

Disk Diffusion Susceptibility Testing of Ticarcillin Plus Clavulanic Acid

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Disk diffusion susceptibility testing of ticarcillin plus the beta-lactamase inhibitor clavulanic acid was performed on 489 clinical isolates, and the results were compared with reference broth microdilution susceptibilities. Four different disks containing 75 µg of ticarcillin plus 2.5, 5, 10, and 15 µg of clavulanic acid were evaluated. Based on test performance and clavulanic acid stability, the 75-10-µg disk is recommended. Interpretive criteria for ticarcillin as published by the National Committee for Clinical Laboratory Standards appear to be satisfactory for the combination drug, but because the number of ticarcillin-clavulanic acid-resistant isolates was small, this proposal must be considered only tentative.

The combination of ticarcillin with the potent beta-lactamase inhibitor clavulanic acid (CA) has been demonstrated to significantly enhance the wide antimicrobial spectrum of ticarcillin in vitro (4, 8). Ticarcillin-CA is a formulation of ticarcillin and CA currently being provided to clinical investigators by Beecham Laboratories, Bristol, Tenn., in recommended intravenous doses of 1 to 4 g of ticarcillin and 0.2 g of CA. It is appropriate, therefore, to determine in vitro susceptibility testing criteria for this combination. The susceptibility testing of a combination of a beta-lactam antibiotic and a beta-lactamase inhibitor by the disk diffusion method has been shown in the case of amoxicillin plus CA (Augmentin) to reliably correlate with dilution susceptibility testing (1). The purpose of the current study was to determine the most suitable disk antibiotic content and interpretive criteria for disk diffusion susceptibility of ticarcillin-CA. Since the current standardized disk antibiotic content for ticarcillin susceptibility testing is 75 µg and since ticarcillin is the primary antimicrobial component of ticarcillin-CA, it seemed prudent to maintain 75 µg of ticarcillin in the combination disk. Since the appropriate disk content of CA has not been determined, four disks containing 75 µg of ticarcillin and four different concentrations of CA were evaluated.

Ticarcillin and CA were supplied as reference powders by Beecham Laboratories. The bacteria tested were clinical isolates and included the following: *Citrobacter diversus*, 10 isolates; *Citrobacter freundii*, 10 isolates; *Enterobacter aerogenes*, 20 isolates; *Enterobacter agglomerans*, 10 isolates; *Enterobacter cloacae*, 20 isolates; *Escherichia coli*, 25 isolates; *Klebsiella pneumoniae*, 25 isolates; *Morganella morganii*, 9 isolates; *Proteus mirabilis*, 25 isolates; *Proteus vulgaris*, 10 isolates; *Providencia rettgeri*, 11 isolates; *Providencia stuartii*, 20 isolates; *Serratia marcescens*, 25 isolates; *Acinetobacter anitratus*, 15 isolates; *Pseudomonas aeruginosa*, 50 isolates; *Pseudomonas* species, 31 isolates; *Staphylococcus aureus*, 60 isolates, including 29 beta-lactamase-positive and 9 methicillin-resistant strains; *Staphylococcus*

epidermidis, 25 isolates, including 14 beta-lactamase producers; *Streptococcus faecalis*, 24 isolates; *Streptococcus pyogenes*, 22 isolates; *Streptococcus agalactiae*, 20 isolates; and *Streptococcus pneumoniae*, 22 isolates. MICs were determined by broth microdilution methods as previously described (3, 6). For ticarcillin-CA testing, the concentration of CA was kept constant at 2 µg/ml, and ticarcillin was serially diluted from 256 to 0.5 µg/ml, as recently recommended (2). The disk diffusion susceptibility test was performed by the method of the National Committee for Clinical Laboratory Standards (7). For ticarcillin-CA, the four disks tested contained 75 µg of ticarcillin and 2.5, 5, 10, and 15 µg of CA, respectively. These disks were prepared by Beecham Laboratories. The susceptibility results by the two methods were plotted as scattergrams and analyzed by the error rate-bounded method (5).

With the 75-15-µg disk, marked antagonism (producing the major false-resistant errors listed in Table 1) was apparent with 20 (9%) members of the *Enterobacteriaceae* family tested, including 9 of 10 *C. diversus*, 6 of 10 *Enterobacter agglomerans*, and 5 of 20 *Providencia stuartii*. Most of these were ticarcillin resistant but susceptible to the combination drug by dilution testing and to disks with smaller CA content. With the 75-2.5-, 75-5-, and 75-10-µg disks (Fig. 1), the zone diameters correlated equally well with the MICs. By error rate-bounded analysis, several breakpoints yielded acceptable results (Table 1). The lowest error rates occurred with the 75-10- and 75-5-µg disks, and the differences between these two disks were minimal. A slightly higher error rate occurred with the 75-2.5-µg disk. We recommend the 75-10-µg disk for ticarcillin-CA susceptibility testing because the stability of CA is poorer than that of ticarcillin and the loss of up to 50% of a 10-µg CA disk would have no effect on endpoint interpretation. In fact, a 75% loss in potency would affect disk performance only minimally. This margin of safety is also a factor to consider in light of the recent deregulation of the contents of susceptibility testing disks.

It should be noted that a weakness in this analysis is the relatively few ticarcillin-CA-resistant strains represented in the bacterial population studied. The strains studied were routine clinical isolates from five different geographical areas

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TABLE 1. Error rates for representative disk diffusion susceptibility testing breakpoints of ticarcillin-CA

Disks	Error types	Total error rate (%) ^a				
		11 (12-14) 15 ^b	11 (12-16) 17	11 (12-17) 18	12 (13-15) 16	13 (14-16) 17
75-2.5 ^c	Very major	0.8	0.8	0.8	0.8	0.8
	Major	1.4	1.4	1.4	2.4	2.0
	Minor	2.0	3.6	5.8	2.4	3.6
75-5	Very major	1.0	0.6	0.6	0.6	0.6
	Major	0.2	0.2	0.2	1.6	1.0
	Minor	2.6	4.2	5.6	2.2	2.8
75-10	Very major	1.2	0.4	0.4	0.4	0.8
	Major	0.8	0.8	0.8	1.8	1.6
	Minor	2.0	4.0	5.0	2.2	3.0
75-15	Very major	1.2	0.6	0.6	0.3	1.2
	Major	3.4	3.4	3.4	5.0	4.4
	Minor	4.0	6.2	6.6	4.2	4.4

^a N = 489.

^b Numbers indicate resistant, intermediate (in parentheses), and susceptible breakpoints in millimeters.

^c Disk content of ticarcillin-CA in micrograms.

and were representative of the resistance patterns normally encountered in these areas. Thus, only 13% of the isolates were ticarcillin resistant, and a mere 4% were ticarcillin-CA resistant. This paucity of resistant strains requires us to be only tentative in our recommendations for zone size interpretive criteria. Since the standard ticarcillin disk content recommended by the National Committee for Clinical Laboratory Standards (7) is 75 µg, as is the ticarcillin content of the combination drug disk, and since the antibacterial activity of the combination drug is due to the ticarcillin component, then logically the zone diameter interpretive criteria should be equivalent. Our data support this assumption,

since the total error rate was lowest with the recommended ticarcillin breakpoints of ≤11 mm (resistant), 12 to 14 mm (intermediate), and ≥15 mm (susceptible). However, the major and very major error rates bordered on the 1% acceptable range at these breakpoints. These could be reduced substantially by increasing the intermediate range (see Table 1) but at the expense of increasing the minor and total error rates. Until a greater number of resistant strains have been tested, we believe that the ticarcillin breakpoints can be used.

All of the 60 *Staphylococcus aureus* isolates tested were susceptible to ticarcillin-CA by MIC and disk diffusion

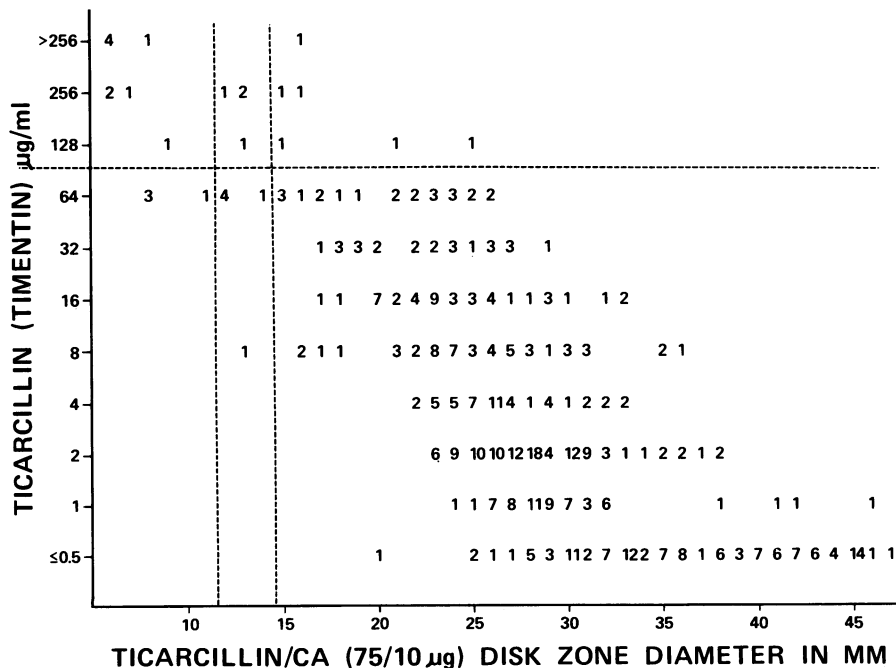


FIG. 1. Scattergram of ticarcillin-CA MIC-disk diffusion susceptibility correlates against 489 clinical isolates. MICs of ticarcillin with 2 µg of CA per ml versus zone diameter with 75-10-µg disks of ticarcillin-CA are shown.

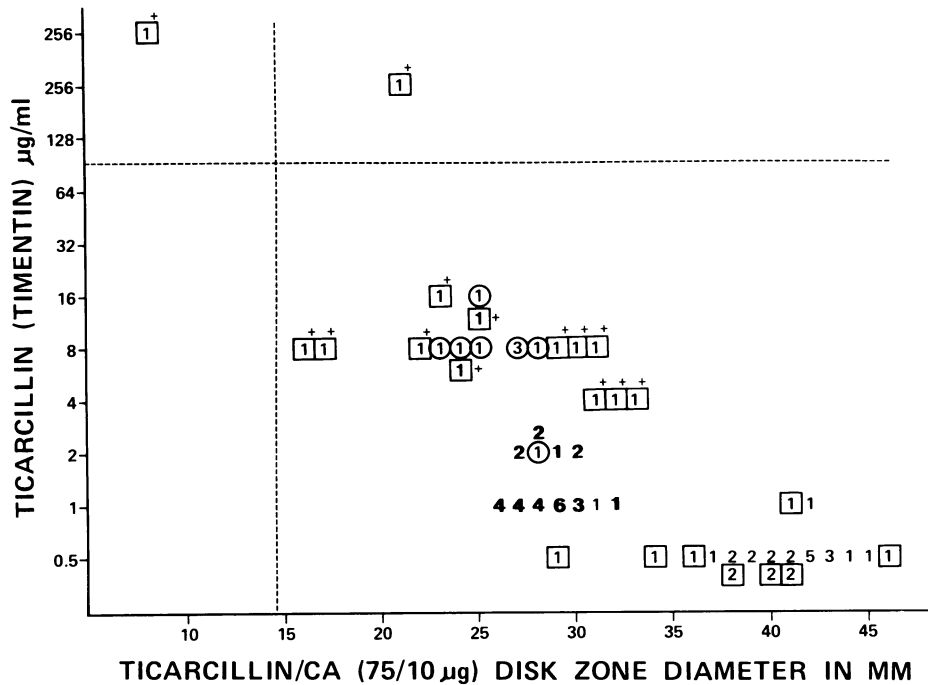


FIG. 2. Scattergram of ticarcillin-CA MIC-disk diffusion susceptibility correlates against 85 staphylococcal isolates. MICs of ticarcillin with 2 µg of CA per ml versus zone diameters with 75-10-µg disks of ticarcillin-CA. Symbols: □, beta-lactamase-negative *Staphylococcus epidermidis*; □+, beta-lactamase-positive *Staphylococcus epidermidis*; ○, methicillin-resistant *Staphylococcus aureus*. Unenclosed numbers, *Staphylococcus aureus*; heavy numbers, beta-lactamase-positive *Staphylococcus aureus*.

criteria (Fig. 2). Any of the susceptible breakpoints listed in Table 1 would encompass all these isolates, including the nine methicillin-resistant strains. In light of the accumulated experience of poor correlation between beta-lactam susceptibility testing of methicillin-resistant *Staphylococcus aureus* and clinical efficacy, it would be prudent to currently consider these strains to be resistant to ticarcillin-CA. Beta-lactamase-negative, coagulase-negative staphylococci were highly susceptible to ticarcillin-CA (MICs of 0.5 to 1.0 µg/ml), whereas beta-lactamase-producing strains were more erratic, with ticarcillin-CA MICs of 4 to >256 µg/ml. With a ≥15-mm susceptible breakpoint, all but one of these strains were correctly categorized. One strain with a ticarcillin-CA MIC of 256 had a false-susceptible zone diameter of 20 mm.

We conclude that the breakpoints recommended by the National Committee for Clinical Laboratory Standards for ticarcillin (7) can be satisfactorily used at this time for ticarcillin-CA disk diffusion susceptibility testing of clinical isolates of nonfastidious gram-negative bacilli, streptococci, and staphylococci (excluding methicillin-resistant strains). This conclusion is tentative and awaits further studies with greater numbers of ticarcillin-CA-resistant strains.

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