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In Vitro Susceptibility of Gram-Negative Bacilli from Pediatric Patients to Moxalactam, Cefotaxime, Ro 13-9904, and Other Cephalosporins

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Moxalactam, Ro 13-9904, cefotaxime, cefoperazone, older cephalosporins, and four aminoglycosides were tested in vitro against 432 strains of gram-negative bacteria isolated from pediatric patients. The new drugs were uniformly active against coliform bacilli obtained from patients with meningitis and against aminoglycoside-resistant coliform bacilli.

Numerous reports have attested to the enhanced potency of the "third generation" cephalosporin derivatives against gram-negative bacteria in comparison with older β-lactam antibiotics. These investigational drugs include cefotaxime (HR 756), cefoperazone (T 1551), Ro 13-9904, and an oxa-β-lactam derivative, moxalactam (LY 127935 [6059S]). In the field of pediatrics, they are of particular interest as candidate drugs for treatment of coliform meningitis, infections due to β-lactamase-producing *Haemophilus influenzae* type b, and nosocomial infections caused by bacilli that are multiply resistant to aminoglycoside antibiotics.

Accordingly, we have tested the above-mentioned drugs, cefoxitin, cefamandole, cefuroxime, ampicillin, and four aminoglycosides (gentamicin, kanamycin, amikacin, and netilmicin) against a variety of gram-negative bacilli isolated from pediatric patients. The meningitis strains of coliform bacilli were from patients enrolled in the Neonatal Meningitis Cooperative Study (3). and the multiply aminoglycoside-resistant coliforms were mainly strains sent to our laboratory from many institutions in North and South America. The *Pseudomonas* strains were from the Parkland Memorial Hospital Pediatric Burn Unit, Dallas, Tex., and 45% were resistant to carbenicillin, ticarcillin, gentamicin, tobramycin, and kanamycin, as determined in the clinical laboratory. Although there were no duplicate strains from the same patient, it is likely that some *Pseudomonas* strains were identical since they represented nosocomial infections. There was no known epidemiological connection between other patients.

Laboratory standards of designated potency were obtained from the manufacturers. In preliminary trials with moxalactam and Mueller-Hinton broth, it was determined that there was an inoculum effect on the minimal inhibitory concentration (MIC) and minimal bactericidal concentration of four dilutions in the inoculum range of 10^2 to 10^6 colony-forming units. With an inoculum of 10^8 colony-forming units, coliform bacilli were resistant to more than 8 μg of moxalactam per ml.

In other preliminary trials with 15 strains of coliform bacilli from patients with meningitis, Mueller-Hinton broth and agar dilution results were compared. Moxalactam MICs were identical or within one dilution by the two methods, and minimal bactericidal concentrations in broth were the same as or within one dilution of the MIC.

Subsequent testing of *Enterobacteriaceae* and *Pseudomonas* spp. was done by agar dilution on Mueller-Hinton agar with 10^5 colony-forming units of inoculum diluted from overnight Mueller-Hinton broth seed cultures after 16 h of incubation at 35°C. A Steers-Foltz replicating device was used. In all tests, an inoculum control was performed with each strain, and the actual range of inocula was from 5×10^4 to 1×10^5 colony-forming units. *Haemophilus* strains were tested with 1% supplement C (Difco Laboratories) added to the media. Gonococci were tested on proteose no. 3 agar (Difco) with 1% hemoglobin (Difco) and 1% IsoVitaleX (BBL Microbiology Systems) and incubated in a candle extinction jar. Freshly prepared antibiotic standards were incorporated into the agar. With cefotaxime, cefoperazone, moxalactam, and Ro 13-9904, twofold dilutions from 32 μg/ml were used; with other antibiotics, the twofold dilutions were from 20 μg/ml. Cefoperazone was tested only against the coliform meningitis strains. The MIC was determined as the lowest concentration causing no growth or fewer than 10 discrete colonies, which represented more than 99% inhibition. Resistance was defined as an MIC of more than 8 or 10 μg/ml.

Modal MICs are shown in Table 1, and MICs for 90% of the strains are shown in Table 2.

TABLE 1. *MICs of antibiotics tested against various gram-negative bacilli*

Bacteria	No. of strains tested	Modal MIC (μg/ml)[a]										
		LY	Ro	CTX	CFT	CFM	CFX	GEN	KAN	AMI	NET	AMP
Salmonella spp.	49	0.12	0.06	0.06	2.5	0.62	10	1.25	5	2.5–5	0.62	0.62
Shigella spp. (ampicillin-resistant)	44	0.06	0.015	0.03	2.5	2.5–5	5	1.25	5	5	1.25	>20
Shigella spp. (ampicillin-susceptible)	50	0.06	0.015	0.03	2.5	0.31	2.5	1.25	5	5	0.62	5
Haemophilus spp. (β-lactamase-negative)	33	0.03	≤0.004	0.008	1.25	0.31	0.31	NT	NT	NT	NT	0.31
Haemophilus spp. (β-lactamase-positive)	27	0.015	≤0.004	≤0.004	0.62	0.31	0.31	NT	NT	NT	NT	10
Gonococci (β-lactamase-negative)	25	0.008–0.015	0.008	≤0.008	0.31	NT	0.015	NT	NT	NT	NT	NT
P. aeruginosa	31	8–16	16	16	NT	NT	NT	NT	NT	NT	NT	NT
Coliform bacilli (aminoglycoside-resistant)	57	0.06	0.03	0.06	2.5	1.25–10	2.5	>10	>20	2.5	>10	NT
Coliform bacilli (meningitis strains)	116	0.06	0.03	0.06	2.5	0.62	5	1.25	5	2.5	0.31	2.5

[a] LY, Moxalactam; Ro, Ro 13-9904; CTX, cefotaxime; CFT, cefoxitin; CFM, cefamandole; CFX, cefuroxime; GEN, gentamicin; KAN, kanamycin; AMI, amikacin; NET, netilmicin; AMP, ampicillin; NT, not tested.

TABLE 2. *MICs for 90% of strains*

Bacteria	No. of strains tested	MIC (μg/ml)[a]										
		LY	Ro	CTX	CFT	CFM	CFX	GEN	KAN	AMI	NET	AMP
Salmonella spp.	49	0.12	0.06	0.12	5	2.5	10	5	5	5	1.25	2.5
Shigella spp. (ampicillin-resistant)	44	0.25	0.03	0.06	5	10	5	1.25	10	10	1.25	>20
Shigella spp. (ampicillin-susceptible)	50	0.25	0.03	0.03	5	0.31	5	1.25	10	5	1.25	5
Haemophilus spp. (β-lactamase-negative)	33	0.03	≤0.004	0.008	2.5	0.62	0.62	NT	NT	NT	NT	0.31
Haemophilus spp. (β-lactamase-positive)	27	0.03	≤0.004	0.008	2.5	0.62	0.62	NT	NT	NT	NT	10
Gonococci (β-lactamase-negative)	25	0.06	≤0.008	≤0.008	0.31	NT	0.16	NT	NT	NT	NT	NT
P. aeruginosa	31	>16	>16	>16	NT	NT	NT	NT	NT	NT	NT	NT
Coliform bacilli (aminoglycoside-resistant)	57	0.12	0.12	0.12	10	>20	20	>10	>20	5	>10	NT
Coliform bacilli (meningitis strains)	116	0.25	0.06	0.12	10	>20	>20	2.5	20	5	0.62	>20

[a] See Table 1, footnote *a*.

Salmonella spp., *Shigella* spp. and β-lactamase-negative gonococci were uniformly susceptible to low concentrations of moxalactam, Ro 13-9904, and cefotaxime. One *Salmonella* strain was resistant to cefoxitin, three strains were resistant to cefamandole, four strains were resistant to ampicillin and cefuroxime, and two strains were resistant to kanamycin. Ampicillin-resistant and -susceptible *Shigella* strains were comparably inhibited by cefoxitin, cefuroxime, and the aminoglycosides. However, with cefamandole, ampicillin-susceptible *Shigella* spp. were inhibited by 1 μg/ml or less, but 21 ampicillin-resistant *Shigella* spp. required from 2 to 8 μg/ml for inhibition, and two strains were resistant.

Most *Pseudomonas aeruginosa* strains, including the 14 strains resistant to carbenicillin and the aminoglycosides, were inhibited by 8 or 16 μg of moxalactam, Ro 13-9904, or cefotaxime per ml, but 11, 8, and 9 strains, respectively, required 32 μg/ml for inhibition. One strain of *Pseudomonas cepacia* and one of *Pseudomonas maltophilia* were susceptible to the three drugs.

Susceptibilities of the coliform bacteria from infants with meningitis are shown in Fig. 1. Of 116 strains, 84 were *Escherichia coli*. None was resistant to moxalactam, Ro 13-9904, cefotaxime, or cefoperazone. For other drugs, the numbers of resistant strains were as follows: cefoxitin, 8; cefamandole, 18; cefuroxime, 15; gentamicin, 2; kanamycin, 15; amikacin, 5; netilmicin, 1; and ampicillin, 32. Additionally, there were two strains of *Flavobacterium* resistant to all antibiotics.

Of the multiply resistant coliform bacilli, 73% were resistant to gentamicin, 97% were resistant to kanamycin, 64% were resistant to netilmicin, and only 4% were resistant to amikacin. The number of resistant strains included 23 *Klebsiella* spp., 17 *E. coli*, 9 *Enterobacter* spp., 7 *Serratia* spp., and 1 *Salmonella* sp. All were inhibited by less than 1 μg of moxalactam, Ro 13-9904, and cefotaxime per ml. Cefamandole was somewhat less active than cefuroxime or cefoxitin (Fig. 2).

With the exception of ampicillin, β-lactamase-positive and -negative *H. influenzae* type b were comparably inhibited by each drug, but the three new drugs were most active, and cefoxitin was somewhat less active than cefuroxime or cefamandole (Fig. 3).

In summary, the new cephalosporin derivatives were uniformly more active against coliform bacilli, *Haemophilus* spp., and gonococci than cefuroxime, cefamandole, cefoxitin, and the aminoglycosides. Their activity against *P. aeruginosa* was considerably less, but they were active against carbenicillin- and aminoglycoside-resistant strains. All coliform bacilli from patients with meningitis and all aminoglycoside-resistant coliform bacilli were susceptible to extremely low concentrations of these investigational drugs. The order of activity was as follows: Ro 13-9904 > cefotaxime > moxalactam > cefoperazone. However, these differences would probably be of little clinical importance, in view of the great potency of all of them. With aminoglycoside therapy, an average of 3.5 days is required to sterilize the cerebrospinal fluid in infants with coliform meningitis (3). The possibility that the greatly increased in vitro activity of these new agents will result in more rapid eradication of bacteria is supported by experiments with moxalactam in the infant rat (2) and rabbit (4) experimental meningitis models and

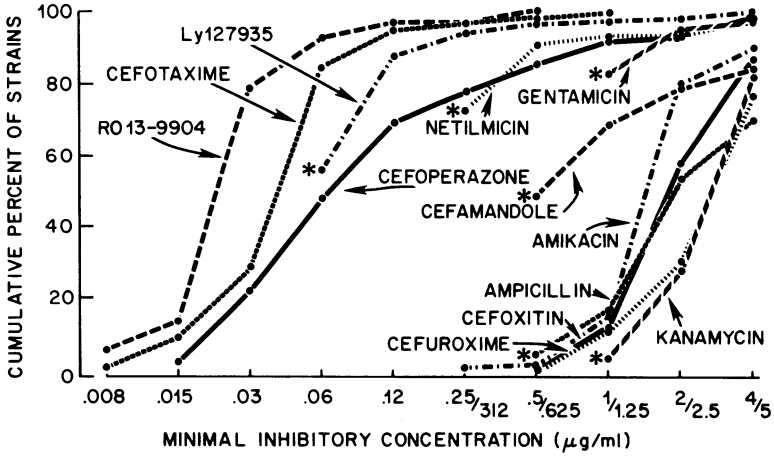

FIG. 1. *Cumulative susceptibilities to the indicated antibiotics of isolates from patients with meningitis. Asterisks indicate the lowest concentration tested.*

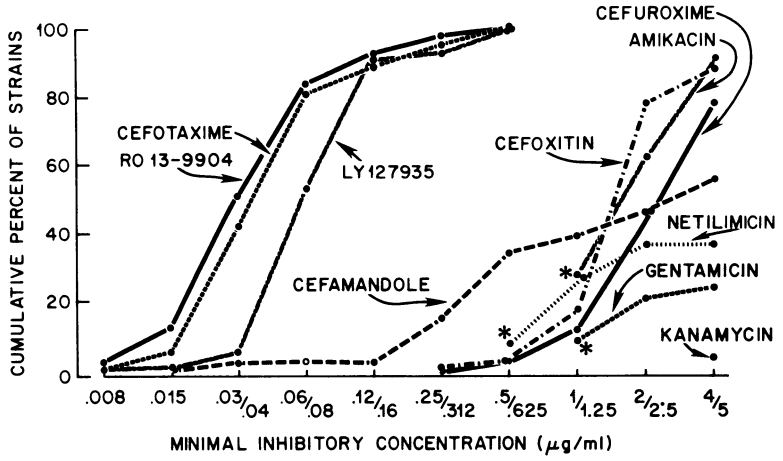

FIG. 2. *Cumulative susceptibilities of 57 coliform bacilli resistant to two or more aminoglycoside antibiotics. Asterisks indicate the lowest concentration tested.*

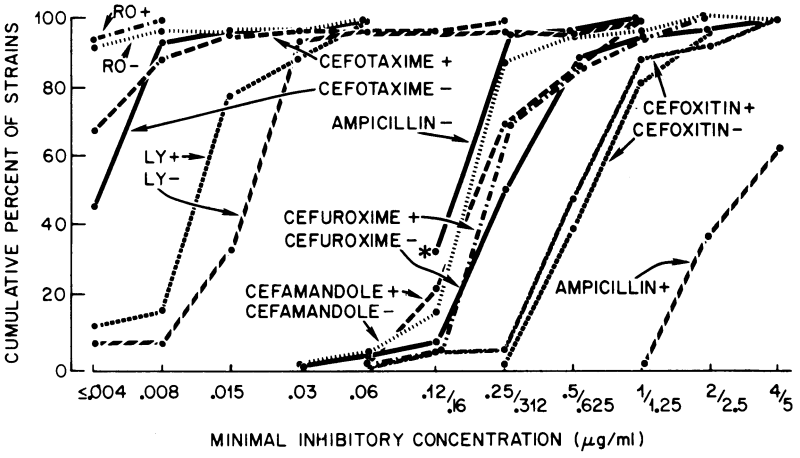

FIG. 3. *Cumulative susceptibilities of 33 β-lactamase-negative (−) and 27 β-lactamase-positive (+) H. influenzae type b strains. The asterisk indicates the lowest dilution of ampicillin tested. RO, Ro 13-9904; LY, LY127935.*

by the preliminary experience with five neonates and one child treated for coliform meningitis with cefotaxime (1). Moxalactam has greater penetrability into cerebrospinal fluid than the other three new cephalosporins (4), and this feature makes it a particularly attractive drug for therapy of coliform meningitis. Clinical trials are proceeding apace.

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