Antimicrobial Susceptibility of *Haemophilus parainfluenzae*

JOAN B. MAYO AND LAURENCE R. McCARTHY

Infectious Disease Service, and the Diagnostic Microbiology Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and Cornell University Medical College, New York, New York 10021

Received for publication 22 November 1976

Fifty random clinical isolates of *Haemophilus parainfluenzae* were tested for their susceptibility to 10 antibiotics by a microtiter broth dilution method. Three of the strains tested were resistant to ampicillin, whereas eight were resistant to tetracycline. All strains tested were susceptible to chloramphenicol, kanamycin, gentamicin, cephalothin, and colistin. The ranges of minimal inhibitory concentrations for the three remaining antibiotics were: 0.5 to ≥128 μg of penicillin G per ml, 0.03 to 4 μg of carbenicillin per ml, and 1 to 16 μg of erythromycin per ml. Elevated minimal inhibitory concentrations for penicillin and carbenicillin were noted for the three ampicillin-resistant strains. Tests for beta-lactamase production demonstrated the presence of this enzyme in each of the three ampicillin-resistant strains.

The importance of *Haemophilus influenzae*, especially type b, in human disease is well established (1, 21). As a consequence, a large body of research has focused on this species of the genus *Haemophilus*. It has been generally thought that the other three common species (*H. parainfluenzae*, *H. parahaemolyticus*, and *H. haemolyticus*) are a part of the upper respiratory tract normal flora and rarely cause disease. With increasing frequency, however, *H. parainfluenzae* has been cited as the causative agent of meningitis, epiglottitis, brain abscess, bacteremia, and endocarditis (8, 9, 12). Despite these reports, establishment of *H. parainfluenzae* as a pathogen, little is known about its susceptibility to antimicrobial agents.

Studies on the antimicrobial susceptibility of *H. influenzae* have been well documented (19, 22). Recently, strains of *H. influenzae* have been isolated that demonstrate resistance to ampicillin (6, 14). As a consequence, ampicillin is no longer recommended as the drug of choice in those areas of the United States where resistant strains have been found (2).

In view of these findings and the increasing role of *H. parainfluenzae* in human disease, this study was undertaken to determine the susceptibility of *H. parainfluenzae* to 10 antibiotics and to determine if ampicillin resistance is also present in this species.

(This work constitutes part of a thesis in the Department of Microbiology, College of Dentistry, in partial fulfillment of the requirements for the Degree of Master of Science at New York University.)

MATERIALS AND METHODS

Organisms. The 50 strains of *H. parainfluenzae* used in this study represented random clinical isolates recovered from respiratory tract specimens taken from 50 different patients seen at the Memorial Sloan-Kettering Cancer Center between May and November 1974. Identity of the strains was confirmed by their demonstration of a V-factor requirement on Columbia agar (BBL) and their inability to produce hemolysis on 5% defibrinated rabbit blood agar. All isolates were preserved for study by freezing of bacterial suspensions in sterile defibrinated rabbit blood at −70°C.

Preparation of inocula. A standardized inoculum was prepared by first streaking strains of *H. parainfluenzae* onto chocolate agar (BBL). After 24 h of incubation at 35°C in 10% CO₂, colonies of the test bacteria were suspended in 1.0 ml of Mueller-Hinton broth (BBL) enriched with 5% supplement B (Difco). The suspension was adjusted to contain 10⁶ colony-forming units per ml by comparison with a 0.5 MacFarland standard and subsequently diluted in the supplemented Mueller-Hinton broth to yield a final inoculum concentration of 10⁴ colony-forming units.

Susceptibility tests. Each isolate was tested for its susceptibility to chloramphenicol, tetracycline, gentamicin, kanamycin, erythromycin, colistin, penicillin, ampicillin, carbenicillin, and cephalothin. Techniques used were identical to those described by Thornberry and Kirven (19) for antibiotic susceptibility testing of *H. influenzae* except that Mueller-Hinton broth was enriched with 5% supplement B rather than 5% Fildes reagent. Stock solutions of the 10 antibiotics were prepared, and 14 serial twofold dilutions were made in sterile flat-
bottom 120-well microtiter trays (Ames Co.) using the supplemented Mueller-Hinton broth. The last well in each row of each microtiter tray contained broth without added antibiotic and served as the growth control. All antibiotics except carbenicillin were tested over a concentration range of 0.015 to 128 µg/ml. Carbenicillin concentrations used ranged from 0.03 to 256 µg/ml.

The inoculated trays were sealed with sterile plastic strips (Ames Co.) and incubated at 35°C without CO₂ for 24 h. The minimal inhibitory concentration (MIC) was determined as the lowest concentration of an antibiotic with which there was no macroscopic evidence of growth.

To assure sterility, all preparation of antibiotic dilutions and inoculation of the microtiter trays were performed under a laminar flow hood. Two selected strains of *H. parainfluenzae* were tested simultaneously with each new series of antibiotic dilutions to check the reproducibility of the method.

To test for beta-lactamase production by ampicillin-resistant strains of *H. parainfluenzae* were performed using both a rapid capillary tube method (20) and an iodometric test (4). Positive controls for beta-lactamase activity for both test methods used a penicillinase-producing strain of *Staphylococcus aureus* and two ampicillin-resistant *H. influenzae* strains provided by Clyde Thornberry of the Center for Disease Control. Ampicillin-susceptible strains of *H. parainfluenzae* served as negative controls.

**RESULTS**

The MICs of the 10 antibiotics for the strains of *H. parainfluenzae* tested in this study are shown in Table 1. All 50 strains were susceptible to chloramphenicol. MICs ranged between 0.25 and 2.0 µg/ml with a median MIC of approximately 0.5 µg/ml. The results for tetracycline demonstrated that 84% of the isolates were susceptible to 0.5 µg/ml or less, whereas 16% were resistant with MICs of 8 µg/ml.

Gentamicin was the more active of the two aminoglycoside antibiotics tested. All strains of *H. parainfluenzae* yielded MICs of 1 µg or less per ml. Results for kanamycin demonstrated that 94% of the strains were susceptible with MICs less than 6 µg/ml, and 6% were moderately susceptible with MICs of 8 or 16 µg per ml.

MICs for erythromycin ranged between 1 and 16 µg/ml. Using the National Committee for Clinical Laboratory Standards breakpoint of ≤2.0 µg/ml (15) to indicate susceptibility, it appears that only 28% of the strains were susceptible, 70% were moderately susceptible, and 2% were resistant. All strains tested were susceptible to colistin with MICs of 1 µg/ml or less.

MICs for penicillin ranged between 0.5 and 128 µg/ml. Fifty-four percent of the strains of *H. parainfluenzae* were susceptible with MICs less than 1.5 µg/ml; 40% were moderately susceptible; and 6% were resistant with MICs in excess of 32 µg/ml. MICs for ampicillin ranged between 0.25 and 128 µg/ml. Ninety-four percent of the strains were susceptible with MICs less than 1.5 µg/ml, and 6% resistant with MICs of 8 to 128 µg/ml.

All isolates including those demonstrating resistance to ampicillin displayed sensitivity to the beta-lactam antibiotics, carbenicillin and cephalothin. MICs for carbenicillin ranged between 0.03 and 4 µg/ml, whereas MICs for cephalothin ranged between 0.5 and 2 µg/ml. Slightly elevated MICs for carbenicillin were observed with those strains displaying resistance to penicillin and ampicillin.

**Correlation between ampicillin resistance and beta-lactamase production.** The three ampicillin-resistant strains produced beta-lactamase as determined by both test methods. In contrast, 11 ampicillin-sensitive strains of *H. parainfluenzae*, randomly selected from the strains used in the study, showed no beta-lactamase activity with either method.

**Table 1. MICs of 10 antibiotics for 50 strains of Haemophilus parainfluenzae**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of strains inhibited (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>17 (34)</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>44 (88)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td></td>
</tr>
</tbody>
</table>

* Minimal inhibitory concentration (micrograms per milliliter).
DISCUSSION

The reported pathogenicity of H. parainfluenzae, as well as the prevalence of strains of H. influenzae resistant to ampicillin, prompted us to examine the antimicrobial susceptibility of 50 strains of H. parainfluenzae. All strains were isolates from respiratory specimens of patients admitted to Memorial Sloan-Kettering Cancer Center. All isolates appeared to be a part of the normal respiratory flora for each of these patients and were not related to any disease process.

All strains tested were susceptible to chloramphenicol, gentamicin, kanamycin, colistin, carbenicillin, and cephalothin and demonstrated variable susceptibilities for tetracycline and erythromycin. Three strains of H. parainfluenzae demonstrated resistance to ampicillin and penicillin. One of these strains and seven ampicillin-susceptible strains were found to be resistant to tetracycline. These findings correlate with the incidence of tetracycline resistance in H. influenzae noted recently (22).

In this study, the enzyme produced by these ampicillin-resistant H. parainfluenzae was characterized as a beta-lactamase. Thus, the mechanism of resistance appears similar to the described ampicillin-resistant strains of H. influenzae (4, 14, 20). The beta-lactamase produced by strains of H. influenzae has been characterized as a constitutive plasmid-mediated enzyme which exhibits high activity against penicillin, ampicillin, and cephaloridine substrates, low activity against penicillins such as oxacillin and methicillin, and intermediate activity against carbenicillin and cephalothin (10, 14, 18). Although oxacillin and methicillin were not tested in our study, all ampicillin-resistant strains of H. parainfluenzae demonstrated resistance to penicillin and slightly elevated MICs for carbenicillin and cephalothin. This observation suggests some similarity between the beta-lactamase enzymes of H. influenzae and H. parainfluenzae. During our study of H. parainfluenzae, we also determined the susceptibility of 14 clinical isolates of H. parahaemolyticus to ampicillin and found one strain to demonstrate a high level of ampicillin resistance (>128 μg/ml), which was also mediated by a beta-lactamase. Our results and the recent reports of diseases caused by ampicillin-resistant strains of H. parainfluenzae, H. haemolyticus, and H. paraphrophilus (11, 13) suggest that ampicillin resistance occurs throughout the genus.

In the past ampicillin has been the drug of choice for treating meningitis due to H. influ-
Three Hemophilus species. Am. J. Dis. Child. 121:35-
37.
lin-resistant Haemophilus paraphrophilus laryngo-
K. Sax. 1974. Haemophilus influenzae type b resistant
Assoc. 229: 298-301.
15. National Committee for Clinical Laboratory Stan-
dards. 1975. Performance standards for antimicrobial
disc susceptibility tests, p. 1-11. National Committee
for Laboratory Standards,
1972. Treatment of Hemophilus influenzae meningi-
tis: a comparison of chloramphenicol and tetracy-
1971. A comparison of ampicillin and chlorampheni-
col therapy in Haemophilus influenzae meningitis.
1975. R-factor mediated beta-lactamase production by
Haemophilus influenzae. J. Med. Microbiol. 8:437-
441.
susceptibility of Haemophilus influenzae. Antimi-
resistance in Haemophilus influenzae as determined
by a rapid test for beta-lactamase production. Anti-
21. Türk, D. C., and J. R. May. 1967. Haemophilus influ-
enzae: its clinical importance in modern medicine.
English University Press, Ltd., London.
1:134-137.