

Evaluation of Mannitol Salt Agar for Detection of Oxacillin Resistance in *Staphylococcus aureus* by Disk Diffusion and Agar Screening

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Mannitol salt agar was evaluated for detection of oxacillin resistance in 136 *Staphylococcus aureus* isolates. All *mecA*-positive isolates ($n = 54$) were correctly categorized as oxacillin resistant by the disk diffusion test (1- μg disk; zone diameter, <16 mm); the specificity was 97.6%. Agar screening (2 μg of oxacillin per ml) revealed a sensitivity of 98.1% and a specificity of 95.1%.

In the United States and Europe, nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) remain a prominent problem (17, 33) resulting in the performance of many surveillance cultures for detection and monitoring of MRSA. Several body sites of MRSA index patients, contact patients, and nursing and medical staff members may require investigation, depending on the surveillance scheme in a hospital (9). Many methods to detect oxacillin resistance in *S. aureus* have been evaluated and are widely used in bacteriological laboratories, including the following: agar dilution on Mueller-Hinton agar with 6 μg of oxacillin per ml (26), disk diffusion (4 μg of oxacillin) on Mueller-Hinton agar (15), disk diffusion (1 μg of oxacillin) on Mueller-Hinton or Iso-Sensitest agar (10), MIC determination by broth dilution (18), oxacillin broth screening test (10), automatic systems such as the VITEK (11), or even flow cytometry (24). Mannitol salt agar (MSA) has been used since 1945 as a selective medium for the isolation of pathogenic staphylococci (3, 4) and was found to be valuable for use with specific specimens such as sputum from patients with cystic fibrosis (22). MSA was first investigated as a medium for susceptibility testing in 1985 (12). Its value as a screening medium for MRSA has been investigated in several studies (13, 16). It has been further evaluated as a primary isolation medium for recovery of MRSA when containing 6 μg of oxacillin per ml (28). Recently, it has been suggested that MSA might be a promising medium for use in the disk diffusion method with a 1- μg oxacillin disk or for agar screening, especially for surveillance cultures (10). Therefore, we evaluated MSA for use in disk diffusion and agar screen tests.

MATERIALS AND METHODS

Bacterial strains, genotyping, and identification. One hundred fifty-one clinical isolates previously identified as *S. aureus* by agglutination tests were initially included in the study (71 MRSA isolates; 80 methicillin-susceptible *S. aureus* [MSSA] isolates). Five MRSA isolates originally came from Argentina, 8 were from Belgium, 24 were from Canada, 29 were from Germany, and 5 were from Switzerland; all 80 MSSA isolates were from Germany. All MRSA isolates were screened for clonal identity by pulsed-field gel electrophoresis (PFGE) (*Sma*I digest) prior to evaluation experiments (19). Patterns were compared by three investigators, and isolates were grouped in accordance with the criteria reported by Tenover et al. (25). Two isolates from Belgium and four isolates from Canada were excluded from further experiments because their PFGE patterns were found to be indistinguishable from those of other isolates. The remaining 65 MRSA isolates were found to be either distinct clones ($n = 54$) or epidemiologically related ($n = 11$). Identification of the species *S. aureus* was confirmed

by detection of the coagulase gene by PCR (21) and by tube coagulation (rabbit plasma; bioMérieux, Nürtingen, Germany) by using the criteria of Sperber and Tatini (23). Eight of the remaining 145 isolates did not have a coagulase gene, and one of the coagulase-gene-positive isolates failed to show plasma coagulation; all nine of these isolates were excluded. This resulted in a total of 136 *S. aureus* isolates that were used for further experiments.

Disk diffusion test. A sterile swab was dipped in an *S. aureus* suspension (McFarland standard 0.5) and plated onto MSA (CM 85; Oxoid, Basingstoke, England). Oxacillin disks (1 μg ; Becton Dickinson, Heidelberg, Germany) were applied by using a sterile forceps. Agar plates were incubated at $36 \pm 1^\circ\text{C}$ for 24 h. The zone of inhibition was documented in millimeters (no inhibition zone was noted as 0 mm). An isolate was classified as resistant to oxacillin when the inhibition zone was less than 16 mm in diameter (10).

Agar screen test. An aliquot of 10 μl of a 1:100 dilution of a bacterial suspension (McFarland standard 0.5) was placed on MSA with 2 μg of oxacillin per ml (18). All plates were incubated for 24 h at $36 \pm 1^\circ\text{C}$ and assessed for the presence of colonies. An isolate was regarded as resistant to oxacillin when growth of at least one typical *S. aureus* colony was detected at the inoculation site on the agar plate.

MIC of oxacillin (E-test). The MICs (of oxacillin) were determined by using the E-test. A sterile swab was dipped into an inoculum suspension (McFarland standard 0.5). Excess fluid was removed by rotating and pressing the swab firmly against the inside wall of the test tube. The entire surface of a Mueller-Hinton agar plate (2% NaCl supplement) was swabbed three times by rotating the plate each time to ensure an even distribution of the inoculum (2). An E-test strip was placed aseptically onto the agar plate. After incubation at $36 \pm 1^\circ\text{C}$ for 18 to 24 h, the MIC was read at the point of intersection between the zone edge and the E-test strip.

PCR for detection of the *mecA* gene. All *S. aureus* isolates were investigated for the presence of the *mecA* gene (PCR product, 533 bp) by using PCR with the *mecA1* and *mecA2* primers as described before (10).

Statistics. Sensitivity and specificity rates were calculated for the disk diffusion and agar screen tests. To choose the best cutoff for the zone diameter of the disk diffusion test, sensitivity was plotted versus $1 - \text{specificity}$ in the form of a receiver operating characteristic (ROC) curve (1).

RESULTS

Of all 136 *S. aureus* isolates included in the study, 54 were found to be *mecA* positive and 82 were found to be *mecA* neg-

TABLE 1. Evaluation of the disk diffusion (1 μg of oxacillin) and agar screen (2 μg of oxacillin per ml) tests on MSA for detection of oxacillin resistance in *S. aureus* isolates ($n = 136$)

Presence of the <i>mecA</i> gene	Total no. of isolates	No. of isolates			
		Disk diffusion on MSA with zone diam of:		Agar screening on MSA	
		<16 mm	≥ 16 mm	Growth	No growth
<i>mecA</i> positive	54	54	0	53	1
<i>mecA</i> negative	82	2	80	4	78

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TABLE 2. Disk diffusion zone diameters (1- μ g oxacillin disk) on MSA relative to the presence of the *mecA* gene in 136 *S. aureus* isolates

Zone diam (mm)	No. of isolates		Total
	<i>mecA</i> gene negative	<i>mecA</i> gene positive	
0	0	53	53
15	2	1	3
16	2		2
18	1		1
19	1		1
20	4		4
21	3		3
22	9		9
23	6		6
24	13		13
25	14		14
26	11		11
27	5		5
28	4		4
29	4		4
30	1		1
32	2		2
Total	82	54	136

ative. The presence or absence of the *mecA* gene was regarded as the reference to detect oxacillin resistance (27).

All 54 *mecA*-positive isolates were correctly identified as oxacillin resistant by disk diffusion on MSA (sensitivity, 100%), and 80 of the 82 *mecA*-negative isolates were found to be oxacillin susceptible (specificity, 97.6%), resulting in a positive predictive value of 96.4% and a negative predictive value of 100% (Table 1). The agar screen test on MSA was found to be highly sensitive, too (98.1%), with a lower specificity (95.1%), resulting in a positive predictive value of 93.0% and a negative predictive value of 98.7% (Table 1).

The inhibition zone diameters (in millimeters) relative to the number of isolates with the *mecA* gene present are shown in Table 2. A ROC curve was plotted (Fig. 1). A zone diameter of <15 mm results in a 100% specificity rate and a 98.1% sensitivity rate of detecting oxacillin resistance, whereas a zone

diameter of <16 mm results in a 100% sensitivity rate and a 97.6% specificity rate.

The zone diameters (in millimeters) relative to the MICs (of oxacillin) are demonstrated in Fig. 2 as a scattergram with a linear regression curve. The correlation coefficient was -0.802 (Pearson's correlation coefficient, two-tailed). The breakpoint MICs (of oxacillin) for *S. aureus* are ≤ 2 μ g/ml (susceptible) and ≥ 4 μ g/ml (resistant) (18). For the resulting ROC curve, any isolate for which the MIC was ≤ 2 μ g/ml was regarded as susceptible and the remaining isolates were regarded as resistant to oxacillin. Figure 3 shows that only with a zone diameter of <15 mm could oxacillin resistance be detected at a specificity rate of 100% and that zone diameters of ≤ 16 mm result in detection at a 100% sensitivity rate.

DISCUSSION

MSA was developed in 1945 as a selective medium for the isolation of pathogenic staphylococci (4). It is regarded as a valuable medium for the isolation of *S. aureus* from water, milk, the skin, respiratory tract secretions, and the nose (6, 20, 32), although subinhibitory concentrations of a beta-lactam compound have been reported to result in failure of *S. aureus* to grow on MSA (14). Since 1985, MSA has been studied with regards to its suitability as a medium for susceptibility testing (12).

In the present investigation, the disk diffusion test (1 μ g of oxacillin) on MSA was found to be an excellent screening method to detect oxacillin resistance in *S. aureus* (sensitivity, 100%; specificity, 97.6%). Our data indicate that an *S. aureus* isolate with an inhibition zone diameter on MSA of <16 mm (oxacillin disk, 1 μ g) should be reported as resistant. Four strains were *mecA* negative with zone diameters of ≤ 16 mm. It may be useful to consider isolates with a zone diameter of between 14 and 16 mm as intermediately resistant and to confirm the result by an alternate test (e.g., E-test). The agar screen test (2 μ g of oxacillin per ml) on MSA was almost as sensitive (98.1%) and specific (95.1%) as the disk diffusion test. In another study, direct plating of swabs onto methicillin-containing MSA (4 μ g/ml) revealed a lower detection rate of MRSA after 18 h of incubation (sensitivity, 66%) (5).

Lipovitellin-salt-mannitol agar has been evaluated for disk

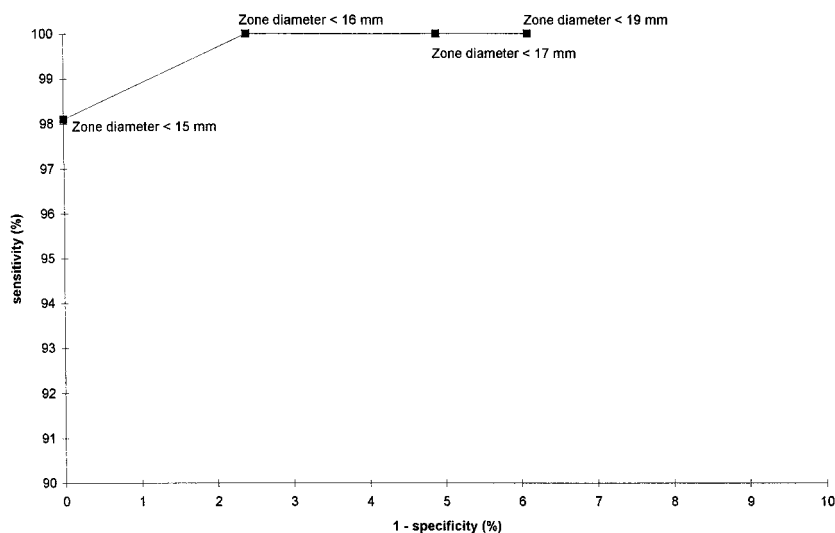


FIG. 1. ROC curve based on specificity and sensitivity rates of the disk diffusion test on MSA (oxacillin, 1 μ g) for *S. aureus* isolates positive for the *mecA* gene.

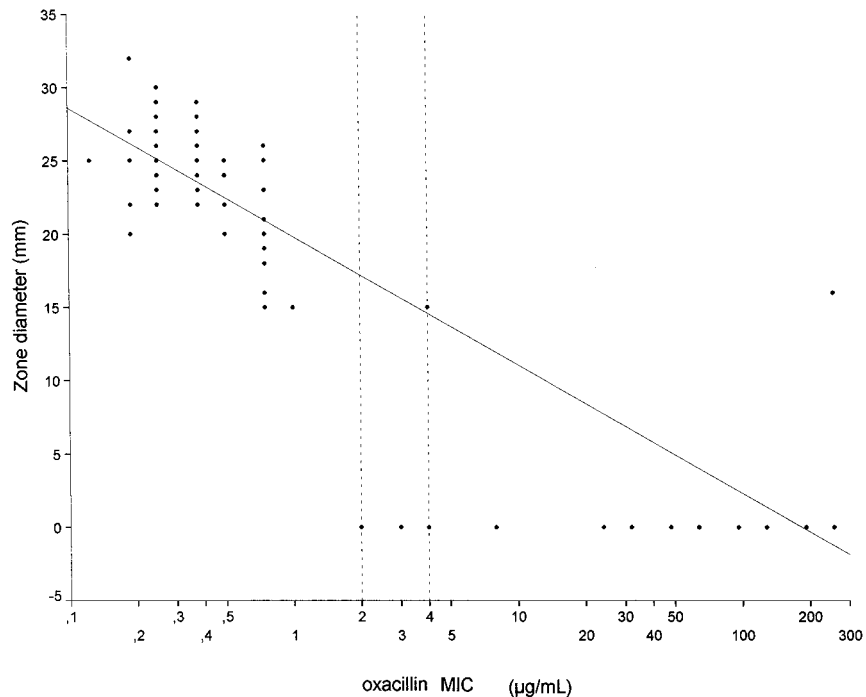


FIG. 2. Scattergram with a linear regression curve resulting from plotting inhibition zone diameters for oxacillin resistance on MSA and the E-test oxacillin MICs. The National Committee for Clinical Laboratory Standards breakpoints for oxacillin resistance are shown as dotted lines.

diffusion (1 µg of oxacillin) by using 97 MRSA (MIC breakpoint, ≤ 4 µg/ml) and 56 MSSA isolates. A zone diameter of < 13 mm was interpreted as evidence of oxacillin resistance. The medium was found to have a sensitivity of 100% and a specificity of 70% in detecting MRSA isolates (31). In a previous report on 89 *mecA*-positive *S. aureus* isolates, the disk diffusion test on MSA was found to have a 100% sensitivity (zone diameter of < 16 mm = resistant) (10). Oxacillin-MSA has been used as a screening medium in national and international studies on the frequency of MRSA (29, 30) and is recommended by the National Committee for Clinical Laboratory Standards (18).

The salt concentration in the medium is known to be of great importance for *S. aureus*. It has been reported that salt supplements of 2 or 4% to Iso-Sensitest agar significantly improve the sensitivity of disk diffusion testing with oxacillin (8, 10). MSA contains 7.5% NaCl. This salt concentration is unique in a medium for testing oxacillin susceptibility. It might be worthwhile to evaluate the use of Iso-Sensitest agar with an even larger salt supplement (e.g., 6 or 7.5%) as the medium for disk diffusion testing. Although Mueller-Hinton agar is still recommended and used, it has the disadvantage of a variable reproducibility of results (7).

In summary, our study shows that MSA is a suitable medium

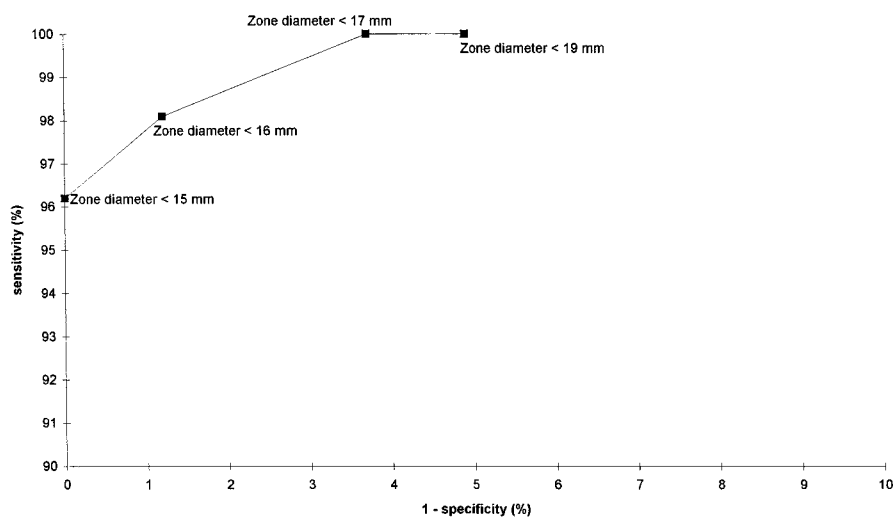


FIG. 3. ROC curve based on specificity and sensitivity rates of the disk diffusion test on MSA (oxacillin, 1 µg) for *S. aureus* isolates relative to MICs (oxacillin susceptible = MIC of ≤ 2 µg/ml; oxacillin resistant = MIC of ≥ 4 µg/ml); two isolates with MICs of 3 µg/ml were excluded from this analysis.

for disk diffusion and agar screen testing to detect oxacillin resistance in *S. aureus*.

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