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

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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 \( \beta \)-lactamase. LY127935 inhibited 99% of the strains at a concentration of 0.125 \( \mu \)g/ml; the remaining strain was inhibited by this antibiotic at 0.25 \( \mu \)g/ml. \( \beta \)-Lactamase-producing strains were inhibited by ampicillin at \( \geq 2 \) \( \mu \)g/ml. \( \beta \)-Lactamase-negative strains were all inhibited by ampicillin at \( \leq 1 \) \( \mu \)g/ml, except for one nontypable strain which required 2 \( \mu \)g of ampicillin per ml for inhibition. All strains were susceptible to chloramphenicol at \( \leq 4 \) \( \mu \)g/ml. \( \beta \)-Lactamase-producing strains were less susceptible (geometric mean = 4.702 \( \mu \)g/ml) to ticarcillin than were strains which did not produce \( \beta \)-lactamase (geometric mean = 0.331 \( \mu \)g/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 \( \beta \)-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of *H. influenzae* have indicated uniform susceptibility among ampicillin-resistant and ampicillin-susceptible strains (unpublished data).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 \( \beta \)-lactamase and frozen at \(-70^\circ\)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 confirmed by gram stain, microscopic morphology, requirement for nicotinamide adenine dinucleotide, and lack of ability to synthesize porphobilinogen and porphyrins from \( \delta \) 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 \( \beta \)-lactamase was assessed by the acidometric method of Escamilla (6). Susceptibility studies were performed by the agar dilution method, using Mueller-Hinton agar supplemented with nicotinamide adenine dinucleotide and hemin (each at 10 \( \mu \)g/ml; Sigma Chemical Co.) (2). The agar plates, containing log10 concentrations of antibiotic, were inoculated by using a Steers-Foltz type replicator (16). The replicator delivered an inoculum of approximately 10^2 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. The minimal inhibitory concentration (MIC) was
defined as the concentration of antibiotic which al-
lowed 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. Mi-
crobroth 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 com-

ination with compound LY127935 in a "checkerboard"
distribution. H. influenzae strains were added to wells
(100-μl volumes) in both a high inoculum consisting of
10^8 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
e xist when the MIC of one of the antibiotics in com-
bination 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 antag-
onistic if the MIC of one of the antibiotics was in-
creased 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 cellu-
ritis, 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

cause 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. influenzae isolates,
86 (82.7%) were serotype b.

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

The susceptibility of 104 strains of H. influ-
enzae to compound LY127935, ampicillin, chlor-
amphenicol, 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, suscepti-
ility to ampicillin was correlated directly with
β-lactamase production in all but one strain. All
of the strains which did not elaborate β-lacta-
mase were inhibited by ampicillin at ≤1 μg/ml,
except for one nontypable isolate from a tracheal
aspirate. All strains which elaborated β-lacta-
mase 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.

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**Table 1. MIC susceptibility of H. influenzae to four antibiotics**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of strains inhibited by the following concn (μg/ml):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>LY127935</td>
<td>6</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>2</td>
</tr>
</tbody>
</table>

* MIC determined after incubation at 35°C for 48 h.

* 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 chloramphenicol remains an effective drug for treatment of *H. influenzae*, it is associated with hematopoietic depression, and recently, chloramphenicol-resistant *H. influ-

<table>
<thead>
<tr>
<th>Organism no.; inoculum*</th>
<th>MIC (µg/ml)</th>
<th>Synergy†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicillin alone</td>
<td>Ampicillin + LY127935</td>
</tr>
<tr>
<td>44; Low</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>64</td>
<td>1.0</td>
</tr>
<tr>
<td>50; Low</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>0.25</td>
</tr>
<tr>
<td>21; Low</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>32</td>
<td>0.25</td>
</tr>
<tr>
<td>70; Low</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>8.0</td>
<td>0.5</td>
</tr>
<tr>
<td>53; Low</td>
<td>64</td>
<td>4.0</td>
</tr>
<tr>
<td>High</td>
<td>32</td>
<td>8.0</td>
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<tr>
<td>78; Low</td>
<td>2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>49; Low</td>
<td>4.0</td>
<td>0.25</td>
</tr>
<tr>
<td>High</td>
<td>16.0</td>
<td>4.0</td>
</tr>
<tr>
<td>57; High</td>
<td>8.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Low inoculum indicates $1 \times 10^4$ CFU; high inoculum indicates $1 \times 10^6$ CFU.
† First and second values in each pair represent ampicillin and LY127935 MICs, respectively.
‡ S, Synergy; PS, partial synergy.

<table>
<thead>
<tr>
<th>Organism no.; inoculum*</th>
<th>Chloramphenicol alone</th>
<th>Chloramphenicol + LY127935</th>
<th>LY127935 alone</th>
<th>Synergy†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50; Low</td>
<td>0.25</td>
<td>0.25</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>High</td>
<td>0.5</td>
<td>0.5</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>21; Low</td>
<td>0.25</td>
<td>0.25</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>High</td>
<td>0.25</td>
<td>0.25</td>
<td>0.0156</td>
<td>0.031</td>
</tr>
<tr>
<td>53; Low</td>
<td>0.5</td>
<td>0.25</td>
<td>0.0156</td>
<td>0.0625</td>
</tr>
<tr>
<td>High</td>
<td>0.5</td>
<td>0.25</td>
<td>0.031</td>
<td>0.0625</td>
</tr>
</tbody>
</table>

* Low inoculum indicates $10^6$ CFU; high inoculum indicates $10^8$ CFU.
† First and second values in each pair represent chloramphenicol and LY127935 MICs, respectively.
‡ 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 carbencillin with ampicillin in the therapy of meningitis caused by ampicillin-susceptible H. influenzae and found them similar. These authors found the median CSF carbencillin concentration to be 0.85 μg/ml. Many β-lactamase-producing H. influenzae are not susceptible to carbencillin or ticarcillin at concentrations <4 μg/ml (9); we have confirmed these findings for ticarcillin. Thus, ticarcillin or carbencillin 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.

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SUSCEPTIBILITY OF H. INFLUENZAE 473

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