

## Use of a Predictor Panel To Evaluate Susceptibility Testing Methods for Ampicillin-Sulbactam

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A predictor panel of clinical isolates that produce a variety of types and amounts of  $\beta$ -lactamases was used to assess the accuracies of a variety of susceptibility tests for ampicillin-sulbactam. Combinations of ampicillin-sulbactam in ratios of 1:1 and 2:1 and with sulbactam held constant at concentrations of 4 and 8  $\mu\text{g/ml}$  were examined in dilution tests performed in agar and broth. In addition, disks containing 10/10, 20/10, 20/20, and 20/30  $\mu\text{g}$  of ampicillin-sulbactam were examined in diffusion tests. The results indicated that the MICs obtained in broth microdilution tests performed with each of the four combinations differed, on average, less than twofold. Of the disks tested, the 20/10- $\mu\text{g}$  ampicillin-sulbactam disk provided the best separation between susceptible and resistant strains when interpretive criteria for resistance was a zone size of  $\leq 16$  mm and that for susceptibility was a zone size of  $\geq 21$  mm. This disk also gave the highest overall agreement with MICs, regardless of the combination used in the broth microdilution test. Discrepancies between agar and broth microdilution MICs were greater than twofold, on average, and this necessitated recommendation of separate criteria for the two methods. Thus, a predictor panel was very useful in identifying the parameters of susceptibility tests that were most accurate in identifying strains that were susceptible and resistant to ampicillin-sulbactam.

Since the combination of ampicillin plus sulbactam has been available for clinical use in the United States, there have been three sets of criteria proposed for interpretation of results from disk diffusion tests performed with the 10/10- $\mu\text{g}$  ampicillin-sulbactam disk (1, 3, 11, 13). In addition, the interpretive criteria for disk diffusion tests established by the National Committee for Clinical Laboratory Standards (NCCLS) and those listed by the U.S. Food and Drug Administration in the package insert for ampicillin-sulbactam are not the same (11, 13, 14). The correlation between these various criteria and clinical outcome has not been clearly established, and questions concerning the accuracy of results of both disk diffusion and dilution tests have arisen (6, 7). Furthermore, earlier studies that attempted to set interpretive criteria for the ampicillin-sulbactam disk included both ampicillin-susceptible and -resistant strains. Since ampicillin-sulbactam would not be a relevant therapeutic choice for infections caused solely by ampicillin-susceptible bacteria, the inclusion of such strains in analyses to set interpretive criteria could bias the selection process. Therefore, the present study was undertaken in an attempt to identify reliable procedures for assessing the in vitro susceptibilities of clinical isolates of ampicillin-resistant, nonfastidious bacteria to ampicillin-sulbactam. Since a previous study showed the predictor panel approach to be an effective means of identifying reliable susceptibility tests for cefoperazone-sulbactam (4), a similar approach was used in the present study.

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### MATERIALS AND METHODS

**Bacterial strains.** In the present study, the predictor panel consisted of 150 strains of *Staphylococcus aureus* and mem-

bers of the family *Enterobacteriaceae*, many of which had well-characterized mechanisms of resistance (Table 1). The panel contained a majority of species that would be considered to be within the clinically useful antimicrobial spectrum of ampicillin-sulbactam. All strains were resistant to ampicillin alone as determined by agar dilution, broth dilution, or disk tests, and the results were interpreted by using the current NCCLS criteria (12, 13). The panel of strains included strains which should be susceptible (e.g., low-level producers of most plasmid-mediated  $\beta$ -lactamases) and re-

TABLE 1. Strains of the predictor panel

Organism	No. of strains	Ampicillin-sulbactam MIC range ( $\mu\text{g/ml}$ ) <sup>a</sup>
<i>Citrobacter freundii</i> <sup>b</sup>	9	64-128
<i>Enterobacter cloacae</i> <sup>b</sup>	13	64-128
<i>Escherichia coli</i> <sup>c</sup>	60	2->256
<i>Klebsiella oxytoca</i> <sup>d</sup>	3	128
<i>Klebsiella ozaenae</i>	2	4-8
<i>Klebsiella pneumoniae</i> <sup>e</sup>	23	2->256
<i>Morganella morganii</i> <sup>b</sup>	3	32-64
<i>Proteus vulgaris</i> <sup>b</sup>	3	32-64
<i>Providencia retgerii</i> <sup>b</sup>	2	16
<i>Providencia stuartii</i> <sup>b</sup>	2	32
<i>Serratia marcescens</i> <sup>b</sup>	10	32->256
<i>Staphylococcus aureus</i> <sup>f</sup>	20	2-32

<sup>a</sup> Performed in broth with a 2:1 ratio.

<sup>b</sup> Group 1  $\beta$ -lactamase, high- and low-level producers.

<sup>c</sup> Includes quality control strains ATCC 25922 and ATCC 35218. High- and low-level producers of TEM-1, TEM-2, TEM-3, TEM-4, TEM-5, TEM-7, TEM-9, TEM-10, TEM-12, TEM-101, SHV-1, SHV-2, SHV-3, SHV-4, SHV-5, OXA-1, OXA-2, OXA-3, OXA-4, OXA-5, OXA-6, OXA-7, PSE-1, PSE-2, PSE-3, PSE-4, HMS-1, OHIO-1, CAZ-2, SAR-1, and LXA-1  $\beta$ -lactamases.

<sup>d</sup> High- and low-level producers of K1  $\beta$ -lactamase (group 2b').

<sup>e</sup> High- and low-level producers of TEM-1, SHV-1, and SHV-2  $\beta$ -lactamases.

<sup>f</sup> Methicillin susceptible and methicillin resistant.

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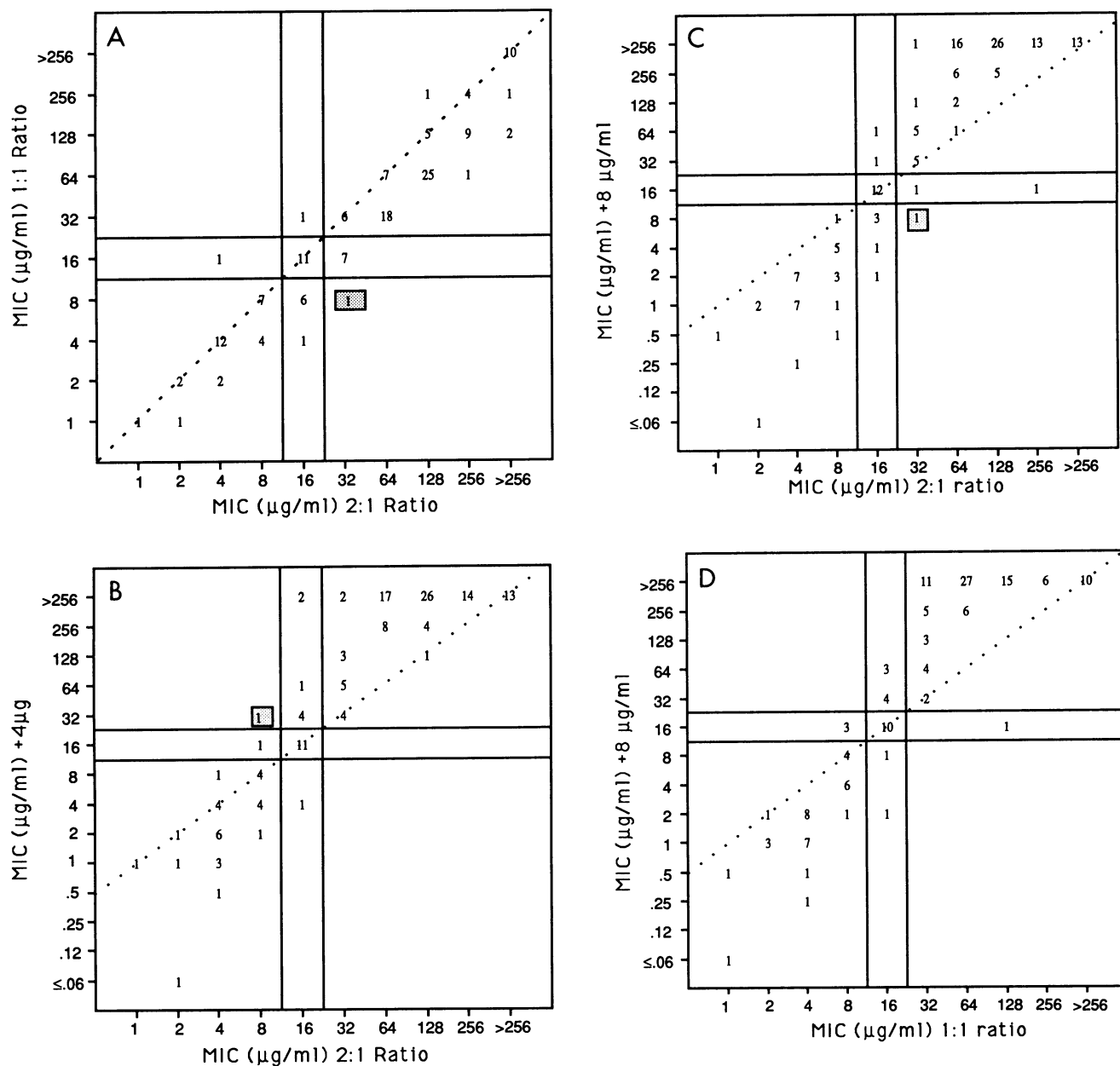


FIG. 1. Comparison of MICs obtained in broth dilution tests with various combinations of ampicillin-sulbactam. (A) 1:1 ratio versus 2:1 ratio; (B) +4  $\mu\text{g/ml}$  versus 2:1 ratio; (C) +8  $\mu\text{g/ml}$  versus 2:1 ratio; (D) +8  $\mu\text{g/ml}$  versus 1:1 ratio; (E) +4  $\mu\text{g/ml}$  versus +8  $\mu\text{g/ml}$ . —, MIC breakpoints; ···, line of equivalence; 1, number of very major discrepancies. Numbers in figures indicate numbers of strains.

sistant (e.g. certain hyperproducers of plasmid-mediated and Bush group 1  $\beta$ -lactamases) to ampicillin-sulbactam. The type and amount of  $\beta$ -lactamase was determined as described previously (4, 15–17).

**Susceptibility tests.** All susceptibility tests were performed by standard procedures (12, 13). Serial twofold dilutions of ampicillin-sulbactam (provided by Roerig Division, Pfizer Inc., New York, N. Y.) were tested in 2:1 and 1:1 fixed ratios and also with sulbactam at constant concentrations of 4  $\mu\text{g/ml}$  (+4  $\mu\text{g/ml}$ ) and 8  $\mu\text{g/ml}$  (+8  $\mu\text{g/ml}$ ). Agar dilution and broth microdilution tests were performed simultaneously. The MIC was defined as the lowest concentration that

prevented growth after 18 h of incubation at 35°C. MIC interpretive criteria for ampicillin-sulbactam were those of the NCCLS (12).

Disk diffusion tests were performed with commercially prepared disks containing 10  $\mu\text{g}$  of ampicillin plus 10  $\mu\text{g}$  of sulbactam (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and were interpreted according to current NCCLS guidelines (13). Investigational disks containing 20  $\mu\text{g}$  of ampicillin plus 20  $\mu\text{g}$  of sulbactam, 20  $\mu\text{g}$  of ampicillin plus 30  $\mu\text{g}$  of sulbactam (Difco Laboratories, Detroit, Mich.), and 20  $\mu\text{g}$  of ampicillin plus 10  $\mu\text{g}$  of sulbactam were also tested.

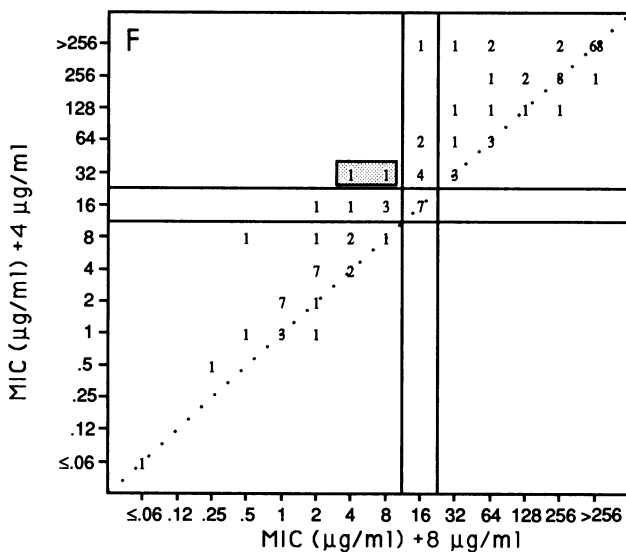
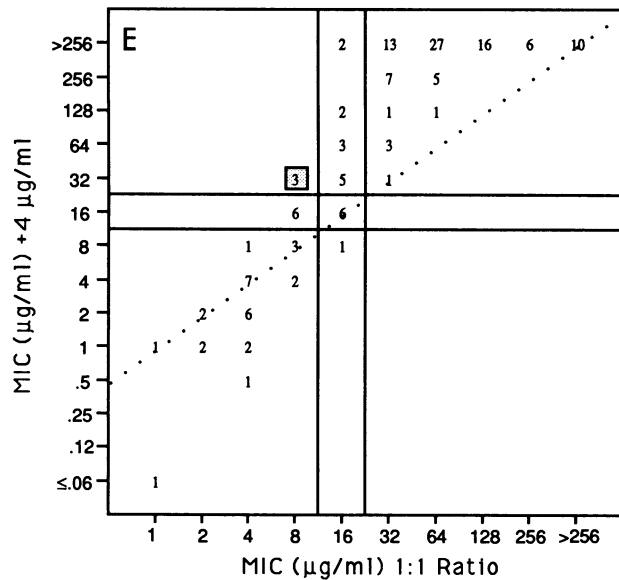


FIG. 1—Continued

The quality control strains *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 were included on each day of testing.

**Analysis.** Regression analysis was performed by the least-squares method. In addition, results obtained with various dilution methods were compared by Student's *t* test for paired data. All MICs that were off-scale (above or below the actual concentrations tested) were excluded from the calculations for both regression analysis and Student's *t* test. For MIC-versus-MIC comparisons, differences of twofold or less were considered to be insignificant. The MICs obtained in the dilution tests and the zone diameters obtained in the diffusion tests were compared by using the error rate-bounded analysis method described by Metzler and DeHaan (9) and modified by Bradford and Sanders (4). In the

TABLE 2. Comparison of MICs of ampicillin-sulbactam determined in agar and broth dilution tests

Combination	Mean difference between agar and broth MICs		<i>P</i> value <sup>a</sup>	No. of very major discrepancies	Strains (no.) involved in very major discrepancies
	Log 2	Anti-Log 2			
2:1 ratio	1.158	2.2	0.0001	5	<i>E. coli</i> (3) <i>K. pneumoniae</i> (2)
1:1 ratio	0.757	1.7	0.0001	7	<i>S. aureus</i> , methicillin resistant (1) <i>E. coli</i> (5) <i>C. freundii</i> (1)
+4 µg/ml	1.423	2.7	0.0001	10	<i>S. aureus</i> , methicillin resistant (2) <i>E. coli</i> (6) <i>K. pneumoniae</i> (1) <i>E. cloacae</i> (1)
+8 µg/ml	1.690	3.2	0.0001	9	<i>E. coli</i> (5) <i>K. pneumoniae</i> (1) <i>E. cloacae</i> (1) <i>C. freundii</i> (1) <i>P. stuartii</i> (1)

<sup>a</sup> From Student's *t* test.

comparisons, the MIC was always considered the reference test.

## RESULTS

**Comparisons between MICs determined in broth.** In initial analyses, MICs were determined with ampicillin and sulbactam in combinations containing a 1:1 ratio, a 2:1 ratio, and sulbactam at constant concentrations of 4 µg/ml (+4 µg/ml) and 8 µg/ml (+8 µg/ml). The MICs determined with one combination were compared with those determined with each of the other combinations by regression analysis (Fig. 1) and Student's *t* test. Although Student's *t* test indicated that MICs differed significantly (*P* < 0.05) when results obtained in tests performed with the 2:1 ratio and the 1:1 ratio, the 2:1 ratio and +8 µg/ml, the 2:1 ratio and +4 µg/ml, the 1:1 ratio and +8 µg/ml, the 1:1 ratio and +4 µg/ml, and +8 µg/ml and +4 µg/ml were compared, the geometric mean differences between MICs were not greater than two-fold (range, 1.0- to 1.7-fold) for any of the six pairs. The number

TABLE 3. Error rate-bounded analysis for the ampicillin-sulbactam 10/10-µg disk with MICs determined with a 2:1 ratio in broth microdilution tests

Error	Previous NCCLS criteria <sup>a</sup>			Current NCCLS criteria <sup>b</sup>		
	No.	P (%)	RC (%)	No.	P (%)	RC (%)
Very major	7	5	7	13	10	14
Major	0			0		
Minor	15	11		22	17	
Total	22	17		34	27	

<sup>a</sup> Previous NCCLS criteria (11) for resistant, moderately susceptible, and susceptible were ≤13, 14 to 16, and ≥17 mm, respectively. P, population rate, error rate for the entire population tested; RC, risk corrected rate, error rate for only strains at risk for this error. For calculations, refer to reference 4.

<sup>b</sup> Current NCCLS criteria (13) for resistant, moderately susceptible, and susceptible were ≤11, 12 to 14, and ≥15 mm, respectively.

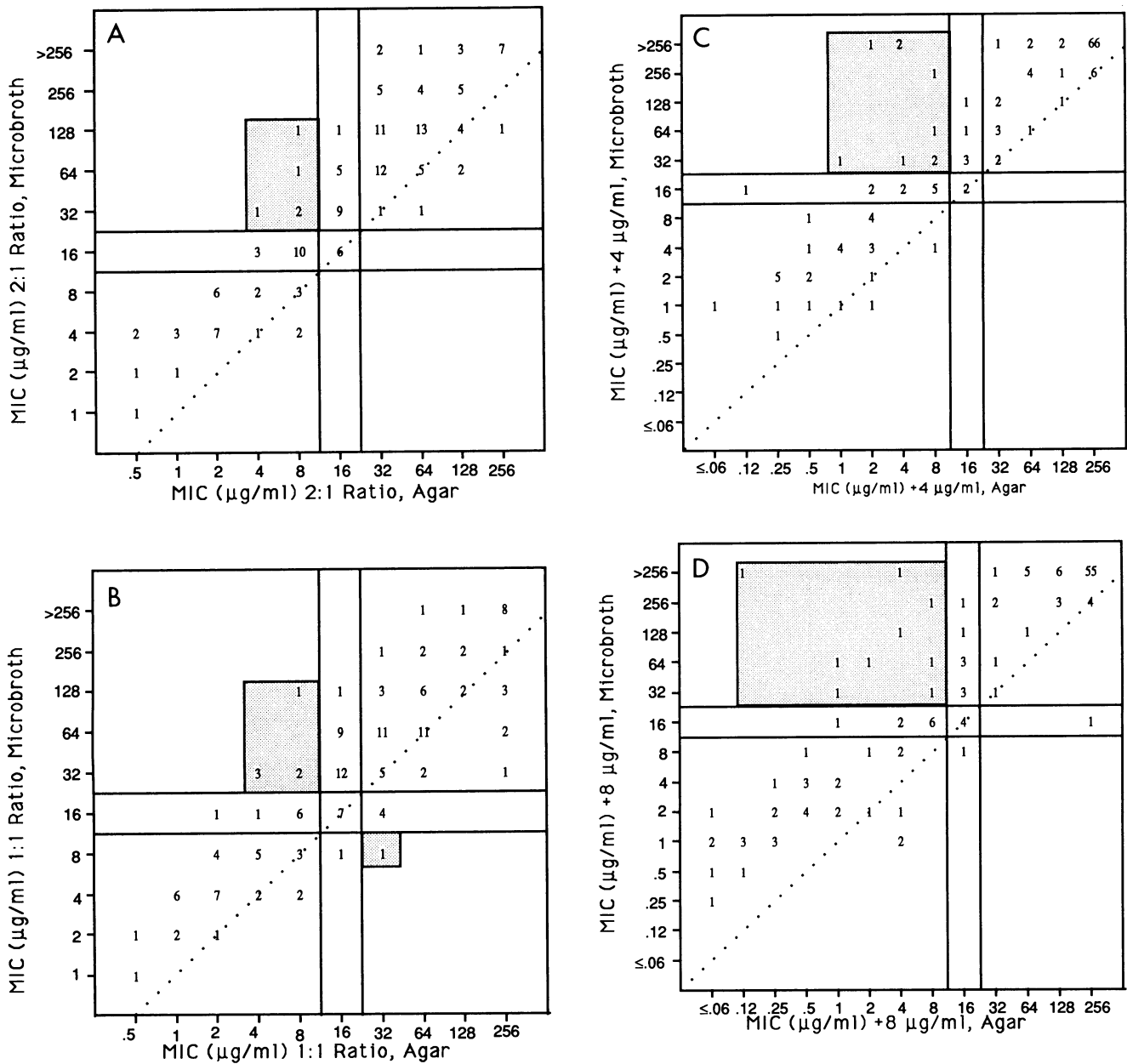


FIG. 2. Comparison of agar and broth MICs for various combinations of ampicillin-sulbactam. The MICs obtained by broth microdilution are plotted on the vertical axis, while the MICs obtained in agar are plotted on the horizontal axis. (A) 2:1 ratio; (B) 1:1 ratio; (C) +4 µg/ml; (D) +8 µg/ml. —, MIC breakpoints; ···, line of equivalence; [shaded], number of very major discrepancies. Values in the figure are numbers of strains.

of very major discrepancies (in which the MIC in one test indicated resistance while that in the other test indicated susceptibility) varied from 0 to 3 (Fig. 1).

There was a tendency for most strains to appear more susceptible in tests performed with the 1:1 ratio than in tests performed with the 2:1 ratio (Fig. 1A). For strains inhibited by ampicillin-sulbactam combinations containing ampicillin at 8 µg/ml or less, MICs were lower when tests were performed with +4 µg/ml than when tests were performed with the 2:1 ratio (Fig. 1B). However, at ampicillin concentrations of greater than 8 µg/ml, MICs were greater in tests performed with +4 µg/ml. Similarly, when the MICs deter-

mined in tests with the 2:1 ratio were compared with those determined in tests with +8 µg/ml (Fig. 1C), many strains appeared more susceptible with the +8 µg/ml combination when ampicillin concentrations were 16 µg/ml or less. However, strains appeared more resistant to the +8 µg/ml combination at higher concentrations of ampicillin. Both of these trends were probably due to the presence of more sulbactam in the +8- or +4-µg/ml combinations in comparison with that in the 2:1 ratio at the lower ampicillin concentrations and less sulbactam in the +8- or +4-µg/ml combinations at the higher ampicillin concentrations. Similar sulbactam-dependent trends were seen in comparisons of

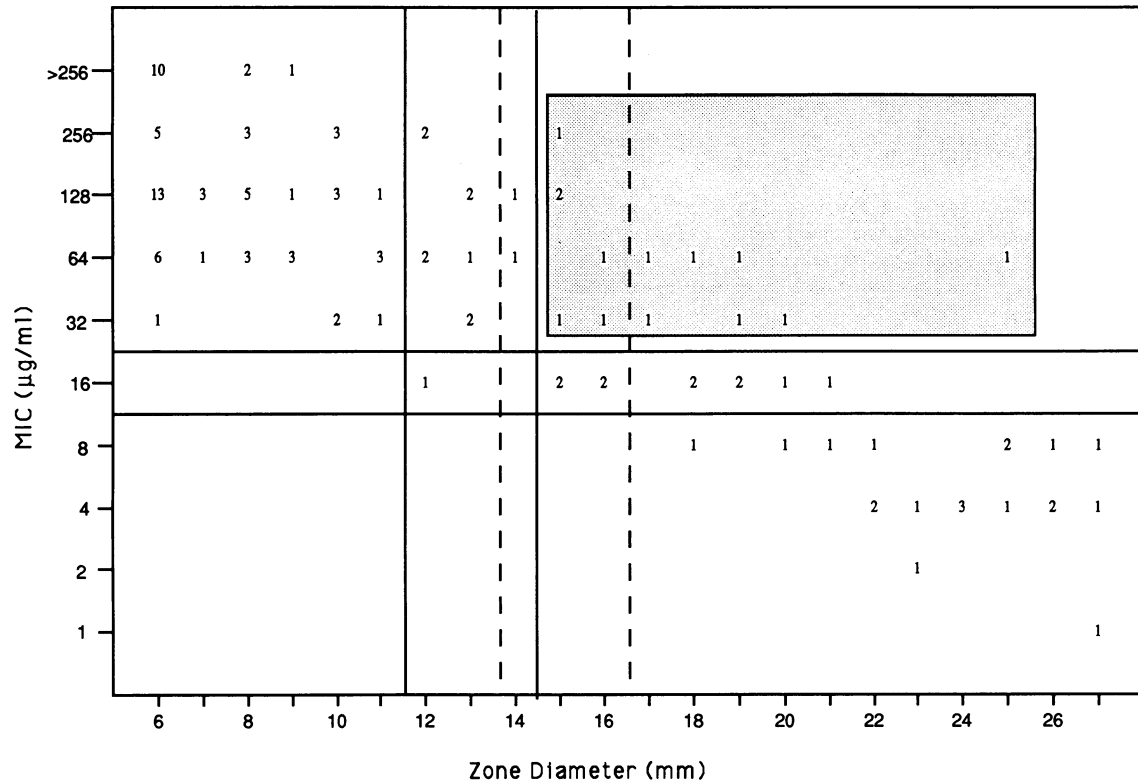


FIG. 3. Error rate-bounded analysis for 10/10-µg ampicillin-sulbactam disk. MICs obtained by broth microdilution at a 2:1 ratio are plotted on the vertical axis, while zone diameters are plotted on the horizontal axis. —, Current NCCLS MIC breakpoints (12) and zone diameter (13) interpretive criteria. ···, previous NCCLS zone diameter interpretive criteria (11); [shaded area], number of very major errors. Values in the figure are numbers of strains.

MICs obtained in tests performed with the +8-µg/ml combination and the 1:1 ratio (Fig. 1D) and with the +4-µg/ml combination and the 1:1 ratio (Fig. 1E). MICs determined in tests performed with the +4-µg/ml combination were generally higher than those obtained with the +8-µg/ml combination (Fig. 1F).

**Comparisons between MICs obtained in agar and broth.** Because previous studies showed significant discrepancies between MICs obtained in broth and those obtained in agar tests with cefoperazone-sulbactam (4), analyses were performed in the present study to determine whether similar discrepancies existed for ampicillin-sulbactam. From Stu-

TABLE 4. Error rate-bounded analysis of data for MICs and zones obtained with investigative disks<sup>a</sup>

Disk	2:1 Ratio			1:1 Ratio			+4 µg/ml			+8 µg/ml		
	No.	P (%)	RC (%)	No.	P (%)	RC (%)	No.	P (%)	RC (%)	No.	P (%)	RC (%)
20/10 µg (≤16, 17–20, ≥21) <sup>b</sup>												
Very major	1	0.8	1.1	1	0.8	1.1	2	1.6	2.0	1	0.8	1.1
Major	0			1	0.8	4.2	0			1	0.8	4.2
Minor	10	8.0		17	13.6		11	8.8		14	11.2	
Total	11	8.8		19	15.2		13	10.4		16	12.8	
20/20 µg (≤18, 19–21, ≥22)												
Very major	1	0.8	1.1	3	2.4	3.0	2	1.6	2.0	1	0.8	1.1
Major	0			0			0			0		
Minor	14	11.2		18	14.4		17	13.6		16	12.8	
Total	15	12.0		21	16.8		19	15.2		17	13.6	
20/30 µg (≤20, 21–25, ≥26)												
Very major	1	0.8	1.1	1	0.8	1.1	2	1.6	2.0	1	0.8	1.1
Major	0			0			0			0		
Minor	16	12.8		25	20.0		17	13.6		22	17.6	
Total	17	13.6		26	20.8		19	15.2		23	18.4	

<sup>a</sup> MICs were determined in microbroth dilution tests. For definitions of P and RC, see footnote a of Table 3.

<sup>b</sup> Values in parentheses are interpretive criteria for resistant, moderately susceptible, and susceptible, respectively, and are given in millimeters.

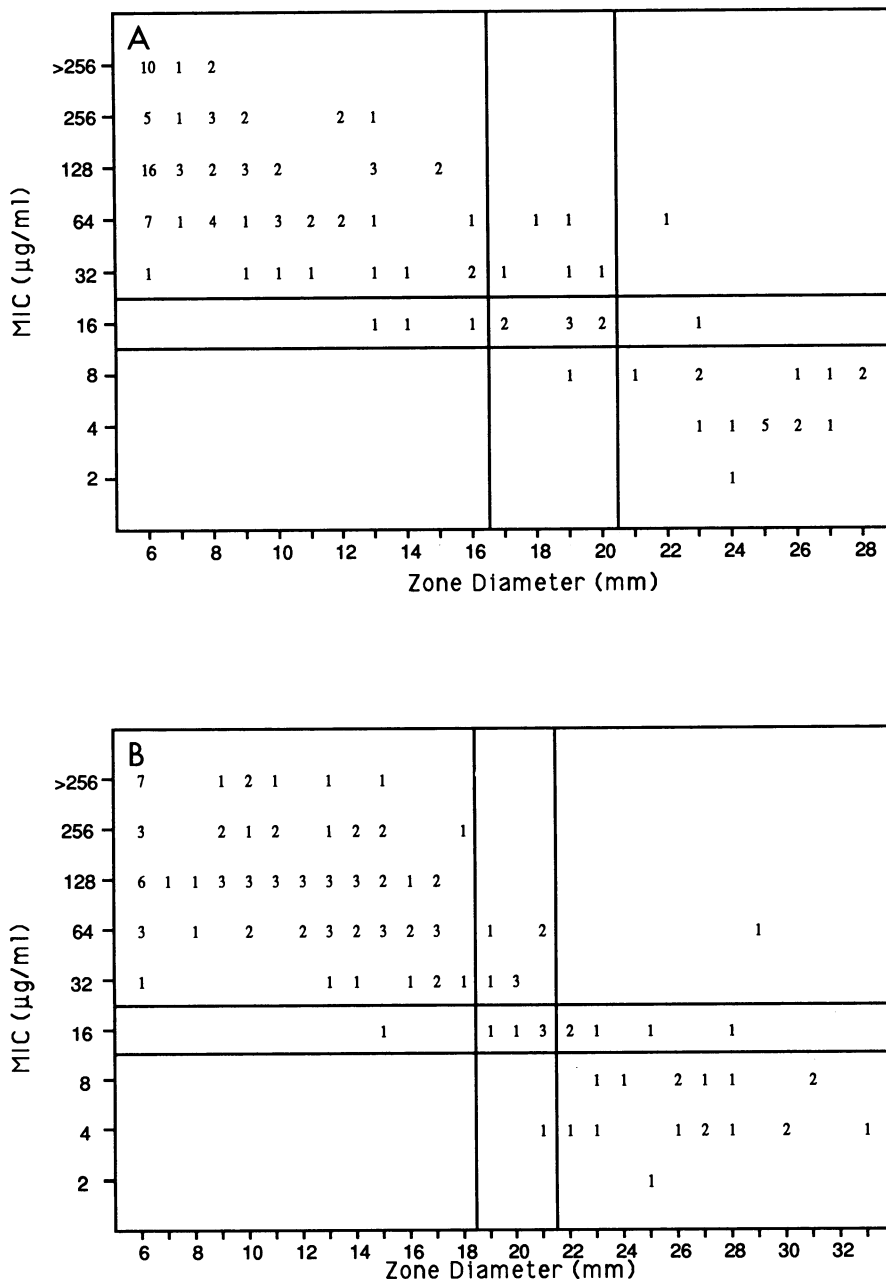


FIG. 4. Error rate-bounded analysis for ampicillin-sulbactam investigational disks. MICs obtained by broth microdilution in a 2:1 ratio are plotted on the vertical axis, while zone diameters are plotted on the horizontal axis. (A) 20/10-µg disk; zone sizes of ≤16 mm indicated resistance, 17 to 20 mm indicated moderate susceptibility, and ≥21 mm indicated susceptibility; (B) 20/20-µg disk; zone sizes of ≤18 mm indicated resistance, 19 to 21 mm indicated moderate susceptibility, and ≥22 mm indicated susceptibility; (C) 20/30-µg disk; zone sizes of ≤20 mm indicated resistance, 21 to 23 mm indicated moderate susceptibility, and ≥26 mm indicated susceptibility. —, MIC breakpoints and zone diameter interpretive criteria. Values in the figure are number of strains.

dent's *t* test, there was a significant difference between results obtained in agar and broth tests for each of the ampicillin-sulbactam combinations tested (Table 2). The geometric mean difference between MICs was greater than twofold for each of the combinations except the 1:1 ratio. The number of very major discrepancies varied from 5 to 10, and all but one of these involved an agar MIC that indicated susceptibility while the broth MIC indicated resistance (Fig. 2). The one strain that appeared to be resistant in agar tests

but susceptible in broth tests was a methicillin-resistant *S. aureus* (Fig. 2B).

**Comparisons between MICs and disk diffusion tests.** In initial analyses, data obtained with the 10/10-µg ampicillin-sulbactam disk currently in use were compared with MICs obtained with the 2:1 ratio currently recommended for broth dilution tests (8, 12). Results for staphylococci were analyzed separately. Methicillin-susceptible and -resistant staphylococci were not separated by broth dilution or disk

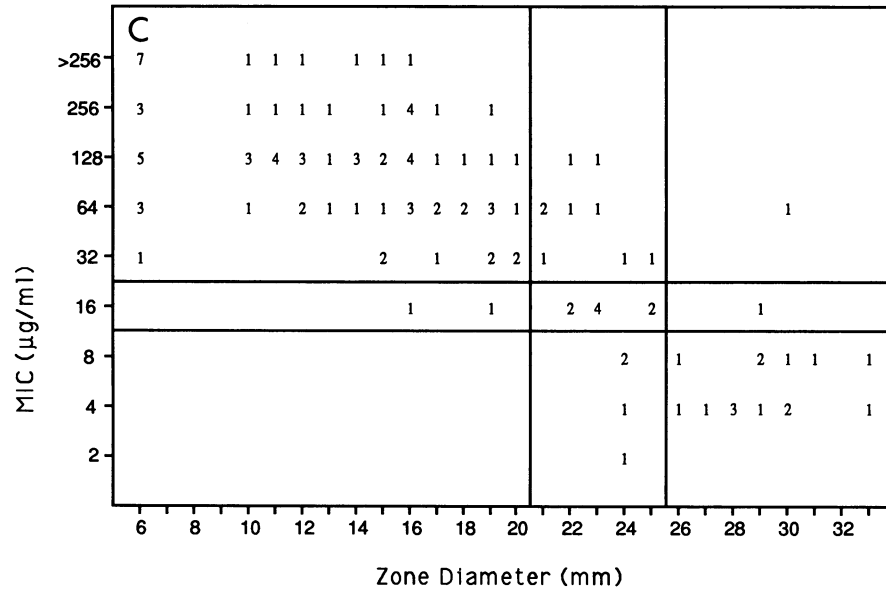


FIG. 4—Continued

diffusion tests. Several strains of methicillin-resistant staphylococci appeared to be susceptible by both methods (data not shown). Thus, the data obtained with the staphylococci were excluded from subsequent analyses.

For the remainder of the strains, zone diameters obtained with the 10/10-µg ampicillin-sulbactam disk were plotted against the MICs obtained from broth microdilution tests performed with a 2:1 ratio of ampicillin to sulbactam (Fig. 3). Error rate-bounded analyses were performed by using two sets of criteria: the current NCCLS criteria (13) and the criteria listed in a previous NCCLS document (11). Results are summarized in Table 3. With the previous NCCLS criteria, the seven very major errors consisted of three strains of *E. coli* that produced low levels of TEM-1 β-lactamase, one strain of *E. coli* that produced TEM-5 β-lactamase, one strain of *Klebsiella pneumoniae* that produced SHV-1 and TEM-1 β-lactamases, and one strain each of *Enterobacter cloacae* and *Citrobacter freundii*, each of which possessed the wild-type inducible group 1 β-lactamase. The numbers of both very major errors and minor errors increased when results were analyzed by using the criteria recently adopted by the NCCLS (13). The additional errors raised the risk-corrected error rate for very major errors to 14% and the total population error rate to 27%. The additional very major errors included one *E. coli* strain that produced low levels of TEM-1 β-lactamase, one strain each of *E. coli* and *K. pneumoniae*, each of which produced low levels of SHV-1 β-lactamase, one *K. pneumoniae* that produced TEM-1 and SHV-1 β-lactamases, and two strains of *Providencia stuartii* that possessed the wild-type inducible group 1 β-lactamase.

From an examination of Fig. 3, it is clear that for each MIC there was a very broad range of zone sizes obtained with the 10/10-µg disk. This broad range made it difficult to select interpretive criteria for diffusion tests that would adequately separate susceptible from resistant strains. On the basis of the data presented in Fig. 3, the best zone size for the susceptibility breakpoint would be ≥21 mm and that for the resistance breakpoint would be ≤16 mm. These

criteria would eliminate all but one very major error and would produce only two major errors.

The results obtained with three investigational disks of 20/10, 20/20, and 20/30 µg of ampicillin-sulbactam were then examined to determine whether the correlation of zone diameter with MIC obtained in broth microdilution tests could be improved by changing masses of drug and inhibitor in the disk. Zone diameter breakpoints were subjectively set to, first, minimize very major errors and, second, to minimize all other errors. The results obtained by error rate-bounded analyses are summarized in Table 4.

Regardless of the combination used to obtain MICs, the fewest very major errors and best overall agreement with results were obtained with the 20/10-µg disk. The largest number of errors were obtained in comparisons with the 20/30-µg disk. The correlation between MICs obtained with the 2:1 ratio and the investigative disks is shown in Fig. 4. One strain of *E. coli* that expressed SHV-5 β-lactamase produced a very major error with all three disks.

**Quality control.** *E. coli* ATCC 25922 and ATCC 35218 have been recommended by the NCCLS (12, 13) for use as quality control strains for susceptibility testing in the clinical laboratory. In the present study, zone diameters were within the limits for both strains with the 10-µg ampicillin disk and the 10/10-µg ampicillin-sulbactam disk in ≥90% of replications (data not shown). In addition, the MIC for *E. coli* ATCC 25922 was within the MIC limits of both the drug and drug-inhibitor combinations. However, the MIC for the β-lactamase-positive strain *E. coli* ATCC 35218 was out of the control range for 67% of the replications when MICs were determined by the broth microdilution method. In addition, for *E. coli* ATCC 35218 there were very major discrepancies between the MICs determined in agar and broth with three of the MIC combinations for ampicillin-sulbactam. This would result in a very major error for disk tests on the days when the MIC was 32 µg/ml. For this reason, a strain of *E. coli* designated BAS was identified as an alternative to *E. coli* ATCC 35218 for use as a quality control strain in susceptibility tests for ampicillin-sulbactam.

Results for susceptibility tests with this strain were as follows. Disk diffusion results for the 10- $\mu$ g ampicillin disk and the 10/10-, 20/10-, 20/20- and 20/30- $\mu$ g ampicillin-sulbactam disks were  $6 \pm 0$ ,  $26 \pm 2.7$ ,  $27 \pm 1.4$ ,  $29 \pm 1.8$ , and  $30 \pm 2.2$  mm (mean  $\pm$  standard deviation; 15 replications), respectively. MICs with the 1:1 and 2:1 ratios were both  $2 \pm 0.5$   $\mu$ g/ml (geometric mean  $\pm$  standard deviation; 45 replications). For strain BAS, MICs obtained in broth were only twofold greater than those obtained in agar (data not shown). Strain BAS was selected because it (i) consistently produced a zone diameter of 6 mm when tested with the 10- $\mu$ g ampicillin disk, (ii) produced zone diameters with all the ampicillin-sulbactam disks tested that were in the susceptible range and that were easily measured, and (iii) produced very reproducible results by both disk and dilution tests for ampicillin-sulbactam.

### DISCUSSION

In the present study, a predictor panel was used to assess both dilution and disk diffusion tests for ampicillin-sulbactam. Several combinations of both drug and inhibitor were also examined in an attempt to identify a combination that would accurately discriminate between susceptible and resistant strains.

Results indicated that the MICs obtained in broth microdilution tests with the different ampicillin-sulbactam combinations did not vary more than twofold, on average. Thus, theoretically, any of the four combinations could be used for dilution tests. Jones and Barry (8) have advocated the use of a 2:1 ratio, while Jenkins (7) has suggested that a 1:1 ratio be used. This latter recommendation was based on reports that in certain body fluids, ampicillin-sulbactam may reach levels that approximate a 1:1 ratio (7). However, levels in serum maintain a 2:1 ratio throughout the dosing interval, and the ratios found in many other body fluids, like alveolar lining fluid, approximate a 2:1 ratio (5, 10, 18). In view of the observation in the present study that the MICs obtained with the 1:1 ratio tend to be lower than those obtained with the 2:1 ratio and the fact that a 2:1 ratio is more representative of the levels achieved overall, it would seem prudent to use a 2:1 ratio in dilution susceptibility tests.

The predictor panel identified discrepancies between the MICs obtained in agar and the MICs obtained in broth for all combinations of ampicillin-sulbactam tested. Because very major discrepancies were found with strains which should be considered clinically resistant, the results from the broth microdilution method were considered to be correct. If dilution tests for ampicillin-sulbactam must be performed in agar, the MIC breakpoints may need to be changed to  $\leq 4$   $\mu$ g/ml for susceptibility, 8  $\mu$ g/ml for moderate susceptibility, and  $\geq 16$   $\mu$ g/ml for resistance to promote better interpretive agreement between the MICs obtained in agar and broth.

Of the disks tested, the 20/10- $\mu$ g ampicillin-sulbactam disk gave the best agreement for MICs obtained in broth microdilution tests with gram-negative organisms. For staphylococci, none of the disks examined in the present study accurately identified susceptible and resistant strains when the interpretive criteria developed with gram-negative bacteria were used. This finding agrees with those of Barry and Jones (2). Therefore, it seems reasonable that staphylococci continue to be assessed by procedures that will accurately determine susceptibility to penicillin and oxacillin. Strains resistant to oxacillin should be presumed to be resistant to ampicillin-sulbactam without direct testing.

The  $\beta$ -lactamase-positive strain of *E. coli* ATCC 35218

which is currently used for quality control in susceptibility tests with  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations does not provide an adequate assessment of the validity of susceptibility tests for ampicillin-sulbactam because it appears to be resistant in broth microdilution tests but susceptible in disk diffusion tests. *E. coli* BAS would provide a reasonable alternative for use as a quality control organism because it is resistant to ampicillin alone but appears to be susceptible to ampicillin-sulbactam, regardless of the method in which the test is performed.

All in all, the use of a predictor panel of ampicillin-resistant bacteria helped to identify those parameters of diverse susceptibility tests most likely to correctly identify isolates that are susceptible and resistant to ampicillin-sulbactam. On the basis of the results of the present study, we recommend that a 2:1 ratio be used in dilution tests and that a 20/10- $\mu$ g ampicillin-sulbactam disk be used in diffusion tests. If an agar dilution method is to be used, lower breakpoints for susceptibility should be used. Finally, if the 10/10- $\mu$ g disk continues to be used, new interpretive criteria should be adopted to eliminate the unacceptably high very major error rate that exists with the current criteria.

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