

Comparative Susceptibilities of *Pseudomonas aeruginosa* to 1-Oxacephalosporin (LY127935) and Eight Other Antipseudomonal Antimicrobial Agents (Old and New)

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In vitro susceptibilities of 53 clinical isolates of *Pseudomonas aeruginosa* to nine antipseudomonal antibiotics were determined. From 96 to 100% of the isolates were susceptible to piperacillin, ticarcillin, and 1-oxacephalosporin (LY127935). Of the aminoglycosides, 89, 82, 79, and 29% were susceptible to amikacin, tobramycin, gentamicin, and netilmicin, respectively. A total of 96% and 78% of the isolates were susceptible to 1-oxacephalosporin (LY127935) and cefotaxime, respectively, at concentrations of 62.5 µg/ml. Supplementation of testing media by calcium and magnesium not only markedly increased the minimal inhibitory concentrations of the aminoglycosides, but also raised those of cefotaxime and the penicillins; no significant effect was noted with 1-oxacephalosporin. Synergy was not demonstrated consistently in vitro with 1-oxacephalosporin combined with either carbenicillin, ticarcillin, gentamicin, or tobramycin.

1-Oxacephalosporin (LY127935) is a new beta-lactam antimicrobial agent with an extended spectrum of coverage (5). An important characteristic of this drug is its excellent in vitro activity against *Pseudomonas aeruginosa*. Because comparative susceptibility studies of 1-oxacephalosporin and other antibiotics are lacking, we performed parallel in vitro susceptibility tests with 1-oxacephalosporin and eight other antimicrobial agents which have in vitro activity against *P. aeruginosa*. Five of the other drugs are commonly used for *P. aeruginosa* infections (carbenicillin, gentamicin, tobramycin, amikacin, ticarcillin), and four are still undergoing clinical evaluation (netilmicin, piperacillin, cefotaxime, 1-oxacephalosporin [LY127935]).

In addition, we compared the effect of calcium and magnesium supplementation in the testing media on the minimal inhibitory concentration (MIC) obtained with *P. aeruginosa* and the nine antimicrobial agents surveyed. Finally, we sought to determine whether 1-oxacephalosporin could be combined in vitro with other antibiotics to achieve a synergistic effect against isolates resistant to carbenicillin or gentamicin.

Fifty-three isolates of *P. aeruginosa* were obtained from infected patients at Presbyterian-University Hospitals or the Pittsburgh Veterans Administration Medical Center. Forty-one were isolated from urine, four were from respiratory secretions, and eight came from blood. MICs were determined by a microtiter broth dilution method using a 12-channel dispenser, 50-µl mi-

crodiluters, and 9 by 12 microtiter plates (Flow Laboratories) (1). Tests were run in parallel using Mueller-Hinton broth (lot KODHAR, Becton, Dickinson & Co.) and Mueller-Hinton broth supplemented with 65.5 mg of calcium per liter and 22.1 mg of magnesium per liter (MHB-S) (7). 1-Oxacephalosporin was supplied by Eli Lilly and Co., netilmicin came from Schering Corp., piperacillin came from Lederle Laboratories, and cefotaxime was provided by Hoechst-Roussel Pharmaceuticals. A checkerboard microtiter titration method using MHB-S was used to detect synergy against 13 isolates resistant to carbenicillin, gentamicin, or both (6). Fractional inhibitory concentrations and indices were calculated as described by Elion et al (2).

Before 1969, there was a paucity of antimicrobial agents that were clinically useful against serious infections caused by *P. aeruginosa*. Subsequently, gentamicin and carbenicillin were introduced for clinical use as antibiotics with significant antipseudomonal activity. In the last several years we have witnessed a dramatic increase in the introduction of promising antimicrobial agents with activity against *P. aeruginosa*. Despite numerous susceptibility studies published attesting to the in vitro activity of each of the individual antibiotics, there are no available susceptibility studies with *P. aeruginosa* which compare the mainstay antibiotics (e.g., carbenicillin and gentamicin) and the newer antibiotics (e.g., ticarcillin and amikacin) with the newest experimental antibiotics (e.g.,

TABLE 1. Susceptibilities of 53 *P. aeruginosa* isolates to nine antimicrobial agents^a

Drug	% Susceptible ^b	Median MIC	Cumulative % of isolates inhibited at dose ($\mu\text{g/ml}$):								
			≤ 1	2	3.9	7.8	15.6	31.3	62.5	125	≥ 250
Piperacillin	100	7.8		7	31	55	74	94	98	100	
1-Oxacephalosporin (LY127935)	96	15.6				12	69	88	96	100	
Ticarcillin	96	62.5					9	50	87	96	100
Amikacin	89	7.8	2		15	59	89	96	100		
Tobramycin	82	3.9	6	39	70	82	88	88	88	94	100
Gentamicin	79	7.8	2	4	50	79	88	88	88	88	100
Cefotaxime	78	31.3				10	43	63	78	98	100
Carbenicillin	69	125						6	36	69	100
Netilmicin	29	15.6	2			29	68	86	94	94	100

^a Using Mueller-Hinton broth supplemented with calcium and magnesium.

^b Isolates were considered susceptible if MIC was $\leq 125 \mu\text{g}$ of carbenicillin, ticarcillin, or piperacillin per ml; $\leq 62.5 \mu\text{g}$ of 1-oxacephalosporin and cefotaxime per ml; $\leq 15.6 \mu\text{g}$ of amikacin per ml; $\leq 7.8 \mu\text{g}$ of gentamicin, tobramycin, and netilmicin per ml.

TABLE 2. Effect of calcium and magnesium supplementation in the testing media for nine antipseudomonal antimicrobial agents against 53 clinical isolates of *P. aeruginosa*

Drug	MIC decreased by 2 log ₂ (%)	MIC unchanged or increased or decreased by 1 log ₂ (%)	MIC increased by 2 log ₂ (%)	MIC increased by 3 log ₂	MIC increased by 4 log ₂ or greater (%)
Tobramycin	2	2	10	15	71
Amikacin	2	6	8	45	39
Netilmicin	0	10	2	12	76
Gentamicin	0	12	16	37	35
Carbenicillin	2	57	31	6	4
Piperacillin	2	78	16	2	2
Ticarcillin	2	86	12	0	0
Cefotaxime	2	76	20	0	2
1-Oxacephalosporin (LY127935)	0	93	7	0	0

piperacillin, cefotaxime, 1-oxacephalosporin and [LY127935]. Carbenicillin, ticarcillin, and piperacillin are semisynthetic penicillins, whereas gentamicin, tobramycin, amikacin, and netilmicin are aminoglycosides with in vitro activity against *P. aeruginosa*. Cefotaxime and 1-oxacephalosporin (LY127935) are new semisynthetic beta-lactam antibiotics with a broad spectrum of activity including activity against *P. aeruginosa*.

Table 1 ranks the nine antibiotics in descending order on the basis of percentage of isolates susceptible at achievable serum concentrations. Piperacillin was the most active penicillin in vitro. Tobramycin was the most active aminoglycoside in vitro with 39% of the *P. aeruginosa* isolates susceptible to 2 $\mu\text{g/ml}$ or less (compared to 2, 4, and 2% for amikacin, gentamicin, and netilmicin, respectively). However, overall resistance to amikacin was only 11% as opposed to 18% for tobramycin.

The cutoff MICs for susceptibility of *P. aeruginosa* to cefotaxime and 1-oxacephalosporin are uncertain to date; however, serum concentrations of 125 $\mu\text{g/ml}$ are achievable with

TABLE 3. Results of combination testing for synergy against 13 isolates of *P. aeruginosa* resistant to carbenicillin, gentamicin, or both

Combination	% Synergistic ^a	% Indeterminate ^b	% Antagonistic ^c
Carbenicillin plus gentamicin	8	77	15
1-Oxacephalosporin plus carbenicillin	8	84	8
1-Oxacephalosporin plus ticarcillin	0	92	8
1-Oxacephalosporin plus gentamicin	15	77	8
1-Oxacephalosporin plus tobramycin	15	77	8

^a Fractional inhibitory concentration (FIC) ≤ 0.5 .

^b $0.5 < \text{FIC} \leq 1.5$.

^c FIC > 1.5 .

either antibiotic. Since 96 and 78% of the isolates were susceptible to 62.5 μg or less of 1-oxacephalosporin or cefotaxime per ml, both of these new antibiotics may be useful for serious *P. aeruginosa* infections.

Susceptibility testing was performed in par-

allel using MHB-S and unsupplemented media. Table 2 shows the striking effect of divalent cation supplementation for the nine antimicrobial agents tested. The increase in MICs obtained for aminoglycosides using supplemented media is well documented (7, 8), but a significant increase in MICs of cefotaxime and the semisynthetic penicillins was also observed with 12 to 41% of the isolates. Clearly, divalent cation content of the testing media must be controlled before valid comparisons between antipseudomonal antibiotics can be made.

Synergy with the combination of carbenicillin and gentamicin is usually not demonstrable with *P. aeruginosa* isolates that are highly resistant to gentamicin (3, 4). Using selected isolates resistant to carbenicillin, gentamicin, or both, we were unable to demonstrate consistent synergy between 1-oxacephalosporin (LY127935) and carbenicillin, ticarcillin, gentamicin, or tobramycin (Table 3).

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