Resistance to Ticarcillin-Potassium Clavulanate among Clinical Isolates of the Family Enterobacteriaceae: Role of PSE-1 β-Lactamase and High Levels of TEM-1 and SHV-1 and Problems with False Susceptibility in Disk Diffusion Tests

CHRISTINE C. SANDERS,1* JOSEPH P. IACONIS,1 GERALD P. BODEY,2 AND GEORGE SAMONIS3

Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178, 1 and University of Texas, M. D. Anderson Cancer Center, Houston, Texas 770302

Received 7 March 1988/Accepted 20 June 1988

Thirty-four clinical isolates of the family Enterobacteriaceae from the University of Texas M. D. Anderson Cancer Center appeared resistant to ticarcillin-potassium clavulanate in agar dilution and broth macrodilution tests. Among those isolates producing a single non-class β-lactamase, resistance was due to production of high levels of TEM-1, SHV-1, or class IV enzymes. In five Escherichia coli isolates, production of low levels of PSE-1 was responsible for resistance which seemed due to rapid hydrolysis of ticarcillin rather than diminished susceptibility of PSE-1 to inhibition by potassium clavulanate. Comparisons of dilution and disk diffusion tests revealed major discrepancies, with 65% false susceptibility in the disk test. Revision of the interpretive criteria used for disk diffusion tests from \( \leq 11 \) to \( \leq 18 \) mm for resistance is proposed to resolve these discrepancies until clinical data are obtained which can be used to determine which in vitro test is most predictive of therapeutic outcome. These new criteria would diminish false susceptibility without introducing false resistance.

Combinations containing a β-lactamase inhibitor and an enzyme-labile drug have been shown to be highly effective both in vitro and in vivo against many β-lactamase-producing bacteria (2, 16). Potassium clavulanate is a suicide inhibitor of many β-lactamases and, in combination with ticarcillin, restores the activity of the latter against members of the family Enterobacteriaceae producing enzymes of essentially all Richmond and Sykes classes except class I, the chromosomal cephalosporinases (5, 10, 22). However, in a recent survey of piperacillin-resistant members of the Enterobacteriaceae, a number of ticarcillin-potassium clavulanate (Timentin)-resistant strains were found among isolates recovered from patients at the University of Texas M. D. Anderson Cancer Center. Many of these organisms belonged to genera that characteristically produce the plasmid-mediated enzymes of Richmond and Sykes classes III and V that are usually highly susceptible to inhibition by potassium clavulanate. Thus, their resistance to ticarcillin-potassium clavulanate was unexpected.

Therefore, a study was designed to assess the mechanisms responsible for resistance to ticarcillin-potassium clavulanate in 34 clinical isolates of the Enterobacteriaceae. During the study, questions arose concerning the validity of results obtained in certain types of susceptibility tests with ticarcillin-potassium clavulanate, and these are addressed as well.

MATERIALS AND METHODS

Bacterial strains. Fifty-five strains of piperacillin-resistant members of the Enterobacteriaceae were collected from patients at the University of Texas M. D. Anderson Cancer Center, Houston. Attempts were made to exclude (i) isolates representing genera (e.g., Enterobacter) or species (e.g., Serratia marcescens and Morganella morgani) commonly possessing Richmond and Sykes class I enzymes and (ii) multiple isolates from the same patient unless differences in β-lactam susceptibility were noted. These 55 isolates included 26 Escherichia coli, 18 Klebsiella pneumoniae, 2 K. oxytoca, 8 Citrobacter spp., and 1 Serratia sp. Initial susceptibility tests were performed by a broth microdilution procedure in one laboratory (G.P.B.). All isolates were then sent to a second laboratory (C.C.S.) for retesting and determination of β-lactamase content.

Susceptibility tests. All susceptibility tests except agar dilution were performed by standard procedures (14, 15). For all dilution procedures, the antibiotics, kindly provided by their respective manufacturers, were prepared on the day of use. Agar dilution tests were performed in Mueller-Hinton with an inoculum of \( 10^5 \) CFU per spot applied with a Steers replicator (21). Broth macrodilution tests were performed in Mueller-Hinton (3-ml final volume) with an inoculum of \( 10^5 \) CFU/ml. The MIC was defined as the lowest concentration preventing growth after incubation for 18 h at 35°C. The MIC of ticarcillin-potassium clavulanate was determined with serial twofold dilutions of ticarcillin, each containing a constant concentration of 2 μg of potassium clavulanate per ml. MICs were interpreted according to the National Committee for Clinical Laboratory Standards guidelines, with \( \geq 128 \) μg of ticarcillin per ml indicative of resistance, 32 to 64 μg/ml indicative of moderate susceptibility, and \( \leq 16 \) μg/ml indicative of susceptibility (15). Control strains were included in each run to monitor reproducibility, which was found to be \( \pm 1 \) dilution. Disk diffusion tests were performed with commercially prepared disks containing 75 μg of ticarcillin and 10 μg of potassium clavulanate. Interpretive criteria were zones of \( \geq 11 \) mm for resistance, 12 to 14 mm for intermediate susceptibility, and \( \geq 15 \) mm for susceptibility (5). Antibiotic susceptibility profiles were determined for each isolate, using commercially prepared disks containing ticarcillin, piperacillin, cefoxitin, cephalothin, cefoperazone, cefamandole, cefotaxime, and ceftazidime.

* Corresponding author.
Characterization of β-lactamases. For organisms producing β-lactamases constitutively, sonic extracts were prepared from overnight cultures in Mueller-Hinton broth. For organisms producing inducible β-lactamases, sonic extracts were prepared following a 2-h induction by cefoxitin at one-fourth its MIC (18). All sonic extracts were frozen at –70°C until tested. β-Lactamases were characterized initially by determining their (i) pi in polyacrylamide gels (19); (ii) susceptibility to inhibition by clavulanic acid and potassium clavulanate after focusing (19); (iii) ability to hydrolyze penicillin G, cephalothin, and nitrocefin; and (iv) inducibility by cefoxitin. These characteristics were then compared with those obtained with enzyme standards prepared from strains possessing well-characterized β-lactamases. These included P99 (Enterobacter cloacae P99 kindly provided by L. Koulup of Merck Sharp & Dohme); class IV enzyme (K. pneumoniae A20346 kindly provided by F. Leitner of Bristol Laboratories), and various plasmid-mediated β-lactamases all kindly provided by A. A. Medeiros of Brown University. The latter included TEM-1 (E. coli RTEM (R6K)), TEM-2 (E. coli 1725E-RP1), OXA-1 (E. coli 1357-RGN238), OXA-2 (E. coli 1573-R46), OXA-3 (E. coli 1894E-R57b), OXA-4 (E. coli 7259), OXA-5 (E. coli J53-pMG54), OXA-6 (Pseudomonas aeruginosa PAO38-pMG30), OXA-7 (E. coli J781), OHO-1 (E. coli C600-pOHO), SHV-1 (E. coli J53 R1010), HMS-1 (E. coli J53-R997), PSE-1 (P. aeruginosa PU21-RPL11), PSE-2 (P. aeruginosa PU21-R151), PSE-3 (P. aeruginosa PU21-RMS149), PSE-4 (P. aeruginosa PU21-pMG19), and SAR-1 (E. coli J62.2-pUK657). Additional substrates were examined as necessary whenever these initial characteristics did not provide a definitive identification. For example, any enzyme appearing to be SHV-1 was tested for activity against ceftazidime and cefotaxime since SHV-1 and SHV-2 have the same pi but differ in their substrate profile (9). Similarly, any enzyme appearing to be class IV was tested for its ability to hydrolyze cefotaxime and aztreonam since certain plasmid-mediated enzymes (e.g., OXA-7) have a similar pi but different substrate profiles. Very low levels of constitutively produced chromosomal enzyme present in most gram-negative bacteria were ignored for the purposes of this investigation.  

β-Lactamase characterization. Hydrolysis of cephalosporins and aztreonam was determined in UV spectrophotometric assays monitoring the λmax, associated with the β-lactam ring (18). Hydrolysis of penicillins was determined in microdifferential and spectrophotometric assays (20, 24). The amount of β-lactamase in sonic extracts was quantified with nitrocefin as a substrate. One unit of specific activity was defined as the amount of enzyme hydrolyzing 1 nmol of nitrocefin at pH 7.0 in 37°C per min per mg of protein in sonic extracts. The susceptibility of β-lactamases to inhibition by potassium clavulanate was determined quantitatively by preincubating enzyme with various concentrations of inhibitor for 10 min. Nitrocefin (100 μM) was then added as the substrate, and residual enzyme activity was measured. The concentration of potassium clavulanate required to inhibit 50% of enzyme activity (I50) was then determined by probit analysis.  

Kinetic parameters were established for the enzyme in sonic extracts prepared from PSE-1-producing E. coli 45. Nitrocefin was used as the substrate, and potassium clavulanate and clavulanic acid were used as inhibitors. Km and Vmax were determined from Lineweaver-Burk plots for various substrate concentrations. K was determined from a secondary replot of slopes of Lineweaver-Burk plots for various inhibitor concentrations (4). The enzyme in sonic extracts from E. coli 45 was concentrated by dialysis in membranes.

<table>
<thead>
<tr>
<th>MIC from agar dilution (μg/ml)</th>
<th>No. of isolates</th>
<th>No. (%) with indicated susceptibility in disk diffusion tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>≥128</td>
<td>34</td>
<td>22 (65)</td>
</tr>
<tr>
<td>32-64</td>
<td>16</td>
<td>15 (94)</td>
</tr>
<tr>
<td>≤16</td>
<td>5</td>
<td>5 (100)</td>
</tr>
</tbody>
</table>

*Interpreted by using criteria in references 5, 14, and 15.

RESULTS

Susceptibility tests. The susceptibility of the 55 piperacillin-resistant isolates to ticarcillin, potassium clavulanate, and ticarcillin-potassium clavulanate was determined in agar dilution tests. MICs for all isolates were >128 μg of ticarcillin per ml and between 16 and 64 μg of potassium clavulanate per ml. The isolates fell into these groups based on susceptibility to ticarcillin-potassium clavulanate (the latter at a constant concentration of 2 μg/ml). Group 1 consisted of 34 resistant isolates (11 with MICs of 128 μg and 23 with MICs of >128 μg of ticarcillin per ml); group 2 consisted of 16 moderately susceptible isolates (MIC, 32 to 64 μg/ml); and group 3 consisted of 5 susceptible isolates (MIC, ≤16 μg/ml). Disk diffusion tests showed all group 3 and all but one group 2 isolates to be susceptible to ticarcillin-potassium clavulanate (Table 1). The remaining group 2 isolate was of intermediate susceptibility. However, for group 1 isolates, only 2 (6%) had zones indicative of susceptibility, while 22 (65%) had zones indicative of susceptibility. The remaining 10 (27%) appeared to be of intermediate susceptibility. Mean zone sizes (mm) ± 1 standard deviation were 15.4 ± 2.7 for group 1, 19.2 ± 2.8 for group 2, and 22.8 ± 1.6 for group 3 isolates.

Since there was a great disparity between results of disk diffusion and agar dilution susceptibility tests, broth macrodilution tests were also performed on group 1 isolates. Results indicated that 30 of the 34 isolates were resistant, 3 were moderately susceptible, and 1 was susceptible to ticarcillin-potassium clavulanate. After raising the potassium clavulanate concentration in broth macrodilution tests from 2 to 5 μg/ml, 21 of the 34 (62%) group 1 isolates still appeared resistant; 9 (26%) appeared moderately susceptible, and 4 (12%) appeared susceptible to ticarcillin-potassium clavulanate.

Types of enzymes responsible for ticarcillin-potassium clavulanate resistance. Each of the 34 group 1 isolates produced at least one β-lactamase. From substrate and inhibitor profiles and pi's, the identity of these enzymes could be determined (Table 2). Thirteen isolates produced TEM-1 only, two produced SHV-1 only, and two produced TEM-1 plus SHV-1. These included 10 E. coli and 7 K. pneumoniae. Two isolates produced a chromosomal enzyme plus TEM-1. These included one Serratia sp. with an inducible class I enzyme and one Citrobacter freundii with only low constitutive levels of class I enzyme. Seven isolates produced only a chromosomal enzyme (Table 2). These included two K. oxytoca producing high constitutive levels of class IV en-
enzyme and five C. freundii producing high constitutive levels of class I enzyme. Five E. coli appeared to produce only TEM-1. Two K. pneumoniae produced both TEM-1 and SHV-1. One remaining E. coli produced TEM-1 plus a second enzyme with a pI of 5.15 which focused consistently between HMS-1 (pI 4.86) and PSE-4 (pI 5.19).

**Table 2.** Types of β-lactamases found in 34 clinical isolates resistant to ticarcillin-potassium clavulanate (group 1)

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>No. of isolates (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM-1 only</td>
<td>13 (10 E. coli, 3 K. pneumoniae)</td>
</tr>
<tr>
<td>SHV-1 only</td>
<td>2 (K. pneumoniae)</td>
</tr>
<tr>
<td>TEM-1 plus SHV-1</td>
<td>2 (K. pneumoniae)</td>
</tr>
<tr>
<td>Chromosomal enzyme plus TEM-1</td>
<td>2 (1 Serratia sp., 1 Citrobacter sp.)</td>
</tr>
<tr>
<td>Chromosomal enzyme only</td>
<td>7 (2 K. oxytoca, 5 Citrobacter sp.)</td>
</tr>
<tr>
<td>PSE-1 only</td>
<td>5 (E. coli)</td>
</tr>
<tr>
<td>PSE-1 plus SHV-1</td>
<td>2 (K. pneumoniae)</td>
</tr>
<tr>
<td>Unknown plus TEM-1</td>
<td>1 (E. coli)</td>
</tr>
</tbody>
</table>

* Class IV.  
* Class I.

The relationship between the quantity of TEM-1 or SHV-1 produced and susceptibility to ticarcillin-potassium clavulanate is shown in Table 3. The mean quantity of TEM-1 or SHV-1 produced was highest for isolates in group 1, intermediate for isolates in group 2, and lowest for isolates in group 3. This direct association between enzyme quantity and MIC of ticarcillin-potassium clavulanate suggested that production of high levels of a potassium clavulanate-susceptible enzyme might be responsible for the resistance of the group 1 isolates. To test this hypothesis, the susceptibility of the enzymes in E. coli 34 and K. pneumoniae 17 to inhibition by potassium clavulanate was assessed and compared with that of E. coli RTEM(R6K), which produces a well-characterized TEM-1 enzyme. These strains were chosen for study because they all produced large quantities of enzyme (>1,400 U) and were resistant to ticarcillin-potassium clavulanate in all dilution tests. The I<sub>50</sub> for TEM-1 in E. coli 34 and RTEM(R6K) were 0.030 and 0.039 μM, respectively, while that for SHV-1 in K. pneumoniae 17 was 0.022 μM. Since the intrinsic susceptibility of the enzyme to inhibition by clavulanate was unchanged, it appeared that production of high levels of a potassium clavulanate-susceptible enzyme was responsible for the resistance observed in group 1 isolates possessing either TEM-1 or SHV-1. This was further supported when agar dilution tests with ticarcillin were repeated, using 5 μg of potassium clavulanate per ml. MICs obtained with this higher concentration of potassium clavulanate decreased for isolates in both groups 1 and 2 (Table 3).

Two isolates of K. oxytoca produced large quantities of class IV enzyme (11,329 and 12,923 U). The enzyme in one isolate (strain 21) was tested for susceptibility to inhibition by potassium clavulanate, and the I<sub>50</sub> was found to be 0.065 μM. Thus, the ticarcillin-potassium clavulanate resistance in these isolates also appeared to be due to production of high levels of a potassium clavulanate-susceptible enzyme.

The five remaining group 1 isolates that had a single non-class I β-lactamase appeared to produce PSE-1. Each of these isolates produced a relatively low level of enzyme (68 to 176 U). The mean enzyme level was 111 ± 40 μM and more closely resembled group 3 isolates producing TEM-1 or SHV-1 (Table 3) than group 1 isolates. Thus, additional studies were performed to analyze the enzyme in these strains further.

**Studies with PSE-1.** E. coli 45 was chosen for further study because it had one of the higher enzyme levels (139 U) of the five isolates appearing to produce PSE-1. The substrate profile of the enzyme from E. coli 45 clearly identified it as a carbenicillin-hydrolyzing β-lactamase. In microiodometric assays, it hydrolyzed carbenicillin at 75% the rate of penicillin G, similar to PSE-1. Relative rates of hydrolysis obtained in spectrophotometric assays showed a clear penicillinase profile for the enzyme hydrolyzing cephalothin at a rate of 0.4% of that for penicillin G. Like PSE-1, it was poorly inhibited by cloxacillin (K<sub>i</sub>, 191.4 μM) but was highly susceptible to potassium clavulanate (I<sub>50</sub>, 0.012 μM). To assess whether potassium clavulanate was acting as an irreversible inhibitor, enzyme from E. coli 45 was incubated for a total of 24 h with 0.01, 0.05, 0.075, and 0.1 μM potassium clavulanate. At 10 min, 1 h, 4 h, and 24 h, samples were removed and enzyme activity was determined, using 100 μM nitrocefin as the substrate. The percentage of enzyme activity inhibited over this 24-h period (Table 4) indicates the irreversible nature of the inhibition once saturating concentrations of the inhibitor are achieved. The apparent molecular weight of the enzyme in E. coli 45 was 31,500 by both Sephadex G-75 chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The results obtained in tests with E. coli 45 suggested that the ticarcillin-potassium clavulanate resistance in this strain was due to the rapid hydrolysis of ticarcillin by PSE-1 rather than to any lack of susceptibility of the enzyme for the inhibitor. Thus, MIC determinations were repeated with all
five *E. coli* strains that appeared to produce PSE-1, using 5 μg of potassium clavulanate per ml to determine whether MICs of ticarcillin could be brought into the clinically achievable range with a higher concentration of inhibitor. With 5 μg of potassium clavulanate per ml, MICs of ticarcillin decreased to 16 to 64 μg/ml for the five isolates producing PSE-1.

**DISCUSSION**

The mechanisms responsible for resistance to ticarcillin-potassium clavulanate among clinical isolates of the *Enterobacteriaceae* that do not produce high levels of class I β-lactamase have not been examined extensively. Clearly, such strains exist among *E. coli* and *Klebsiella* spp., although they are usually a small minority of total isolates examined (5, 8, 17, 22, 23). Thus, the availability of a fairly large collection of ticarcillin-potassium clavulanate-resistant members of the *Enterobacteriaceae* presented the first opportunity to examine this aspect in detail.

The results of this study showed an inverse relationship between quantity of β-lactamase produced and susceptibility to ticarcillin-potassium clavulanate which has not been previously documented. Joly et al. (8) reported an association between hyperproduction of penicillinase in several strains and ticarcillin-potassium clavulanate resistance; however, no quantitative data were shown. That there did appear to be a strong association between amount of TEM-1 or SHV-1 produced and MIC of ticarcillin-potassium clavulanate was somewhat surprising. Permeability as well as amount and type of β-lactamase determine the final susceptibility of a gram-negative organism to β-lactam antibiotics. Since a large number of different strains and two different species were involved, major differences in permeability might have masked the relationship between quantity of enzyme produced and resistance. This appears not to have been the case. The results presented in this study clearly indicate that, even for enzymes highly susceptible to potassium clavulanate, elevated levels may be reached in the periplasmic space that cannot be efficiently inactivated by this inhibitor. This applies not only to TEM-1 and SHV-1, but also to the chromosomal class IV enzymes found in *K. oxytoca*.

The role of PSE-1 in resistance to ticarcillin-potassium clavulanate among members of the *Enterobacteriaceae* has not been investigated previously. PSE-1 was once thought to be restricted to *P. aeruginosa* (12). However, in 1982, Medeiros et al. described isolates of the *Enterobacteriaceae* that produced this enzyme (13). Nevertheless, since PSE-1 is still most prevalent in *P. aeruginosa* and is only rarely found in members of the *Enterobacteriaceae*, all of the studies concerning the effects of potassium clavulanate on strains possessing this enzyme specifically have been performed in *P. aeruginosa*. Such studies have shown that MICs or MBCs of penicillins for *P. aeruginosa* with PSE-1 may be reduced by potassium clavulanate; however, they usually do not fall into the clinically achievable range (3, 6).

The results obtained with *E. coli* 45 indicated that the enzyme responsible for ticarcillin-potassium clavulanate resistance in five isolates of *E. coli* was PSE-1. The identical pl and molecular weight, as well as the substrate profile and resistance to cloxacillin, clearly identified this enzyme as PSE-1 (12, 13). PSE-1 has been shown to be susceptible to inhibition by potassium clavulanate in previous studies by Li et al. (11), and it also appeared highly susceptible in a variety of tests in the current study. This susceptibility to potassium clavulanate was reflected by the ability of this inhibitor at higher concentrations to reduce the MIC of ticarcillin against the five *E. coli* producing PSE-1 to clinically achievable concentrations. Thus, for PSE-1-producing *E. coli*, it appears that rapid hydrolysis of ticarcillin by low levels of a potassium clavulanate-susceptible enzyme is responsible for the ticarcillin-potassium clavulanate resistance in these strains.

In addition to identifying mechanisms responsible for resistance to ticarcillin-potassium clavulanate, a major disagreement between results of dilution and diffusion susceptibility tests was also noted in this study. Many strains appearing to be resistant to ticarcillin-potassium clavulanate in agar and broth dilution susceptibility tests appeared to be susceptible in disk diffusion assays. The most likely explanation for this disparity was the difference in the concentration of potassium clavulanate in the two types of tests. Disks contained 10 μg of the inhibitor, while dilution tests were performed with only 2 μg/ml (5, 14, 15). These fixed concentrations were derived from results of several studies indicating that (i) MICs were comparable whether 1, 2, or 4 μg of potassium clavulanate per ml was used in dilution tests; (ii) correlation was similar between MICs determined with ticarcillin plus 2 μg of potassium clavulanate per ml and zone sizes obtained with disks containing ticarcillin (75 μg) plus 10, 5, or 2.5 μg of potassium clavulanate; and (iii) levels in serum and tissue of 2 μg of potassium clavulanate per ml were easily achieved and maintained for up to 2 h after standard doses of ticarcillin-potassium clavulanate were administered to humans (1, 5, 7). However, even in these earlier studies, several problems were noted.

Fuchs et al. recommended the interpretive criteria for zone sizes currently used (5). However, they did note a high "very major" error rate (false susceptibility) with all disks regardless of their concentration of potassium clavulanate. In fact, for 17 nonstaphylococcal isolates with ticarcillin-potassium clavulanate MICs of >64 μg/ml, only 8 (47%) gave zones indicative of resistance while 5 (29%) gave zones indicative of susceptibility. This 29% false sensitivity was smaller than the 65% rate observed in the current study. However, the current study was biased toward collecting isolates that were resistant to ticarcillin-potassium clavulanate. Thus, these differences may merely reflect the larger number of resistant strains analyzed here. They may also reflect differences in β-lactamases responsible for the resistance since isolates with class I enzymes appeared resistant in all tests including disk diffusion while those with TEM-1 or SHV-1 were likely to show discrepancies between the various tests.

In the absence of good clinical correlates, it is difficult to assess which test reflects the true susceptibility of these isolates to ticarcillin-potassium clavulanate. One could argue that, given the in vitro instability of potassium clavulanate, results in diffusion tests would be more accurate than

---

**TABLE 4. Inactivation of PSE-1 produced by *E. coli* 45 during prolonged incubation with potassium clavulanate**

<table>
<thead>
<tr>
<th>Time of preincubation</th>
<th>% of enzyme activity inhibited after incubation with potassium clavulanate at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01 μM</td>
</tr>
<tr>
<td>10 min</td>
<td>43</td>
</tr>
<tr>
<td>1 h</td>
<td>46</td>
</tr>
<tr>
<td>4 h</td>
<td>29</td>
</tr>
<tr>
<td>24 h</td>
<td>18</td>
</tr>
</tbody>
</table>
dilution assays since the former have a larger potassium clavulanate content and would thus be less affected by loss of potency (5). Conversely, one could argue that, if significant degradation does not occur, the diffusion test could overpredict susceptibility due to the presence of high, clinically unachievable concentrations of potassium clavulanate. We tend to support the latter contention since all of our dilution tests were performed with fully potent potassium clavulanate (as assayed by F. Barr at Beecham Laboratories and monitored by us) prepared immediately before use. In fact, if the interpretive criteria for results of diffusion tests were altered, results of dilution and diffusion tests would agree quite well. From the regression analyses of MICs and zone sizes for ticarcillin-potassium clavulanate by Joly et al. (8) and the mean zone sizes observed in the current study, interpretive criteria more closely reflecting MICs would be zones of ≥23 mm for susceptibility, 19 to 22 mm for intermediate susceptibility, and ≤18 mm for resistance. By these criteria, none of the 34 resistant isolates in this study and only 1 of 17 resistant isolates in the study by Fuchs et al. (5) would show false susceptibility results. Thus, between the two studies, the very major error rate would decrease from 53% (27 of 51) to 2% (1 of 51) for isolates for which MICs are ≥128 μg/ml. These new interpretive criteria would not increase major errors (false resistance) among isolates for which MICs are ≤16 μg/ml in the two studies and would only increase “minor” errors (susceptible/intermediate or intermediate/resistant) from 3 to 6%. Thus, until clinical data are gathered that show the superiority of one procedure over another in predicting clinical outcome, it might be prudent to alter the interpretive criteria used for the diffusion test to those that more closely reflect MICs. This conservative approach would minimize the possible fatal error of false susceptibility but would not introduce a problem with false resistance.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Beecham Laboratories, Bristol, Tenn., and by the Health Future Foundation, Omaha, Nebr.

We thank Ellen S. Moland for excellent technical assistance.

LITERATURE CITED