Use of a Primary Isolation Medium for Recovery of Methicillin-Resistant Staphylococcus aureus

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Clinical specimens frequently contain methicillin-resistant Staphylococcus aureus (MRSA) isolates in low numbers or mixed with methicillin-susceptible staphylococci, which can obscure MRSA on nonselective media. By using an oxacillin-containing mannitol-salt-based selective and differential medium on 936 respiratory specimens, we recovered 45% more MRSA isolates (29 versus 20) than on nonselective media alone.

Strains of Staphylococcus aureus which are resistant to β-lactamase stable penicillins (methicillin-resistant Staphylococcus aureus [MRSA]) are being isolated with more frequency (3, 6) and are easily transferred among hospital personnel and patients (1, 2). It is in the interest of hospitals to limit the spread of MRSA by identifying and treating patients and employees who are colonized or infected with MRSA, because infections caused by these strains can be reliably treated only with the expensive antibiotic vancomycin (4, 8).

Within each MRSA population only a minority (10^-3 to 10^-5) of cells express the resistant phenotype unless resistance is selected by prior antibiotic exposure (5). Most clinical laboratory protocols involve plating specimens on nonselective media and then selecting a few staphylococcal colonies for susceptibility testing. Low-level colonization may be missed because of overgrowth of normal flora or the presence of a mixture of MRSA with methicillin-susceptible strains. By using a selective and differential medium, we have identified MRSA isolates in specimens when conventional methodology failed to detect any S. aureus isolates or failed to detect MRSA among methicillin-susceptible colonies.

Thirty-nine MRSA strains recovered from Loyola University Medical Center patients and 30 bacterial and 5 yeast strains from laboratory stock cultures were used to test the medium. For a prospective medium evaluation, 936 consecutive respiratory specimens (sputum, tracheal aspirate, throat, nasal, and nasopharyngeal) were plated on selective and conventional media. Medium constituents included mannitol, agar, and phenol red broth base (Difco Laboratories, Detroit, Mich.), oxacillin (Bristol-Myers), and polymyxin B (Roerig/Pfizer). The final medium consisted of the following (per liter): phenol red broth base (16 g), NaCl (60 g), mannitol (10 g), agar (15 g), oxacillin (6 mg), and polymyxin B (10 mg). Other reagents used in the study included Staphaurex (Wellcome Diagnostics, Inc.) and rabbit coagulase plasma (BBL Microbiology Systems, Cockeysville, Md.).

Although staphylococci are salt tolerant, they grow more slowly on high-salt media. We wished to devise a medium which would be selective for MRSA but yield visible growth in 24 h. Nineteen strains of MRSA were serially diluted and plated on oxacillin-mannitol-salt agar (OMSA) containing 4.5, 6.5, or 7.5% NaCl. OMSA with 7.5% NaCl (the concentration in commercial mannitol-salt agar) inhibited the visible growth of most MRSA isolates at 24 h. The 7.5% formulation was considered too inhibitory for use as a rapid screening medium. The 6.5% NaCl medium supported the growth of 18 of 19 strains at 24 h, the majority of which also showed visible fermentation of mannitol.

To determine whether NaCl reduced the number of colonies or only the rate of colony growth, 20 MRSA strains were quantitatively plated in duplicate on noninhibitory sheep blood agar, 4.5% NaCl OMSA, and 6.5% NaCl OMSA. The arithmetic mean number of colonies per plate was 96 on sheep blood agar, 95 on 4.5% NaCl OMSA, and 90 on 6.5% NaCl OMSA. This difference between means was not significant by Student's t test of means (P, 0.05). The colony size was largest on the blood agar by approximately twofold, and it was approximately the same on the 4.5% and 6.5% NaCl media.

The growth of non-MRSA normal flora on OMSA was tested by spot inoculating 30 bacterial strains and 5 yeast species on the 4.5% and 6.5% NaCl OMSA media. The strains used included the following: Aeromonas hydrophila, Alcaligenes faecalis, Acinetobacter calcoaceticus var. anitratus, A. calcoaceticus var. lwoffi, Bordetella bronchiseptica, Citrobacter diversus, Citrobacter freundii, Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis (two strains), Klebsiella oxytoca, Klebsiella pneumoniae, Micrococcus luteus, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas cepacia, Salmonella typhi, Shigella flexneri, Shigella sonnei, Serratia marcescens, S. aureus (one methicillin-resistant strain and one methicillin-susceptible strain), Staphylococcus epidermidis, Staphylococcus xylosus, Streptococcus bovis, Streptococcus pyogenes, Yersinia interococlitica, Candida albicans, Candida krusei, Candida tropicalis, Cryptococcus laurentii, and Trichosporon capitatum. Seven bacterial and one yeast species grew on the 6.5% NaCl medium at 24 h. MRSA isolates grew luxuriantly, but the methicillin-susceptible S. aureus isolates did not grow even after 72 h. Nineteen bacterial and two yeast species grew on the 4.5% NaCl medium. The 4.5% NaCl formulation was not considered sufficiently selective for use as a screening medium.

To inhibit the enteric organisms which grew on the 6.5% NaCl OMSA at 24 h, polymyxin B (10 μg/ml) was added to the medium. Only MRSA, S. xylosus, C. tropicalis, and P. mirabilis grew on this medium. C. tropicalis grew slowly,
and _P. mirabilis_ did not ferment mannitol. Both strains were easily distinguished from the mannitol-fermenting staphylococci. Eighteen MRSA strains were then plated on the OMSA medium, with and without polymyxin B to determine whether polymyxin B inhibited any MRSA strains. Polymyxin B had no effect on the colony size or number.

The medium was prospectively evaluated by plating 936 respiratory specimens (throat cultures, expectorated and induced sputa, tracheal aspirates, and bronchial washings) on OMSA in addition to routine culture media (blood, chocolate, and MacConkey agars). The institution occasionally performs nasal cultures to screen for MRSA during outbreaks, but no such outbreaks occurred during this study and no MRSA nasal screen cultures were included. The OMSA plates were incubated separately and read at 24, 48, and 72 h by one investigator. The OMSA culture results were not revealed to the technologists reading the routine plates. All colonies which grew on OMSA and fermented mannitol were subcultured to blood agar and tested by Gram stain, catalase, tube coagulase, and Staphaurex tests.

Of 936 specimens processed, 105 specimens grew mannitol-fermenting colonies on OMSA. One of the 105 cultures grew yeast cells, 1 grew gram-negative rods, 101 grew staphylococci, and 2 grew both yeast and staphylococci. Of the 103 staphylococci, 29 were _S. aureus_ (coagulase and Staphaurex positive). Seventeen (59%) of the MRSA isolates were detected in 24 h, and 28 (97%) were detected by 48 h. The coagulase-negative staphylococci tended to require longer incubation: 15 (20%) grew at 24 h; the remainder required 48 or 72 h.

MRSA isolates were recovered from sputum and tracheal aspirate cultures only, not throat cultures or bronchial washings. MRSA isolates made up 22% of all _S. aureus_ isolates recovered by the laboratory during the period of the study. The number recovered from respiratory cultures (20 of 936 [2.1%]) suggests that sources other than the respiratory tract were the major reservoirs of MRSA.

Nine of the 29 MRSA strains were detected on OMSA and missed on conventional cultures. In some specimens, the overgrowth of normal respiratory flora on nonselective media obscured the small number of staphylococcal colonies; in others, _S. aureus_ was recovered by conventional culture, but the colonies tested were methicillin susceptible. The number of MRSA isolates recovered in these nine specimens was relatively low (moderate in one specimen and few in five specimens; three specimens grew five or fewer colonies).

On the basis of our studies, we recommend using an oxacillin-containing selective and differential medium for MRSA screening and as a primary plating medium if it is important to detect low levels of MRSA. Our experience indicates that approximately 30% of MRSA-containing clinical specimens may be missed if only nonselective media are used for primary specimen plating. We currently use the medium for nasal screen cultures on patients and staff. Its components are all inexpensive, but the medium must be formulated locally, since no commercial source exists.

OMSA is not completely selective for MRSA. Several species of coagulase-negative staphylococci may ferment mannitol (7), and many are resistant to oxacillin. These colonies may be confused with MRSA until they are subcultured and tested for coagulase. Some investigators have reported that Staphaurex may be used on colonies taken directly from a selective medium similar to ours (9). We found that colonies tested directly gave inconclusive results (stringy clumps with milky background), and catalase activity was slow and weak. MRSA tended to appear sooner than coagulase-negative staphylococci, but since more specimens contained coagulase-negative staphylococci, a mannitol-fermenting colony at 24 h was as likely to be a coagulase-negative staphylococcus (15 of 936) as an MRSA (17 of 936) isolate.

It still remains necessary to confirm the identity of MRSA isolates with conventional tests and their susceptibility to oxacillin with National Committee for Clinical Laboratory Standards-approved methods. The primary benefit of using OMSA to identify MRSA isolates in clinical specimens, therefore, remains its substantially increased sensitivity rather than the elimination of steps.

**REFERENCES**


