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Comparative Activities of Selected Beta-Lactam Antibiotics Against *Haemophilus influenzae*

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The comparative activities of ampicillin, cefamandole, cefoxitin, cefaclor, and cefatrizine against both beta-lactamase-producing and non-beta-lactamase-producing isolates of *Haemophilus influenzae* were determined by using an agar dilution susceptibility test procedure. Ampicillin was the most active drug tested against non-beta-lactamase-producing isolates, whereas cefamandole was most active against beta-lactamase-producing strains.

The emergence of ampicillin-resistant (6, 11) and, now, chloramphenicol-resistant (7) strains of *Haemophilus influenzae* has prompted a search for alternative chemotherapeutic agents showing greater activity against these organisms. Certain of the newer cephalosporins, such as cefamandole, cefatrizine, and cefaclor, have shown greater activity against *H. influenzae* than did earlier cephalosporins such as cephalothin and cephalexin (4, 5, 12). Cefoxitin, a member of the cephamycin class of antibiotics, has been shown to offer extreme resistance to degradation by gram-negative bacterial beta-lactamases (8, 12).

The greater cerebrospinal fluid penetration of certain of the newer cephalosporins may allow their use for treatment of meningitis. However, there is also a need for effective chemotherapeutic agents against serious *H. influenzae* infections other than meningitis, for example, otitis media and acute epiglottitis (1, 9, 10). Neither cephalothin nor cephalexin has been found previously to be effective in the treatment of these infections (12).

The purpose of this study was to compare the in vitro activities of cefamandole, cefatrizine, cefaclor, cefoxitin, and ampicillin against 100 *H. influenzae* isolates, including 31 beta-lactamase producers. Most of the test strains were type b, although certain strains were non-typable. Susceptibility tests were performed by using an agar dilution technique with Mueller-Hinton agar and 5% supplement C (2). An inoculum of approximately 10^4 microorganisms was applied to the surface of the agar plates with a Steers-Foltz inoculum-replicating device. All susceptibility tests were incubated for 24 h at 35°C in a 3 to 5% CO_2 atmosphere. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that either prevented visible growth on the surface of the agar plates or allowed growth of no more than three colonies. Production of beta-lactamase by the test strains was determined by using a recently described rapid paper strip test (3).

The results of testing 68 non-beta-lactamase-producing isolates of *H. influenzae* are shown in Fig. 1. Ampicillin was the most active of the five antibiotics tested, with all strains inhibited by 1 μg/ml or less. Cefamandole was next most active, with 99% of strains inhibited by 2 μg/ml. A concentration of 8 μg/ml of cefoxitin was re-

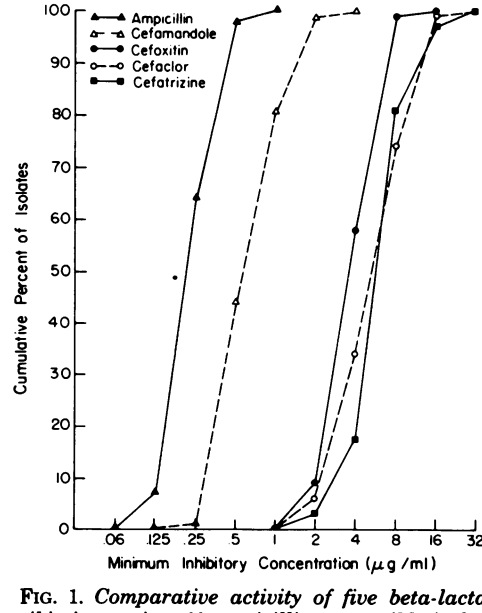

FIG. 1. *Comparative activity of five beta-lactam antibiotics against 68 ampicillin-susceptible isolates of H. influenzae.*

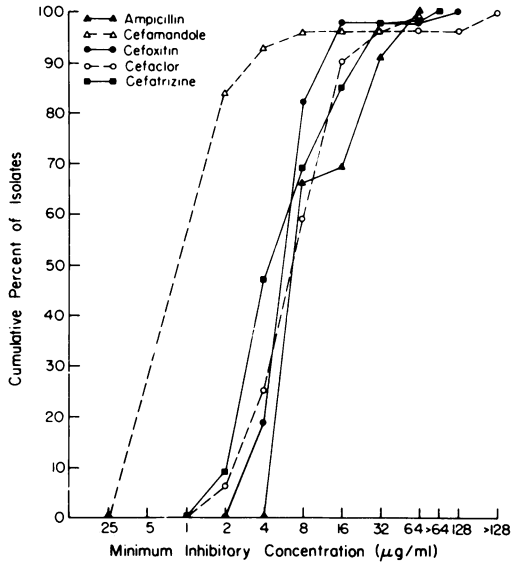

FIG. 2. *Comparative activity of five beta-lactam antibiotics against 32 ampicillin-resistant isolates of Haemophilus influenzae.*

quired to inhibit 99% of isolates tested. Cefaclor and cefatrizine showed essentially identical activities by inhibiting more than 95% of isolates at 16 μg/ml.

When 32 ampicillin-resistant *H. influenzae* isolates were tested (Fig. 2), cefamandole was found to be the most active agent; 84% of strains were inhibited by 2 μg/ml, which is similar to data observed with the non-beta-lactamase-producing strains. Cefoxitin was only slightly more active than the two remaining cephalosporins; 82% of the strains were inhibited by 8 μg/ml of cefoxitin, and 98% were inhibited by 16 μg/ml. Cefaclor and cefatrizine again were quite similar in activity, with 85 to 90% of the isolates inhibited at 16 μg/ml. As expected, ampicillin was least active against these strains, with 32 μg/ml required to inhibit >90% of the test strains.

It was noted that all of the cephalosporin-type drugs except cefamandole were at least one two-fold concentration increment less active against the beta-lactamase-producing strains than against the non-beta-lactamase group. One of the isolates included in this group was an ampicillin-resistant, but non-beta-lactamase-producing strain. The MIC of ampicillin for this strain was 8 μg/ml; the MIC of cefamandole was 64 μg/ml; that of cefoxitin was 128 μg/ml; that of cefaclor was >128 μg/ml; and that of cefatrizine was >64 μg/ml. The mechanism of resistance of this organism to beta-lactam drugs is unclear and bears further investigation.

In this study we compared the activities of several recently developed semisynthetic cephalosporin or cephamycin antibiotics against isolates of *H. influenzae*. These drugs showed superior inhibitory activities as compared with previous cephalosporins, especially against beta-lactamase-producing bacteria (4, 5, 12). Two new oral cephalosporins, cefaclor and cefatrizine, also show good in vitro activity against beta-lactamase-producing *H. influenzae*.

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LITERATURE CITED

1. **Jacobson, J. A., J. B. McCormick, P. Hayes, C. Thornesberry, and L. Kirven.** 1976. Epidemiologic characteristics of infections caused by ampicillin-resistant *Haemophilus influenzae*. Pediatrics **58**:388–391.
2. **Jorgensen, J. H., and P. M. Jones.** 1975. Simplified medium for ampicillin susceptibility testing of *Haemophilus influenzae*. Antimicrob. Agents Chemother. **7**:186–190.
3. **Jorgensen, J. H., J. C. Lee, and G. A. Alexander.** 1977. Rapid penicillinase paper strip test for detection of beta-lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **11**:1087–1088.
4. **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.
5. **Kattan, S., P. Cavanaugh, and J. D. Williams.** 1975. Relationship between β-lactamase production by *Haemophilus influenzae* and sensitivities to penicillin and cephalosporins. J. Antimicrob. Chemother. **1**:79–84.
6. **Khan, W., S. Ross, W. Rodriguez, G. Controni, and A. K. Saz.** 1974. *Haemophilus influenzae* type B resistant to ampicillin. J. Am. Med. Assoc. **229**:298–301.
7. **Manten, A., B. Van Klingersen, and M. Dessens-Kroon.** 1976. Chloramphenicol resistance in *Haemophilus influenzae*. Lancet **i**:702.
8. **Neu, H. C.** 1974. Cefoxitin, a semisynthetic cephamycin antibiotic: antibacterial spectrum and resistance to hydrolysis by gram-negative beta-lactamases. Antimicrob. Agents Chemother. **6**:170–176.
9. **Shurin, P. A., S. I. Pelton, D. Scheifele, and J. O. Klein.** 1976. Otitis media caused by non-typable, ampicillin-resistant strains of *Haemophilus influenzae*. J. Pediatr. **88**:646–649.
10. **Todd, J. K., and F. W. Bruhn.** 1975. Severe *Haemophilus influenzae* infections: spectrum of disease. *Am. J. Dis. Child.* **129**:607–611.
11. **Tomeh, M. O., S. E. Starr, J. E. McGowan, Jr., P. M. Terry, and A. J. Nahmias.** 1974. Ampicillin-resistant *Haemophilus influenzae* type B infection. J. Am. Med. Assoc. **229**:295–297.
12. **Washington, J. A., II.** 1976. The in vitro spectrum of the cephalosporins. Mayo Clin. Proc. **51**:237–250.