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Ticarcillin and Ticarcillin-Clavulanic Acid Susceptibility Tests: Error Rates for Disk Tests with Consecutively Isolated Members of the Family *Enterobacteriaceae*

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A five-laboratory coordinated study was undertaken to determine whether ticarcillin and ticarcillin-clavulanic acid disk susceptibility tests could accurately detect resistance among enteric bacilli. Each facility performed disk tests and broth microdilution susceptibility tests with reagents distributed from a common source, and appropriate controls were included in order to ensure methodologic uniformity. Each institution tested 500 consecutively isolated enteric bacilli against ticarcillin and ticarcillin-clavulanic acid. The prevalence of discrepancies between disk tests and dilution tests with 2,435 unselected enteric bacilli provided a valid estimate of the true error rate for tests with the two disks. The current interpretive criteria for susceptibility tests with ticarcillin and ticarcillin-clavulanic acid disks were found to be reliable; i.e., there were major or very major discrepancies between MIC categories and disk test results for less than 1% of all strains and minor discrepancies for only 5 to 10%. Even lower error rates would occur if the zone size-interpretive criteria were modified, but at this time it is difficult to determine whether the practical problems created by making such changes can be justified by the magnitude of the problem that is being resolved.

In 1988, Sanders et al. (6) described enteric bacilli that were resistant to ticarcillin-clavulanic acid as determined by antibiotic dilution tests, but the majority (65%) of those strains were susceptible by disk diffusion methods. Most of those isolates were capable of producing high levels of β-lactamase enzymes, and a few produced β-lactamase enzymes that were able to resist inhibition by clavulanic acid. *Escherichia coli* and *Klebsiella* spp. comprised the majority of these exceptionally resistant strains. Sanders et al. (6) proposed a modification of the zone size-interpretive criteria for ticarcillin-clavulanic acid disk tests in order to minimize false-susceptible disk test results that may occur when enteric bacilli that produce high levels of plasmid-mediated β-lactamases are tested.

In 1989, Fuchs et al. (2) evaluated the situation in order to consider altering the zone size criteria for ticarcillin-clavulanic acid disk tests. They concluded that the modified zone size criteria of Sanders et al. (≤18 mm for resistant and ≥23 mm for susceptible) would indeed eliminate most very major errors with the unusually resistant strains, but that change was not recommended because minor error rates would be markedly increased because of the large proportion of truly susceptible strains that would be placed in the intermediate or moderately susceptible category. The decision to modify zone size-interpretive criteria for ticarcillin-clavulanic acid disk tests will depend on the prevalence of strains that give false-susceptible disk test results compared with the prevalence of susceptible strains that would be miscategorized as being moderately susceptible. In this report, we describe the results of a five-laboratory survey that was designed to document the true prevalence of interpretive discrepancies when 75-μg ticarcillin or 75-μg ticarcillin–10-μg clavulanic acid disk tests were performed against consecutively isolated members of the family *Enterobacteriaceae*. The survey provided a data base that was unbiased by prior selection of unusually resistant strains or by the disproportionate distribution of species that are normally selected for evaluating disk tests.

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MATERIALS AND METHODS

Testing facilities. The directors of each laboratory that collaborated in this study and the five clinical laboratories were as follows: Peter C. Fuchs, St. Vincent Hospital and Medical Center, Portland, Oreg.; E. Hugh Gerlach, St. Francis Regional Medical Center, Wichita, Kans.; Dwight J. Hardy, University of Rochester Medical Center, Rochester, N.Y.; James C. McLaughlin, University of New Mexico Medical Center, Albuquerque; and Michael Pfaller, University of Iowa Hospitals and Clinics, Iowa City. The study was coordinated by the Clinical Microbiology Institute, Tualatin, Oreg.

Antimicrobial susceptibility tests. The disk diffusion test and broth microdilution procedures were as outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (4, 5). Broth microdilution trays were prepared and distributed to the participating laboratories by Prepared Media Laboratories, Tualatin, Oreg. The broth microdilution trays contained twofold dilutions of ticarcillin (256 to 2.0 μg/ml). The ticarcillin was tested alone, with 2 μg of clavulanic acid per ml, and with 16 μg of clavulanic acid per ml. The 16-μg/ml test system was included in an effort to identify strains that were resistant to the combination by virtue of a β-lactamase-mediated system that could be neutralized by the excess inhibitor. Antimicrobial disks included 75-μg ticarcillin or 75-μg ticarcillin–10-μg clavulanic acid disks and were distributed from a common source.

Throughout the study, each participant performed at least 20 replicate tests with each of three quality control strains (*E. coli* ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853). *Staphylococcus aureus* ATCC 25923 was also tested by the disk method. Furthermore, to document comparability among testing facilities, 20 reference strains were tested in all five clinical laboratories and by the coordinating facility. These reference strains included 10 *E. coli* (5 ticarcillin-resistant), 5 *Klebsiella pneumoniae* (3 ticarcillin-resistant and 2 moderately susceptible), and 5 *Citrobacter freundii* (4 ticarcillin-resistant) strains. Because these control data documented comparability, data from the five testing facilities were combined for this report.

Study design. Each testing facility performed broth microdilution and disk diffusion susceptibility tests with all isolates belonging to the family *Enterobacteriaceae* that were judged significant enough to be identified and subjected to routine susceptibility tests. The number of patients from whom these isolates were recovered was not considered for this type of microorganism sample. The studies in each institution continued until at least 500 consecutive isolates were evaluated. This provided an unbiased collection of clinical isolates that were encountered from November 1990 through January 1991. The majority (65%) of all isolates were obtained from urine cultures, 11% were from respiratory tract specimens, 7% were from blood cultures, and 18% were from other sources. As expected, *E. coli* predominated in this type of sample. Discrepancies between broth microdilution and disk diffusion test results were then expressed in terms of error rates that would be expected in the clinical laboratory rather than those that might be expected with the extremely biased challenge sets of microorganisms that are appropriately used in the initial evaluation of disk diffusion susceptibility testing criteria.

RESULTS

Repeatability. Data accumulated with 20 reference strains were evaluated before the studies began. All six testing laboratories reported similar test results. For each strain, the range of zone diameters recorded by six laboratories varied from 1 to 7 mm and the average range was 3.4 mm (with ticarcillin) or 4.4 mm (with ticarcillin-clavulanic acid). Reported MICs varied over ranges of 2 or 3 doubling dilutions, averaging 1.2 dilution intervals for ticarcillin-clavulanic acid and 2.5 dilution steps for ticarcillin. The commonly accepted variation of ±1 doubling dilution (2-dilution interval ranges) was met with the majority of isolates. Regression analysis demonstrated that such a 2-dilution interval range in MICs was approximately comparable to an 8-mm range in zone diameters. In that sense, agar diffusion disk tests were more reproducible than broth microdilution susceptibility tests.

Quality control data reported throughout the studies also documented an acceptable level of reproducibility. Two quality control strains of *E. coli* (ATCC 25922 and ATCC 35218) were tested 108 times, and >95% of all reported MICs were within the acceptable limits that are defined for tests with ticarcillin or ticarcillin-clavulanic acid (4). The standard *P. aeruginosa* ATCC 27853 control strain was tested 31 times, and all but one MIC fell within acceptable limits (4). Those three control strains also gave satisfactory results when the disk diffusion test was used. Tests with *S. aureus* ATCC 25923 were also evaluated by the disk test. Zone size limits for the latter strain are not available because the results were originally found to be too variable to be of value for quality control purposes, but in the current study, reliable results were obtained; i.e., 95% of 104 zone diameters fell into a 7-mm range (30 to 37 mm).

Overall susceptibility patterns. The 2,435 enteric bacilli that were evaluated included 1,335 *E. coli* isolates, 444 *Klebsiella* spp., and 242 *Enterobacter* spp. Table 1 summarizes the results of broth microdilution tests with the three dominant genera and with all strains combined. At 16 μg/ml, ticarcillin inhibited 61% of all enteric bacilli, and the addition of 2 μg of clavulanic acid per ml added another 23% to the susceptible category. When the concentration of clavulanic acid was further increased to 16 μg/ml, nearly all enteric bacilli were susceptible to 16 μg of ticarcillin per ml. The 30 strains that were resistant to 128 μg of ticarcillin per ml with 16 μg of clavulanic acid per ml included 23 *Enterobacter cloacae*, 4 *C. freundii*, and 2 *Serratia liquefaciens* strains and 1 *Morganella morganii* strain. All but 2 of those 30 strains that were resistant to the combination with 16 μg/ml were also very resistant to ticarcillin and ticarcillin-clavulanic acid as determined by MIC testing and by disk test. The two exceptions were *E. cloacae* strains that were susceptible to both drugs as determined by MIC testing and by disk test. At 16 μg/ml, clavulanic acid appeared to be antagonistic for those two isolates, but they were not available for us to confirm that antagonism. We concluded that among enteric bacilli, resistance to ticarcillin is nearly all due to β-lactamase enzymes that can be neutralized in vitro when enough clavulanic acid is added to the system.

Among the 1,335 *E. coli* isolates, 25% were resistant to ticarcillin but only 6% were resistant to ticarcillin-clavulanic acid. The latter isolates are presumed to be the hyperproducers of β-lactamase that Sanders et al. described (6), because they were rendered susceptible by increasing the amount of clavulanic acid. MICs for strains in the moderately susceptible category occurred with 11% of all *E. coli* isolates tested against ticarcillin-clavulanic acid. It is difficult to know whether these strains will be clinically responsive to therapy with the combination, but all of those strains were rendered susceptible when the concentration of inhibitor was increased. The latter strains were previously considered susceptible (MIC, ≤64 μg/ml-2 μg/ml for ticarcillin-clavulanic acid) (1, 3), and no clinical problems with the previous categorization have yet been reported.

Klebsiella species tended to be resistant to ticarcillin, but 92% were susceptible to ≤16 μg of ticarcillin per ml with 2 μg of clavulanic acid per ml. At 64 and 2 μg/ml, ticarcillin-clavulanic acid inhibited another 4% of the *Klebsiella* species. All but three strains were susceptible to 16 μg/ml when the amount of clavulanic acid was increased.

The *Enterobacter* species presented a somewhat different picture, since the addition of clavulanic acid (2 μg/ml) had very little effect on the overall susceptibility to ticarcillin. Approximately 80% of all *Enterobacter* spp. were susceptible or moderately susceptible to ticarcillin and to ticarcillin-clavulanic acid. Further increase in clavulanic acid left only 9% resistant to ticarcillin. For the purposes of this study, the role of type I β-lactamase enzymes that are typically produced by these microorganisms is unclear, since they should not be inhibited by the clavulanic acid.

Ticarcillin disk tests. The interpretive agreements between ticarcillin MIC categories and disk test results are illustrated in Fig. 1 and summarized in Table 2. For tests with 75-μg ticarcillin disks, strains with zones ≤14 mm in diameter were resistant, those with zones 15 to 19 mm in diameter were moderately susceptible, and those with zones ≥20 mm in

TABLE 1. In vitro activity of ticarcillin with and without clavulanic acid against consecutively isolated strains of the *Enterobacteriaceae*[a]

Species (no. tested) and antimicrobial agent(s)[b]	No. of strains inhibited by a MIC[c] (μg/ml) of:						% of strains[d]		
	≤8.0	16	32	64	128	≥256	S	MS	R
All *Enterobacteriaceae* (2,435)									
Ticarcillin	1,450	36	54	95	156	644	61	6	33
Ticarcillin + 2 μg of CA/ml	1,935	119	107	108	88	78	84	9	7
Ticarcillin + 16 μg of CA/ml	2,278	60	43	24	22	8	96	3	1
E. coli (1,335)									
Ticarcillin	971	10	10	5	5	334	74	1	25
Ticarcillin + 2 μg of CA/ml	1,036	71	76	73	54	25	83	11	6
Ticarcillin + 16 μg of CA/ml	1,325	7	1	2	0	0	>99	<1	0
Klebsiella spp. (444)[e]									
Ticarcillin	14	8	28	55	115	224	5	19	76
Ticarcillin + 2 μg of CA/ml	391	17	11	7	4	14	92	4	4
Ticarcillin + 16 μg of CA/ml	443	2	3	0	0	0	99	1	0
Enterobacter spp. (242)[f]									
Ticarcillin	160	3	7	18	17	37	67	10	22
Ticarcillin + 2 μg of CA/ml	163	5	10	16	21	27	69	11	20
Ticarcillin + 16 μg of CA/ml	173	15	16	15	16	7	78	13	9

[a] Tests took place in five medical centers from November 1990 through January 1991.
[b] Twofold dilutions of ticarcillin combined with clavulanic acid (CA) at 2 or 16 μg/ml.
[c] MICs are expressed as the concentrations of ticarcillin in the ticarcillin-clavulanic acid combinations.
[d] Susceptible (S), MIC = ≤16 μg/ml; moderately susceptible (MS), MIC = 32 or 64 μg/ml; resistant (R), MIC = ≥128 μg/ml.
[e] Includes 365 *K. pneumoniae* and 79 *K. oxytoca* isolates.
[f] Includes 73 *E. aerogenes*, 8 *E. agglomerans*, 2 *E. amnigenus*, 153 *E. cloacae*, and 4 *E. hafniae* isolates and 2 unidentified species.

diameter were susceptible, according to current NCCLS criteria. False-susceptible disk test results (very major errors) occurred with less than 1% of all enteric bacilli and only 0.2% of the *E. coli* isolates tested. Since very major errors can occur only with strains that are resistant as determined by MIC testing, the very major error rate might be appropriately described as the percentage of resistant strains (strains for which MICs are ≥128 μg/ml). That would have

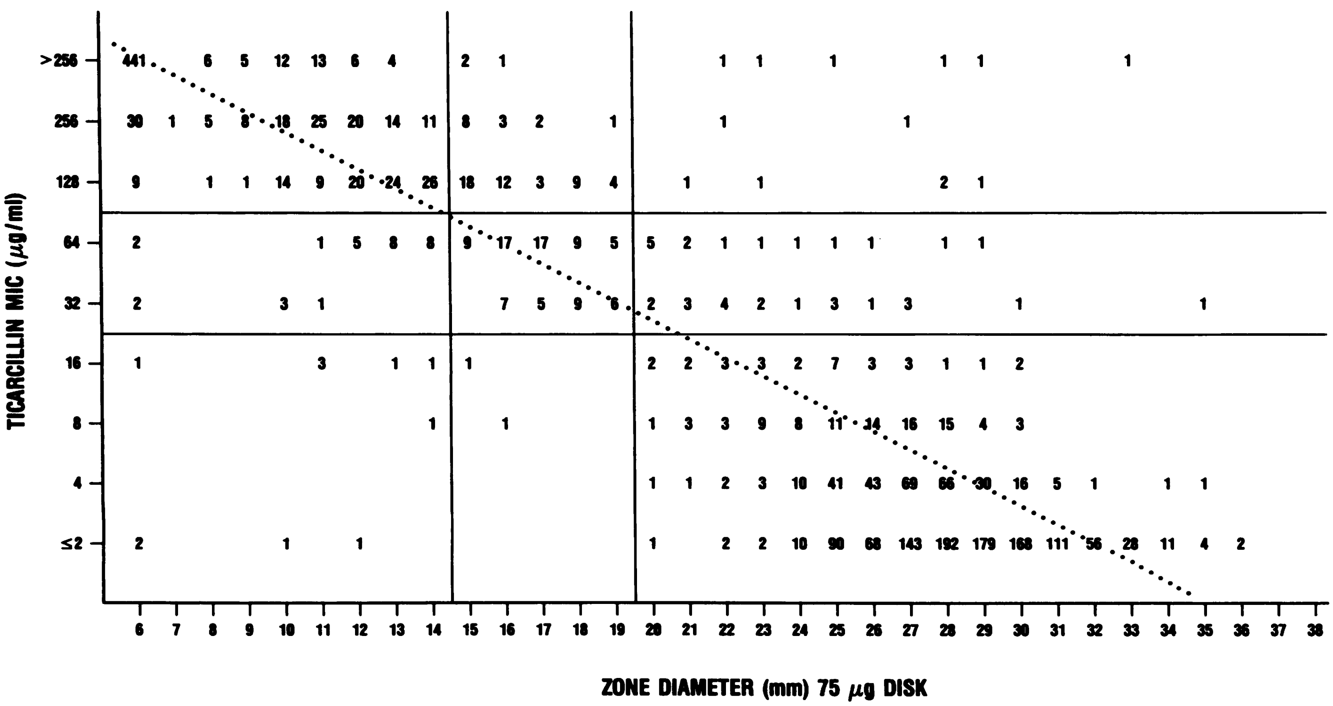

FIG. 1. Results of ticarcillin susceptibility tests performed at five institutions with 2,435 strains of the *Enterobacteriaceae*. Broth microdilution MICs are compared with zone diameters obtained with 75-μg disks. Horizontal and vertical lines represent current breakpoints for categorization of test results.

TABLE 2. Interpretive agreement between ticarcillin MIC categories and disk test results when consecutively isolated strains of the *Enterobacteriaceae* were tested in five laboratories

Species and MIC category[a]	No. (%) of strains in each category as determined by MIC testing	No. (%) in each category as determined by disk test[b] with zone of:			Error rate (%)[c]		
		≤14 mm, R	15–19 mm, MS	≥20 mm, S	Major	Minor	Very major
All *Enterobacteriaceae*							
R	799 (33)	723 (90)	63 (8)	13 (2)			0.5
MS	149 (6)	30 (20)	84 (56)	35 (23)		5.3	
S	1,486 (61)	11 (<1)	2 (<1)	1,473 (99)	0.4		
Total	2,434	764 (31)	149 (6)	1,521 (63)			
E. coli							
R	339 (25)	329 (97)	1 (<1)	9 (3)			0.2
MS	15 (1)	1 (7)	5 (33)	9 (60)		0.8	
S	981 (73)	4 (<1)	0	977 (>99)	0.3		
Total	1,335	334 (25)	6 (<1)	999 (75)			
Klebsiella spp.							
R	338 (76)	287 (85)	51 (15)	0			0
MS	83 (19)	18 (22)	57 (69)	8 (10)		17.8	
S	23 (5)	6 (26)	2 (9)	15 (65)	1.4		
Total	444	311 (70)	110 (25)	23 (5)			
Enterobacter spp.							
R	54 (22)	48 (89)	3 (6)	3 (6)			1.2
MS	25 (10)	5 (20)	9 (36)	11 (44)		7.8	
S	163 (67)	1 (1)	0	162 (99)	0.4		
Total	242	54 (22)	12 (5)	176 (73)			

[a] For definitions of categories, see Table 1, footnote *d*.
[b] Percentages calculated for the number of tests in the designated MIC category, not necessarily the total number tested. Disks contained 75 μg of ticarcillin.
[c] Expressed as a percentage of the total number of strains tested.

resulted in a very major error rate of 2% for all enteric bacilli or 3% for *E. coli* isolates only. No very major errors occurred with *Klebsiella* spp., and *Enterobacter* spp. produced only three very major errors (1.2% of the 242 strains or 6% of the 54 resistant strains). False-resistant disk test results were also relatively uncommon (≤1% of all enteric bacilli to 1.4% of the *Klebsiella* spp.). Minor discrepancies (categorization of strains as moderately susceptible by one method and resistant or susceptible by the other method) occurred with 5% of all enteric bacilli but with 18% of the *Klebsiella* spp. A moderately susceptible MIC category was recorded for 6% of all enteric bacilli but 19% of the *Klebsiella* spp. Those moderately susceptible strains are the ones that were previously considered susceptible but were recently reclassified (1, 3, 4).

Ticarcillin-clavulanic acid disk tests. Figure 2 presents the results of tests with all enteric bacilli, comparing ticarcillin-clavulanic acid MICs and zone size categories. Table 3 summarizes discrepant results with two zone size criteria (≤14 and ≥20 mm contrasted with ≤18 and ≥23 mm). By broth microdilution tests, 84% of all enteric bacilli were susceptible and 7% were resistant. With the proposed breakpoints of ≤18 and ≥23 mm, 84% were susceptible and 8% were resistant. By the current zone site criteria, 90% of all enteric bacilli were susceptible and only 3% were resistant.

Very major error rates, expressed as the percentage of 166 strains that were determined to be resistant by MIC testing, were reduced from 10% with the current criteria to 2% with the proposed criteria. For *E. coli* isolates, very major errors dropped from 6 to 0%. At the same time, the proportion of strains with zone sizes that put them in the moderately susceptible category increased from 6% by the current method to 9% by the proposed method. Among *E. coli* isolates, that figure changed from 8 to 11%. With *Klebsiella* spp. and *Enterobacter* spp., the proportion of strains with intermediate zone sizes did not change greatly.

Error rates for tests with 75-μg ticarcillin–10-μg clavulanic acid disks are summarized in Table 4. If very major errors are recorded as the percentage of all strains tested, the rate for strains of the *Enterobacteriaceae* is 0.8% and modification of the zone size standards would reduce that to 0.2%. At the same time, major error rates would be increased from 0.05 to 0.2% and the proportion of strains with moderately susceptible disk test results would increase from 6 to 9%. If the very major error rates are expressed as the percentage of strains in the resistant category as determined by MIC tests, the rate is 10% by the current zone standards but only 2% by the proposed standards. For *E. coli* isolates alone, the same very major error rates are 6 and 0%. For *Enterobacter* spp., very major error rates would be reduced from 8 to 6% (1.6 and 1.2% of the total number of strains tested).

DISCUSSION

In the first edition of the NCCLS standard for antimicrobial dilution susceptibility tests (3), microorganisms for which the MICs are ≤64 μg/ml were defined as susceptible to ticarcillin or ticarcillin with 2 μg of clavulanic acid per ml.

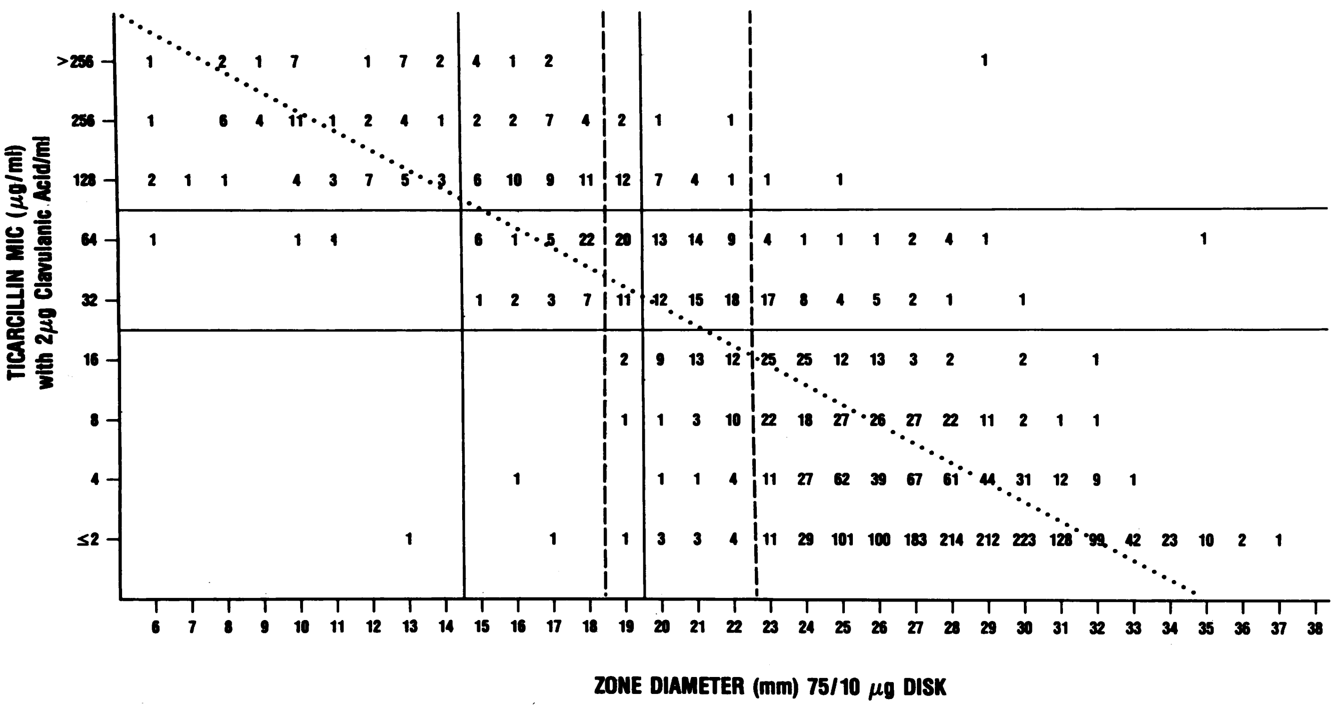

FIG. 2. Results of ticarcillin-clavulanic acid susceptibility tests performed at five institutions with 2,435 strains of the *Enterobacteriaceae*. Broth microdilution MICs are compared with zone diameters obtained with 75-μg ticarcillin–10-μg clavulanic acid disks. Horizontal and vertical solid lines represent the current breakpoints for categorization of test results. The broken lines represent alternative zone size breakpoints that are under consideration.

In the second edition of that standard (4), susceptible strains of the *Enterobacteriaceae* were further divided into two categories, i.e., susceptible (MIC ≤16 μg/ml) and moderately susceptible (MIC, 32 or 64 μg/ml). Strains in the moderately susceptible category are not resistant, but they may not be as responsive to therapy as those strains that fall into the susceptible category. There are currently no clinical data that will document the efficacy or lack of efficacy of ticarcillin or ticarcillin-clavulanic acid against moderately susceptible enteric bacilli. For some species, MICs are normally ≤16 μg/ml, but some β-lactamase-producing strains may require slightly elevated MICs, and those are the strains that fall into the moderately susceptible category. The strains that produce high levels of β-lactamase that were described by Sanders et al. (6) may appear resistant or moderately susceptible to ticarcillin-clavulanic acid by standard methods, but they may appear susceptible if the amount of clavulanic acid is increased. According to Sanders et al. (6) and Fuchs et al. (2), some of those β-lactamase-producing strains may be categorized as susceptible by the disk test unless zone size breakpoints are modified. We are left with two unanswered questions: which in vitro test gives the clinically correct category with these strains, and what is the true prevalence of false-susceptible disk test results among contemporary isolates? The present report addresses the latter question but not the former.

The distribution of MICs of ticarcillin with and without clavulanic acid against consecutively isolated strains of the *Enterobacteriaceae* was first evaluated to determine how the changes in MIC-interpretive criteria affected the overall picture. Among the 2,435 enteric bacilli that we tested, 33% were resistant to ticarcillin, but with the addition of clavulanic acid only 7% were resistant and 9% were moderately susceptible. With a further increase of clavulanic acid (to 16 μg/ml), 96% of all enteric bacilli were susceptible and 3% were moderately susceptible. Most of the strains producing high levels of β-lactamase which were reported by Sanders et al. (6) belong to the species *E. coli*. Among our 1,335 *E. coli* isolates, 25% were ticarcillin resistant and only 20 strains (1%) were moderately susceptible. The majority of *E. coli* strains were inhibited by ≤2.0 μg of ticarcillin per ml, and those susceptible strains were not influenced by the addition of clavulanic acid. On the other hand, *E. coli* strains with elevated MICs of ticarcillin were influenced by the addition of clavulanic acid. With 2 μg of clavulanic acid per ml, only 6% of our *E. coli* strains were resistant and another 11% were moderately susceptible. Nearly all (92%) of the *Klebsiella* spp. were rendered fully susceptible to ticarcillin when 2 μg of clavulanic acid was added. *Enterobacter* spp., on the other hand, were not markedly affected by the β-lactamase inhibitor.

The primary reason for doing this study was to determine the prevalence of discrepancies between broth microdilution and disk diffusion tests when consecutively isolated strains of the *Enterobacteriaceae* were evaluated. When ticarcillin was tested alone, major and very major error rates were <1% and minor discrepancies involved 5% of the strains (18% of the 444 *Klebsiella* spp.). The discrepancies with the greatest clinical importance are perceived to be the false-susceptible disk test results (very major errors); that perception is based on the assumptions that the MIC is always correct clinically and that discrepant disk test results must be incorrect. It is also possible that very major errors might represent false-resistant MIC results rather than false-susceptible disk test results. Since very major errors can occur only with strains that are determined to be resistant by MIC

TABLE 3. Interpretive agreement between ticarcillin-clavulanic acid MIC categories and results of disk test with two zone size criteria when consecutively isolated strains of the *Enterobacteriaceae* were tested[a]

Species and MIC category[b]	No. (%) of strains in each category as determined by MIC testing	No. (%) of strains in each category as determined by disk test[c] with zone of:					
		≤14 mm, R	15–19 mm, MS	≥20 mm, S	≤18 mm, R	19–22 mm, MS	≥23 mm, S
All *Enterobacteriaceae*							
R	166 (7)	77 (46)	72 (43)	17 (10)	135 (81)	28 (17)	3 (2)
MS	215 (9)	3 (1)	78 (36)	134 (62)	50 (23)	112 (52)	53 (25)
S	2,053 (84)	1 (<1)	6 (<1)	2,046 (>99)	3 (<1)	68 (3)	1,982 (97)
Total	2,434	81 (3)	156 (6)	2,197 (90)	188 (8)	208 (9)	2,038 (84)
E. coli							
R	79 (6)	11 (14)	55 (70)	13 (16)	53 (67)	26 (33)	0
MS	149 (11)	1 (1)	54 (36)	94 (63)	29 (19)	94 (63)	26 (17)
S	1,107 (83)	0	1 (<1)	1,106 (>99)	0	29 (3)	1,078 (97)
Total	1,335	12 (1)	110 (8)	1,213 (91)	82 (6)	149 (11)	1,104 (83)
Klebsiella spp.							
R	17 (4)	6 (35)	11 (65)	0	17 (100)	0	0
MS	18 (4)	0	11 (61)	7 (39)	8 (44)	8 (44)	2 (11)
S	409 (92)	0	4 (1)	405 (99)	2 (<1)	24 (6)	383 (94)
Total	444	6 (1)	26 (6)	412 (93)	27 (6)	32 (7)	385 (86)
Enterobacter spp.							
R	48 (20)	42 (87)	2 (4)	4 (8)	44 (92)	1 (2)	3 (6)
MS	26 (11)	1 (4)	7 (27)	18 (69)	7 (27)	4 (15)	15 (58)
S	168 (69)	1 (1)	0	167 (99)	1 (1)	3 (2)	164 (98)
Total	242	44 (18)	9 (4)	189 (78)	52 (21)	8 (3)	182 (75)

[a] Abbreviations: R, resistant; MS, moderately susceptible; S, susceptible.
[b] MIC categories are as defined in Table 1, footnote *d*, except that 2 μg of clavulanic acid per ml was added to the ticarcillin.
[c] Percentages calculated for the number of tests in the designated MIC category, not necessarily the total number tested. Disks contained 75 μg of ticarcillin and 10 μg of clavulanic acid.

testing, it seems logical to define the error rate as the percentage of resistant strains rather than the percentage of all strains tested. When those two definitions were applied, very major error rates were either 0.8 or 2% for all enteric bacilli and, for *E. coli* strains alone, the error rates were either 0.2 or 3%. Most investigators feel comfortable with very major error rates of ≤1% (based on the total number of strains tested). An acceptable level of errors based on the number of resistant strains alone has not yet been established.

The prevalence of minor errors is a measure of the proportion of strains near or within the moderately susceptible or intermediate category as determined by MIC testing. With *Klebsiella* spp., 19% of the strains were categorized as moderately susceptible to ticarcillin (MIC, 32 or 64 μg/ml) and another 28% required MICs that were only 1 doubling dilution above or below the breakpoints (16 or 128 μg/ml). Since dilution tests often vary ±1 doubling dilution when repeated, 47% (19% + 28%) of our *Klebsiella* spp. are capable of changing ticarcillin categories if the dilution tests are repeated. Only 2% of our *E. coli* strains fall into that group of strains for which there are potentially variable results. For the same reason, minor discrepancies between two types of testing systems would be expected to occur more frequently with *Klebsiella* spp. than with *E. coli* strains. We concluded that the current zone size criteria were satisfactory for tests with 75-μg ticarcillin disks compared with broth microdilution tests using the current MIC breakpoints.

Disks containing ticarcillin and clavulanic acid (75 and 10 μg, respectively) were also evaluated with two zone size criteria. The extended zone size criteria proposed by Sanders et al. (6) did indeed reduce the total number of very major errors from 0.8 to 0.2% (10% of resistant strains to 2% of resistant strains). At the same time, the number of strains falling into the moderately susceptible disk test category increased from 6 to 9% (by dilution tests, 9% of enteric bacilli were moderately susceptible). Among the strains that were fully susceptible as determined by MIC testing, 6 were moderately susceptible and 1 was resistant by the current zone standards, whereas 68 would be moderately susceptible and 3 would be resistant if the proposed standards were used. Among *E. coli* strains, the 1% very major error rate was completely eliminated but the number of moderately susceptible strains increased from 8 to 11%. With *Klebsiella* spp., there were no very major errors with either disk criterion but minor errors were 5 and 8% with the two zone size criteria.

The overall agreement between MIC categories and disk susceptibility test results was slightly improved by applying the interpretive zone size criteria of Sanders et al. (6). When there were discrepancies, it was difficult to determine which of the two tests would better predict the clinical outcome. In the absence of compelling clinical data which document failure of the current criteria, we are reluctant to recommend yet another change in interpretive breakpoints. There is a very real need for clinical data that will help to evaluate the utility of ticarcillin or ticarcillin-clavulanic acid in treating

TABLE 4. Effect of altering zone size-interpretive criteria for ticarcillin-clavulanic acid disk tests against consecutively isolated strains of the *Enterobacteriaceae*[a]

Species and zone size criteria (mm)	Error rate as % of total			No. (%) of MS strains[b]
	Minor	Major	Very major[c]	
All *Enterobacteriaceae*				
R ≤ 14 and S ≥ 20	10.5	0.05	0.8 (10)	156 (6)
R ≤ 18 and S ≥ 23	9.7	0.2	0.2 (2)	208 (9)
E. coli				
R ≤ 14 and S ≥ 20	11.3	0	1.0 (6)	110 (8)
R ≤ 18 and S ≥ 23	8.2	0	0 (0)	149 (11)
Klebsiella spp.				
R ≤ 14 and S ≥ 20	5.0	0	0 (0)	6 (1)
R ≤ 18 and S ≥ 23	7.7	0.4	0 (0)	32 (7)
Enterobacter spp.				
R ≤ 14 and S ≥ 20	8.7	0.4	1.6 (8)	9 (4)
R ≤ 18 and S ≥ 23	10.7	0.4	1.2 (6)	8 (3)

[a] Disks contained 75 μg of ticarcillin and 10 μg of clavulanic acid. MS, moderately susceptible; R, resistant; S, susceptible.
[b] Having zones 15 to 19 or 19 to 22 mm in diameter.
[c] Percentages in parentheses represent calculations based on the total number of resistant strains rather than the total number tested.

infections due to microorganisms which were initially considered susceptible but are now categorized as being moderately susceptible (MIC, 32 or 64 μg/ml). If such microorganisms are truly nonresponsive, they should be categorized as resistant and revised zone size criteria will be needed. However, at this time, there is no reason to believe that enteric bacilli for which MICs are ≤64 μg/ml will fail to respond to therapy. False-susceptible ticarcillin-clavulanic acid disk test results do occur, but this happens with less than 1% of all enteric bacilli. That low error rate could be further reduced by changing the zone size breakpoints, but it is difficult to determine whether the practical problems created by making changes in interpretive criteria can be justified by the magnitude of the problem that is being resolved.

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