Critical Evaluation of Amdinocillin Disk Susceptibility Tests Correlated with Agar Dilution Tests

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Tests with 10-μg amdinocillin disks were performed in parallel with agar dilution tests and three different ranges of inoculum density. A resistance breakpoint of ≥13 mm and an intermediate category of 14 to 16 mm are recommended. Susceptible strains provided zones ≥17 mm in diameter, but many appeared to be resistant by the agar dilution methods if the inoculum exceeded 10⁴ CFU per spot.

Amdinocillin (formerly known as mecillinam) is an amdinopenicillin which is active against most Enterobacteriaceae. It may be used alone for treating lower urinary tract infections or it may be combined with other β-lactams for a synergistic effect (3–6, 10). In vitro susceptibility tests with amdinocillin are complicated by significant instability of the drug under ordinary testing conditions (9, 12). In vitro tests are also greatly influenced by the conductivity or osmolarity of the test medium (9, 11, 12). Minor changes in inoculum density can dramatically affect the results of dilution tests (9). Because of these difficulties in determining MICs, it is difficult to select an appropriate MIC breakpoint for separating susceptibility and resistance categories. Based on pharmacokinetic data, strains with MICs ≤4.0 μg/ml are often considered susceptible.

Anderson et al. (1) evaluated the performance of tests with 10-μg amdinocillin disks and recommended a 16-mm breakpoint for the susceptible category (MIC, ≤4.0 μg/ml). However, their published scattergram revealed an inordinately high rate of discrepancies. The reliability of a 16-mm cutoff point for susceptibility was confirmed by comparing zone diameters with bacteriological and clinical efficacy during therapeutic trials with humans (4, 5).

Intepretable standards for dilution tests or disk diffusion tests have not been established by the National Committee for Clinical Laboratory Standards because of the apparent high rate of discrepancy between the two types of test. In this study, I reevaluated the correlation between standard agar dilution and disk diffusion tests by studying a limited number of enteric bacilli with carefully controlled inocula for both tests. In addition, zone diameters with a modified disk test (reduced inoculum) were correlated with MICs.

Tests were performed with 3 Citrobacter diversus, 7 C. freundii, 12 Enterobacter aerogenes, 3 E. agglomerans, 6 E. cloacae, 19 Escherichia coli (11 ampicillin resistant), 3 Klebsiella oxytoca, 22 K. pneumoniae, 1 Morganella morganii, 2 Providencia rettgeri, 5 P. stuartii, 1 Proteus vulgaris, 2 Salmonella enteritidis, and 2 Serratia marcescens. Amdinocillin was incorporated into Mueller-Hinton agar in twofold dilutions ranging from 0.06 to 64 μg/ml and tested within 3 days after preparation. No loss of potency could be detected by testing reference strains on agar plates that were refrigerated for as long as 5 days; storage for longer periods was not evaluated. For each strain, a log-phase broth culture was prepared and then diluted to match the turbidity to a MacFarland 0.5 standard. With an inoculum-replicating device (13), the test plates were inoculated with 1:2, 1:4, 1:20, and 1:40 dilutions of each adjusted suspension. At the same time, two disk diffusion tests were performed. One was the standard National Committee for Clinical Laboratory Standards procedure (7), and the other used a 1:10 dilution of the adjusted cell suspension. Three lots of 10-μg amdinocillin disks (BBL Microbiology Systems lot no. 405568 and C118862-01 and Difco Laboratories lot no. C11872-01) were tested on each plate. The average of the three zone diameters was rounded to the nearest whole number, and that value was used for calculating regression statistics. Significant differences between the three lots of disks were not observed.

The total number of viable cells in each adjusted inoculum suspension was determined when the tests were prepared. The actual inoculum deposited on each agar dilution plate was calculated by assuming that 2-μl volumes were deposited by the inoculum replicator (8). Each strain provided four MICs with different inocula. Three inoculum categories (≥5 × 10⁷ to <1 × 10⁸, ≥1 × 10⁸ to <5 × 10⁹, and ≥5 × 10⁹ to <1 × 10¹⁰ CFU per spot) could be identified for all 88 strains. The National Committee for Clinical Laboratory Standards (8) recommends an inoculum of approximately 10⁹ CFU per spot, but even under the most strictly controlled test conditions this could easily vary from 5 × 10⁸ to 5 × 10⁹ CFU per spot (the first two categories). The third category (≥5 × 10⁹ to <1 × 10¹⁰ CFU per spot) represents a slightly excessive inoculum which could be achieved by the standard methods.

Table 1 summarizes the results of agar dilution tests with the three different inocula. When the inoculum was less than 1.0 × 10⁹ CFU per spot, 84% of the enteric bacilli were susceptible to 4.0 μg of amdinocillin per ml. However, when the inoculum was in the upper portion of the acceptable range, only 54% were susceptible. With an even greater inoculum, only 33% were susceptible. It should be noted that the 88 isolates included a disproportionate number of strains belonging to species most likely to be affected by changes in inoculum density, i.e., Enterobacter spp., Klebsiella spp., Citrobacter spp., ampicillin-resistant E. coli, etc.

Zone diameters obtained with both types of disk test (standard and 1:10 dilution of inoculum) were correlated with agar dilution MICs (≥5 × 10⁷ to <1 × 10⁹ CFU per spot). The regression formulas are presented in Table 2. By regression analysis, an MIC of 8.0 μg/ml correlated with the zone size breakpoint of ≥16 mm recommended by Anderson et al. (1). The calculated zone size breakpoints for the standard method were ≥17 mm for susceptibility (MIC, ≤4.0 μg/ml) and ≤13 mm for resistance (MIC, ≥16 μg/ml). Only 3 of the 88 strains produced 16-mm zones (2 susceptible and 1 resistant); thus, a change from ≥16 to ≥17 mm for the susceptibility category should have little impact on the
The error rates were calculated by assuming that 2-μl spots were deposited onto agar plates.

Table 1 shows the overall accuracy of the test. When the diluted inoculum was used, the breakpoints were 3 mm larger and the correlation coefficient was only slightly improved. MICs obtained with denser inocula did not correlate with either set of zone diameters (correlation coefficients, \(\leq 0.5\)). Those regression statistics are not shown.

Table 3 compares the interpretive agreements between the three types of MIC and both disk diffusion tests (scored by the zone size interpretive standards defined in Table 2). Of the 73 strains that were susceptible with the standard disk method, 1 was resistant by MIC with a light inoculum, 25 were resistant with a slightly greater inoculum, and 42 were resistant with an even larger inoculum. Similar results were obtained when the inoculum for the disk test was reduced 10-fold and zone size standards were adjusted accordingly. The error rates would not be changed substantially if a susceptibility breakpoint of \(\geq 16\) or \(\geq 17\) mm were applied to the modified disk test.

The number of viable cells I achieved after adjusting turbidity to match a MacFarland 0.5 standard varied from 5.2 \(\times\) 10^7 to 2.1 \(\times\) 10^8 CFU/ml (mean, 10^8 CFU/ml). That range of variability is well within the range of viable cell counts that were previously documented for repeated tests with standard control strains (2). Agar diffusion tests are normally performed by simply preparing a fixed dilution of the adjusted inoculum, assuming that all cultures contain 10^8 viable cells per ml. If a 1:20 dilution is prepared, the plates should receive 10^7 CFU per 2-μl spot, but the actual inoculum could vary from 5 \(\times\) 10^6 to 2 \(\times\) 10^6 CFU per spot. Realistically, an even greater degree of variability might be expected because of the lack of precision in adjustment of turbidity. This range of variability does not markedly affect tests with most drugs (2), but it can dramatically affect the amdinocillin MICs of many strains. With the disk diffusion test, the normal variability in inoculum density does not appear to be as critical.

In conclusion, the 10-μg amdinocillin disk does correlate with agar dilution MICs if the inoculum is held to \(<10^6\) CFU per spot. However, a significant proportion of strains that are susceptible by the standard disk test can appear to be resistant by MIC methods if the inoculum is increased slightly (within the range of normal variability). The disk diffusion test (zone, \(\geq 16\) mm) has been found to correlate with clinical and bacteriological efficacy data (4, 5; data on file at Hoffman-LaRoche Inc.). To establish a reasonable intermediate range, I adjusted the susceptibility breakpoint to \(\geq 17\) mm rather than \(\geq 16\) mm. Because the disk test does not appear to be as sensitive as diffusion tests to inoculum variability, tests with 10-μg amdinocillin disks are more likely to provide reproducible results. Presumably, broth dilution tests would be subject to the same type of inoculum-dependent variability. The disk test appears to be the preferred method for testing for susceptibility to amdinocillin.

**LITERATURE CITED**


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**TABLE 1. Amdinocillin agar dilution susceptibility test results with three different inoculum densities**

<table>
<thead>
<tr>
<th>Inoculum range (CFU/spot)</th>
<th>Cumulative % inhibited by concen (μg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>(\geq 5 \times 10^2)–&lt;1.0 (\times 10^4)</td>
<td>19</td>
</tr>
<tr>
<td>(\geq 1.0 \times 10^4)–&lt;5 (\times 10^4)</td>
<td>14</td>
</tr>
<tr>
<td>(\geq 5 \times 10^4)–&lt;1 (\times 10^5)</td>
<td>8</td>
</tr>
</tbody>
</table>

* Each of 88 Enterobacteriaceae was tested with three different inocula calculated by assuming that 2-μl spots were deposited onto agar plates.

**TABLE 2. Regression analysis comparing 10-μg amdinocillin disk test results (two methods) with agar dilution MICs obtained with \(\geq 5 \times 10^2\) to \(<1.0 \times 10^4\) CFU per spot**

<table>
<thead>
<tr>
<th>Disk test procedure</th>
<th>Mean zone diam*</th>
<th>Regression formula*</th>
<th>Correlation coefficient</th>
<th>Zone correlates (mm) for an MIC (μg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(\geq 16) (\geq 4.0)</td>
</tr>
<tr>
<td>Standard method</td>
<td>21.4</td>
<td>(y = 37.5 – 1.8x)</td>
<td>0.79</td>
<td>(\geq 13) (\geq 17)</td>
</tr>
<tr>
<td>Inoculum at 1:10</td>
<td>24.3</td>
<td>(y = 42.1 – 2.0x)</td>
<td>0.82</td>
<td>(\geq 16) (\geq 20)</td>
</tr>
</tbody>
</table>

* Overall mean zone diameter in millimeters; 88 strains were tested.

* Calculated interpretive breakpoints for resistance (MIC, \(\geq 16\) μg/ml) and susceptibility (MIC, \(\leq 4.0\) μg/ml) categories for each disk method.