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Revised Interpretation of Oxacillin MICs for *Staphylococcus epidermidis* Based on *mecA* Detection

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In 1992 and 1993, at The Ohio State University Medical Center, a larger proportion of *Staphylococcus epidermidis* strains required oxacillin MICs of 1 to 2 μg/ml than did *Staphylococcus aureus* strains. *mecA* genotype was correlated with antimicrobial susceptibility for selected clinical *S. epidermidis* strains. All 14 strains that required oxacillin MICs of ≤0.25 μg/ml and 2 of 5 strains that required oxacillin MICs of 0.5 μg/ml were susceptible by 1-μg oxacillin disk test and were *mecA* negative. Three of 5 strains that required oxacillin MICs of 0.5 μg/ml and all 18 strains that required oxacillin MICs of ≥1.0 μg/ml were resistant by oxacillin disk test and were *mecA* positive. Current National Committee for Clinical Laboratory Standards MIC interpretive criteria may underestimate methicillin resistance among *S. epidermidis* strains.

Oxacillin resistance among staphylococci, a serious clinical problem, also presents a challenge in the laboratory (8, 17, 19). Differences in the susceptibility patterns of staphylococcal species have been noted. *Staphylococcus aureus* is typically either very susceptible or very resistant to oxacillin (MICs of ≤0.5 μg/ml or ≥8 μg/ml, respectively), but for *Staphylococcus epidermidis*, the distribution of oxacillin MICs is not as clearly bimodal, and the natural breakpoint appears to be lower. Relative to that of *S. aureus* strains, a larger proportion of *S. epidermidis* strains require oxacillin MICs in the range of 1 to 2 μg/ml, just below the recommended breakpoint for identifying susceptible strains (13), yet many of these strains appear to be oxacillin resistant by the disk test (3, 14).

Several investigators (1, 2, 7, 11, 14, 15, 20–22) have correlated the oxacillin susceptibility phenotype with the *mecA* contents of staphylococci. These data are somewhat difficult to interpret, as susceptibility testing was not usually conducted according to National Committee for Clinical Laboratory Standards (NCCLS) protocols, correlation data were not expressed in easily comparable formats, or only a small number of strains for which MICs were close to recommended breakpoints were examined. In general, however, *mecA*-MIC susceptibility correlations have been good (resistant strains have been *mecA* positive and susceptible strains have been *mecA* negative), but discrepancies between *mecA* genotype and susceptibility phenotype have been noted. *mecA*-positive strains for which MICs were indicative of oxacillin susceptibility or methicillin susceptibility were observed more often among *S. epidermidis* strains than among *S. aureus* strains (1, 4, 11, 14, 21).

To gain more insight into the relationship between MIC results and true methicillin resistance, we examined the oxacillin broth dilution MICs for a large number of clinical *S. aureus* and *S. epidermidis* isolates and the *mecA* genotypes of a group of *S. epidermidis* strains selected for their oxacillin broth dilution MICs (obtained according to NCCLS guidelines [13]). We have found that *S. epidermidis* strains for which oxacillin MICs are 0.5 to 2 μg/ml are frequently isolated in the clinical laboratory and that such strains are likely to be resistant by oxacillin disk test and *mecA* positive. Routine use of the current NCCLS breakpoint (i.e., ≤2 μg/ml) for determining the oxacillin susceptibilities of all staphylococcal species deserves serious scrutiny.

At The Ohio State University Medical Center, determinations of MICs are routinely performed for all clinical isolates that meet screening criteria suggestive of infection. If strains are isolated from the same site of a given patient within a 3-day period, MIC testing is not repeated. MICs are determined by the NCCLS-recommended, standardized broth microdilution test (13). The oxacillin MICs for *S. epidermidis* and *S. aureus* strains isolated in 1992 and 1993 by the Microbiology Laboratory of The Ohio State University Medical Center are shown in Fig. 1. These MIC distributions were qualitatively different, as were the natural, visually determined breakpoints. There were many more *S. epidermidis* strains than *S. aureus* strains for which the MIC was in the range of 1 to 2 μg/ml (1,289 of 3,744 [31.2%] versus 123 of 3,792 [3.2%] [$P = <10^{-6}$ by chi-square test]).

Clinical *S. epidermidis* strains were selected for *mecA* content-susceptibility phenotype correlation. Oxacillin broth dilution MICs were measured (13), and disk diffusion susceptibilities to oxacillin were assessed according to NCCLS guidelines (12). Strains with zones of inhibition indicative of susceptibility were also read at 48 h, but these second readings did not change the results. *mecA* content was determined by a nonradiometric dot blot assay as outlined below.

Bacterial DNA was isolated as previously described (9), with some modifications. Treatment of *S. epidermidis* strains with lysostaphin (80 to 100 μg/ml) and 0.5% sodium dodecyl sulfate (SDS) yielded inconsistent lysis of bacterial cells, with parallel variations in the amounts of DNA recovered. We achieved consistent cell lysis by treatment of bacterial cell pellets with cold acetone, as described by Heath et al. (5), and then sequential incubations of the bacterial cell suspension with lysostaphin and lysozyme at concentrations of 100 μg/ml and 4 mg/ml, respectively (37°C for 30 min), and the addition of SDS to a final concentration of 0.5% (wt/vol).

The hybridization probe, a fragment of the *mecA* gene, had been previously cloned into pBluescript and maintained in *Eschericia coli* DH5α as plasmid pSA12. This was kindly pro-

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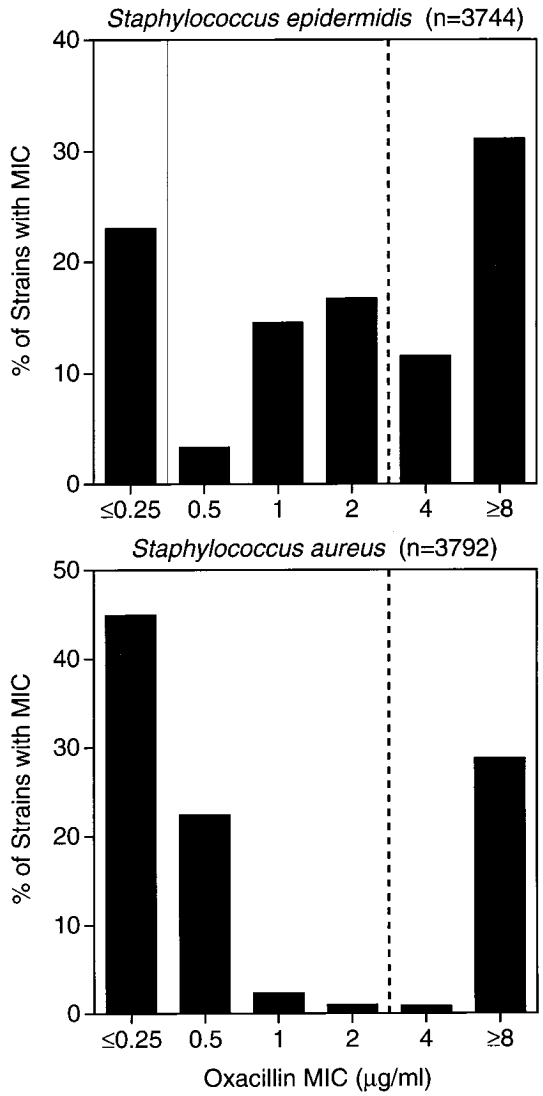

FIG. 1. Frequency distribution bar graphs of oxacillin MICs for *S. epidermidis* and *S. aureus* strains isolated by The Ohio State University Medical Center Microbiology Laboratory in 1992 and 1993. Vertical broken lines indicate the breakpoints for distinguishing resistant from susceptible strains recommended by the NCCLS. The vertical solid line represents the proposed breakpoint for *S. epidermidis* strains.

vided by Roberta Fontana, Institute of Microbiology, University of Verona, Verona, Italy (7). Crude plasmid DNA (10) was treated with RNase A (200 μg/ml) and RNase T_1 (200 U/ml), extracted with phenol-$CHCl_3$, and precipitated with ethanol (9). This more-purified plasmid DNA was then digested with *Bam*HI-*Eco*RI, and an approximately 530-bp fragment was recovered from the agarose gel slice via a commercially available DNA purification kit (Elu-Quik; Schleicher & Schuell, Keene, N.H.) (18). Approximately 1 μg of this purified *mecA* fragment DNA was then labeled by photoderivatization with psoralen biotin (6, 16) by utilizing a commercially available kit (Rad-Free; Schleicher & Schuell).

Purified staphylococcal DNA was denatured and then spotted onto a nylon filter (Nytran; Schleicher & Schuell). Prehybridization, hybridization, and posthybridization conditions were those specified by the manufacturer of the DNA labeling and detection kit (Rad-Free; Schleicher & Schuell). Hybridization was detected by a 1,2-dioxetane-based chemiluminescence system (Lumi-Phos 530; Lumigen, Inc., Detroit, Mich.) with a streptavidin-linked alkaline phosphatase conjugate (Rad-Free; Schleicher & Schuell). Emitted light was captured with Ilford RCRapid polycontrast (MGR 1M) photographic paper, which was developed with a 1:4 dilution of a stock solution of Kodak Dektol and with Kodak Rapid Fixer. Typical exposure times were 1 to 2 h, taken 22 to 24 h after assembly of the Lumi-Phos 530 substrate sheet-blot sandwich.

TABLE 1. Correlation of susceptibility testing and *mecA* genotype for *S. epidermidis* clinical strains

Oxacillin MIC (μg/ml)	No. of strains	24-h inhibition zone (mm)	*mecA* probe
≤0.25	14	18–28	Negative
0.5	2	21–28	Negative
0.5	3	6[a]	Positive
1.0	14	6[b]	Positive
≥16	4	6	Positive

[a] All three strains had larger zones of inhibition but had colonies within the zone to the diameter indicated (heteroresistance).
[b] Heteroresistance was noted for 8 of these 14 strains.

By using approximately 0.5 μg of DNA per spot, hybridization yielded completely dichotomous results. Spots either were dark black or showed no signal at all. When serial twofold dilutions of 0.5 μg of DNA from a positive control strain (ATCC 33591 [methicillin-resistant *S. aureus*]) were examined, spot intensity faded with dilution, but a signal was clearly visible at a dilution of 1:32. Similarly, a negative control strain (ATCC 29213 [methicillin-susceptible *S. aureus*]) yielded a completely negative spot despite increasing the amount of DNA spotted from 0.5 to 4 μg. Variations in times for exposure and photographic development had only minor impact on the results, yielding variable detection of the 1:32 dilution of the positive control but no additional positives among those strains previously negative.

The oxacillin MICs, corresponding inhibitory-zone diameters, and *mecA* dot blot assay results for 37 *S. epidermidis* strains are summarized in Table 1. All 14 *S. epidermidis* isolates for which oxacillin MICs were ≤0.25 μg/ml and 2 of 5 isolates for which oxacillin MICs were 0.5 μg/ml were susceptible by the disk diffusion test and were *mecA* negative. Three of 5 isolates for which oxacillin MICs were 0.5 μg/ml and all 18 isolates for which oxacillin MICs were ≥1 μg/ml were resistant by the disk diffusion test and were *mecA* positive. All positive dot blot spots were strongly positive (i.e., regardless of oxacillin MICs), and all negative spots showed no signal at all. A representative section of a dot blot is shown in Fig. 2.

Our comparison of oxacillin MICs for a large group of clinical *S. aureus* and *S. epidermidis* strains yielded results that were consistent with those of previous works (3, 14). The major discriminating features appeared to be the relatively large number of strains that required oxacillin MICs of 1 to 2 μg/ml and the obviously lower breakpoint in the distribution for *S. epidermidis* strains. The distribution of oxacillin MICs for *S. aureus* strains, on the other hand, was again bimodal, with a clear-cut visual breakpoint that was consistent with current NCCLS recommendations (Fig. 1).

Among *S. epidermidis* strains subjected to *mecA* assays, all those for which oxacillin MICs were ≤0.25 μg/ml were *mecA* negative. Among strains for which oxacillin MICs were 0.5 μg/ml, some were probe negative while others were probe positive. Finally, those strains for which oxacillin MICs were ≥1 μg/ml were uniformly *mecA* positive. Oxacillin disk diffu-

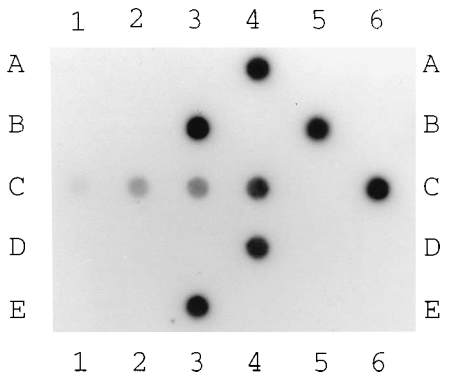

FIG. 2. Dot blot of bacterial DNA probed with the *mecA* fragment derived from pSA12 (7). DNA was spotted on a nylon membrane and hybridized with the *mecA* probe; hybridization was detected by chemiluminescence. DNAs (0.5 μg each) from *S. epidermidis* strains for which oxacillin MICs were ≤0.25 μg/ml were spotted at positions A-2, A-6, B-1, D-2, D-6, and E-5. Strains for which oxacillin MICs were 1 μg/ml were spotted at A-4, B-5, D-4, and E-3. DNA from one *S. epidermidis* strain for which the oxacillin MIC was >16 μg/ml was spotted at B-3. All DNAs spotted in row C were derived from methicillin-resistant *S. aureus* ATCC 33591; 0.5, 0.25, 0.13, 0.06, and 0.03 μg were spotted at positions C-6, C-4, C-3, C-2, and C-1, respectively. Positions not specified above were blank.

sion testing corroborated these findings completely. That is, only the most sensitive group of isolates (i.e., those that required oxacillin MICs of ≤0.25 μg/ml) could be expected to be devoid of the *mecA* gene and thus truly methicillin susceptible or oxacillin susceptible. Although the MICs for them fell within the current NCCLS range for oxacillin susceptibility, some isolates that required oxacillin MICs of 0.5 μg/ml (which are uncommon) and all isolates that required MICs of ≥1 μg/ml contained the *mecA* gene.

Because 31% of our hospital's *S. epidermidis* strains required oxacillin MICs in the range of 1 to 2 μg/ml and because all of the strains tested that required MICs of 1 μg/ml contained the *mecA* gene, we suspect that the current NCCLS breakpoint is an inadequate measure of oxacillin resistance for *S. epidermidis* strains. For our institution's *S. epidermidis* strains, oxacillin broth dilution susceptibility and resistance would be more appropriately defined by respective breakpoints of ≤0.25 and ≥0.5 μg/ml.

We cannot exclude the possibility that strain selection bias or some other local phenomenon affected our data, but these findings should be striking enough to motivate the NCCLS to reexamine the genetic basis of breakpoints for *S. epidermidis* strains and consider similar *mecA*-MIC correlations for other staphylococcal species. At the very least, clinical laboratories should seriously consider routine oxacillin disk susceptibility testing to confirm sensitivity for *S. epidermidis* strains for which oxacillin MICs are in the range of 0.5 to 2 μg/ml.

REFERENCES

1. **Archer, G. L., and E. Pennell.** 1990. Detection of methicillin resistance in staphylococci by using a DNA probe. Antimicrob. Agents Chemother. **34:** 1720–1724.
2. **Brakstad, O. G., J. A. Maeland, and Y. Tveten.** 1993. Multiplex polymerase chain reaction for detection of genes for *Staphylococcus aureus* thermonuclease and methicillin resistance and correlation with oxacillin resistance. APMIS **101:**681–688.
3. **Fass, R. J., V. L. Helsel, J. Barnishan, and L. W. Ayers.** 1986. In vitro susceptibilities of four species of coagulase-negative staphylococci. Antimicrob. Agents Chemother. **30:**545–552.
4. **Geha, D. J., J. R. Uhl, C. A. Gustaferro, and D. H. Persing.** 1994. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. J. Clin. Microbiol. **32:**1768–1772.
5. **Heath, L. S., G. L. Sloan, and H. E. Heath.** 1986. A simple and generally applicable procedure for releasing DNA from bacterial cells. Appl. Environ. Microbiol. **51:**1138–1140.
6. **Levenson, C., R. Watson, and E. L. Sheldon.** 1990. Biotinylated psoralen derivative for labeling nucleic acid hybridization probes. Methods Enzymol. **184:**577–583.
7. **Ligozzi, M., G. M. Rossolini, E. A. Tonin, and R. Fontana.** 1991. Nonradioactive DNA probe for detection of gene for methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35:**575–578.
8. **Madiraju, M. V. V. S., D. P. Brunner, and B. J. Wilkinson.** 1987. Effects of temperature, NaCl, and methicillin on penicillin-binding proteins, growth, peptidoglycan synthesis, and autolysis in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **31:**1727–1733.
9. **Maher, W. E., M. Kobe, and R. J. Fass.** 1993. Restriction endonuclease analysis of clinical *Pseudomonas aeruginosa* strains: useful epidemiologic data from a simple and rapid method. J. Clin. Microbiol. **31:**1426–1429.
10. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Murakami, K., W. Minamide, K. Wada, E. Nakamura, H. Teraoka, and S. Watanabe.** 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J. Clin. Microbiol. **29:**2240–2244.
12. **National Committee for Clinical Laboratory Standards.** 1993. Approved standard M2-A5. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **National Committee for Clinical Laboratory Standards.** 1993. Approved standard M7-A3. Dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Laboratory Standards, Villanova, Pa.
14. **Olsson-Liljequist, B., P. Larsson, S. Ringertz, and S. Lofdahl.** 1993. Use of a DNA hybridization method to verify results of screening for methicillin resistance in staphylococci. Eur. J. Clin. Microbiol. Infect. Dis. **12:**527–533.
15. **Predari, S. C., M. Ligozzi, and R. Fontana.** 1991. Genotypic identification of methicillin-resistant coagulase-negative staphylococci by polymerase chain reaction. Antimicrob. Agents Chemother. **35:**2568–2573.
16. **Saffran, W. A., J. T. Welsh, R. M. Knobler, F. P. Gasparro, C. R. Cantor, and R. L. Edelson.** 1988. Preparation and characterization of biotinylated psoralen. Nucleic Acids Res. **16:**7221–7231.
17. **Sierra-Madero, J. G., C. Knapp, C. Karaffa, and J. A. Washington.** 1988. Role of β-lactamase and different testing conditions in oxacillin-borderline-susceptible staphylococci. Antimicrob. Agents Chemother. **32:**1754–1757.
18. **Thompson, J. D., K. K. Cuddy, D. S. Haines, and D. Gillespie.** 1990. Extraction of cellular DNA from crude lysate with glass. Nucleic Acids Res. **18:** 1074.
19. **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.
20. **Tokue, Y., S. Shoji, K. Satoh, A. Watanabe, and M. Motomiya.** 1992. Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **36:**6–9.
21. **Ubukata, K., S. Nakagami, A. Nitta, A. Yamane, S. Kawakami, M. Sugiura, and M. Konno.** 1992. Rapid detection of the *mecA* gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain reaction products. J. Clin. Microbiol. **30:**1728–1733.
22. **Unal, S., J. Hoskins, J. E. Flokowitsch, C. Y. Wu, D. A. Preston, and P. L. Skatrud.** 1992. Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. J. Clin. Microbiol. **30:**1685–1691.