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Reassessment of Susceptibility Test Interpretive Criteria for Ticarcillin and Ticarcillin-Clavulanic Acid

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There are at least four different existing or proposed interpretive criteria for the disk diffusion susceptibility testing of ticarcillin and ticarcillin plus clavulanic acid (T/C). To assess these criteria, 570 gram-negative bacillary isolates were tested for susceptibility to ticarcillin and T/C by both disk diffusion and broth microdilution methods. These included 53 strains of the family *Enterobacteriaceae* selected for ticarcillin resistance and high-level β-lactamase production. The broth microdilution test results were more influenced by increased β-lactamase production than were disk diffusion results. In the absence of published data indicating which of the two standardized test methods better predicts clinical response, we conclude that until such data are available the more conservative National Committee for Clinical Laboratory Standards tentative criteria for tests with members of the *Enterobacteriaceae* are appropriate. Our data do not support the use of separate T/C interpretive criteria for *Pseudomonas* spp. and members of the *Enterobacteriaceae*. The appropriateness of different interpretive criteria needs further evaluation.

The combination of clavulanic acid (CA), a potent β-lactamase inhibitor, with ticarcillin has proven to be an effective therapeutic agent (4, 11). However, there is some recent controversy regarding the in vitro susceptibility testing interpretive criteria for ticarcillin plus CA (T/C), as well as ticarcillin alone. In 1984, we recommended the 75/10-μg T/C disk for disk diffusion susceptibility testing and tentatively suggested the same interpretive zone diameter breakpoints that were recommended for ticarcillin alone (2). The National Committee for Clinical Laboratory Standards (NCCLS) adopted these recommendations for disk susceptibility testing of T/C (5). At least two problems exist with these NCCLS criteria. (i) The MIC breakpoints for dilution susceptibility testing of T/C, ticarcillin, and related penicillins found in one NCCLS document (6) differ from the MIC correlates of the disk diffusion breakpoints found in another NCCLS document (5). (ii) There appears to be a high frequency of discrepancies between disk diffusion and broth dilution susceptibility test results with T/C when members of the family *Enterobacteriaceae* that produce high levels of plasmid-mediated β-lactamases are tested (10).

The NCCLS recently published new tentative interpretive criteria for this group of drugs which are designed to minimize the discrepancies between the disk diffusion and dilution susceptibility test documents (7, 8). These tentative criteria provide different breakpoints for *Pseudomonas* spp. and non-*Pseudomonas* gram-negative bacteria (NPGNB). Sanders et al. proposed yet another set of criteria for T/C disk diffusion tests designed to reduce discrepant categorization of members of the *Enterobacteriaceae* producing high levels of β-lactamase (10). The purpose of the present study was to evaluate the cited interpretive criteria by simultaneously testing a large number of clinical isolates by disk diffusion and broth microdilution methods. To further evaluate the criteria proposed by Sanders et al. (10), additional tests were performed with strains of *Enterobacteriaceae* selected because of their increased β-lactamase production and resistance to pseudomonas-active penicillins.

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MATERIALS AND METHODS

Organisms. A total of 570 gram-negative bacterial isolates, consisting of 96 *Pseudomonas* spp. and 474 NPGNB (including 53 ticarcillin-resistant members of the *Enterobacteriaceae* selected for high-level production of β-lactamase), were tested.

Antibiotic susceptibility testing. MICs were determined by the broth microdilution method described by the NCCLS (6). Ticarcillin and potassium clavulanate were provided as standardized powders by Beecham Laboratories, Bristol, Tenn. Each broth microdilution tray contained three series of ticarcillin twofold dilutions with concentrations ranging from 512 to 1 μg/ml in cation-supplemented Mueller-Hinton broth. In one of these was 2 μg of CA per ml in each well; the second series contained 4 μg of CA per ml in each well; and the third series of dilutions contained no CA. β-Lactamase-producing isolates of *Salmonella* and *Shigella* spp. were collected and tested by the laboratory directed by C. Thornsberry, Centers for Disease Control, Atlanta, Ga. Disk diffusion susceptibility tests were conducted according to the recommendations of the NCCLS (5). Commercial 75-μg ticarcillin and 75/10-μg T/C disks manufactured by Difco Laboratories, Detroit, Mich., were used. Standard quality control strains were included on each day of testing.

RESULTS

The MICs of ticarcillin and T/C for the 517 clinical isolates not selected for high-level production of β-lactamase are summarized in Table 1. The susceptibility patterns for the different species were similar to those previously reported (1, 3). When the disk diffusion inhibitory zone diameters were plotted against the MICs for this group of organisms, the correlation coefficients for ticarcillin were 0.87 and 0.76 for members of the *Enterobacteriaceae* and *Pseudomonas* spp., respectively; corresponding values for T/C were 0.83 and 0.90.

Table 2 summarizes the effect of 2 versus 4 μg of CA per ml in the susceptibility testing of T/C against members of the *Enterobacteriaceae*. No significant difference was observed

TABLE 1. Ticarcillin and T/C MICs for 517 gram-negative isolates[a]

Organism (no. of isolates)	MIC (μg/ml)[b]					
	Ticarcillin		T/C (2 μg/ml)[c]		T/C (4 μg/ml)[c]	
	Range	50%	Range	50%	Range	50%
Acinetobacter anitratus (30)	2.0–16	8.0	≤1.0–16	4.0	≤1.0–8.0	2.0
Citrobacter diversus (14)	64–128	128	2.0–4.0	4.0	2.0–4.0	2.0
Citrobacter freundii (13)	2.0–>512	64	2.0–>512	8.0	2.0–>512	8.0
Enterobacter aerogenes (31)	2.0–256	4.0	2.0–256	4.0	2.0–256	4.0
Enterobacter agglomerans (10)	2.0–512	128	2.0–512	8.0	4.0–512	8.0
Enterobacter cloacae (32)	≤1.0–>512	4.0	2.0–>512	4.0	2.0–>512	4.0
Escherichia coli (50)	≤1.0–>512	8.0	≤1.0–512	8.0	≤1.0–512	4.0
Klebsiella spp. (32)	32–>512	256	2.0–512	4.0	2.0–512	4.0
Morganella morganii (20)	≤1.0–64	4.0	≤1.0–128	16	≤1.0–64	8.0
Proteus mirabilis (31)	≤1.0–128	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0
Proteus vulgaris (20)	2.0–64	16	≤1.0–2.0	≤1.0	≤1.0	≤1.0
Providencia spp. (54)	≤1.0–>512	≤1.0	≤1.0–32	≤1.0	≤1.0–16	≤1.0
Pseudomonas acidovorans (5)	32_1, 64_1, 128_2, 256_1		$≤1_4$, 2_1		$≤1_4$, 2_1	
Pseudomonas aeruginosa (55)	8.0–>512	32	8.0–>512	32	8.0–>512	32
Pseudomonas cepacia (8)	2.0–>512	16	4.0–>512	16	4.0–>512	16
Pseudomonas fluorescens (8)	64–>512	256	64–>512	256	64–>512	512
Pseudomonas maltophilia (10)	2.0–64	16	2.0–64	4.0	4.0–128	8.0
Pseudomonas putida (5)	128_2, 256_2, 512_1		128_1, 256_4		128_2, 256_2, 512_1	
Pseudomonas stutzeri (5)	$≤1_1$, 4_2, 8_2		$≤1_1$, 2_2, 4_1, 8_1		$≤1_2$, 2_3	
Serratia spp. (33)	2.0–>512	8.0	2.0–>512	8.0	2.0–>512	16
Shigella spp. (20)	≤1.0–>512	2.0	≤1.0–64	2.0	≤1.0–64	2.0
Yersinia enterocolitica (10)	4.0–512	256	≤1.0–8.0	4.0	≤1.0–8.0	4.0
Other NPGNB[d] (21)	≤1.0–>512	16	≤1.0–>512	8.0	≤1.0–>512	8.0

[a] MICs of T/C reflect the concentration of ticarcillin in the combination.
[b] 50%, MIC for 50% of isolates tested. When fewer than six isolates were tested, individual MICs are shown. The inferior number is the number of isolates with the indicated MIC.
[c] Concentration of CA.
[d] Other NPGNB included: four *Achromobacter xylosoxidans*, three *Aeromonas* spp., two *Cedecea lapagei*, three *Citrobacter amalonaticus*, three *Enterobacter sakazaii*, two *Flavobacterium* spp., and three *Hafnia alvei* isolates and one *Salmonella typhi* isolate.

with routine clinical isolates, but a significant (fourfold) reduction in T/C MICs was seen with 4 μg of CA per ml in the test system when producers of high levels of β-lactamase were tested.

Scattergrams for ticarcillin MICs plotted against inhibitory zone diameters are displayed in Fig. 1 for these organisms, illustrating the two NCCLS breakpoints. Table 3 provides the calculated error rates for each subset of organisms when each of the sets of interpretive criteria was applied: criteria A, original NCCLS-recommended criteria (5); criteria B, tentative NCCLS criteria for *Pseudomonas* spp. (7); criteria C, tentative NCCLS criteria for NPGNB (7); and criteria D, proposed criteria of Sanders et al. for members of the *Enterobacteriaceae* (10).

Application of criteria A to ticarcillin yielded high very major error rates (false-susceptibility disk diffusion results) for NPGNB (2.6%) and *Pseudomonas* spp. (3.1%). These were reduced to acceptable levels with the application of the tentative NCCLS criteria, i.e., to 0.5% for NPGNB and to 1.0% for *Pseudomonas* spp. Application of criteria B yielded the lowest error rates for both NPGNB and *Pseudomonas* spp.

The corresponding scattergrams for T/C are given in Fig. 2. NPGNB (Fig. 2A and B) had an unacceptably high very major error rate with criteria A (1.4%), but these errors were eliminated by the other criteria. However, the total error rate was highest with criteria D (10.0%), owing exclusively to minor discrepancies.

No very major errors occurred with T/C testing of *Pseudomonas* spp. by any of the criteria evaluated. Criteria A and C yielded the lowest total error rates with this genus (13.5%), but criteria A and B gave a 2.1% major error rate compared with no major errors with criteria C (Table 3).

Table 4 summarizes the T/C error rates obtained when the same interpretive criteria were applied to the 382 isolates of *Enterobacteriaceae* among the 517 bacteria tested, as well as the 53 members of the *Enterobacteriaceae* producing high levels of β-lactamase. Scattergrams for these organisms with

TABLE 2. Effects of 2 and 4 μg of CA per ml on susceptibility to ticarcillin of isolates of *Enterobacteriaceae*

Enterobacteriaceae (no. of isolates)	Agent (CA concn; μg/ml)	Cumulative % susceptible at ticarcillin concn (μg/ml) of:										
		≤1.0	2.0	4.0	8.0	16	32	64	128	256	512	>512
Unselected (382)	Ticarcillin	18	29	46	54	58	63	68	74	81	84	100
	T/C (2)	23	36	57	72	80	83	87	90	96	98	100
	T/C (4)	23	43	59	73	82	86	92	95	97	98	100
High-level-β-lactamase producers (53)	Ticarcillin	0	0	0	0	0	0	0	0	4	8	100
	T/C (2)	0	0	0	0	0	0	13	34	70	92	100
	T/C (4)	0	0	0	4	8	21	51	75	96	100	

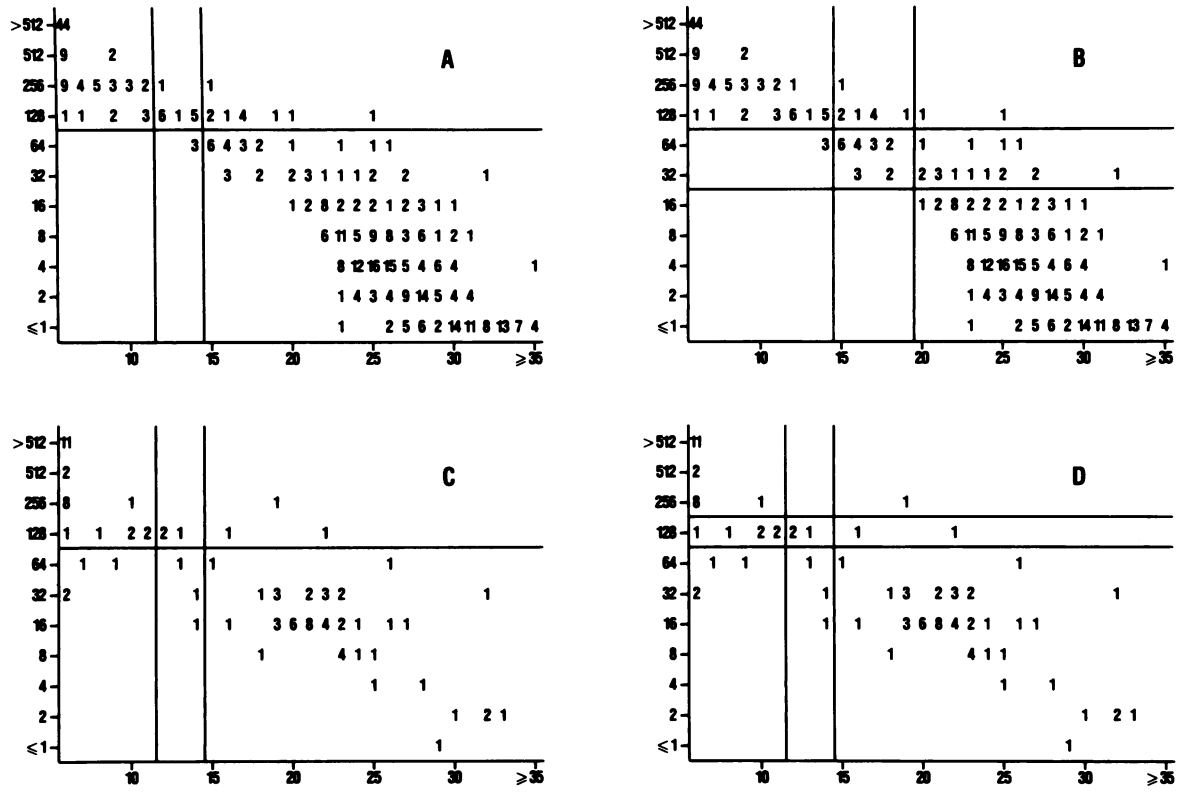

FIG. 1. Ticarcillin scattergrams. (A and B) NPGNB with former (A; criteria A) and proposed (B; criteria C) NCCLS breakpoints; (C and D) *Pseudomonas* spp. with former (C; criteria A) and proposed (D; criteria B) NCCLS breakpoints. *x* Axis, Zone diameters (millimeters); *y* axis, MICs (micrograms per milliliter).

T/C are shown in Fig. 3. No major errors occurred with any of the criteria. The very major error rates were ≤1% for all criteria with the 382 unselected clinical strains. For the 53 producers of high levels of β-lactamase, the very major error rates were extremely high for criteria A, B, and C (77, 59, and 9%, respectively), but no very major errors occurred with criteria D. The minor and total error rates were more than twice as high for criteria D (10.5%) than for any of the

TABLE 3. Ticarcillin and T/C disk susceptibility error rates with 517 gram-negative isolates using four interpretive criteria

Criteria[a]	Organisms (no. of isolates)	No. of interpretive errors (% of total tested)[b]							
		Ticarcillin				T/C			
		V. Major	Major	Minor	Total	V. Major	Major	Minor	Total
A (S = ≤64 μg/ml, ≥15 mm; I = 12–14 mm; R = ≥128 μg/ml, ≤11 mm)	*Pseudomonas* spp. (96)	3 (3.1)	4 (4.2)	6 (6.3)	13 (13.5)	0	2 (2.1)	11 (11.5)	13 (13.5)
	NPGNB (421)	11 (2.6)	0	16 (3.8)	27 (6.4)	6 (1.4)	0	14 (3.3)	20 (4.8)
	Total (517)	14 (2.7)	4 (0.8)	22 (4.2)	40 (7.7)	6 (1.2)	2 (0.4)	25 (4.8)	33 (6.4)
B (S = ≤64 μg/ml, ≥15 mm; MS = 128 μg/ml, 12–14 mm; R = ≥256 μg/ml, ≤11 mm)	*Pseudomonas* spp. (96)	1 (1.0)	4 (4.2)	11 (11.5)	16 (16.7)	0	2 (2.1)	12 (12.5)	14 (14.6)
	NPGNB (421)	1 (0.2)	0	21 (5.0)	22 (5.2)	0	0	19 (4.5)	19 (4.5)
	Total (517)	2 (0.4)	4 (0.8)	32 (6.2)	38 (7.4)	0	2 (0.4)	31 (6.0)	33 (6.4)
C (S = ≤16 μg/ml, ≥20 mm; MS = 32–64 μg/ml, 15–19 mm; R = ≥128 μg/ml, ≤14 mm)	*Pseudomonas* spp. (96)	1 (1.0)	1 (1.0)	22 (22.9)	24 (25.0)	0	0	13 (13.5)	13 (13.5)
	NPGNB (421)	2 (0.5)	0	29 (6.8)	31 (7.4)	1 (0.2)	0	21 (5.0)	22 (5.2)
	Total (517)	3 (0.6)	1 (0.2)	51 (9.9)	55 (10.6)	1 (0.2)	0	34 (6.6)	35 (6.8)
D (S = ≤16 μg/ml, ≥23 mm; MS = 32–64 μg/ml, 19–22 mm; R = ≥128 μg/ml, ≤18 mm)	*Pseudomonas* spp. (96)	0	3 (3.1)	35 (36.5)	38 (39.6)	0	0	33 (34.4)	33 (34.4)
	NPGNB (421)	1 (0.2)	0	52 (12.4)	53 (12.6)	0	0	42 (10.0)	4 (10.0)
	Total (517)	1 (0.2)	3 (0.6)	87 (16.8)	91 (17.6)	0	0	75 (14.5)	75 (14.5)

[a] Criteria are described in Results. S, susceptible; I, intermediate; R, resistant; MS, moderately susceptible.

[b] V. Major, Susceptibility zone diameters with resistance MICs; Major, resistance zone diameters with susceptibility MICs; Minor, zone diameter or MIC (but not both) indicating moderate susceptibility or intermediate susceptibility.

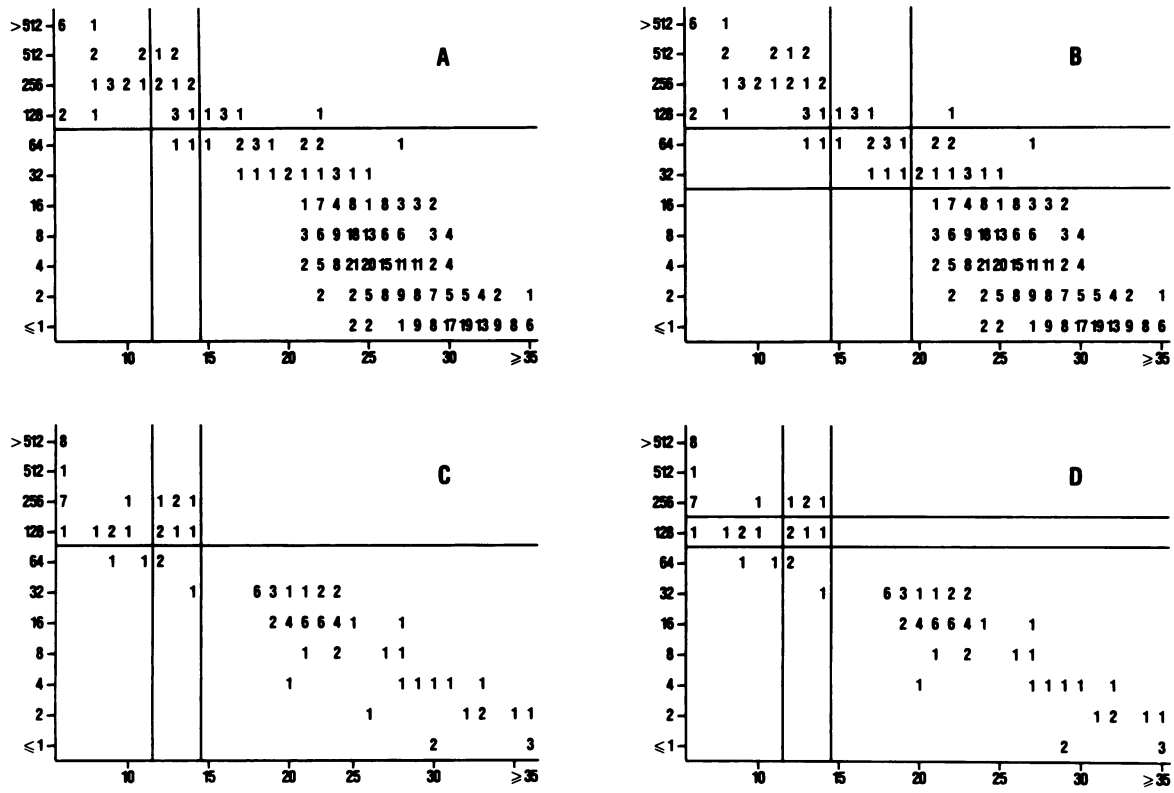

FIG. 2. T/C scattergrams. See the legend to Fig. 1.

other criteria with the 382 unselected strains. With the 53 producers of high levels of β-lactamase, however, the total error rate for criteria D (32%) was less than half that of any of the other criteria.

Antagonism with the combination of ticarcillin and CA (defined as a fourfold-or-greater increase in ticarcillin MIC) was observed with 12 of the 517 isolates studied (Table 5), all members of the *Enterobacteriaceae*. Only with two organisms did the disk diffusion test demonstrate a significantly smaller zone diameter with T/C.

DISCUSSION

None of the cited tentative or proposed interpretive criteria for the disk diffusion susceptibility testing of T/C is strongly supported by the data in this study. On the other hand, these data do raise a number of questions that should be resolved before definitive interpretive criteria for T/C and related drugs are selected.

Microbiologically, our data provide no support for the concept of different interpretive criteria for *Pseudomonas*

TABLE 4. T/C disk diffusion susceptibility error rates with unselected and ticarcillin-resistant members of the *Enterobacteriaceae* with four different interpretive criteria

Criteria[a]	Organisms (no. of isolates)	No. of T/C errors (rate)[b]			
		V. Major	Major	Minor	Total
A (S = ≤64 μg/ml, ≥15 mm; I = 12–14 mm; R = ≥128 μg/ml, ≤11 mm)	Unselected (382)	4 (1.0)	0	14 (3.7)	18 (4.7)
	High-level-β-lactamase producers (53)	41 (77.4)	0	4 (7.5)	45 (84.9)
B (S = ≤64 μg/ml, ≥15 mm; MS = 128 μg/ml, 12–14 mm; R = ≥256 μg/ml, ≤11 mm)	Unselected (382)	0	0	17 (4.5)	17 (4.5)
	High-level-β-lactamase producers (53)	31 (58.5)	0	13 (24.5)	44 (83.0)
C (S = ≤16 μg/ml, ≥20 mm; MS = 32–64 μg/ml, 15–19 mm; R = ≥128 μg/ml, ≤14 mm)	Unselected (382)	1 (0.3)	0	18 (4.7)	19 (5.0)
	High-level-β-lactamase producers (53)	5 (9.4)	0	41 (77.4)	46 (86.8)
D (S = ≤16 μg/ml, ≥23 mm; MS = 32–64 μg/ml, 19–22 mm; R = ≥128 μg/ml, ≥18 mm)	Unselected (382)	0	0	40 (10.5)	40 (10.5)
	High-level-β-lactamase producers (53)	0	0	17 (32.1)	17 (32.1)

[a] See footnote *a* to Table 3.
[b] See footnote *b* to Table 3.

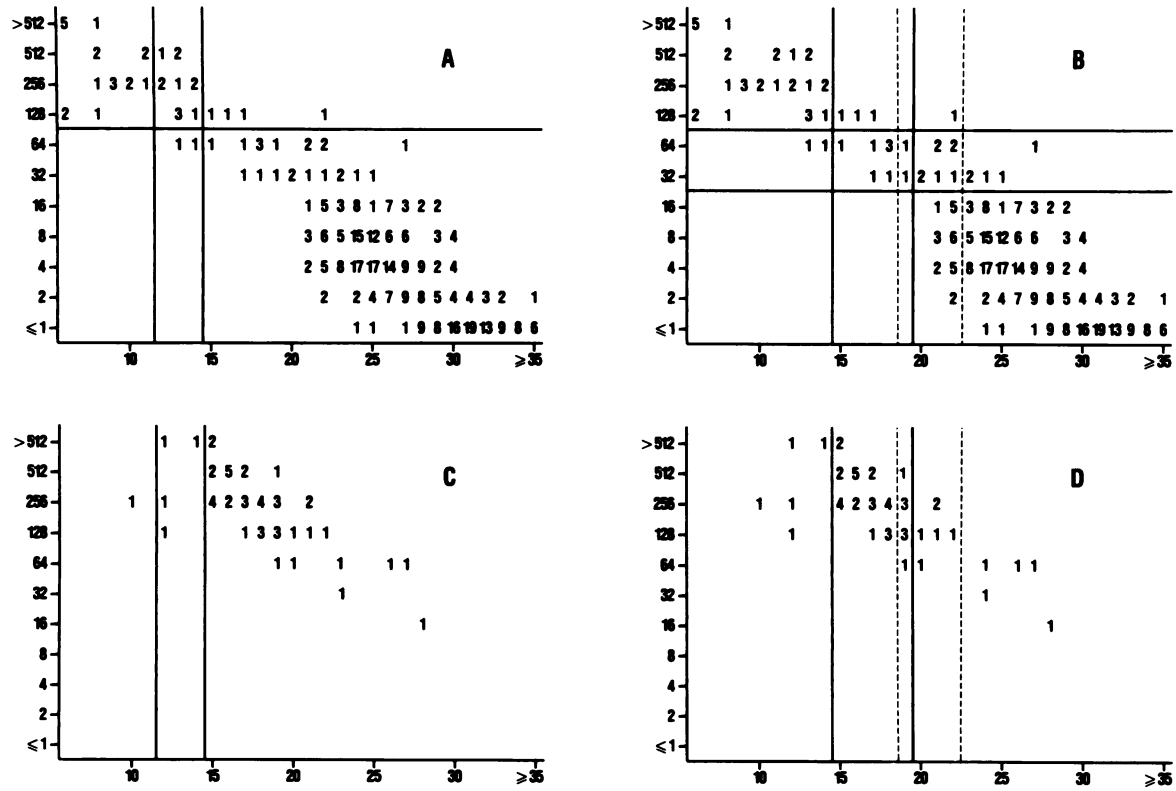

FIG. 3. T/C scattergrams for members of the *Enterobacteriaceae*. (A and B) All 382 strains of *Enterobacteriaceae* from current study with former (A) and proposed (B) NCCLS breakpoints. Zone diameter breakpoints (broken lines) in panels B and D are those proposed by Sanders et al. (10). (C and D) Strains of *Enterobacteriaceae* (n = 53) selected for high-level β-lactamase production with the same breakpoints as in panels A and B, respectively. x Axis, Zone diameters (millimeters); y axis, MICs (micrograms per milliliter).

spp. and NPGNB (Table 3). We recognize that the MICs of the drugs tend to be higher for *Pseudomonas* spp. than for NPGNB and that in the absence of susceptibility data for the specific infecting organisms it is appropriate to treat with higher doses when *Pseudomonas* spp. are present or suspected. However, we are unaware of any data to support the assumption that *Pseudomonas aeruginosa* is more susceptible than *Escherichia coli* when the MIC for each is the same; e.g., with a T/C MIC of 64 μg/ml, *P. aeruginosa* would be considered susceptible but *E. coli* would be moderately susceptible according to the latest NCCLS proposal (7). This double standard is potentially confusing to both the microbiologist and the clinician and, in our opinion, should be avoided unless and until there are clinical outcome data to support it.

TABLE 5. MICs and zone diameters of ticarcillin and T/C against 12 members of the *Enterobacteriaceae* with which the combination drug gave antagonistic results[a]

Organism and isolate no.	MIC (μg/ml)			Zone diam (mm)	
	Ticar	T/C-2	T/C-4	Ticar	T/C
Enterobacter aerogenes 86	4.0	16	32	25	24
E. aerogenes K75	16	64	64	22	14
E. agglomerans 365-82	4.0	16	32	28	29
E. cloacae 69	≤1.0	2.0	8.0	28	28
E. sakazakii 125	32	128	128	23	6
Morganella morganii					
179	≤1.0	4.0	8.0	28	28
180	≤1.0	8.0	8.0	27	30
184	2.0	8.0	4.0	27	27
286-82	2.0	16	8.0	28	28
F46	≤1.0	16	4.0	28	28
F47	2.0	8.0	8.0	28	27
Serratia marcescens 442-82	4.0	16	16	26	26

[a] Ticar, Ticarcillin with no CA; T/C-2, ticarcillin with 2 μg of CA per ml; T/C-4, ticarcillin with 4 μg of CA per ml.

Production of high levels of β-lactamase by certain members of the *Enterobacteriaceae* may prove to be a significant problem for T/C susceptibility testing. Both microdilution and disk diffusion methods correctly recognized all 53 strains producing high levels of β-lactamase to be resistant to ticarcillin. On the other hand, significant interpretive discrepancies occurred with those strains between the two methods of testing when T/C was tested, which confirms the findings of Sanders et al. (10). That the increased β-lactamase production is a major contributor to the discrepant results is supported by the fact that ticarcillin MICs for the strains producing high levels of β-lactamase were fourfold lower when ticarcillin was combined with 4 μg of CA per ml than when it was combined with the currently recommended 2 μg of CA per ml (Table 2). A similar effect of higher concentrations of CA on ticarcillin MICs against ticarcillin-resistant enteric bacilli was previously reported (9). By contrast, the T/C MICs for the unselected members of the *Enterobacteriaceae* were reduced less than one-quarter of a twofold dilution when 4 μg of CA was used in lieu of 2 μg/ml (Tables 1 and 2). It appears that increased β-lactamase production has a greater effect on the broth microdilution susceptibility test than on the disk diffusion test under the

currently accepted standard test conditions. Unfortunately, there are no published clinical data to indicate which of the two test systems will better predict the clinical response of infection by these organisms to treatment with T/C. Such clinical information is essential if reliable in vitro susceptibility test conditions and criteria are to be established.

Preliminary data from ongoing clinical outcome studies with T/C therapy collected by Beecham Laboratories suggest that there is a good correlation between susceptibility of members of the *Enterobacteriaceae* by disk diffusion tests (with criteria A, B, and C but not D) and favorable bacteriological and clinical response. MIC data are not available. This is based on 17 patients with bacteremia caused by ticarcillin-resistant members of the *Enterobacteriaceae* who were all treated successfully with T/C. All isolates were considered to be susceptible to T/C by criteria A then in use, but eight isolates had T/C inhibitory zone diameters of 15 to 22 mm, which would be interpreted as indicating resistance to moderate susceptibility by criteria D (personal communication, W. James Alexander).

With respect to *Pseudomonas* spp., the best interpretive correlation between T/C MIC and zone diameters occurs with criteria C (Table 3). On the other hand, preliminary data collected by Beecham Laboratories on nine patients with *P. aeruginosa* bacteremia treated with T/C tend to favor criteria A or B. Of the nine isolates, eight were susceptible and one was moderately susceptible by criteria A and B; six were susceptible, two were moderately susceptible, and one was resistant by criteria C. The only failure occurred in a patient with an isolate susceptible by all three criteria (personal communication, W. James Alexander). Since the moderate susceptibility category is not a contraindication to therapy but merely implies the need for higher doses, these preliminary data do not argue strongly against criteria C for *Pseudomonas* spp.

Until interpretive criteria can be established and justified by clinical data, it appears prudent to assume a conservative position, i.e., to assume that the dilution test results are the standards and inhibitory zone diameter criteria should be adjusted to best correlate with the dilution categories. Based strictly on the distribution of endpoints with the 517 unselected gram-negative bacteria, the NCCLS tentative criteria for *Pseudomonas* spp. (criteria B) and NPGNB (criteria C) provided good correlation of zone diameter and MIC susceptibility categories (Table 3). The total error rates for this group of organisms with these two criteria were 6.4 and 6.8%, respectively. With criteria D, the error rates were essentially doubled. On the other hand, criteria D yielded the lowest error rates when members of the *Enterobacteriaceae* producing high levels of β-lactamase were tested, albeit the rates were quite high at 32% (Table 3). More importantly, criteria D eliminated very major errors with this uniquely resistant group of organisms; very major error rates were 59 and 9%, respectively, for criteria B and C.

It should be noted, however, that the prevalence of members of the *Enterobacteriaceae* producing high levels of β-lactamase is probably quite low among clinical isolates, probably well below 1% of the *Enterobacteriaceae*. The 53 strains in the current study included 19 strains of *Salmonella* spp. collected at the Centers for Disease Control because of their resistance to piperacillin and related β-lactams and the 34 members of the *Enterobacteriaceae* producing high levels of β-lactamase reported by Sanders et al. (10) and provided by Beecham Laboratories. Those 34 strains were originally isolated from patients at M. D. Anderson Hospital, Houston, Tex., and specifically selected for their resistance to antipseudomonal penicillins (personal communication, G. Bodey). Although we are unaware of any prevalence studies in which these producers of high levels of β-lactamase were specifically sought, it is our strong impression that their occurrence is very low among current clinical isolates.

Assuming this to be true, it would be difficult to justify the use of criteria D at this time for T/C disk diffusion testing of members of the *Enterobacteriaceae* since the error rates for the usual isolates of *Enterobacteriaceae* are twice as high as those obtained with the other criteria. Since the error rates for criteria B and C are comparable, the selection of either should depend upon whether ≥256 or ≥128 μg/ml is the more appropriate MIC resistance breakpoint clinically. In the absence of clinical data to help clearly decide between these two MIC breakpoints, it could be argued that criteria C is preferable because it is much less likely to yield very major errors with the rare producers of high levels of β-lactamase.

Although CA is a potent β-lactamase inhibitor, it can also be a β-lactamase inducer (12). There is no evidence that this property has any role in the aforementioned problem with members of the *Enterobacteriaceae*. However, it may play a role in the few instances of antagonism observed (Table 5). If type I β-lactamases induced by CA resulted in production of high levels seen in the ticarcillin-resistant strains, this could explain the antagonism. Of the 12 instances detected by the microdilution test, only 2 were also detected by the standard disk diffusion test. However, if the disk diffusion plates were held an extra 24 h, many developed colonies within the original T/C zone of inhibition. This suggests the presence of a resistant subpopulation that is capable of growth once the antibiotic is inactivated.

In summary, we agree that the susceptibility criteria for ticarcillin, T/C, and related penicillins, as published by the NCCLS (5, 6), need revision and better correlation between the two major testing methods. On the other hand, an essential element for establishing appropriate standards (viz., clinical data) is lacking. This is particularly true of clinical response to T/C of infections with the rare members of the *Enterobacteriaceae* producing high levels of β-lactamase. Once the clinical responses are better characterized, one can standardize the in vitro susceptibility methods and criteria to better reflect clinical response. Until then, the tentative NCCLS criteria (7, 8) appear to be appropriate.

LITERATURE CITED

1. **Fuchs, P. C., A. L. Barry, C. Thornsberry, and R. N. Jones.** 1984. In vitro activity of ticarcillin plus clavulanic acid against 632 clinical isolates. Antimicrob. Agents Chemother. **25**:392–394.
2. **Fuchs, P. C., R. N. Jones, A. L. Barry, and C. Thornsberry.** 1984. Disk diffusion susceptibility testing of ticarcillin plus clavulanic acid. J. Clin. Microbiol. **19**:555–557.
3. **Henry, D., A. G. Skidmore, J. Ngui-Yen, A. Smith, and J. A. Smith.** 1985. In vitro activities of enoxacin, ticarcillin plus clavulanic acid, aztreonam, piperacillin, and imipenem and comparison with commonly used antimicrobial agents. Antimicrob. Agents Chemother. **28**:259–264.
4. **Holloway, W. S.** 1985. Treatment of infections in hospitalized patients with ticarcillin plus clavulanic acid: a comparative study. Am. J. Med. **79**(Suppl. 5B):168–171.
5. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobial disk susceptibility tests. M2-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A. National Committee for

Clinical Laboratory Standards, Villanova, Pa.
7. **National Committee for Clinical Laboratory Standards.** 1988. Performance standards for antimicrobial disk susceptibility tests, 4th ed. M2-T4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. M7-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Paisley, J. W., and J. A. Washington II.** 1978. Combined activity of clavulanic acid and ticarcillin against ticarcillin-resistant, gram-negative bacilli. Antimicrob. Agents Chemother. **14**:224–227.
10. **Sanders, C. C., J. P. Iaconis, G. P. Bodey, and G. Samonis.** 1988. 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. Antimicrob. Agents Chemother. **32**:1365–1369.
11. **Siebert, W. T., and P. E. Kopp.** 1985. Ticarcillin plus clavulanic acid versus moxalactam therapy of osteomyelitis, septis arthritis, and skin and soft tissue infections. Am. J. Med. **79**(Suppl. 5B):141–145.
12. **Stobberingh, E. E.** 1988. Induction of chromosomal β-lactamases by different concentrations of clavulanic acid in combination with ticarcillin. J. Antimicrob. Chemother. **21**:9–16.