Proposed Quality Control and Interpretive Criteria for Disk Diffusion Susceptibility Testing with Enoxacin

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The standardized disk diffusion test, in which a 10-μg enoxacin disk is used, was performed and microbroth dilution MICs were determined to establish individual test control values with Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and S. aureus ATCC 29213. In addition, regression analysis correlating inhibitory zone diameter with MICs for approximately 400 gram-negative clinical isolates was performed. Based on linear regression and error rate-bounded analyses, criteria for the category calls of isolates are proposed.

Enoxacin, formerly called AT-2266 or CI-919, is a new pyridone carboxylic acid derivative originally described by Matsumoto and co-workers (5) as an oral antipseudomonal drug. Subsequent in vitro data suggested that enoxacin had a spectrum of activity which covered not only Pseudomonas aeruginosa, but also members of the family Enterobacteriaceae and Staphylococcus spp. (1, 2, 4, 7). In this study, quality control values for performing disk diffusion susceptibility testing and determining microbroth dilution MICs are proposed. In addition, linear regression analysis correlating MICs and inhibitory zone diameters (determined with a 10-μg enoxacin disk) for various gram-negative organisms was performed.

MATERIALS AND METHODS

**Disk quality control values.** Quality control values for the standard assay strains Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923 were determined by the standard disk diffusion test (9). A single lot of Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) and a single lot of 10-μg enoxacin disks prepared by BBL Microbiology Systems, Cockeysville, Md., were used for all studies. Tests with the three assay strains were performed on 100 consecutive days and read each day by one of 12 different technologists. The inhibitory zone diameters were measured with sliding calipers. Several other antimicrobial agents, including ampicillin, carbenicillin, chloramphenicol, tetracycline, gentamicin, tobramycin, and cephalothin, were simultaneously tested with enoxacin to serve as controls. Daily quality control ranges were defined as the median zone diameter ± two times the standard deviation rounded off to the nearest whole number (95% confidence limits).

**MICs and regression line analysis.** MIC and inhibitory zone diameter methodologies were performed simultaneously on 402 gram-negative clinical isolates. MICs were determined by the standardized microdilution broth method (8) in 0.1-ml volumes with cation-supplemented Mueller-Hinton broth (Difco). Microdilution panels, prepared with the MIC-2000 system (Dynatech Laboratories Inc.), were stored at −70°C until used. Enoxacin at final concentrations ranging from 0.06 to 16 μg/ml in log2 dilutions was used. Inhibitory zone diameters were determined by using the standardized disk diffusion test (9). Growth from four to five isolated colonies of the test organisms incubated for 4 to 6 h at 37°C in brain heart infusion broth was adjusted to the turbidity equivalent to that of a 0.5 McFarland standard. This suspension was used for inoculating Mueller-Hinton agar plates for disk diffusion testing and further diluted for microbroth diffusion testing. Microdilution panels were inoculated by using disposable inoculators (Dynatech) so that each well contained ca. 5 × 10^5 CFU/ml. The MIC was defined as the lowest concentration of drug that prevented visible growth. Disk diffusion zone diameters were measured to the nearest 0.1 mm with calipers. Quality control for each method was performed with each set of inoculations, using P. aeruginosa ATCC 27853, E. coli ATCC 25922, S. aureus ATCC 29213, or S. aureus ATCC 25923.

Inhibitory zone diameters and MICs for each drug-organism combination were plotted for linear regression analysis (y = mx + b; where y = log2 MIC, m = zone diameter in millimeters, and b = y intercept). The results were analyzed by the error rate-bounded method of Metzler and DeHaan (6). When an MIC did not have an endpoint (<0.06 or >16 μg/ml), data points from that MIC-zone diameter combination were not plotted or included in the calculations.

RESULTS AND DISCUSSION

**Quality control.** The data obtained by disk diffusion for the three quality control strains are summarized in Table 1. Tentative values for individual daily quality control are also shown. A range of daily control values for microdilution broth determination of MICs is shown in Table 2.

**Regression line analysis.** Table 3 shows the MICs and zone diameter data for the 402 gram-negative clinical isolates used for linear regression and error rate-bounded analyses. Data from eight drug-organism combinations were excluded from analysis because discrete MIC endpoints were not determined. Figure 1 is a scattergram of MICs versus zone diameters for 394 gram-negative clinical isolates, showing the results of both regression line and error rate-bounded

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classification analyses. Recently, a great deal of pharmacokinetic data on enoxacin has been compiled by several investigators. Tsuei et al. (R. Wolf, R. Eberl, A. Dunky, N. Mertz, T. Chang, J. R. Goulet, and J. Latts, J. Antimicrob. Chemother., in press) showed that steady-state levels of enoxacin after a 400-mg dose averaged 3.53 μg/ml (standard deviation, 0.92). Similar studies by Wolf et al. (S. E. Tsuei, A. S. Darraugh, and L. Brick, J. Antimicrob. Chemother., in press) demonstrated steady-state levels of 4.53 μg/ml (standard deviation, 0.81) and 5.90 μg/ml (standard deviation, 2.07) after twice a day administration of 400 and 600 mg of enoxacin, respectively. Based on these data, organisms requiring for inhibition MICs of >4 μg/ml were considered to be resistant to enoxacin. On the basis of linear regression, zone diameters of ≤21 mm are resistant (MIC of >4 μg/ml), and zone diameters of ≥22 mm are susceptible (MIC of ≤4 μg/ml). However, use of linear regression for determining category call breakpoints may be subject to a variety of problems, including an uneven distribution of observations with more susceptible than resistant isolates, use of discrete MICs, failure to include extreme answers (discrete MIC not determined or no zone diameter around disk), and a curvilinear rather than linear relationship between the values (6). For these reasons, the error rate-bounded method of Metzler and DeHaan is frequently considered to be a more appropriate technique for determining breakpoints. When the latter method was applied to our data, zone diameter breakpoints of ≤21 mm for the resistant category and ≥22 mm for the susceptible category were determined. These breakpoints correspond to error rates of 0.5% false-susceptible and 1.5% false-resistant.

Two other groups of investigators have performed linear regression and error rate-bounded analyses for enoxacin with different results. Using the agar dilution technique and a breakpoint of 3.1 μg/ml, Chin and Neu (1) reported zone diameters of ≤14 and ≥18 mm for the resistant and susceptible categories, respectively, when testing 345 isolates. Others (P. C. Fuchs, R. N. Jones, A. L. Barry, and C. Thornsberry, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemther. 23rd, Las Vegas, Nev., abstr. no. 694, 1983) tested 487 isolates by the microdilution technique and obtained the same results when using 4 μg/ml as the resistant breakpoint. Several possible explanations for the discrepancy between our data and those of the other investigators exist. One obvious difference is the type of isolates tested. Our data represent gram-negative clinical isolates exclusively, whereas they tested both gram-positive and gram-negative clinical isolates. Chin and Neu used a different breakpoint and a different technique for determining MICs. Gerlach (3) described a comparative study in which agar dilution results were frequently lower than microdilution and macrodilution MICs. Approximately half of our microdilution MICs are 1 log2 dilution higher than the agar dilution results of Chin and Neu. However, when our data are compared with those of Fuchs et al., many of our MICs are still 1 log2 dilution higher than theirs. The disk diffusion quality control limits determined by Fuchs et al. were, in general, larger than ours: E. coli, 28 to 36 mm; P. aeruginosa, 22 to 28 mm; and S. aureus, 22 to 28 mm (see Table 1 for comparison). Although neither group included MICs for the three control organisms, it would appear that the discrepancy in our data can be explained by a 1 log2 dilution difference in MICs for many of the organisms tested. This difference may: (i) reflect the acceptable interlaboratory deviation in reproducibility of MIC testing, (ii) represent geographic differences in the susceptibility of various organisms to enoxacin, or (iii) reflect a bias due to the inclusion of several multiply resistant organisms in our study. Since the published susceptibility data on enoxacin (1–3) are also not in complete agreement, it is difficult to pinpoint a single factor responsible for these differences. Until more data are available from other investigators, the results of this and previous studies must be regarded as preliminary and should be updated as more information becomes available.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Range</th>
<th>Mode</th>
<th>Mean</th>
<th>SD</th>
<th>Daily quality control range</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>26–33</td>
<td>29</td>
<td>28.7</td>
<td>1.6</td>
<td>26–32</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>21–26</td>
<td>23</td>
<td>23.5</td>
<td>1.1</td>
<td>21–26</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>20–25</td>
<td>22</td>
<td>22.4</td>
<td>1.1</td>
<td>20–25</td>
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* Sample size of 100.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone diam (mm)</th>
<th>Daily quality control range</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>26–33</td>
<td>29</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>21–26</td>
<td>23</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>20–25</td>
<td>22</td>
</tr>
</tbody>
</table>

* Sample size of 15.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. tested</th>
<th>Zone diam range (mm)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobacter spp.</td>
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<td>16–18</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Alcaligenes spp.</td>
<td>3</td>
<td>18–23</td>
<td>4–8</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>26</td>
<td>26–34</td>
<td>0.125–1</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>39</td>
<td>26–35</td>
<td>0.125–2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>53</td>
<td>27–37</td>
<td>0.06–1</td>
</tr>
<tr>
<td>Flavobacterium spp.</td>
<td>2</td>
<td>18–22</td>
<td>4–8</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>45</td>
<td>16–34</td>
<td>0.125–8</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>12</td>
<td>27–33</td>
<td>0.125–0.5</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>35</td>
<td>27–38</td>
<td>0.25–1</td>
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<tr>
<td>Providencia spp.</td>
<td>16</td>
<td>18–36</td>
<td>0.25–8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>59</td>
<td>16–31</td>
<td>0.5–16</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>12</td>
<td>15–31</td>
<td>0.25–16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. tested</th>
<th>Zone diam range (mm)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>11</td>
<td>29–34</td>
<td>0.25–0.5</td>
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<tr>
<td>Serratia spp.</td>
<td>42</td>
<td>11–33</td>
<td>0.25–16</td>
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<td>Shigella spp.</td>
<td>9</td>
<td>28–33</td>
<td>0.125–0.25</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>3</td>
<td>30–32</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Pseudomonas species not aeruginosa or maltophilia.
FIG. 1. Correlation between MICs and zone diameters with 10-μg enoxacin disks. The solid horizontal line is the proposed MIC breakpoint of 4 μg/ml; the broken vertical line represents the proposed critical zone diameter for susceptible organisms (22 mm) determined by the error rate-bounded method. The solid vertical line denotes the resistant breakpoint (21 mm) determined by linear regression analysis.

Regression line: \( y = 9.03 - 0.343x \); correlation coefficient = 0.69. Letters A through K represent 1 to 11 observations, respectively.

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LITERATURE CITED


