (1)
Piperacillin/tazobactam	64	32 (2)	>512 (<0.125)	2 (32)	128 (0.5)
Ticarcillin/clavulanate	64	32 (2)	>512 (<0.125)	16 (4)	256 (0.25)
Tobramycin	4	4 (1)	16 (0.25)	2 (2)	32 (0.125)

^aNumber of isolates with BICs tested when different from reported total: meropenem (N=68), piperacillin/tazobactam (N=68), and ciprofloxacin (N=67).

^bBIC₅₀ was defined as the 50th percentile of BIC values, with MIC₅₀ similarly defined for MIC values. BIC₉₀ was defined as the 90th percentile of BIC values, with MIC₉₀ similarly defined for MIC values. BIQ₅₀ was defined as achievable serum concentration divided by the BIC₅₀. BIQ₉₀ was defined as achievable serum concentration divided by the BIC₉₀. MIQ₅₀ and MIQ₉₀ are similarly defined relative to MIC₅₀ and MIC₉₀ values.

Table 3

Antibiotic regimens by study group.

	BIC Group (N = 20)	MIC Group (N = 19)	TOTAL (N = 39)
Ceftazidime / Amikacin	0	1 (5.3%)	1 (2.6%)
Ciprofloxacin / Azithromycin	1 (5.0%)	0	1 (2.6%)
Meropenem / Amikacin	1 (5.0%)	0	1 (2.6%)
Meropenem / Ciprofloxacin	5 (25.0%)	5 (26.3%)	10 (25.6%)
Meropenem / Tobramycin	5 (25.0%)	4 (21.0%)	9 (23.1%)
Piperacillin-Tazobactam / Amikacin	1 (5.0%)	0	1 (2.6%)
Piperacillin-Tazobactam / Azithromycin	1 (5.0%)	0	1 (2.6%)
Piperacillin-Tazobactam / Ciprofloxacin	0	2 (10.5%)	2 (5.1%)
Piperacillin-Tazobactam / Tobramycin	2 (10.0%)	4 (21.0%)	6 (15.4%)
Ticarcillin-Clavulanate / Ciprofloxacin	2 (10.0%)	2 (10.5%)	4 (10.3%)
Ticarcillin-Clavulanate / Tobramycin	1 (5.0%)	0	1 (2.6%)
Tobramycin / Ciprofloxacin	1 (5.0%)	1 (5.3%)	2 (5.1%)

Table 4Changes in *Pseudomonas* sputum density and FEV₁, by study group.

	BIC group	MIC group	Mean difference between groups, adjusted for baseline (95% CI)	p-value
Change in <i>P. aeruginosa</i> sputum density (log₁₀ CFU/g)^a				
Subjects with follow-up data, n	17	14		
Baseline density, mean (SD)	7.26 (1.04)	7.68 (1.20)		
Follow-up density, mean (SD)	4.32 (3.04)	4.42 (3.17)		
Change in density, mean (SD)	-2.94 (2.83)	-3.27 (3.09)	0.28 (-1.98, 2.54)	0.80
Change in FEV₁ (L)				
Subjects with follow-up data, n	18	16		
Baseline FEV ₁ , mean (SD)	2.47 (1.06)	2.39 (1.39)		
Follow-up FEV ₁ , mean (SD)	2.66 (1.07)	2.51 (1.35)		
Change in FEV ₁ , mean (SD)	0.18 (0.20)	0.12 (0.22)	0.07 (-0.08, 0.22)	0.34

Samples with a total density of zero were assigned a value of 1 so that the transformed (log₁₀) value would be equal to zero.

^aTotal density of *P. aeruginosa* was calculated by summing CFU/g for all *P. aeruginosa* morphotypes isolated, and then transforming the data to the logarithmic (log₁₀) value.