ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1989, p. 1368–1372
0066-4804/89/081368-05$02.00/0

Vol. 33, No. 8

Cross-Resistance of *Pseudomonas aeruginosa* to Ciprofloxacin, Extended-Spectrum β-Lactams, and Aminoglycosides and Susceptibility to Antibiotic Combinations

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The susceptibilities of 270 clinical isolates of *Pseudomonas aeruginosa* from diverse sources (82 burn patients, 76 cystic fibrosis [CF] patients, and 112 other sources) to ciprofloxacin and three other quinolones, nine extended-spectrum beta-lactams, and three aminoglycosides were determined by an agar dilution method in cation-supplemented Mueller-Hinton medium. Ciprofloxacin, ceftazidime, imipenem, and aztreonam were the most active. MICs for burn isolates were consistently higher than those for other isolates for most antibiotics, whereas those for CF strains were consistently lower. Multidrug resistance to aminoglycosides and beta-lactams occurred in 21% of the burn isolates, 2.6% of the CF isolates, and 8.9% of the other isolates. Ninety percent of these strains remained susceptible to ciprofloxacin. Seven percent of the isolates were resistant to ciprofloxacin (MIC, ≥2 μg/ml). Concurrent resistance to ciprofloxacin and beta-lactams or aminoglycosides was rare (1.8 to 4%). Analysis by Spearman rank correlation revealed a high degree of correlation of MICs among antibiotics within the same class, except for imipenem. An inoculum effect was observed for all antibiotics between 10^6 and 10^4 CFU ($P < 0.05$), with those for piperacillin and cefoperazone being the most pronounced (16-fold and 8-fold differences, respectively), and was least apparent for the quinolones, aminoglycosides, imipenem, and aztreonam (twofold differences). Selected strains for which there were high MICs of ciprofloxacin (≥1 μg/ml) were tested against ciprofloxacin in combination with other agents in a checkerboard agar dilution assay. Synergistic (summated fractional inhibitory concentration, ≤0.5) interactions at clinically achievable concentrations were most frequent with mezlocillin (33%), piperacillin (21%), and cefoperazone (17%), less frequent with ceftazidime and imipenem (12% each), and infrequent with cefsulodin (7.6%), aztreonam (3.7%), and the aminoglycosides (3.7%). Antagonism (summated fractional inhibitory concentration, ≥4) was observed in only one instance (with gentamicin).

Ciprofloxacin, a DNA gyrase inhibitor, is among the most active fluoroquinolones, with broad-spectrum activity in vitro against both gram-positive and gram-negative bacteria (7, 12, 29). Its activity against *Pseudomonas aeruginosa*, however, is less consistent, and resistance has been encountered, particularly during therapy (2, 11, 22, 23). In this study, we examined the in vitro activity of ciprofloxacin by an agar dilution method against 270 clinical isolates of *P. aeruginosa* from various sources, including isolates from cystic fibrosis (CF) patients and burn wound isolates with multidrug resistance. Comparative activity and cross-resistance were determined with three other quinolones, nine antipseudomonal beta-lactams, and three aminoglycosides. The potential effects of inoculum density on their in vitro activities were also compared. Finally, selected strains with high MICs of ciprofloxacin were tested for possible synergy of ciprofloxacin in combination with other agents by a checkerboard agar dilution technique. Demonstration of synergistic activity in vitro with these antimicrobial combinations may suggest useful therapeutic regimens for multidrug-resistant *P. aeruginosa* infections and for preventing the emergence of resistance during therapy.

MATERIALS AND METHODS

Organisms. A total of 270 *P. aeruginosa* isolates obtained from patients of Vancouver General Hospital were identified by standard techniques. Multiple isolates from the same patient were excluded. Eighty-two and 76 isolates were from burn and CF patients, respectively. The other 112 isolates were from wound specimens (30 isolates), urine (27 isolates), blood (19 isolates), sputum (19 isolates), and miscellaneous sources (17 isolates). Twenty-seven isolates for which there were high MICs of ciprofloxacin (MIC, ≥1 μg/ml; MIC for 90% of the isolates, 4 μg/ml) were selected for combination studies. A reference strain, *P. aeruginosa* ATCC 27853, was included in each experiment to assure reproducibility.

Susceptibility testing. Antibiotic powders for susceptibility testing were kindly supplied by the respective pharmaceutical manufacturers. The MIC of each antimicrobial agent was determined by the standard agar dilution method described by Barry (1), using inocula of 10^4 and 10^6 CFU. Inocula were prepared from an overnight broth culture of the test organism in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), either undiluted (10^9 CFU/ml) or adjusted to a 0.5 McFarland standard and diluted 1:20 with saline (10^7 CFU per ml). A Steers replicator was used to deliver 0.0025 ml of inoculum onto the surface of Mueller-Hinton agar (BBL) supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) and containing twofold serial dilutions of the test antibiotics. Plates without antibiotics served as controls. All plates were incubated for 24 h at 37°C. The MIC was considered the lowest concentration of antibiotic which permitted no visible growth.

Combination studies. Combinations of ciprofloxacin with

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TABLE 1. Comparative susceptibilities of *P. aeruginosa* from different clinical sources to quinolones, extended-spectrum beta-lactams, and aminoglycosides

Antibiotic	MIC (μg/ml) with inoculum of 10^4 CFU[a]								Median ratio[b] (270 isolates), MIC (10^6 CFU)/ MIC (10^4 CFU)
	Burn (82)		CF (76)		Other (112)		Total (270)		
	50%	90%	50%	90%	50%	90%	50%	90%	
Ciprofloxacin	0.5	1[c]	0.5	2	0.5	1	0.5	1	2
Norfloxacin	2	4[c]	1	4[d]	1	4	1	4	2
Difloxacin	4	8[c]	2	4[d]	2	8	4	8	4
A-56620	2	2[c]	0.5	4	1	2	1	4	2
Ceftazidime	2	64	2	4[d]	2	32	2	32	4
Cefoperazone	8	≥128	4	16[d]	8	128	8	≥128	8
Cefsulodin	4	64[c]	2	8[d]	4	32	4	32	4
Piperacillin	8	512[c]	4	16[d]	8	64	8	128	16
Ticarcillin	32	256	16	64[d]	32	256	32	256	3
Carbenicillin	64	256	32	256[d]	64	256	64	256	2
Imipenem	8	32	8	32	8	64	8	32	2
S-34343	≥128	≥128	≥128	≥128[d]	≥128	≥128	≥128	≥128	
Aztreonam	8	64[c]	2	16[d]	4	32	4	32	2
Gentamicin	≥400	≥400[c]	6.2	12.5[d]	6.2	50	12.5	≥400	2
Tobramycin	100	≥400[c]	3.1	6.2[d]	3.1	12.5	3.1	≥400	2
Amikacin	25	100[c]	12.5	25[d]	12.5	50	12.5	50	4

[a] The number of isolates is shown in parentheses. 50% and 90%, MIC for 50 and 90% of the isolates, respectively.

[b] MICs with inoculum of 10^6 CFU were significantly higher than with 10^4 CFU ($P < 0.05$, Wilcoxon signed rank test, two tailed) for all agents tested except S-34343, for which the ratio could not be accurately determined since most strains were highly resistant to this antibiotic at either inoculum.

[c] Significantly higher by rank sum test ($P < 0.05$ [two tailed]) compared with value for "Other" isolates.

[d] Significantly lower by rank sum test ($P < 0.05$ [two tailed]) compared with value for "Other" isolates.

seven extended-spectrum beta-lactams (imipenem, aztreonam, piperacillin, mezlocillin, ceftazidime, cefoperazone, and cefsulodin) and three aminoglycosides (gentamicin, tobramycin, and amikacin) were examined by a two-dimensional checkerboard agar dilution method as previously described (10). Briefly, twofold serial dilutions of antibiotics were prepared to give an initial concentration of four times the MICs of the respective antibiotics alone as determined in individual susceptibility testing. Combinations of antibiotics were added, with drug A diluted along the abscissa and drug B diluted along the ordinate. Thus, for a given range of dilutions every possible combination of drug concentrations was achieved. Plates were incubated with 10^4 CFU per inoculum and read as described above.

In the evaluation of combination effects, the ratio of the MIC of one antibiotic in the combination to the MIC of that antibiotic used alone, termed the fractional inhibitory concentration (FIC), was calculated for each antibiotic in each combination, and the FICs were then summated (ΣFIC). Synergy and antagonism were defined as a minimum ΣFIC of ≤0.5 and a maximum ΣFIC of >4.0, respectively. Interaction indices were noted, particularly if they occurred at antibiotic concentrations that can be readily achieved in serum clinically.

Statistical methods. Comparison of MICs between different groups of isolates was by the rank sum test (two tailed). The effect of inoculum density with 10^6 versus 10^4 CFU was examined by the Wilcoxon signed rank test (two tailed). Cross-resistance to antibiotic pairs was determined by Spearman rank correlations with correction for ties as described by Hollander and Wolfe (20).

RESULTS

The in vitro susceptibilities of 270 *P. aeruginosa* strains to ciprofloxacin and 15 other antimicrobial agents tested singly are given in Table 1. The MICs for the reference strain were all within the expected ranges for each antibiotic and did not vary more than one twofold dilution in 10 separate experiments. Overall, the most potent agents were the quinolones, among which ciprofloxacin was the most active and difloxacin was the least active. Among the extended-spectrum beta-lactams, imipenem, aztreonam, ceftazidime, and cefsulodin were the most active agents. The penem S-34343 was inactive against these isolates. Not surprisingly, amikacin was the most active agent among the aminoglycosides. Isolates from burn wounds and from CF patients were separately analyzed (Table 1). The MICs for burn isolates were consistently higher than for other isolates for all antibiotics except ceftazidime, cefoperazone, ticaricillin, carbenicillin, imipenem, and S-34343 ($P < 0.05$, rank sum test, two tailed). Similarly, CF isolates were consistently more sensitive than other isolates to all antibiotics except ciprofloxacin, A-56620, and imipenem ($P < 0.05$, rank sum test). The MICs of all antibiotics were significantly higher when tested with an inoculum of 10^6 CFU than with an inoculum of 10^4 CFU ($P < 0.05$, Wilcoxon signed rank test, two tailed). However, the effect of inoculum density was most prominent for piperacillin and cefoperazone (16-fold and 8-fold differences, respectively), intermediate for difloxacin, ceftazidime, cefsulodin, ticarcillin, and amikacin (three- to fourfold differences), and least apparent for ciprofloxacin, norfloxacin, A-56620, imipenem, aztreonam, carbenicillin, gentamicin, and tobramycin (twofold differences) (Table 1). The inoculum effect for S-34343 could not be accurately determined since most strains were highly resistant at either inoculum density.

The relationships between different antibiotic pairs with respect to their activities against the 270 *P. aeruginosa* isolates were examined by Spearman rank correlation (Table 2). The higher the values of the correlation coefficients (r) between the MICs of drug pairs, the higher the probability of cross-resistance or cross-susceptibility. There was a positive correlation between all 120 possible drug combinations.

TABLE 2. Coefficients of correlation for the MICs of antibiotic pairs against 270 isolates of *P. aeruginosa*

Antibiotic[a]	Spearman correlation coefficient (r)[b]														
	CFX	NFX	DFX	A20	GTM	TBM	AMK	CTZ	CFP	CSL	PCL	TCL	CCL	AZT	S43
NFX	**0.71**														
DFX	**0.63**	**0.76**													
A20	**0.69**	**0.74**	**0.59**												
GTM	0.30	0.40	0.33	0.37											
TBM	0.23	0.34	0.28	0.31	**0.89**										
AMK	0.30	0.43	0.35	0.36	**0.81**	**0.81**									
CTZ	0.21	0.29	0.29	0.20	0.38	0.37	0.40								
CFP	0.28	0.39	0.39	0.32	0.46	0.45	0.47	**0.74**							
CSL	0.26	0.32	0.38	0.23	0.40	0.40	0.39	**0.73**	**0.73**						
PCL	0.23	0.32	0.34	0.21	**0.54**	**0.53**	0.48	**0.62**	**0.72**	**0.73**					
TCL	0.18	0.34	0.35	0.17	0.35	0.37	0.37	**0.65**	**0.75**	**0.78**	**0.73**				
CCL	0.19	0.37	0.37	0.23	0.36	0.36	0.34	**0.65**	**0.74**	**0.80**	**0.74**	**0.88**			
AZT	0.24	0.39	0.39	0.25	0.36	0.35	0.38	**0.78**	**0.73**	**0.75**	**0.61**	**0.74**	**0.76**		
S43	0.19	0.37	0.36	0.17	0.30	0.29	0.22	0.24	0.40	0.35	0.48	0.44	0.45	0.40	
IMP	0.02	0.05	0.03	0.07	0.08	0.13	0.23	0.20	0.17	0.10	0.01	0.08	0.09	0.14	0.19

[a] Abbreviations: CFX, ciprofloxacin; NFX, norfloxacin; DFX, difloxacin; A20, A-56620; GTM, gentamicin; TBM, tobramycin; AMK, amikacin; CTZ, ceftazidime; CFP, cefoperazone; CSL, cefsulodin; PCL, piperacillin; TCL, ticarcillin; CCL, carbenicillin; AZT, aztreonam; S43, S-34343; IMP, imipenem.

[b] $r \geq 0.16$, significant with $P \leq 0.01$ (two tailed); $r \geq 0.20$, significant with $P \leq 0.001$. The correlation coefficients of those antibiotic combinations with $r \geq 0.5$ are indicated in boldface type.

Only 11 of the 120 correlation coefficients showed values less than 0.16 (all in imipenem combinations) and therefore were not statistically significant at the 1% level ($P < 0.01$). In general, high correlation coefficients were observed among agents within the same antibiotic class (i.e., quinolones, aminoglycosides, or beta-lactams). There was a high degree of intercorrelation among the penicillins, cephalosporins, and aztreonam ($r \geq 0.5$) but not the penems (Table 2). Piperacillin also demonstrated moderate correlation with gentamicin and tobramycin ($r \geq 0.5$). Ciprofloxacin correlated poorly with the beta-lactams or aminoglycosides. Imipenem demonstrated the least correlation with all other agents tested.

Twenty-nine of the 270 isolates were found to be resistant to both gentamicin (MIC, ≥6 μg/ml) and piperacillin (MIC, ≥128 μg/ml) and were separately analyzed (Table 3). These included 17 of 82 (21%) burn isolates, 2 of 76 (2.6%) CF isolates, and 10 of 112 (8.9%) other isolates. In addition to gentamicin and piperacillin, these strains were also frequently resistant to other agents, including extended-spectrum cephalosporins and other aminoglycosides. However, 90% of these isolates remained susceptible to ciprofloxacin and to A-56620.

Twenty of the 270 isolates were resistant to ciprofloxacin (MIC, ≥2 μg/ml) (Table 3). They included 5 of 82 (6%) burn isolates, 9 of 76 (12%) CF isolates, and 6 of 112 (5.3%) other isolates. The majority of these strains (≥80%) remained susceptible to ceftazidime, cefoperazone, cefsulodin, piperacillin, and aztreonam. Only 60% were susceptible to imipenem.

Twenty-seven isolates for which there were relatively high MICs of ciprofloxacin (MIC for 90% of the isolates, 4 μg/ml) were selected for combination studies with ciprofloxacin (Table 4). Synergistic interactions (minimum ΣFIC, ≤0.5) at clinically achievable concentrations were most frequent with mezlocillin (33%), piperacillin (21%), and cefoperazone (17%), less frequent with ceftazidime and imipenem (12% each), and infrequent with cefsulodin (7.6%), aztreonam (3.7%), and the aminoglycosides (3.7%). Antagonism (maximum ΣFIC, ≥4) was rare, occurring only with gentamicin and one isolate.

DISCUSSION

The in vitro susceptibility of *P. aeruginosa* reported herein is in general agreement with results obtained by other investigators and demonstrates the excellent antipseudomonal activity of ciprofloxacin, as well as of ceftazidime, imipenem, and aztreonam (3, 7, 11–13, 22, 23, 25, 28, 29). Our data are unique in that a relatively large number of clinical isolates were tested and results were analyzed according to the patient population from which they were isolated, including burn wound and CF patients. These data also demonstrate the frequency of multidrug resistance of *P. aeruginosa* isolates from our burn unit. Importantly, these isolates remained susceptible to ciprofloxacin, although the MICs of this agent were also significantly higher than those

TABLE 3. Susceptibilities of selected strains of *P. aeruginosa* resistant to gentamicin and piperacillin or to ciprofloxacin

Antibiotic	Breakpoint (μg/ml)	Strains resistant to:			
		Gentamicin and piperacillin (n = 29)		Ciprofloxacin (n = 20)	
		MIC_{90}[a] (μg/ml)	% Susceptible at breakpoint	MIC_{90} (μg/ml)	% Susceptible at breakpoint
Ciprofloxacin	1	1	90	4	0
Norfloxacin	4	4	86	16	45
Difloxacin	4	8	38	32	40
A-56620	2	2	90	4	25
Ceftazidime	32	≥128	27	128	80
Cefoperazone	32	≥128	7	32	90
Cefsulodin	32	≥128	38	128	85
Piperacillin	64	>500	0	≥500	85
Ticarcillin	64	>500	17	500	70
Carbenicillin	64	>500	3	500	55
Imipenem	16	32	59	64	60
S-34343	16	≥128	0	≥128	10
Aztreonam	16	≥128	17	128	80
Gentamicin	6	≥400	0	100	40
Tobramycin	6	≥400	17	100	75
Amikacin	25	100	38	100	75

[a] MIC_{90}, MIC for 90% of the isolates.

TABLE 4. Ciprofloxacin interactions with other antibiotics against 27 strains of *P. aeruginosa*

Antibiotic	No. of isolates tested	No. of isolates with:	
		ΣFIC ≤ 0.5 (synergistic)	ΣFIC ≥ 4 (antagonistic)
Mezlocillin	24	8 (8)[a]	0
Piperacillin	24	5 (5)	0
Cefoperazone	24	4 (4)	0
Ceftazidime	24	4 (3)	0
Imipenem	24	3 (3)	0
Cefsulodin	26	3 (2)	0
Aztreonam	27	1 (1)	0
Gentamicin	27	2 (1)	1
Amikacin	27	1 (1)	0
Tobramycin	26	1 (1)	0

[a] Shown in parentheses are the numbers of isolates which demonstrated synergy at clinically achievable concentrations of the antibiotic combinations (for breakpoints, see Table 3; the breakpoint for mezlocillin was 64 μg/ml).

for non-burn isolates. CF isolates remained highly susceptible to ciprofloxacin, as was reported by Klinger and Aronoff (22) and Bosso et al. (3). Interestingly, in contrast to other agents tested, no significant difference in MICs of ciprofloxacin was noted in our study between CF and non-CF isolates.

Using cation-supplemented Mueller-Hinton agar, we demonstrated a significant effect of inoculum density on the MICs for all agents tested, although this was less apparent for the quinolones, aminoglycosides, imipenem, and aztreonam than for other beta-lactam agents. The inoculum effect was most prominent for piperacillin and cefoperazone. Eng et al. (14) reported similar findings and described three groups of antipseudomonal agents according to their inoculum effect and antibacterial activity: group I antibiotics (typified by piperacillin and cefoperazone) demonstrated the largest inoculum effect, were poorly bactericidal, and produced aberrant, elongated bacilli during antibiotic exposure; group II antibiotics (typified by ceftazidime and ticarcillin) demonstrated moderate inoculum effect, were slowly bactericidal, and caused minimal formation of aberrant, elongated bacilli; and group III antibiotics (typified by imipenem and tobramycin) were bactericidal and did not cause the formation of elongated bacilli and regrowth. Our data suggest that quinolones, particularly ciprofloxacin, belong in the group III category of antibiotics against *P. aeruginosa*.

It has been reported by several investigators that the cation concentration and type of medium used for susceptibility testing greatly influence the in vitro activities of the quinolones, aminoglycosides, and extended-spectrum beta-lactams against *P. aeruginosa* (9, 15, 19, 30). We supplemented the test medium in our studies since the lot of Mueller-Hinton agar we used was low in calcium and magnesium and required supplementation to attain physiologic concentrations (calcium, 2.25 to 2.75 mM; magnesium, 0.75 to 1.25 nM). We believe that it is prudent to routinely determine the calcium and magnesium concentrations of the medium to be used to appropriately assess the need for supplementation, since variation in media between lots and from different manufacturers is not uncommon (26).

The occurrence of cross-resistance or concurrent resistance of *P. aeruginosa* to related or unrelated antibiotics has not been studied extensively. Without identifying the mechanisms of resistance, it is impossible to determine whether true cross-resistance to two agents is present. Our analysis by the Spearman rank correlation technique indicates a high degree of correlation among antibiotics within the same class (i.e., quinolones, extended-spectrum beta-lactams, or aminoglycosides) and suggests that cross-resistance to these agents might be present. Multidrug resistance to gentamicin and piperacillin, as well as to tobramycin, aztreonam, and extended-spectrum cephalosporins, was found in approximately 10% of our isolates. Importantly, these strains remained susceptible to ciprofloxacin. Coresistance to ciprofloxacin and aminoglycosides (1.8 to 4% of strains), imipenem (3%), or other extended-spectrum beta-lactams (0.7 to 4%) was observed infrequently. Multidrug resistance involving ciprofloxacin, imipenem, ceftazidime, and aminoglycosides was not found. Selection of multidrug resistance to quinolones, beta-lactams, and aminoglycosides is being increasingly recognized among nosocomial pathogens (27). These data underscore the need to examine carefully the frequency with which resistance to any new antibiotic develops, as well as the patterns of multidrug resistance which may occur simultaneously. Such determinations are particularly important in areas of high selection pressure due to intensive antimicrobial use, such as burn and intensive care units, and among CF patients.

Since 7% of our strains were resistant to ciprofloxacin and multidrug resistance to aminoglycosides and extended-spectrum beta-lactams was not infrequent, the activity of ciprofloxacin in combination with other agents was of particular interest. Synergy was most frequent with mezlocillin (33%) and piperacillin (21%), moderately frequent with cefoperazone, ceftazidime, and imipenem (12 to 16%), and least frequent with aztreonam and aminoglycosides (3.7%). Similar data have been obtained by other investigators using either the checkerboard microdilution technique or the time-kill curve method (4, 5, 8, 16–18, 21, 24). In addition to synergistic interactions of ciprofloxacin with mezlocillin and piperacillin, frequent synergistic interactions of ciprofloxacin with azlocillin against *P. aeruginosa* have been noted by other workers both in vitro and in vivo (21, 24). The mechanism(s) responsible for the enhanced activity of these agents in combination are presently unknown. Several investigators have pointed out the relative lack of agreement between the checkerboard and time-kill curve techniques in demonstrating antimicrobial synergy against pseudomonads (6, 18, 24). These differences could be due to variations in media, including divalent cation content, or to the selection and regrowth of aberrant forms of *P. aeruginosa*, which can be more readily demonstrated in broth during the time-kill assay than in an agar medium. Nevertheless, the in vitro data demonstrating synergistic but not antagonistic interactions with ciprofloxacin combinations suggest that these agents may prove useful for the management of multidrug-resistant *P. aeruginosa* infections. Combination therapy with ciprofloxacin may also deter the emergence of resistance, as has been demonstrated in earlier studies (23). The clinical relevance of these observations, however, must await careful evaluation of controlled therapeutic trials in vivo.

LITERATURE CITED

1. **Barry, A. L.** 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations, p. 1–26. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
2. **Blaser, J., M. N. Dudley, D. Gilbert, and S. H. Zinner.** 1986. Influence of medium and method on the in vitro susceptibility of *Pseudomonas aeruginosa* and other bacteria to ciprofloxacin and enoxacin. Antimicrob. Agents Chemother. **29**:927–929.
3. **Bosso, J. A., J. E. Allen, and J. M. Matsen.** 1989. Changing susceptibility of *Pseudomonas aeruginosa* isolates from cystic

fibrosis patients with the clinical use of newer antibiotics. Antimicrob. Agents Chemother. **33**:526–528.

4. **Bustamante, C. I., G. L. Drusano, R. C. Wharton, and J. C. Wade.** 1987. Synergism of the combinations of imipenem plus ciprofloxacin and imipenem plus amikacin against *Pseudomonas aeruginosa* and other bacterial pathogens. Antimicrob. Agents Chemother. **31**:632–634.
5. **Chalkley, L. J., and H. J. Koornhof.** 1985. Antimicrobial activity of ciprofloxacin against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* determined by the killing curve method: antibiotic comparisons and synergistic interactions. Antimicrob. Agents Chemother. **28**:331–342.
6. **Chan, E. L., and R. Z. Zabransky.** 1987. Determination of synergy by two methods with eight antimicrobial combinations against tobramycin-susceptible and tobramycin-resistant strains of pseudomonas. Diagn. Microbiol. Infect. Dis. **6**:157–164.
7. **Chin, N.-X., and H. C. Neu.** 1984. Ciprofloxacin, a quinolone carboxylic acid compound active against aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. **25**:319–326.
8. **Chin, N. X., and H. C. Neu.** 1987. Synergy of imipenem—a novel carbapenem, and rifampin and ciprofloxacin against Pseudomonas aeruginosa, Serratia marcescens and Enterobacter species. Chemotherapy **33**:183–188.
9. **Chow, A. W., and K. H. Bartlett.** 1981. Comparative in vitro activity of ceftazidime (GR 20263) and other betalactamase stable cephalosporins against Pseudomonas. Effect of inoculum size and divalent cation supplementation. J. Antimicrob. Chemother. **8**(Suppl. B):345–350.
10. **Chow, A. W., J. Wong, and K. H. Bartlett.** 1988. Synergistic interactions of ciprofloxacin and extended-spectrum β-lactams or aminoglycosides against multiply drug-resistant *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. **32**:782–784.
11. **Daikos, G. L., V. T. Lolans, and G. E. G. Jackson.** 1988. Alterations in outer membrane proteins of *Pseudomonas aeruginosa* associated with selective resistance to quinolones. Antimicrob. Agents Chemother. **32**:785–787.
12. **Eliopoulous, G. M., A. Gardella, and R. C. Moellering, Jr.** 1984. In vitro activity of ciprofloxacin, a new carboxyquinolone antimicrobial agent. Antimicrob. Agents Chemother. **25**:331–335.
13. **Eliopoulous, G. M., A. E. Moellering, E. Reiszner, and R. C. Moellering, Jr.** 1985. In vitro activities of the quinolone antimicrobial agents A-56619 and A-56620. Antimicrob. Agents Chemother. **28**:514–520.
14. **Eng, R. H. K., S. M. Smith, and C. Cherubin.** 1984. Inoculum effect of new β-lactam antibiotics on *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **26**:42–47.
15. **Fuchs, P. C., R. N. Jones, A. L. Barry, T. L. Gavan, and the Collaborative Antimicrobial Susceptibility Test Group.** 1989. Ofloxacin susceptibility testing quality control parameters for microdilution and disk diffusion, and confirmation of disk diffusion interpretive criteria. J. Clin. Microbiol. **27**:49–52.
16. **Fuursted, K., and P. Gerner-Smidt.** 1987. Analysis of the interaction between piperacillin and ciprofloxacin or tobramycin against thirteen strains of Pseudomonas aeruginosa, using killing curves. Acta Pathol. Microbiol. Immunol. Scand. **95**:193–197.
17. **Giamarellou, H., and G. Petrikkos.** 1987. Ciprofloxacin interactions with imipenem and amikacin against multiresistant *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **31**:959–961.
18. **Haller, I.** 1985. Comprehensive evaluation of ciprofloxacin-aminoglycoside combinations against *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains. Antimicrob. Agents Chemother. **28**:663–666.
19. **Hirschhorn, L., and H. C. Neu.** 1986. Factors influencing the in vitro activity of two aryl-fluoroquinolone antimicrobial agents, difloxacin (A-56619) and A-56620. Antimicrob. Agents Chemother. **30**:143–146.
20. **Hollander, M., and D. A. Wolfe.** 1973. Nonparametric statistical methods. John Wiley & Sons, Inc., New York.
21. **Johnson, M., P. Miniter, and V. T. Andriole.** 1987. Comparative efficacy of ciprofloxacin, azlocillin, and tobramycin alone and in combination in experimental pseudomonas sepsis. J. Infect. Dis. **155**:783–788.
22. **Klinger, J. D., and S. C. Aronoff.** 1985. In vitro activity of ciprofloxacin and other antibacterial agents against Pseudomonas aeruginosa and Pseudomonas cepacia from cystic fibrosis patients. J. Antimicrob. Chemother. **15**:679–684.
23. **Michea-Hamzehpour, M., J. C. Pechere, B. Marchou, and R. Auckenthaler.** 1986. Combination therapy—a way to limit emergence of resistance? Am. J. Med. **80(6B)**:138–142.
24. **Moody, J. A., D. N. Gerding, and L. R. Peterson.** 1987. Evaluation of ciprofloxacin's synergism with other agents by multiple in vitro methods. Am. J. Med. **82**(Suppl. 4A):44–54.
25. **Ng, W. W. S., P. Y. Chau, Y. K. Leung, and D. M. Livermore.** 1985. In vitro activities of Ro 17-29301 and aztreonam compared with those of other new β-lactam antibiotics against clinical isolates of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **27**:872–873.
26. **Pollock, H. M., A. L. Barry, T. L. Gavan, P. C. Fuchs, S. Hansen, C. L. Thornsberry, H. Frankel, and S. B. Forsythe.** 1986. Selection of a reference lot of Mueller-Hinton agar. J. Clin. Microbiol. **24**:1–6.
27. **Sanders, C. C., W. E. Sanders, Jr., R. V. Goering, and V. Werner.** 1984. Selection of multiple antibiotic resistance by quinolones, β-lactams, and aminoglycosides with special reference to cross-resistance between unrelated drug classes. Antimicrob. Agents Chemother. **26**:797–801.
28. **Thornsberry, C.** 1985. Review of in vitro activity of third-generation cephalosporins and other newer betalactam antibiotics against clinically important bacteria. Am. J. Med. **79**(Suppl. 2A):14–20.
29. **Van Caekenberghe, D. L., and S. R. Pattyn.** 1984. In vitro activity of ciprofloxacin compared with those of other new fluorinated piperazinyl-substituted quinolone derivatives. Antimicrob. Agents Chemother. **25**:518–521.
30. **Zauravleff, J. J., V. L. Yu, R. B. Yee, M. K. Zaphyr, W. Diven, and F. B. Taylor.** 1982. Effect of calcium, magnesium, and zinc on ticarcillin and tobramycin alone and in combination against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **22**: 839–843.