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Screening Method for Recovery of Methicillin-Resistant *Staphylococcus aureus* from Primary Plates

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A study designed to screen for the presence of methicillin-resistant *Staphylococcus aureus* from primary plates was conducted from 1 January to 1 September 1985 in a small community hospital. The screening method used a plate of lipovitellin salt mannitol agar and a 4-μg oxacillin disk incubated at 30°C. Growth of yellow colonies, typical of *S. aureus*, around the disk without a zone of inhibition was called presumptive methicillin-resistant *S. aureus*. All susceptibilities were confirmed by using the National Committee for Clinical Laboratory Standards macrodilution technique. Of 224 cultures containing *S. aureus*, 118 (53%) were positive for methicillin-resistant *S. aureus* isolates. Of these 118, 111 (94%) were correctly identified from the primary plates as methicillin-resistant *S. aureus*. Of the 224 isolates, 14 could not be categorized from the primary plates as methicillin-resistant *S. aureus* due to the small amounts of *S. aureus* recovered.

Methicillin-resistant *Staphylococcus aureus* was first isolated in Great Britain in 1961 (2). In the late 1970s and early 1980s, it became a rapidly emerging clinical and epidemiological problem in the United States, seen most frequently in patients in tertiary-care hospitals (1, 5). Since that time, these patients have filtered through the health care system, so that methicillin-resistant *S. aureus* is now commonly recovered from nursing home patients treated at small community hospitals. At our 100-bed hospital, a large number of patients transferred from nursing homes and colonized with methicillin-resistant *S. aureus* precipitated a need for a more rapid identification of the organism. This study evaluated the use of lipovitellin salt mannitol (LSM) agar with a 4-μg oxacillin disk as an effective single-plate method for the isolation and presumptive identification of methicillin-resistant *S. aureus* from a primary plate.

MATERIALS AND METHODS

Evaluation of screening media. Two media were originally considered for the experiment: Mueller-Hinton agar supplemented with 4% NaCl and 6 μg of oxacillin per ml (MHA-S) (16) and LSM agar (4) with a 4-μg oxacillin disk (10) (Bristol Laboratories, Syracuse, N.Y.). LSM agar is a mannitol salt agar base with 20 mg of egg yolk added per ml, which provides an additional lecithinase reaction. An initial test with swabs from the decubitus ulcers of 10 patients was performed to evaluate the general characteristics of each medium. The criteria for medium evaluation were colony morphology, selectivity, and ease of isolation. As shown below, the LSM agar with the 4-μg disk was found to be better suited for our patient population.

Specimens. The specimens used in the study were routine bacterial cultures received in our laboratory on Culturettes (Marion Scientific Div. Marion Laboratories, Inc., Kansas City, Mo.) and contained *S. aureus*. The sources included decubitus ulcers, surgical wounds, draining wounds, and eye, sputum, and blood cultures. Nasal and axillary swabs were also obtained from patients admitted from one particular nursing home. During the 8-month study, 224 of these cultures were found to contain *S. aureus*.

Antimicrobial agents. Following the recommendations of McDougal and Thornsberry (10), a 4-μg oxacillin disk was chosen as the antimicrobial agent. Because disks of this potency are not available for purchase, oxacillin powder suitable for susceptibility testing was obtained from Bristol Laboratories. The disks were prepared by adding to each blank 6-mm filter paper disk (3) 0.025 ml of antibiotic solution (11) with a concentration 40 times that of the desired final concentration (10, 11). These disks were cold-vacuum desiccated and stored at −30°C. Because a −70°C freezer was not available, disks were made in small quantities and discarded after 2 months if unused. Quality control of disk potency by using known strains of methicillin-resistant and non-methicillin-resistant *S. aureus* was performed weekly.

Screening procedure. For the study, LSM agar was added to the regimen of media routinely set up on all bacterial wound cultures. On all other cultures, however, when the initial Gram stain revealed organisms suggestive of staphylococci, an LSM agar was set up. A 4-μg oxacillin disk was placed in quadrant 1 of the LSM agar plate, which was then incubated at 30°C (12, 17). The remaining plates were incubated at 35 to 36°C, consistent with our standard microbiology procedures. Plates were examined at 24 and 48 h, with an additional reading of the LSM agar plates at approximately 30 h. LSM agar plates were examined for growth of yellow colonies typical of *S. aureus* around the oxacillin disk. Occasionally, a few *S. aureus* colonies that were not recovered from the other media were found on the LSM agar plate. These colonies were presumptively identified as methicillin-resistant *S. aureus* by catalase and coagulase reactions, colony morphology, and growth up to the oxacillin disk when adequate growth was present.

Susceptibility tests. MICs were determined by the macrodilution broth method as described by the National Committee for Clinical Laboratory Standards (11), except that 2% NaCl was added to the cation-supplemented Mueller-Hinton broth (16). The Micro-Media Gram Positive Fox Panel (Micro-Media Systems, Inc., Potomac, Md.), a commercially available microdilution method, is used routinely in our laboratory. Although this method does not

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TABLE 1. Growth characteristics of organisms on LSM agar and MHA-S

Organism	Growth characteristic(s) on:	
	LSM agar	MHA-S
Staphylococcus aureus	Yellow colonies; yellow agar, agar precipitate[a]	White or yellow colonies
Staphylococcus epidermidis	White colonies; pink agar	White colonies
Streptococcus faecalis	Clear colonies; yellow agar	Pinpoint colonies
Diphtheroids	Pinpoint colonies; pink agar	No growth
Pseudomonas aeruginosa	No growth	Large spreading colonies
Proteus mirabilis	No growth	Large spreading colonies
Candida albicans	No growth	Pinpoint colonies

[a] Agar precipitate, Clouding occurred in the medium surrounding the colony.

supplement the oxacillin broth dilutions with salt and has been found to recover only 71% of the methicillin-resistant *S. aureus* isolates (6), it was also used on all presumptive methicillin-resistant *S. aureus* isolates. The microdilution panel provided epidemiological data helpful in determining nosocomial infections and indicating strains with multiple resistance. All isolates determined to be methicillin-resistant *S. aureus* yielded MICs for methicillin of $>$128 μg/ml by National Committee for Clinical Laboratory Standards methods and $>$16 μg/ml by the microdilution technique.

Bacteriophage typing. All isolates were sent to the Illinois Department of Public Health for phage typing. Because the patients came from several nursing homes, phage typing information was obtained to determine how many strains were involved and whether different homes harbored different strains.

RESULTS AND DISCUSSION

The initial selection of LSM agar over MHA-S was due primarily to the nature of the specimens involved. Of the cultures that yielded *S. aureus*, 42% came from decubitus ulcers, which often contain multiple organisms both gram positive and gram negative. Therefore, the need for a selective medium became obvious. A wide variety of expression was present in the cultures from the 10 sample swabs that were originally plated to determine growth characteristics of the two media (Table 1).

The major problem with the MHA-S was that growth of gram-negative rods, particularly the swarming *Proteus* sp., made the recognition of methicillin-resistant *S. aureus* or its recovery difficult. When a *Proteus* sp. or even a *Pseudomonas* sp. was found growing from the culture, any staphylococci present were overgrown and indistinguishable. Taking into consideration the source of the specimens, we decided that the medium was not selective enough for our purposes.

The LSM agar, on the other hand, gave good growth of all the staphylococci while inhibiting the gram-negative rods. It turns yellow from mannitol-positive organisms and gives an opaque zone around colonies that are lecithinase positive. Colonies of most *S. aureus* strains also take on a bright yellow pigment. When the culture was mixed with coagulase-positive and coagulase-negative staphylococci, it was relatively easy to differentiate the two strains. Zone sizes on the LSM agar plates for non-methicillin-resistant *S. aureus* organisms ranged from 20 to 28 cm on Petri dishes (100 by 15 mm) containing 25 ml of medium. The methicillin-resistant *S. aureus* always grew right to the edge of the disk when there was confluent growth. The only organism that presented any confusion was *Streptococcus faecalis* which, although it ferments mannitol, never took on the distinct yellow appearance of the *S. aureus* colony but grew instead as a tiny translucent colony.

To test the screening method, 224 *S. aureus*-containing cultures were studied, 118 (53%) of which were positive for methicillin-resistant *S. aureus*. Of these, 111 (94%) were correctly identified as methicillin-resistant *S. aureus* from the LSM agar. Of the 224 isolates, 14 (6%) could not be categorized as methicillin-resistant *S. aureus* from the primary plates because of the small amounts of *S. aureus* recovered. Identification methods included Gram stain and slide and tube coagulase, catalase, mannitol, and lecithinase reactions.

Growth of *S. aureus* on LSM agar was compared with growth on the corresponding tryptic soy agar with 5% sheep blood which was inoculated with *S. aureus* at the same time. Obvious growth differences between the two plates were apparently caused by the differences in incubation temperatures. The LSM agar plate was incubated at 30°C for maximum recovery of methicillin-resistant *S. aureus* (17).

TABLE 2. Resistance and susceptibility patterns[a] and phage types of methicillin-resistant *S. aureus* isolates

Resistance	Susceptibility	No. of isolates	Phage type (no. of isolates)
Met, Ery	Clin, Gen, Tet	1	75 (1)
Met, Clin, Ery	Gen, Tet	21	47 (1)
			47/75 (2)
			75 (3)
			6/47//81 (1)
			47/54/75 (4)
			6/42E/47/53/54/75/79 83A/85//81 (2)
			6/47/54/75//81 (1)
			47/53/54/75/83A//85 (1)
			81 (2)
			83A (1)
			Failed to type (3)
Met, Clin, Ery, Gen		12	6/47/54/75//81 (6)
			75 (4)
			6/47/54//81 (1)
			Failed to type (1)
Met, Clin, Ery, Tet	Gen	4	6/47/54//81 (3)
			81 (1)
Met, Clin, Ery, Gen, Tet		4	6/47/54/75//81 (2)
			Failed to type (2)

[a] Met, Methicillin; Ery, erythromycin; Clin, clindamycin; Gen, gentamicin; Tet, tetracycline.

No *S. aureus* isolates that did not demonstrate methicillin resistance at 35°C were recovered.

According to Hartman and Tomasz (7) and Sabath (12), the phenotypic expression of methicillin-resistant *S. aureus* can be categorized into three groups. Group 1 demonstrates heterogeneity, having only 1 of 10^5 or 10^6 cells able to grow in high concentrations of methicillin. Group 2 shows this same heterogeneous nature at 37°C but expresses methicillin resistance homogeneously at 30°C. Group 3 is homogeneously resistant at both temperatures. The methicillin-resistant *S. aureus* isolates recovered in our laboratory appeared to be of group 3. This was shown by the results obtained from the Micro-Media microdilution panels. Oxacillin resistance was recorded for all methicillin-resistant *S. aureus* isolates from this panel, even though the panel was incubated at 35 to 36°C. The easy identification of the methicillin-resistant *S. aureus* may be unique to the strains seen at this hospital. However, this method has proven very useful in our laboratory and may also be useful on strains found at other institutions.

The 118 cultures containing methicillin-resistant *S. aureus* originated from 42 patients ranging in age from 50 to 101 years. Only three patients were found to have two strains of *S. aureus* mixed together, one strain being methicillin-resistant and the other being non-methicillin-resistant *S. aureus*. Multiple resistance was found in all strains, some being resistant to more antibiotics than others.

The resistance mechanism for methicillin appears to be chromosome as opposed to plasmid mediated, although the resistance to the other antibiotics noted has been traced to plasmids (9). This fact could account for the variation in susceptibility patterns for the methicillin-resistant *S. aureus* isolates. When phage typing data were compared with susceptibility data, little correlation was observed (Table 2). It is evident that these organisms neither originated from a single source nor were involved in a single outbreak. Susceptibility patterns did not match up with a single phage type, as has been the case at other institutions (13, 14). Individual strains were also not confined to a single nursing home, although many residents of one particular home did have the same strain. Previous hospitalization at numerous institutions was common for many of these patients. Many also had lived in more than one nursing home.

On the average, the LSM agar plates permitted the presumptive identification of methicillin-resistant *S. aureus* 12 to 16 h sooner than do routine isolation and susceptibility techniques. The 30°C temperature slowed overall growth, so the colonies on the LSM agar plates were always much smaller. The most important step involved in speeding up the identification was reexamination of the LSM agar plates before we left at the end of the day. The additional 8 h of growth for the organisms was enough to determine clearly the presence of a zone of inhibition. It was also necessary to compare the growth on the plate with tryptic soy agar and 5% sheep blood with that on the LSM agar plate, because an occasional mannitol-negative strain of methicillin-resistant *S. aureus* may be recovered (13). It resembled the typical growth of *Staphylococcus epidermidis*. Resistance, however, could still be predicted from the LSM agar plate, regardless of the mannitol reaction.

We believe that LSM agar is more advantageous than the mannitol salt agar used in other studies (8). The lecithinase reaction was sometimes helpful for differentiation in a mixture of methicillin-susceptible and methicillin-resistant *S. aureus*, which otherwise appeared similar. The egg yolk reaction is a definite asset to the medium. As seen in earlier work (3, 12, 15), the growth of methicillin-resistant *S. aureus* was enhanced by a neutral pH, increased salt concentration, and decreased temperature. This medium was well suited to these characteristics and proved very useful for the isolation and presumptive identification of methicillin-resistant *S. aureus* from cultures with mixed organisms.

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