Evaluation of MRSASelect™ chromogenic medium for the early detection of methicillin-resistant Staphylococcus aureus from blood cultures

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INTRODUCTION: Staphylococcus aureus bacteremia is associated with considerable morbidity and mortality. In theory, reducing the turnaround time in reporting of methicillin-resistant S. aureus (MRSA) among patients with bacteremia could assist with the rapid optimization of antimicrobial therapy.

OBJECTIVE: To evaluate the sensitivity and specificity of MRSASelect (Bio-Rad Laboratories, USA), a chromogenic medium, in the early detection of MRSA from blood cultures growing Gram-positive cocci in clusters, and to confirm that routine use of this medium would, in fact, reduce turnaround time for MRSA identification.

METHODS: The present study was conducted at three microbiology laboratories in Manitoba. Between April 2010 and May 2011, positive blood cultures with Gram-positive cocci in clusters visualized on Gram stain were subcultured to both MRSASelect and routine media. MRSA isolates were identified using conventional microbiological methods from routine media and using growth with the typical colony morphology (pink colony) on MRSASelect medium.

RESULTS: A total of 492 blood cultures demonstrating Gram-positive cocci in clusters on Gram stain were evaluated. S. aureus was recovered from 274 blood cultures, with 51 S. aureus isolates (51 of 274 [18.6%]) identified as MRSA. MRSASelect medium had a sensitivity of 98%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 99.8% for the recovery and identification of MRSA directly from positive blood culture bottles. In addition, use of MRSASelect medium was found to improve turnaround time in the detection of MRSA by almost 24 h relative to conventional methods.

DISCUSSION: These data support the utility of MRSASelect medium for the rapid identification of MRSA from positive blood cultures. Further clinical studies are warranted to determine whether the improvement in turnaround time will result in a measurable reduction in suboptimal antimicrobial therapy and/or improvement in patient outcome.

Key Words: Blood cultures; Chromogenic agar; MRSA; Staphylococcus aureus

Staphylococcus aureus is an important cause of both community and nosocomially acquired bloodstream infection (1,2). In a recent Canadian surveillance study (the Canadian Ward [CANWARD] surveillance study), S. aureus was the second most common cause of bacteremia among patients admitted to, or evaluated at, Canadian hospitals (3). S. aureus bacteremia may be complicated by the development of endocarditis and/or vertebral osteomyelitis, and a mortality rate ranging from approximately 10% to 50% has been described in various publications (1,4,5). Methicillin resistance among S. aureus bloodstream isolates has been reported to be associated with increased patient mortality (4,6). Of concern, methicillin-resistant S. aureus (MRSA) has been detected with increasing frequency in various surveillance studies (7). Among patients with bacteremia evaluated at Canadian hospitals, approximately 24% of

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TABLE 1
Gram-positive cocci in clusters isolated from 490 positive blood cultures

<table>
<thead>
<tr>
<th>Organism identification</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin-susceptible S aureus</td>
<td>223 (45.5)</td>
</tr>
<tr>
<td>Methicillin-resistant S aureus</td>
<td>51 (10.4)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci (not further identified)</td>
<td>132 (26.9)</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>55 (11.2)</td>
</tr>
<tr>
<td>Staphylococcus hominis</td>
<td>10 (2.0)</td>
</tr>
<tr>
<td>Staphylococcus capitis</td>
<td>5 (1.0)</td>
</tr>
<tr>
<td>Staphylococcus lugdunensis</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Staphylococcus schleiferi</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Staphylococcus warneri</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Micrococcus species</td>
<td>8 (1.6)</td>
</tr>
<tr>
<td>Total</td>
<td>490</td>
</tr>
</tbody>
</table>

S aureus isolates are reported to be MRSA (3). In addition, in the United States, MRSA was found to account for 49.1% of inpatient S aureus bloodstream isolates recovered in a study conducted by the Surveillance Network (7).

In view of the significant morbidity and mortality associated with S aureus bloodstream infections, particularly those caused by MRSA, it is critical that clinicians initiate appropriate antimicrobial therapy in a timely fashion. Indeed, inadequate empirical antimicrobial therapy for S aureus bacteremia has been associated with increased mortality and prolonged time to defervescence in some, although not all, clinical studies (8-14). Conventional confirmation of MRSA from a blood culture growing Gram-positive cocci in clusters typically takes approximately 48 h. The thermostable DNase test and tube coagulase test permit rapid preliminary identification of S aureus from blood cultures; however, these tests do not allow for the differentiation of MRSA from methicillin-susceptible S aureus (MSSA) (15).

To assist clinicians with the rapid optimization of antimicrobial therapy, various investigators have evaluated novel methods for the detection of MRSA from positive blood cultures. Among these methods, molecular testing for rapid MRSA detection appears quite promising (16,17). Unfortunately, molecular methods are expensive and, therefore, may not be suitable for many smaller clinical microbiology laboratories that have limited resources. MRSASelect (Bio-Rad Laboratories, USA) is a chromogenic medium that has been developed for the detection of MRSA from screening specimens (eg, nares swab) (18). The direct inoculation of blood cultures growing Gram-positive cocci onto a chromogenic medium such as MRSASelect has the potential to reduce time to MRSA detection. The possible advantages of this method over molecular testing include both simplicity of use and lower cost. The purpose of the present study was to evaluate the sensitivity and specificity of MRSASelect for the early detection of MRSA from blood cultures, and to confirm that routine use of this medium for blood cultures growing Gram-positive cocci in clusters would, in fact, reduce turnaround time for MRSA identification.

METHODS

The present study was conducted at three clinical microbiology laboratories located in Manitoba. These laboratories provide diagnostic bacteriology services to all hospitals in the cities of Winnipeg and Brandon. The study was performed between April 2010 and May 2011, with some variability in the study months between the three sites. All blood culture bottles received at the three participating sites are routinely loaded onto a BacT/ALERT 3D instrument (BioMérieux, USA). When growth in a blood culture bottle is detected, the bottle is flagged as positive and a Gram stain is performed. All blood cultures with a Gram stain demonstrating Gram-positive cocci in clusters recovered during the study period were included. Thermostable DNase or tube coagulase testing is routinely used by all sites for rapid detection of S aureus. MRSASelect plates used in the present study were purchased from Bio-Rad Laboratories. An aliquot of the positive blood culture bottle was inoculated onto both an MRSASelect plate and routine media. MRSASelect plates were incubated at 35°C in an aerobic atmosphere and examined 18 h to 28 h following inoculation. MRSA was identified by the appearance of pink colonies on the chromogenic medium. Conventional identification of MRSA was performed using a VITEK2 instrument (BioMérieux, USA) once a suspect isolate was recovered from subculture of a blood culture bottle onto blood agar. The results obtained using inoculation of MRSASelect plates were compared with those obtained using routine identification.

Turnaround time for MRSA detection using MRSASelect and conventional identification was also recorded for each MRSA isolate recovered in the study.

RESULTS

In total, 490 blood cultures demonstrating Gram-positive cocci in clusters on Gram stain were evaluated. The bacteria recovered by conventional culture and identification included 223 MSSA isolates, 208 coagulase-negative staphylococci isolates, 51 MRSA isolates and eight Micrococcus species isolates (Table 1). MRSA accounted for 18.6% (51 of 274) of S aureus isolates and 10% (51 of 490) of all Gram-positive cocci in clusters. Fifty of the 51 MRSA isolates recovered in the present study grew on the MRSASelect medium. The one MRSA isolate that failed to grow on the chromogenic medium was recovered on repeat testing. A plate inoculation error was believed to explain the original lack of growth of this isolate on MRSASelect. All MRSA isolates demonstrated the typical colony morphology (ie, small pink colonies) on MRSASelect plates. Bacteria other than MRSA were not recovered on subculture of the positive blood culture bottles to MRSASelect. Overall, MRSASelect had a sensitivity of 98%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 99.8% for the recovery of MRSA directly from positive blood culture bottles.

In the present study, the average turnaround time using MRSASelect for identification of MRSA from a blood culture bottle growing Gram-positive cocci in clusters was 24 h (range of 18 h to 28 h). Used in conjunction with a thermostable DNase test (or tube coagulase), a report of MRSA or MSSA would be available within 24 h of a positive blood culture result. In contrast, the turnaround time for conventional testing to detect MRSA from a positive blood culture was 42 h (range 36 h to 48 h). The cost for an MRSASelect plate is approximately $1.50 to $5.00. Based on the data generated in the present study (testing of 490 isolates, with 50 MRSA isolates recovered on the chromogenic medium), it would cost between $14.70 and $49.00 to improve the turnaround time for one MRSA culture by approximately 24 h. The cost for any given institution would vary depending on the prevalence of MRSA and the exact medium price. The cost could further be reduced if testing was limited to Gram-positive cocci in clusters that demonstrated a positive thermostable DNase test, although this may not be optimal for workflow.

DISCUSSION

In the present study, MRSASelect demonstrated excellent sensitivity and specificity for the identification of MRSA from blood cultures growing Gram-positive cocci in clusters. Additionally, turnaround time was reduced by almost 24 h in comparison with conventional methods for MRSA identification. The results of the present study are largely confirmatory. Use of chromogenic media for MRSA recovery from blood cultures has previously been described in the literature. Riedel et al (19) evaluated MRSASelect for the detection of MRSA from positive blood cultures. The authors reported a sensitivity and specificity of 99% for the identification of MRSA using MRSASelect (19). Chromogenic agar produced by other manufacturers has also been evaluated and similarly found to be both sensitive and specific for the detection of MRSA from positive blood cultures (20,21). While all MRSA isolates recovered in the current study demonstrated the typical colony morphology on MRSASelect agar, isolates that are not
MRSA may occasionally grow as faintly pink colonies on this medium. Indeed, in a study by Riedel et al (19), 10 isolates subsequently demonstrated not to be MRSA grew as faint pink colonies on the chromogenic agar. To ensure that isolates growing on MRSASelect are in fact S aureus, confirmation with a tube coagulase test or a thermostable DNAse test should be performed, particularly for faintly pink colonies with atypical morphology.

In theory, reduced turnaround time for MRSA and MSSA detection from blood cultures has the potential to contribute to the rapid optimization of antimicrobial therapy, thereby improving patient outcome and antibiotic utilization. However, it should be noted that clinical data indicating that improved outcomes will indeed occur with more rapid MRSA/MSSA reporting are extremely limited. At present, much of the available data come from studies assessing molecular methods for rapid MRSA/MSSA detection. Frye et al (17) evaluated the clinical impact of real-time polymerase chain reaction (PCR) for rapid detection of MRSA and MSSA from blood cultures growing Gram-positive cocci in clusters. These investigators were unable to demonstrate an improvement in time to patient receipt of optimal antimicrobial therapy, despite a significant improvement in turnaround time (17). Carroto et al (22) also investigated the utility of real-time PCR for rapid identification of S aureus from blood cultures and determination of methicillin resistance, in comparison with identification and susceptibility testing by conventional phenotypic methods. The implementation of PCR testing did not improve patient clinical outcome at 12 weeks of follow-up (22). Bauer et al (16) did report a reduction in the mean time to switch from empirical vancomycin to cefazolin or nafcillin among patients with MSSA bacteremia following the introduction of PCR testing on blood cultures growing Gram-positive cocci in clusters. However, implementation of the PCR test was not independently associated with a reduction in patient mortality (16).

There are several possible reasons why the above cited studies have demonstrated only minimal clinical benefit from the more rapid differentiation of MRSA from MSSA in patients with bacteremia. Empirical therapy at many institutions may routinely cover both MRSA and MSSA, obscuring any potential benefit from earlier MRSA identification. Additionally, some clinical studies have not found an association between the appropriateness of initial antimicrobial therapy and clinical outcome among patients with S aureus bacteremia (5,8,14,23). It is likely that patient outcome depends on a number of factors in addition to antimicrobial therapy, including community versus hospital onset, source of bacteremia, patient comorbidities and severity of illness. Hence, in an unselected population with S aureus bacteremia, it may be difficult to demonstrate improved outcomes by optimizing antimicrobial therapy one or two days earlier. Finally, earlier reporting of MRSA versus MSSA may not improve outcome if clinicians are not aware of the testing performed by the microbiology laboratory and acting on the results in a timely fashion. To date, no study has evaluated the clinical impact of chromogenic media, including MRSASelect, in the rapid identification of MRSA from blood cultures. However, extrapolating from the results of molecular studies, one may expect that the major benefit would likely be small at best, and probably limited to improvement in time to optimization of antimicrobial therapy.

**SUMMARY**

The results of the present study reveal that MRSASelect is both sensitive and specific for identification of MRSA from blood cultures growing Gram-positive cocci in clusters. Use of MRSASelect reduced the turnaround time to reporting of MRSA from blood cultures. The major limitation to the present study is the lack of clinical data indicating that the faster turnaround time for MRSA detection actually contributes to the optimization of antimicrobial usage and improvement in patient outcome. Further clinical studies are warranted to determine whether the improved turnaround time does, in fact, lead to a measurable reduction in suboptimal antimicrobial therapy and/or improvement in patient care, thereby justifying the additional expense.

**REFERENCES**


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