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Activity of β-Lactam Antibiotics Against *Pseudomonas aeruginosa* Carrying R Plasmids Determining Different β-Lactamases

GEORGE A. JACOBY* AND LORRAINE SUTTON

Massachusetts General Hospital, Boston, Massachusetts 02114

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Azlocillin, carbenicillin, cefsulodin, mezlocillin, nocardicin A, piperacillin, pirbenicillin, sulbenicillin, and ticarcillin, but not HR756, showed reduced activity against *Pseudomonas aeruginosa* strains producing seven types of β-lactamase.

New β-lactam antibiotics have been developed that possess greater potency than carbenicillin against *Pseudomonas aeruginosa* (1, 2, 11-13, 19, 20). Some have the additional virtue of relative or absolute resistance to hydrolysis by at least some of the β-lactamases produced by R plasmids in this organism (3, 14). R plasmids found in *P. aeruginosa* determine at least seven types of β-lactamase that can be distinguished by substrate profile, molecular weight, analytic isoelectric focusing, and other properties (6, 8, 10, 16, 17). Four of these β-lactamases have been termed *Pseudomonas*-specific enzymes (PSE-1, PSE-2, PSE-3, and PSE-4) since they have not yet been found in other genera (4). The other β-lactamase types (TEM-1, TEM-2, OXA-3) are more widely distributed among gram-negative organisms (9). Each of these enzymes can hydrolyze carbenicillin, and use of carbenicillin has led to the emergence of β-lactamase-producing, plasmid-containing *P. aeruginosa* strains (7). To determine whether newer β-lactam antibiotics are also susceptible to *Pseudomonas* plasmid β-lactamases, they have been tested for activity against a set of *P. aeruginosa* strains isogenic except for the presence of plasmids determining these seven β-lactamase types.

Antibiotics were obtained from the following sources: azlocillin and mezlocillin from Delbay Pharmaceuticals, carbenicillin from Roerig, cefsulodin and sulbenicillin from Takeda Chemical Industries, HR756 from Hoechst-Roussel Pharmaceuticals, nocardicin A from the Fujisawa Pharmaceutical Co., piperacillin from Lederle Laboratories, pirbenicillin from Pfizer Inc., and ticarcillin from Beecham Laboratories. The plasmid host was *P. aeruginosa* strain PU21 (5). The properties of the plasmids and of the β-lactamases they produce have been described elsewhere (6). The minimal inhibitory concentration (MIC) was determined by agar dilution, using Mueller-Hinton medium (Difco) plates containing graded concentrations of antibiotics and an innoculum of 10^4 and 10^5 organisms from an overnight culture applied by a replica-plating device.

Table 1 shows the activities of azlocillin, carbenicillin, cefsulodin (SCE-129), HR756, mezlocillin, nocardicin A, piperacillin (T-1220), pirbenicillin, sulbenicillin, and ticarcillin against derivatives of *P. aeruginosa* strain PU21 carrying plasmids determining seven types of β-lactamase. TEM-1- and TEM-2-type enzymes usually provided the highest levels of β-lactam resistance and OXA-3- and PSE-2-type enzymes generally provided the lowest levels. All the plasmid-containing strains had carbenicillin MICs of 1,000 μg/ml or more, except for PU21 (R151) producing PSE-2-type β-lactamase that had a carbenicillin MIC of 200 μg/ml. MICs of sulbenicillin and ticarcillin were comparable to those of carbenicillin, and those of pirbenicillin were also equivalent or only slightly reduced. MICs of mezlocillin, nocardicin A, and azlocillin were 1,000 μg/ml or less, but plasmid-containing strains were more resistant than R^- PU21, except for three R^+ strains, against nocardicin A. Piperacillin MICs were 400 μg/ml or less, but those for all R^+ derivatives were ≥10-fold more than that for strain PU21. Cefsulodin MICs were 200 μg/ml or less, but 12 of 15 R^+ derivatives had enhanced resistance. Only with HR756 were MICs for R^+ and R^- strains equal.

The activity of a β-lactam antibiotic on *P. aeruginosa* depends on its accessibility and affinity for the target enzyme(s) involved in cell wall biosynthesis and on its stability to *Pseudomonas* β-lactamases. Virtually all isolates of *P. aeruginosa* produce an inducible β-lactamase that is active against cephaloridine and penicillin G but not against carbenicillin (15, 18). One mechanism for carbenicillin resistance is the production of an enzyme able to hydrolyze the drug.

TABLE 1. *Comparative activity of β-lactam antibiotics against plasmid-containing P. aeruginosa*

Plasmid	Incompatibility group	β-Lactamase type	MIC (μg/ml) of:								
			Carbenicillin	Sulbenicillin	Ticarcillin	Pirbenicillin	Mezlocillin	Nocardicin A	Azlocillin	Piperacillin	Cefsulodin	HR756
R^-			25	20	20	10	100	50	5	5	20	20
R527	P-1	TEM-1	2,000	2,000	2,000	2,000	400	400	200	200	50	20
Rms165	P-2	TEM-1	1,000	800	1,000	800	200	100	100	100	20	20
pMG20	P-2	TEM-1	2,000	2,000	2,000	800	200	400	100	100	50	20
R2	P-9	TEM-1	4,000	4,000	4,000	2,000	800	800	400	400	100	20
pMG18	P-9	TEM-1	4,000	4,000	4,000	2,000	400	800	200	200	100	20
RP1	P-1	TEM-2	8,000	≥4,000	4,000	4,000	800	800	400	400	100	20
Rm16b	P-1	TEM-2	8,000	≥4,000	4,000	4,000	800	800	800	400	200	20
R91	P-10	TEM-2	4,000	4,000	4,000	4,000	800	800	400	400	100	20
RP1-1	P-11	TEM-2	4,000	4,000	4,000	2,000	400	800	400	200	100	20
RIP64	P-3	OXA-3	2,000	2,000	2,000	800	400	50	100	100	50	20
RPL11	P-2	PSE-1	4,000	≥4,000	4,000	1,000	800	50	200	100	50	20
Rms139	P-2	PSE-1	4,000	≥4,000	4,000	2,000	1,000	400	800	400	100	20
R151	?	PSE-2	200	1,000	200	200	200	800	50	50	20	20
Rms149	P-6	PSE-3	4,000	≥4,000	4,000	800	200	100	100	50	20	20
pMG19	?	PSE-4	8,000	≥4,000	4,000	1,000	800	50	200	100	100	20

Such additional β-lactamases are determined by R plasmids and are produced constitutively (6, 8, 10, 16–18). The seven currently known plasmid-determined β-lactamases found in *P. aeruginosa* thus comprise the genetic repertoire for one type of β-lactam resistance that new anti-*Pseudomonas* antibiotics must overcome.

The plasmids studied in Table 1 all determine β-lactamases active against carbenicillin. The high MICs of carbenicillin, pirbenicillin, sulbenicillin, and ticarcillin indicate that these drugs are all good substrates for plasmid-determined β-lactamases. MICs of azlocillin, mezlocillin, nocardicin A, and piperacillin were lower than that of carbenicillin, but plasmids found in *Pseudomonas* increased the MICs of each drug, all of which are known to be β-lactamase susceptible (1, 2, 20). MICs of cefsulodin were even lower but, again, were increased by plasmid carriage. This cephalosporin derivative is known to be relatively resistant to β-lactamase hydrolysis (14). Only with HR756 were MICs for R^+ and R^- strains equal. Fu and Neu (3) have shown that this novel cephalosporin is resistant to inactivation by several types of β-lactamase produced by gram-negative organisms. Evidently HR756 is also resistant to attack by the seven β-lactamase types studied here and hence is the most promising of the compounds tested to overcome plasmid-determined resistance in *P. aeruginosa*.

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