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Three-Way Comparison of BBL CHROMagar MRSA II, MRSA*Select*, and Spectra MRSA for Detection of Methicillin-Resistant *Staphylococcus aureus* Isolates in Nasal Surveillance Cultures

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired and life-threatening infections. Active surveillance programs for MRSA utilize either molecular or culture-based methods. A prospective study was performed to compare the performance of selective and differential chromogenic media, BBL CHROMagar MRSA II (CMRSA II; BD Diagnostics, Sparks, MD), MRSA*Select* (Bio-Rad Laboratories, Redmond, WA), and Spectra MRSA (Remel, Lenexa, KS), for the detection of MRSA in nasal swab specimens. A total of 515 compliant remnant nasal swab specimens were sequentially used to inoculate BBL Trypticase soy agar with 5% sheep blood (TSA II) and each chromogenic medium. After 24 h of incubation, colony color reactions and morphology on chromogenic media were compared to suspicious colonies on nonselective TSA II. MRSA on TSA II was confirmed by Gram staining, a coagulase test, and a cefoxitin disk test. The overall prevalence of MRSA and methicillin-susceptible *S. aureus* (MSSA) on TSA II was 12.4% (64/515) and 9.7% (50/515), respectively. When each chromogenic medium was compared to the standard culture method, the sensitivity and specificity, respectively, were as follows: CMRSA II, 87.7% and 98.6%; MRSA*Select*, 89.0% and 93.4%; and Spectra MRSA, 83.6% and 92.1%. The positive predictive values were highest for CMRSA II (91.4%), followed by MRSA*Select* (69.1%) and Spectra MRSA (63.5%). False-positive results on chromogenic media were mainly due to color interpretation. The negative predictive values for all three media were greater than 97%. In conclusion, CMRSA II gave the best overall results for detecting MRSA from nasal specimens.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common cause of hospital-associated infections. To control the transmission of MRSA in health care facilities, targeted active surveillance cultures and an active infection control program to eliminate or control the spread of MRSA in hospitals are recommended (1, 2). The introduction of molecular methods (e.g., PCR) for the detection of MRSA in nasal specimens has improved the reporting time of MRSA and utilization of infection control resources (3–5). However, not all laboratories can justify the added expense of molecular methods. An alternative, cost-effective approach is the use of chromogenic media for screening nasal specimens for MRSA carriage. BBL CHROMagar MRSA II (CMRSA II, BD), MRSA*Select* (Bio-Rad), and Spectra MRSA (Remel) are selective and differential chromogenic media for the qualitative detection of MRSA from nasal surveillance specimens. Each contains specific chromogenic substrates, antimicrobials, and selective agents for the suppression of Gram-negative organisms, yeasts, and other Gram-positive cocci. Identification of MRSA from the primary culture is based on the cleavage of chromogenic substrate by a specific enzymatic activity of *S. aureus*, leading to coloration of colonies. A three-way comparison study was conducted to determine the performance characteristics of these commercially available chromogenic media for the detection of MRSA nasal colonization.

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MATERIALS AND METHODS

Clinical samples. Surveillance swab specimens referred to our laboratory for screening for MRSA carriage were collected and tested in accordance with institutional review board-approved protocols. Anterior nasal swabs were collected using a BBL CultureSwab liquid Stuart double swab (catalog no. 220109) collection and transport system (BD Diagnostics, Sparks, MD). Both swabs were inserted simultaneously and rotated inside each anterior naris approximately five times, with pressure applied to ensure adequate sample collection. All specimens were collected by nursing personnel. Specimens were transported to our laboratory by a pneumatic tube transport system and stored at 2 to 8°C until processed within 24 h after collection.

Culture media. Chromogenic BBL CHROMagar MRSA II (CMRSA II) plates, an improved version of CHROMagar MRSA approved for 24-h reading, were obtained from BD Diagnostics (Sparks, MD), MRSA*Select* plates were obtained from Bio-Rad Laboratories (Redmond, WA), and Spectra MRSA plates were obtained from Remel (Lenexa, KS). The standard culture plate was nonselective Trypticase soy agar with 5% sheep blood (TSA II; BD Diagnostics, Sparks, MD). Each chromogenic plate was handled according to the manufacturer's package insert instructions. All chromogenic media were also stored in the dark prior to inoculation and during incubation for this study. Quality control testing was performed on each chromogenic medium daily using a standardized inoculum of *S. aureus* ATCC 43300 and ATCC 25923.

Study design. (i) Workflow. A total of 515 compliant nasal surveillance swabs were evaluated in this prospective study. One nasal swab from the double swab collection and transport system was used for routine screening of MRSA using our laboratory's current method and not used for comparison purposes in this evaluation. The remaining swab was used

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TABLE 1 Performance of three chromogenic media for recovery of MRSA

Medium	No. of[a]:				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	TP	FN	FP	TN				
CMRSA II	64	9	6	436	87.7	98.6[b]	91.4	98.0
MRSA*Select*	65	8	29	413	89.0	93.4	69.1	98.1
Spectra MRSA	61	12	35	407	83.6	92.1	63.5	97.1

[a] TP, true positive; FN, false negative; FP, false positive; TN, true negative. A true-positive MRSA isolate was defined as a MRSA isolate recovered from TSA II or no MRSA on TSA II but confirmed on CMRSA II, MRSA*Select*, or Spectra MRSA.

[b] CMRSA II was significantly more specific than MRSA*Select* and Spectra MRSA ($P = 0.0002$).

for recovery of MRSA on each chromogenic medium, which was then compared to recovery of MRSA on TSA II. Identification of presumptive *S. aureus* colonies on TSA II was confirmed by the coagulase test, and MRSA was confirmed by the cefoxitin disk test.

(ii) Inoculation and incubation. Swabs were first plated on TSA II and then rotated onto CMRSA II, MRSA*Select*, or Spectra MRSA in a randomized order. On each test day, approximately 10 to 20 swabs were planted on chromogenic media in an alternating and sequential order. Plates were streaked for semiquantitative isolation and incubated in air at 35 to 37°C for 24 h (±10 min). All plates were kept in a dark incubator for the entire incubation period.

(iii) Interpretation. Interpretation of growth on chromogenic media and TSA II was performed by two and three laboratory staff members after approximately 24 h (±10 min) incubation. Interpretation of colony size and color on the chromogenic media was confirmed by two staff members and as described by the manufacturer's instructions. In this study, mauve colonies of any size morphologically resembling staphylococci on CMRSA II plates were interpreted as a positive result for MRSA. On MRSA*Select* plates, any small pink colonies ranging from light pink to strong pink were interpreted as a positive result for MRSA. On Spectra MRSA plates, any small to medium colonies ranging from light to dark denim blue were interpreted as a positive result for MRSA. Colonies giving a questionable color interpretation or discrepant results compared to results on TSA II were further identified. Uncolored or white colonies were not further investigated.

(iv) Identification and confirmation of *S. aureus* and MRSA. TSA II plates were examined for the presence of white, yellow, or golden colonies with or without beta-hemolysis and selected for further testing. *S. aureus* was identified by Gram staining and a slide coagulase test. The tube coagulase test was used to provide a conclusive identification. Susceptibility testing for MRSA was done by the disc diffusion test (6) using BBL Sensi-Disc cefoxitin (30 μg) susceptibility test discs (BD Diagnostics, Sparks, MD). Colonies growing on the chromogenic media with no growth of MRSA on TSA II were reisolated onto TSA II and identified, followed by identification and susceptibility testing as described above for the reference method.

(v) Data analysis. For calculating recovery, a true-positive MRSA isolate was defined as an *S. aureus*-like isolate recovered from either nonselective TSA II or chromogenic media that was confirmed as coagulase positive and cefoxitin resistant. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each chromogenic medium were calculated compared to this reference standard. Logistic regression was used to determine if the differences in performance for the three chromogenic media relative to the standard culture method were statistically significant. The *P* values from multiple comparisons were adjusted by Hommel's method (7). Growth on all study plates was semiquantified as less than 10, 10 to 100, 100 to 300, and more than 300 colonies to estimate the concentration of MRSA present in each patient specimen.

RESULTS

Overall recovery. A total of 515 nasal swabs were analyzed. The prevalence of colonization by methicillin-susceptible *S. aureus* (MSSA) and MRSA was 9.7% ($n = 50$) and 14.2% ($n = 73$), respectively. Semiquantitative culture results of MRSA from nasal swabs ($n = 73$) demonstrated the spectrum of nasal colonization in the patient population studied. The numbers of specimens exhibiting colony counts in the four categories (<10, 10 to 100, 100 to 300, and >300 colonies) were 13 (17.8%), 39 (53.4%), 6 (8.2%), and 15 (20.5%). The frequency of specimens exhibiting 10 or more MRSA colonies on TSA II plates or chromogenic media was 82%. Of the 13 nasal cultures that grew fewer than 10 MRSA colonies, 9 demonstrated 1 colony only. The cultures that grew fewer than 10 MRSA isolates were randomly distributed among TSA II and chromogenic media as single colonies (TSA II, $n = 1$; CMRSA II, $n = 3$; MRSA*Select*, $n = 3$; Spectra MRSA, $n = 2$) or a combination of TSA II and chromogenic media ($n = 4$).

Chromogenic medium performance versus the standard culture method. Table 1 summarizes the results of the comparison of the CMRSA II, Spectra MRSA, and MRSA*Select* plates to standard culture after 24 h based on initial color interpretation. The sensitivity, specificity, PPV, and NPV for CMRSA II were 87.7%, 98.6%, 91.4%, and 98.0%, respectively. Isolates from four specimens recovered on CMRSA II alone and not on TSA II were confirmed as MRSA and considered true positives (TP). Of these four specimens, three also failed to grow MRSA on MRSA*Select* and Spectra MRSA. Six specimens grew mauve colonies on CMRSA II only, but these were confirmed as bacteria other than MRSA. The false-positive (FP) results were caused by *Serratia marcescens* ($n = 1$) and coagulase-negative staphylococci ($n = 5$). The sensitivity, specificity, PPV, and NPV for MRSA*Select* were 89.0%, 93.4%, 69.1%, and 98.1%, respectively. Isolates from four specimens confirmed as MRSA but not detected on TSA II were recovered on MRSA*Select* alone. Of these four specimens, three also failed to grow MRSA on CMRSAII and Spectra MRSA. MRSA isolates recovered on MRSA*Select* alone differed from those recovered on CMRSA II alone. There were pink to light pink colonies from 29 samples which were identified as bacteria other than MRSA. The false-positive results were caused by *S. marcescens* ($n = 1$), coagulase-negative staphylococci ($n = 27$), and MSSA ($n = 1$). Colonies from *S. marcescens* ($n = 1$), coagulase-negative staphylococci ($n = 21$), and MSSA ($n = 1$) were interpreted as light pink. Colonies from six coagulase-negative staphylococci were interpreted as strong pink, similar in color intensity to MRSA colonies. The sensitivity, specificity, PPV, and NPV after 24 h for MRSA Spectra were 83.6%, 92.1%, 63.5%, and 97.1%, respectively. Isolates from three specimens confirmed as MRSA but not detected on TSA II were recovered on Spectra MRSA alone. Of these three specimens, two also failed to grow MRSA on CMRSAII and MRSA*Select*. MRSA isolates recovered on MRSA Spectra alone differed from those recovered on the other chromogenic media. There were

light denim blue shades of isolate colonies from 35 samples which were confirmed as bacteria other than MRSA. The false-positive results were caused by *S. marcescens* ($n = 1$), *Bacillus* spp. ($n = 2$), coagulase-negative staphylococci ($n = 31$), and MSSA ($n = 1$). Colonies from *S. marcescens* ($n = 1$), coagulase-negative staphylococci ($n = 28$), and MSSA ($n = 1$) were interpreted as light denim blue. Colonies from five coagulase-negative staphylococci and one *Bacillus* species were interpreted as dark denim blue, similar in color intensity to MRSA colonies. One specimen grew MRSA on all chromogenic media but failed to grow on TSA II. With the exception of this specimen, the instances of discrepant chromogenic-agar-positive and TSA II-negative MRSA results were random, and only one or two colonies were detected (data not shown).

It was found that the sensitivity of the three chromogenic media compared to standard culture was not statistically significantly different ($P = 0.3480$) at the 0.05 significance level. However, the specificity of the three chromogenic media compared to standard culture was found to be statistically significantly different (P value < 0.0001) at the 0.05 significance level. The difference was attributable to the statistically significant difference in specificity between CMRSA II and MRSA*Select* ($P = 0.0002$) and to the statistically significant difference in specificity between CMRSA II and Spectra MRSA ($P = 0.0002$). The number of true positives was similar for all culture media examined with heavy growth of more than 10 colonies per plate; however, there was a pronounced difference in the number of false-positive (FP) results. The ability to suppress non-MRSA growth was clearly superior with CMRSA II (6 FP). The majority of FP results were found on MRSA*Select* (29 FP) and Spectra MRSA (35 FP) and attributed to color interpretation. When all light-colored colonies on MRSA*Select* and Spectra MRSA were interpreted as non-MRSA, the PPVs of all three chromogenic media were essentially equivalent (91.4% to 92.4%).

DISCUSSION

This study evaluated the performance of CMRSA II, MRSA*Select*, and Spectra MRSA for the direct detection of MRSA isolates from nasal surveillance specimens. To our knowledge, this is the first three-way comparison of chromogenic media for the detection of MRSA directly from a single swab. Our data showed that CMRSA II was more specific than Spectra MRSA and MRSA*Select*, and this difference was statistically significant. These results differ from those of previous studies, which, after 24 h incubation, showed sensitivity and specificity of 95.4% and 99.7%, respectively, for Spectra MRSA (8) and sensitivity and specificity of 98.3% and 98.2%, respectively, for MRSA*Select* (9). False-positive results on Spectra MRSA and MRSA*Select* resulting in poor PPVs was attributed to color interpretation of colonies on an opaque background. Colonies appearing light pink on MRSA*Select* and light denim blue on Spectra MRSA could not be ruled out as possible MRSA. Although the colony sizes were similar on all three chromogenic media, the color intensity of positive colonies on CMRSA II was consistently mauve.

A potential limitation of this comparative study is the variation in specimen collection and inoculum size. Isolation of MRSA by semiquantitative culture demonstrated a wide spectrum of recovery by use of single swabs. Nonetheless, the overall specificity for all three chromogenic media was high, ranging from 92.1 to 98.6%. Recovery of MRSA from the patient's anterior nares was improved by using chromogenic media compared to TSA II alone by detecting low concentrations of MRSA (one or two colonies). Although impractical, the use of multiple nonselective and selective plates in this study improved the overall detection of MRSA. The recommended use of sampling multiple body sites and use of enrichment broth cultures have been shown to increase the sensitivity for MRSA carriage (10–13). These recommendations however, often result in increased cost and processing time. The presence of a single colony of MRSA on chromogenic medium presents the dilemma as to whether a patient actually carries a bacterial load of MRSA that can potentially be transmissible to other patients. A high concentration of MRSA on chromogenic media most certainly represents a true transmission concern and needs to be immediately reported to infection control. Several factors may contribute to the recovery of MRSA, such as interfering substances (e.g., nasal sprays), patient age and immunosuppression, body site, and proper specimen collection, However, Mermel et al. (11) showed that the quantity of MRSA in the nares correlated inversely with the likelihood of MRSA infection.

As observed in this study, experience is needed for the recognition of suspected colonies on chromogenic media. We recommend follow-up confirmation of questionable colonies by a coagulase test or latex agglutination test and Gram stain before reporting detection of MRSA. These steps should increase the specificity of MRSA interpretation and not substantially increase the time to the final report. The use of a latex agglutination test directly from the chromogenic medium has been shown to rule out false-positive results and increase specificity to 99% (14). The manufacturers' inserts for MRSA*Select* and Spectra MRSA recommend coagulase and latex agglutination tests, respectively, directly from the plate to confirm the identification of MRSA. The advantages of using chromogenic media over nonselective TSA II are shorter time to detection of MRSA, enhanced recovery, minimal technician time, and no additional antimicrobial susceptibility or screening tests for *mecA*-mediated oxacillin resistance.

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