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New Recommendations for Disk Diffusion Antimicrobial Susceptibility Tests for Methicillin-Resistant (Heteroresistant) Staphylococci

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The agar disk diffusion susceptibility test was reevaluated for its ability to discriminate between susceptible and resistant *Staphylococcus aureus* (128 strains) and coagulase-negative staphylococci (19 strains) when tested with methicillin, oxacillin, and nafcillin. The results show that the current recommendations for disk potencies and interpretive zone diameters do not fit well with MIC correlates that we now recommend. Based on data from this study, we suggest that these parameters of the test be changed. For methicillin, we recommend a 10-μg disk with breakpoints of ≤11 mm (≥16 μg/ml) to indicate resistance and ≥15 mm (≤4 μg/ml) to indicate susceptibility. For oxacillin and nafcillin, we recommend 4-μg disks with breakpoints of ≤12 mm (≥8 μg/ml) to indicate resistance and ≥16 mm (≤2 μg/ml) to indicate susceptibility. MIC breakpoints were from a broth microdilution system which used a medium containing salt. If one of these three penicillins were to be selected for routine tests, we would recommend oxacillin, based on our data, but we recognize that this may depend upon the population of staphylococci within a particular hospital.

Methicillin-resistant strains of *Staphylococcus aureus* were isolated soon after methicillin was introduced (9). These "heteroresistant" bacteria were also recognized as problem organisms for antimicrobial susceptibility tests (2, 7). The problems are basically due to the occurrence of two populations, one susceptible and one resistant to methicillin, in every culture (13, 14). The resistant population generally grows much slower than the susceptible population, and thus, a culture may appear susceptible because the resistant population has not grown enough to be seen macroscopically. The resistant organisms grow better at lower incubation temperatures or if the salt content of the medium is increased (1, 2, 4, 5, 7, 14, 16). These findings resulted in the following various recommendations for improving the susceptibility tests with these organisms: (i) incubation at 30 or 35°C, instead of 37°C, (ii) incubation for 48 h, (iii) increasing the salt content of the medium, and (iv) using a larger inoculum. We felt (14), as did others (4), that the Bauer-Kirby agar disk diffusion method (3), described in the National Committee for Clinical Laboratory Standards (NCCLS) disk diffusion standard (11), was reliable if incubated at 35°C, and thus, changes were not necessary in the "standard method." This may still be true for many strains, but we recently received a number of isolates that were difficult to test reliably by the standard method.

Among the problems that we recently encountered in disk diffusion susceptibility tests with these bacteria were failure to correlate with results of other methods and failure to show cross-resistance with methicillin, oxacillin, and nafcillin. Another problem with the present disk diffusion test is that, in our opinion, the MIC correlates for the present zone diameter interpretations are too low, considering the pharmacokinetics of these drugs. If the higher MIC correlates are used with the present zone breakpoints (as is done in the NCCLS Standard M2-A2-S2), they do not correlate well. Still another problem that has not been generally recognized is the slow inactivation of these penicillins by β-lactamase which causes smaller zones of inhibition and may result in interpretations of intermediates for these drugs. Finally, the small zone diameters used as breakpoints, i.e., the 9 or 10 mm presently used to indicate resistance with these penicillins, are a problem since they reduce the reproducibility of the test and make measurement of zones more difficult.

Since the incidence of these heteroresistant (methicillin-resistant) bacteria has increased in the United States in recent years (6), we reevaluated the reliability of the methods for testing their susceptibility. We first evaluated the methods for determining the MIC of methicillin, oxacillin, nafcillin, and cephalothin for staphylococci, since the disk diffusion test must be correlated with the MIC test and because the microdilution MIC test did not always yield accurate results with these drugs and bacteria. These MIC studies show that the broth microdilution MIC method is accurate and reliable if 2% NaCl is added to the cation-supplemented Mueller-Hinton broth containing these four drugs (16). Preliminary studies, however, showed that we could not add NaCl to the Mueller-Hinton agar for the disk diffusion test because it interfered with growth of some organisms and susceptibility results of other drug-organism combinations. We concentrated our studies, therefore, on reevaluating the concentration of drugs needed in the disks and the zone diameter breakpoints that would yield the most accurate results. This is a report of the results of those studies.

MATERIALS AND METHODS

Bacteria. The 128 strains of *S. aureus* and the 19 strains of coagulase-negative staphylococci, isolated from various kinds of infections, were sent to our laboratory for susceptibility studies. We selected strains with several patterns of resistance and β-lactamase production. Of the 128 *S. aureus*

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strains, 53 were methicillin susceptible, β-lactamase positive; 51 were methicillin resistant (heteroresistant), β-lactamase positive; 1 was methicillin intermediate, β-lactamase negative; and 23 were methicillin susceptible, β-lactamase negative. Of the 19 coagulase-negative strains, 3 were methicillin susceptible, β-lactamase positive; 4 were methicillin resistant (heteroresistant), β-lactamase positive; 3 were methicillin intermediate, β-lactamase positive; and 9 were methicillin susceptible, β-lactamase negative. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were tested as standard control strains.

Antimicrobial agents. Methicillin, oxacillin, nafcillin, and cephalothin were obtained from the appropriate pharmaceutical companies and were powders suitable for susceptibility tests. The antibiotic disks were purchased (BBL Microbiology Systems) if available, but most were made in our laboratory by adding 0.025 ml of antibiotic solution (which contained enough drug [40 times] to give the final desired concentration) to each blank 6-mm disk. These disks were dried and stored at −70°C until used.

Susceptibility tests. MICs were determined by microdilution as described by the NCCLS dilution standard M7-T (12), except that 2% NaCl was added to the cation-supplemented Mueller-Hinton broth as described previously (16). Agar disk diffusion studies were carried out as described in the NCCLS disk diffusion standard M2-A2-S2 (11), except that different concentrations of drug per disk were used, and the inoculum was prepared by suspending enough growth from a 24-h agar plate in Mueller-Hinton broth to yield turbidity equivalent to a 0.5 McFarland standard (11).

To determine the effect of varying disk potency on size of the zone, we tested several concentrations of methicillin, oxacillin, and nafcillin. For methicillin we prepared and tested disks containing 4, 6, 8, 10, 16, 32, and 64 μg of methicillin, and also tested the commercial 5-μg disk. For oxacillin and nafcillin, the disk potencies tested were 1, 2, 4, 6, 8, 16, and 32 μg; we also tested the commercial 1-μg disks. Agar disk diffusion tests were performed with these disks with a selected set of eight staphylococci: six *S. aureus* and two coagulase-negative staphylococci. Two of the *S. aureus* strains were methicillin resistant and β-lactamase positive, one was methicillin intermediate and β-lactamase negative, two were methicillin susceptible and β-lactamase positive, and one was methicillin susceptible and β-lactamase negative (this was the control strain *S. aureus* ATCC 25923). One of the coagulase-negative strains was methicillin intermediate and β-lactamase positive; the other was methicillin susceptible and β-lactamase negative. Each organism was tested in triplicate with these disks.

After these studies, disks containing 5, 10, and 15 μg of methicillin; 1, 2, and 4 μg of oxacillin; 1, 2, and 4 μg of nafcillin; and 30 μg of cephalothin were selected for agar disk diffusion tests (11) against the 147 staphylococci.

Breakpoints. The MIC breakpoints used for this study were for methicillin, ≤4 μg/ml for susceptibility and ≥16 μg/ml for resistance and for oxacillin and nafcillin, ≤2 μg/ml for susceptibility and ≥8 μg/ml for resistance. These breakpoints are valid only if MICs are determined in the presence of 2% NaCl as described previously (16).

Analysis of data. The MICs and zone diameters for the 147 strains and each disk were plotted on scattergrams, and linear regression statistics were calculated. The zone diameter interpretive breakpoints were determined by the regression statistics and error rate bounding (10). Discrepancies between MICs and zone diameter interpretations were categorized as very major (resistant by MIC and susceptible by zone), major (susceptible by MIC and resistant by zone), and minor (one of the results was intermediate), as described previously (15).

RESULTS AND DISCUSSION

MICs of the four drugs obtained by this method with 2% NaCl (16) (Table 1) were, for the most part, well distributed into our MIC breakpoint categories and could be used as a basis for reevaluating the agar disk diffusion test for these drugs and staphylococci (Fig. 1 through 4).

Increasing the concentrations of the four drugs in the disks resulted in progressively larger zones, but the greater part of the change in zone diameters occurred with concentrations of ≤32 μg for methicillin and ≤16 μg for oxacillin and nafcillin. The slopes and shapes of the curves from the data for the six organisms were essentially the same. The only differences were the comparative diameters of the zones of inhibition that resulted from the differences in activity of the drug on the different organisms. Based on these results, we selected disk potencies of 5, 10, and 15 μg of methicillin and 1, 2, and 4 μg of oxacillin and nafcillin for further study. Only the recommended 30-μg cephalothin disk was included because we did not intend to modify the recommendation for this drug. Both the presence of β-lactamase and the drug concentration on the disk affected the size of the zone (Fig. 5). β-lactamase-negative strains had larger zones than did β-lactamase-positive strains at the same MIC, particularly with the smaller disk potencies (i.e., 5 μg for methicillin and 1 μg for nafcillin and oxacillin). Strains tested with higher antibiotic concentrations had larger zones than when tested with lower concentrations.

Regression statistics for the MICs and zone diameters and the error rates (discrepancies) obtained with the four drugs and the 10 different disk potencies were calculated (Table 2). Scattergrams of these data and the linear regression lines were plotted (Fig. 1 through 4) and examined to determine the disk potencies and interpretive criteria giving the most accurate results. The disk potencies and zone diameter breakpoints now recommended by NCCLS (11) did not fit well with the MIC breakpoints we had chosen. This lack of fit occurred with all three penicillins, but particularly so for oxacillin and nafcillin, the reason being that these interpretive zone criteria were made for lower MIC correlates, e.g., ≤0.6 μg/ml was the susceptible MIC correlate for oxacillin. Unfortunately, in the second revision of the NCCLS disk standard (11) the MIC correlates for these penicillins were raised without the determination of whether they were compatible with the recommended disk potencies and zone interpretive breakpoints.

Since we think the higher MIC breakpoints are pharmacologically correct, and based on our data, we chose the 10-μg

TABLE 1. Susceptibility of *S. aureus* and coagulase-negative staphylococci to four antibiotics by MICs[a]

Category[b]	No. of strains susceptible to the following antibiotics:			
	Methicillin	Oxacillin	Nafcillin	Cephalothin
Resistant	55	56	55	48
Intermediate	4	1	0	5
Susceptible	88	90	92	92

[a] MICs were determined by the method described in reference 16.

[b] Methicillin, resistant, ≥16 μg/ml; susceptible, ≤4 μg/ml. Oxacillin, resistant, ≥8 μg/ml; susceptible, ≤2 μg/ml. Nafcillin, resistant, ≥8 μg/ml; susceptible, ≤2 μg/ml. Cephalothin: resistant, ≥32 μg/ml; susceptible, ≤8 μg/ml.

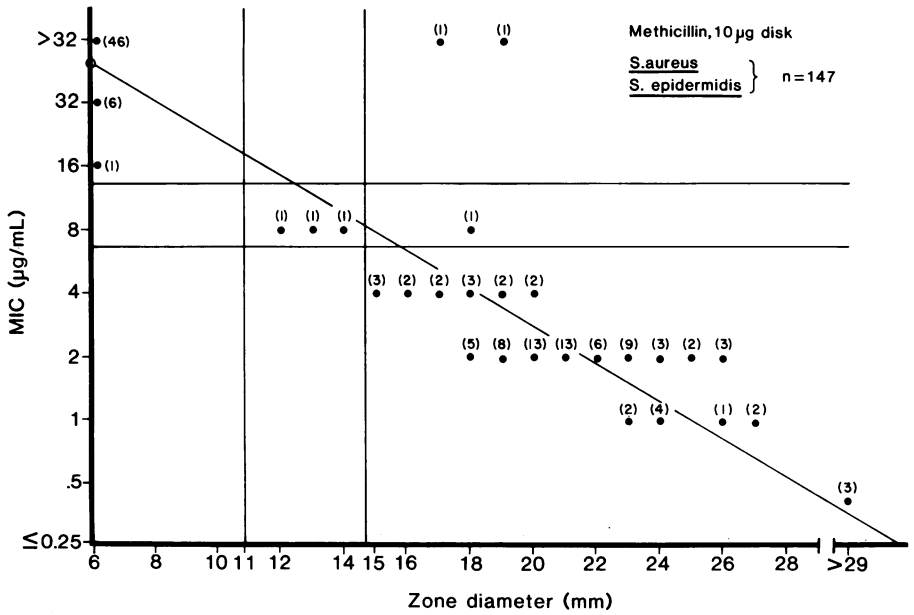

FIG. 1. Scattergram, regression line, and breakpoints for staphylococci tested by disk diffusion with a 10-μg methicillin disk. Numbers in parentheses indicates number of strains at that point. Vertical lines indicate zone diameter breakpoints, and horizontal lines indicate MIC breakpoints.

methicillin, the 4-μg oxacillin, and the 4-μg nafcillin disks as the ones which best discriminated between susceptible, intermediate, and resistant staphylococci (Fig. 1 through 4). An examination of the distribution of the organisms on the scattergram and the error rate data (Table 2) led us to recommend the zone diameter breakpoints shown in Fig. 1 through 4 and Table 3. With these criteria, there were no very major errors with oxacillin and nafcillin and 1.4% very major errors (two strains) with methicillin.

The two strains that caused discrepancies with methicillin represent two groups of organisms that deserve further explanation. These organisms were from outbreaks in two hospitals in different parts of the United States. Both of these unusual strains reacted differently in the disk diffusion test than did other strains. In the first group of these strains, depending upon the concentration of drug, we obtained unusual MICs and unusual zones for methicillin. In the MIC test, skips occurred at 4 or 8 μg/ml or both at 24 h (i.e., no

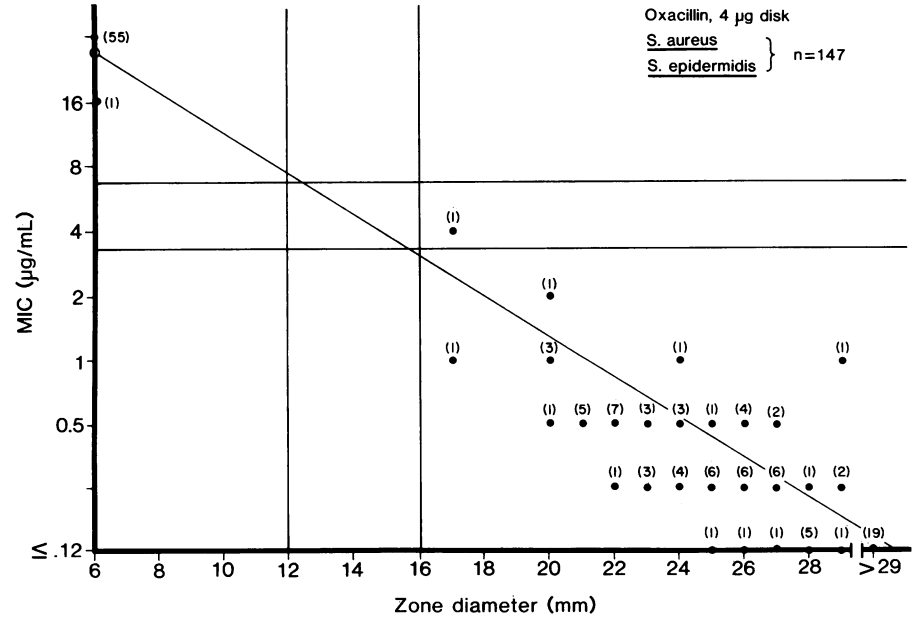

FIG. 2. Scattergram, regression line, and breakpoints for staphylococci tested by disk diffusion with a 4-μg oxacillin disk. Numbers in parentheses indicate number of strains at that point. Vertical lines indicate zone diameter breakpoints, and horizontal lines indicate MIC breakpoints.

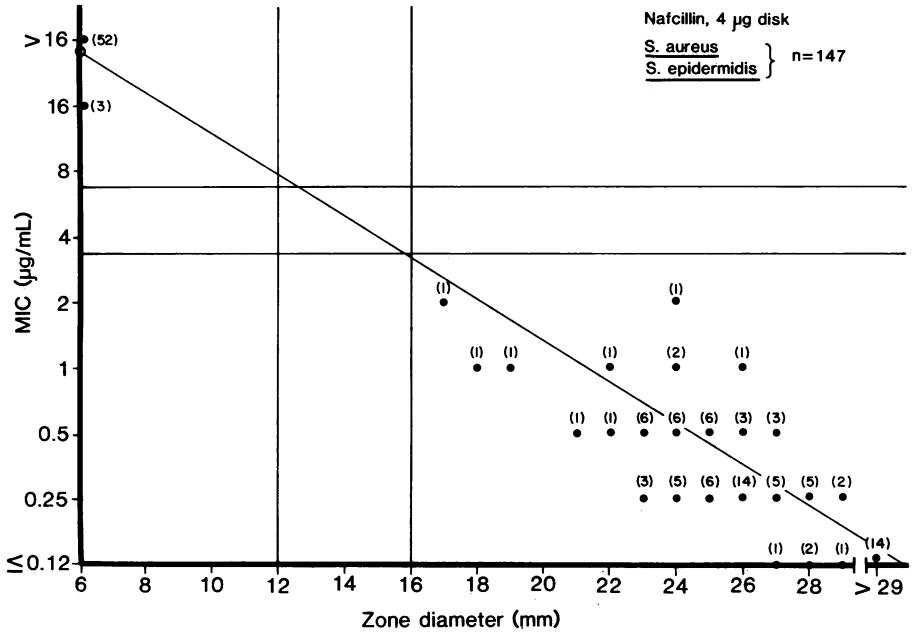

FIG. 3. Scattergram, regression line, and breakpoints for staphylococci tested by disk diffusion with a 4-μg nafcillin disk. Numbers in parentheses indicate number of strains at that point. Vertical lines indicate zone diameter breakpoints, and horizontal lines indicate MIC breakpoints.

visible growth in the well), although there may have been slight growth at 48 h. In the disk potency studies with these organisms, 32- or 64-μg disks resulted in "target" zones that were characterized by heavy growth around the disk, then a clear zone, and then heavy growth again. With 8- or 16-μg disks, however, there would be clear zones which would be erroneously interpreted as susceptible, and with the 5-μg disk, the zone could be either clear or slightly "fuzzy" (Fig. 6). These strains also gave unusual results with nafcillin, but they were less a problem than with methicillin (Fig. 7). In the

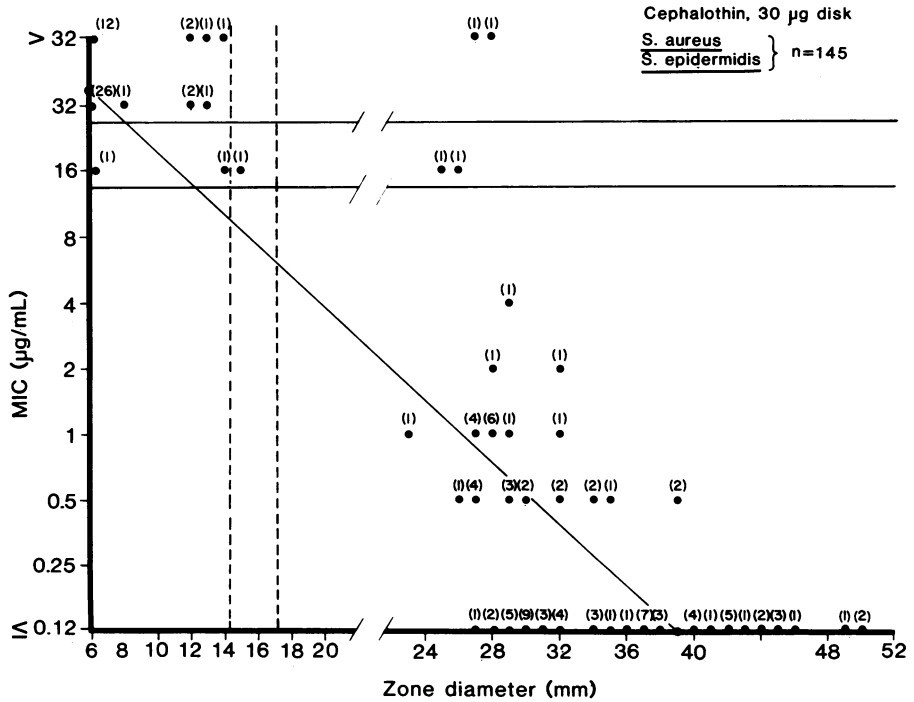

FIG. 4. Scattergram, regression line, and breakpoints for staphylococci tested by disk diffusion with a 30-μg cephalothin disk. Numbers in parentheses indicate number of trains at that point. Vertical lines indicate zone diameter breakpoints, and horizontal lines indicate MIC breakpoints.

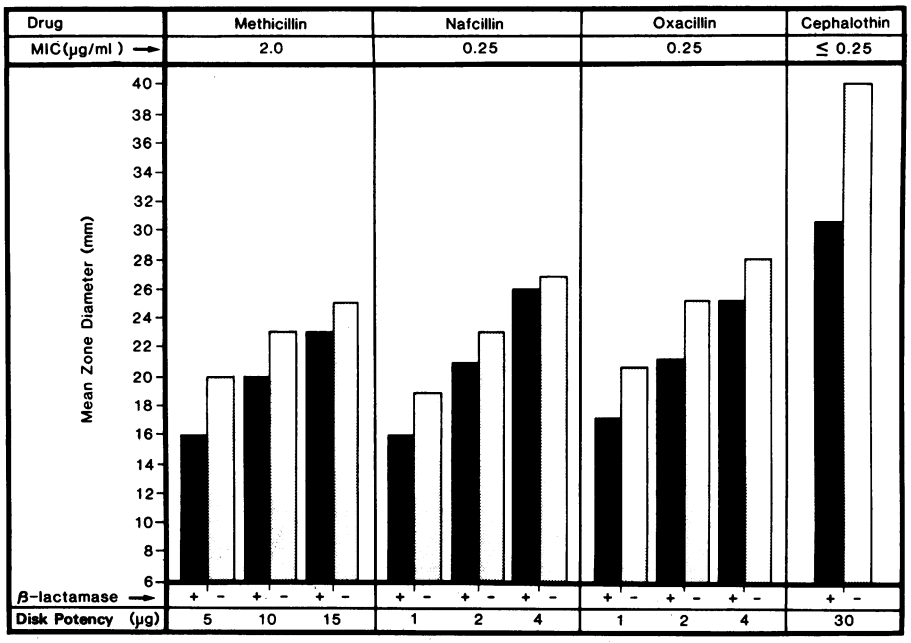

FIG. 5. Comparison of zone diameters obtained with β-lactamase-positive and -negative staphylococci with a selected MIC when tested against disks with three concentrations of methicillin, nafcillin, oxacillin, or cephalothin.

MIC test, there was reduced growth of these strains at 0.25 and 0.5 μg/ml; in the disk test, there was heavy growth around the disk, then a zone of light growth, and then heavy growth. There were no problems with oxacillin in either test (Fig. 8), i.e., there were no skips in the MIC, and the organisms grew heavily up to the disk. With these organisms, then, one could easily miss the resistance if methicillin was used and the appropriate concentrations of drug were not tested. In the MIC test, concentrations higher than 8 μg/ml must be tested to detect the 8 μg/ml skips and get a resistant MIC, i.e., ≥16 μg/ml. The methicillin disk test does not discriminate between these resistant strains and other susceptible strains with either the 5- or 10-μg disk. Oxacillin is undoubtedly the best choice for testing these currently rare organisms.

The organisms in the second unusual group reacted differently than those in the first group. With the methicillin MIC test, there was heavy growth at 2 or 4 μg/ml but only pinpoint colonies at 8 and 16 μg/ml, thus making them more difficult to read. These pinpoint colonies increased in size and number with longer incubation. At 16 to 18 h of incubation, the colonies generally could not be seen, but at 24 h the colonies could be seen with special effort, and at 48 h the colonies could readily be observed. With the methicillin disk diffusion test, zones of inhibition were essentially clear at 24 h or had one or two small colonies within the zone; at 48 h there were many pinpoint colonies up to the disk. There were no problems in detecting resistance in these strains when testing oxacillin and nafcillin. These strains are also rarely encountered.

It is clear that with these two unusual groups of heteroresistant organisms, methicillin is not a good choice, and it is equally clear that oxacillin is a good choice. Thus, based on our data, the selection of oxacillin as the preferred test agent

TABLE 2. Regression statistics and error rates (discrepancies) for different disk masses for methicillin, oxacillin, nafcillin, and cephalothin

Antibiotic and disk potency (μg)	y $\log_2$ intercept	slope	Discrepancies (%)[a]		
			Very major	Major	Minor
Methicillin					
5	13.54	−0.361	0	0	6.8
10	13.42	−0.293	1.4	0	0.7
15	13.37	−0.258	2.0	0	2.0
Nafcillin					
1	12.8	−0.471	0	4.1	2.7
2	12.8	−0.374	0	0.7	0.7
4	12.65	−0.312	0	0	0
Oxacillin					
1	13.0	−0.455	0	2.0	2.0
2	12.8	−0.372	0	0	1.4
4	12.66	−0.317	0	0	0.7
Cephalothin					
30	12.74	−0.250	1.4	0	2.1

[a] Discrepancies are classified as very major, susceptible by disk diffusion, resistant by MIC broth dilution; major, resistant by disk diffusion, susceptible by MIC broth dilution; minor, intermediate by only one of the two methods (disk diffusion, MIC broth dilution).

TABLE 3. Recommendations for zone diameter and MIC interpretive criteria for methicillin, oxacillin, and nafcillin

Antibiotic	Disk potency μg)	Zone diam (mm) (MIC [μg/ml]) for following classification:		
		Resistant	Intermediate	Susceptible
Methicillin	10	≤11 (≥16)	12–14	≥15 (≤4)
Oxacillin	4	≤12 (≥8)	13–15	≥16 (≤2)
Nafcillin	4	≤12 (≥8)	13–15	≥16 (≤2)

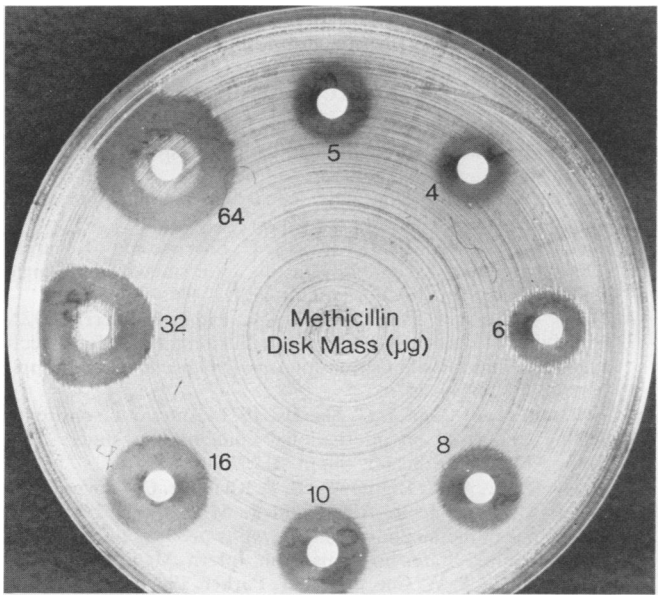

FIG. 6. Zones of inhibition obtained with an unusual strain of methicillin-resistant *S. aureus* tested against disks with various concentrations of methicillin, nafcillin, and oxacillin. Target zones occur at the higher disk masses.

is no longer just for its stability (4, 11), but also for its reliability. We should point out, however, that in a recent report (P. M. Terry, D. R. Lonsway, and J. E. McGowan, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C330, p. 366), heteroresistant *S. aureus* from one hospital could be better tested with methicillin than oxacillin. Their test with oxacillin disks did, however, show the organism to be resistant if the zones were examined with transmitted light and are similar to some strains we reported on previously (14). Selection of a test agent may, therefore, depend upon the characteristics of the endemic population of the organisms.

Although we successfully detected resistance to cephalothin in these bacteria, this test should be still classified as difficult with some strains. The growth is often scanty, and special efforts must be made to examine the tests closely for this growth. We are not sure whether coagulase-negative staphylococci are all resistant, since we showed that only some strains were resistant by MIC, but we suggest that all these strains be considered resistant to cephalothin until there is clinical efficacy data on which to make a decision. We also do not know whether these heteroresistant staphylococci are resistant to all cephalosporins; the third generation cephalosporins are generally less active than cephalothin against these strains. In a recent study, MICs for HR 810, a very active new broad-spectrum cephalosporin which some have called fourth generation, were determined for heteroresistant staphylococci with cation-supplemented Mueller-Hinton broth with and without 2% NaCl. The MICs obtained with 2% NaCl were two to four times higher than the MICs without NaCl and indicated that most strains were resistant to HR 810 (R. N. Jones, A. L. Barry, and C. Thornsberry, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. No. 573, 1983). The results and discrepancies for all three penicillinase-resistant antibiotics and these suggested breakpoints are well within acceptable limits and could be recommended for routine use in clinical laboratories. In the meantime, however, we still recommend that any strain of *Staphylococcus* found to be resistant to any of these three penicillins should also be considered and reported to be resistant to cephalothin.

The regression line for cephalothin was calculated with the data obtained with these staphylococci (Fig. 4), but as mentioned previously, the breakpoints are those recommended by NCCLS (11). These NCCLS breakpoints and this regression line for cephalothin do not match well, but we have not suggested a change because the regression line on which the NCCLS breakpoints are made was obtained with several other species of bacteria in addition to staphylococci.

In these recommendations we have provided for an intermediate category, which some authorities do not agree should exist for these drugs and organisms (12). We think that there are staphylococci that are truly intermediate in their susceptibility to these drugs, but they rarely occur (16). We have, however, no objection to the intermediate strains being called resistant for clinical purposes.

It is not likely that all three of these penicillins will be used in routine tests in a clinical microbiology laboratory since it is still rare to find a lack of complete cross-resistance, i.e., if a strain is resistant to one of these penicillins, it is highly likely to be resistant to the other two, and the same is true for a susceptible result. Thus, a decision as to which one drug to test routinely will have to be made in most laboratories. These data show that for most strains now encountered, any of the three drugs should yield accurate results, but we recommend that oxacillin be the choice for the following reasons: (i) the strains in this study that gave target zones with methicillin were uniformly resistant to oxacillin, and we think the resistant result is the correct category; (ii) there has a been a suggestion in the literature that blood in agar media can affect the zones obtained with nafcillin (8), and we have some data to support this; and (iii) the greater

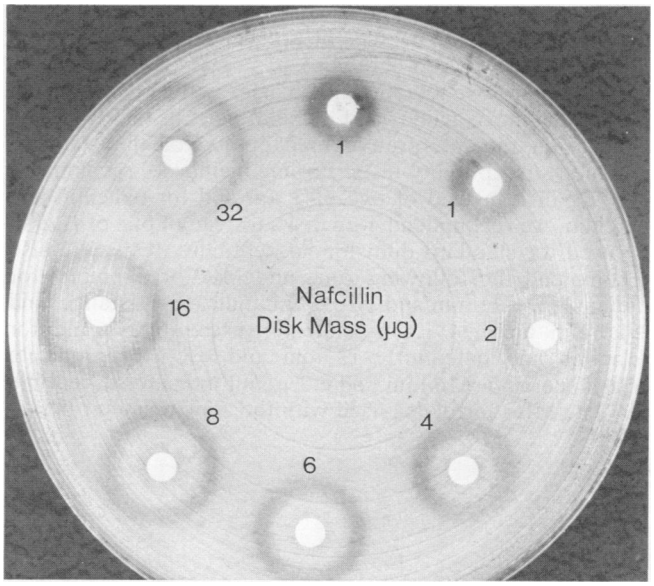

FIG. 7. Zones of inhibition obtained with the unusual strain (same strain as described in the legend to Fig. 6) of methicillin-resistant *S. aureus* tested against disks with various concentrations of nafcillin. Target zones occur at most concentrations.

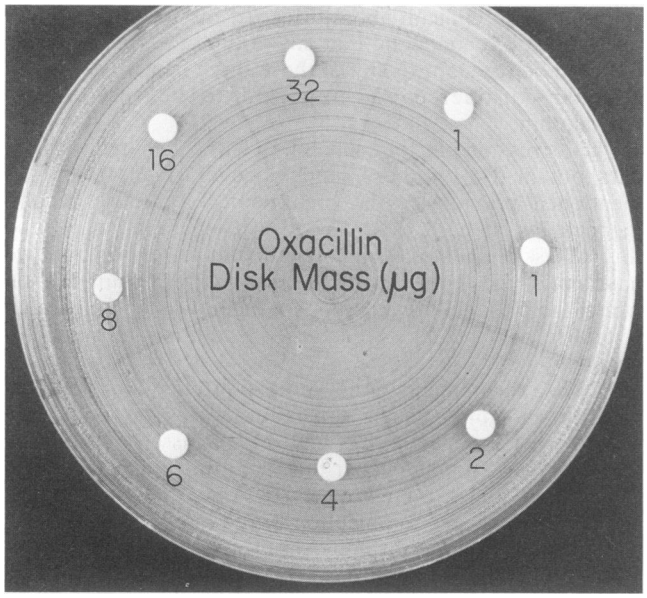

FIG. 8. Disk diffusion test with the unusual strain (same strain as described in the legends to Fig. 6 and 7) of a methicillin-resistant strain of *S. aureus* tested with disks with various concentrations of oxacillin. No zones of inhibition (including target zones) occurred.

stability of oxacillin, as compared with methicillin, has been reported previously (4).

We suggest that the NCCLS Disk Diffusion Subcommittee and the Food and Drug Administration consider these recommendations for changes in disk potencies and breakpoints. Such changes in disk potency and zone size interpretive criteria for older drugs is not without precedent, since it has been done previously for carbenicillin and amikacin.

It should be emphasized that these data were purposely developed with one medium. Further studies should include collaborative studies of quality control and the effect of different brands and sources of Mueller-Hinton agar on the diffusion test reported here.

In summary, we recommend changes in the concentration of methicillin, oxacillin, and nafcillin used in the disks and changes in the interpretive breakpoints for the standard diffusion susceptibility tests. For methicillin we recommend a 10-μg disk instead of one of 5 μg, and for oxacillin and nafcillin, we recommend 4-μg disks instead of one of 1 μg to be used for standard diffusion susceptibility tests. We also recommend the following zone and MIC breakpoints: for methicillin, ≤11 mm and ≥16 μg/ml indicate resistance, and ≥15 mm and ≤4 μg/ml indicate susceptibility; and for oxacillin and nafcillin, ≤12 mm and ≥8 μg/ml indicate resistance, and ≥16 mm and ≤2 μg/ml indicate susceptibility. The MIC correlates used with the zone diameter breakpoint must be obtained in tests with cation-supplemented Mueller-Hinton broth further supplemented with 2% NaCl.

LITERATURE CITED

1. **Annear, D. I.** 1968. The effect of temperature on the resistance of *Staphylococcus aureus* to methicillin and some other antibiotics. Med. J. Aust. **1**:444–446.
2. **Barber, M.** 1964. Naturally occurring methicillin-resistant staphylococci. J. Gen. Microbiol. **35**:183–190.
3. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493–496.
4. **Drew, W. L., A. L. Barry, R. O'Toole, and J. C. Sherris.** 1972. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of *Staphylococcus aureus*. Appl. Microbiol. **24**:240–247.
5. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing: report of an international collaborative study. Acta Pathol. Microbiol. Scand. Suppl. **217**:1–90.
6. **Haley, R. W., A. W. Hightower, R. F. Khabbaz, C. Thornsberry, W. J. Martone, J. R. Allen, and J. M. Hughes.** 1982. The emergence of methicillin-resistant *Staphylococcus aureus* infections in United States hospitals. Ann. Intern. Med. **97**:297–308.
7. **Hewitt, J. H., A. W. Coe, and M. T. Parker.** 1969. The detection of methicillin resistance in *Staphylococcus aureus*. J. Med. Microbiol. **2**:443–456.
8. **Jacobs, M. R., Y. Mithal, R. M. Robins-Browne, M. N. Gaspar, and H. J. Koornhof.** 1979. Antimicrobial susceptibility testing of pneumococci: determination of Kirby-Bauer breakpoints for penicillin G, erythromycin, clindamycin, tetracycline, chloramphenicol, and rifampin. Antimicrob. Agents Chemother. **16**:190–197.
9. **Jevons, M. P.** 1961. "Celbenin"-resistant staphylococci. Br. Med. J. **1**:124–125.
10. **Metzler, C. M., and R. M. DeHaan.** 1974. Susceptibility tests of anaerobic bacteria: statistical and clinical considerations. J. Infect. Dis. **130**:588–594.
11. **National Committee for Clinical Laboratory Standards.** 1979. Approved standard M2-A2. Performance standards for antimicrobic disc susceptibility tests, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **National Committee for Clinical Laboratory Standards.** 1983. Tentative Standard M7-T. Standard methods for dilution antimicrobial tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **Parker, M. T.** 1971. Methicillin-resistant staphylococci. *In* Proceedings of International Conference on Nosocomial Infections, p. 112–116. Center for Disease Control, Atlanta, Ga.
14. **Thornsberry, C., J. Q. Caruthers, and C. N. Baker.** 1973. Effect of temperature on the in vitro susceptibility of *Staphylococcus aureus* to penicillinase-resistant penicillins. Antimicrob. Agents Chemother. **4**:263–269.
15. **Thornsberry, C., T. L. Gavan, J. C. Sherris, A. Balows, J. M. Matsen, L. D. Sabath, F. Schoenknecht, L. D. Thrupp, and J. A. Washington II.** 1975. Laboratory evaluation of a rapid, automated susceptibility testing system: report of a collaborative study. Antimicrob. Agents Chemother. **7**:466–480.
16. **Thornsberry, C., and L. K. McDougal.** 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. J. Clin. Microbiol. **18**:1084–1091.