Interpretive Standards and Quality Control Limits for Susceptibility Tests with Ampicillin-Sulbactam Combination Disks

ARTHUR L. BARRY,1* RONALD N. JONES,2 AND CLYDE THORNSBERRY3

Clinical Microbiology Institute, Tualatin, Oregon 97062; Kaiser-Permanente Regional Laboratories, Clackamas, Oregon 97015; and Centers for Disease Control, Atlanta, Georgia 30333

Received 29 August 1983/Accepted 18 October 1983

In vitro studies were performed to evaluate susceptibility tests with disks containing 10 μg of ampicillin plus 10 μg of sulbactam. Interpretive zone standards of ≤11 mm (resistant), 12 to 13 mm (intermediate), and ≥14 mm (susceptible) are proposed. A nine-laboratory coordinated study was performed to establish tentative zone size limits for quality control of ampicillin-sulbactam disks. This included data with a new control strain selected to monitor performance of such combination disks.

Sulbactam (CP-45, 899) is a sulfone which irreversibly inhibits several important β-lactamases (1, 2, 4). By itself, sulbactam has little antibacterial activity against species other than Acinetobacter and Neisseria. However, when coadministered with a penicillin such as ampicillin, strains which are resistant to ampicillin by virtue of their ability to produce certain β-lactamases could be rendered susceptible to ampicillin (2, 9).

For parenteral use, ampicillin and sulbactam are combined for coadministration. The pharmacokinetic properties of the two drugs are similar (3). For purposes of in vitro susceptibility testing, ampicillin and sulbactam are normally tested in a fixed 1:1 ratio. For the disk diffusion test, disks containing 10 μg of ampicillin plus 10 μg of sulbactam are to be recommended (data on file; Pfizer Central Research, Grotton, Conn.).

The current study was designed to evaluate ampicillin-sulbactam disks containing 10 μg of each drug in comparison with 10-μg ampicillin disks. The results of such in vitro studies were analyzed to develop interpretive standards. In addition, the results of a nine-laboratory coordinated study are presented to establish tentative quality control parameters for ampicillin-sulbactam disks. This includes tests with a recently recommended control strain of Escherichia coli, selected to monitor disks which contain β-lactamase inhibitors combined with a β-lactam (5).

MATERIALS AND METHODS

Bacterial isolates. Tests were performed with 623 isolates collected from seven geographically separate institutions. The number of strains of species represented include 21 Citrobacter freundii, 20 Citrobacter diversus, 30 Eschericha coli, 20 Enterobacter aerogenes, 20 Enterobacter agglomerans, 20 Enterobacter cloacae, 30 Klebsiella pneumoniae, 10 Klebsiella oxytoca, 30 Serratia marcescens, 26 Proteus mirabilis, 17 Proteus vulgaris, 19 Morganella morgani, 17 Providencia rettgeri, 20 Providencia stuartii, 19 Acinetobacter calcoaceticus, 25 Pseudomonas aeruginosa, 5 Pseudomonas acidovorans, 8 Pseudomonas cepacia, 10 Pseudomonas stutzeri, 8 Pseudomonas putida, 11 Pseudomonas fluorescens, 10 Pseudomonas maltophilia, 31 ampicillin-resistant Haemophilus influenzae, 29 ampicillin-susceptible Haemophilus influenzae, 58 Staphylococcus aureus (9 methicillin resistant), 25 coagulase-negative staphylococci (5 methicillin resistant), 25 Streptococcus faecalis, 20 Streptococcus pyogenes, 17 Streptococcus agalactiae, 17 Streptococcus pneumoniae, and 5 Neisseria meningitidis.

Susceptibility tests. Minimal inhibitory concentrations (MICs) were determined by standardized microdilution procedures, as outlined by the National Committee for Clinical Laboratory Standards (8). Briefly, twofold dilutions of ampicillin or ampicillin plus equal concentrations of sulbactam were prepared in cation-supplemented Mueller-Hinton broth and dispensed into microdilution trays. The trays were stored at −40°C or less until needed (maximum of 6 weeks). After the trays were thawed at room temperature, each well was inoculated with ca. 5 × 10⁶ CFU/ml. MICs were determined after 16 to 18 h of incubation at 35°C. For testing H. influenzae, the broth was supplemented with 5% Fildesol solution. MICs for the ampicillin-sulbactam combination were defined as the lowest concentrations of ampicillin which inhibited growth in the presence of equal concentrations of sulbactam.

Disk diffusion tests were performed by the standardized procedure of the National Committee for Clinical Laboratory Standards (7). Disks containing 10 μg of ampicillin plus 10 μg of sulbactam were provided by Pfizer Central Research, and 10-μg ampicillin disks were prepared by BBL Microbiology Systems, Cockeysville, Md.

MIC interpretive breakpoints. Ampicillin MIC correlates for tests with enteric bacilli and enterococci are currently ≤8.0 μg/ml for the susceptible category and >16 μg/ml for the resistant category (7). If either drug is to be used orally, another category of susceptibility would be appropriate, owing to the lower blood levels that might be anticipated, i.e., an MIC of ≤1.0 μg/ml for the susceptible category (8). For the purpose of this analysis, only parenteral therapy was considered. The MIC breakpoints that were applied to tests with ampicillin were also applied to tests with ampicillin-sulbactam. However, different criteria are appropriate for tests with staphylococci and Haemophilus spp. Since β-lactamase-producing strains are assumed to be resistant to all penicillins, even though they may produce relatively low MICs, the susceptible category was defined as a zone size or MIC that best separates β-lactamase producers from nonproducers. These breakpoints are not related to achievable blood levels. Entirely different criteria were needed for defining the susceptible category for ampicillin-sulbactam, since the β-lactamase should be inhibited by sulbactam, rendering such strains susceptible to the ampicillin component.

* Corresponding author.
Quality control parameters. A nine-laboratory coordinated study was performed to evaluate quality control parameters for tests with ampicillin and ampicillin-sulbactam disks. Tests were performed with Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, and E. coli ATCC 35218. The latter strain (formerly Beecham no. 1532) is a β-lactamase-producing strain that has been proposed for monitoring the β-lactamase inhibitor component in such combination disks (5).

Participants in this phase of the study included the three authors, S. Brown (Good Samaritan Hospital, Portland, Ore.), P. C. Fuchs (St. Vincent Hospital, Portland, Ore.), T. L. Gavan (The Cleveland Clinic Foundation, Cleveland, Ohio), E. H. Gerlach (St. Francis Hospital, Wichita, Kans.), J. M. Matsen (University of Utah Medical Center, Salt Lake City), L. B. Reller (University of Colorado Medical Center, Denver), and H. Sommers (Northwestern Medical Center, Chicago, Ill.).

Each participant received the three control strains as well as three separate lots of ampicillin-sulbactam disks (prepared by General Diagnostics, Div. of Warner-Lambert Co., Morris Plains, N.J.) and one lot of ampicillin disks (BBL control no. 205036). Nine lots of Mueller-Hinton agar from four different manufacturers were involved; each investigator used a different medium. Each of the three control strains were tested 50 separate times in each laboratory. For further protocol control, five additional tests were performed in each laboratory with a 10th lot of Mueller-Hinton agar that was common to all laboratories (data not shown). This exercise generated 1,350 zone diameters with each of the control strains tested against ampicillin-sulbactam disks and 450 zones with the single lot of ampicillin disks. The data were analyzed by the medians statistic method of Gavan et al. (6). With this approach, the median value from the data of the nine laboratories is taken as the best estimate of central tendency, and the median of the ranges of zone diameters obtained in each of the individual laboratories is used as the estimate of test variability, i.e., median ± one half of the median range. This statistic is especially appropriate when the recorded zone diameters are not normally distributed.

RESULTS

Quality control limits. Data accumulated from the nine-laboratory collaborative study are summarized in Table 1. When the single ampicillin disk was used, zone diameters were generally within the established control limits, thus confirming that all nine laboratories were performing the tests satisfactorily. The β-lactamase-positive strain of E. coli (ATCC 35218) was fully resistant to ampicillin alone but gave 13- to 19-mm zones around the ampicillin-sulbactam disks. This strain monitors both components of the combination disk, whereas E. coli ATCC 25922 monitors only the ampicillin component. With the Seattle strain of E. coli ATCC 25922, ampicillin-sulbactam disks gave zones which were 2 to 3 mm larger than those with ampicillin alone, a difference not readily explained. S. aureus ATCC 25923 was more variable in its performance with both types of disks, and the zone diameters tended to be skewed toward smaller zones.

We tentatively recommend the control limits defined in Table 1 for ampicillin-sulbactam disks. Both E. coli strains should be tested to monitor the two drugs in the combination disks. Ampicillin disks should be tested routinely against E. coli ATCC 35218, and, if zones are produced, one should suspect that the strain has lost its ability to produce β-lactamase and a fresh culture should be obtained. If the sulbactam component of the combination disk loses potency, ATCC 35218 will produce smaller zones but ATCC 25922 zones will not be affected. The latter will be affected only by the ampicillin component in the disk.

Ampicillin versus ampicillin-sulbactam disks. Figure 1 presents data obtained with 396 gram-negative bacilli when zone diameters observed with ampicillin disks were compared with those observed with ampicillin-sulbactam disks. When sulbactam was added to the 10-μg ampicillin disk, there was a significant increase in zone diameters with ampicillin-resistant gram-negative bacilli, but the very susceptible strains gave equally large zones with both types of disks. Of the 396 gram-negative bacilli, 117 were resistant to ampicillin (zones, ≤11 mm) but susceptible to the ampicillin-sulbactam combination (zones, ≥14 mm). Six members of the family Proteaeae were resistant to the combination but susceptible to ampicillin alone (possible antagonism). Acinetobacter spp. displayed rather marked differences in zone diameters with the two types of disks, possibly owing to the fact that some strains are quite susceptible to sulbactam alone. Our strains of Acinetobacter spp. provided MICs of 0.5 to 2.0 μg/ml with sulbactam alone and 0.25 to 2.0 μg/ml with the ampicillin-sulbactam combination. Additional analysis was carried out to establish the validity of the interpretive zone size breakpoints for different types of microorganisms.

Gram-negative bacilli and enterococci. Figure 2 presents the results of tests with ampicillin disks against 300 members of the family Enterobacteriaceae, 96 nonenteric bacilli, and 25 Streptococcus faecalis strains. Acinetobacter and Pseudomonas spp. were included despite the fact that there are currently no zone size standards for tests with ampicillin bacilli. For members of the family Enterobacteriaceae and enterococci, zone size breakpoints of ≤11 and ≥14 mm are currently recommended for tests with 10-μg ampicillin disks. We observed 20 (4.7%) major or very major discrepancies among the 421 isolates. Ten very major errors (false-susceptible disk tests) occurred with the following numbers of strains: K. pneumoniae, 3; M. morganii, 2; Providencia stuartii, 1; C. diversus, 1; C. freundii, 1; Enterobacter agglomerans, 1; and Escherichia coli, 1. Ten major errors (false-resistant disk tests) occurred with 6 strains of Acinetobacter spp. and 1 Enterobacter agglomerans strain, 1 Enterobacter cloacae strain, 1 K. pneumoniae strain, and 1 Providencia rettgeri strain. The six errors with strains of Acinetobacter spp. might be disregarded since the procedure was not standardized for testing nonenteric gram-negative bacilli. An intermediate ampicillin MIC of 16 μg/ml was.

<table>
<thead>
<tr>
<th>Control strain (ATCC no.)</th>
<th>Zone diameter (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Ampicillin-sulbactam disks (10 μg - 10 μg)</td>
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<tr>
<td></td>
<td>Median value</td>
</tr>
<tr>
<td>E. coli (25922)</td>
<td>22</td>
</tr>
<tr>
<td>E. coli (35218)</td>
<td>16</td>
</tr>
<tr>
<td>S. aureus (25923)</td>
<td>33</td>
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* Based on 1,350 zone size determinations with each control strain.
* Based on 450 zone size determinations with each strain, using a single lot of ampicillin disks. No zone was obtained with E. coli ATCC 35218.
* Data are from reference 7, Table 3.
obtained with 56 (13.3%) of the isolates: 16 were susceptible and 35 were resistant by the disk test. Complete interpretive agreement was observed with 81% of the 421 isolates or 78% of the members of the family Enterobacteriaceae. The 25 S. faecalis strains were susceptible to ampicillin by both MIC and disk test criteria.

Figure 2 also presents data obtained when ampicillin-sulbactam disk zones were compared with ampicillin-sulbactam MICs. An intermediate MIC of 16 μg/ml was obtained with 50 isolates: 32 produced zones of ≥11 mm and 10 produced zones of ≥14 mm. If zone size standards for ampicillin were applied to tests with ampicillin-sulbactam disks, nine (2%) very major and eight (2%) major errors would be recorded (all with members of the family Enterobacteriaceae). Overall category agreement was seen with 85% of the 421 isolates or 79% of the 300 members of Enterobacteriaceae, a result that compares favorably with the percent agreement obtained with ampicillin disk tests.

Streptococcus spp. and N. meningitidis. In addition to the 25 S. faecalis isolates, tests were performed with 20 S. pyogenes, 20 S. agalactiae, and 17 S. pneumoniae strains. All streptococci were susceptible (zones, ≥19 mm) to both types of disks, and all produced MICs of ≤0.5 μg/ml. Five N. meningitidis strains also produced very large zones with both types of disks, and all were inhibited by ≤0.12 μg of ampicillin-sulbactam per ml. They were also inhibited by 0.25 to 2.0 μg of sulbactam alone per ml.

**H. influenzae.** Ampicillin-susceptible *H. influenzae* strains produced ampicillin zones of >20 mm, whereas β-lactamase-producing strains produced zones of <20 mm. When sulbactam was added to ampicillin, both types of *H. influenzae* were susceptible, i.e., MICs of ≤1.0 μg/ml and zones of >20 mm. No *H. influenzae* strain was resistant to the ampicillin-sulbactam combination.

**Staphylococcus spp.** Tests were performed with 58 S. aureus strains and 25 coagulase-negative staphylococci. With rare exception, β-lactamase-negative strains produced zones of ≥29 mm around ampicillin disks, and most β-lactamase-positive strains produced zones of ≥20 mm. Although MICs with the latter were as low as 0.5 μg/ml, they are considered to be resistant to ampicillin. Staphylococcal data with ampicillin-sulbactam disks are presented in Fig. 3. With the exception of some methicillin-resistant strains (4 of 14 strains), sulbactam reduced all ampicillin MICs to ≤2.0 μg/ml; MICs for all but one β-lactamase-negative strain were ≤0.25 μg/ml. Early clinical experience in the treatment of penicillin-resistant staphylococcal infections with the ampicillin-sulbactam combination has been encouraging, thus suggesting that methicillin-susceptible, penicillinase-producing strains might be considered susceptible to the combination (A. K. Knirsch and D. L. Gibbs, personal communication). They all produced zones of ≥20 mm and MICs of ≤2.0 μg/ml. Methicillin-resistant strains, however, were variable in their in vitro susceptibilities to ampicillin-sulbactam: 4 of...
14 strains were resistant to 2.0 μg/ml and 6 strains produced zones of <20 mm. All but one methicillin-resistant S. aureus strain produced ampicillin-sulbactam zones of ≥14 mm. Clinical experience with methicillin-resistant staphylococcal infections is currently inadequate to determine whether they are likely to be responsive to the ampicillin-sulbactam combination. Since we found no methicillin-susceptible staphylococci with zones of <20 mm, the susceptible category could be established at ≥20 or ≥14 mm with equal efficacy, providing that methicillin-resistant strains are assumed to be resistant, regardless of in vitro test results.

**DISCUSSION**

Data presented in this report define the increased antimicrobial spectrum of ampicillin when combined with sulbactam. Presence of the β-lactamase inhibitor significantly improved the in vitro activity of ampicillin against some strains that are known to produce β-lactamases. The effect was particularly dramatic with β-lactamase-producing H. influenzae and Staphylococcus aureus-strains. Acinetobacter spp. were also susceptible to the combination, largely because most strains are inhibited by the sulbactam component.

For tests with members of the family Enterobacteriaceae, the zone size interpretive standards that are being used for ampicillin disks may be utilized for tests with ampicillin-sulbactam disks. Consequently, we recommend the following breakpoints for ampicillin-sulbactam disks containing 10 μg of each drug: susceptible, ≥14 mm (MIC, ≤8.0 μg/ml); intermediate, 12 to 13 mm; and resistant, ≤11 mm (MIC, >16 μg/ml).

Although the intermediate category for ampicillin is defined to include strains with MICs of 16 μg/ml, the disk test erroneously reports most of those strains as being either resistant or susceptible. Strains with ampicillin MICs of 16 μg/ml but with zones of ≥14 mm are generally susceptible to ampicillin-sulbactam, whereas those which are resistant by
the ampicillin disk test tend to be resistant to ampicillin-sulbactam disks. The majority of strains with intermediate ampicillin-sulbactam MICs of 16 μg/ml appear to be resistant by the disk test.

With ampicillin and ampicillin-sulbactam disks, an intermediate zone size (12 to 13 mm) is truly an equivocal result which has no predictive value. The overall accuracy of tests with both disks might be improved if the intermediate category were enlarged to include strains with zone sizes of 12 to 14 or 12 to 15 mm, but this would also increase the number of equivocal disk test results.

Ampicillin and ampicillin-sulbactam breakpoints should be reassessed when data becomes available to permit correlation of clinical responsiveness to in vitro susceptibility test data. Such clinical data may support the need for redefining susceptibility categories for both drugs; i.e., a more conservative MIC breakpoint of <8.0 μg/ml for the susceptible category might be preferred to the current MIC breakpoint of ≤8.0 μg/ml. This change would have little effect on the overall accuracy of tests with ampicillin, since only 16 of our 421 isolates provided MICs of 8.0 μg/ml. In the case of ampicillin-sulbactam, a much larger proportion of strains (58 of 421) produced MICs of 8.0 μg/ml, and most of these isolates produced zones of ≥14 mm. If MIC breakpoints for ampicillin and ampicillin-sulbactam disks were to be redefined as <8.0 μg/ml (susceptible) and ≥16 μg/ml (resistant), the appropriate zone size breakpoints for either disk would be ≥18 mm for the susceptible category and ≥12 mm for the resistant category. If that were done, the intermediate range of 13 to 17 mm would include 58 of 300 members of the family Enterobacteriaceae with ampicillin-sulbactam disks and 35 of 300 strains with ampicillin disks.

When testing H. influenzae or staphylococci, the ampicillin-sulbactam susceptible category could be defined as a zone of ≥14 mm, providing that methicillin-resistant staphylococci are assumed to be resistant. Since we observed no strains that were resistant to the combination disks, it is not possible to firmly establish zone size breakpoints. As a matter of fact, one could seriously question the need for ampicillin-sulbactam disk tests with Haemophilus spp. or with staphylococci. For the sake of simplicity, we propose that the zone size standards developed for enteric bacilli may also be applied to tests with Haemophilus and Staphylococcus spp. The alternative is to use ≥20 mm for the susceptible category and ≤19 mm for the resistant category. Strains with zones of ≤19 mm should be retested, checked for purity, and reidentified before being reported as being resistant.

The foregoing discussion is based on the conservative decision that methicillin-resistant staphylococci should be assumed to be resistant to the ampicillin-sulbactam combination regardless of the results of in vitro tests. This decision is tentative, because there are insufficient clinical data to determine the efficacy of ampicillin-sulbactam against this group of microorganisms. When such clinical data become available, interpretable standards for staphylococci should be reevaluated.

In summary, the interpretive criteria currently accepted for testing ampicillin disks against members of the family Enterobacteriaceae may be used for ampicillin-sulbactam disks (all species). The accuracy of ampicillin-sulbactam disk tests was found to be comparable or superior to that observed with ampicillin disks. Additional data have been presented to establish tentative zone size limits for quality control of ampicillin-sulbactam disks. This includes data with a new control strain which was selected for monitoring such combination disks.

**LITERATURE CITED**


5. Fuchs, P. C., A. L. Barry, C. Thornberry, T. L. Gavan, and...