Effect of Source of Mueller-Hinton Agar on Detection of Oxacillin Resistance in *Staphylococcus aureus* Using a Screening Methodology

J. A. HINDLER* and N. L. WARNER

Department of Pathology, Clinical Microbiology Section, University of California at Los Angeles Medical Center, Los Angeles, California 90024

Received 17 October 1986/Accepted 9 January 1987

Dehydrated Mueller-Hinton agar from five different manufacturers was used to prepare plates containing 4% NaCl and 6 μg of oxacillin per ml to screen for oxacillin resistance in *Staphylococcus aureus*. A total of 137 isolates which included 109 oxacillin-resistant isolates obtained from at least eight geographic areas were examined. Results were compared to MICs obtained by using a standard broth microdilution method. Results obtained with screening plates prepared with dehydrated media from three of the manufacturers showed 100% correlation with MICs in detecting oxacillin-resistant *S. aureus*, and plates prepared with the remaining two media identified oxacillin resistance in 90 and 99% of the resistant isolates, respectively. None of the oxacillin-susceptible isolates grew on any of the screening plates. This study demonstrated that oxacillin screening plates are reliable for detecting oxacillin-resistant *S. aureus*; however, the source of Mueller-Hinton agar can influence the results.

Accurate and timely detection of oxacillin-resistant *Staphylococcus aureus* continues to be a significant concern for clinical laboratorians. Thornsberry and McDougall (5) have shown that an agar screening method using Mueller-Hinton agar (MHA) with 4% NaCl and 6 μg of oxacillin per ml can be reliably used for this purpose. We have previously reported that the source of MHA can influence the detection of oxacillin-resistant *S. aureus* when disk diffusion and agar dilution methodologies are used (1). This led us to evaluate the ability of MHA from five different sources to detect oxacillin-resistant *S. aureus* in the oxacillin screening test.

Ninety-two oxacillin-resistant *S. aureus* isolates were obtained from eight different geographic areas including Los Angeles, San Francisco, Chicago, Minneapolis, Houston, Iowa City, Boston, and Atlanta. An additional 17 oxacillin-resistant *S. aureus* isolates were obtained from Vitek Systems, Inc., Hazelwood, Mo.; the origin of these isolates was not known. Three *S. aureus* isolates fitting criteria for borderline oxacillin resistance (2) were also included as were 25 isolates of oxacillin-susceptible *S. aureus* obtained from specimens submitted to three clinical microbiology laboratories in California. Each isolate was from a different patient, and the identity of these isolates was established by a positive coagulase test which was performed by the tube method with citrated rabbit plasma (Difco Laboratories, Detroit, Mich.). The isolates were stored at −70°C in brucella broth–15% glycerol and subcultured to Trypticase soy agar containing 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) twice before testing.

Screening plates containing 4% NaCl and 6 μg of oxacillin per ml were prepared as previously described (5) and used within 2 weeks from the date of preparation. All media were prepared simultaneously, and control plates containing MHA and 4% NaCl without oxacillin were included to monitor adequate growth on the base media. Dehydrated MHA was kindly supplied by the following manufacturers: Acumedia Manufacturers, Inc., Baltimore Md.; BBL; Scott Laboratories, Inc., Fiskeville, R.I.; Difco; and GIBCO Laboratories, Grand Island, N.Y. Inocula were prepared by suspending colonies obtained from an 18- to 24-h agar plate culture in Mueller-Hinton broth and standardizing the turbidity to match that of a 0.5 McFarland standard. As in the standard Kirby-Bauer procedure (3), a swab was dipped into the inoculum suspension, and the excess liquid was expelled by pressing the swab against the side of the tube. This swab was then used to streak an area of approximately 5 by 30 mm on the test plates. One suspension was used as the inoculum source for the five different media; however, a fresh swab was used for each inoculation. The plates were examined after 24 h of incubation at 35°C in a non-CO₂-supplemented atmosphere. Any growth was considered evidence that the isolate was oxacillin resistant. The plates were incubated for an additional 24 h and reexamined at the end of the 48-h period.

Oxacillin MICs were determined by a broth microdilution method as described by the National Committee for Clinical Laboratory Standards (4) with trays manufactured in house. Log₂ dilutions of oxacillin at concentrations of 0.25 to 16 μg/ml were prepared in Mueller-Hinton broth (Difco) supplemented with 50 mg of calcium per liter, 25 mg of magnesium per liter, and 2% NaCl. Inocula were prepared by suspending colonies from an 18- to 24-h agar plate culture in Mueller-Hinton broth, further diluting in water containing 0.02% Tween 80, and inoculating the oxacillin dilutions to obtain a final concentration of approximately 5 × 10⁸ CFU/ml. MICs were determined after 24 h of incubation at 35°C in a non-CO₂-supplemented atmosphere. The MIC was defined as the lowest concentration that inhibited growth as determined visually. Isolates for which the MICs were ≤2.0 μg/ml were considered susceptible, those for which the MIC was 4.0 μg/ml were considered borderline, and those for which the MICs were >4.0 μg/ml were considered resistant.

All test isolates grew on all 4% NaCl control plates. Of the 109 oxacillin-resistant *S. aureus* isolates, the MICs for 101 were >16 μg/ml, the MIC for 5 was 16.0 μg/ml, and the MIC for 3 was 8.0 μg/ml. All 109 isolates grew after 24 h of

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* Corresponding author.
incubation on screening plates prepared with MHA 1, MHA 2, and MHA 4 (Table 1). However, only 98 oxacillin-resistant *S. aureus* isolates grew on MHA 3, and 108 oxacillin-resistant *S. aureus* isolates grew on MHA 5. Thus, the last two media were successful in accurately categorizing 90 and 99% of the oxacillin-resistant *S. aureus* isolates, respectively. Of the 11 isolates that failed to grow on MHA 3 at 24 h, 6 did grow after 48 h, as did the one isolate that initially failed to grow on MHA 5. The oxacillin MICs for the isolates that failed to grow on one or both of these media were >16 μg/ml (seven isolates), 16 μg/ml (two isolates), and 8 μg/ml (two isolates).

Of the three borderline-resistant isolates, one grew on all five MHA media and one grew on MHA 1 and MHA 2. The third borderline isolate failed to grow on any of the screening plates at 24 h.

After 24 h of incubation, none of the 25 oxacillin-susceptible isolates, of which the MIC for 8 of these was 2 μg/ml, grew on any of the screening plates. However, five of these (MIC for each of 2 μg/ml) showed scant growth on various media after 48 h of incubation.

These results demonstrate that the source of MHA can play a role in detecting oxacillin-resistant *S. aureus* and deficiencies of some media, as previously noted (1), may not be corrected by the addition of 4% NaCl. With an optimal medium, the screening test accurately identifies oxacillin-resistant *S. aureus* after 24 h of incubation. Extending incubation to 48 h is not always helpful in detecting oxacillin-resistant *S. aureus* when a medium that is less efficient in detection at 24 h is used. Additionally, since any growth on the screening plate is considered indicative of an oxacillin-resistant *S. aureus* isolate, extended incubation may falsely categorize susceptible isolates as resistant. In this study, only oxacillin-susceptible isolates for which the MIC was 2 μg/ml demonstrated growth on screening plates after 48 h of incubation. Although the MIC for the three borderline isolates included in this study was 4 μg/ml, as determined by broth microdilution, two of these isolates grew on at least one screening plate containing 6 μg of oxacillin per ml. We cannot comment on these results because of the small number of borderline isolates surveyed. It should be noted that the five sources of agar used in this study were the same as the five included in our initial study (1) and we did not examine more than one lot from any of the manufacturers.

Oxacillin screening plates incubated for 24 h offer a practical method for identifying oxacillin-resistant *S. aureus* in the clinical laboratory; however, media from certain sources (or perhaps certain lots) may present problems in detecting some oxacillin-resistant *S. aureus* isolates. Like other clinical laboratory tests, effective quality control can increase confidence in the results obtained. Although it is impractical to suggest quality control with a strain affected by deficiencies of the media, as identified in this study, at a minimum, quality control should be performed on oxacillin screening plates by using a confirmed oxacillin-resistant *S. aureus* isolate and an oxacillin-susceptible *S. aureus* isolate (ATCC 29213 or ATCC 25923). The negative control would help to minimize reporting false resistance as plates get older and deterioration of the drug may occur. Respect for appropriate storage recommendations and the expiration date should eliminate this type of error. Finally, it is recommended that all potential oxacillin-resistant *S. aureus* isolates identified on the basis of the oxacillin screening test be confirmed with a standard disk diffusion (3) or MIC (4) test.

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**LITERATURE CITED**


