Comparison of Culture Screening Methods for Detection of Nasal Carriage of Methicillin-Resistant *Staphylococcus aureus*: a Prospective Study Comparing 32 Methods

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Screening for carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is fundamental to modern-day nosocomial infection control, both for epidemiologic investigation and day-to-day decisions on barrier isolation. Numerous microbiologic techniques have been advocated for screening for nasal carriage of MRSA, including the use of charcoal rather than rayon swabs, preincubation of swabs in Stuart’s medium, preincubation of swabs in salt-containing trypticase soy broth (TSB), use of mannitol-salt agar (MSA), use of MSA containing oxacillin (MSAOx), use of Mueller-Hinton agar containing oxacillin (MHAOx), and the use of MSA containing lipovitellin with an oxacillin disk (MSALOx). We report a prospective clinical trial undertaken to test all of these methods concurrently. Patients at high risk for MRSA carriage were screened with eight consecutive nasal swabs (four standard rayon, four charcoal-coated rayon), which were processed by primary plating on MSA, MSAOx, MHAOx, and MSAOLOx; Stuart’s preincubation for 72 h followed by plating on the solid media; overnight enrichment in salt-containing TSB followed by plating; and Stuart’s preincubation for 72 h followed by overnight enrichment in TSB and plating. All of the above methods were repeated with charcoal swabs. Each patient was screened by 32 culture methods. Forty-three (42%) of 102 patients studied were positive for MRSA by one or more methods. Among the four media evaluated with direct plating, MSAOx was 11 to 25% more sensitive for detecting MRSA (MSA Ox versus MSAOx, MHAOx, and MSAOLOx; Stuart’s preincubation for 72 h did not enhance recovery of MRSA. Enrichment in salt-containing TSB further increased yield 9%. MSAOx also showed the best specificity, 93%. Charcoal swabs showed no advantage over standard rayon swabs. Our results suggest that the highest yield will be achieved by using standard rayon swabs that are enriched overnight in TSB with inoculation onto MSALOx medium. Direct inoculation of swabs onto MSALOx allows detection of 90% of MRSA carriers.

Since the first report of methicillin-resistant *Staphylococcus aureus* (MRSA) as a major nosocomial pathogen in the 1960s, the incidence of infections caused by this organism continues to rise (13). Data from the National Nosocomial Infection Surveillance study from the Centers for Disease Control and Prevention show that by 2000, nosocomial infections in intensive-care unit patients caused by MRSA in the 300 participating National Nosocomial Infection Surveillance hospitals had increased to 54.5% of all *S. aureus* infections (28). Infections caused by MRSA increase the length of hospital stays, are responsible for rising health care costs, and have a high attributable mortality (5, 22).

Numerous studies have shown that clinical infections by multidrug-resistant organisms such as MRSA represent a minute fraction of the vast population of asymptomatic colonized patients—most of whom are unrecognized—which constitutes the institutional reservoir for spread of these organisms on the hands, apparel, or equipment of health care workers to uncolonized but susceptible patients (30). Reliable detection of MRSA carriage is essential as part of epidemiologic investigation but is also necessary for the prompt implementation of barrier isolation of colonized patients (i.e., the use of gowns and gloves for all direct contact) (3).

A distinctive feature of methicillin resistance is its heterogeneous nature; the expression of resistance varies according to the culture media, the salt and antibiotic concentration of the media, and the temperature of incubation; typically, only about 1% of an MRSA population expresses resistance at any given time (6). Selective media are necessary to enhance the recovery of MRSA in surveillance specimens (20, 34). However, no standards exist that define the most effective microbiological media, and although numerous selective media and techniques have been reported to enhance the detection of MRSA carriage (1, 4, 8, 9, 11, 12, 16–21, 23, 24, 25, 29, 32–35; A. Krenz-Weinrich and U. Weller, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-100, 2002), none has been shown to be clearly superior in a large prospective study that used clinical specimens rather than laboratory strains. The American Society for Microbiology recommends as primary screening media salt-containing trypticase soy broth (TSB) in conjunction with a blood agar plate and mannitol-salt agar (MSA) containing 4 μg of oxacillin per ml (14).

We report a prospective study of 32 different microbiologic techniques for detection of MRSA nasal carriage in hospitalized patients at high risk for MRSA carriage.
MATERIALS AND METHODS

Specimen collection. Patients known to be MRSA positive or to be at high risk for MRSA carriage (e.g., patients that were undergoing interinstitutional transfer, were in intensive care, or were subject to prolonged hospitalization [30]) formed the study population. Each patient had eight nasal specimens collected from anterior nares by using dry, unmoistened swabs—four with charcoal swabs (Copan Diagnostics, Corona, Calif.) and four with rayon swabs (BBL culture swab; BD, Sparks, Md.). To prevent inoculum bias, the eight swabs obtained from each patient were assigned to the culture scheme in a random manner by using a table of random numbers. A preliminary study with demonstrated S. aureus carriers showed little diminution in quantitative positivity with the use of up to 12 consecutive nasal swabs.

Swabs were transported in Stuart’s medium and processed within 2 h of collection in the following manner: primary plating was carried out on MSA, MSA containing oxacillin (MSA0x), Mueller-Hinton agar containing oxacillin (MHAox), and MSA containing lipovitellin and an oxacillin disk (MSALox); Stuart’s preincubation was carried out at 4°C for 72 h followed by plating on the four media; overnight enrichment in salt-containing TSB followed by plating was performed; and finally, Stuart’s preincubation at 4°C for 72 h followed by overnight enrichment in TSB followed by plating was performed. All of the above techniques were also done with charcoal swabs.

Media preparation. All media except MSAL Ox were purchased (Remel, Lenexa, Kans.), stored at 4°C, and used as recommended by the manufacturer. MSAL Ox was made by adding 5% (vol/vol) lipovitellin to commercial MSA before pouring it into the plates. MSAL Ox was stored at 4°C, and fresh lots were prepared each month. A 1-µg oxacillin disk was applied to the plate on the area of maximum expected growth and interpreted according to NCCLS guidelines for oxacillin susceptibility testing (27): a zone size of ≤10 mm was considered resistant; a zone size of ≥13 mm was considered susceptible.

S. aureus strain ATCC 25923 was used as a quality control strain to monitor antibiotic potency. All media studied were tested for capacity to support growth of MRSA with a generous inoculum.

Laboratory protocols. All media (except Stuart’s preincubation) were incubated at 37°C. Inoculated plates were screened at 24, 48, 72, and 96 h for typical staphylococcal colonies. S. aureus was identified by colonial morphology, catalase production (Remel), and a positive-tube coagulase test (Remel) (2). On lipovitellin-MSA, most strains of S. aureus produce an opaque zone around the colony due to lipovitellin lipase activity, thus enhancing visual identification (2).

Broth enrichment. Swabs were immersed in a tube containing 0.5 ml of TSB (Remel) supplemented with 6.5% sodium chloride and incubated overnight at 37°C.

Definition of MRSA carriers. If MRSA was detected by one or more of the 32 microbiologic techniques studied, the patient was considered an MRSA carrier.

Identification and susceptibility testing. Methicillin resistance was confirmed by disk diffusion (Remel) by using the NCCLS criteria (27) and detection of PBP2a produced by MRSA by latex agglutination (Oxoid, Inc., Ogdensburg, N.Y.) (26).

Statistical analysis. McNemar’s test for correlated proportions was used to analyze the data with SAS software (version 8.2; SAS Institute, Cary, N.C.). P values of <0.05 on a two-sided test were considered significant.

RESULTS

Over a 1-year period of study, 816 nasal swabs were obtained from 102 patients; 43 patients (42%) were found to be MRSA positive by one or more methods. Comparing primary plating on the four solid media with rayon swabs, the highest yield was achieved by using MSAL Ox, which detected 90% of all carriers (sensitivity, 90%; P < 0.01 compared to MSA, MHAox, and MSAox) (Table 1). Of 59 patients that were negative for MRSA, MSAL Ox correctly identified 55; there were four false positives, yielding a specificity with MSAL Ox of 93%, which is higher than that seen with MSA (88%) and MSAox (89%).

When broth enrichment followed by primary plating (with the rayon swabs) was compared with primary plating on solid media alone, broth enrichment detected an additional 9% (TSB enrichment with MSAL Ox, 100%) of specimens, with no loss of specificity (93% for TSB enrichment with MSAL Ox; P = 0.01) (Table 1).

No enhanced recovery was found with charcoal swabs directly plated on solid media (85 to 87% sensitivity), preincubation in Stuart’s medium followed by plating on solid media (87% sensitivity), or broth enrichment following preincubation in Stuart’s medium (90% sensitivity) (P > 0.05 for all comparisons).

Primary plating on solid media and broth enrichment followed by plating detected growth that was suggestive of MRSA at 48 h in 98% of positive cultures. Very few cultures showed MRSA positivity by any technique at 24 h. Preincubation with Stuart’s medium followed by plating and preincubation in Stuart’s medium for 72 h followed by broth enrichment and plating both took 5 days on average before MRSA could be reliably detected (Table 2).

DISCUSSION

Although many different microbiologic media and techniques have been studied for the recovery of MRSA from

<table>
<thead>
<tr>
<th>Microbiologic technique</th>
<th>No. of cultures positive by this method (n = 43)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSA</td>
<td>Direct plating</td>
<td>34</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>TSB → MSA</td>
<td>37</td>
<td>86</td>
</tr>
<tr>
<td>MHAox</td>
<td>Direct plating</td>
<td>28</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>TSB → MHAox</td>
<td>34</td>
<td>79*</td>
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<tr>
<td>MSALox</td>
<td>Direct plating</td>
<td>39</td>
<td>90*</td>
</tr>
<tr>
<td></td>
<td>TSB → MSALox</td>
<td>43</td>
<td>100</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Microbiologic method</th>
<th>Time (h) to detection</th>
</tr>
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<tbody>
<tr>
<td>Direct plating</td>
<td>24  48  72 &gt;96</td>
</tr>
<tr>
<td>MSA</td>
<td>5  98  100</td>
</tr>
<tr>
<td>MHAox</td>
<td>2  98  100</td>
</tr>
<tr>
<td>MSAox</td>
<td>8  96  100</td>
</tr>
<tr>
<td>MSALox</td>
<td>5  100 100</td>
</tr>
<tr>
<td>Preincubation in Stuart’s medium for 72 h followed by plating</td>
<td>100</td>
</tr>
<tr>
<td>Overnight TSB enrichment followed by plating</td>
<td>90  100</td>
</tr>
<tr>
<td>Preincubation in Stuart’s medium for 72 h followed by TSB enrichment followed by plating</td>
<td>100</td>
</tr>
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* Data are percentages of cumulative MRSA detected.
clinical specimens, no consensus exists as to the most sensitive and accurate method (1, 4, 8, 9, 11, 12, 16–21, 23, 24, 25, 29, 32–35; Krenz-Weinrich and Weller, 42nd ICAAC). Most prior studies used stocked clinical isolates of MRSA for comparison of media, which is not comparable to surveillance specimens in which a mixed population of bacteria is present and the numbers of MRSA may be very low—especially numbers expressing phenotypic resistance. Other studies have used laboratory-passaged organisms (4), which are also unlike the isolates cultured directly from human carriers. Among solid media used for primary plating, MSAOx and MSAL Ox have been reported to be superior to MSA in small studies (20, 21, 24, 33). On lipovitellin-MSA, most strains of S. aureus produce an opaque zone around the colony due to the presence of lipovitellin lipase activity. Our study with clinical specimens showed that MSA with lipovitellin and an oxacillin disk was the most sensitive (90%) and specific (93%) primary plating medium for recovery of MRSA.

Prior studies have reported enhanced sensitivity and an additional yield of 14 to 25% for detection of MRSA with preenrichment (preincubation) of the specimen in salt-containing TSB for 24 h before plating on solid media (7, 8, 12, 15, 31). The concentration of salt in the broth, however, has varied widely in different studies; too high a concentration may inhibit growth of MRSA and too low a concentration may result in overgrowth of gram-negative bacteria (18). Using 6.5% NaCl (10), we found an enhanced yield by using preenrichment rather than direct plating of the swab, thereby identifying four more MRSA isolates and permitting 100% sensitivity with enrichment followed by plating on MSA containing lipovitellin with an oxacillin disk. (Table 2). Although preenrichment prolongs the time needed to conclusively identify MRSA carriers, the sensitivity gained ensures that virtually all carriers will be detected.

In a study of nasal S. aureus carriage in 91 healthy laboratory volunteers, Eriksen et al. (10) reported that charcoal swabs had a fourfold higher yield for detection of S. aureus than did unmoistened cotton swabs, although the differences were not statistically significant (15 versus 3%; P = 0.07). In the same study, the investigators found that preenrichment of nasal swabs in Stuart’s medium for 7 days also increased the recovery of S. aureus (38 versus 31%; P = 0.01). In our much larger study with colonized patients, we did not find a higher yield with the use of charcoal swabs or preenrichment in Stuart’s medium.

Recently, studies conducted by using PCR techniques to detect MRSA DNA in clinical specimens have shown high sensitivity and specificity (16, 17). Jayaratne and Rutherford reported 100% sensitivity and 97% specificity with PCR compared with MSAOx in a study of 645 surveillance specimens (16); yellow colonies growing on MSAOx were used as the DNA template for PCR amplification. PCR was much more rapid and, further, was more cost-effective than conventional cultures on MSA. In contrast, another recent report comparing detection of MRSA by growth on oxacillin resistance-screening agar base (Oxoid) with triplex PCR of mecA, nuc, and 16S-rRNA on colistin-oxacillin-salt enrichment broth found that PCR had a sensitivity of only 55% but a specificity of 99% (O. Denis, N. Maes, M. Menart, S. Rottiers, B. Byl, and M. J. Struelens, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-101, 2002). Further study is needed to evaluate the promising role of PCR technology for rapid and accurate detection of MRSA in surveillance specimens, but we believe that these techniques should be compared by using salt-containing TSB enrichment combined with MSAL Ox, the technique found to be most sensitive in our study and which is clearly superior to MSAOx and MHAOx (Table 1).

In summary, we found that the most sensitive and accurate culture method to detect MRSA in a nasal culture is to collect the specimen with a rayon swab, which is then preenriched in salt-containing TSB for 24 h, after which it is plated onto MSAL Ox. New molecular techniques to detect MRSA in clinical specimens, such as PCR, may provide more rapid results, but further studies are needed to determine their true sensitivity and specificity compared with the most sensitive conventional culture methods, such as those found in our study.

REFERENCES