Performance of BD Max StaphSR for Screening of Methicillin-Resistant *Staphylococcus aureus* Isolates among a Contemporary and Diverse Collection from 146 Institutions Located in Nine U.S. Census Regions: Prevalence of *mecA* Dropout Mutants

Rodrigo E. Mendes, Amy A. Watters, Paul R. Rhomberg, David J. Farrell, Ronald N. Jones

JMI Laboratories, North Liberty, Iowa, USA

This study determined the performance of BD Max StaphSR and the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) with an unrecognized staphylococcal cassette chromosome *mec* (SCCmec) right-extremity junction (MREJ) region among 907 methicillin-resistant *S. aureus* (MRSA) and 900 methicillin-susceptible *S. aureus* (MSSA) isolates. The rate of *mecA/mecC* dropout mutants was also evaluated. Only three MRSA isolates (99.7% sensitivity; 904/907) were classified as MSSA by the BD Max StaphSR assay, due to negative results for MREJ. Eight MSSA isolates (99.1% sensitivity; 892/900) were assigned as MRSA. However, six of these MSSA isolates had the *mecA* gene confirmed by PCR and sequencing (99.8% sensitivity; 898/900). Overall, 7.1% (64/900) of MSSA isolates showed results compatible with a *mecA* dropout genotype.

Several studies have reported a decline in the incidence of hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) and invasive infections in US and European hospitals (1–6). However, the incidence of community-onset (CO) MRSA infection has varied according to geographic region (7–10). Despite variability in the occurrence of CO-MRSA and HA-MRSA invasive diseases, *S. aureus* persists as the most common organism responsible for human infections, and methicillin resistance remains the most commonly identified resistance in medical institutions (11). Therefore, proper infection control practices and antimicrobial stewardship strategies play important roles in controlling MRSA infections (12, 13).

Screening for MRSA carriers has become an important tool for early detection and to help prevent MRSA spread (14). Early generations of molecular assays targeting the *mecA* gene may provide false-positive results due to the copresence of methicillin-resistant staphylococci other than *S. aureus* (i.e., coagulase-negative staphylococci [CoNS]) (15). Performance evaluations of second-generation assays targeting the staphylococcal cassette chromosome *mec* (SCCmec)-orfX right-extremity junction (MREJ) region reported the presence of *S. aureus* carrying a genetic element that lacked the *mecA* (so-called dropout) mutant, again resulting in false-positive reports (16). Newer approaches targeting both *mec* and MREJ region sequences have been developed to minimize the likelihood of false-positive results, thus minimizing unnecessary isolation precautions (17). However, a false-positive reaction can still occur in the presence of mixed populations of methicillin-resistant CoNS and a dropout *S. aureus* mutant.

This study aimed to (i) determine the relative percentage rate of *mecA/mecC* dropout mutants among methicillin-susceptible *S. aureus* (MSSA) isolates collected from U.S. hospitals and (ii) determine the relative percentage rate of MRSA with unrecognized MREJ region sequences. A total of 907 MRSA and 900 MSSA isolates were included (at least 100 MRSA and 100 MSSA from each U.S. Census region). Isolates were collected from 146 U.S. hospitals during the 2013 SENTRY Antimicrobial Surveillance Program (see Table S1 in the supplemental material). Diversity within this collection was provided by the selection of isolates from multiple medical centers within each US Census region and selection of isolates displaying distinct antimicrobial susceptibility profiles. Isolates were also recovered from multiple different clinical specimen types (>30 types).

Antimicrobial susceptibility testing for oxacillin and cefoxitin was performed by disk diffusion (18, 19) and broth microdilution (20), according to CLSI recommendations. These isolates were defined as MRSA or MSSA by the oxacillin and/or cefoxitin susceptibility results obtained by the reference broth microdilution and/or disk diffusion method (18–20). Isolates were subjected simultaneously to the BD Max StaphSR assay kit according to the manufacturer’s instructions with a small modification. As nasal samples are the primary specimen type used for MRSA screening, swabs were artificially prepared by placing them in fresh bacterial suspensions containing \(~1 \times 10^6\) CFU/ml. The extra inoculum was removed and the swab placed in the manufacturer’s sample buffer tube. The remaining steps followed the manufacturer’s recommendation for specimen preparation. The BD Max StaphSR assay targets the *nuc* and *mecA/C* genes and the MREJ region. Dropout mutants were defined as those reactive for the targeted *nuc* gene (*S. aureus*) and MREJ region and *mecA/C* negative by the BD Max StaphSR assay. Isolates showing discrepant results regarding bacterial identification or the methicillin (oxacillin) status...
TABLE 1 | BD Max StaphSR assay performance compared with phenotypic methicillin (oxacillin and cefoxitin) susceptibility results

<table>
<thead>
<tr>
<th>Isolates (no. tested)* (n = 1,807)</th>
<th>Distribution of isolates by BD Max StaphSRb</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (907)</td>
<td>MRSA</td>
</tr>
<tr>
<td>904</td>
<td>3</td>
</tr>
<tr>
<td>MSSA (900)</td>
<td>882</td>
</tr>
</tbody>
</table>

* Methicillin-resistant (MRSA) and -susceptible (MSSA) S. aureus clinical isolates defined by the oxacillin and/or cefoxitin susceptibility results obtained by the reference broth microdilution and/or disk diffusion methods according to CLSI guidelines (M02-A12, M07-A10, and M100-S25).

b Sensitivity and specificity of 99.7% (904/907) and 99.1% (892/900), respectively.

TABLE 2 | Distribution of dropout mutants among MSSA clinical isolates included in the study

<table>
<thead>
<tr>
<th>U.S. Census region</th>
<th>No. of isolates</th>
<th>Mutantsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. New England</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>2. Mid-Atlantic</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>3. East North Central</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>4. West North Central</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>5. South Atlantic</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>6. East South Central</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>7. West South Central</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>8. Mountain</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>9. Pacific</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>900</td>
<td>64</td>
</tr>
</tbody>
</table>

a The dropout mutants were defined as isolates with a negative signal from the carboxy-X-rhodamine (ROX) channel (mecA/C negative) and a reactive signal from the 6-carboxyfluorescein (FAM) channel (MREJ region positive).
different methodologies, previous studies documented a prevalence of 4.6% for dropout mutants in a worldwide collection of isolates (15), with 3.5% to 3.8% in Canada (31, 32), 5.1% in Germany (33), and 8.3% among isolates collected from arrestees in a correctional institution in the United States (34). The results described herein and elsewhere emphasize the importance of correctly identifying dropout mutants to minimize false-positive results and thus limit unnecessary expenses of infection control practices.

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REFERENCES


