Antibiotic Susceptibilities of *Pseudomonas aeruginosa* Isolates Derived from Patients with Cystic Fibrosis under Aerobic, Anaerobic, and Biofilm Conditions

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Recent studies have determined that *Pseudomonas aeruginosa* can live in a biofilm mode within hypoxic mucus in the airways of patients with cystic fibrosis (CF). *P. aeruginosa* grown under anaerobic and biofilm conditions may better approximate *in vivo* growth conditions in the CF airways, and combination antibiotic susceptibility testing of anaerobically and biofilm-grown isolates may be more relevant than traditional susceptibility testing under planktonic aerobic conditions. We tested 16 multidrug-resistant isolates of *P. aeruginosa* derived from CF patients using multiple combination bactericidal testing to compare the efficacies of double and triple antibiotic combinations against the isolates grown under traditional aerobic planktonic conditions, in planktonic anaerobic conditions, and in biofilm mode. Both anaerobically grown and biofilm-grown bacteria were significantly less susceptible ($P < 0.01$) to single and combination antibiotics than corresponding aerobic planktonically grown isolates. Furthermore, the antibiotic combinations that were bactericidal under anaerobic conditions were often different from those that were bactericidal against the same organisms grown as biofilms. The most effective combinations under all conditions were colistin (tested at concentrations suitable for nebulization) either alone or in combination with tobramycin ($10 \mu\text{g ml}^{-1}$), followed by meropenem combined with tobramycin or ciprofloxacin. The findings of this study illustrate that antibiotic sensitivities are dependent on culture conditions and highlight the complexities of choosing appropriate combination therapy for multidrug-resistant *P. aeruginosa* in the CF lung.

Infection of the respiratory tract by *Pseudomonas aeruginosa* is the major cause of morbidity and mortality in adult patients with cystic fibrosis (CF) (15). Recent advances in antimicrobial therapy against lung pathogens have dramatically contributed to increased life expectancy of CF patients. However, frequent and prolonged antibiotic courses are likely to be a major factor in the selection of highly antibiotic-resistant *P. aeruginosa* strains.

Dual antibiotic treatment (usually with a β -lactam and an aminoglycoside) is now standard for the intravenous treatment of CF lung exacerbations, and combination therapy is increasingly utilized for out-of-hospital maintenance (15). However, selection of effective combination therapy can present many challenges. Conventional antibiotic susceptibility testing evaluates the efficacy of single agents against planktonic cultures of *P. aeruginosa* under aerobic conditions. This approach is unsuitable for the treatment of chronic CF lung infection for several reasons. In the first instance, many isolates are resistant to most if not all suitable antibiotics. Second, it has been shown that *P. aeruginosa* deposited on thickened mucus can penetrate into steep oxygen gradients within the mucus plaques of intraluminal material of the CF airway. The proliferation of the

organism under these conditions generates an anaerobic environment (13, 33) which reduces the efficacy of most antipseudomonal antibiotics. Third, in addition to its planktonic lifestyle, *P. aeruginosa* forms dense communities encased in an exopolysaccharide matrix (5, 28, 33, 34). MICs and minimal bactericidal concentrations can be 100 to 1,000 times greater in these biofilms than for the equivalent planktonic population (4, 14, 19). The long-term persistence of *P. aeruginosa* in the CF lung is likely to reflect genetic mechanisms of drug resistance, decreased efficacy of antibiotics under anaerobic conditions, and biofilm-specific resistance mechanisms.

In the absence of a suitable system for evaluating susceptibilities of isolates to antibiotics under conditions approaching those *in vivo*, selection of combination therapy is generally made empirically. This approach can result in loss of antibiotic efficacy due to antagonism, undesirable side effects, and emergence of further resistance. Multiple combination bactericidal testing (MCBT) allows simultaneous testing of the efficacy of double and triple combinations of antibiotics, providing a more rational basis for the treatment of multiresistant infections (1, 17). MCBT performed on aerobic, planktonic cultures is currently available to guide therapy for CF and non-CF patients in North America, in several European centers, and in eastern Australia (1, 17, 22). An international multicenter randomized controlled trial to evaluate the clinical effectiveness of MCBT is in progress.

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Adaptation of MCBT for biofilm cultures has shown that *P. aeruginosa* is significantly less susceptible to double and triple drug combinations of antibiotics than planktonically grown strains (2). As far as we are aware, the efficacy of combinations of antibiotics against *P. aeruginosa* has not been tested under anaerobic conditions.

In this study, the MICs of antibiotics for multidrug-resistant *P. aeruginosa* from CF sputa grown planktonically under aerobic conditions were first determined. MCBT was then used to compare the efficacies of combinations of antibiotics against the isolates grown planktonically under anaerobic conditions and in biofilm mode with those using conventional aerobic planktonic cultures. We tested the hypothesis that among isolates of multidrug-resistant *P. aeruginosa*, culture conditions influence the probability of detecting bactericidal activity of antibiotic combinations.

MATERIALS AND METHODS

Investigations were carried out on 16 multidrug-resistant *P. aeruginosa* isolates from the sputa of 16 CF patients between 2002 and 2003; 8 were treated at Royal Prince Alfred Hospital, Sydney, and 8 at The Prince Charles Hospital, Brisbane. Isolates were identified as *P. aeruginosa* by growth on ceftrimide fucidin cephaloridine agar (Oxoid), oxidase-positive reaction (MedVet Science), growth on Columbia horse blood agar (HBA; Oxoid) at 42°C, and resistance to 9-chloro-9-[4-diethylaminophenyl]-10-phenylacridan (Dutec Diagnostics). Multidrug resistance is defined as resistance to all of the drugs in at least two of the following three classes: beta lactams, aminoglycosides, and quinolones (6).

Antibiotics. The 10 antibiotics used in the study were potentially useful both for out-of-hospital maintenance and for inpatient treatment of pulmonary exacerbations. Concentrations used in the MCBT for the following antibiotics were based on average peak serum levels one hour after standard single-dose intravenous or oral administration (20): amikacin, tobramycin, and azithromycin (Pfizer) (32 $\mu\text{g ml}^{-1}$, 10 $\mu\text{g ml}^{-1}$ and 0.4 $\mu\text{g ml}^{-1}$, respectively); cefepime (Bristol-Myers Squibb) (32 $\mu\text{g ml}^{-1}$); ceftazidime and ticarcillin-clavulanate (Timentin) (GlaxoSmithKline) (32 $\mu\text{g ml}^{-1}$ and 32/2 $\mu\text{g ml}^{-1}$, respectively); ciprofloxacin (Bayer) (2 $\mu\text{g ml}^{-1}$); cotrimoxazole (trimethoprim-sulfamethoxazole) (Roche) (10/2 $\mu\text{g ml}^{-1}$); and meropenem (Astra Zeneca) (32 $\mu\text{g ml}^{-1}$). Azithromycin was included despite acknowledged inactivity against planktonically grown *Pseudomonas* because of several reports of the potential role of macrolides in CF patients (11, 25, 32). Tobramycin and colistin (Sigma Pharmaceuticals, St. Louis, Mo.) were tested at levels suitable for nebulized use (200 $\mu\text{g ml}^{-1}$ [10] and 32 $\mu\text{g ml}^{-1}$, respectively).

MIC. Susceptibility testing for all single antibiotics except azithromycin and cotrimoxazole was performed on aerobic, planktonic cultures using the broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (31). The breakpoint of 2 μg for colistin was based on that described for polymyxin E and on assays using the ATCC reference strain *P. aeruginosa* 27853. There have been no clinical studies to correlate the local concentration of colistin to MICs, and colistin concentrations in sputum are difficult to determine, since the inhaled agent is the prodrug methanesulphonate rather than colistin itself (27).

MCBT. The bactericidal activities of nine single antibiotics (tobramycin was tested at 10 $\mu\text{g ml}^{-1}$ and 200 $\mu\text{g ml}^{-1}$ and azithromycin and cotrimoxazole were not tested as single agents) and 22 double and 15 triple combinations were tested in Mueller-Hinton II cation adjusted broth (MHB II) (Becton Dickinson, Microbiology Systems, Cockeysville, MD) according to our previously described protocols (1). All planktonic and biofilm tests were performed in triplicate at 35°C in 96-well round-bottomed microtiter plates (Greiner Bio-one). Antibiotics were added sequentially to the wells prior to the addition of the bacteria. The final inoculum in each well was approximately 5×10^5 CFU.

Aerobic planktonic MCBT. The plates were examined for turbidity at 24 and 48 h using a plate reader (Titertek). The contents of nonturbid wells at 24 h were subcultured onto HBA. Following incubation at 35°C for 24 h, the plates were examined for 99.9% kill. Growth and sterility controls (no antibiotics and no organism inoculum, respectively) were run in each MCBT.

Anaerobic planktonic MCBT. *P. aeruginosa* grows under anaerobic conditions in the CF airways using either nitrate or nitrite as a terminal electron acceptor (33). Our preliminary experiments determined that *P. aeruginosa* grew in MHB

II anaerobically without added nitrates to at least 10^8 CFU ml^{-1} within 48 h. These concentrations were suitable for use as test inocula. The MHB II used to dilute the inoculum to the required concentration was placed under anaerobic conditions in an anaerobic jar for 2 h prior to use to reduce the oxygen tension. Since growth rates for *P. aeruginosa* were found to be slower under anaerobic than aerobic conditions, the MCBT plates were read and nonturbid wells plated onto HBA to determine 99.9% kill at 48 h rather than at 24 h as described for the aerobic cultures.

Biofilm MCBT. Biofilms were established using a modification of the Calgary biofilm device (4) as described by Aaron et al. (2). This technique involved the generation of biofilms on plastic pegs attached to the lids of 96-well microtiter plates. All plates were incubated aerobically at 35°C for 18 h. The growth of biofilms on the pegs was confirmed by scanning electron microscopy. Preliminary experiments showed that the number of biofilm-forming bacteria across different isolates ranged from 10^4 to 10^6 , which falls within published limits (24). A rocking table was used to produce shear forces across each peg. Following exposure to the antibiotics overnight at 35°C, biofilms were removed from each peg using ultrasonication into a corresponding sterile broth well. The viability of biofilm bacteria was assessed by incubating the plates at 35°C for 24 h and then examining them for turbidity in the well using a plate reader. This does not guarantee 100% kill of the biofilm, since there may be a few "persister" bacteria in the wells (30), but not enough to cause turbidity. Thus, for planktonic cultures bactericidal effect was assessed as defined by NCCLS guidelines for planktonic cultures (31), and for biofilms bactericidal effect was evaluated as defined by Ceri et al. (4).

Statistical analysis. The study hypothesis was tested by comparing the number of antibiotic combinations that were found to be bactericidal in each isolate under the three alternative culture conditions. This was tested by repeated measures analysis of variance in which the dependent variable was the number of antibiotic combinations demonstrating bactericidal activity for each isolate and the within-subject (repeated) variable was the culture condition. The analysis was implemented using SPSS (version 11.5) software.

RESULTS

The mean age of the 16 CF patients who provided isolates was 22.2 years, and the male-to-female ratio was 12:4. Patients had been chronically infected with *P. aeruginosa* for a minimum of 4 years. Ten of the 16 isolates were mucoid.

MIC testing. The 16 isolates were all multiresistant based on individual antibiotic MIC testing. Over 75% of the isolates tested were resistant to tobramycin, cefepime, ceftazidime, ticarcillin-clavulanate, and cotrimoxazole at NCCLS breakpoints. None of the isolates was sensitive to amikacin or ciprofloxacin. Fifty percent of the isolates were sensitive to colistin at the chosen breakpoint of 2 $\mu\text{g ml}^{-1}$, and 25% were sensitive to ticarcillin-clavulanate and meropenem.

MCBT. Single antibiotic susceptibilities. Antibiotics were tested singly to allow assessments of antagonism. Colistin (32 $\mu\text{g ml}^{-1}$) was bactericidal most often, killing all 16 (100%), 12 of 16 (75%), and 4 of 16 (25%) of isolates tested under aerobic, anaerobic, and biofilm conditions, respectively (Fig. 1). Meropenem, the second-most-effective agent, was bactericidal against 11 (69%) of isolates aerobically and 5 (31%) of isolates anaerobically, but under biofilm conditions this figure dropped to 3 (19%). Amikacin was not bactericidal for any isolate under any test conditions. Among other antibiotics, ticarcillin-clavulanate, cefepime, ceftazidime, and tobramycin (10 $\mu\text{g ml}^{-1}$) were not bactericidal against any isolates under anaerobic conditions, while ceftazidime was not effective against any isolate in biofilm mode.

In all, 37 double and triple antibiotic combinations were tested. The number of combinations of antibiotics that demonstrated bactericidal activity varied depending upon culture conditions. The mean number of combinations bactericidal for the 16 isolates was highest under aerobic conditions (mean =

FIG. 1. The percentages of isolates for which each single antibiotic was bactericidal under aerobic, anaerobic, and biofilm conditions are shown. Col, colistin ($32 \mu\text{g ml}^{-1}$); Mem, meropenem ($32 \mu\text{g ml}^{-1}$); T200, tobramycin ($200 \mu\text{g ml}^{-1}$); Tim, ticarcillin-clavulanate ($32/2 \mu\text{g ml}^{-1}$); Cip, ciprofloxacin ($2 \mu\text{g ml}^{-1}$); Cef, cefepime ($32 \mu\text{g ml}^{-1}$); Caz, ceftazidime ($32 \mu\text{g ml}^{-1}$); T10, tobramycin ($10 \mu\text{g ml}^{-1}$); Amk, amikacin ($32 \mu\text{g ml}^{-1}$).

25/37), and this was significantly more than the number of bactericidal combinations under anaerobic conditions (mean = 14/37; $P < 0.0001$) and under biofilm conditions (mean = 13/37; $P < 0.0001$). There was no significant difference in the mean number of bactericidal combinations between anaerobic and biofilm conditions. As shown in Table 1 and Fig. 2, the antibiotic combinations that were bactericidal under anaerobic conditions were markedly different from those that were effective against biofilm growth. None of the 16 isolates showed the same susceptibilities to combinations of antibiotics under aerobic, anaerobic, or biofilm conditions. There was no association between mucoidy and bactericidal activity.

Susceptibilities to double antibiotic combinations. Among the 22 double antibiotic combinations, those containing colistin were bactericidal against most isolates under the three growth conditions (Table 1). Of the double combinations that did not contain colistin, meropenem-tobramycin ($10 \mu\text{g ml}^{-1}$) and meropenem-ciprofloxacin were most effective under the three growth conditions. Double combinations containing tobramycin ($10 \mu\text{g ml}^{-1}$) or amikacin or cotrimoxazole were most adversely affected by anaerobic conditions. Only one combination, azithromycin-cefepime, showed no bactericidal activity against any isolates under any conditions. Under anaerobic conditions, amikacin-ticarcillin-clavulanate, tobramycin ($10 \mu\text{g ml}^{-1}$)-cotrimoxazole, and azithromycin-ticarcillin-clavulanate were not bactericidal against any isolates. Under biofilm conditions, cotrimoxazole-ciprofloxacin and cotrimoxazole-tobramycin ($10 \mu\text{g ml}^{-1}$) were not bactericidal against any isolates.

Susceptibilities of triple antibiotic combinations. Among the 15 triple combinations, those containing both meropenem and tobramycin ($10 \mu\text{g ml}^{-1}$) were bactericidal against the highest percentage of isolates under all conditions (Fig. 2). Triple combinations of meropenem plus tobramycin ($10 \mu\text{g ml}^{-1}$), together with cefepime, ciprofloxacin, or ticarcillin-clavulanate, were bactericidal against 100% of isolates tested under aerobic conditions.

TABLE 1. Percentage of isolates for which the double antibiotic combination was bactericidal in aerobic, anaerobic, or biofilm MCBT

Antibiotic combination ^a	% Isolates susceptible		
	Aerobic	Anaerobic	Biofilm
Col, Cip	100	81	81.3
Col, Sxt	100	81.3	81.3
Col, Caz	100	93.8	75
Col, Azm	94	75	75
Mem, T10	93.8	56.3	43.8
Mem, Cip	87.5	68.8	50
Mem, Sxt	81.3	18.8	12.5
Mem, Caz	68.8	43.8	25
Mem, Azm	62.5	31.3	18.8
T10, Caz	62.5	18.8	6.3
T10, Tim	62.5	6.3	12.5
T10, T/S	62.5	37.5	50
T200, Cip	56.3	43.8	43.8
T200, Azm	50	25	25
T10, Cef	50	12.5	18.8
Amk, Cip	43.8	12.5	6.3
T10, Cip	37.5	12.5	12.5
Amk, Tim	37.5	0	6.3
Sxt, Cip	25	6.3	0
T10, Sxt	18.8	0	0
Azm, Tim	18.8	0	12.5
Azm, Cef	0	0	0

^a Col, colistin ($32 \mu\text{g ml}^{-1}$); Mem, meropenem ($32 \mu\text{g ml}^{-1}$); T200, tobramycin ($200 \mu\text{g ml}^{-1}$); Tim, ticarcillin-clavulanate ($32/2 \mu\text{g ml}^{-1}$); Cip, ciprofloxacin ($2 \mu\text{g ml}^{-1}$); Cef, cefepime ($32 \mu\text{g ml}^{-1}$); Caz, ceftazidime ($32 \mu\text{g ml}^{-1}$); T10, tobramycin ($10 \mu\text{g ml}^{-1}$); Amk, amikacin ($32 \mu\text{g ml}^{-1}$); Sxt, cotrimoxazole ($10/2 \mu\text{g ml}^{-1}$); Azm, azithromycin ($0.4 \mu\text{g ml}^{-1}$).

meropenem, and cotrimoxazole showed growth. Similarly, tobramycin ($10 \mu\text{g ml}^{-1}$)-ciprofloxacin-ticarcillin-clavulanate showed antagonism for three of the isolates. Under anaerobic conditions, antagonism occurred in three isolates for each of the meropenem-cotrimoxazole, ciprofloxacin-cotrimoxazole, and tobramycin ($200 \mu\text{g ml}^{-1}$)-azithromycin-ciprofloxacin combinations. Under biofilm conditions, tobramycin ($200 \mu\text{g ml}^{-1}$)-azithromycin was antagonistic for 19% of isolates tested. Some combinations, e.g., meropenem-ceftazidime, were bactericidal against one particular isolate and antagonistic for another.

DISCUSSION

As far as we are aware, this is the first report of the efficacy of antibiotic combinations against *P. aeruginosa* grown under

FIG. 2. The percentages of isolates bactericidal for 11 triple combinations of antibiotics under aerobic, anaerobic, and biofilm conditions are shown. Azm, azithromycin ($0.4 \mu\text{g ml}^{-1}$); Sxt, cotrimoxazole (trimethoprim-sulfamethoxazole) ($10/2 \mu\text{g ml}^{-1}$). Other abbreviations are as defined in the legend to Fig. 1.

anaerobic conditions. Our findings also extend previous studies using MCBT for CF *P. aeruginosa* grown under aerobic planktonic conditions and in biofilm mode by incorporating colistin combinations into the protocols. The emergence of high levels of resistance to many antipseudomonal antibiotics has seen a resurgence of interest in nebulized colistin for CF lung infection.

The increased resistance of *P. aeruginosa* to antibiotics in advanced CF lung disease has been attributed to genetic mechanisms including the emergence of hypermutable *P. aeruginosa* (23), the generation of biofilms, and exposure of the organisms to an environment which is anaerobic, acidic, and nutrient depleted (13). It is believed that *P. aeruginosa* is able to grow under anaerobic conditions in CF lung sputum using NO_3 , NO_2 , or nitrous oxide as a terminal electron acceptor. Anaerobic growth has been achieved in vitro by the addition of nitrates to conventional media (33). However, in this study MHB II supported the anaerobic growth of all isolates to 10^8 CFU ml^{-1} within 48 h without supplementation. It is assumed that MHB II contains sufficient nitrates for anaerobic growth. The major ingredient of MHBII, beef extract, is the likely source.

The first-line antipseudomonal antibiotics, the beta-lactams, aminoglycosides, and fluoroquinolones, act along different pathways, but all are most effective against rapidly dividing bacteria. Theories that these agents would be less effective

against *P. aeruginosa* growing more slowly in the static, anaerobic mucus in the CF lung are supported by the results of this study. Various other processes are known to underpin the adverse effect of anaerobic respiration on the efficacy of antibiotics, e.g., the activity of aminoglycosides is dependent on internalization by specific transport mechanisms requiring oxidative phosphorylation (13). In one recent study it was reported that addition of nitrate decreased susceptibilities of the organisms to antibiotics, suggesting that local oxygen deficiencies and the presence of nitrate contribute to the reduced susceptibilities (3).

There is only limited literature addressing the susceptibilities of *P. aeruginosa* grown anaerobically to antimicrobial agents. Davey and colleagues reported that the minimal bactericidal concentration of ciprofloxacin for *P. aeruginosa* under anaerobic conditions was only slightly raised, while that for gentamicin was raised eightfold (7). In the current study all antibiotics were found to be less effective under anaerobic and biofilm conditions than under aerobic conditions. Colistin and meropenem were the most effective single agents under all conditions; at the other extreme amikacin was ineffective against all isolates under all conditions. Interestingly, there was no clear correlation between the class of drug or mode of growth and bactericidal activity. Colistin, meropenem, and ciprofloxacin were bactericidal against more isolates under anaerobic conditions than in biofilm mode, whereas tobramycin, ticarcillin-clavulanate, and ceftazidime were more effective under biofilm than anaerobic conditions. Resistance to colistin has rarely been reported even in the face of the selection pressure of a daily dose by inhalation (8). Although the efficacy of colistin was markedly diminished when tested under anaerobic conditions, it remained the most effective single agent. Meropenem had bactericidal activity against more isolates than high-dose tobramycin under planktonic aerobic conditions. These two agents, however, were equally effective under anaerobic conditions, and high-dose tobramycin was bactericidal for more isolates under biofilm conditions. Notably, three of the four most effective dual combinations under all conditions (colistin with ciprofloxacin or cotrimoxazole or azithromycin) do not involve parenteral administration or the need for hospitalization and are therefore potentially useful for out-of-hospital maintenance.

It was interesting that the proportion of *P. aeruginosa* isolates susceptible to single and combination antibiotics under anaerobic conditions was not significantly different from that in the biofilm mode of growth. Since colistin was effective against all isolates as a single agent, it was not surprising that colistin combinations were the most effective under all conditions. Aside from colistin, combinations containing meropenem were the most effective under aerobic as well as anaerobic and biofilm conditions.

Biofilm-specific resistance is now accepted as a complex physiological process. It has been reported that *P. aeruginosa* responds to anaerobic conditions by increased production of alginate, which poses a physical barrier to antibiotics (33). The adsorption of positively charged antibiotics, such as the aminoglycosides, to the negatively charged alginate polymers also retards penetration (12, 21). Hypoxic conditions within the deeper layers of the biofilm and the overexpression of efflux pumps combined with the slower growth rates in anaerobic and

biofilm cultures are also likely to contribute to resistance. It has been reported that antibiotic-resistant phenotypic variants of *P. aeruginosa* with an enhanced ability to form biofilms arise at high frequency both in vitro and in the lungs of CF patients (9). There is also evidence that the bacteria residing within biofilms employ other genetic mechanisms to resist the action of antibiotics. Mah and colleagues recently proposed a model wherein periplasmic glucans bind to and sequester antibiotics including tobramycin (18). Tolerance to antibiotics in biofilm cultures has also been attributed to the presence of "persister" cells (30), although the underlying mechanisms are unknown. Our data also provide further evidence that the use of antagonistic combinations may contribute to the persistence of infection.

There is evidence to suggest that the apparent activity of macrolides against *P. aeruginosa* in vivo may relate to its anti-inflammatory activity and/or modulation of the production of alginate or other virulence factors including elastase and rhamnolipid by quorum-sensing systems. These quorum-sensing systems are activated under stationary phase and under anaerobic conditions (16). The effect may also be due to a sub-MIC effect that interferes with the biofilm matrix. Macrolides paired with other agents have shown synergistic activity against multidrug-resistant, planktonically grown *P. aeruginosa* (1, 26). Surprisingly, in this study, antibiotic combinations containing azithromycin proved to be more effective against aerobic, planktonically grown isolates than against the corresponding isolates grown under anaerobic conditions or in biofilms. Furthermore, among anaerobically or biofilm-grown isolates, antagonism was observed in up to 19% of combinations containing azithromycin. This finding may have implications for the widespread use of azithromycin in the CF population (29).

Increased evidence indicating that the CF lung contains *P. aeruginosa* living in both planktonic and biofilm mode in conditions varying from aerobic through hypoxic to anaerobic has important implications for selection of optimal antibiotic combinations, particularly since the relative size of each population is not known. There is evidence from this study that antibiotic combinations bactericidal under aerobic or anaerobic planktonic conditions in vitro are not necessarily bactericidal against organisms growing in biofilm mode and may even be antagonistic or vice versa. It is also possible that anaerobic cultures and biofilms generated in vitro do not accurately reflect the hostile environment of the CF lung and/or that the concentrations of antibiotics used in our study may not reflect the concentrations found in CF airway mucus. Eradication of *P. aeruginosa* from the chronically infected CF lung is rarely achieved and may not be a realistic goal using current antimicrobials. Nonetheless, selections of antibiotic combinations that are commonly bactericidal against clinical isolates grown under aerobic planktonic, anaerobic planktonic, and biofilm conditions may prove to be more effective in reducing bacterial load. Clinical trials will be needed to determine whether this approach leads to improvements in lung function and thus quality and longevity of life for CF patients.

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