Disk Diffusion Susceptibility Testing and Broth Microdilution Quality Control Guidelines for BMY-28100, a New Orally Administered Cephalosporin

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The BMY-28100 30-μg-disk test was evaluated by using 615 clinical isolates. Regression analyses and error rates were determined, leading to the recommendation of ≥18-mm zone diameters (MIC correlate, ≥8.0 μg/ml) for susceptibility and ≤14-mm zone diameters (MIC correlate, ≥32 μg/ml) for resistance. Nearly all false-susceptible disk test results were among the Providencia spp. and the beta-lactamase-positive Haemophilus influenzae strains. Susceptibility disk test results for these species should be interpreted with caution. The following broth microdilution MIC quality control guidelines were determined from results of a collaborative trial: Escherichia coli ATCC 25922, 1.0 to 4.0 μg/ml; Enterococcus faecalis ATCC 29212, 4.0 to 16 μg/ml; Staphylococcus aureus ATCC 29213, 0.25 to 1.0 μg/ml; and Pseudomonas aeruginosa ATCC 27853, >32 μg/ml.

BMY-28100 is a recently studied, orally administered cephalosporin that has structural similarities with cefadroxil (position 7 p-hydroxy group) and cefixime (position 3 cis-propenyl group) (2-5, 7, 12, 13). These modifications produce a spectrum of antimicrobial activity most similar to that of cefaclor, i.e., against Staphylococcus spp., beta-hemolytic streptococci, Streptococcus pneumoniae, beta-lactamase-producing Haemophilus influenzae, Neisseria gonorrhoeae, Escherichia coli, Klebsiella spp., Proteus mirabilis, and some strains of enteric pathogens, such as Salmonella spp. and Shigella spp. (3, 4, 7, 11-13). In addition, measurable activity was observed against Enterococcus faecalis and cephalosporin-susceptible MIC breakpoint of ≥8.0 μg/ml (data on file; Bristol-Myers Co., Wallingford, Conn.). This contrasts with levels in serum observed with some new oral cephalosporins that may require susceptible breakpoints as low as ≤1.0 μg/ml (5, 6, 11).

In this report we present the results of studies with 30-μg BMY-28100 disks that correlate the observed zones of inhibition with MICs. An additional quality control (QC) collaborative investigation determined preliminary guidelines for BMY-28100 tested by the broth microdilution method.

BMY-28100 was provided by Bristol-Myers. Other diagnostic and reagent antimicrobial agents were supplied by their respective domestic manufacturers. The BMY-28100 30-μg disk and a control 30-μg cefuroxime disk were manufactured by BBL Microbiology Systems, Cockeysville, Md. The study used over 600 clinical isolates that were tested by the National Committee for Clinical Laboratory Standards standardized procedures for the broth microdilution and disk diffusion methods (9, 10). Regression analysis was performed by the method of least squares, and error rates were calculated (8). The collaborative MIC QC study used the study design previously described (1).

Table 1 and Fig. 1 present the regression comparisons of BMY-28100 MICs and 30-μg-disk zone diameters. The scattergram in Fig. 1 and regression line analysis suggest that

**TABLE 1.** Regression analyses correlating BMY-28100 MICs and zones of inhibition around 30-μg disks

<table>
<thead>
<tr>
<th>Organisms (no. tested)</th>
<th>Regression formula*</th>
<th>Correlation coefficient</th>
<th>Zone diam (mm)</th>
<th>% Interpretive error†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>All strains (615)</td>
<td>y = 17.1 - 0.29x</td>
<td>0.80</td>
<td>≥18</td>
<td>≤14</td>
</tr>
<tr>
<td>Without Providencia and Haemophilus species (544)</td>
<td>y = 17.2 - 0.30x</td>
<td>0.88</td>
<td>≥18</td>
<td>≤14</td>
</tr>
<tr>
<td>Providencia spp. (29)</td>
<td>y = 16.5 - 0.17x</td>
<td>0.59</td>
<td>≥18</td>
<td>≤14</td>
</tr>
<tr>
<td>H. influenzae (42)</td>
<td>y = 11.9 - 0.08x</td>
<td>0.09</td>
<td>≥18</td>
<td>≤14</td>
</tr>
</tbody>
</table>

* y = MIC as log5₀ + 9; y = zone diameter in millimeters.
† Interpretive zone options.
‡ Very major, false-susceptible by disk diffusion results; major, false-resistant by disk diffusion results; minor, intermediate by either the MIC or disk test.
§ Error rate among the 20 beta-lactamase-producing strains only.

*L. monocytogenes. BMY-28100 is 45% protein bound, bactericidal, and relatively stable to the most frequently isolated beta-lactamases (3, 4, 7, 12, 13). Preliminary human pharmacokinetic information indicates sufficient drug concentration in serum after oral doses to apply the traditional diagnostic and reagent antimicrobial agents were supplied by their respective domestic manufacturers. The BMY-28100 30-μg disk and a control 30-μg cefuroxime disk were manufactured by BBL Microbiology Systems, Cockeysville, Md. The study used over 600 clinical isolates that were tested by the National Committee for Clinical Laboratory Standards standardized procedures for the broth microdilution and disk diffusion methods (9, 10). Regression analysis was performed by the method of least squares, and error rates were calculated (8). The collaborative MIC QC study used the study design previously described (1).

Table 1 and Fig. 1 present the regression comparisons of BMY-28100 MICs and 30-μg-disk zone diameters. The scattergram in Fig. 1 and regression line analysis suggest that
TABLE 2. Broth microdilution MIC results for BMY-28100 tested by six medical centers against four recommended QC strains

<table>
<thead>
<tr>
<th>QC organism</th>
<th>No. of reports at MIC (µg/ml) of:</th>
<th>Mode (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 29213</td>
<td>(1)</td>
<td>101</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Includes common lot results. See reference 1.
b Parentheses indicate recommended MIC range.

FIG. 1. Scattergram comparing the BMY-28100 MICs with zones of inhibition around a 30-µg disk. A total of 615 bacterial isolates was tested, and the preliminary interpretive breakpoints were acceptable. The MIC values were represented by the vertical lines. The regression line is drawn for the MIC interval of 0.12 to 32 µg/ml. Numbers surrounded by circles and squares were the false-negative errors contributed by the Providencia spp. and the beta-lactamase-positive H. influenzae strains, respectively.

the best zone criterion correlating with a susceptible MIC of ≤8.0 µg/ml would be ≥18 mm. This zone was identical to those for many other cephalosporins (9, 11). The intermediate range would be 15 to 17 mm, correlating with a BMY-28100 MIC of 16 µg/ml. A zone for resistant isolates would be ≥14 mm, or an MIC of ≥32 µg/ml. However, the false-susceptible rate was unacceptably high (2.8% very major errors), although the false-resistant (major errors) and minor interpretive error rates were acceptable (Table 1). Two genera, Haemophilus and Providencia, contributed the vast majority of false-susceptible zone diameters. When Providencia rettgeri, Providencia stuartii, and H. influenzae strains (71 organisms) were deleted from the analyses, the very major errors were reduced from 2.8 to only 0.7%. The very major interpretive error rates among these problem genera ranged from 10% for beta-lactamase-positive H. influenzae to 38% for the Providencia spp. Results with the control 30-µg cefuroxime disk produced results comparable to those in previous reports (8).

The broth microdilution QC study required from each of six laboratories 20 MICs derived from trays prepared in their own facility. In addition, each laboratory performed five MICs on a broth microdilution tray common to all investigators. Four routinely used QC organisms were tested, and the results are summarized in Table 2. Modes were clearly established for all four organisms.

BMY-28100 is an oral cepham with a spectrum very comparable to those of many currently utilized cephalosporins (2-4, 7, 11-13). Other investigational orally administered cephalosporins appear to have superior activity compared with that of BMY-28100, especially against members of the family Enterobacteriaceae and against H. influenzae (3-6, 14). The development of in vitro diagnostic tests for some of these newer oral cephalos has been difficult because of lower susceptibility MIC breakpoints and high false-susceptible disk results produced by some members of the family Proteaeae (5, 6). We found that BMY-28100 disk diffusion results were compromised by a very high incidence of very major interpretive errors among the Providencia spp. This contrasts with the finding of high error rates among the Morganella morganii isolates tested against cefixime and cefetamet (5, 6). It appears that some strains of beta-lactamase-producing H. influenzae could also be interpreted as susceptible by the disk test, yet they have BMY-28100 MICs of ≥32 µg/ml. All other tested species had an absolute correlation between the MIC and disk zone interpretation of 92.8%, with only 0.9% very major and major interpretive errors. We recommend interpretive zones for the 30-µg BMY-28100 disk as follows: susceptible, ≤18 mm (MIC correlate, ≤8.0 µg/ml), and resistant, ≤14 mm (MIC correlate, ≥32 µg/ml).

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LITERATURE CITED

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