Alternative Use for Spectra MRSA Chromogenic Agar in Detection of Methicillin-Resistant Staphylococcus aureus from Positive Blood Cultures

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Spectra MRSA agar (Remel, Lenexa, KS), a novel chromogenic medium originally developed to detect methicillin-resistant Staphylococcus aureus (MRSA) from nasal swabs, was evaluated in this multicenter study for the detection of MRSA from positive blood cultures exhibiting Gram-positive cocci upon initial Gram staining.

In recent decades, hospital-acquired (HA) and community-acquired (CA) methicillin-resistant Staphylococcus aureus (MRSA) infections have caused elevated morbidity and mortality rates and substantially increased health care costs (5, 8, 14). First isolated in the 1960s, HA-MRSA is now endemic in most U.S. hospitals and long-term-care facilities (9, 12). Classically associated with skin and soft tissue infections, CA-MRSA has been involved in an increasing number of cases involving bacteremia and sometimes fatal invasive disease (15). Regardless of origin, a major financial burden has been placed on the health care industry due to MRSA-related bacteremia that stems from extended hospital stays, the cost of isolation, and intensive-care-unit expenses (1, 5). This multicenter study compared the performance of a novel chromogenic medium, Spectra MRSA agar (Remel, Lenexa, KS), with that of traditional culture to screen for MRSA from positive blood cultures.

Blood specimens were inoculated into aerobic and anaerobic blood culture bottles (site A, BacT/Alert FA [aerobic] and BacT/Alert FN [anaerobic]; site B, Standard Aerobic/F and Peds Plus/F [aerobic] and Lytic/10 Anaerobic/F [anaerobic]; site C, VersaTREK Redox 1 [aerobic] and VersaTREK Redox 2 [anaerobic]; site D, Plus Aerobic/F and Lytic/10 Anaerobic/F), followed by incubation in an automated blood culture system (site A, bioMérieux BacT/Alert; sites B and D, Becton Dickinson BACTEC; site C, TREK Diagnostics VersaTREK) for up to 5 days. Positive blood cultures, defined as Gram-positive cocci upon Gram staining, were subcultured onto Spectra MRSA agar and blood agar, the “gold standard.” Following aerobic incubation at 35°C, Spectra MRSA plates were observed for denim blue colony growth at 24- and 48 h-time intervals, indicating MRSA. Blood agar plates were observed for up to 48 h for suspected S. aureus colonies that were typically yellow, catalase positive, and composed of Gram-positive cocci often demonstrating beta-hemolysis (2).

Suspected MRSA colonies from Spectra MRSA and blood agar plates were tested with the Oxoid PBP2’ latex agglutination test (Remel) and the oxacillin Etest (bioMérieux, Marcy l’Etoile, France) according to the protocol described by Peterson et al. (11) for MRSA confirmation. The MICs were determined as defined by the CLSI guidelines (3), and the values were interpreted as follows: ≤2 μg/ml, oxacillin sensitive; ≥4 μg/ml, oxacillin resistant; and 2 to 4 μg/ml, intermediate oxacillin resistance (3). PBP2’-negative S. aureus strains with an oxacillin MIC of 4 to 6 μg/ml were reported as oxacillin resistant as described in the CLSI guidelines (3). True positives (TP) are defined as denim blue colonies on Spectra MRSA agar that were identified as MRSA by the Oxoid PBP2’ test and oxacillin Etest (≥4 μg/ml) or S. aureus PBP2’-negative colonies with an oxacillin MIC of 4 to 6 μg/ml. False positives (FP) are defined as denim blue methicillin-susceptible S. aureus (MSSA) colonies on Spectra MRSA agar. True negatives (TN) are defined as the absence of denim blue colonies on Spectra MRSA agar that was confirmed negative for MRSA by blood agar and standard techniques. False negatives (FN) are defined as confirmed MRSA colonies on blood agar that did not grow on Spectra MRSA agar.

The combined sensitivity and specificity of Spectra MRSA agar for the detection of MRSA were 96% and 99.6% at 24 h and 99.4% and 98.5% at 48 h (see Table 1 for positive predictive values [PPV] and negative predictive values [NPV]). A total of seven FN isolates were reported at 24 h: four due to the absence of growth and three resulting from MRSA colonies expressing light-blue pigmentation. However, six of the seven FN isolates eventually produced denim blue colonies at 48 h. A total of two FP isolates were reported at 24 h: one isolate produced a single denim blue colony, and the other isolate produced pinpoint denim blue colonies (both confirmed as PBP2’-negative MSSA). Individual trial site analyses are shown in Table 2. A total of six borderline oxacillin-resistant S. aureus (BORSAs) strains were identified (S. aureus PBP2’-negative colonies with an oxacillin MIC of 4 to 6 μg/ml):
three strains produced denim blue colonies at 24 h, and the remaining three strains (previously included with the FN results) demonstrated no growth at 24 h. However, two of the three strains eventually produced denin blue colonies at 48 h, and the remaining FN strain failed to produce any growth at 48 h.

Currently, the “gold standard” diagnostic test for bacteremia includes the use of automated blood culture systems that detect bacterial growth from submitted blood specimens in aerobic/anerobic bottles (6). Once bacterial growth has been confirmed, the broth is subcultured and organisms are identified using traditional and/or novel techniques. Traditional methods include standard bacteriological culture followed by identification via analysis of biochemical characteristics, morphological assessment, and determination of antimicrobial susceptibilities. However, obtaining definitive results for identification and susceptibility testing usually requires 2 to 4 days. Automated bacterial identification systems allow for rapid and highly accurate bacterial identification, with the added benefit of a susceptibility report. The advantages of these systems are significant time savings and automation (13). However, the turnaround time for determining susceptibilities once bacterial identification has been confirmed is prolonged an additional 16 to 18 h and thus fails to provide the timely susceptibility results needed for optimal patient care.

With rapid turnaround time and good accuracy, real-time PCR (RT-PCR) has proven to be ideal in a clinical setting where MRSA is moderately endemic and where large numbers of screens need to be processed on a daily basis. However, the implementation of RT-PCR in small, community-based hospitals may not be feasible due to limited work space, up-front costs, and the cost per test (7). Fluorescently labeled oligonucleotide probes (i.e., those used in peptide nucleic acid fluororesent in situ hybridization [PNA-FISH]) were developed for hybridization detection of common pathogens in bacteremic patients (6). Unfortunately, probes cannot distinguish between MRSA and MSSA.

Currently, only BBL CHROMagar MRSA medium (10) and MRSA ID chromogenic medium (4) have been evaluated for the detection of MRSA-associated bacteremia; both media provide MRSA detection from positive blood cultures that is highly sensitive and specific in comparison to that provided by conventional methods. The data presented in this report demonstrate that Spectra MRSA agar can, with high sensitivity and specificity, also rapidly identify and differentiate MRSA from other Gram-positive cocci recovered from positive blood cultures, thus providing guided antimicrobial therapy in a timely fashion.

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REFERENCES


