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Determination of Activities of Levofloxacin, Alone and Combined with Gentamicin, Ceftazidime, Cefpirome, and Meropenem, against 124 Strains of *Pseudomonas aeruginosa* by Checkerboard and Time-Kill Methodology

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A total of 124 *Pseudomonas aeruginosa* strains were tested for synergy between levofloxacin and cefpirome, ceftazidime, gentamicin, and meropenem. Checkerboards yielded synergistic fractional inhibitory concentration (FIC) indices (≤0.5) with 25 of 496 possible combinations. All other FIC indices were >0.5 to 2 (additive or indifferent), with no antagonism. Time-kill studies with 12 strains showed that levofloxacin (0.06 to 0.5 μg/ml) was synergistic with cefpirome, ceftazidime, gentamicin, and meropenem in 10, 9, 4, and 11 strains, respectively.

Standard therapy for *Pseudomonas aeruginosa* infections includes broad-spectrum cephalosporins, such as cefpirome (not available in the United States) and ceftazidime; aminoglycosides, such as gentamicin; and carbapenems, such as imipenem and meropenem (3, 5–7, 10, 11, 13–17). Levofloxacin, the *l*-isomer of ofloxacin, is also active against this organism (9, 19, 20). The current study investigated the activity of levofloxacin, alone and in combination with cefpirome, ceftazidime, gentamicin, and meropenem, against 124 *P. aeruginosa* strains with different susceptibilities to the latter four agents.

One hundred twenty-four strains of *P. aeruginosa*, recently isolated from clinical specimens and identified by conventional methodology (12), were tested. Strains resistant to cephalosporins and meropenem only were obtained from David Livermore (Central Public Health Laboratories, London, United Kingdom). Strains included 30 susceptible to ceftazidime, cefpirome, gentamicin, and meropenem; 26 resistant to ceftazidime only; 21 resistant to gentamicin only; 24 resistant to meropenem only; and 23 with various susceptibility patterns. Laboratory powders of known potency were obtained from their various manufacturers.

MICs of each agent alone were determined by broth microdilution testing according to standard National Committee for Clinical Laboratory Standards (NCCLS) methodology (18). Breakpoints for ceftazidime and gentamicin were those recommended by NCCLS (18). Breakpoints used for meropenem were identical to those of imipenem (18), as recently approved (but not yet published) by NCCLS. No cefpirome breakpoints are available. Strains with intermediate susceptibility (18) to ceftazidime, gentamicin, and meropenem were classified as resistant. Less than 5% of resistant strains were intermediate to ceftazidime and gentamicin, but 48% were intermediate to meropenem: all of the latter, however, were resistant (MICs of ≥16 μg/ml) to imipenem. Additionally, because serious *P. aeruginosa* infections caused by strains with intermediate resistance are treated as if fully resistant, we elected to combine the two groups.

Checkerboard synergy was performed as described previously (2). Fractional inhibitory concentrations (FICs) were calculated as (MIC of drug A or B in combination)/(MIC of drug A or B alone), and the FIC index was obtained by adding the FIC values. FIC indices were interpreted as synergistic if values were ≤0.5, additive or indifferent if >0.5 to 4.0 and antagonistic if >4.0 (1, 2, 8).

Three strains from each of the above four susceptibility groups were tested by time-kill as described previously (1, 2). All compounds were tested alone, and levofloxacin was tested in combination with cefpirome, ceftazidime, gentamicin, and meropenem. Viability counts were performed at 0, 6, 12, and 24 h. Drug carryover was addressed by dilution, as described previously (1, 2). In view of regrowth in many strains (which could have been selected in vitro) after 24 h, synergy was defined as a ≥2-log decrease in the viable count of the combination at 12 h compared to the more active of the two agents alone (8).

Results of microbroth MIC testing of each agent alone for the four organism groups as well as the miscellaneous group are presented in Table 1. As can be seen, high-level resistance to levofloxacin (≥8 μg/ml) was only seen in gentamicin-resistant strains; in other strains, MICs at which 90% of the isolates are inhibited (MIC_{90}s) were ≤4 μg/ml.

Checkerboard titration results are listed in Table 2. Synergistic FIC indices (≤0.5) were found in nine strains (7.3%) (three fully susceptible, three resistant to ceftazidime, three miscellaneous) with levofloxacin-cefpirome, eight strains (6.5%) (three ceftazidime resistant, four meropenem resistant, one miscellaneous) with levofloxacin plus ceftazidime, one ceftazidime-resistant strain (0.8%) with levofloxacin-gentamicin, and seven strains (5.6%) (two fully susceptible, two ceftazidime resistant, one meropenem resistant, two miscellaneous) with levofloxacin plus meropenem. All other FIC indices were >0.5 to 2 (additive or indifferent), and no antagonism (FIC indices of >4) was found.

The results of time-kill synergy tests are listed in Table 3. Checkerboard titrations with these strains showed that one strain showed synergy with levofloxacin plus ceftazidime, and one showed synergy with levofloxacin plus meropenem. Time-

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TABLE 1. Broth microdilution MIC_{50}s and MIC_{90}s of each agent alone

Group (*n*)	MIC (μg/ml)[a]									
	Levofloxacin		Cefpirome		Ceftazidime		Gentamicin		Meropenem	
	50%	90%	50%	90%	50%	90%	50%	90%	50%	90%
Susceptible (30)[b]	0.5	4	8	16	2	8	2	4	1	2
Resistant										
Ceftazidime (26)	2	4	64	>128	128	>128	0.5	4	1	4
Gentamicin (21)	8	>32	8	16	8	8	32	>64	1	4
Meropenem (24)	1	2	8	8	4	8	1	4	8	16
Miscellaneous (23)[c]	4	32	128	>128	128	>128	32	64	4	32

[a] 50% and 90%, MIC_{50}R and MIC_{90}, respectively.
[b] Susceptible to cephalosporins, gentamicin, and meropenem.
[c] Resistant to cephalosporins, gentamicin, and meropenem ($n = 10$); resistant to gentamicin and cephalosporins and susceptible to meropenem ($n = 12$); gentamicin susceptible, resistant to cephalosporins and meropenem ($n = 1$).

kill synergy assays showed that levofloxacin, at sub-MIC concentrations of 0.06 to 0.5 μg/ml, showed synergy with cefpirome, ceftazidime, gentamicin, and meropenem in 10, 9, 4, and 11 strains, respectively.

Levofloxacin yields MICs for all organisms which are 1 to 2 dilutions lower than those for ofloxacin (9, 19, 20). Our study confirms these findings. Of note in our study were the higher levofloxacin MICs for strains resistant to gentamicin only. Recently, NCCLS has approved breakpoints of ≤2.0 μg/ml (susceptible), 4.0 μg/ml (intermediate), and ≥8.0 μg/ml (18). Recent studies have documented MIC_{50}s of 0.5 to 1.0 μg/ml and MIC_{90}s of 2.0 to 8.0 μg/ml for *P. aeruginosa* (9, 20). Of broad-spectrum cephalosporins with activity against *P. aeruginosa*, cefpirome has been reported to have a MIC_{50} of 2.0 to 16.0 μg/ml and a MIC_{90} of 8.0 to 16.0 μg/ml (5, 10, 14). Gargalianos et al. (10) have demonstrated the range of cefpirome MICs to be 1.0 to 16.0 μg/ml in *P. aeruginosa* strains with increased non-β-lactamase-mediated resistance to carbenicillin, plasmid-mediated β-lactamase production, and partially derepressed chromosomal β-lactamase expression. Two strains with totally derepressed chromosomal β-lactamase expression yielded cefpirome MICs of 16.0 and 32.0 μg/ml, respectively. In all of the latter resistance groups, ceftazidime MIC ranges were <0.5 to 32.0 μg/ml (10).

Ceftazidime MICs for *P. aeruginosa* generally correspond with those of cefpirome (3, 5, 10, 14). This was also the case in our study. Although a small percentage of *P. aeruginosa* strains are resistant to ceftazidime, widespread use of this compound in the United States has not led to a significant rise in ceftazidime resistance (3). Although gentamicin was originally very active against *P. aeruginosa* strains, resistance is common in most hospital settings (6, 11, 15, 16).

Meropenem, a recently developed parenteral carbapenem, is very active against *P. aeruginosa*, with MIC_{50}s of 0.25 to 0.5 μg/ml and MIC_{90}s of 1.0 to 4.0 μg/ml for imipenem-susceptible strains. The in vitro activity of meropenem is greater than that of imipenem (7, 13, 16). Against a series of *P. aeruginosa* strains with well-characterized resistance mechanisms, meropenem retained high-level activity against strains with the more common types of resistance mechanisms known to affect other β-lactams. Resistance to meropenem may not arise as readily in *P. aeruginosa* as it does with most other β-lactams (16).

Our findings that time-kill tests for synergy were more discriminatory than the checkerboard methodology reflect findings by our group and others for other organisms (1, 2, 4). Our study shows that levofloxacin, in sub-MIC concentrations of ≤0.5 μg/ml, was synergistic at 12 h, when combined with cefpirome, ceftazidime, or meropenem in 9 to 11 strains, and had lower synergy rates when combined with gentamicin. Clinical studies are necessary to test the validity of these in vitro findings, as well as the significance of regrowth after 24 h.

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TABLE 2. Results of checkerboard synergy testing[a]

Group (*n*)	Result for combination with FIC index of[b]:							
	Levofloxacin + cefpirome		Levofloxacin + ceftazidime		Levofloxacin + gentamicin		Levofloxacin + meropenem	
	≤0.5	>0.5–4	≤0.5	>0.5–4	≤0.5	>0.5–4	≤0.5	>0.5–4
Susceptible (30)[c]	3	27	0	30	0	30	2	28
Resistant								
Ceftazidime (26)	3	23	3	23	1	25	2	24
Gentamicin (21)	0	21	0	21	0	21	0	21
Meropenem (24)	0	24	4	20	0	24	1	23
Miscellaneous (23)	3	20	1	22	0	23	2	21

[a] No antagonistic FIC indices (>4) were found).
[b] Results for FIC indices of ≤0.5 are synergistic, and those of >0.5 to 4.0 are additive or indifferent.
[c] Susceptible to all compounds.

TABLE 3. Comparison of synergy testing by checkerboard and time-kill methodologies

Strain	MIC (μg/ml)					Result for drug combination by method[a]							
	Levofloxacin	Cefpirome	Ceftazidime	Gentamicin	Meropenem	Levofloxacin + cefpirome		Levofloxacin + ceftazidime		Levofloxacin + gentamicin		Levofloxacin + meropenem	
						C	T	C	T	C	T	C	T
Susceptible to all compounds													
1	8	8	2	4	1	Ad	Ad	Ad	Sy (0.5/8.0)	Ad	Ad	Ad	Sy (0.5/0.5)
2	0.5	8	2	2	1	Ad	Sy (0.125/2)	Ad	Sy (0.125/1)	Ad	Ad	Ad	Sy (0.125/0.25)
3	1	8	4	2	1	Ad	Sy (0.125/4)	Ad	Ad	Ad	Ad	Ad	Sy (0.125/0.25)
Resistant to cefpirome (≤8.0 μg/ml) and ceftazidime													
4	0.5	64	64	0.5	1	Ad	Sy (0.125/16)	Ad	Sy (0.125/32)	Ad	Ad	Ad	Sy (0.125/0.5)
5	2	64	128	2	1	Ad	Sy (0.5/16)	Ad	Sy (0.5/16)	Ad	Ad	Ad	Sy (0.5/0.5)
6	0.5	128	128	0.5	2	Ad	Sy (0.25/64)	Ad	Sy (0.25/64)	Ad	Sy (0.25/0.25)	Ad	Sy (0.25/1)
Resistant to gentamicin													
7	2	8	8	64	0.5	Ad	Sy (0.5/8)	Ad	Sy (0.5/2)	Ad	Ad	Ad	Sy (0.5/0.25)
8	1	8	4	8	4	Ad	Sy (0.25/4)	Ad	Sy (0.25/1)	Ad	Sy (0.25/1)	Ad	Sy (0.25/0.25)
9	0.5	8	8	64	0.5	Ad	Sy (0.06/2)	Ad	Sy (0.06/4)	Ad	Sy (0.06/16)	Ad	Sy (0.06/0.06)
Resistant to meropenem													
10	1	8	2	4	8	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad
11	2	8	8	2	16	Ad	Sy (0.25/8)	Sy	Sy (0.25/4)	Ad	Sy (0.25/0.25)	Ad	Sy (0.25/2)
12	0.125	8	1	1	16	Ad	Sy (0.25/2)	Ad	Ad	Ad	Ad	Sy	Sy (0.25/0.125)

[a] C, checkerboard titration; T, time-kill; Sy, synergistic; Ad, additive or indifferent. Values in parentheses are MICs and indicate the lowest concentration (micrograms per milliliter) of each compound that yielded sustained bactericidal activity (≥100-CFU/ml drop) at 12 h compared to that of the more active drug.

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