Evaluation of a New Selective Medium, BD BBL CHROMagar MRSA II, for Detection of Methicillin-Resistant Staphylococcus aureus in Different Specimens


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The sensitivity of screening for methicillin-resistant Staphylococcus aureus (MRSA) can be improved by adding other specimen sites to nares. We describe an evaluation of a new selective medium, BD BBL CHROMagar MRSA II (CMRSAII), for its ability to detect MRSA from different specimen types. CMRSAII is a chromogenic medium which incorporates cefoxitin for the detection of MRSA. A study was performed at four clinical laboratories with the following specimens: 1,446 respiratory, 694 stool, 1,275 skin, and 948 wound specimens and 688 blood culture bottles containing Gram-positive cocci. The recovery of MRSA on traditional culture media was compared to results with CMRSAII. S. aureus was tested by cefoxitin disk diffusion. CMRSAII was interpreted as positive for MRSA at 24 h (range, 18 to 28 h) based solely on the visualization of mauve-colored colonies and at 48 h (range, 36 to 52 h) based on detection of mauve colonies with subsequent confirmation as S. aureus (by coagulase or latex agglutination testing). MRSA was recovered more frequently on CMRSAII (89.8% at 24 h and 95.6% at 48 h) than on traditional culture plates (83.1% at 24 h and 79.8% at 48 h) for all specimen types combined (P < 0.001). The percent sensitivities of CMRSAII at 24- and 48-h reads, respectively, were 85.5 and 92.4% for respiratory specimens, 87.9% and 98.3% for stool specimens, 88.4% and 96.1% for skin specimens, 92.1% and 94.6% for wound specimens, and 100% and 100% for positive blood cultures. The specificity was 99.8% for respiratory specimens and 100% for all others. In conclusion, CMRSAII is a reliable screening medium for multiple specimen types.

Controlling the spread of multidrug-resistant microorganisms and especially methicillin-resistant Staphylococcus aureus (MRSA) has become a major infection control objective in the United States (4) and many European countries (3, 4, 21). A part of most programs to control the spread of MRSA is screening of patients (4, 8, 14), and screening has even become mandatory in some countries (11, 31).

Traditionally, MRSA screening included mainly the culturing of naris swabs. However, it has been demonstrated that up to 35% of MRSA carriers may be colonized only from sites other than the nares, for example, the throat or the rectum (1, 2, 16).

Usage of chromogenic media can improve the sensitivity and pace of MRSA detection (5, 6, 9, 10, 12, 13, 15, 17, 19, 20, 22–24, 26–30); however, currently available media that have been marketed at this time are recommended only for nasal specimens.

This study was designed to compare the performance of BD BBL CHROMagar MRSA II (CMRSAII), a chromogenic medium which incorporates cefoxitin, with traditional culture media in the recovery and identification of MRSA isolates from clinical specimens, including respiratory, lower gastrointestinal, and skin specimens as well as wound cultures and blood culture bottles with Gram-positive cocci. In addition, it was designed to determine whether CMRSAII results may be reported as presumptive or definitive with no (or one) confirmatory test at 24 and 48 h of incubation. (These data were presented in part at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 25 to 28 October 2008.)

MATERIALS AND METHODS

Participating centers. Four centers participated in the study, two in the United States (Hospital of St. Raphael, New Haven, CT [RAPH] and Rhode Island Hospital and Brown Albert Medical School, Providence, RI [RIH]) and two in Germany (University Hospital Heidelberg, Heidelberg, Germany [HYG] and Labor Limbach, Heidelberg, Germany [LIMB]). The study began in October 2007 and ended in March 2008.

The sponsor trained technologists performing testing at each site. Emphasis was placed on appropriate storage and examination of the medium, as well as proper performance of antimicrobial susceptibility tests. All sites and participating technologists were required to pass a proficiency panel prior to participation in the study.

The study sites followed their own laboratory approved methods and procedures for collection and transport of all clinical specimens. The following collecting devices were used: uni-Ter Ames CLR, sterile tubes, Bactec media (Plus Aerobic, Plus Anaerobic, and PES Plus) (HYG); Ames agar with charcoal Copan 114, Ames flexible wire Copan 190C, Bectec media (Plus Aerobic, Plus Anaerobic, and PES Plus) (LIMB); culture swab with Stuarts, Copan 139CQ, sterile cups, Trek media (standard aerobic, 80 ml; standard anaerobic, 80 ml) (RIH; used double swabs); sterile culturette Starplex Scientific 2162, sterile cup, BacT/Alert media (standard aerobic, standard anaerobic) (RAPH).

Media and inoculation. The study sites followed their own laboratory approved procedures and order of inoculation of specimens onto standard culture media. All sites inoculated specimens to a traditional reference blood agar plate in addition to the CMRSAII plate. Each site chose the placement of

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the CMRSAII plate, but each specimen was plated first to the reference medium and then to the CMRSAII plate. The following reference media were used: HYG, Columbia agar with 5% sheep blood for all specimens; LIMB, Columbia agar with colistin, nalidixic acid, and 5% sheep blood for stool specimens and Columbia agar with 5% sheep blood for all other specimens; RIH and RAPH, Columbia agar with colistin, nalidixic acid, and 5% sheep blood for stool specimens and Trypticase soy agar (TSA) with 5% sheep blood for all other specimens.

All media were streaked for isolation and incubated at 35 to 37°C. All CMRSAII plates were required to be read at 18 to 28 h for the 24-h read range. If mauve colonies were observed during this reading range, no further incubation or reading was performed. If no mauve colonies were observed at the 24-h read range, plates were required to be reincubated and read again at 36 to 52 h for the 48-h read range.

**Detection and identification of S. aureus and MRSA.** Recovery and identification of MRSA from traditional media was considered the reference method. Following incubation, the traditional media were examined for colonies suggestive of *S. aureus*, identified using conventional laboratory tests, including coagulase testing (HYG, Pastorex Staph plus [Bio-Rad]; LIMB, SliedexStaph Plus [bioMerieux]) and/or coagulase plasma (rabbit with EDTA); RIH and RAPH, Staphaurex [Remel]); and Vitek (RIH), Vitek 2 (HYG and LIMB), or MicroScan (RAPH). Methicillin resistance was determined by the criteria specified in the standard test method documents (CLSI M2-A9 [7]) for cefoxitin disk diffusion.

Recovery and identification of MRSA on CMRSAII were considered the test method. Interpretive categories for the CMRSAII medium were the following. Growth of one or more mauve-colored colonies was considered positive for MRSA; a coagulase test was performed to confirm each CMRSAII mauve colony as positive for *S. aureus* during the study. Nonmauve colony growth or the absence of growth was considered negative for MRSA. MRSA was confirmed from the reference plate by cefoxitin disk diffusion testing. If the reference plate did not indicate any *S. aureus* colonies (e.g., due to no growth or overgrowth by other normal flora) and the CMRSAII plate recovered a presumptively positive MRSA (mauve colony, coagulase positive), then the cefoxitin disk diffusion testing was performed using the CMRSAII plate. Confirmation of MRSA using either a reference plate and/or CMRSAII was considered a true positive.

**Data analysis.** Sample size estimation was done separately for each specimen group (respiratory, stool, and skin specimens and wound and positive blood culture bottles containing Gram-positive cocci). Depending on the study site and the specimen type, an estimated *S. aureus* isolation rate of 5 to 25% was expected. Of these *S. aureus* isolates, approximately 5 to 40% were expected to be MRSA depending on the site and specimen type. The goal was to recover approximately 350 to 475 total MRSA clinical isolates across specimen types and all participating sites with a target of approximately 50 to 75 MRSA isolates positive for each of respiratory, stool, and skin samples, as well as 100 to 125 MRSA-positive wounds and approximately 100 to 125 MRSA isolates from positive blood cultures containing Gram-positive cocci. Addressing the prevalence of MRSA and the probable recovery of MRSA isolates from the various specimen sites was necessary to allow a statistically powered study for the comparison of the new media.

Only those specimens that were compliant with the protocol were included in the data analysis. For study subjects with multiple positive MRSA specimens over the study period, only up to two MRSA-positive isolates from each specimen type per subject were included. Subsequent multiple MRSA isolates from the same specimen type and subject (3 or more) were excluded.

Data entry was performed primarily through the use of automated case report forms (CRF) using the Cardiﬀ TeleForm System. Data were stored in a Microsoft SQL server database. Data verification was performed during data entry, using both visual inspection and programming.

### RESULTS

A total of 5,148 specimens were enrolled in the study, of which 97 had to be excluded due to noncompliance with the study protocol, e.g., specimen types that were not included in the protocol or repeated positive samples from the same patient. From the evaluated 5,051 specimens, 1,186 *S. aureus* isolates were recovered, of which 778 (65.6%) were MRSA. The overall MRSA rate was 15% and differed between 13% and 18% by study site and between 14 and 17% by type of specimen (Table 1).

**Poolability.** For respiratory and stool specimens and blood, data were poolable across sites ($P$ value = 0.1092, 1.0000, and 1.0000, respectively) at the 0.05 significance level. For skin and wound specimens, an outlier position was detected for one site (RIH). However, this was probably attributable to the low numbers of skin specimens and the perfect performance for wound specimens at this clinical site. The 95% confidence intervals of the overall agreement for this site demonstrate the overlapping with the 95% confidence intervals of the overall agreement for the other three sites. Therefore, the skin and wound data were deemed poolable across the study sites, and the data for all sites were included in the evaluation.

**Accuracy of identification as MRSA with and without a confirmatory test.** After 24 h (18 to 28 h) of incubation time, 99.7% (671/673) of mauve colonies on CMRSAII were confirmed as MRSA. Of 243 additional plates with mauve colonies at 48 h (36 to 52 h) of read time, 170 were not *S. aureus* as revealed by a recommended confirmatory test (coagulase or *Staphylococcus* latex agglutination test). Based on these data, it was determined that mauve-colored colonies visible at 24 h (read range, 18 to 28 h) on CMRSAII need not be confirmed as *S. aureus* by a coagulase or *Staphylococcus* latex agglutination test. Conversely, it was determined that mauve-colored colonies visible at 48 h (read range, 36 to 52 h) should be

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>HYG</th>
<th>LIMB</th>
<th>RIH</th>
<th>RAPH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory, e.g., nares, sputum, throat</td>
<td>738 (11)</td>
<td>325 (32)</td>
<td>223 (11)</td>
<td>160 (18)</td>
<td>1,446 (16)</td>
</tr>
<tr>
<td>Stool samples</td>
<td>5 (0)</td>
<td>41 (41)</td>
<td>355 (12)</td>
<td>293 (21)</td>
<td>694 (17)</td>
</tr>
<tr>
<td>Skin, e.g., groin, axilla, perineum, perineal</td>
<td>599 (13)</td>
<td>629 (14)</td>
<td>5 (20)</td>
<td>42 (17)</td>
<td>1,275 (14)</td>
</tr>
<tr>
<td>Wound</td>
<td>234 (23)</td>
<td>565 (9)</td>
<td>61 (23)</td>
<td>88 (11)</td>
<td>948 (14)</td>
</tr>
<tr>
<td>Blood</td>
<td>207 (8)</td>
<td>256 (23)</td>
<td>133 (16)</td>
<td>92 (17)</td>
<td>688 (16)</td>
</tr>
<tr>
<td>Total</td>
<td>1,783 (13)</td>
<td>1,816 (18)</td>
<td>777 (13)</td>
<td>675 (18)</td>
<td>5,051 (15)</td>
</tr>
</tbody>
</table>
confirmed as *S. aureus* by a confirmatory coagulase or latex agglutination test.

**Positive and negative percent agreement of CMRSAII versus traditional reference media.** The positive percent agreement between CMRSAII and traditional reference media was 87.8% (545/621) at the 24-h read time without a confirmatory test (coagulase or *Staphylococcus* latex agglutination test) and 94.5% (587/621) at the 48-h read time with a confirmatory test. Negative percent agreement was 97.1% (4,302/4,430) at the 24-h read time with no confirmatory test and 96.4% (4,271/4,430) at the 48-h read time with the confirmatory test. Overall agreement with the reference media was 96% (4,847/5,051) when colony color (alone) was used to report MRSA at 24 h and 96.2% (4,858/5,051) with a confirmatory *S. aureus* test at 48 h (coagulase or latex agglutination at 48 h).

**Sensitivity and specificity of CMRSAII result compared to cefoxitin disk diffusion.** The sensitivities of CMRSAII compared to the cefoxitin disk test at the 24-h (without confirmation test) and 48-h (with confirmation test) reads were between 85.5% and 100%, with the lowest sensitivity for respiratory specimens and the highest sensitivity for blood cultures containing Gram-positive cocci. The specificity was 99.8% for respiratory specimens and 100% for all others.

**Recovery of MRSA on CMRSAII versus traditional culture reference plate and cefoxitin.** Overall recovery of MRSA from 5,051 compliant specimens for CMRSAII was 95.6% (744/778), compared to recovery on reference blood plates of 79.8% (621/778) (*P* < 0.001) (Table 2). For each specimen type group, recovery of MRSA on CMRSAII was better than recovery of MRSA from the traditional culture method (or equal in the case of positive blood cultures containing Gram-positive cocci, where both were 100%). Only 2 false positives were encountered on the CMRSAII plate at 24 h and none at 48 h when a confirmatory test was used. The false-positive rates were 0.05% at 24 h (2 of 4,340) and 48 h (2/4,273).

**DISCUSSION**

According to the manufacturer, CMRSAII is a selective medium that is specifically designed for screening for MRSA from various kinds of microbial specimens, including sputum, wound swabs, and stool. This implies that the composition of the medium allows suppression of a broad range of normal flora. Otherwise, it is likely that high numbers of fast-growing microorganisms overgrow the MRSA colonies. In this multicenter study, we evaluated the medium and tested its ability to detect MRSA even from high-flora samples with mixed flora, such as stool and rectal samples.

Apart from positive blood cultures, the highest sensitivity and recovery rate were found for stool samples, from which the broadest range of microbial flora is expected. In this kind of material, *S. aureus* colonies can be difficult to detect among other microorganisms on the nonselective reference media. The lowest sensitivity and recovery rates were found for respiratory samples, although the difference was not significant. The fraction of *S. aureus* in the normal flora of the respiratory tract is certainly higher than that in other body sites. In addition, fast growing Gram-negative rods are less often detected from respiratory specimens, and thus, *S. aureus* or MRSA, respectively, can be more easily detected on the nonselective reference media even if they are present in small numbers.

The recovery of MRSA from different kinds of samples has been compared in a few other studies (19, 22, 26, 29). For some types of specimens, the recovery rate was as low as 50% (29), but this does not seem to be equally distributed for different brands of selective media. For example, Nahimana et al. (22) compared three different chromogenic media for the detection of MRSA and found the lowest recovery from throat specimens for some media, which is comparable with our results, but for another medium, the lowest level of recovery was detected for perineal specimens. The differences between the sensitivities of different chromogenic media may be caused by additives in the media that inhibit growth of the concomitant microorganisms but that also inhibit the growth of MRSA (25). However, groups that compared different media usually did not compare the recovery rates for different specimens on selective media to those on nonselective media (19, 22, 26, 29).

Since there is a lack of a gold standard for MRSA screening, most authors compared the recovery rate of a specific medium with the combined result of all media or methods that have been used (5, 6, 9, 10, 12, 13, 15, 19, 20, 21–24, 26–30). This prevents the misclassification of true-positive results, i.e., detection of MRSA classified as false positive if the comparison method failed to detect MRSA. However, this method is prone to considerable bias, because it depends on the quality and characteristics of the studied medium itself and all media and methods that have been included. In addition, recovery rates for a single test medium seem to be lower if the number of included reference media is high (22, 26) or if a direct PCR is included in the methods (28, 29). Nahimana et al. (22) and Stoakes et al. (26) both compared 4 different selective media and found recovery rates of approximately 80% after 48 h of incubation. Van Hal et al. (28, 29), who used PCR detection of MRSA for comparison in two studies, found recovery rates of around 75% for the selective media. Groups that compared only one selective medium with nonselective media reported recovery rates above 90% (9, 12, 19, 24, 27). Hence, comparison of results gained from different studies can only be performed very cautiously.

CHROMagar MRSA is the predecessor of the medium that has been studied here. Flayhart et al. (12) compared this me-

### TABLE 2. MRSA recovery from traditional culture and CMRSAII

<table>
<thead>
<tr>
<th>Specimen category</th>
<th>Read time (h)</th>
<th>% MRSA recovery (no. positive by indicated test/total no. positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional culture</td>
<td>CMRSAII</td>
</tr>
<tr>
<td>Respiratory</td>
<td>24</td>
<td>79.8 (182/228)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>76.8 (182/237)</td>
</tr>
<tr>
<td>Stool samples</td>
<td>24</td>
<td>86.9 (93/107)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>77.5 (93/120)</td>
</tr>
<tr>
<td>Skin</td>
<td>24</td>
<td>68.6 (118/172)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>66.3 (118/178)</td>
</tr>
<tr>
<td>Wound</td>
<td>24</td>
<td>90.6 (115/127)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>88.5 (115/130)</td>
</tr>
<tr>
<td>Blood</td>
<td>24</td>
<td>100 (113/113)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100 (113/113)</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>83.1 (621/747)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>79.8 (621/778)</td>
</tr>
</tbody>
</table>
diurn in a multicenter study to a nonselective medium and found a recovery rate for CHROMagar MRSA of 95%, compared to 86% on TSA blood agar for nasal specimens. The recovery rate of CMRSAII for respiratory swabs, which included nasal swabs, was 92%, compared to 77% for the reference media, and was thus in the same range.

Recovery rates of MRSA from positive automated blood culture bottles with Gram-positive cocci were 100% for CMRSAII, identical to those that have been previously described for another chromogenic medium (9). Positive blood cultures bottles with Gram-positive cocci were 100% for CMRSAII, identical to those that have been previously described for another chromogenic medium (9). Positive blood cultures are mostly positive for only one species, and the number of microorganisms is high, making lack of sensitivity or overgrowth with other microorganisms unlikely. On the other side, false-positive results may occur due to growth of susceptible organisms that have been inoculated on the medium in high numbers (9). This phenomenon was not observed in this study for CMRSAII.

Similar to most chromogenic media containing cefoxitin, the specificity of CMRSAII was very high. At the 24-h read time, the total number of false positives was only 2 of 673 (0.02%), allowing for the possibility of reporting mauve colonies as MRSA after a 24-h read time without further confirmation. Compernolle et al. (10) found 2% false-positive results on chromogenic media (MRSA ID [bioMérieux] and CMRSA [BD Diagnostics]) after 24 h of incubation time in their institution and suggested performing confirmatory testing at the 24-h read time. They included a large number of samples for surveillance cultures that were taken from patients in the intensive care unit, where resistant organisms that may grow on the selective media are more prevalent than in other departments. We did not collect data on the location of the patients; however, a high number of specimens were taken from university hospitals that often have higher rates of multiresistant organisms without impairing the specificity.

Because 9.8% of MRSA isolates (73/744) were detected only after 48 h of incubation, the extension of the incubation time is valuable for every specimen except positive blood cultures. After 48 h of incubation, 170 out of 243 specimens with mauve colonies on CMRSAII did not grow MRSA on the reference plate, but all of these mauve colonies could be easily proven to be MRSA by using a confirmatory test, such as coagulase or cefoxitin disk diffusion testing. When a confirmatory test was included, the specificity of CMRSAII was 100% and was comparable to or better than those described for other chromogenic media (9, 10, 12, 19, 22, 23, 26). In conclusion, CMRSAII is a reliable screening medium for multiple specimen types.

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