Susceptibilities of β-Lactamase-Producing and -Nonproducing Ampicillin-Resistant Strains of *Haemophilus influenzae* to Cefituben, Cefaclor, Cefuroxime, Cefixime, Cefotaxime, and Amoxicillin-Clavulanic Acid

ARTHUR L. BARRY,1,* PETER C. FUCHS,2 AND MICHAEL A. PFALLER3

The Clinical Microbiology Institute, P.O. Box 947, Tualatin, Oregon 970621; St. Vincent Hospital and Medical Center, Portland, Oregon 972252; and Oregon Health Sciences University, Portland, Oregon 972013

Received 23 April 1992/Accepted 12 October 1992

In *in vitro* studies we evaluated the susceptibilities of β-lactamase-producing and -nonproducing, ampicillin-resistant strains of *Haemophilus influenzae* and compared them with those of ampicillin-susceptible strains. Ampicillin, amoxicillin-clavulanic acid, cefituben, cefaclor, cefuroxime, cefixime, and cefotaxime were evaluated by broth microdilution tests and disk diffusion tests. The disk diffusion tests accurately categorized β-lactamase-producing strains and ampicillin-susceptible strains as being susceptible to the study drugs other than ampicillin. Ampicillin-resistant, β-lactamase-nonproducing strains were relatively resistant to all seven study drugs, but the disk diffusion test did not always predict that resistance. The clinical relevance of the decreased susceptibility to various agents remains unclear, but to be conservative, all ampicillin-resistant, β-lactamase-nonproducing strains might be assumed to be resistant to other β-lactams. After excluding that small group of isolates, reliable susceptibility test results were obtained with lots of Haemophilus Test Medium that met quality assurance criteria.

Ampicillin is the drug of choice for treating many infections caused by *Haemophilus influenzae*, but its usefulness has been compromised by the increasing prevalence of ampicillin-resistant strains. Different medical centers throughout the world report that 15 to 30% of their *H. influenzae* isolates are resistant to ampicillin (4, 9, 18). Nearly all of the ampicillin-resistant *H. influenzae* isolates are capable of producing a TEM-like β-lactamase enzyme which can inactivate ampicillin. That enzyme can also inactivate other penicillins and some cephalosporins, but the rate of inactivation may vary with different drugs. A β-lactamase inhibitor such as clavulanic acid irreversibly binds to the β-lactamase enzymes, thus rendering the microorganism susceptible to a coadministered penicillin. Amoxicillin and clavulanic acid have been combined for clinical use, and that combination is effective against β-lactamase-producing, ampicillin-resistant strains of *H. influenzae* as well as ampicillin-susceptible, β-lactamase-nonproducing strains.

Some strains of *H. influenzae* have developed resistance to ampicillin by virtue of a mechanism other than β-lactamase production. Such ampicillin-resistant, β-lactamase-nonproducing (Amp³ NBLP) strains probably represent <1% of all *H. influenzae* isolates recovered in the United States (4, 9, 18), but they are becoming prevalent in the United Kingdom (19). Amp³ NBLP strains are thought to have altered penicillin-binding proteins with decreased affinities for ampicillin and other β-lactams (13, 14, 18). Because of the decreased affinity or diminished permeability for β-lactam molecules, a greater concentration of antibiotic is required to inhibit growth of Amp³ NBLP strains. Against those strains, MICs of ampicillin and other β-lactams are increased, and that relative resistance may or may not predict clinical resistance. Although the clinical importance of elevated β-lactam MICs for Amp³ NBLP strains is not always understood, at least one cefuroxime treatment failure resulting from infection with a strain with altered penicillin-binding proteins has been reported (12).

In the study described here, we evaluated the activities of five cephalosporins and amoxicillin-clavulanic acid against Amp³ NBLP strains of *H. influenzae* and compared them with those against other strains of *H. influenzae*. In the process, we evaluated disk diffusion and broth microdilution susceptibility testing procedures, both using Haemophilus Test Medium.

MATERIALS AND METHODS

**Microorganisms.** *H. influenzae* isolates were selected from our stock culture collection of clinical isolates that were initially recovered in medical centers throughout the continental United States. Studies were performed with 60 ampicillin-susceptible strains, 20 β-lactamase-producing strains, and 20 Amp³ NBLP strains. Ampicillin MICs were ≥2.0 µg/ml for the two types of ampicillin-resistant strains, whereas the susceptible strains were all inhibited by ≤1.0 µg/ml. β-Lactamase activity was detected by use of a nitrocefin-based filter paper spot test (Cefinase; Becton-Dickinson Microbiology Systems, Cockeysville, Md.).

**Ampicillin susceptibility tests.** Broth microdilution and disk diffusion tests were performed by the procedures recommended by the National Committee for Clinical Laboratory Standards (16, 17). All tests were performed with broth or agar versions of the Haemophilus Test Medium of Jorgensen et al. (10). Mueller-Hinton broth and agar media were selected after initial screening tests with standard control strains identified lots that fulfilled National Committee for Clinical Laboratory Standards performance criteria (2, 3, 8, 16, 17). Interpretive criteria for microdilution tests and disk diffusion tests were those described in the current National Committee for Clinical Laboratory Standards documents (16, 17). Interpretive criteria for cefituben are not yet

---

* Corresponding author.
included in those documents, and thus, tentative interpretive criteria were those described previously (1, 6) for testing other species on unsupplemented Mueller-Hinton agar.

RESULTS

Susceptibility test results with Amp’ NBLP, β-lactamase-producing, and ampicillin-susceptible strains of H. influenzae are contrasted in Fig. 1 and 2. As expected, the β-lactamases produced by H. influenzae strains inactivated ampicillin, resulting in elevated MICs; the other study drugs did not appear to be affected, since MICs for β-lactamase-positive and -negative strains were essentially identical. Strains in those two categories are not separated in the scattergrams, but Amp’ NBLP strains are designated separately; they were relatively resistant to each of the seven drugs. For Amp’ NBLP strains, the MICs of amoxicillin with clavulanic acid and ampicillin alone ranged from 2.0 to 16 µg/ml; in contrast, the MICs for ampicillin-susceptible strains ranged from 0.12 to 2.0 µg/ml. Cefotaxime MICs for Amp’ NBLP strains were at least 10 times greater than those for susceptible strains. However, all Amp’ NBLP strains were inhibited by ≤1.0 µg of cefotaxime per ml, and that concentration is well below the susceptibility breakpoint that has been defined for tests with other microorganisms. On the other hand, ceftibuten, cefixime, cefaclor, and cefuroxime MICs for some but not all Amp’ NBLP strains exceeded the MIC breakpoint currently used to define clinical resistance for tests with other species.

Ampicillin disk test criteria have recently been modified (10, 16), and the new zone size breakpoints were applied to these data (Fig. 1). All 20 β-lactamase-producing strains and 18 of 20 Amp’ NBLP strains were resistant to ampicillin by disk tests (2 strains were intermediate in susceptibility by both methods), and all 60 ampicillin-susceptible strains were susceptible by the disk diffusion test procedure. As expected, β-lactamase-producing and NBLP strains were equally susceptible to amoxicillin-clavulanic acid by both methods (Fig. 1). Against amoxicillin-clavulanic acid, Amp’ NBLP strains were relatively resistant (MICs, 2.0/1.0 to 16/8.0 µg/ml, respectively) but all strains were susceptible by the current disk test criterion. All but one strain in the normally susceptible population were inhibited by ≤1.0/0.5 µg/ml, respectively, and gave zones of inhibition of ≥25 mm in diameter. Cefaclor and cefuroxime disk tests and broth microdilution tests identified most Amp’ NBLP strains as being resistant or intermediate in susceptibility (Fig. 1). It seems reasonable to assume that, for practical purposes, all Amp’ NBLP strains are actually resistant to cefaclor, cefuroxime, and ampicillin-clavulanic acid, regardless of the in vitro test results.

Half of the 20 Amp’ NBLP strains were also fairly resistant to cefixime and cefetibuten by disk diffusion and broth microdilution tests. For the other half, zone sizes were larger and MICs were lower, but most MICs were two to four times greater than those recorded for the normal susceptible population. That low-level resistance (decreased susceptibility) among the latter Amp’ NBLP strains was not reliably detected by disk diffusion tests (Fig. 2). Six of 10 such strains were resistant to 5-µg cefixime disks, and 8 of 9 strains were susceptible to 30-µg cefetibuten disks. Cefotaxime MICs and 30-µg disk tests categorized all 20 Amp’ NBLP strains as being susceptible (all MICs were ≤1.0 µg/ml and zone sizes were ≥31 mm). However, other H. influenzae isolates were even more susceptible to cefotaxime (MIC, ≤0.06 µg/ml). The decreased susceptibility of Amp’ NBLP strains to cefotaxime does not necessarily indicate that such strains will be nonresponsive to chemotherapy or that cure rates will be diminished.

DISCUSSION

The prevalence of ampicillin-resistant H. influenzae has increased in recent years, and consequently, it is becoming difficult to justify the empiric use of ampicillin in treating infections caused by H. influenzae. Because ampicillin resistance is primarily due to the production of TEM-like β-lactamase enzymes, there are two possible solutions to the problem of selecting alternative chemotherapeutic agents. First, a β-lactamase inhibitor may be combined with a penicillin in order to expand the antimicrobial spectrum to include β-lactamase-producing strains. There are several combinations available; amoxicillin-clavulanic acid was the combination evaluated in the present study. Second, cephalosporins that are relatively resistant to hydrolysis by the TEM-like β-lactamases should be effective if they are active against ampicillin-susceptible strains. Most cephalosporins included in the present study are relatively resistant to hydrolysis by such β-lactamase enzymes (6), and consequently, β-lactamase-producing strains should be just as susceptible as ampicillin-susceptible NBLP strains. For that reason, any of the six study drugs would theoretically be viable alternatives to ampicillin alone.

Infrequently encountered Amp’ NBLP strains of H. influenzae present an entirely different challenge to chemotherapeutic logistics. Since most of those strains are ampicillin resistant because they have developed altered penicillin-binding proteins with decreased affinities for a variety of β-lactams, the alternative drugs also demonstrate decreased in vitro activity (14). The clinical importance of the decreased susceptibility of Amp’ NBLP strains is unknown. Conservatively, one could assume that all Amp’ NBLP strains are likely to be resistant to amoxicillin-clavulanic acid, cefaclor, cefuroxime, cefixime, cefetibuten, and cefotaxime. Cefotaxime might represent an exception, since MICs for Amp’ NBLP strains are not greatly elevated, but we conservatively assume that the strains are resistant until proven otherwise.

If Amp’ NBLP strains of H. influenzae can be identified accurately, there would be no need for clinical laboratories to test strains for susceptibility to the other six drugs that we tested, since they are uniformly effective against strains other than Amp’ NBLP H. influenzae. Broth microdilution tests with ampicillin coupled with a nitrocefin-based β-lactamase test should be sufficient. Appropriate procedural controls and quality assurance tests of the medium are of utmost importance since MICs are very method dependent (18). β-Lactamase-negative strains for which ampicillin MICs are ≥4.0 µg/ml are categorized as Amp’ NBLP H. influenzae strains, and they may be assumed to be relatively resistant to most other β-lactams. Strains for which ampicillin MICs are intermediate (2.0 µg/ml) are difficult to categorize and should be retested.

Heelan et al. (5) found ampicillin to be unreliable since most NBLP strains that appear to be resistant or intermediate in susceptibility by disk diffusion tests have proven to be susceptible by MIC determinations. In our experience, all Amp’ NBLP and all β-lactamase-positive strains were resistant or intermediate in susceptibility and all of our ampicillin-susceptible strains were susceptible by the disk diffusion test. That is in conflict with the results of Mendelman et al. (11, 15), who reported that 10-µg ampicillin disks fail to
FIG. 1. Scattergrams comparing broth microdilution MICs with zones of inhibition for each of four different antimicrobial agents against 100 H. influenzae isolates. Tests with 20 β-lactamase-producing strains (ampicillin MIC, ≥4.0 µg/ml) and 60 ampicillin-susceptible strains (ampicillin MIC, ≤1.0 µg/ml) are combined and designated as the number of strains at each x-y intercept. For drugs other than ampicillin, those two types of strains were equally susceptible. Results of tests with each of 20 Amp' NBLP strains are represented by closed circles.
detect Amp’ NBLP strains consistently. However, they used previously recommended interpretive criteria and performed their studies before the current quality assurance criteria were defined (2, 3, 8). The 20/10-μg amoxicillin-clavulanic acid disks misclassified all Amp’ NBLP strains as being susceptible, even though for 7 of 20 strains MICs were ≥8.0 μg/ml. That error would be diminished if the breakpoints for susceptibility were changed to ≥25 mm (MIC, ≤1.0 μg/ml). Preferably, all Amp’ NBLP strains should be excluded because they may be assumed to be resistant, regardless of the in vitro test results.

Ceftibutene disk diffusion tests failed to identify resistance among 8 of 9 Amp’ NBLP strains, for which MICs were ≤2.0 μg/ml, but correctly identified 10 of 11 Amp’ NBLP strains, for which MICs were >8.0 μg/ml. Tests with 5-μg cefixime disks were more reliable than those with 30-μg ceftibuten disks. Jones and Erwin (7) recently described cefixime data similar to those reported here. However, they assumed that

FIG. 2. Scattergrams comparing broth microdilution MICs with zones of inhibition for each of three cephalosporins against 100 H. influenzae isolates. Tests with 20 β-lactamase-producing strains and 60 ampicillin-susceptible strains are designated as the number at each x-y intercept. The two types of strains were equally susceptible to all three drugs. Results of tests with each of 20 Amp’ NBLP strains are represented by closed circles.
all of their Amp' NBLP strains were actually susceptible, because the MICs were ≤1.0 μg/ml, and thus, they proposed decreasing the zone size breakpoint to ≥21 mm for the susceptible category. In our hands, 9 of 20 Amp' NBLP strains were unequivocally resistant to cefixime (MIC, ≥4.0 μg/ml) but had inhibition zone diameters of ≥21 mm. With the current breakpoint of ≥30 mm for susceptibility, 16 of 20 Amp' NBLP strains were resistant to cefixime, and we judge that categorization to be the most appropriate. Whether that in vitro resistance is clinically relevant remains to be seen.

Some Amp' NBLP strains are likely to show clinical resistance because the MICs for the strains exceed the MIC interpretive breakpoints for susceptibility, but strains for which MICs are increased only slightly are difficult to evaluate. There is relatively little information about the clinical importance of in vitro observations with Amp' NBLP strains. In the United States, Amp' NBLP strains of H. influenzae are very uncommon, but they may become more prevalent in the future, and then the problem of interpreting in vitro data will become much more critical. In the interim, it might be prudent to assume that all Amp' NBLP strains are resistant to all β-lactam antimicrobial agents, regardless of the results of in vitro tests with those agents. With the exclusion of Amp' NBLP strains, reliable disk diffusion and broth microdilution susceptibility tests were obtained when using lots of Haemophilus Test Medium that meet quality assurance criteria.

ACKNOWLEDGMENT

This work was supported by a grant from Schering-Plough Corporation.

REFERENCES