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In Vitro Antimicrobial Activity of Cefoperazone, Cefotaxime, Moxalactam (LY127935), Azlocillin, Mezlocillin, and Other β-Lactam Antibiotics Against *Neisseria gonorrhoeae* and *Haemophilus influenzae*, Including β-Lactamase-Producing Strains

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Minimum inhibitory concentrations and agar disk diffusion tests were determined on clinical isolates of β-lactamase-positive and β-lactamase-negative *Neisseria gonorrhoeae* and *Haemophilus influenzae* with the newer β-lactam antibiotics, cefoperazone, cefotaxime, moxalactam (LY127935), azlocillin, mezlocillin, and piperacillin, and with seven older β-lactam antibiotics. All the drugs were active against β-lactamase-negative strains of *N. gonorrhoeae* and *H. influenzae*. The drug most active against β-lactamase-positive *N. gonorrhoeae* was cefotaxime, followed closely by cefoperazone, moxalactam, piperacillin, and mezlocillin. The drugs most active against β-lactamase-positive strains of *H. influenzae* were cefotaxime, moxalactam, cefoperazone, and cefamandole.

The β-lactamase-producing strains of *Neisseria gonorrhoeae* and *Haemophilus influenzae* constitute a portion of the clinical isolates in some geographical areas of the world (2, 8-12, 14, 15). Their discovery has stimulated a search for more effective antibiotics among the currently available agents (1, 2, 4, 11, 14-16) and for the synthesis of new compounds. Prominent in the former group are cefamandole, cefuroxime, and cefoxitin. In the latter group, β-lactam antibiotics receiving the greatest attention are cefoperazone (T-1551), cefotaxime (HR756), moxalactam (LY127935 or 6059-S), azlocillin, mezlocillin, and piperacillin (3, 5, 7, 13, 17; T. Yoshida, M. Narisada, S. Matsuura, W. Nagata, and S. Kuwahara, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 151, 1978).

In this study, the in vitro antimicrobial activity of 13 β-lactam antimicrobial drugs against recent clinical isolates of *N. gonorrhoeae* and *H. influenzae* was determined.

The antimicrobial agents tested were received from Pfizer Inc., New York, N. Y. (cefoperazone); Hoechst-Roussel Pharmaceuticals, Somerville, N.J. (cefotaxime); Eli Lilly & Co., Indianapolis, Ind. (moxalactam); and Delbay Research Corporation, Florham Park, N.J. (azlocillin and mezlocillin). The *N. gonorrhoeae* and *H. influenzae* strains were randomly selected from isolates referred to the Antimicrobics Investigation Section, Center for Disease Control, Atlanta, Ga. in 1979. Equal numbers of β-lactamase-positive (β-lac$^+$) and β-lactamase-negative (β-lac$^-$) bacteria were tested. The minimum inhibitory concentrations (MICs) were determined by methods previously described (1, 2, 4, 13). The disk diffusion tests for *H. influenzae* and *N. gonorrhoeae* were performed on Mueller-Hinton agar plus 1% hemoglobin and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) and on GC agar base plus 1% IsoVitaleX, respectively (1, 2, 13). The *Haemophilus* test plates were incubated at 35°C for 24 h in ambient air, and the gonococcal test plates were incubated at 35°C for 24 h in a candle extinction jar. Diameters of zones of inhibition were measured with a ruler to the nearest millimeter. Cefoperazone disks with concentrations of 30, 50, 75, and 100 μg were prepared by the investigators, and the other disks were obtained from a commercial source (BBL). Some of these disks were made for purposes of investigation and are not available otherwise.

Table 1 shows the zone diameters and MICs obtained with the 13 antibiotics when 25 β-lac$^-$ and 25 β-lac$^+$ strains of *N. gonorrhoeae* were tested.

The β-lac$^-$ strains of gonococci were very susceptible to all the antibiotics, as indicated by the MICs and disk diffusion results. However, the three cephalosporins under investigation (cefotaxime, moxalactam, and cefoperazone) were more active than the four that are available. In

TABLE 1. *Comparison of the antimicrobial activity of seven cephalosporins and six semisynthetic penicillins against 50 strains of N. gonorrhoeae, measured by disk diffusion and MIC methods*

Antibiotic	Disk concn (μg)	β-lac⁻				β-lac⁺			
		Inhibitory zone diam (mm)		MIC (μg/ml)		Inhibitory zone diam (mm)		MIC (μg/ml)	
		Range	Median	Range	Mode	Range	Median	Range	Mode
Cefoperazone	30	40->50	>50	≤0.004-0.06	≤0.004	31-46	38	≤0.004-0.25	0.03
Cefotaxime	30	46->50	>50	≤0.004-0.015	≤0.004	41->50	50	≤0.004-0.12	≤0.004
Moxalactam	30	40->50	>50	≤0.004-0.06	≤0.004	34->50	43	0.015-0.25	0.03
Cefamandole	ND[a]			≤0.004-1	0.03			0.015-8	0.25
Cefazolin	ND			0.12-2	0.12			0.015-8	1
Cefoxitin	ND			0.015-0.5	0.12			≤0.004-4	0.5
Cephalothin	30	30->50	40	0.06-1	0.12	16-36	30	≤0.004-16	0.5
Azlocillin	30	44->50	>50	≤0.004-0.12	≤0.004	6-40	28	0.25-1	0.5
Mezlocillin	75	50->50	>50	≤0.004-0.12	≤0.004	7-40	31	0.03-0.25	0.03, 0.12
Ampicillin	ND			≤0.004-0.25	≤0.004			0.25-16	8
Carbenicillin	100	38->50	>50	0.008-0.5	0.015	17-33	21	0.5-16	8
Piperacillin	ND			≤0.004-0.06	≤0.004			0.03-0.25	0.06
Ticarcillin	ND			0.008-0.5	0.015			1-8	4, 8

[a] ND, Tests not done.

general, they were at least 10 times as active as cefamandole and at least 100 times as active as cefazolin, cefoxitin, and cephalothin. Piperacillin, mezlocillin, azlocillin, and ampicillin were slightly more active against the β-lac⁻ strains than carbenicillin and ticarcillin, which were equal in activity.

Many of the β-lac⁺ strains of gonococci were also very susceptible to these drugs, but, in most cases, the MICs were considerably higher. The modal MIC of cefotaxime remained the same (≤0.004 μg/ml), but the modes of the other cephalosporins were 5 to 10 times higher. The effect on the penicillins was much more marked on ampicillin, carbenicillin, and ticarcillin, with increases of approximately 1,000-fold, and on azlocillin, with increases of approximately 100-fold. The effect on piperacillin and mezlocillin was increased approximately 10-fold.

Even though the median zone diameters with the cephalosporins remained large when β-lac⁺ strains were tested, they were lower with all seven drugs tested than those obtained with β-lac⁻ strains. The differences in zone diameters were more marked with the penicillins than with the cephalosporins. There was no overlap of the penicillin zone diameters obtained with the β-lac⁻ and β-lac⁺ strains. Therefore, the penicillins discriminated between β-lac⁻ and β-lac⁺ strains better than the cephalosporins did when the disk diffusion test was performed.

A closer comparison of the activity of these 13 β-lactam drugs against β-lac⁻ and β-lac⁺ *N. gonorrhoeae* is shown by geometric means in Table 2 and by cumulative percent inhibition in Fig. 1. The drugs under investigation, cefotaxime, moxalactam, piperacillin, azlocillin, cefoperazone, and mezlocillin, were essentially equal in their activities against β-lac⁻ strains and were more active than the available drugs, ampicillin, cefamandole, carbenicillin, ticarcillin, cefoxitin, cephalothin, and cefazolin. Although ampicillin was not as active overall as the new drugs (Table 2), most strains were about as susceptible to ampicillin as to the new drugs (Fig. 1). With the β-lac⁺ strains, the order of activity determined on the basis of geometric means (Table 2) and cumulative MICs (Fig. 1) was different. Cefotaxime was essentially unchanged and was the most active drug; it was followed, in decreasing order, by cefoperazone, moxalactam, piperacillin, mezlocillin, cefoxitin, cefamandole, cephalothin, cefazolin, azlocillin, ticarcillin, carbenicillin, and ampicillin. The largest change in MICs between β-lac⁻ and β-lac⁺ strains was with ampicillin, with the geometric mean MIC against the β-lac⁺ strains being 375 times greater than the geometric mean MIC against the β-lac⁻ strains. Other marked changes in MICs were observed with carbenicillin, ticarcillin, and azlocillin.

Thus, cefotaxime, moxalactam, and cefoperazone were more active than other cephalosporins that have been tested against *N. gonorrhoeae* (1, 2, 4, 11, 13, 15). Among the cephalosporins that are now being marketed, cefuroxime has been shown to be the most active against β-lac⁻ and β-lac⁺ strains of gonococci (10, 11, 15). Piperacillin and mezlocillin have marked activity against both β-lac⁻ and β-lac⁺ strains of gonococci. This confirms a previous report from our laboratory on the unique activity of piperacillin on gonococci (13).

Table 3 shows the MICs and disk diffusion results obtained with these 13 antibiotics when 20 β-lac⁻ and 20 β-lac⁺ strains of *H. influenzae* were tested. All of the β-lac⁻ strains were sus-

ceptible to the lowest concentrations of the drugs tested except cefamandole, cefoxitin, cephalothin, and cefazolin. With the β-lac$^+$ strains, cefotaxime and moxalactam were still active at the lowest dilutions tested, as was cefoperazone with most strains. The other cephalosporins were similar in activity, but in some cases they were slightly more active on these strains than on the β-lac$^-$ strains. The penicillins, however, were affected by the β-lactamase, with increases in MICs of at least 512-fold in some cases. The greatest change was again with ampicillin. The greater activity of the β-lactamase on the penicillins was also reflected by the greater change in the disk diffusion results. The zone diameters obtained with the penicillins for β-lac$^+$ *H. influenzae* were considerably smaller than for the β-lac$^-$ strains. In contrast to the results with gonococci, there was an overlap of zone diameter ranges between the β-lac$^-$ and β-lac$^+$ strains with all three penicillins tested by disk diffusion, but these overlaps were minimal.

TABLE 2. *Geometric mean MICs of 13 antibiotics for 25 β-lac$^-$ and 25 β-lac$^+$ strains of N. gonorrhoeae*

Antibiotic	Geometric mean MICs (μg/ml) for:		
	β-lac$^-$	β-lac$^+$	β-lac$^+$/β-lac$^-$[a]
Cefotaxime	0.0043	0.0064	1.5
Moxalactam	0.0056	0.0564	10.1
Piperacillin	0.0062	0.0774	12.4
Azlocillin	0.0064	0.4900	76.6
Cefoperazone	0.0068	0.0256	3.7
Mezlocillin	0.0085	0.0798	9.3
Ampicillin	0.0120	4.5200	375.4
Cefamandole	0.0492	0.2200	4.5
Carbenicillin	0.0540	4.3600	80.7
Ticarcillin	0.0564	4.0000	70.9
Cefoxitin	0.1350	0.2000	1.5
Cephalothin	0.2250	0.3900	1.7
Cefazolin	0.3000	0.4175	1.4

[a] Geometric mean of β-lac$^+$ strains divided by geometric mean of β-lac$^-$ strains.

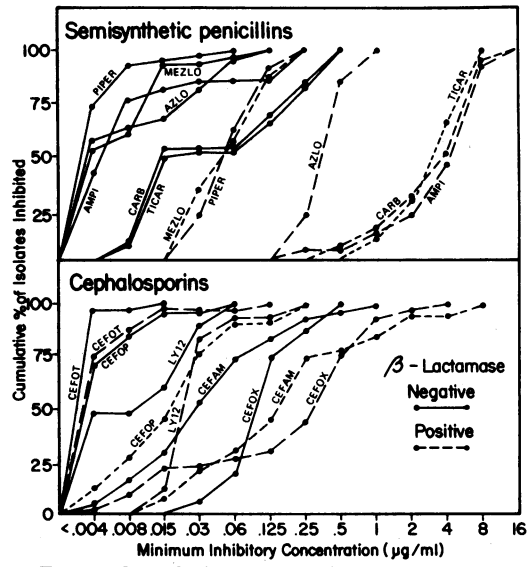

FIG. 1. *Cumulative percent of N. gonorrhoeae isolates inhibited by various concentrations of antibiotics. Piper, piperacillin; azlo, azlocillin; mezlo, mezlocillin; ampi, ampicillin; carb, carbenicillin; ticar, ticarcillin; cefot, cefotaxime; cefop, cefoperazone; LY12, moxalactam; cefam, cefamandole; cefox, cefoxitin. Symbols: ●---●, β-lac$^+$ strains; ●—●, β-lac$^-$ strains.*

TABLE 3. *Comparison of the antimicrobial activity of seven cephalosporins and six semisynthetic penicillins against 40 strains of H. influenzae, measured by disk diffusion and MIC methods*

Antibiotic	Disk concn (μg)	β-lac$^-$				β-lac$^+$			
		Inhibitory zone diam (mm)		MIC (μg/ml)		Inhibitory zone diam (mm)		MIC (μg/ml)	
		Range	Median	Range	Mode	Range	Median	Range	Mode
Cefoperazone	30	27–40	30	≤0.25	≤0.25	30–40	34	≤0.25–0.5	≤0.25
Cefotaxime	30	28–38	35	≤0.06	≤0.06	33–40	38	≤0.06	≤0.06
Moxalactam	30	27–35	31	≤0.06	≤0.06	30–42	35	≤0.06	≤0.06
Cefamandole	ND[a]			≤0.06–0.25	0.25			≤0.06–8	0.12
Cefazolin	ND			2–16	4			2–32	2
Cefoxitin	ND			0.5–4	2			0.5–2	0.5
Cephalothin	30	19–25	23	0.5–4	2	20–25	24	0.5–8	1
Azlocillin	75	29–45	35	≤0.25	≤0.25	6–27	23	4–128	16
Mezlocillin	75	27–45	35	≤0.25	≤0.25	6–29	23	2–128	4
Ampicillin	ND			≤0.25	≤0.25			16–>128	64
Carbenicillin	100	28–43	33	≤0.25	≤0.25	7–31	26	0.5–128	1
Piperacillin	ND			≤0.25	≤0.25			1–128	1
Ticarcillin	ND			≤0.25	≤0.25			1–128	1, 2

[a] ND, Tests not done.

Thus, the most active cephalosporins on β-lac⁻ and β-lac⁺ strains of *H. influenzae* were cefotaxime, moxalactam, and cefaperazone, and the most active penicillins were piperacillin, carbenicillin, and ticarcillin. However, unlike the results with *N. gonorrhoeae*, piperacillin activity on *H. influenzae* was equal to that of carbenicillin and ticarcillin, thus confirming our previous report (13).

Although cefoxitin, cefotaxime, and moxalactam have been reported to be stable to β-lactamase hydrolysis (3), increases in modal MICs of cefoxitin and moxalactam were obtained (Table 1). There were also increases in cephalosporin MICs for the β-lac⁺ strains when geometric means were examined; for most of these drugs, the change was minimal, with the largest ratio being 10:1 for moxalactam.

The increases in modal and geometric mean MICs of the penicillins were lowest with piperacillin and mezlocillin. The smallest changes in zone diameters were obtained with cefotaxime, and the largest changes in zone diameters were obtained with azlocillin and mezlocillin. These results may indicate that all of these drugs could be at least slightly susceptible to hydrolysis by this β-lactamase. Increased resistance to these drugs, however, may be due to a mechanism such as cell wall permeability or to a combination of mechanisms (6, 13). As both β-lac⁺ *N. gonorrhoeae* and *H. influenzae* produce the TEM type III enzyme, the difference in activity of a β-lactam drug on these two organisms is due to a mechanism other than the action of the enzyme (13). This is the case with piperacillin, which is more active on β-lac⁺ *N. gonorrhoeae* than on β-lac⁺ *H. influenzae*.

The general activity of the drugs under investigation on these two genera is impressive and may indicate that they merit evaluation in clinical trials. Cephalosporins have not been used to treat patients with meningitis because therapeutic levels were not readily obtained in the spinal fluid. However, cefotaxime and moxalactam inhibited all strains of *H. influenzae* (including β-lac⁺) at a concentration of 0.06 μg/ml, and the highest cefoperazone MIC was 0.5 μg/ml. It is possible that these levels can be achieved and that they can be used to treat patients with meningitis. In addition, cephalosporins have not been generally recommended for the treatment of patients with gonorrhea, but the latest Center for Disease Control guidelines include cefoxitin as a suggested alternative, and cefuroxime appears to be promising (8, 11). As the three new cephalosporins are extremely active against both β-lac⁻ and β-lac⁺ gonococci, they deserve expanded clinical trials in the treatment of gonococcal infections.

Piperacillin and mezlocillin are very active against both β-lac⁺ and β-lac⁻ gonococci and appear to be resistant (or at least relatively resistant) to TEM β-lactamase. They deserve to be considered for clinical trials in the treatment of gonorrhea. The use of these penicillins in the treatment of patients with some *H. influenzae* infections would, however, have to be approached with more caution as some strains would require at least 128 μg/ml for inhibition, a level which is not likely to be achieved in the spinal fluid or the middle ear.

In conclusion, these β-lactam antibiotics under investigation are generally more active in vitro on *N. gonorrhoeae* and *H. influenzae* than the more generally available β-lactam drugs used in this study.

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