Identification of Two Borderline Oxacillin-Resistant Strains of *Staphylococcus aureus* From Routine Nares Swab Specimens by One of Three Chromogenic Agars Evaluated for the Detection of MRSA

Blake W. Buchan, PhD,1 and Nathan A. Ledeboer, PhD, D(ABMM)1,2

**Key Words:** Chromogenic agar; Methicillin resistant *Staphylococcus aureus*; MRSA; Borderline resistance

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**Abstract**

*Methicillin-resistant* Staphylococcus aureus (MRSA) is a leading cause of nosocomial infections that result in extended hospital stays and increased mortality. Therefore, rapid, cost-effective techniques for surveillance and detection of MRSA are critical to the containment and prevention of the spread of MRSA within the health care environment. We examined the ability of 3 chromogenic media (Spectra MRSA, Remel, Lenexa, KS; MRSA Select, Bio-Rad, Redmond, WA; and ChromID MRSA, bioMerieux, Marcy l’Etoile, France) to detect MRSA from routine surveillance specimens following 18, 24, and 48 hours of incubation. Our results indicate that detection of MRSA using all 3 chromogenic media is optimal following 24 hours of incubation. Early examination reduced sensitivity, while extended incubation reduced specificity. In addition, Spectra MRSA identified 2 borderline oxacillin-resistant strains of *S* aureus that were not detected by the other 2 chromogenic agars evaluated. These strains demonstrate increased basal and inducible resistance to β-lactam antibiotics.

The usefulness of chromogenic media has been highlighted by studies that demonstrate this type of media to be superior to standard culture methods for the rapid isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from typically polymicrobial specimens such as superficial wounds, ulcers, sputum, and the anterior portion of the nares.1,2 However, the full benefits of chromogenic agar can be realized only if the medium is able to consistently and reliably identify MRSA strains with a high sensitivity and specificity. These parameters can be affected by variables such as incubation time before inspection for typically colored colonies and bacterial load, specifically MRSA, in a given specimen.

A study by Nonhoff et al3 examined the sensitivity and specificity of 3 chromogenic media for the detection of MRSA from mucocutaneous swab specimens following 18 or 48 hours of incubation at 35°C. Their findings demonstrated excellent sensitivity when using MRSA quality control strains but poor sensitivity (44.1%-45.6%) of all 3 media (ChromID MRSA, bioMerieux, Marcy l’Etoile, France; MRSA Select, Bio-Rad, Redmond, WA; and MRSA Screen, Oxoid, Basingstoke, England) for the detection of MRSA in clinical specimens following 18 hours of incubation.3 The sensitivity of all 3 media increased dramatically after 48 hours of incubation; however, this extended incubation time ablates one of the primary advantages of using chromogenic agar for the detection of MRSA. A different study that evaluated yet another type of chromogenic agar (CHROMagar, Paris, France) found a high sensitivity for detecting MRSA from clinical specimens at 24 hours after inoculation (95%) but failed to include any comparator chromogenic medium.3

In our present study, we evaluated the claim that Bio-Rad MRSA Select chromogenic medium can reliably identify MRSA from direct plating of clinical specimens as early as...
Materials and Methods

A total of 364 clinical samples (nares swabs) were obtained from Dynacare Laboratories (Milwaukee, WI) in accordance with an institutional review board–approved protocol. Specimens were eluted in 0.85% saline solution by vortex, concentrated by centrifugation, and resuspended in 100 μL of 0.85% saline. Equal amounts of the concentrate were streaked for isolation to each of the 3 chromogenic media evaluated in this study.

Plates were incubated at 37°C and observed at 18, 24, and 48 hours for typically colored colonies (Spectra MRSA, denim blue; MRSA Select, pink; ChromID MRSA, green) indicative of MRSA. Representative colonies were restreaked for isolation onto trypticase soy agar with 5% sheep blood (BD, Sparks, MD). Identification of MRSA was confirmed by Gram stain, catalase reaction, and latex agglutination (Staphaurex, Remel).

The oxacillin minimum inhibitory concentration (MIC) of confirmed S. aureus strains was determined by using Etest oxacillin (bioMerieux). A 0.5 McFarland standard bacterial suspension was made from an overnight culture, plated to Mueller-Hinton (MH) or MH with 2% sodium chloride agar (as per current Clinical and Laboratory Standards Institute [CLSI] recommendation), and allowed to dry, and an Ettest strip was applied to the plate. The MIC was interpreted following 24 hours of incubation at 37°C. Characterization of identified borderline oxacillin-resistant strains was carried out using Etest ampicillin and Etest ampicillin/sulbactam strips. Latex agglutination for MecA (PBP2a) was conducted using Etest ampicillin and Etest ampicillin/sulbactam strips.

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18 hours after inoculation. As a means for comparison, we also evaluated the performance of Remel Spectra MRSA and bioMerieux ChromID MRSA, both of which recommend a 24-hour incubation for best results.

Results

Of 364 clinical specimens screened, 50 contained colonies identified as MRSA by at least 1 of the chromogenic media and were confirmed to be S. aureus (catalase-positive, Staphaurex agglutination-positive, Gram-positive cocci in clusters). Results, including the sensitivity, specificity, PPV, and NPV at 18, 24, and 48 hours after inoculation for each medium, are summarized in Table 1 and Table 2.

Briefly, the appearance of typically colored colonies at 18 hours after inoculation occurred on 41 (82%) of 50 Spectra MRSA and 43 (86%) of 50 MRSA Select plates containing S. aureus–positive specimens. In contrast, a relatively low number (29/50 [58%]) of S. aureus–positive specimens produced typically colored colonies on ChromID MRSA at 18 hours after inoculation. The sensitivity of all 3 media increased markedly following 24 hours of incubation, when 48 (96%) of 50 specimens containing S. aureus produced typically colored colonies on Spectra MRSA and MRSA Select and 44 (88%) of 50 S. aureus–positive specimens produced typically colored colonies on ChromID MRSA plates (Table 1). It is interesting that 2 specimens contained S. aureus strains (MRSA109 and MRSA141, discussed later) that produced typically colored colonies after 24 hours of incubation and grew only on Spectra MRSA. Following 48 hours of incubation, only Spectra MRSA had a sensitivity of 100%, whereas the other 2 media remained at 96% (Table 1). Based on these data, the PPVs approached 100% at 18 and 24 hours after inoculation for all 3 media (Table 2) but decreased dramatically following longer incubation.

There was a high incidence of false-positive, typically colored colonies at 48 hours after inoculation on Spectra MRSA and MRSA Select media, which was not observed on ChromID MRSA. This high rate of false-positive results following 48 hours of incubation resulted in PPVs of 44.6% (Spectra MRSA), 46.6% (MRSA Select), and 92.3% (ChromID MRSA) at that late time point (Table 2). Specificity was 100% at 18 hours after inoculation for all 3 media and remained at more than 99.6% at 24 hours after inoculation (Table 1). Following 48 hours of incubation, however, the specificities dropped to 80.3% and 82.5% for Spectra MRSA and MRSA Select, respectively, whereas ChromID MRSA retained a high specificity of 98.7% (Table 1). The NPV at all incubation times for all 3 media was more than 97%, with the exception of ChromID MRSA at 18 hours, which was 93.7%. The overwhelming majority of false-positive results after 48 hours of incubation were due to strains having biochemical test results consistent with coagulase-negative Staphylococcus sp. Typically colored colonies confirmed to be S. aureus most often demonstrated oxacillin resistance at an MIC of more than 256 but ranged from 8.0 to more than 256 μg/mL (data not shown).

During the course of this study, 2 strains (MRSA109 and MRSA141) were identified that produced typically colored colonies after 24 hours and grew only on Remel Spectra MRSA and MRSA Select. Comparison of oxacillin MIC by Ettest showed an MIC of 0.38 to 0.5 for each of these strains on MH agar, which is comparable to that of the methicillin-sensitive
**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
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<th>Specificity</th>
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<tr>
<td></td>
<td>18 h</td>
<td>24 h</td>
<td>48 h</td>
<td>18 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Spectra MRSA</td>
<td>82.0 (68-91)</td>
<td>96.0 (86-99)</td>
<td>100 (92-100)</td>
<td>100 (98-100)</td>
<td>99.7 (98-99)</td>
<td>80.3 (75-84)</td>
</tr>
<tr>
<td>MRSA Select</td>
<td>86.0 (72-94)</td>
<td>96.0 (86-99)</td>
<td>96.0 (86-99)</td>
<td>100 (98-100)</td>
<td>100 (98-100)</td>
<td>82.5 (77-86)</td>
</tr>
<tr>
<td>ChromID MRSA</td>
<td>58.0 (43-71)</td>
<td>88.0 (75-95)</td>
<td>96.0 (86-99)</td>
<td>100 (98-100)</td>
<td>99.7 (98-99)</td>
<td>98.7 (96-99)</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*.

* Data are given as percentage (95% confidence interval). A total of 50 true-positive results are included. True-positive results were defined as the appearance of typically colored colonies on at least 1 of the chromogenic media that, on isolation, contained catalase-positive, Staphylococcus agglutination-positive, Gram-positive cocci in clusters, which are consistent with identification of *Staphylococcus aureus*. False-positive results are defined as typically colored colonies at any time point with Gram stain, catalase, or coagulase results inconsistent with *S. aureus*. Sensitivity is defined as True-Positive/(True-Positive + False-Negative) and specificity as True-Negative/(True-Negative + False-Negative).

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>PPV</th>
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<th>NPV</th>
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<tbody>
<tr>
<td></td>
<td>18 h</td>
<td>24 h</td>
<td>48 h</td>
<td>18 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Spectra MRSA</td>
<td>100 (91-100)</td>
<td>98.0 (89-99)</td>
<td>44.6 (35-54)</td>
<td>97.2 (94-98)</td>
<td>99.4 (97-99)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>MRSA Select</td>
<td>100 (99-100)</td>
<td>100 (92-100)</td>
<td>46.6 (36-56)</td>
<td>97.8 (95-99)</td>
<td>99.4 (97-99)</td>
<td>99.2 (97-99)</td>
</tr>
<tr>
<td>ChromID MRSA</td>
<td>100 (88-100)</td>
<td>97.8 (88-99)</td>
<td>92.3 (81-97)</td>
<td>93.7 (90-96)</td>
<td>98.1 (95-99)</td>
<td>99.4 (97-99)</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; NPV, negative predictive value; PPV, positive predictive value.

* Data are given as percentage (95% confidence interval). The PPV is defined as True-Positive/(True-Positive + False-Positive) and NPV as True-Negative/(True-Negative + False-Negative).

**Image 1**

Identification of borderline oxacillin-resistant strains of *Staphylococcus aureus*. Two clinical specimens, MRSA109 (A, B, and C) and MRSA141 (D, E, and F), produced typically colored colonies on Remel Spectra MRSA medium (A and D) that failed to grow on Bio-Rad MRSA Select (B and E) or bioMerieux ChromID MRSA (C and F) from direct and subculture plating. Each strain was streaked for purification, and a 0.5 McFarland standard resuspension was plated to all 3 media. Again, each strain produced typically colored colonies only on Spectra MRSA. MRSA, methicillin-resistant *Staphylococcus aureus*. 

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S. aureus (MSSA) control strain ATCC 29213. However, unlike ATCC 29213, the oxacillin MIC increased to about 4 μg/mL as indicated by a light zone of growth when the Etest assay was repeated on MH agar containing 2% sodium chloride (CLSI-recommended medium for Etest oxacillin assay). This is significant because the resistance breakpoint for S. aureus on oxacillin is 4 μg/mL or more per CLSI recommendations. Further characterization by the PBP2a agglutination assay indicated that these strains were both negative for the oxacillin resistance determinant MecA (PBP2a). Additional experiments demonstrated higher basal resistance of MRSA109 and MRSA141 (MIC 2.0 μg/mL) to ampicillin as compared with ATCC 29213 (MIC 0.5 μg/mL). Again, growth in the presence of 2% sodium chloride resulted in a 3-fold increase in MIC for MRSA109 and MRSA141 (MIC 8 μg/mL) and ATCC 29213 (MIC 1.5 μg/mL) Table 3 and Image 2. Under both growth conditions, resistance to ampicillin was reduced by the presence of the β-lactamase inhibitor sulbactam (Table 3 and Image 2).

Low-level resistance to oxacillin has been attributed to genes carried on a specific plasmid present in some MecA-negative S. aureus isolates. To address this possibility, we attempted to isolate plasmid DNA from each strain by using a protocol similar to that used by Vriesema et al6 (see the “Materials and Methods” section). Duplicate cultures of each strain were grown in trypticase soy broth and treated with lysozyme before lysis and column-based plasmid purification. Concentration of broth cultures by centrifugation and lysozyme before lysis and column-based plasmid purification. Concentration of broth cultures by centrifugation and concentration of column eluate by ethanol precipitation were performed to attain a concentrated sample of plasmid DNA. Two attempts to isolate plasmid DNA failed to produce a detectable amount of nucleic acid using NanoDrop (Thermo Scientific, Waltham, MA) analysis. Because of the high sensitivity of the NanoDrop instrument, this result suggests that there was no plasmid recovered and the mechanism of low-level resistance to oxacillin in these strains is not mediated by plasmid-borne determinants.

Antibiotic profiles (antibiograms) of each strain generated using an automated system revealed several key differences between the 2 strains. Each strain had identical sensitivities to erythromycin (<0.5 μg/mL), clindamycin (<0.5 μg/mL), gentamicin (<2 μg/mL), rifampin (<0.5 μg/mL), and tetracycline (<0.5 μg/mL) but differed in resistance to levofloxacin (MRSA109, 4 μg/mL; MRSA141, <0.5 μg/mL), moxifloxacin (MRSA109, 1 μg/mL; MRSA141, <0.5 μg/mL), and linezolid (MRSA109, 2 μg/mL; MRSA141, 4 μg/mL).

Table 3
Increased Basal and Inducible Resistance to Ampicillin by MRSA109 and MRSA141

<table>
<thead>
<tr>
<th>Strain/Etest Strip</th>
<th>MIC (μg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>MH Agar</td>
</tr>
<tr>
<td>ATCC29213 (MSSA)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.75</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>0.38</td>
</tr>
<tr>
<td>MRSA109</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2.0</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>1.0</td>
</tr>
<tr>
<td>MRSA141</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2.0</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>1.0</td>
</tr>
</tbody>
</table>

MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-sensitive Staphylococcus aureus.

Discussion

Routine screening for asymptomatic carriage of MRSA on hospital admission has been demonstrated in some institutions to reduce the rate of MRSA acquisition and infection. The use of polymerase chain reaction–based technologies for the detection of MRSA provides a rapid means of identifying MRSA in clinical specimens, often with turnaround times of as little as 1.5 hours from time of specimen submission. However, the relatively high per test cost may make polymerase chain reaction–based methods impractical for routine surveillance of a large inpatient population, especially in low endemicity environments. In contrast, standard culture-based techniques for screening are inexpensive but can be laborious, requiring isolation of colonies from mixed cultures followed by biochemical or automated identification. This process can take up to 76 hours, which may delay proper interventional strategies or result in extended, unnecessary isolation of non-colonized patients. Chromogenic media provide a method for rapid, comparatively inexpensive, high-throughput screening. MRSA colonies are easily differentiated from normal flora or among mixed infections on a primary streak plate based on a characteristic color. This allows rapid positive identification of MRSA from clinical specimens with a sensitivity and specificity similar to that of nucleic acid tests.

Our data indicate that identification of MRSA from clinical specimens based on typically colored colonies on chromogenic media should be performed at 24 hours after inoculation to achieve the highest sensitivity. Observation at times earlier than 24 hours after inoculation can lead to reduced sensitivity owing to the occurrence of false-negatives. Specifically, the reduced ability to reliably call typically colored colonies after 18 hours of incubation on all 3 media was especially prevalent in specimens having a low S. aureus burden. Conversely, analysis of the chromogenic media at 48 hours is significant because the resistance breakpoint for S. aureus on oxacillin is 4 μg/mL or more per CLSI recommendations.
Increased basal and inducible resistance to ampicillin by methicillin-resistant Staphylococcus aureus (MRSA)109 and MRSA141 strains as compared with methicillin-sensitive Staphylococcus aureus (MSSA) QC strain ATCC 29213. The MSSA QC strain ATCC 29213 has an ampicillin minimum inhibitory concentration (MIC) of 0.75 μg/mL on MH agar. Both MRSA109 and MRSA141 have an elevated basal ampicillin MIC of 2.0 μg/mL on MH agar. The MIC of all 3 strains is elevated approximately 3-fold in the presence of 2% NaCl, resulting in ampicillin MICs of 1.5 μg/mL for the MSSA QC strain ATCC 29213 and 8.0 μg/mL for MRSA109 and MRSA141. The presence of sulbactam reduces the ampicillin MIC for all strains regardless of NaCl in the growth medium. These results suggest that strains MRSA109 and MRSA141 overexpress an intrinsic β-lactamase, which is further inducible by elevated NaCl concentrations and may be responsible for the observed borderline oxacillin resistance of these strains.
hours after inoculation results in reduced specificity, primarily as a result of coagulase-negative Staphylococcus sp. Because of the observed poor specificity of MRSA Select and Spectra MRSA following extended incubation times, we recommend confirmation of typically colored colonies using catalase and S aureus–specific latex agglutination or other biochemical tests for typically colored colonies observed following more than 24 hours of incubation.

A limitation of our study was the manner in which specimens were plated for comparison. Generally, nares swabs for the surveillance of MRSA are plated directly to a chromogenic agar for screening. In this study, swabs were first resuspended in saline and then concentrated before plating. This method may have reduced the absolute number of bacteria plated to each medium and, thus, may have had a negative effect on sensitivity. However, this step was necessary to ensure that approximately the same number of bacteria was plated to each type of agar for a fair comparison of performance.

Spectra MRSA identified 2 strains of S aureus with elevated resistance profiles that were not detected by MRSA Select or ChromID MRSA. Unlike typical MRSA strains, these strains were both negative for the methicillin-resistance determinant MecA (PBP2a). Increased sodium chloride concentration is one of several environmental cues known to induce expression of genes encoding penicillinases intrinsic to S aureus. Elevated expression of these enzymes in S aureus has been shown to partially hydrolyze penicillinase-resistant β-lactams, including oxacillin, effectively conferring low-level resistance to these strains. So-called borderline oxacillin-resistant S aureus strains have been described in clinical settings and have failed treatment with β-lactam antibiotics commonly used to treat MSSA infections.

Low-level oxacillin resistance among mecA-negative S aureus strains has also been attributed to plasmid-borne determinants, including hyperproduced penicillinases, genes conferring resistance to cadmium, or other gene products; however, we were unable to isolate plasmid from MRSA109 or MRSA141. We were also unable to enrich for or isolate colonies with elevated oxacillin resistance, indicating that the heteroresistance observed on the oxacillin Etest on MH agar containing 2% sodium chloride was the result of altered gene expression rather than a subpopulation of bacteria with intrinsically higher resistance to oxacillin. Furthermore, antibiograms of each strain obtained from Phoenix automated sensitivity tests revealed different resistance patterns, suggesting that these are independent isolates rather than clonal strains.

Hence, our data indicate that the ability of MRSA109 and MRSA141 to grow in the presence of the β-lactam antibiotic present in Spectra MRSA medium, despite being mecA-negative, may be due to elevated basal and inducible expression of intrinsic β-lactamases in these 2 strains. While these strains are not archetypal MRSA, identification of these strains as MRSA by Remel Spectra MRSA medium is of clinical importance because they must be treated as MRSA in a clinical setting owing to the risk of treatment failure following MSSA treatment guidelines.

References


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