

Antimicrobial Susceptibility and Synergy Studies of *Burkholderia cepacia* Complex Isolated from Patients with Cystic Fibrosis[∇]

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Susceptibility (18 antimicrobial agents including high-dose tobramycin) and checkerboard synergy (23 combinations) studies were performed for 2,621 strains of *Burkholderia cepacia* complex isolated from 1,257 cystic fibrosis patients. Minocycline, meropenem, and ceftazidime were the most active, inhibiting 38%, 26%, and 23% of strains, respectively. Synergy was rarely noted (range, 1% to 15% of strains per antibiotic combination).

Since the late 1970s, *Burkholderia cepacia* complex bacteria have been recognized as particularly virulent pathogens in cystic fibrosis (CF) (24, 40). Infection with *B. cepacia* complex can be associated with a rapid decline in lung function and markedly shorter median survival (9, 26, 31, 40). Currently, *B. cepacia* complex bacteria are isolated from 3% to 4% of CF patients in the United States (10–17). *B. cepacia* complex bacteria are multidrug resistant due to innate and acquired mechanisms of resistance (1, 5, 6, 23, 30), and the susceptibility profiles of strains from CF patients may differ from those noted in strains from non-CF patients, presumably since CF patients receive multiple courses of oral, intravenous, and aerosolized antibiotics (20, 22, 29, 35, 41). However, there have been few recent surveys of the antimicrobial susceptibility patterns of these microorganisms in CF patients, particularly in an era when selective media for *Burkholderia* are widely used in clinical microbiology laboratories (1, 5, 33).

We report the in vitro activity of antimicrobial agents against *B. cepacia* complex strains from patients with CF and the potential synergistic activity of these agents in combination as studied at the CF Referral Center for Susceptibility and Synergy Studies (<http://synergy.columbia.edu/>) at Columbia University (36, 37, 39). The Institutional Review Board of Columbia University approved this study.

From 1996 to 2004, the CF Referral Center received an average of 291 isolates (range, 119 to 408) of *B. cepacia* complex per year. Overall, 2,621 *B. cepacia* complex strains from 1,257 patients (range, 1 to 17 strains per patient) from 150 CF care centers located in 46 states were processed. To assess the generalizability of this study's findings, the numbers of patients harboring *B. cepacia* complex that were reported to the U.S. CF Foundation Patient Registry annually (10–18) were compared to the numbers of patients whose *B. cepacia* complex isolates were processed at the CF Referral Center. We estimated that each year, isolates from 34% (range, 21% to 42%

per year) of CF patients with *B. cepacia* complex were processed.

Strains referred as *Burkholderia* spp. were plated within 24 h of receipt on Biplate media (Remel, Lenexa, KS) for purity and oxidative-fermentative-polymyxin B-bacitracin-lactose media (OFPBL; Remel, Lenexa, KS) to verify species phenotype (38). Plates were incubated at 35°C for 18 to 24 h, and if needed, slowly growing strains were reincubated for an additional 18 to 24 h.

Throughout the study period, susceptibility to 18 antimicrobial agents including high-dose tobramycin was determined by broth microdilution assay using frozen commercially prepared microtiter plates (Microtech Medical Systems, Inc., Aurora, CO). This assay has been endorsed by the Clinical and Laboratory Standards Institute (CLSI) to determine the antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from patients with CF (32). Susceptibility panels were incubated at 35°C for 18 h, and slowly growing strains were incubated for an additional 18 to 24 h. Susceptibility and synergy plates were placed on a mirrored surface, and turbidity was visualized using the microtiter test reading mirror (DYNEX Technologies, Chantilly, VA). CLSI interpretive criteria for susceptibility breakpoints for non-*Enterobacteriaceae* were used when breakpoints for *Burkholderia* spp. were unavailable (7, 8). Minocycline, meropenem, and ceftazidime were the most active and inhibited 38%, 26%, and 23% of strains, respectively (Table 1). High concentrations of tobramycin (256 µg/ml), such as could be achieved by aerosolization, inhibited 45% of strains. Overall, 18% (473/2,621) of strains were resistant to all the agents tested. No changes in susceptibility trends for the commonly used agents ceftazidime, minocycline, meropenem, or trimethoprim-sulfamethoxazole were noted during the study period (Fig. 1).

Checkerboard synergy testing of 23 pairs of antimicrobial agents was performed on frozen commercially prepared microtiter plates (Microtech Medical Systems, Inc., Aurora, CO) using the same inoculum as the susceptibility studies (Table 2). The fractional inhibitory concentration (FIC) was calculated as previously described (37). FIC of ≤0.5 was considered synergistic, FIC of >0.5 to ≤4.0 was considered nonsynergistic, and FIC of >4.0 was considered antagonistic (19, 25, 34). Clinically achievable concentrations were generally used in the synergy

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TABLE 1. Activity of selected antimicrobial agents against *Burkholderia cepacia* complex isolated from patients with cystic fibrosis, 1996 to 2004

Antimicrobial agent	No. of strains tested	MIC ($\mu\text{g/ml}$)			Strains tested (%)		
		Range tested	50%	90%	Susceptible ^a	Intermediate	Resistant
Ceftazidime ^b	2,621	1->128	32	128	23	12	65
Chloramphenicol ^c	2,405	2->64	64	>64	5	10	85
Doxycycline ^d	1,903	1->32	16	>32	22	17	61
Meropenem ^c	2,405	0.25->64	8	32	26	26	48
Minocycline ^c	2,405	1->32	8	32	38	25	37
Piperacillin ^b	2,621	4->256	256	>256	11	9	80
Piperacillin-tazobactam ^c	2,405	4->256	256	>256	16	9	75
Trimethoprim-sulfamethoxazole ^c	2,405	0.5->16	>16	>16	5	N/A ^e	95

^a Fewer than 10% of strains were inhibited by cefepime (8%), imipenem (7%), ciprofloxacin (5%), amikacin (4%), ticarcillin-clavulanic acid (3%), ampicillin-sulbactam (2%), aztreonam (2%), ticarcillin (2%), or rifampin (1%).

^b Used from January 1996 to December 2004.

^c Used from August 1997 to December 2004.

^d Used from October 1999 to December 2004.

^e N/A, not applicable.

panels. As a result, the range of concentrations tested in these panels was lower than the range of concentrations tested in the susceptibility panels. Thus, the precise FIC was 'indeterminate' for a given two agents if growth occurred in all the combination wells. Synergistic activity was rare (range, 1% to 15% of isolates per antibiotic combination) as was antagonistic activity

(range, 0% to 9% of isolates per antibiotic combination). In contrast, nonsynergistic or indeterminate activity was observed among 37% (range, 8% to 88%) and 55% (range, 2% to 87%) of isolates per combination, respectively.

This is the largest survey of antibiotic susceptibility of *B. cepacia* complex published to date. Although the CF Referral

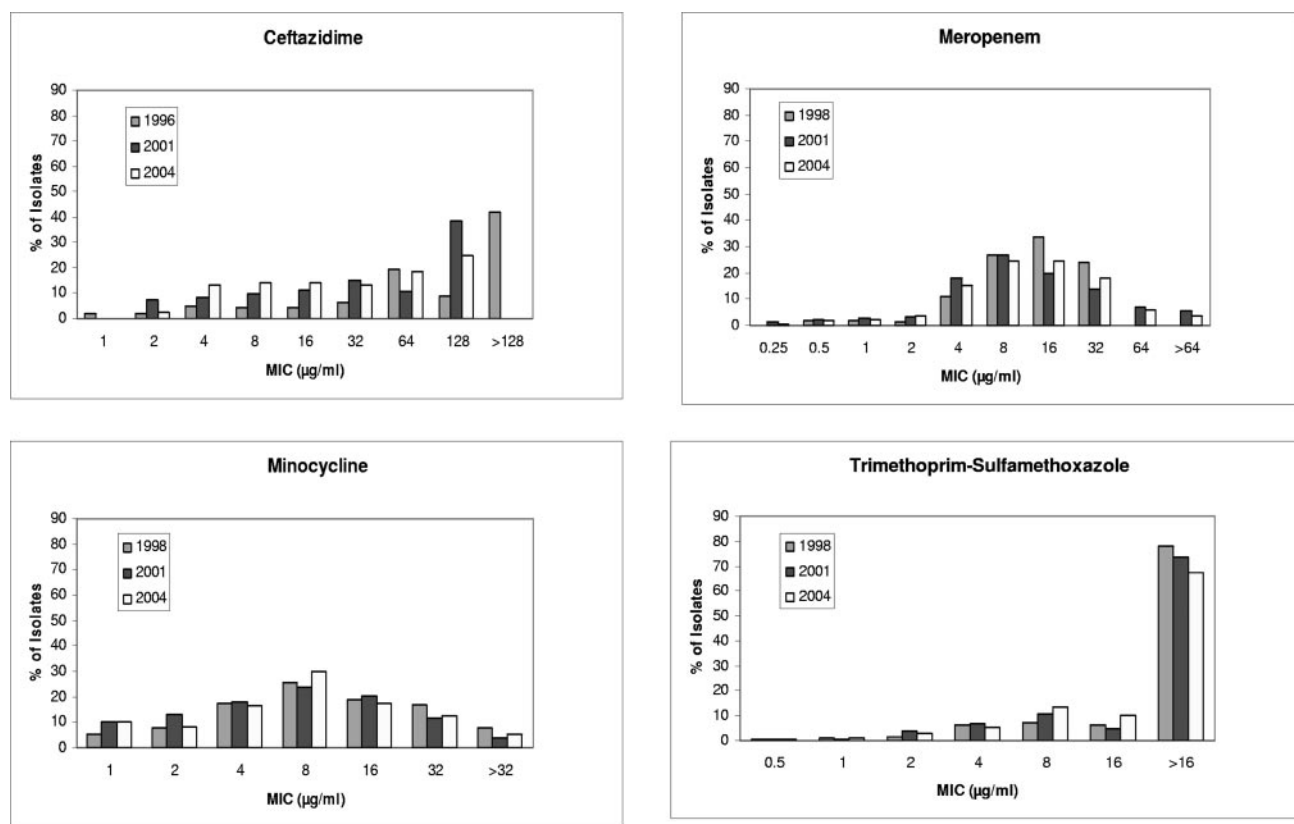


FIG. 1. The distributions of MICs for *Burkholderia cepacia* complex bacteria isolated from patients with CF in 1996 ($n = 187$), 1998 ($n = 239$), 2001 ($n = 382$), and 2004 ($n = 302$) are shown for the selected agents ceftazidime, meropenem, minocycline, and trimethoprim-sulfamethoxazole. The highest concentrations of antibiotics tested varied during the study period for some agents. For ceftazidime, the highest concentration tested in 1996 was 128 $\mu\text{g/ml}$ and in 2001 and 2004 the highest concentration tested was 64 $\mu\text{g/ml}$. Strains not inhibited by 128 $\mu\text{g/ml}$ are depicted as an MIC of >128 $\mu\text{g/ml}$. For meropenem, the highest concentration tested in 1998 was 16 $\mu\text{g/ml}$ and in 2001 and 2004 the highest concentration tested was 64 $\mu\text{g/ml}$. Strains that were not inhibited by 64 $\mu\text{g/ml}$ are depicted as an MIC of >64 $\mu\text{g/ml}$.

TABLE 2. Synergistic activity of pairs of antimicrobial agents against *Burkholderia cepacia* complex isolated from patients with cystic fibrosis, 1996 to 2004

Antimicrobial pair	No. of strains tested	Ranges tested ($\mu\text{g/ml}$)	Strains tested (%)			
			Synergistic	Nonsynergistic	Antagonistic	Indeterminate
Aztreonam + tobramycin ^a	216	4–32/1–8	6	10	2	82
Cefepime + chloramphenicol ^b	1,903	2–16/2–16	4	24	0	72
Ceftazidime + chloramphenicol ^c	2,405	2–16/2–16	10	48	1	41
Ceftazidime + trimethoprim-sulfamethoxazole ^c	2,405	2–16/0.25–2	6	38	2	54
Ceftazidime + amikacin ^a	216	4–128/4–32	15	45	7	33
Ceftazidime + tobramycin ^a	216	4–128/1–8	12	46	9	33
Chloramphenicol + ciprofloxacin ^b	1,903	2–16/0.25–2	1	17	0	82
Chloramphenicol + trimethoprim-sulfamethoxazole ^d	502	4–32/1–32	1	44	1	54
Ciprofloxacin + trimethoprim-sulfamethoxazole ^d	502	0.5–16/1–8	1	27	1	71
Doxycycline + chloramphenicol ^b	1,903	2–16/1–8	7	45	0	48
Doxycycline + trimethoprim-sulfamethoxazole ^b	1,903	1–8/0.5–4	1	43	0	56
Imipenem + ciprofloxacin ^e	718	0.5–16/0.5–4	5	29	1	65
Imipenem + tobramycin ^a	216	0.5–16/1–8	4	17	3	76
Imipenem + amikacin ^a	216	0.5–16/4–32	3	16	3	78
Meropenem + chloramphenicol ^b	1,903	1–8/2–16	4	54	2	40
Meropenem + ciprofloxacin ^c	2,405	1–8/0.5–2	4	57	2	37
Meropenem + doxycycline ^b	1,903	1–8/1–8	7	64	2	27
Meropenem + tobramycin ^b	1,903	1–8/1–8	3	51	2	44
Meropenem + trimethoprim-sulfamethoxazole ^b	1,903	1–8/0.25–2	4	44	2	50
Minocycline + chloramphenicol ^d	502	1–32/4–32	9	88	1	2
Piperacillin + ciprofloxacin ^f	2,621	8–64/0.25–2	10	27	1	62
Piperacillin + tobramycin ^a	216	8–64/1–8	9	19	0	72
Ticarcillin-clavulanate + tobramycin ^a	216	8–64/1–8	5	8	0	87

^a Used from January 1996 to August 1997.

^b Used from October 1999 to December 2004.

^c Used from August 1997 to December 2004.

^d Used from August 1997 to October 1999.

^e Used from January 1996 to October 1999.

^f Used from January 1996 to December 2004.

Center solicits multidrug-resistant organisms, these results may be broadly generalizable to the CF patient population in the United States, as approximately one-third of CF patients harboring *Burkholderia* spp. were studied.

Treatment options for *B. cepacia* complex remain limited. The CLSI recommends testing susceptibility to ticarcillin plus clavulanic acid, ceftazidime, meropenem, minocycline, levofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole (8). Similarly, treatment recommendations for *B. cepacia* complex include meropenem, ciprofloxacin, minocycline, trimethoprim-sulfamethoxazole, and chloramphenicol (21, 27). However, in this study of isolates from CF patients, only 3% to 38% of strains were susceptible to these agents. Somewhat surprisingly, the relative proportion of strains resistant to the commonly used agents ceftazidime, meropenem, minocycline, and trimethoprim-sulfamethoxazole did not change during the 9 years of the study. This observation may reflect the solicitation of multidrug-resistant strains by the CF Referral Center coupled with the extensive use of these agents in the CF population throughout the study period.

Aaron et al. have published in vitro synergy studies for *B. cepacia* complex isolated from CF patients using the multiple combination bactericidal test (MCBT) (1). In contrast to the synergy methods described in our study, the MCBT methodology tests the activity of peak serum concentrations of antimicrobial agents. In the MCBT assays, meropenem combined with minocycline, amikacin, or ceftazidime was bactericidal against 76%, 73%, and 73% of isolates, respectively. In addition, triple antibiotic combinations such as tobramycin, mero-

penem, plus another agent were bactericidal against 81% to 93% of isolates. Unlike the MCBT method, the checkerboard assay used in our study demonstrated few synergistic combinations. These observations may reflect differences in the methods used, including the concentrations of antibiotics tested and/or the use of inhibitory versus bactericidal activity. Differences in the isolates studied, the patient populations, or agents tested may also have contributed to the differing results.

Although two agents are generally recommended for treatment of a CF pulmonary exacerbation (20), there are relatively few published reports describing treatment of *B. cepacia* complex in CF patients. Blumer et al. studied the safety and efficacy of meropenem and tobramycin in 14 subjects with *B. cepacia* complex (4). A reduction in bacterial density ($-1.8 \log_{10}$, $P = 0.02$) was noted, but improvement in lung function was not reported in this subgroup. Aaron et al. explored the efficacy of treatment guided by MCBT versus treatment guided by conventional susceptibility testing in 132 patients including 54 infected with *B. cepacia* complex (2). While subjects in both groups clinically improved, no differences in response to treatment, defined as the time to the next pulmonary exacerbation, or improvement in lung function were noted.

There were some limitations to our study. We studied multidrug-resistant organisms, which could overestimate the relative proportion of resistant *B. cepacia* complex strains in CF patients. We do not know the genomovars of the isolates, although to date, there have been limited studies comparing the antimicrobial susceptibilities of different genomovars of *Burkholderia* (3, 33, 42). It is possible that some strains were

misidentified as *B. cepacia* complex (28). Many of the synergy results were indeterminate; this may reflect the limitations of the panel design as these multidrug-resistant strains were not inhibited by the clinically achievable concentrations tested in the combination wells. Finally, the clinical relevance of synergy studies utilizing the FIC methodology described in this report has not been assessed in patients with CF.

In conclusion, the management of multidrug-resistant *B. cepacia* complex remains challenging in the CF population. Agents used in non-CF patients have limited activity against CF strains, and it is likely that checkerboard synergy studies have limited utility against *B. cepacia* complex strains. Newer treatment options are needed for *B. cepacia* complex.

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