Susceptibility of *Haemophilus influenzae* to Piperacillin-Tazobactam Combinations: Interpretive Criteria and Quality Control Limits for Standardized Tests


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In vitro studies evaluated methods for testing the susceptibility of *Haemophilus influenzae* to piperacillin-tazobactam combinations. Ampicillin-resistant β-lactamase-nonproducing strains of *H. influenzae* may be presumed to be relatively resistant to combinations of piperacillin-tazobactam, even though they frequently appear to be susceptible by disk diffusion methods. Other ampicillin-resistant or -susceptible strains were predictably susceptible; i.e., 130 such strains gave zones of inhibition ≥26 mm in diameter, and MICs for these strains were <0.125/4.0 μg/ml (<1/100 μg/ml when an 8:1 ratio was tested). A resistant category has yet to be defined. For quality control purposes, *H. influenzae* ATCC 49247 should give zones of inhibition 32 to 38 mm in diameter, and broth microdilution MICs should be 0.12/4.0 to 0.5/4.0 μg/ml.

Piperacillin, like many other penicillins, has activity against *Haemophilus influenzae*, but it is readily inactivated by the β-lactamases that are produced by some strains. If a β-lactamase inhibitor were coadministered with piperacillin, it should be equally active against β-lactamase-producing and -nonproducing strains. Tazobactam is the β-lactamase inhibitor that has been combined with piperacillin for clinical application. This combination is active against a broad range of bacterial species, including *H. influenzae* (3, 5). Although piperacillin-tazobactam is not intended to be a primary drug for treating *H. influenzae* infections, it might be appropriate for treating mixed infections that include *H. influenzae*. Clinically resistant strains of *H. influenzae* have not yet been documented, but standardized susceptibility testing criteria are needed in order to detect such resistant strains when or if they do appear. This report describes our efforts to propose interpretive criteria and quality control limits for testing *H. influenzae* against piperacillin-tazobactam.

Some strains of *H. influenzae* have developed resistance to ampicillin by a mechanism that does not involve β-lactamase enzymes (10). Ampicillin-resistant β-lactamase-nonproducing (Amp′ BLNP) strains are relatively resistant to ampicillin because of altered penicillin-binding proteins with diminished affinities for different penicillin molecules (7). Such strains often show reduced susceptibilities to other β-lactam antibiotics, and that decreased potency should not be influenced by the presence of a β-lactamase inhibitor (1). In the United States, Amp′ BLNP strains of *H. influenzae* are currently uncommon (<1% of all isolates), but in the United Kingdom they are beginning to increase in prevalence (5, 10, 12). Our studies included 19 Amp′ BLNP strains in order to determine whether their decreased susceptibilities to piperacillin-tazobactam would be reliably detected by the methods being evaluated.

Most of the previous broth dilution tests with piperacillin-tazobactam (2, 4, 11) were carried out by combining 8 parts piperacillin with 1 part tazobactam (8:1 ratio). That approach provides very little tazobactam in the presence of the low concentrations of piperacillin that inhibit *H. influenzae*. More recently, in vitro tests have been standardized by preparing doubling dilutions of piperacillin with a constant concentration of 4 μg of tazobactam per ml, and that might better reflect the amount of tazobactam at the site of infection during the first few hours, when β-lactamase enzymes are being irreversibly bound. In the present study, broth microdilution tests were performed with both types of drug combinations (8:1 ratios and constant concentrations of 4 μg/ml). All agar diffusion tests were performed with disks containing 100 μg of piperacillin and 10 μg of tazobactam (lot no. 903542; Becton Dickinson Microbiology Systems, Cockeysville, Md.). Ampicillin disks (10 μg) were also evaluated to provide methodological control and to confirm categorization of the study strains. No major or very major errors were recorded with ampicillin disk tests.

Antimicrobial susceptibility tests were performed according to the procedures recommended by the National Committee for Clinical Laboratory Standards for testing *H. influenzae* (8, 9). Haemophilus test medium (HTM), developed by Jorgensen et al. (6), was used throughout. All lots of HTM broth and agar were previously shown to perform satisfactorily when tested with other antimicrobial agents. The inocula were adjusted to provide ca. 5 × 10⁵ CFU/ml. Agar media were incubated in 5 to 7% CO₂, and broth dilution tests were incubated in ambient air (35°C). β-lactamase activity was detected by the nitrocefin-based filter paper spot test (Cefinase; Becton Dickinson Microbiology Systems).

Broth microdilution and disk diffusion tests were carried out with 149 *H. influenzae* isolates, including 41 β-lactamase-producing strains, 19 Amp′ BLNP strains, and 89 ampicillin-susceptible β-lactamase-nonproducing strains.

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Data with the Amp\(^{\text{R}}\) BLPN strains were analyzed separately. Figure 1 contains scattergrams generated with the two types of MICs (8:1 ratios and constant concentrations of 4 \(\mu\)g/ml) compared with zone diameters around 100/10-\(\mu\)g piperacillin-tazobactam disks. The 41 \(\beta\)-lactamase-producing strains were uniformly susceptible to either piperacillin-tazobactam combination. When the two drugs were tested in an 8:1 combination, \(\beta\)-lactamase-producers were inhibited by 0.12/0.016 to 1.0/0.12\(\mu\)g of piperacillin-tazobactam per ml. Of the 89 ampicillin-susceptible isolates, 88 were inhibited by \(\leq0.06/0.008\) \(\mu\)g/ml, and the MIC for one strain was 0.12/0.016\(\mu\)g/ml. When the amount of tazobactam was increased to a constant concentration of 4.0\(\mu\)g/ml, \(\beta\)-lactamase-producing and -nonproducing strains were both inhibited by \(\leq0.12\) \(\mu\)g of piperacillin per ml. Tazobactam alone (4.0\(\mu\)g/ml) failed to inhibit any of the strains included in this report. More than 99% of the zones of inhibition were \(\geq29\) mm in diameter (one outlying strain had a 26-mm-diameter zone).

The results of tests with Amp\(^{\text{R}}\) BLPN strains were examined separately to determine whether the diminished susceptibilities of these strains could be identified by the two testing procedures (microdilution and disk diffusion tests). In Fig. 1, the results of tests with each Amp\(^{\text{R}}\) BLPN strain are indicated by open circles. All 19 strains produced large zones of inhibition (31 to 40 mm in diameter). As might be expected, broth microdilution tests were not influenced by the amount of tazobactam in the test system: with either combination, MICs of piperacillin ranged from \(\leq0.06\) to 2.0 \(\mu\)g/ml. When piperacillin was tested with a constant concentration of 4.0 \(\mu\)g of tazobactam per ml, the other 130 strains were inhibited by \(\leq0.12/4.0\) \(\mu\)g/ml, but 15 of 19 Amp\(^{\text{R}}\) BLPN strains were not inhibited by those concentrations. All three types of strains gave equally large zones of inhibition around 100/10-\(\mu\)g piperacillin-tazobactam disks, and thus the diminished susceptibilities of Amp\(^{\text{R}}\) BLPN strains were not recognized by disk diffusion tests.

Because there were no unequivocally resistant strains (for which the MIC was \(>64\) \(\mu\)g/ml), we could only define a susceptible category. An arbitrary breakpoint was defined as a zone 3 mm smaller than the 29-mm low end of the 99% population or 1 doubling dilution greater than the highest MIC recorded for 99% of the strains. For the 8:1 ratio, the susceptible category would include strains for which the MIC was \(\leq4.0/0.5\) \(\mu\)g/ml. With the currently recommended constant concentration of tazobactam, a susceptible category would include strains for which the MIC was \(\leq0.25/4.0\) or \(\leq4.0/4.0\) \(\mu\)g/ml, depending on whether Amp\(^{\text{R}}\) BLPN strains are included or excluded. When 100/10-\(\mu\)g disks are tested on HTM, strains with zones \(\geq26\) mm in diameter can be defined as susceptible. Resistant categories cannot be defined at this time.

Clinical experience in treating infections due to Amp\(^{\text{R}}\) BLPN strains is not currently available because these strains are not frequently encountered (5, 10, 12). Without such experience, it is not possible to declare them as either susceptible or resistant to piperacillin-tazobactam. We would recommend initial screening tests for ampicillin resistance and for \(\beta\)-lactamase production. Conservatively, Amp\(^{\text{R}}\) BLPN strains might be assumed to be relatively resistant to piperacillin-tazobactam and most other \(\beta\)-lactams (1). Other H. influenzae isolates may be assumed to be susceptible to piperacillin-tazobactam. All strains should give zones \(\geq26\) mm in diameter, or all MICs for strains should be \(\leq4.0/4.0\) \(\mu\)g/ml. Isolates that are not susceptible by those criteria should be very uncommon, and such test results must be confirmed before being reported. Although some strains are relatively resistant, they are not necessarily resistant clinically. In practice, \(\beta\)-lactamase-nonproducing strains (ampicillin susceptible or resistant) would not be treated with any \(\beta\)-lactamase inhibitor-\(\beta\)-lactam combination, and at this point, all \(\beta\)-lactamase-producing strains can be assumed to be susceptible to the inhibitor combinations. Although not included in the present study, strains of H. influenzae that produce an extended-spectrum ROB-1 \(\beta\)-lactamase have been found by one of us (F.C.T.) to be susceptible to the drug combination. Continued surveillance is needed in order to detect any changes in microbial populations, and for that reason, clinical microbiology laboratories might be asked to evaluate piperacillin-tazobactam against selected strains.

Two different multilaboratory studies were undertaken in order to evaluate the reproducibilities of disk diffusion and broth microdilution tests with the standard Amp\(^{\text{R}}\) BLPN control strain of H. influenzae (ATCC 49247). Each study involved tests with a different lot of agar or broth assigned to each of five participants and a sixth lot that was common to all participants. Each laboratory performed replicate tests, and the distribution of MICs or zone diameters was evaluated in order to define the range of acceptable results. The overall distribution of zone measurements around 100/10-\(\mu\)g piperacillin-tazobactam disks is displayed in Table 1. Control limits that accept zone diameters of 32 to 38 mm were calculated by using the median statistic of Gavan et al. (3).

Table 2 describes the results of replicate broth microdilution tests with piperacillin and tazobactam combined in an 8:1 ratio and with a constant concentration of 4 \(\mu\)g of tazobactam per ml. Each laboratory generated 30 MICs (20 MICs with microdilution trays that they prepared and 10 MICs with a control lot of trays common to all participants).
TABLE 1. Zones of inhibition with piperacillin-tazobactam disks against *H. influenzae* ATCC 49247a

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>No. of times the following zone diam (mm) was recorded</th>
<th>Range (mm)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 32 33 34 35 36 37 38 39 40 41</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1 5 31 25 7 12 6 3</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>1 9 32 37 11</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>2 4 20 30 28 6</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>8 42 30 10</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>2 4 16 34 23 11</td>
<td>5</td>
</tr>
</tbody>
</table>

All 0 3 14 67 144b 110 57 35 17 3 0

a The five participating laboratories reported 75 zones of inhibition on the five different lots of HTM agar and another 15 zones on a sixth lot common to all participants. This table combines all 90 zones reported by each laboratory.

b The median (target value) for all laboratories was 35 mm, and the median of the ranges was 5 mm. The target value ±1/2 median range (rounded up) defined zone-size limits of 32 to 38 mm.

That exercise resulted in 150 MICs for both drug combinations. The distribution of MICs defined the modal target value, and control limits were identified as the mode ±1 doubling dilution (Table 2). The Amp" BLNP control strain (ATCC 49247) was not greatly influenced by the presence of tazobactam, and thus the same MIC control limits (0.12 to 0.25 μg/ml) apply to either combination. Additional tests with other control strains will be needed to assure the user that appropriate concentrations of tazobactam are present in the microdilution trays or in the susceptibility testing disks. In unsupplemented broth, *Escherichia coli* ATCC 35218 and ATCC 25922 are used for that purpose.

TABLE 2. Distribution of broth microdilution MICs for *H. influenzae* ATCC 49247a

<table>
<thead>
<tr>
<th>Proportion of tazobactam tested</th>
<th>No. of times each of the following MICs (μg/ml) was reported in 150 testsb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.03 0.06 0.12 0.25 0.5 1.0</td>
</tr>
<tr>
<td>Constant 4-μg/ml concn</td>
<td>6c [56 86 1]</td>
</tr>
<tr>
<td>Fixed 8:1 ratio</td>
<td>6c [42 92 1]</td>
</tr>
</tbody>
</table>

a MICs were determined in a five-laboratory collaborative effort to evaluate two combinations of piperacillin-tazobactam in HTM broth.

b Brackets enclose MICs that are within the proposed control limits (0.12 to 0.25 μg/ml). MICs shown are of piperacillin.

c All MICs outside the proposed lower limits were reported with the broth medium assigned to laboratory B; that laboratory reported acceptable results with the common-lot trays.

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REFERENCES


