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Evaluation of Mannitol Salt Agar with Oxacillin as a Screening Medium for Methicillin-Resistant *Staphylococcus aureus*

RICHARD T. LALLY,* MARY N. EDERER, AND BERT F. WOOLFREY

Clinical Microbiology Section, Department of Anatomic and Clinical Pathology, St. Paul-Ramsey Medical Center, St. Paul, Minnesota 55109

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We evaluated the use of mannitol salt agar with oxacillin for use as a primary screening medium for the simultaneous detection and identification of methicillin-resistant *Staphylococcus aureus* in clinical surveillance specimens. Oxacillin agar dilution susceptibility tests with mannitol salt agar and Mueller-Hinton agar were performed in parallel with disk-agar diffusion testing on 95 oxacillin-susceptible and 105 oxacillin-resistant *S. aureus* stock isolates. MICs were found to be comparable, showing distinct separation of susceptible and resistant isolates into two groups with MICs of ≤2 and ≥32 μg/ml, respectively. In accord with these findings, 4 μg of oxacillin per ml was selected for use in the screening medium. For performance evaluation, mannitol salt agar with 4 μg of oxacillin per ml was compared with mannitol salt agar without oxacillin by performing parallel screening tests on 153 clinical surveillance specimens. For detection of methicillin-resistant *S. aureus*, mannitol salt agar with 4 μg of oxacillin per ml was as sensitive as mannitol salt agar without oxacillin and required significantly fewer confirmatory tests. For primary identification of methicillin-resistant *S. aureus*, mannitol salt agar with 4 μg of oxacillin per ml was 6.4% false-positive and 1.1% false-negative, with a 93.6% positive predictive value. These findings indicate that mannitol salt agar with 4 μg of oxacillin per ml can be used as a reliable and cost-effective screening medium for the simultaneous detection and identification of methicillin-resistant *S. aureus* in clinical surveillance specimens.

The incidence of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* continues to increase in the United States, especially in burn and intensive-care units of large hospitals affiliated with medical schools. Screening programs for detection and monitoring of methicillin-resistant *S. aureus* outbreaks commonly involve the surveillance of many patients with cultures derived from several body sites. The present study evaluates the use of mannitol salt agar (MSA; BBL Microbiology Systems, Cockeysville, Md.) with oxacillin (Bristol Laboratories, Syracuse, N.Y.) as a cost-effective single screening medium for detection and identification of methicillin-resistant *S. aureus*.

MATERIALS AND METHODS

Experimental design. (i) Evaluation of MSA for oxacillin susceptibility testing of *S. aureus*. Agar dilution tests with MSA were performed in parallel and compared with the results of both agar dilution and modified disk-agar diffusion testing with Mueller-Hinton agar (MHA; BBL) for 95 oxacillin-susceptible *S. aureus* and 105 oxacillin-resistant *S. aureus* stock clinical isolates. MSA susceptibility tests were incubated at 35°C to judge the performance of MSA at the temperature most likely to be used in most laboratories for a screening program. MHA susceptibility tests were incubated at 30°C to provide optimum detection of methicillin-resistant *S. aureus* (1). Susceptibility tests were interpreted at both 24 and 48 h. The results for MSA and MHA were then compared to assess the performance of MSA as an oxacillin susceptibility test medium, to ensure agreement between agar dilution and disk-agar diffusion test results, and to select an optimum oxacillin concentration for use with MSA as a screening medium. As shown in Results and discussed below, 4 μg of oxacillin per ml was selected.

* Corresponding author.

(ii) Evaluation of MSA with MSA-4 Ox as a screening medium for detection of methicillin-resistant *S. aureus* in clinical surveillance specimens. Parallel screening cultures with MSA containing 4 μg of oxacillin per ml (MSA-4 Ox) and MSA without oxacillin were performed on 153 Culturette swab (Marion Scientific, Kansas City, Mo.) surveillance specimens originating from patients in the Burn Unit of St. Paul-Ramsey Medical Center. Swab specimens were processed and plated on the two media in a manner designed to eliminate inoculum bias. Plates were incubated in air at 35°C for 48 h and were examined for the presence of colonies with the morphotypic features of *S. aureus* on MSA. All morphotypes on each plate were tested for oxacillin susceptibility by the disk-agar diffusion test with MHA incubated at 35°C and evaluated at 24 h. Gram stain, catalase, and tube coagulase tests were performed from growth on the disk-agar diffusion test plates to confirm *S. aureus* identification. MSA-4 Ox and MSA results were then compared to assess their relative efficacy and cost effectiveness.

***S. aureus* isolates.** For the first portion of the study, 95 oxacillin-susceptible and 105 oxacillin-resistant *S. aureus* clinical isolates were randomly selected from the St. Paul-Ramsey Medical Center Clinical Microbiology Laboratory stock culture collection. Each isolate had previously been identified as *S. aureus* by Gram stain, catalase reaction, and tube coagulase reaction, and oxacillin susceptibility had been determined by the standard disk-agar diffusion test performed according to the recommendations of the National Committee for Clinical Laboratory Standards (9). Each isolate was retrieved from −70°C by two 24-h passages on blood agar plates incubated in air at 35°C. Material selected from several well-isolated colonies on the second stock retrieval plate was used to prepare inoculum suspensions for the parallel MSA and MHA dilution and MHA disk-agar diffusion susceptibility tests.

TABLE 1. Comparison of MSA and MHA oxacillin susceptibility test results as determined by agar dilution and disk-agar diffusion tests performed on 200 *S. aureus* isolates

Disk-agar diffusion test results (no. of isolates)	Medium (conditions)	No. of isolates assigned to MIC (μg/ml) category:							
		<1	1	2	4	8	16	32	>32
Resistant (105)[a]	MHA-Ox[c] (30°C, 48 h)			1	1	1	1	9	92
	MSA-Ox[d] (35°C, 48 h)							99	6
Susceptible (95)[b]	MHA-Ox (30°C, 48 h)	93	1	1					
	MSA-Ox (35°C, 48 h)	76	15	4					

[a] Zone diameter, ≤10 mm.
[b] Zone diameter, ≥13 mm.
[c] MHA-Ox, MHA with oxacillin.
[d] MSA-Ox, MSA with oxacillin.

For the second portion of this study, 153 routine methicillin-resistant *S. aureus* clinical surveillance specimens were plated on both MSA-4 Ox and MSA plates. Specimens were received as Culturette swabs that had been maintained in transport broth in the Culturette tube for not more than 2 h after patient sampling. Each swab was placed in a tube containing 0.5 ml of tryptic soy broth and was vigorously swirled to remove and disperse specimen material into the broth for use as a common inoculum suspension. Separate swabs saturated with the common inoculum suspension were then used to inoculate the MHA-4 Ox and MSA screening plates.

MSA and MHA dilution tests. MSA and MHA were prepared according to the instructions of the manufacturer and were used to prepare agar dilution plates containing oxacillin concentrations in twofold dilution steps from 1.0 through 32 μg/ml. Plates were allowed to gel for 1 h at room temperature and were then stored in plastic bags at 4°C for not more than 1 week before use. Quality control with *S. aureus* ATCC 29213 was performed at the time of plate preparation and with each experimental batch. Agar dilution plates were inoculated with a replicator device designed to deliver approximately 10^4 CFU to each inoculum point by inoculum suspensions prepared with colony material selected from the second stock retrieval plates. MSA dilution plates were incubated in air at 35°C, and MHA dilution plates were incubated in air at 30°C. The plates were examined and interpreted at both 24 and 48 h. Oxacillin susceptibility and resistance for MHA testing was defined as MICs of ≤2 and >2 μg/ml, respectively.

MHA disk-agar diffusion test. MHA disk-agar diffusion plates were prepared according to the instructions of the manufacturer and filled to a depth of 4 mm. Plates were allowed to gel at room temperature for 1 h and were then stored in plastic bags at 4°C. Procedural steps for the disk-agar diffusion tests, including the use of 1-μg oxacillin disks, corresponded exactly with the recommendations of the National Committee for Clinical Laboratory Standards (9), except for the use of 30°C incubation. Inhibition zone susceptibility (≥13 mm) and resistance (≤10 mm) breakpoints for MHA corresponded to the recommendations of the National Committee for Clinical Laboratory Standards (9). Quality control with *S. aureus* ATCC 25923 was performed at the time of medium preparation and with each test batch.

MSA-4 Ox and MSA screening plates. Screening plates containing 4 μg of oxacillin per ml were prepared as described above for the MSA dilution plates. MSA screening plates were prepared according to the instructions of the manufacturer. Parallel MSA-4 Ox and MSA screening plates were inoculated by separate swabs saturated with material from the common inoculum suspension prepared from the clinical specimen. The plates were incubated in air at 35°C and were examined at 24 and 48 h for the presence of colonies with morphotypic features that have been described for *S. aureus* on MSA (4). Specifically, regardless of size, colonies were considered to be potentially *S. aureus* if they were circular, smooth, and opaque and showed signs of mannitol utilization as indicated by lemon-yellow colony color and the presence of a yellow zone in the adjacent medium. In addition, we included large (≥1 mm), circular, smooth, opaque, yellow or white colonies having no yellow zone, since our experience with MSA over a 9-year period has indicated that these morphotypes are consistently identified as *S. aureus* (unpublished data). Each morphotype on each plate was selected for oxacillin disk-agar diffusion susceptibility testing. Growth from the susceptibility plates was used to confirm the presence of gram-positive, catalase-positive, and tube coagulase-positive cocci.

RESULTS

Table 1 presents a comparison of MSA and MHA oxacillin susceptibility results for 200 *S. aureus* stock isolates. Results for MHA dilution and disk-agar diffusion testing were identical at 24 and 48 h of incubation; however, MSA dilution results were uninterpretable until 48 h because of insufficient growth at 24 h of incubation. For the 95 isolates susceptible by disk-agar diffusion testing, all had MICs of ≤2 μg/ml by both MSA and MHA dilution. For the 105 isolates resistant by MHA disk-agar diffusion testing, all had MICs of ≥32 μg/ml by MSA dilution. MSA and MHA dilution MICs were generally comparable, with the latter showing slightly lower values for a few isolates. For MSA and MHA dilution MICs, a total of 103 (98%) were within ±2 twofold dilution steps for the resistant isolates, and 100% were within ±2 twofold dilution steps for the susceptible isolates. In accord with the comparability of MSA and MHA MICs and the clear bimodal separation of susceptible and resistant isolates, 4 μg of oxacillin per ml was selected for use in the methicillin-resistant *S. aureus* screening study.

The results of screening clinical specimens for the presence of methicillin-resistant *S. aureus* with MSA-4 Ox and MSA without oxacillin are presented in Table 2. Of the 72 (47%) clinical specimens that yielded methicillin-resistant *S. aureus* from the MSA-4 Ox or the MSA plates or both, MSA-4 Ox detected 71 (98.6%). The single positive specimen not detected by MSA-4 Ox grew only two methicillin-resistant *S. aureus* colonies (MIC, ≥32 μg/ml) on the corresponding MSA plate. Testing of all 162 colony morphotypes, including those <1 mm in diameter, selected from the 153 MSA screening plates resulted in false-positive and false-negative rates of 47.5 and 0%, respectively, for methicillin-resistant *S. aureus* detection and identification. When morphotype selection was limited to the 110 large (>1 mm) colonies with characteristic *S. aureus* morphotype, false-positive and false-negative methicillin-resistant *S. aureus* rates became 22.7 and 0%, respectively. For the 115 morphotypes, including those <1 mm in diameter selected from the 153 MSA-4 Ox screening plates, false-positive and false-negative rates were 23.5 and 1%, respectively, for methicillin-resistant *S. aureus* detection and identification. When morphotype selection was limited to the 94 large (>1

TABLE 2. Comparison of MSA-4 Ox and MSA used as primary screening media for detection of methicillin-resistant *S. aureus* in 153 clinical surveillance specimens

Media (all morphotypes)	No. of selected morphotypes[a]	No. of isolates			Sensitivity[b]	Predictive +[c]
		Methicillin-resistant *S. aureus*	False-positive	False-negative		
MSA (162)	110	85	25	0	100	77.3
MSA-Ox (115)	94	88	6	1	98.9	93.6

[a] Colonies were yellow or white, ≥ mm only.
[b] (True-positive/true-positive + false-negative) for detection of methicillin-resistant *S. aureus* by colony morphology.
[c] (True-positive/true-positive + false-positive) for detection of methicillin-resistant *S. aureus* by colony morphology.

mm) colonies with characteristic *S. aureus* morphotype, false-positive and false-negative methicillin-resistant *S. aureus* detection rates were 6.4 and 1.1%, respectively. With all morphotypes, predictive values for finding methicillin-resistant *S. aureus* in a screening specimen were 52.5 and 76.5%, respectively, for MSA and MSA-4 Ox plates. When only characteristic *S. aureus* morphotypes were selected, predictive values for detecting methicillin-resistant *S. aureus* improved and were 77.3 and 93.6% for MSA and MSA-4 Ox, respectively. A total of 13 specimens from three patients with no methicillin-resistant *S. aureus* yielded no growth or no methicillin-resistant *S. aureus* morphotypes on MSA-4 Ox plates. For the same specimens, six required additional testing, because of the appearance of characteristic *S. aureus* morphotypes, to rule out methicillin-resistant *S. aureus* on MSA plates.

DISCUSSION

The epidemiological monitoring of methicillin-resistant *S. aureus* has become an essential component in the control of nosocomial infections in a number of large hospitals in the United States (2, 3, 8, 11, 12). The identification of *S. aureus* and the determination of methicillin resistance by routine isolation media and methods is generally too cumbersome and expensive for use in most surveillance programs. Although acid production from mannitol has been described as a means for differentiation of coagulase-positive and coagulase-negative staphylococci (5–7), the use of MSA plates as methicillin-resistant *S. aureus* screening media requires secondary confirmatory and susceptibility testing for all isolates thought to be *S. aureus* on primary isolation. In the present investigation, we attempted to define a single medium that would be capable of both detection and identification of methicillin-resistant *S. aureus* on primary culture.

As a basis of test development, we first demonstrated the similarity of oxacillin susceptibility test results with MSA and MHA as agar dilution test media. Both MSA and MHA susceptibility testing separated randomly chosen oxacillin-susceptible and oxacillin-resistant *S. aureus* stock isolates into two distinct groups with MICs of ≤2 and ≥32 μg/ml, respectively. In accord with the comparability of MSA and MHA susceptibility results, and on the basis of the distinct bimodal pattern of the susceptible and resistant isolates, 4 μg of oxacillin per ml was selected for use with MSA as a potential single medium to be used for detection and identification of methicillin-resistant *S. aureus* in primary cultures of surveillance specimens. As the lowest concentration in the range between our susceptible and resistant isolates, and conforming with the National Committee for Clinical Laboratory Standards (10) breakpoint (≥4 μg/ml) for oxacillin resistance, the 4-μg/ml concentration might be expected to err on the side of producing a small percentage of false-positive results, a situation acceptable for most screening procedures. In our investigation of its use as a primary screening medium, MSA-4 Ox detected the presence of methicillin-resistant *S. aureus* with essentially the same sensitivity as did MSA without oxacillin, and significantly decreased the number of isolates (115 versus 162) requiring confirmatory identification and susceptibility tests. When testing was limited to characteristic *S. aureus* morphotypes, the numbers of false-positive results for methicillin-resistant *S. aureus* from MSA and MSA-4 Ox plates were, respectively, 25 (22.7%) and 6 (6.4%). As expected, the use of MSA-4 Ox resulted in a small number of false-positive (6) and false-negative (1) results, with positive and negative predictive values of 93.7 and 96.4%, respectively. If the false-positive rate of 6.4% was deemed acceptable, as is the case for most screening tests, no secondary confirmatory identification and susceptibility tests would be required for any potential methicillin-resistant *S. aureus* isolates selected from MSA-4 Ox screening plates. If a 0% false-positive rate was deemed desirable, all MSA-4 Ox isolates could be submitted to confirmatory identification and susceptibility testing, but would still obviate a significant portion of the follow-up work necessitated by the use of MSA without oxacillin. The false-negative rate for MSA-4 Ox resulted from one surveillance specimen that had no *S. aureus*-compatible colonies on MSA-4 Ox and only two colonies on MSA without oxacillin, a situation suggesting that the false-negative rate was probably related more to the small numbers of methicillin-resistant *S. aureus* within the sureillance specimen than to the characteristics of the two screening media. The small false-negative rate, even if caused by media differences, would be diminished and insignificant in most screening programs since specimens from several body sites are usually submitted. In accord with our findings, we suggest that MSA-4 Ox can be used as a reliable and cost-effective screening medium for detection and identification of methicillin-resistant *S. aureus* in surveillance specimens.

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