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Correlation of Penicillin Minimum Inhibitory Concentrations and Penicillin Zone Edge Appearance with Staphylococcal Beta-Lactamase Production

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Production of staphylococcal beta-lactamase was shown to be correlated with penicillin G minimum inhibitory concentrations (MICs) of greater than 0.05 μg/ml for 97% of the *Staphylococcus aureus* and 99% of the *Staphylococcus epidermidis* strains tested. However, it is important to note that of the isolates for which MICs were equal to or less than 0.05 μg/ml, a significant percentage (16% of *S. aureus* and 5% of *S. epidermidis*) were beta-lactamase producers. Thus, lack of beta-lactamase production, which implies susceptibility to penicillin, cannot be presumed solely on the basis of low MICs. Beta-lactamase production can be easily predicted from disk diffusion susceptibility tests by observing the appearance of the penicillin inhibition zone edge. A sharply demarcated edge was correlated with beta-lactamase production for 100% of the *S. aureus* and 93% of the *S. epidermidis* strains tested. The presence of this type of zone edge when a penicillin zone measures in the intermediate or susceptible range indicates that the isolate should be checked for beta-lactamase production.

Most laboratories perform penicillin susceptibility testing of staphylococcal isolates either by an agar disk diffusion or a broth dilution method. However, it is generally thought that the best indicator of resistance to penicillinase-sensitive penicillins is the production of beta-lactamase. Our laboratory uses a broth microdilution susceptibility testing method, and we were interested in establishing the correlation between penicillin G minimum inhibitory concentrations (MICs) and the production of staphylococcal beta-lactamase, that is, in determining at what MIC level an isolate could be assumed to be a beta-lactamase producer.

Various investigators have suggested that beta-lactamase production can be predicted by the appearance of the inhibitory zone edge around a penicillin G (or ampicillin) disk (1, 2). There is, however, little information available as to the reliability of this phenomenon. We have attempted to provide information on the usefulness of both the penicillin MIC and the appearance of the penicillin zone edge as predictors of beta-lactamase production for staphylococci.

MATERIALS AND METHODS

Staphylococcal isolates. *Staphylococcus aureus* and *Staphylococcus epidermidis* strains were obtained from clinical specimens submitted to the Microbiology Service Laboratory, National Institutes of Health, Bethesda, Md. Isolates which gave a positive coagulase test were identified as *S. aureus*, whereas those which gave a negative coagulase test were identified as *S. epidermidis*. All strains had microdilution antibiotic susceptibility tests performed, and all were also checked for beta-lactamase production.

Microdilution susceptibility testing. Antibiotic susceptibility determinations were done by using microdilution methods. A change in laboratory procedures occurred during these studies, so that some organisms were tested with the method described by MacLowry et al. (3) and others were tested with the method described by Witebsky et al. (5).

Determination of zone edge appearance. In the performance of the microdilution procedures described above, a purity check plate was always included. For purposes of this study, the check plate consisted of an unsupplemented Mueller-Hinton agar plate which had been flooded with the same inoculum used for the microdilution test (approximately 10^6 organisms per ml). Excess fluid was aspirated off, and a penicillin G disk (10 U) and an oxacillin disk (1 μg) were placed on the surface. These disk diffusion plates were then incubated overnight at 35°C. The appearance of the penicillin zone edge was used to predict whether an isolate was a beta-lactamase producer. Organisms which showed a sharp, well-demarcated edge, either slightly heaped up or with discrete full-size individual colonies right at the edge, were considered positive for beta-lactamase production, whereas those showing a gradual decrease in growth (tapered edge) were considered negative. Figures 1 and 2 show examples of a positive and a negative zone edge, re-

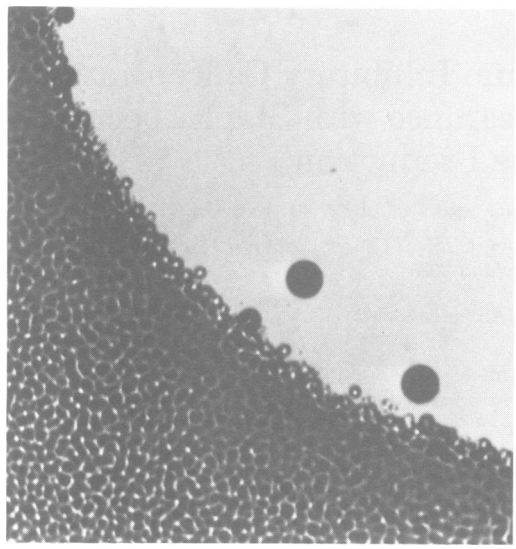

FIG. 1. *Sharply demarcated zone edge with fully developed individual colonies within the inhibition zone. Zone edges with this appearance suggest that the organism tested is a beta-lactamase producer (magnification, ×6).*

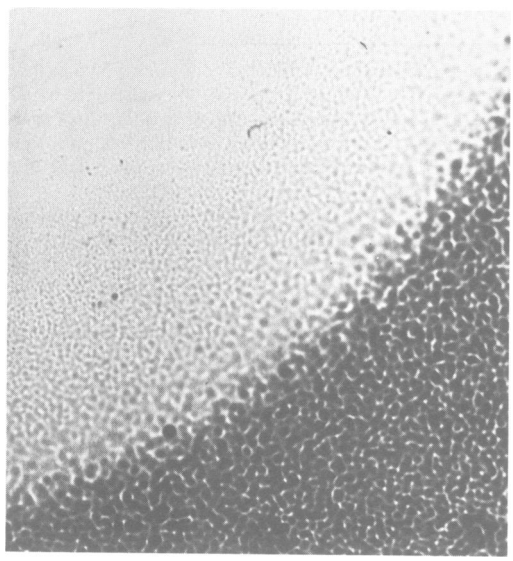

FIG. 2. *Zone edge showing gradual tapering of growth. Zone edges with this appearance suggest that the organism tested is not a beta-lactamase producer (magnification, ×6).*

spectively. When the reader could not decide which type of edge an isolate had, it was classified as uninterpretable. These interpretations were made without knowledge of the penicillin MIC or of the results of the beta-lactamase test. Size of the zone was not considered in the interpretation of the zone edge. Isolates with no zones were not included in this study.

Because our inoculum is significantly lighter than the standard inoculum used for the Kirby-Bauer disk diffusion technique, 10 *S. aureus* and *S. epidermidis* strains were run in parallel with both inoculum methods. These were read in a blind fashion, and no differences were found in the interpretations of the zone edges.

Beta-lactamase production. Detection of beta-lactamase was done with a rapid acidometric method (4). The substrate was dispensed in 0.1-ml amounts into 0.5-ml sample cups (Acculab, Norwood, N.J.), which were covered, sealed with Parafilm (American Can Co., Greenwich, Conn.), and then stored at −60°C. These were brought to room temperature just before use. Since beta-lactamase is inducible in staphylococci, growth from around an oxacillin disk (see above) was used as the inoculum. Although most positive results were obtained within the first 15 min, test reactions were held for 1 h before being regarded as negative. Known positive and negative control strains were tested with each batch of staphylococci.

RESULTS

Table 1 summarizes the results for *S. aureus*. For 7 of 234 (3%) beta-lactamase producers, MICs were equal to or less than 0.05 μg of penicillin G per ml. For an additional 71 (30%), penicillin MICs were between 0.05 and 1.0 μg/ml. For all 38 beta-lactamase-negative organisms, penicillin MICs were equal to or less than 0.05 μg/ml. Interpretation of the zone edge for both beta-lactamase-positive and beta-lactamase-negative organisms showed a 100% correlation for the 276 organisms tested.

The results of similar studies done on *S. epidermidis* are shown in Table 2. For 3 of 213 (1%) beta-lactamase producers, penicillin MICs were equal to or less than 0.05 μg/ml, whereas for 75 (35%), MICs were between 0.05 and 1.0 μg/ml. Interpretation of the zone edge for beta-lactamase production showed correlation for 188 beta-lactamase-positive and 55 beta-lactamase-negative organisms. However, 17 of 260 *S. epidermidis* isolates (7%) had zone edges which were considered uninterpretable by the reader.

TABLE 1. *Penicillin MICs, appearance of the zone edge, and beta-lactamase production for S. aureus*

Penicillin MIC (μg/ml) and zone edge interpretation	No. tested	No. (%) beta-lactamase positive	No. (%) beta-lactamase negative
MIC			
≤0.05	45	7 (3)	38 (100)
>0.05 ≤ 1.0	71	71 (30)	0
>1.0	156	156 (67)	0
Zone edge			
Negative	34	0	34 (100)
Positive	242	242 (100)	0

TABLE 2. *Penicillin MICs, appearance of the zone edge, and beta-lactamase production for S. epidermidis*

Penicillin MIC (μg/ml) and zone edge interpretation	No. tested	No. (%) beta-lactamase positive	No. (%) beta-lactamase negative
MIC			
≤0.05	61	3 (1)	58 (100)
>0.05 ≤ 1.0	75	75 (35)	0
>1.0	135	135 (64)	0
Zone edge			
Negative	55	0	55 (95)
Positive	188	188 (93)	0
Uninterpretable	17	14 (7)	3 (5)

Of these, 14 were beta-lactamase positive and 3 were beta-lactamase negative.

DISCUSSION

Penicillin MICs and beta-lactamase production. The results for both *S. aureus* and *S. epidermidis* showed that from 1 to 3% of isolates can be very susceptible to penicillin at MICs of equal to or less than 0.05 μg/ml and yet still be beta-lactamase producers. These *S. aureus* isolates were retested by using growth from around an oxacillin disk as inoculum to provide beta-lactamase induced cells; these then showed MICs of greater than 0.05 μg/ml. After this study was completed, a review of additional staphylococcal isolates tested between January 1978 and July 1980 showed that 0.8% of 2,028 *S. aureus* and 1.4% of 861 *S. epidermidis* isolates had MICs of equal to or less than 0.05 μg/ml and yet were beta-lactamase producers.

In the present study, approximately 30 to 35% of both *Staphylococcus* spp. showed MICs of between 0.05 and 1.0 μg/ml. It is significant that all of these isolates were beta-lactamase producers. No organisms which were beta-lactamase negative gave MICs which were greater than 0.05 μg/ml.

It has been previously recommended that all *S. aureus* strains tested by microdilution should also be tested for beta-lactamase by a chemical test (4). Our results indicate that laboratories using a microdilution procedure should at least check all staphylococcal isolates for which MICs are equal to or less than 0.05 μg/ml because 7 of 45 (16%) *S. aureus* and 3 of 61 (5%) *S. epidermidis* strains for which MICs were equal to or less than 0.05 μg/ml were beta-lactamase producers. There should be some mechanism in the laboratory reporting system to indicate that staphylococcal isolates for which penicillin MICs are equal to or less than 0.05 μg/ml but are positive for beta-lactamase production should be considered penicillin resistant. Chemical testing of isolates with MICs greater than 0.05 μg/ml would not be necessary since these all appear to be penicillinase producers. The reporting system should specify that isolates for which penicillin MICs are greater than 0.05 μg/ml should be considered beta-lactamase producers which are resistant to penicillin.

Appearance of the zone edge and beta-lactamase production. The appearance of the penicillin zone edge for staphylococci can be a simple, accurate, and useful quality control procedure for detecting beta-lactamase producers which may, on occasion, show intermediate or even susceptible zone sizes by disk diffusion testing. Our results, particularly with *S. aureus*, show that the test was highly reliable. Although we did not use the standard Kirby-Bauer disk test, a limited comparision of zone edge interpretations made from our routine plates and from standard Kirby-Bauer plates showed no difference in the interpretations. This suggests that laboratories using the Kirby-Bauer technique can utilize this zone edge phenomenon. At the least, it can be an inexpensive screen for the detection of beta-lactamase production in isolates showing susceptible or intermediate zone sizes around a penicillin G disk.

Interpretation of the appearance of the zone edge is not difficult once the difference has been shown to those reading disk results. This is particularly true for *S. aureus*. In the present study, greater than 90% of *S. epidermidis* zone edges were also read without difficulty. However, 7% of *S. epidermidis* strains had edges which were uninterpretable; these consisted of both beta-lactamase-positive and beta-lactamase-negative organisms. This may in part be related to the heterogeneity in coagulase-negative staphylococci, the majority of which are *S. epidermidis*, but a small percentage of which (from clinical specimens) may be other species. Our experience with these other species is limited, but recent tests with several reference strains have shown that the zone edges for certain *Staphylococcus* spp., such as *S. cohnii*, may be difficult to interpret; one strain each of *S. xylosus* and *S. saprophyticus* yielded penicillin zone edges which were unambiguously read as beta-lactamase positive, but were negative by the acidometric test. These preliminary results suggest that, probably, for a very small percentage of coagulase-negative staphylococci (most of which would be called *S. epidermidis* by clinical laboratories), one cannot rely on the appearance of the zone edge to predict beta-lactamase production. However, this should not offset the

general usefulness of noting the appearance of the zone edge.

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