ORIGINAL ARTICLE

Evaluation of MRSA*Select*™ 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 bactermia could assist with the rapid optimization of antimicrobial therapy.

OBJECTIVE: To evaluate the sensitivity and specificity of MRSA*Select* (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 MRSA*Select* 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 MRSA*Select* medium.

RESULTS: A total of 490 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. MRSA*Select* 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 MRSA*Select* 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 MRSA*Select* 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

L'évaluation du milieu de culture chromogène MRSA*Select*MD pour le dépistage précoce du *Staphylococcus aureus* résistant à la méthicilline à partir de cultures sanguines

HISTORIQUE : La bactériémie à *Staphylococcus aureus* s'associe à une morbidité et une mortalité considérables. En théorie, la réduction du délai à confirmer un *S aureus* résistant à la méthicilline (SARM) chez les patients ayant une bactériémie pourrait contribuer à l'optimisation rapide de la thérapie antimicrobienne.

OBJECTIF : Évaluer la sensibilité et la spécificité du MRSA*Select* (Bio-Rad Laboratories, États-Unis), un milieu de culture chromogène, pour déceler rapidement le SARM dans des cultures sanguines contenant des coccobacilles à Gram positif en grappes et confirmer que l'utilisation systématique de ce milieu de culture réduit véritablement le délai de dépistage du SARM.

MÉTHODOLOGIE : Les chercheurs ont mené la présente étude dans trois laboratoires de microbiologie du Manitoba. Entre avril 2010 et mai 2011, les cultures sanguines positives aux coccobacilles à Gram positif en grappes visualisées sur une coloration de Gram ont été repiquées à la fois dans un milieu de culture MRSA*Select* et dans un milieu de culture habituel. Les chercheurs ont repéré des isolats de SARM au moyen des méthodes microbiologiques classiques dans les milieux de culture habituels et des milieux de culture ayant la morphologie classique des colonies (colonie rose) sur les milieux de culture MRSA*Select*.

RÉSULTATS : Au total, les chercheurs ont évalué 490 cultures sanguines démontrant des cocccobacilles à Gram positif en grappes sur une coloration de Gram. Ils ont prélevé le *S aureus* sur 274 analyses sanguines et déterminé que 51 isolats de *S aureus* (51 sur 274 [18,6 %]) étaient un SARM. Le milieu de culture MRSA*Select* avait une sensibilité de 98 %, une spécificité de 100 %, une valeur prédictive positive de 100 % et une valeur négative prédictive de 99,8 % à l'égard de la récupération et du dépistage du SARM directement dans les fioles de culture sanguine. De plus, l'utilisation du milieu de culture MRSA*Select* raccourcissait de près de 24 heures le délai de dépistage du SARM par rapport aux méthodes classiques.

EXPOSÉ : Ces données appuient l'utilité du milieu de culture MRSA*Select* pour dépister rapidement le SARM dans les cultures sanguines positives. D'autres études cliniques s'imposent pour déterminer si l'obtention plus rapide des résultats s'associera à une réduction mesurable de la thérapie antimicrobienne sous-optimale ou à une amélioration de l'issue des patients.

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

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

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. MRSA*Select* (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 MRSA*Select* 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 MRSA*Select* 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*. MRSA*Select* 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 MRSA*Select* plate and routine media. MRSA*Select* 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 MRSA*Select* plates were compared with those obtained using routine identification. Turnaround time for MRSA detection using MRSA*Select* 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 MRSA*Select* 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 MRSA*Select*. All MRSA isolates demonstrated the typical colony morphology (ie, small pink colonies) on MRSA*Select* plates. Bacteria other than MRSA were not recovered on subculture of the positive blood culture bottles to MRSA*Select*. Overall, MRSA*Select* 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 MRSA*Select* 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 MRSA*Select* 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, MRSA*Select* 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 MRSA*Select* for the detection of MRSA from positive blood cultures. The authors reported a sensitivity and specificity of 99% for the identification of MRSA using MRSA*Select* (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 MRSA*Select* 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 to not be MRSA grew as faint pink colonies on the chromogenic agar. To ensure that isolates growing on MRSA*Select* 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). Cattoir 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 MRSA*Select*, 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 MRSA*Select* is both sensitive and specific for identification of MRSA from blood cultures growing Gram-positive cocci in clusters. Use of MRSA*Select* 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.

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