

Susceptibility of Ampicillin-Resistant *Haemophilus influenzae* to Seven Penicillins

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Sixty-seven clinical isolates of *Haemophilus influenzae* from various sections of the United States, England, and Germany were tested for susceptibility to penicillin, ampicillin, amoxicillin, epicillin, carbenicillin, ticarcillin, and methicillin. Fifty-three of the strains had previously been judged to be ampicillin resistant and 14 had been determined to be ampicillin susceptible. Fifty-two of the 53 resistant strains produced β -lactamase, but none of the susceptible strains produced it. On the basis of minimal inhibitory concentrations, the most active compounds were ticarcillin and carbenicillin. Whether this greater activity is useful clinically has not been established.

Ampicillin-resistant *Haemophilus influenzae* strains have recently been isolated from clinical specimens (4, 9, 13). Most of these strains have been shown to produce a β -lactamase (4, 12), which was responsible for their resistance to ampicillin. This has created a dilemma for the physician who must treat patients with life-threatening infections caused by these organisms, since ampicillin is the drug of choice and many physicians are reluctant to use chloramphenicol, the alternate drug of choice.

The purpose of this study was to determine the susceptibility of ampicillin-resistant and susceptible strains of *H. influenzae* to six other penicillins. The penicillins studied were: penicillin G; methicillin (β -lactamase resistant); ampicillin and two similar compounds, amoxicillin and epicillin; and carbenicillin and a similar compound, ticarcillin.

MATERIALS AND METHODS

Organisms. Sixty-seven strains (53 ampicillin resistant, 14 ampicillin susceptible) of *H. influenzae* which had been isolated from clinical specimens were examined. These organisms had been submitted to the Center for Disease Control (CDC) for antimicrobial susceptibility testing and/or typing and had been stored in rabbit blood at -70°C . For these studies, they were thawed and subcultured on chocolate agar supplemented with IsoVitaleX (BBL) or supplement B (Difco).

Antimicrobial agents. The antimicrobial agents used in these studies were kindly supplied by the following companies: penicillin G and carbenicillin, Pfizer, Inc., New York, N.Y.; ampicillin, Wyeth Laboratories, Philadelphia, Pa.; methicillin, Bristol Laboratories, Syracuse, N.Y.; ticarcillin and amoxicillin, Beecham, Inc., Bristol, Tenn.; and epicillin, Squibb Institute, Princeton, N.J.

Susceptibility tests. The susceptibility tests were performed by a microdilution procedure described previously (13), except that the tests were performed in parallel with Schaedler broth (BBL) and Mueller-Hinton broth (BBL). For each test the medium was supplemented with 5% peptic digest of blood (Fildes reagent). The inoculum used was 10^4 colony-forming units/ml. The test trays were incubated for 24 h at 35°C and then were read. The results were recorded as minimal inhibitory concentrations (MICs). The MIC was the lowest concentration of antimicrobial agent that prevented growth of the organism as judged macroscopically.

Typing. The organisms were serologically typed by a slide agglutination procedure (3) with CDC antisera, types a, b, c, d, e, and f.

RESULTS

The anatomic sites from which these *H. influenzae* strains were isolated and their serological types are listed in Table 1. The geographic distribution is given in Table 2.

Designation of ampicillin resistance was based not only on the MIC but also on the production of β -lactamase. The distribution of the 67 strains according to ampicillin MICs and beta-lactamase production is shown in Table 3. These figures indicate that 53 (79.1%) of the 67 strains were ampicillin resistant and 14 (20.9%) were ampicillin susceptible. The ampicillin susceptible strains had MICs of 0.25 $\mu\text{g}/\text{ml}$ or less and did not produce β -lactamase. The ampicillin-resistant strains (in Schaedler broth) had MICs of 4 to 128 $\mu\text{g}/\text{ml}$, and all but one of them produced β -lactamase.

The susceptibility of these strains to various concentrations of ampicillin, as determined in Mueller-Hinton and Schaedler broths, is presented in Table 4. The MICs are similar in the

TABLE 1. Site of isolation and serological types of 67 strains of *H. influenzae*

Source	No. of strains of:	
	Type b	Untypable
Spinal fluid	30	2
Blood	5	1
Respiratory tract	7	16
Ear	0	5
Eye	0	1

TABLE 2. Geographic distribution of 67 strains of *H. influenzae*

Strain	State or country
Ampicillin resistant	Alabama
	Arizona
	Arkansas
	California
	Colorado
	District of Columbia
	Florida
	Georgia
	Illinois
	Indiana
	Maryland
	Missouri
	North Carolina
	New Hampshire
	Oregon
	Texas
	Utah
	Virginia
	England
	Germany
Ampicillin sensitive	Arizona
	Florida
	Georgia
	Illinois
	New York

TABLE 3. Distribution of 67 strains of *H. influenzae* by ampicillin MICs and β -lactamase production

No. of strains	MIC (μ g/ml)	β -Lactamase production
48	≥ 8	All +
5	4	4+; 1-
14	≤ 0.25	All -

two broths, but growth was much better in the Schaedler broth, which made the tests easier to read. These results have led us to routinely use Schaedler broth, supplemented with 5% peptic digest of blood, for performing susceptibility tests on *H. influenzae* strains by the microdilution procedure.

The distribution of the MICs (Schaedler broth) of the seven penicillins is shown in Table 5, and the cumulative percentage of strains inhibited by each penicillin is shown in Fig. 1.

On the basis of MICs, the order of the penicillins according to greatest activity is: ticarcillin, carbenicillin, methicillin, amoxicillin, ampicillin, epicillin, and penicillin. Ticarcillin and carbenicillin had very similar activity. Amoxicillin was slightly more active than ampicillin and epicillin, and none of the three was much more active than penicillin. On the basis of

TABLE 4. MICs of ampicillin obtained with 67 strains (53 ampicillin resistant, 14 ampicillin susceptible) of *H. influenzae* grown in two different media

MIC (μ g/ml)	Distribution (%) in:	
	Mueller-Hinton broth	Schaedler broth
>128	0	0
128	6.0	6.0
64	11.9	10.4
32	11.9	10.4
16	20.9	25.4
8	13.4	19.4
4	7.5	7.5
2	7.5	0
1	0	0
0.5	0	0
0.25	6.0	7.5
0.12	11.9	13.4
0.06	3.0	0
0.03	0	0

TABLE 5. Results of susceptibility tests of 67 strains (53 ampicillin resistant, 14 ampicillin susceptible) of *H. influenzae* to seven penicillins^a

MIC (μ g/ml)	Distribution (%) to 7 penicillins ^b						
	Pen	Amp	Amox	Epi	Carb	Ticar	Meth
128	7.5	6.0	0	7.5	0	0	1.5
64	14.9	10.4	4.5	11.9	1.5	1.5	0
32	16.4	10.4	16.4	17.9	0	0	19.4
16	26.9	25.4	31.4	23.9	10.4	3.0	13.4
8	13.4	19.4	19.4	17.9	16.4	13.4	28.4
4	0	7.5	7.5	0	6.0	13.4	16.4
2	0	0	0	0	31.4	19.4	0
1	0	0	0	0	13.4	22.3	11.9
0.5	3.0	0	4.5	7.5	0	6.0	9.0
0.25	13.4	7.5	14.8	13.4	4.5	3.0	0
0.12	4.5	13.4	1.5	0	11.9	9.0	0
0.06	0	0	0	0	4.5	7.5	0
0.03	0	0	0	0	0	1.5	0

^a Tests were performed using Schaedler broth. Strains with MICs shown above the horizontal line are ampicillin resistant; those below are ampicillin susceptible.

^b Abbreviations: Pen, penicillin G; Amp, ampicillin; Amox, amoxicillin; Epi, epicillin; Carb, carbenicillin; Ticar, ticarcillin; and Meth, methicillin.

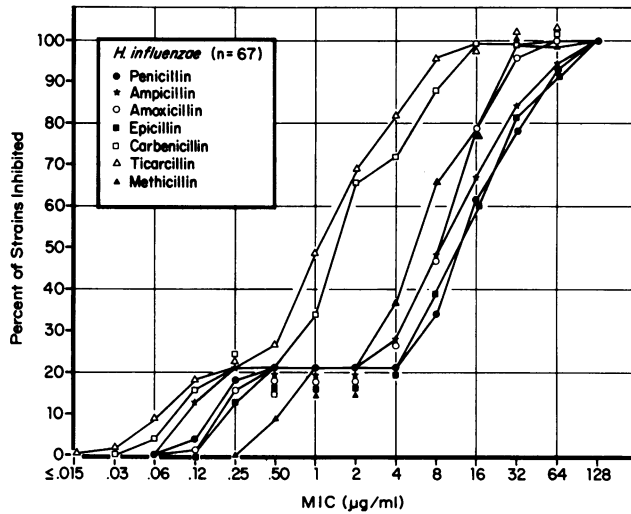


FIG. 1. Cumulative percentage of 67 strains (53 ampicillin resistant, 14 ampicillin susceptible) of *H. influenzae* inhibited by seven penicillins (tests performed in Schaedler broth).

MICs, methicillin was least active on the ampicillin-susceptible strains, but was more active on the ampicillin-resistant strains than penicillin, ampicillin, amoxicillin, or epicillin.

These strains were also tested for susceptibility to chloramphenicol. The MICs ranged from 0.12 to 1.0 $\mu\text{g/ml}$, with the majority being either 0.25 or 0.5 $\mu\text{g/ml}$, indicating that all the strains were very susceptible to chloramphenicol.

DISCUSSION

The distribution of ampicillin-resistant and susceptible strains reported in this study does not, in any way, represent the normal distribution of these strains in the population of this country. The extent to which these organisms occur or are involved in disease is unknown, but it is probable that the incidence is low. However, it is now clear that ampicillin-resistant *H. influenzae* can be found in most states within this country. In addition to the states listed in Table 2, we have also confirmed the presence of ampicillin-resistant strains in Oklahoma, Michigan, Mississippi, New York, and Washington. We also have reliable reports of ampicillin-resistant strains in Massachusetts and South Carolina. It is probably safe to assume that such strains could be isolated anywhere within the continental United States. Therefore, all physicians who treat life-threatening infections caused by *H. influenzae* should be aware of this possibility. We have not seen, nor are we aware of, a strain of *H. influenzae* that is resistant to chloramphenicol. For this

reason, it has been suggested that chloramphenicol should be included in the primary treatment regimen (3, 7). A rapid β -lactamase test, performed on the first growth of the organism on an agar plate, can be used to determine whether chloramphenicol should be continued or deleted from the treatment regimen. All ampicillin-resistant strains we have studied produced β -lactamase, except the two strains we reported previously (12), one of which was included in this study. All β -lactamase results should be confirmed by susceptibility tests. In recent studies in our laboratories we have shown that the β -lactamase test we routinely use (12) and two other rapid methods (2, 11) can be used equally well with *H. influenzae* strains.

The lower MICs obtained in this study for carbenicillin and a similar compound, ticarcillin, indicate that these two compounds are more active than the other penicillins on the ampicillin-resistant strains of *H. influenzae*. More than 40% of the MICs of carbenicillin and ticarcillin are either 1 or 2 $\mu\text{g/ml}$, whereas none of the MICs of the other penicillins were this low for the ampicillin-resistant strains.

Whether the differences can be useful clinically remains to be proven. As with ampicillin, the amount of carbenicillin that enters the spinal fluid apparently varies considerably. Levels of 2 to 38 $\mu\text{g/ml}$ of spinal fluid, representing 2 to 20% of the corresponding serum levels, have been reported in patients with and without meningeal disease (1, 5, 6, 10).

The effect of inoculum size on the MICs of the

ampicillin-resistant strains must also be considered. We have previously reported (13), and it has been confirmed by Madeiros and O'Brien (8), that increasing the inoculum greatly increases the ampicillin MIC, and the latter authors have also reported the same effect on carbenicillin and cephalothin MICs. The clinical implications of the inoculum size are unknown but might well be the determining factor in the outcome of therapy, if the infecting organism was a β -lactamase producer.

We know of only one case in which meningitis caused by an ampicillin-resistant strain of *H. influenzae* was apparently cured by treatment with ampicillin, although other cures of this type may have occurred. Madeiros and O'Brien (8) reason that such cures could occur because of the greater permeability of the *H. influenzae* cell to the penicillins, as compared with *Escherichia coli* containing a similar β -lactamase. However, clinical histories of many other patients who had ampicillin-resistant *H. influenzae* meningitis indicate that the patients did not respond well until they were treated with chloramphenicol.

It is apparent that these questions can be answered only if clinical trials are performed. Until such studies are done, clinicians will probably be reluctant to use a different penicillin to treat the infection, even though it may be hydrolyzed more slowly than ampicillin or penicillin (8).

ADDENDUM

Since this manuscript was originally written, we have confirmed the ampicillin resistance of strains from Connecticut, New Hampshire, New Jersey, Pennsylvania, and South Dakota. Ampicillin-resis-

tant strains have also been reported in Canada, Australia, and Israel.

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