ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1980, p. 470-473
0066-4804/80/03-0470/04$02.00/0

Vol. 17, No. 3

In Vitro Susceptibility of 104 Clinical Isolates of *Haemophilus influenzae* to Moxalactam (LY127935), Ampicillin, Chloramphenicol, and Ticarcillin

EDWARD O. MASON, JR.,[1,2*] SHELDON L. KAPLAN,[1,2] DONALD C. ANDERSON,[1,2] DAVID B. HINDS,[3] AND RALPH D. FEIGIN[1]

Department of Pediatrics, Baylor College of Medicine,[1] Charles T. Parker Memorial Laboratory[2] and Microbiology Section, Department of Pathology,[3] Texas Children's Hospital, Houston, Texas 77030

A total of 104 strains of *Haemophilus influenzae* isolated from pediatric patients over a 1-year period were tested for susceptibility to moxalactam (LY127935), ampicillin, chloramphenicol, and ticarcillin. Of these strains, 30 produced β-lactamase. LY127935 inhibited 99% of the strains at a concentration of 0.125 μg/ml; the remaining strain was inhibited by this antibiotic at 0.25 μg/ml. β-Lactamase-producing strains were inhibited by ampicillin at ≥2 μg/ml. β-Lactamase-negative strains were all inhibited by ampicillin at ≤1 μg/ml, except for one nontypable strain which required 2 μg of ampicillin per ml for inhibition. All strains were susceptible to chloramphenicol at ≤4 μg/ml. β-Lactamase-producing strains were less susceptible (geometric mean = 4.702 μg/ml) to ticarcillin than were strains which did not produce β-lactamase (geometric mean = 0.331 μg/ml). LY127935 susceptibility was not influenced by increasing inoculum size, as was ampicillin susceptibility. Combinations of LY127935 and chloramphenicol or ampicillin were not antagonistic in vitro.

The emergence in 1974 (3, 4) of ampicillin-resistant *Haemophilus influenzae* type b dictated the use of chloramphenicol for the treatment of serious infections due to this organism (10). The prevalence of ampicillin-resistant strains varies in different regions of the country (3, 4); in our pediatric population, the incidence of ampicillin-resistant *H. influenzae* type b has risen from 1.6% in 1974 to 14.3% in 1978. Recently, several centers have reported *H. influenzae* isolates resistant to chloramphenicol (5, 6). Studies at Shionogi Research Laboratories of the new semisynthetic β-lactam, moxalactam (LY127935), on a small number of strains of *H. influenzae* have indicated uniform susceptibility among ampicillin-resistant and ampicillin-susceptible strains (unpublished data). In addition, this antibiotic may adequately penetrate into the cerebrospinal fluid (CSF) of newborn infants (personal communication, R. Kammer, Lilly Research Laboratories). Thus, LY127935 appears to be a promising new antibiotic for the treatment of systemic infections caused by *H. influenzae*.

This study summarizes our experience with clinical strains of *H. influenzae* that were isolated over a 1-year period at Texas Children's Hospital. The in vitro susceptibility of the strains to compound LY127935, ampicillin, chloramphenicol, and ticarcillin also are reported.

MATERIALS AND METHODS

Bacterial strains. *H. influenzae* strains were isolated by the Clinical Microbiology Laboratory, Department of Pathology, Texas Children's Hospital, Houston. After isolation on chocolate agar, each strain was tested for the elaboration of β-lactamase and frozen at −70°C in glycerol-tryptic soy broth until further studies could be performed. A total of 120 strains of *H. influenzae* were isolated from unrelated pediatric patients during the period 1 July 1978 to 30 June 1979. Complete biochemical and antibiotic susceptibility data were available on 104 of these strains. The identification of the strains of *H. influenzae* was reconfirmed by gram stain, microscopic morphology, requirement for nicotinamide adenine dinucleotide, and lack of ability to synthesize porphobilinogen and porphyrins from δ aminolaevulinic acid (11). The strains were serotyped, using overnight broth cultures, by countercurrent immunoelectrophoresis with antisera obtained from Hyland Laboratories (type b) and Burroughs-Wellcome Laboratories (types a, c, d, e, and f) (13).

Antibiotic susceptibility studies. Production of β-lactamase was assessed by the acidometric method of Escamilla (8). Susceptibility studies were performed by the agar dilution method, using Mueller-Hinton agar supplemented with nicotinamide adenine dinucleotide and hemin (each at 10 μg/ml; Sigma Chemical Co.) (2). The agar plates, containing $\log_2$ concentrations of antibiotic, were inoculated by using a Steers-Foltz type replicator (16). The replicator delivered an inoculum of approximately 10^5 colony-forming units (CFU). In some experiments, the concentration of bacteria was varied to determine the effect of increas-

ing inoculum. Plates were incubated at 35°C without CO_2. The minimal inhibitory concentration (MIC) was defined as the concentration of antibiotic which allowed no visible growth of *H. influenzae* after 48 h of incubation. Compound LY127935 (Lilly Laboratories), ampicillin (Bristol Laboratories), chloramphenicol (Warner Lambert/Parke, Davis & Co.), and ticarcillin (Beecham Laboratories) were obtained as standard powders from the manufacturers.

Susceptibility to antibiotic combinations. Microbroth dilution synergy tests were performed in 96-well U-bottomed sterile plastic microtiter plates (Cooke Engineering Co., Alexandria, Va.). Ampicillin and chloramphenicol were tested alone and in combination with compound LY127935 in a "checkerboard" distribution. *H. influenzae* strains were added to wells (100-μl volumes) in both a high inoculum consisting of 10^6 CFU and a low inoculum consisting of 10^4 CFU. The plates were incubated at 35°C overnight, and the MIC was recorded as the lowest concentration of antibiotic which inhibited the growth of the organism as determined by the lack of visible turbidity. Synergy was considered present if the MIC of each antibiotic in combination was one-fourth or less of the MIC of that antibiotic alone. Partial synergy was judged to exist when the MIC of one of the antibiotics in combination was one-fourth or less of the MIC of that antibiotic alone but the MIC of the second antibiotic was only one-half of the MIC when used alone. The antibiotics were considered indifferent if the MIC of either antibiotic alone was unchanged when used in combination. The combination was considered antagonistic if the MIC of one of the antibiotics was increased over the MIC of that antibiotic when used alone (12).

RESULTS

Bacterial strains. Fifty-nine strains (49.2% of all strains) were isolated from the CSF of children with bacterial meningitis. Thirty-three (27.5% of all strains) were isolated from the blood of children with a variety of systemic infections (6 pneumonia, 3 meningitis, 11 cellulitis, 7 septicemia, 4 epiglottitis, and 2 septic arthritis). In addition, strains isolated as pure cultures from sites of infections included: three bone/joint, one lung aspirate, one skin aspirate (cellulitis), four middle ear aspirates, and one ventricular-peritoneal shunt CSF. Eighteen strains could not be definitively classified as causative agents of infection. Of the CSF isolates and the blood isolates 29 and 30%, respectively, elaborated β-lactamase. Isolation of strains of *H. influenzae* elaborating β-lactamase was 0% in the July–September quarter and 39.5, 27.0, and 25.6% in subsequent quarters. β-Lactamase was elaborated by 29.2% of all the strains over the one-year period. Of the 104 *H. influezae* isolates, 86 (82.7%) were serotype b.

Antibiotic susceptibility. The results of agar dilution susceptibility tests were determined after 48 h of incubation because the distinction between growth and initial inoculum was clearer then than after 24 h of incubation. Effect of inoculum size on agar dilution susceptibility was tested with 36 strains, of which 12 elaborated β-lactamase. There was no effect on the concentration of LY127935, chloramphenicol, or ticarcillin required to inhibit any of these 36 strains when the inoculum size was increased from 10^4 to 10^7 CFU. However, ampicillin susceptibility, particularly of β-lactamase-producing strains, was definitely altered by increasing inoculum concentrations. At 10^5 CFU, all β-lactamase-producing strains were resistant to ampicillin at ≥2 μg/ml. Based on these results, an inoculum size of 10^5 CFU was used for all studies.

The susceptibility of 104 strains of *H. influenzae* to compound LY127935, ampicillin, chloramphenicol, and ticarcillin is shown in Table 1. All strains, regardless of capacity to produce β-lactamase, were inhibited by compound LY127935 at 0.25 μg/ml. In contrast, susceptibility to ampicillin was correlated directly with β-lactamase production in all but one strain. All of the strains which did not elaborate β-lactamase were inhibited by ampicillin at ≤1 μg/ml, except for one nontypable isolate from a tracheal aspirate. All strains which elaborated β-lactamase required ≥2 μg of ampicillin per ml for inhibition. Chloramphenicol inhibited all strains of *H. influenzae* at ≤4 μg/ml. Of 104 isolates, 97 (93.3%) were inhibited by ≤8 μg of ticarcillin per ml. The geometric mean MIC of ticarcillin for β-lactamase-positive strains was 4.702 μg/ml, and that for β-lactamase-negative strains was 0.331 μg/ml.

TABLE 1. *MIC[a] susceptibility of H. influenzae to four antibiotics*

Antibiotic	No. of strains inhibited by the following concn (μg/ml):														
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
LY127935	6	48	34	15	1										
Ampicillin			1	21	28	19	4	3[b]	0	3	0	2	8	10	5
Chloramphenicol			1	0	7	48	35	11	2						
Ticarcillin			2	22	32	16	5	3	5	12	7				

[a] MIC determined after incubation at 35°C for 48 h.

[b] One nontypable, β-lactamase-negative strain isolated from sputum; ampicillin MIC = 2 μg/ml.

Antibiotic combination studies. The results of the combination synergy studies are outlined in Table 2. With a low inoculum of 10^4 CFU, ampicillin and LY127935 were synergistic against two of seven β-lactamase-producing isolates. Partial synergy was noted with the five remaining isolates. With a high inoculum (10^7 CFU), partial synergy was demonstrated against seven isolates and synergy was noted in one instance. Inoculum size did not alter the results of these combination studies. Antagonism between ampicillin and LY127935 was not demonstrated by these studies.

Three organisms were tested by using a combination of chloramphenicol and LY127935 (Table 3). With a low inoculum, partial synergy was demonstrated with two of these three strains, and indifference was demonstrated with one strain. With the high inoculum, indifference was demonstrated with all three strains. Again, no antagonism was demonstrated between chloramphenicol and LY127935 against three isolates of ampicillin-resistant *H. influenzae* type b.

DISCUSSION

H. influenzae type b is the most common organism responsible for bacterial meningitis in children between 2 months and 5 years of age. In addition, septic arthritis, cellulitis, epiglottitis, and pneumonia in children commonly are due to *H. influenzae*. Since 1974, when ampicillin-resistant strains were first reported (3, 4), chloramphenicol has been recommended as one of the antibiotics to be used in the initial management of severe infections due to *H. influenzae* (10). Although choramphenicol remains an effective drug for treatment of *H. influenzae*, it is associated with hematopoietic depression, and recently, chloramphenicol-resistant *H. influ-*

TABLE 2. *MICs of LY127935 and ampicillin alone and in combination against H. influenzae type b*

Organism no.; inoculum[a]	MIC (μg/ml)				Synergy[c]
	Ampicillin alone	Ampicillin	+ LY127935[b]	LY127935 alone	
44; Low	2.0	0.25	0.008	0.031	S
High	64	1.0	0.0156	0.031	PS
50; Low	2.0	0.25	0.0156	0.031	PS
High	16	0.25	0.0156	0.031	PS
21; Low	2.0	0.25	0.0156	0.031	S
High	32	0.25	0.0156	0.031	PS
70; Low	2.0	0.25	0.008	0.0156	PS
High	8.0	0.5	0.008	0.031	S
53; Low	64	4.0	0.031	0.0625	PS
High	32	8.0	0.031	0.0625	PS
78; Low	2.0	0.25	0.008	0.0156	PS
High	4.0	2.0	0.008	0.031	PS
49; Low	4.0	0.25	0.008	0.0156	PS
High	16.0	4.0	0.008	0.031	PS
57; High	8.0	1.0	0.0156	0.031	PS

[a] Low inoculum indicates 1×10^4 CFU; high inoculum indicates 1×10^6 CFU.
[b] First and second values in each pair represent ampicillin and LY127935 MICs, respectively.
[c] S, Synergy; PS, partial synergy.

TABLE 3. *MICs of LY127935 and chloramphenicol alone and in combination against H. influenzae type b*

Organism no.; inoculum[a]	MIC (μg/ml)				Synergy[c]
	Chloramphenicol alone	Chloramphenicol	+ LY127935[b]	LY127935 alone	
50; Low	0.25	0.25,	0.031	0.031	I
High	0.5	0.5,	0.031	0.031	I
21; Low	0.25	0.125,	0.0156	0.031	PS
High	0.25	0.25,	0.031	0.031	I
53; Low	0.5	0.25,	0.0156	0.0625	PS
High	0.5	0.25,	0.031	0.0625	I

[a] Low inoculum indicates 10^4 CFU; high inoculum indicates 10^6 CFU.
[b] First and second values in each pair represent chloramphenicol and LY127935 MICs, respectively.
[c] PS, Partial synergy; I, indifference.

enzae isolates have been reported (5, 6). If ampicillin-resistant organisms continue to increase in prevalence and also become resistant to chloramphenicol, alternative antibiotics will be necessary (18). Overturf et al. (15) have compared carbenicillin with ampicillin in the therapy of meningitis caused by ampicillin-susceptible *H. influenzae* and found them similar. These authors found the median CSF carbenicillin concentration to be 0.85 μg/ml. Many β-lactamase-producing *H. influenzae* are not susceptible to carbenicillin or ticarcillin at concentrations <4 μg/ml (9); we have confirmed these findings for ticarcillin. Thus, ticarcillin or carbenicillin cannot be recommended as alternative antibiotics for the therapy of meningitis due to β-lactamase-producing *H. influenzae*.

Neu et al. have reported that 90% of 12 strains of *H. influenzae* were inhibited by LY127935 at 1.6 μg/ml (14). Trager et al. (17) reported 90% of *H. influenzae* strains, including one β-lactamase-producing strain, susceptible to 0.04 μg/ml. Wise and co-workers (19) determined that 90% of 25 strains of *H. influenzae* were inhibited by ≤0.06 μg of LY127935 per ml. They found no difference in the MIC between β-lactamase-positive and β-lactamase-negative strains. We found 99% of 104 *H. influenzae* strains isolated at Texas Children's Hospital over a 1-year period from pediatric patients were inhibited by LY127935 at ≤0.125 μg/ml. This 1-year survey of clinical isolates of *H. influenzae* included 30 strains which were resistant to ampicillin on the basis of β-lactamase production. Our frequency of isolation of β-lactamase-producing *H. influenzae* (29%) exceeds even what the Center for Disease Control considers an exaggerated national incidence (18%) of ampicillin resistance (7). Since LY127935 appears to penetrate the CSF at concentrations which exceed these values (personal communication, R. Kammer), this antibiotic may be a useful alternative antibiotic for the treatment of systemic disease caused by antibiotic-resistant *H. influenzae* and other gram-negative bacilli (1). Our study also shows that LY127935 is not antagonistic in combination with either ampicillin or chloramphenicol against a selected number of strains. Thus, pharmacology trials of LY127935 can be conducted safely during concomitant treatment with ampicillin or chloramphenicol.

ACKNOWLEDGMENTS

This study was supported by grant no. 8420D from Eli Lilly & Co.

We acknowledge the technical assitance of Carol Mitchell, Sally Kvernland, Helen Li and Kyle Womack. We thank Jo Ann Bynes for assistance in preparation of the manuscript and Martha D. Yow for review of the manuscript.

LITERATURE CITED

1. **Barza, M., F. T. Tally, N. V. Jacobus, and S. L. Gorbach.** 1979. In vitro activity of LY127935. Antimicrob. Agents Chemother. **16**:287-292.
2. **Brinkley, A. W., and T. W. Huber.** 1978. Method for evaluating broth culture media: application to *Haemophilus*. J. Clin. Microbiol. **8**:520-523.
3. **Center for Disease Control.** 1974. Ampicillin-resistant *Haemophilus influenzae*—Texas. Morbid. Mortal. Weekly Rep. **23**:99.
4. **Center for Disease Control.** 1974. Ampicillin-resistant *Haemophilus influenzae* meningitis—Maryland, Georgia. Morbid. Mortal. Weekly Rep. **23**:77-78.
5. **Center for Disease Control.** 1976. Chloramphenicol-resistant *Haemophilus influenzae*—Connecticut, Massachusetts. Morbid. Mortal. Weekly Rep. **25**:267.
6. **Center for Disease Control.** 1976. Chloramphenicol-resistant *Haemophilus influenzae*. Morbid. Mortal. Weekly Rep. **25**:385.
7. **Center for Disease Control.** 1979. Bacterial meningitis and meningococcemia—United States, 1978. Morbid. Mortal. Weekly Rep. **28**:277-279.
8. **Escamilla, J.** 1976. Susceptibility of *Haemophilus influenzae* to ampicillin as determined by use of a modified, one minute β-lactamase test. Antimicrob. Agents Chemother. **9**:196-198.
9. **Kammer, R. B., D. A. Preston, J. R. Turner, and L. C. Hawley.** 1975. Rapid detection of ampicillin-resistant *Haemophilus influenzae* and their susceptibility to sixteen antibiotics. Antimicrob. Agents Chemother. **8**: 91-94.
10. **Katz, S. L.** 1975. Ampicillin-resistant *Haemophilus influenzae*. Pediatrics **55**:5-8.
11. **Kilian, M.** 1974. A rapid method for the determination of Haemophilus strains. The porphyrin test. Acta Pathol. Microbiol. Scand. Sect. B. **82**:835-842.
12. **Lau, W. K., L. S. Young, R. E. Black, D. G. Winston, S. R. Linne, R. J. Weinstein, and W. L. Hewitt.** 1977. Comparative efficacy and toxicity of amikacin/carbenicillin versus gentamicin/carbenicillin in leukopenic patients. A randomized prospective trial. Am. J. Med. **62**:959-966.
13. **Myhre, E. B.** 1974. Typing of *Haemophilus influenzae* by counterimmunoelectrophoresis. Acta Pathol. Microbiol. Scand. **82**:164-166.
14. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new-1-oxa cephalosporin compared with that of other β-lactam compounds. Antimicrob. Agents Chemother. **16**:141-149.
15. **Overturf, G. D., E. A. Steinberg, A. E. Underman, J. Wilkins, J. M. Leedom, A. W. Mathies, Jr., and P. F. Wehrle.** 1977. Comparative trial of carbenicillin and ampicillin therapy for purulent meningitis. Antimicrob. Agents Chemother. **11**:420-426.
16. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307-311.
17. **Trager, G. M., G. W. White, V. M. Zimelis, and A. P. Panwalker.** 1979. LY127935: a novel β-lactam antibiotic with unusual antibacterial activity. Antimicrob. Agents Chemother. **16**:297-300.
18. **Ward, J. I., T. F. Tsai, G. A. Filice, and D. W. Fraser.** 1978. Prevalence of ampicillin and chloramphenicol-resistant strains of *Haemophilus influenzae* causing meningitis and bacteremia: national survey of hospital laboratories. J. Infect. Dis. **138**:421-424.
19. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1979. LY127935, a novel oxa β-lactam: an in vitro comparison with other β-lactam antibiotics. Antimicrob. Agents Chemother. **16**:341-345.