

Use of a Predictor Panel for Development of a New Disk for Diffusion Tests with Cefoperazone-Sulbactam

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The proper disk mass for diffusion susceptibility tests with cefoperazone-sulbactam was determined by using a predictor panel of clinical isolates that included staphylococci and gram-negative bacteria intrinsically susceptible, intrinsically resistant, and of various susceptibilities because of the production of different types and amounts of β -lactamase. A primary panel of 24 isolates was used to screen various disk masses of cefoperazone and sulbactam in disk diffusion susceptibility tests. Regression analyses were performed for each combination by comparing MICs to zone diameters. Analysis of each component demonstrated that decreasing the disk mass of cefoperazone shifted the regression line to the left while decreasing the disk mass of sulbactam diminished the slope of the line. Ten candidate disks that adequately separated susceptible and resistant strains among the primary panel were identified, and these 10 disks, along with the previously proposed 75/30- μ g disk, were then tested against an expanded panel of 265 isolates. Results indicated that a 30/20- μ g cefoperazone-sulbactam disk provided the best separation between susceptible and resistant strains when interpretive criteria of ≤ 15 mm for resistance, 16 to 19 mm for moderate susceptibility, and ≥ 20 mm for susceptibility were used. They also identified discrepancies between agar and broth microdilution MICs of sufficient size to warrant separate interpretive criteria for the two methods. Overall, the use of a predictor panel to develop interpretive criteria for susceptibility tests appeared to be a very useful approach, especially when antibiotics designed to be used against drug-resistant organisms are involved.

In the past, interpretive criteria for disk diffusion susceptibility tests have been determined from data generated with large collections of clinical isolates (2, 5-8, 10, 11, 16). Although these collections usually contain most of the major species within the anticipated clinically useful spectrum of the antibiotic, the isolates within each species are usually chosen at random. Therefore, resistant isolates among the species may be underrepresented. This can become a major problem if the antimicrobial agent under study has been developed for its activity against strains resistant to older agents.

The negative impact of having too few resistant strains in the collection used to design disk diffusion tests can be readily seen in previous studies of β -lactamase inhibitor- β -lactam drug combinations. For example, the criteria that were originally used for ticarcillin-potassium clavulanate were found to grossly overpredict susceptibility among members of the family *Enterobacteriaceae* (20). This was due, in part, to the lack of an in-depth assessment of resistant strains during the development of the 75/10- μ g ticarcillin-potassium clavulanate disk (9). Only 17 isolates of ticarcillin-potassium clavulanate-resistant gram-negative bacteria were included in the study that ultimately led to the interpretive criteria originally approved by the National Committee for Clinical Laboratory Standards (15).

There have been additional problems with the design of disks for testing β -lactamase inhibitor- β -lactam drug combinations. In previous studies, the inhibitor has been added to the drug, the latter at the same disk mass as when tested alone, and the interpretive criteria have remained identical to those used for the drug alone (12). The apparent rationale behind this approach is that the inhibitor will neutralize the effect of the β -lactamase, leaving the drug to work alone.

However, this approach fails to recognize differences in diffusion of the two compounds through the medium as well as rates of entry into the bacterial cell. It also fails to recognize that while inhibitors often restore susceptibility to the drug, they may not restore susceptibility to the level of that of a β -lactamase-negative strain.

With these problems as background, a study was undertaken to design a disk for diffusion testing of cefoperazone-sulbactam by utilizing an approach different from those employed previously. In the initial phase, the disk masses of both components of the combination were varied to determine the impact upon zone diameters obtained with a small test panel of organisms spanning the range of possible susceptibilities to cefoperazone-sulbactam. From these initial studies, 10 candidate disks were chosen for analysis against a larger predictor panel of organisms chosen to represent (i) each of the diverse genera for which the new combination may be a potentially useful therapeutic agent; (ii) a complete range of strains, from the most susceptible to the highly resistant; (iii) strains with both natural and acquired resistances to the drug; and (iv) strains with diverse mechanisms of resistance (19). From the data generated with this predictor panel, a disk capable of accurately discriminating between susceptible and resistant strains could be chosen.

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MATERIALS AND METHODS

Bacterial strains. In this study, the predictor panel consisted of two parts, the primary panel and the expanded panel. The primary panel consisted of 24 isolates of staphylococci and gram-negative bacteria (Table 1). These included strains which produced high and low levels of chro-

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TABLE 1. Strains of the primary predictor panel

Organism (no. of strains)	MIC range ^a (μg/ml)	Characteristics
<i>Staphylococcus aureus</i> (5)	64->256	4 Methicillin resistant, penicillinase positive
	2	1 Methicillin susceptible, penicillinase positive
<i>Staphylococcus</i> sp., coagulase negative (1)	1	Methicillin resistant, penicillinase negative
<i>Escherichia coli</i> (6)	8-64	High- and low-level producers of TEM-1 β-lactamase (255-1,623 U)
<i>Escherichia coli</i> (1)	8	SHV-1 β-lactamase (59 U)
<i>Klebsiella pneumoniae</i> (2)	8,256	High- and low-level producers of SHV-1 β-lactamase (119-2721 U)
<i>Klebsiella oxytoca</i> (2)	8,128	High- and low-level producers of class IV β-lactamase (2-17,237 U)
<i>Enterobacter cloacae</i> (2)	0.25-32	Class I β-lactamase wild-type and derepressed mutant pairs ^b
<i>Pseudomonas aeruginosa</i> (4)	4-64	Class I β-lactamase wild-type and partially or fully derepressed mutants ^b
<i>Acinetobacter</i> sp. (1)	1	Susceptible to sulbactam alone

^a MICs of cefoperazone-sulbactam were determined in broth with a 2:1 ratio of cefoperazone to sulbactam.

^b Wild type expresses enzyme inducibly, while mutants express enzyme semiconstitutively or constitutively.

mosomally and plasmid-mediated β-lactamases and had a broad spectrum of susceptibilities to cefoperazone-sulbactam (MIC range, ≤0.06 to >256 μg/ml). The expanded panel consisted of 265 strains from diverse genera, many of which had well-characterized mechanisms of resistance (Table 2). This panel contained a majority of organisms that would be considered to be within the clinically useful antimicrobial spectrum for cefoperazone-sulbactam and included strains with a broad range of susceptibilities to cefoperazone-sulbactam. Of the gram-negative bacteria, 53% were resistant (MIC, ≥64 μg/ml), 9% were moderately susceptible (MIC,

32 μg/ml), and 38% were susceptible (MIC, ≤16 μg/ml) to cefoperazone alone by broth microdilution. These included strains which should be susceptible (i.e., low-level producers of most plasmid-mediated β-lactamases) and resistant (i.e., certain hyperproducers of plasmid-mediated and class I β-lactamases) to cefoperazone-sulbactam. The type and amount of β-lactamase were determined as previously described (20-22). High-level producers of TEM, SHV, and class IV enzymes were defined as those strains which hydrolyzed nitrocefin at a rate of more than 300 nmol/min/mg of cell-free sonic extract. Class I β-lactamases were characterized as either having a wild-type inducible phenotype or a stably or partially derepressed mutant phenotype, producing high levels of β-lactamase constitutively.

TABLE 2. Strains of the expanded panel

Organism (no. of strains)	Cefoperazone-sulbactam MIC range ^a (μg/ml)
<i>Acinetobacter</i> sp. (3)	0.25-16
<i>Aeromonas hydrophila</i> ^b (12)	0.5-32
<i>Flavobacterium</i> sp. (5)	8-128
<i>Escherichia coli</i> ^c (63)	0.125-64
<i>Klebsiella oxytoca</i> ^d (10)	0.25-256
<i>Klebsiella ozaenae</i> (2)	0.25
<i>Klebsiella pneumoniae</i> ^e (24)	0.25->256
<i>Enterobacter cloacae</i> ^b (15)	0.25-64
<i>Serratia marcescens</i> ^b (10)	1-256
<i>Hafnia alvei</i> ^b (1)	64
<i>Citrobacter freundii</i> ^b (12)	1-64
<i>Citrobacter diversus</i> ^b (2)	≤0.06, 32
<i>Providencia</i> sp. ^b (4)	2-32
<i>Morganella morganii</i> ^b (3)	2-4
<i>Proteus vulgaris</i> ^b (4)	2
<i>Staphylococcus aureus</i> ^f (20)	2->256
<i>Staphylococcus</i> spp., coagulase negative ^f (16)	1->256
<i>Pseudomonas aeruginosa</i> ^b (38)	4-256
<i>Pseudomonas fluorescens</i> ^b (1)	8
<i>Pseudomonas cepacia</i> ^b (1)	4
<i>Xanthomonas maltophilia</i> ^b (9)	4-128

^a MICs were determined in broth with a 2:1 ratio of cefoperazone to sulbactam.

^b Class I β-lactamase high- and low-level producers.

^c These strains are 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, SVH-4, SVH-5, OXA-1, OXA-2, OXA-3, OXA-4, OXA-5, OXA-6, OXA-7, PSE-1, PSE-2, PSE-3, PSE-4, HMS-1,1 OHIO-1, CAZ-2, SAR-1, and LXA-1 β-lactamases. Includes quality control strains ATCC 25218 and ATCC 35218.

^d High- and low-level producers of Class IV β-lactamase.

^e High- and low-level producers of TEM-1, SHV-1, and SHV-2 β-lactamases.

^f Methicillin susceptible and methicillin resistant.

Susceptibility tests. All susceptibility tests were performed by standard procedures (17, 18). Agar dilution tests were performed in Mueller-Hinton agar, with an inoculum of 10⁴ CFU per spot applied with a Steers replicator (23). Serial-twofold dilutions of cefoperazone and sulbactam (provided by Roerig Division of Pfizer Inc., New York, N.Y.) were tested in a 2:1 fixed ratio and were prepared on the day of use. Broth microdilution tests were performed with panels (prepared by Pasco Labs, Inc., Wheat Ridge, Colo.) with an inoculum of 10⁴ CFU per well. Agar dilution and broth microdilution tests were performed simultaneously. The MIC was defined as the lowest concentration preventing growth after 18 h of incubation at 35°C. Results obtained with cefoperazone alone were interpreted by using the criteria approved by the National Committee for Clinical Laboratory Standards (18). MICs of cefoperazone-sulbactam were interpreted according to the criteria proposed by Jones et al. (13), with ≥64 μg of cefoperazone indicating resistance and ≤16 μg of cefoperazone indicating susceptibility. Disk diffusion tests were performed with a commercially prepared disk containing 75 μg of cefoperazone and 30 μg of sulbactam (BBL), and results were interpreted by using the criteria proposed by Barry et al. (4). In addition, test disks for cefoperazone (75, 50, 30, 25, 20, and 15 μg per disk) and sulbactam (30, 25, 20, 15, and 10 μg per disk) were prepared the day of use. Quality control strains *Escherichia coli* ATCC 25922 and ATCC 35218 were tested by each method on each day of testing.

Analysis. Regression analysis was performed by using the least-squares method. MICs obtained in dilution tests and zone diameters obtained in diffusion tests were compared by using the error rate-bounded analysis method originally described by Metzler and DeHaan (14). In these compari-

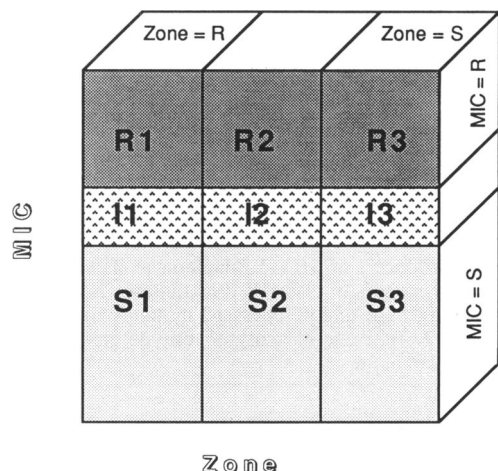


FIG. 1. Error rate-bound analysis. MICs are plotted on the vertical axis, and zone diameters are plotted on the horizontal axis. Horizontal lines indicate susceptible and resistant breakpoints for MICs, while vertical lines indicate breakpoints for zone diameters. Susceptible (S), moderately susceptible or intermediate (I), and resistant (R) areas on the graph are indicated. R1, indicates true resistance; S3, true susceptibility; R3, false susceptibility; S1, false resistance.

sons, the MIC was always considered the standard test. Errors were defined as follows: very major, susceptible by disk diffusion and resistant by MIC testing; major, resistant by disk diffusion and susceptible by MIC testing; minor, resistant by disk diffusion and moderately susceptible by MIC testing, moderately susceptible by disk diffusion and susceptible by MIC testing, susceptible by disk diffusion and moderately susceptible by MIC testing, or moderately susceptible by disk diffusion and resistant by MIC testing.

Error rates were calculated by using two sets of formulae. In the first set, the population error rate (P rate) was defined as the percent of errors occurring in the entire population tested. Thus, as shown in Fig. 1, the P rate for very major errors would be R_3/T , where R_3 is the number of strains in the block representing false susceptibility and T is the total number of strains tested ($R_1 + R_2 + R_3 + I_1 + I_2 + I_3 + S_1 + S_2 + S_3$). The P rate for major errors would be S_1/T , while that for the minor errors would be $(R_2 + I_1 + I_3 + S_2)/T$. Thus, the P rates were dependent on the composition of the total population tested (T). In the second set of formulae, the risk corrected error rate (RC rate) was defined as the percent of errors occurring among only those organisms at risk for that error. Thus, the RC rate for very major errors would be R_1/R_T , where $R_T = R_1 + R_2 + R_3$ (Fig. 1), since only resistant strains have the risk of appearing falsely susceptible. The RC rate for major errors would be S_1/S_T , where $S_T = S_1 + S_2 + S_3$ (Fig. 1). The RC rate for minor errors would be calculated the same as the P rate since all strains tested carry the risk of minor errors.

RESULTS

Primary panel. Initial studies were performed with the primary panel in an attempt to identify 10 candidate disks for further testing with the expanded panel. To accomplish this, cefoperazone masses of 75, 50, 30, 25, 20, and 15 μg per disk and sulbactam masses of 30, 25, 20, 15, and 10 μg per disk in all possible combinations were applied to sterile blank disks.

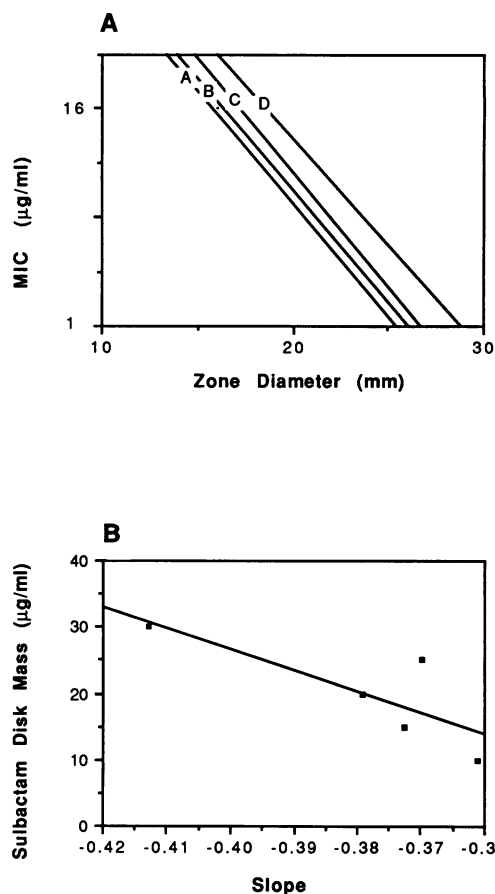


FIG. 2. Effect of altering the mass of each component of the cefoperazone-sulbactam disk. (A) Impact of changing cefoperazone disk masses in the presence of 30 μg of sulbactam. Cefoperazone disk masses were 15 μg (A), 25 μg (B), 30 μg (C), and 75 μg (D). MICs were determined by agar dilution. (B) Impact of changing the sulbactam disk mass in the presence of 30 μg of cefoperazone. Slope is for the regression line obtained when analyzing agar dilution MICs versus zone diameter.

These disks were used in diffusion susceptibility tests with the 24 strains from the primary panel.

Regression analyses of the agar dilution MIC versus the zone diameter for each disk were performed. As shown in Fig. 2, decreasing the cefoperazone disk mass shifted the regression line slightly to the left, while decreasing the sulbactam disk mass decreased the slope of the line. When data for each strain were analyzed separately, it appeared that for most strains, a concentration around 30 μg of cefoperazone produced a maximum zone diameter. Zone diameters increased only a few millimeters when concentrations of sulbactam were held constant and the cefoperazone concentration was increased to more than 30 μg (Fig. 3). This suggested that cefoperazone disk masses of more than 30 μg were beyond the optimal dose response for disk diffusion tests. Thus, cefoperazone disk masses of more than 30 μg were not examined further in this study, except for data generated with the commercial 75/30- μg disk.

Regression analyses were also performed to determine the quality of the separation between susceptible and resistant strains as determined by agar dilution MIC testing. Disks which provided less than 6 mm of separation between

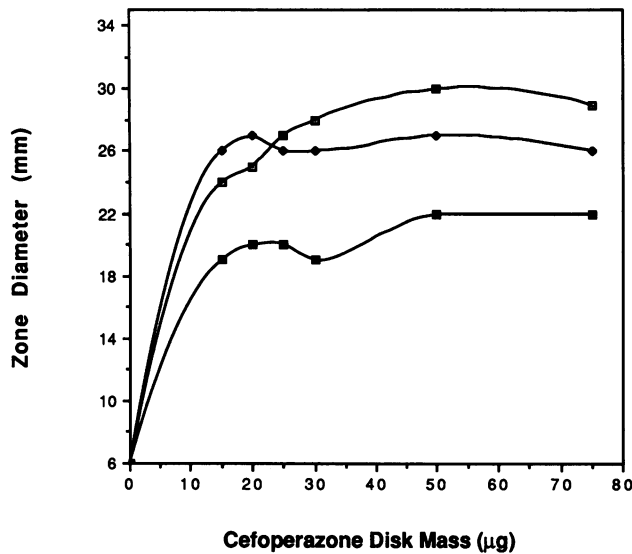


FIG. 3. Effect of increasing the cefoperazone disk mass in the presence of 30 µg of sulbactam. □, *Pseudomonas aeruginosa* 132; ◆, *Enterobacter cloacae* E55; ■, *Escherichia coli* GB 87.

susceptible and resistant strains and those which produced small zones overall were not considered further. Although the commercial 75/30-µg disk provided a 6-mm separation between susceptible and resistant strains, a large range of zone diameters (6 to 17 mm) was obtained with resistant strains and the slope of the regression line was very steep (-0.4126) compared with those obtained with other disks (Table 3). Ten candidate disks from the 24 disks tested were selected for further analysis because they (i) separated susceptible from resistant strains by 6 mm or more, (ii) produced small zones in tests with resistant strains, and (iii) had a less acute regression line compared with the 75/30-µg disk (Table 3).

Expanded panel. The 10 candidate disks and the commercial 75/30-µg disk were tested against the larger panel of 265 organisms. Regression analyses comparing the agar dilution MIC and the broth microdilution MIC to the zone diameter for each combination disk were performed. In initial analyses, data obtained with staphylococci were analyzed separately. Neither the proposed zone diameter criteria (4) for the commercial 75/30-µg disk nor any criteria developed with the 10 candidate disks provided adequate separation of methicillin-susceptible and methicillin-resistant staphylococci (data not shown). Thus, data obtained with the staphylococci were excluded from subsequent analyses.

With the broth microdilution as the standard, data for gram-negative bacteria tested with the commercial 75/30-µg disk were analyzed first. This analysis revealed 7 very major errors, no major errors, and 47 minor errors (Table 4). The very major errors that occurred with the 75/30-µg disk were with an *Escherichia coli* producing a high level of TEM-1 β-lactamase; one each of *Serratia marcescens*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Hafnia alvei* with the wild-type inducible phenotype of class I β-lactamase; and a *Pseudomonas aeruginosa* and an *Enterobacter cloacae* producing class I β-lactamase constitutively. Thus, an attempt was made to alter the interpretive criteria from those of Barry et al. (4) to eliminate the very major errors produced with this disk. Adopting a susceptible breakpoint of ≥24 mm and a resistant breakpoint of ≤18 mm reduced the very

TABLE 3. Candidate disks

Disk concn (µg) of cefoperazone-sulbactam	Interpretive criteria ^a		Slope of regression line ^b	Correlation coefficient
	Resistant zone (mm)	Susceptible zone (mm)		
30/25	≤13	≥21	-0.3699	0.87
30/20	≤12	≥19	-0.3792	0.89
30/15	≤12	≥19	-0.3725	0.90
30/10	≤10	≥18	-0.3612	0.91
25/30	≤10	≥18	-0.3562	0.86
25/25	≤12	≥18	-0.3561	0.76
25/20	≤10	≥18	-0.3617	0.79
20/25	≤12	≥18	-0.3661	0.77
20/20	≤11	≥18	-0.3643	0.79
20/15	≤10	≥20	-0.3593	0.80
75/30	≤15	≥21	-0.4126	0.81

^a Tentative criteria from the primary panel of this study except for the 75/30-µg disk (4).

^b Regression analysis performed by the agar dilution procedure for MIC determination.

major errors to 3 and increased the major and minor errors to 1 and 45, respectively. However, the very major errors could not be completely eliminated.

Error rate-bounded analyses were also performed for the 10 candidate disks by using MICs obtained in the broth microdilution test. For each disk, interpretive zone diameters that gave a very major P rate of <1% and minimized major and minor errors were selected. The error rates for the 75/30-µg disk and the 30/20-µg disk which gave the lowest overall errors for the 10 candidate disks are summarized in Table 4. Data for the two disks are shown in Fig. 4A and B. The very major errors that occurred with the 30/20-µg disk were an *Escherichia coli* producing a high level of TEM-1 β-lactamase and a *S. marcescens*. This disk separated susceptible and resistant strains, produced small zone diameters with many resistant isolates, had an equal distribution of minor errors among the four possible categories (see Materials and Methods), and had a more relaxed regression line than the 75/30-µg disk.

Cefoperazone disk. Since earlier analyses had indicated that disk masses of cefoperazone of more than 30 µg were excessive, data obtained in tests with cefoperazone alone were analyzed to determine whether the 75-µg cefoperazone disk also had excessive drug mass. Error rate-bounded analysis was performed by using the results obtained with a commercially prepared 75-µg disk and broth microdilution assays. In this analysis, there were 33 very major errors, which gave a P rate of 14% and an RC rate of 27%. There were no major errors and 62 minor errors, which gave a total overall P rate of 41%. The majority of the minor errors involved a moderately susceptible zone but resistance by MIC testing. Among organisms resistant to 256 µg of cefoperazone per ml, the zone diameters obtained with the 75-µg disk ranged from 6 to 25 mm.

The number of errors occurring with the 75-µg cefoperazone disk could be reduced by changing the interpretive criteria to ≤20 mm for resistance, 21 to 25 mm for moderate susceptibility, and ≥26 mm for susceptibility. With these criteria, there were three very major errors and three major errors, which gave a P rate of 1% for both categories and RC rates of 2 and 3%, respectively. There were 67 minor errors, which gave a total overall P rate of 32%.

Agar versus broth MIC. Because initial analyses indicated a discrepancy between agar dilution MICs and broth micro-

TABLE 4. Comparison of the 75/30- μg and 30/20- μg cefoperazone-sulbactam disks

Disk concn (μg) of drug	Interpretive criteria ^a	Slope of regression line	Errors	No. of strains ^b	P rate	RC rate
75/30	$\leq 15, 16-20, \geq 21$	-0.2828	Very major	7	3	16
			Major	0	0	0
			Minor	47	21	
			Total	54	24	
30/20	$\leq 15, 16-19, \geq 20$	-0.2710	Very major	2	0.9	5
			Major	1	0.4	0.6
			Minor	37	16	
			Total	40	17	

^a Criteria for the 75/30- μg disk were from Barry et al. (4). Those for the 30/20- μg disk were from this study.

^b By broth microdilution, 44 strains were resistant, 29 strains were moderately susceptible, and 156 strains were susceptible to cefoperazone-sulbactam.

dilution MICs for some strains, these results were compared directly for the strains of the expanded panel (Fig. 5). For the vast majority of strains, MICs ranged from four 4- to 64-fold higher in broth than in agar, with a mean difference of 4-fold. The 18 strains in the box in Fig. 5 represent very major errors, i.e., resistant by microdilution MIC testing but susceptible by agar dilution MIC testing. Nine of these strains were mutants which constitutively produced high levels of class I β -lactamase, and three of these strains produced high levels of TEM-1 or SHV-1. The remaining six strains possessed a variety of resistance mechanisms. Because very major errors were found with strains which should be considered to be clinically resistant, the results from the broth microdilution method were considered to be correct.

Quality control. *Escherichia coli* ATCC 35218, which has been designated for use for quality control in susceptibility tests for other β -lactam- β -lactamase inhibitor combinations, was found to be inappropriate for use with cefoperazone-sulbactam. This strain was resistant to cefoperazone and susceptible to cefoperazone-sulbactam in broth microdilution tests, but it produced large zones with both cefoperazone alone (29 mm) and cefoperazone-sulbactam at 30/20 μg (30 mm), in disk diffusion tests. Thus, a strain of *Escherichia coli*, TIM 35, that could be used as a quality control strain for cefoperazone-sulbactam was identified from the study. It was resistant to cefoperazone, but it was susceptible to cefoperazone-sulbactam in broth microdilution tests, appeared only moderately susceptible to cefoperazone alone (19 mm), and was susceptible to cefoperazone-sulbactam at 30/20 μg (21 mm), respectively, in disk diffusion tests. This strain could also be used for quality control with piperacillin-tazobactam but not for ampicillin-sulbactam, amoxicillin-clavulanate, or ticarcillin-clavulanate because it is resistant to the latter combinations (data not shown).

DISCUSSION

In this study, a predictor panel was used successfully to design a disk for diffusion susceptibility tests that would accurately discriminate between strains susceptible and resistant to cefoperazone-sulbactam. This approach had a major advantage over previous studies in that it assessed the impact of both components of the combination on the zone diameters produced and because it included a relatively large number of resistant strains with diverse mechanisms of resistance. As a result, several problems were identified with the 75/30- μg disk, which had been recommended in previous studies (4).

The currently proposed 75/30- μg cefoperazone-sulbactam disk evolved from studies that examined disks of only two

potencies, 75/30 and 75/15 μg (4). Thus, the impact of each component of the combination on the zone diameter produced was not assessed. Had this been done, it is likely that the presence of excessive cefoperazone would have been detected. Such excessive drug mass can lead to problems with very major errors if the dose response (i.e., change in zone diameter with change in susceptibility) is dampened as a result. Thus, one of the major goals of the current study was to identify a disk with a less acute slope of the regression line that would still accurately separate susceptible from resistant strains. Interestingly, the interpretive criteria for the 30/20- μg disk recommended in the current study vary only 1 mm from those recommended previously for the 75/30- μg disk (4). However, the lower error rates obtained with the 30/20- μg disk show the improved discrimination between susceptible and resistant strains of the lower potency disk due to the more relaxed slope of the regression line.

It is possible that the 75- μg cefoperazone disk currently in use also has an excessive drug mass. This possibility was suggested by results obtained in tests with quality control strains that appeared to be resistant in broth microdilution assays but susceptible or moderately susceptible in disk diffusion assays. The error rate-bounded analysis of the 75- μg cefoperazone disk also showed a very high very major error rate. This was the largest number of very major errors and total errors for any disk tested, suggesting once again an excessive disk mass. Some of these problems would be eliminated by changing the interpretive criteria. Alternatively, a disk containing ≤ 30 μg of cefoperazone may alleviate this problem, although this was not assessed in the current study.

For staphylococci, none of the disks examined in this study accurately identified susceptible and resistant strains when interpretive criteria developed with gram-negative bacteria were utilized. This finding agrees with those of Barry and Jones (3). Therefore, it seems prudent that staphylococci continue to be assessed by utilizing procedures that will accurately determine susceptibility to penicillin and oxacillin. Strains resistant to the latter should be presumed resistant to cefoperazone-sulbactam without direct testing.

For a large number of strains, the MIC determined by using broth microdilution was significantly higher than that determined by using agar dilution. These results suggest that for cefoperazone-sulbactam, different breakpoints may have to be used for the two tests. From the data presented here, it appears that a MIC of 4 $\mu\text{g}/\text{ml}$ determined by using agar corresponds to a broth microdilution MIC of 16 $\mu\text{g}/\text{ml}$. Thus, the former may be a more accurate breakpoint for susceptibility for tests performed with agar. Similarly, an agar

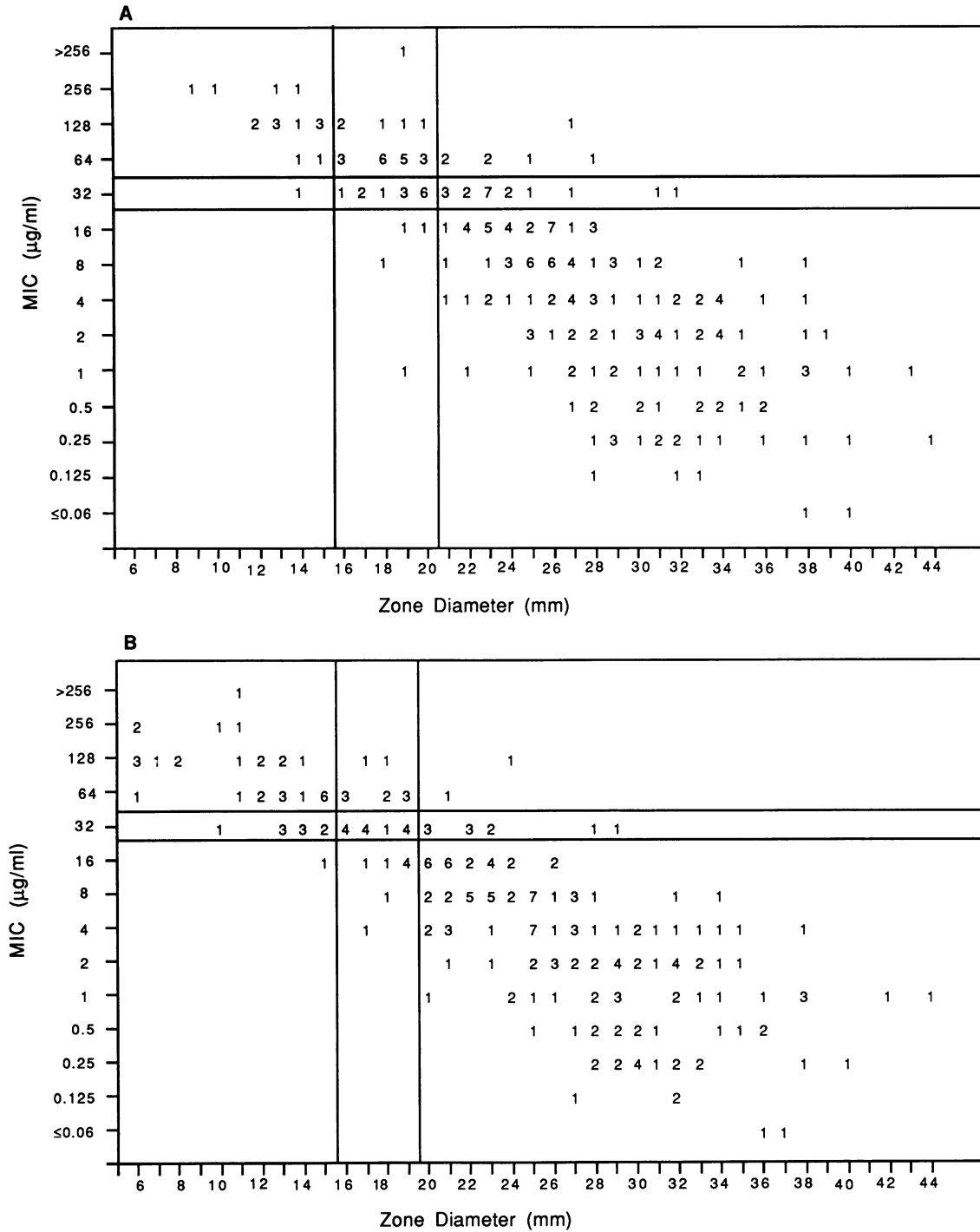


FIG. 4. Error rate-bounded analysis for 229 strains of gram-negative bacteria for cefoperazone-sulbactam disks. (A) 75/30- μ g disk. (B) 30/20- μ g disk. MICs determined by broth microdilution are plotted on the vertical axis versus zone diameter on the horizontal axis. Numbers on the plot represent the number of strains occurring at each point.

dilution MIC of $\geq 16 \mu\text{g/ml}$ would reflect resistance. An alternative solution to this problem would be the use of a constant low concentration (2 or 4 $\mu\text{g/ml}$) of sulbactam rather than a 2:1 ratio for tests performed by agar dilution to prevent false susceptibility from being reported. However, further studies will be required to assess the accuracy of such tests.

Although a preselected panel has been used previously to evaluate a new method for susceptibility testing (1), this is the first use of a panel of organisms to design a new disk to be used for diffusion tests. Also, the predictor panel utilized in this study is unique in that most of the organisms in the panel had a well-characterized mechanism of resistance to β -lactam antibiotics and the panel was chosen specifically

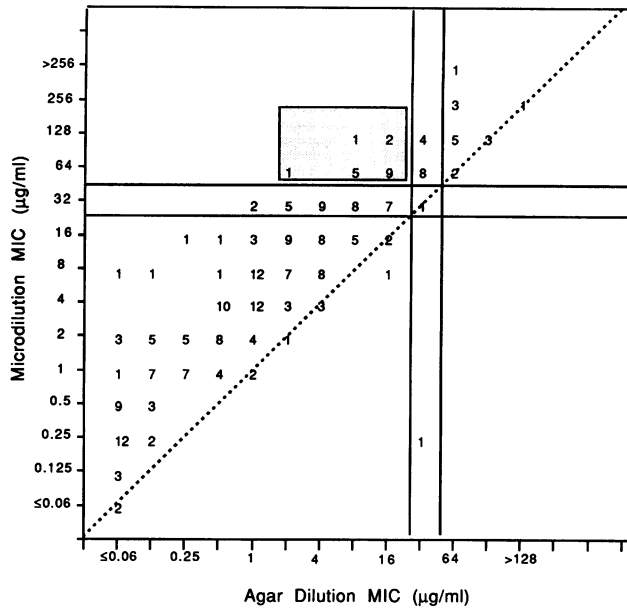


FIG. 5. Agar dilution MICs versus broth microdilution MICs of cefoperazone-sulbactam. Agar dilution MICs are plotted on the horizontal axis, while microdilution MICs are plotted on the vertical axis. The numbers on the graph represent the number of strains occurring at each point. The dotted line represents equivalence of MICs. The solid lines represent the MIC breakpoints for cefoperazone-sulbactam: ≤ 16 $\mu\text{g/ml}$ for susceptible, 32 $\mu\text{g/ml}$ for moderately susceptible, and ≥ 64 $\mu\text{g/ml}$ for resistant strains. The strains in the box represent those appearing susceptible in agar dilution tests but resistant in broth microdilution tests.

for analysis of this inhibitor-drug combination. This approach of using a test-specific or antibiotic-specific panel of organisms has an advantage over the use of a single set panel of organisms (1), as it allows changes in the composition of the panel to fit the needs of the investigation (19). All in all, the use of a predictor panel in the design of disks for diffusion testing appears to be a useful approach that avoids many of the problems that have been encountered in the past in studies utilizing clinical isolates chosen at random.

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