Comparative In Vitro Activity of Cefixime against *Haemophilus influenzae* Isolates, Including Ampicillin-Resistant, Non-β-Lactamase-Producing Isolates, from Pediatric Patients

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The in vitro activity of cefixime was comparatively tested against 232 non-type b and 102 type b isolates of *Haemophilus influenzae* derived from clinical specimens of pediatric patients, including 10 non-type b strains that did not produce β-lactamase and demonstrated resistance to ampicillin. Cefixime was active against the ampicillin-susceptible and ampicillin-resistant, β-lactamase-producing isolates; however, its activity against some non-β-lactamase-producing, ampicillin-resistant isolates appeared to be limited.

More than 15 years have passed since the initial recognition of ampicillin resistance in clinical isolates of *Haemophilus influenzae* type b (9, 20). Currently, between 10 and 30% of type b strains and between 3 and 30% of non-type b strains of *H. influenzae* are resistant to ampicillin (1, 4, 18). The major mechanism of resistance is TEM-1 β-lactamase activity. The other significant mechanism of resistance is the reduced affinity of certain penicillin-binding proteins for ampicillin (14, 20).

An effective alternate therapeutic agent for the treatment of serious ampicillin-resistant *H. influenzae* infections is chloramphenicol. However, the potential hematologic toxicities of chloramphenicol therapy have resulted in the proliferation of newer antimicrobial agents with low toxicities. One orally administered agent, cefixime, has demonstrated broad-spectrum activity and may be useful in the treatment of respiratory tract infections (otitis media and sinusitis), including infections due to ampicillin-resistant *H. influenzae*. It has demonstrated activity against *H. influenzae* in vitro, and its activity in the presence of a wide range of β-lactamases, including TEM-1, is unimpaired (3, 13, 17, 19).

The present study was designed to compare the in vitro activity of cefixime, other oral antimicrobial agents, and ceftriaxone against clinical isolates of *H. influenzae* from pediatric patients in the United States and to compare the activity of cefixime against isolates of non-β-lactamase-producing *H. influenzae* resistant to ampicillin.

Clinical isolates of *H. influenzae* were recovered from pediatric patients at St. Christopher's Hospital for Children, Philadelphia, Pa. (194 isolates); University of Texas School of Medicine, Houston (48 isolates) (kindly provided by M. LaRocco); Primary Children's Hospital, Salt Lake City, Utah (47 isolates) (kindly provided by J. Daly); and Los Angeles Children's Hospital, Los Angeles, Calif. (39 isolates) (kindly provided by C. Inderlied). Organisms were identified as *H. influenzae* by using standard methods. All isolates required β-NAD (V factor) and hemin (X factor) for growth when incubated at 37°C, and the presence of capsular antigen was determined by using *H. influenzae* typing antisera (Difco Laboratories, Detroit, Mich.) and a slide agglutination procedure. The isolates included four non-β-lactamase-producing strains of *H. influenzae*, originally isolated from pediatric patients during a collaborative study (J. E. Mortensen, M. LaRocco, S. L. Himes, C. Inderlied, J. A. Daly, J. M. Campos, and P. M. Mendelman, Diagn. Microbiol. Infect. Dis., in press), and six clinical isolates provided by Paul M. Mendelman (Children's Hospital and Medical Center, Seattle, Wash.), ampicillin MICs for all of which were ≥4 μg/ml when tested at a concentration of 10^6 CFU/ml with supplemented brain heart infusion broth (Difco) in tube broth macrodilution (14-16). These 10 isolates were defined as ampicillin resistant, non-β-lactamase producing (Amp' NBLP).

The ability of the isolates to produce β-lactamase was determined by using a nitrocefin-impregnated disk (BBL Microbiological Systems, Cockeysville, Md.). MICs were determined by using broth microdilution panels (PML Microbiologicals, Tualatin, Oreg.) containing *Haemophilus* test medium (8). The eight antimicrobial agents, or combinations of agents, were tested at the following concentrations: amoxicillin, amoxicillin, and cefaclor, 0.03 to 16 μg/ml; amoxicillin-clavulanic acid, 0.03/0.06 to 16/32 μg/ml; cephalaxin, 0.12 to 32 μg/ml; cefixime, cefuroxime, and ceftriaxone, 0.015 to 8 μg/ml. Test panels were inoculated with 50 μl of a suspension of test organisms to give a final concentration of 1 × 10^5 to 5 × 10^8 CFU/ml. Test panels were incubated for 24 h at 35°C and were examined macroscopically for evidence of growth. An MIC was defined as the lowest concentration of antimicrobial agent which inhibited growth of the organism. *Escherichia coli* ATCC 29522, *Staphylococcus aureus* ATCC 29213, and *H. influenzae* ATCC 10211 were used as controls.

Statistical analysis was carried out by using the Mann-Whitney U test. Significance was defined as *P* < 0.05.

The MICs of the antimicrobial agents tested against *H. influenzae* type b isolates (102 isolates) and non-type b isolates (232 isolates) are given in Tables 1 and 2, respectively. Tables 1 and 2 do not include data from tests of the 10 Amp’ NBLP isolates. The serotype b isolates, as a group, were more susceptible to ampicillin, amoxicillin-clavulanic acid, cephalaxin, cefaclor, cefuroxime, and cefixime (*P* < 0.05) than were the non-type b isolates. There was no statistical difference between serotype b and nontypeable isolates for susceptibility to amoxicillin or ceftriaxone. Ceftriaxone was the most active agent tested; MICs for 90% of

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the isolates (MIC<sub>90</sub>) were ≤0.015 μg/mL. When orally administered agents were compared, ceferoxime was the most active agent (MIC<sub>90</sub> ≤0.03 μg/mL). Ceferoxime demonstrated activity similar to that of ceftriaxone against the Amp<sup>4</sup> and Amp<sup>7</sup> BLP isolates. These results are consistent with those from other studies of Amp<sup>4</sup> and Amp<sup>7</sup> BLP H. influenzae (11-13, 17, 19) but three- to sixfold lower than those observed in some other studies (2, 10).

The results of susceptibility testing of Amp<sup>4</sup> BLP isolates are given in Table 3. As a group, the Amp<sup>4</sup> BLP isolates were significantly more resistant to amoxicillin-clavulanic acid, cefixime, cefuroxime (P < 0.001), and cephalexin (P < 0.05) than were the other four phenotype groups tested. They were also more resistant to amoxicillin and amoxicillin-clavulanic acid than were the two ampicillin-susceptible phenotype groups (P < 0.001) but more susceptible to amoxicillin and amoxicillin-clavulanic acid than were the β-lactamase-producing phenotype groups (P < 0.001) (16). There was no difference in the activity of ceftriaxone when any of the phenotypic groups were compared. Previous reports have noted higher amoxicillin-clavulanic acid and cephalosporin MICs in Amp<sup>4</sup> BLP isolates (14-16).

Cefixime MICs found in this study are similar to those in some previous reports (7, 13, 17) but 2- to 16-fold (2, 19) higher than those in other reports for Amp<sup>4</sup> BLP H. influenzae. Fuchs and associates (5, 6) have defined organisms whose MICs of cefixime were <1 μg/mL as "susceptible" and those whose MICs were >1 but ≤4 μg/mL as "intermediate." Since the achievable concentration of cefixime in serum approaches only 4 μg/mL (5, 6), the therapeutic efficacy of cefixime for these strains may be limited.

In conclusion, ceferoxime demonstrated activity comparable to that of other oral cephalosporins and penicillins in vitro against all of the phenotypic groups tested. However, its activity against some Amp<sup>4</sup> BLP strains is significantly less than that of ceftriaxone, a comparative broad-spectrum cephalosporin included in this study.

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**LITERATURE CITED**


7. Jorgensen, J. H., G. V. Doern, C. Thornberry, D. A. Preston,


