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Synergistic Activities of Macrolide Antibiotics against *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Alcaligenes xylosoxidans* Isolated from Patients with Cystic Fibrosis

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Azithromycin and clarithromycin were paired with other antibiotics to test synergistic activity against 300 multidrug-resistant pathogens isolated from cystic fibrosis (CF) patients. Clarithromycin-tobramycin was most active against *Pseudomonas aeruginosa* and inhibited 58% of strains. Azithromycin–trimethoprim-sulfamethoxazole, azithromycin-ceftazidime, and azithromycin-doxycycline or azithromycin–trimethoprim-sulfamethoxazole inhibited 40, 20, and 22% of *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, and *Achromobacter* (*Alcaligenes*) *xylosoxidans* strains, respectively.

Cystic fibrosis (CF) is the most common autosomal recessive, life-shortening disease among Caucasians (3, 16). The unique predilection of *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex for the CF lung has been well described (4), but other gram-negative bacilli, including *Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans*, are being recovered from CF patients with increasing frequency (1). While the impact of these potentially emerging pathogens on morbidity and mortality remains under study, there are limited therapeutic options available due to intrinsic and acquired resistance to antimicrobial agents.

There has been much interest recently in the treatment of CF patients with macrolide antibiotics (8, 12). In vitro, macrolide agents can inhibit the production of virulence factors by *P. aeruginosa* (13, 14, 23), including the production of alginate, the main component of the biofilm associated with chronic lung disease in CF (2, 7, 9, 10). Macrolides may be antiinflammatory and may decrease cytokine production by neutrophils, monocytes, and bronchial epithelial cells (6). Macrolides paired with other agents can have synergistic activity against *P. aeruginosa* (2). In this study, azithromycin and clarithromycin were paired with several antimicrobial agents to test synergistic activity against multidrug-resistant strains of *P. aeruginosa, B. cepacia* complex , *S. maltophilia*, and *A. xylosoxidans* isolated from CF patients.

The isolates tested were selected from strains sent to the CF Referral Center for Susceptibility and Synergy Testing at Columbia University (18). Fifty mucoid and 50 nonmucoid strains of *P. aeruginosa* from CF patients, 50 strains each of *B. cepacia* complex, *S. maltophilia*, and *A. xylosoxidans* from CF patients, and 50 strains of *P. aeruginosa* from non-CF patients (19) were studied. CF strains with different antimicrobial susceptibilities were selected (Table 1). To confirm identification to the species level, strains were placed on oxidative-fermentative, polymixin, bacitracin, lactose (OFPBL)–DNase and Mueller-Hinton agar plates (Remel, Lenexa, Kans.), and probed for the exotoxin A gene of *P. aeruginosa* as previously described (19, 20). The commercial identification assay API 20 NE (bioMérieux/Vitek, Hazelwood, Mo.) was used to identify *A. xylosoxidans* (20). To determine antimicrobial susceptibility, a broth microdilution assay (Microtech Medical Systems, Inc., Aurora, Colo.) was used to test 13 antimicrobial agents including azithromycin and clarithromycin. To determine synergy, checkerboard dilutions of pairs of antimicrobial agents in microtiter plates were also prepared commercially (Microtech). The following 12 combinations were tested in serial twofold dilutions: azithromycin (0.5 to 8 μg/ml)-tobramycin (1 to 8 μg/ml), azithromycin (0.5 to 8 μg/ml)-ciprofloxacin (0.5 to 4 μg/ml), azithromycin (0.5 to 8 μg/ml)-trovafloxacin (0.5 to 4 μg/ml), azithromycin (0.5 to 8 μg/ml)–ticarcillin-clavulanate (8 to 64 μg/ml), azithromycin (0.5 to 8 μg/ml)-ceftazidime (2 to 16 μg/ml), azithromycin (0.5 to 8 μg/ml)-meropenem (1 to 8 μg/ml), azithromycin (0.5 to 8 μg/ml)-doxycycline (1 to 8 μg/ml), azithromycin (0.5 to 8 μg/ml)–trimethoprim-sulfamethoxazole (0.5 to 4 μg/ml), clarithromycin (0.5 to 8 μg/ml)-tobramycin (1 to 8 μg/ml), clarithromycin (0.5 to 8 μg/ml)-ciprofloxacin (0.5 to 4 μg/ml), clarithromycin (0.5 to 8 μg/ml)-trovafloxacin (0.5 to 4 μg/ml), and clarithromycin (0.5 to 8 μg/ml)-ceftazidime (2 to 16 μg/ml). The fractional inhibitory concentration (FIC) was calculated as described previously (18); an FIC of <0.5 was considered synergistic and an FIC of 0.5 to <1.0 was considered additive. The same inoculum (10^5 CFU/ml) of a given isolate was used in the susceptibility studies and the synergy studies.

As expected, all strains were resistant to azithromycin and clarithromycin (the MIC at which 50% of isolates were inhibited was $\geq$512 μg/ml). However, synergistic and additive activities were noted when macrolide agents were paired with conventional antimicrobial agents (Table 2). This was particularly true for *P. aeruginosa* strains. Overall, combinations were more active against CF isolates than against non-CF isolates and more active against mucoid strains than against nonmucoid strains.

With the increasing life expectancy of CF patients, more courses of antibiotics are used with the adverse consequence of increasing antimicrobial resistance. During the past decade, no

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TABLE 1. Antimicrobial susceptibility patterns of tested isolates

Agent[a]	MIC (μg/ml)[b]		% Susceptible strains
	50%	90%	
P. aeruginosa			
CF, nonmucoid (n = 50)			
CAZ	>32	>32	34
CIP	2	8	78
IMP	>16	>16	42
TOB	8	256	60
ZOS	128	>128	38
CF, mucoid (n = 50)			
CAZ	>32	>32	34
CIP	2	4	70
IMP	8	>16	52
TOB	4	64	76
ZOS	128	>128	36
Non-CF (n = 50)			
CAZ	8	>32	66
CIP	0.5	4	78
IMP	4	>16	62
TOB	1	64	80
ZOS	32	>128	68
B. cepacia complex (n = 50)			
CHL	>32	>32	10
DOX	>32	>32	20
MER	8	>16	58
TMP-SMZ	16	16	14
S. maltophilia (n = 50)			
DOX	2	8	98
TIM	16	>128	66
TMP-SMZ	8	>16	24
A. xylosoxidans (n = 50)			
CAZ	32	>32	34
CHL	32	>32	26
CIP	>8	>8	2
TIM	>128	>128	36
TOB	>256	>256	2

[a] Abbreviations used: CAZ, ceftazidime; CIP, ciprofloxacin; IMP, imipenem; TOB, tobramycin; ZOS, piperacillin-tazobactam; CHL, chloramphenicol; DOX, doxycycline; MER, meropenem; TMP-SMZ, trimethoprim-sulfamethoxazole; TIM, ticarcillin-clavulanate.

[b] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

new antibiotics have been approved for the treatment of pathogens in CF patients. Thus, the CF community has been exploring the novel use of currently available agents such as aerosolized tobramycin (17) and, more recently, macrolide agents (8, 12).

Much of this interest stems from the use of macrolides in the treatment of diffuse panbronchiolitis (DPB), a disease seen primarily in Japanese adults (11, 21). DPB has several clinical features similar to those of CF including chronic, progressive lung disease caused by mucoid *P. aeruginosa.* DPB may be mild CF; patients with DPB do not have the common CFTR mutations seen in Caucasian patients with CF, but they do have mutations of the CFTR gene (K. Yoshimura, S. Iizuka, et al., Abstr. 2000 International Conference of the American Thoracic Society, Am. J. Respir. Crit. Care Med. **161:**A77, 2000). For patients with DPB, the use of macrolides has been associated with marked improvement in morbidity and mortality (11, 21, 22). For patients with CF, small, open-label trials have demonstrated that either clarithromycin or azithromycin improved pulmonary function or increased body weight (M. I. Anstead, R. J. Kuhn, et al., Abstr. 13th North American Cystic Fibrosis Conference, Pediatr. Pulmonol., Suppl. 19, abstr. 421, 1999). A study currently under way in the United States (L. Saiman, personal communication) and another recently completed in the United Kingdom (A. Equi, A. Bush, et al., Abstr. 15th North American Cystic Fibrosis Conference, Pediatr. Pulmonol., Suppl. 22, abstr. 396, 2001) test the safety and efficacy of prolonged therapy with azithromycin in CF patients chronically infected with *P. aeruginosa.*

We confirmed the lack of activity of the macrolide agents azithromycin and clarithromycin against CF pathogens which included multidrug-resistant strains of *P. aeruginosa*, *B. cepacia*, *S. maltophilia*, and *A. xylosoxidans.* However, when they were paired with ceftazidime, quinolones, or agents that interfere with protein synthesis such as chloramphenicol, tetracycline, or tobramycin, modest synergistic and additive activities

TABLE 2. Synergistic and additive effects of macrolide agents paired with conventional antimicrobial agents

Combination of agents tested[a]	Synergistic activity/additive activity[b] against:					
	P. aeruginosa			*B. cepacia* (n = 50)	*S. maltophilia* (n = 50)	*A. xylosoxidans* (n = 50)
	CF, nonmucoid (n = 50)	CF, mucoid (n = 50)	Non-CF (n = 50)			
AZI-TOB	21/14	6/14	0/9	0/0	2/0	2/0
AZI-CAZ	4/14	12/10	6/23	2/18	18/6	0/4
AZI-TIM	2/4	6/6	0/9	0/0	8/12	4/1
AZI-MER	0/0	4/10	3/14	0/16	2/4	6/16
AZI-DOX	14/28	20/20	26/14	2/12	2/4	0/4
AZI-TMP	18/12	28/12	0/0	6/6	32/10	4/18
AZI-CIP	0/2	4/12	0/6	0/0	0/2	0/0
AZI-TRV	0/2	8/12	0/14	0/4	0/2	0/8
CLA-TOB	18/40	14/26	0/2	2/0	0/2	0/0
CLA-CIP	0/26	10/34	0/6	4/2	0/0	0/0
CLA-CAZ	8/16	10/12	6/17	6/22	8/6	0/2
CLA-TRV	8/22	12/32	0/17	0/8	0/2	0/4

[a] Abbreviations used: AZI, azithromycin; TOB, tobramycin; CAZ, ceftazidime; TIM, ticarcillin-clavulanate; MER, meropenem; DOX, doxycycline; TMP, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; TRV, trovafloxacin; CLA, clarithromycin.

[b] Expressed as the percentages of isolates against which activities were demonstrated.

were observed. This compared favorably with the findings of our previous studies in which pairs of conventional agents inhibited between 10% (e.g., imipenem and amikacin) and 37% (e.g., piperacillin and tobramycin) of multidrug-resistant strains of *P. aeruginosa* (18).

There are several limitations to this study. In vitro activity may not predict in vivo efficacy, particularly for the synergy studies. Conventional susceptibility testing is an inadequate methodology for determining the nonbactericidal activities of macrolide agents. Finally, individual macrolide agents may not be comparable in their effects.

In conclusion, macrolide antibiotics may reduce the virulence factors of *P. aeruginosa.* The chronic, progressive lung disease of CF is due in part to the formation of a biofilm (10, 13), and the appearance of the mucoid phenotype of *P. aeruginosa* has been associated with clinical deterioration (5, 15). A strategy that could prevent or disrupt the biofilm within the CF lung could potentially reduce the bacterial burden, promote the activity of conventional antibiotics, and perhaps even prevent chronic infection.

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