Disk Diffusion Susceptibility Tests with Norfloxacin: Confirmation of Proposed Interpretive Criteria

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One hundred and eighty three clinical isolates of aerobic bacteria were tested against norfloxacin by both agar dilution (WHO-ICS) and disk diffusion test procedures (standardized FDA single disk test). Two experimental 10 µg norfloxacin disks were studied. Results were analyzed in terms of recently recommended breakpoints for clinical susceptibility (MIC ≤ 16 µg/ml, zone diameter ≥ 17 mm) and resistance (MIC ≥ 32 µg/ml, zone diameter ≤ 12 mm). Excellent correlation was demonstrated by statistical analysis between paired MIC and zone size values (average value for each MIC; \( r = -0.9782 \)). An MIC of 16 µg/ml was found to correlate with a zone of 10.4 mm. Application of the recommended zone size breakpoints resulted in prediction of 177 isolates as being susceptible while six (3.3 %) were predicted to be either intermediate or resistant. The findings of this study validate the earlier recommendations stated above.

Norfloxacin (MK-0366, AM-715) is a new, orally active antimicrobial agent active both in vitro and in vivo against a number of different aerobic gram-negative pathogens (1, 2). The extended spectrum of norfloxacin together with its pharmacologic properties (3) and the absence of cross-resistance between norfloxacin and other antimicrobial agents (4), suggest that this antimicrobial agent may have a potential role in the treatment of urinary tract infections caused by susceptible organisms.

Recently, tentative guidelines for use in diffusion disk susceptibility tests with a 10 µg norfloxacin disk have been proposed (5). These guidelines, based upon an MIC of 16 µg/ml as being the upper limit of probable clinical susceptibility, are as follows: susceptible (MIC ≤ 16 µg/ml) – a zone diameter of 17 mm or more; resistant (MIC ≥ 32 µg/ml) – a zone diameter of 12 mm or less; intermediate – a zone diameter of 13 to 16 mm. The studies reported here were performed in order to corroborate these recommendations.

Materials and Methods

Microorganisms. A total of 183 clinical isolates of Enterobacteriaceae (138 isolates), Pseudomonas spp. (33 isolates) and gram-positive cocci (12 isolates were tested. Most were of etiological or nosocomial importance. The control organisms Escherichia coli ATCC 25922, Staphylococcus aureus 25923 and Pseudomonas aeruginosa ATCC 27853 were included in all experiments. Inocula for susceptibility tests were prepared from overnight cultures. Inocula for use in MIC determinations were adjusted to contain approximately 10⁵ CFU per replication. Inocula for use in the disk diffusion tests were prepared using a No. 0.5 MacFarland standard.

Susceptibility Testing. Minimal inhibitory concentrations for norfloxacin were determined using the WHO-ICS agar dilution procedure as described by Washington and Sutter (6). Commercial dehydrated Mueller Hinton agar (BBL Microbiology Systems) was used in these tests. Disk diffusion susceptibility tests were performed using the standardized FDA version of the Bauer-Kirby single disk method as discussed by Barry and Thornberry (7). Prepared plates of Mueller Hinton agar (MH II, BBL Microbiology Systems) were used in these latter tests. Two commercially prepared 10 µg norfloxacin disks were tested (Difco Laboratories, lot 691009; BBL Microbiology Systems, lot 2454). In analyzing the data from the disk diffusion tests, the paired results obtained with the two disks were averaged.

Statistical Analysis. As will be seen in the results to be presented, the vast majority of the organisms included in this study (177 or 97 %) had norfloxacin

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MICs of 2 μg/ml or less. Because of the bias such a distribution could exert on the statistical analysis of the data, the correlation between agar dilution MICs and disk diffusion test zone diameters was analyzed by pairing log 2 MIC values with the average zone of inhibition obtained for each MIC value. This correlation was then examined by linear regression analysis.

Results

A summary of the results of this study is presented in Table 1 in terms of norfloxacin MIC values, numbers of organisms inhibited and range and average (± standard deviation) of the corresponding zone size values. As already noted 177 isolates had norfloxacin MICs of 2 μg/ml or less; 102 isolates (56%) had MIC values of 0.13 μg/ml or less. The highest MIC observed was 16 μg/ml (two isolates).

Results of the regression analysis are shown in Figure 1. Correlation between MIC and average zone size values was excellent (r = -0.9782). In the study of Shungu, Weinberg and Gadebusch (5) it was proposed that 16 μg/ml of norfloxacin be regarded as the limiting MIC for probable clinical susceptibility; statistically, 16 μg/ml correlated with a zone diameter of 11 mm (5). In the study reported here, a MIC of 16 μg/ml was found to correlate with a zone of 10.4 mm. The disagreement between these two correlates is minor.

As noted, 177 isolates had norfloxacin MIC values of 2 μg/ml or less. All but two isolates had zones of inhibition of 17 mm or more. Both of the latter were isolates of *Streptococcus fecalis* with norfloxacin MICs of 2 μg/ml. Two isolates (one each of *Pseudomonas* and *Acinetobacter* species) had MICs of 8 μg/ml;

Table 1: Distribution of averaged 10 μg norfloxacin zone size values (diameter) according to corresponding norfloxacin MIC values*.

<table>
<thead>
<tr>
<th>Agar dilution norfloxacin MIC value (μg/ml)**</th>
<th>0.063</th>
<th>0.13</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>&gt; 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of isolates inhibited</td>
<td>48</td>
<td>54</td>
<td>25</td>
<td>12</td>
<td>11</td>
<td>27</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Range of zone size values (mm)</td>
<td>25.60</td>
<td>23.10</td>
<td>21.30</td>
<td>19.40</td>
<td>15.70</td>
<td>16.00</td>
<td>16.70</td>
<td>12.10</td>
<td>6.50</td>
<td>3.16</td>
</tr>
<tr>
<td>Average zone size value (SD)</td>
<td>31.00</td>
<td>(3.16)</td>
<td>27.81</td>
<td>(2.07)</td>
<td>27.80</td>
<td>(3.21)</td>
<td>25.25</td>
<td>(4.16)</td>
<td>22.93</td>
<td>(4.86)</td>
</tr>
</tbody>
</table>

*Data obtained using the standardized FDA version of the single disk diffusion test and two experimental 10 μg norfloxacin disks.

**Data obtained using the standardized WHO-ICS agar dilution procedure.