

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

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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* (SCC*mec*) 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* (SCC*mec*)-*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 ceftiofur 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 ceftiofur 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 $\sim 1 \times 10^4$ 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

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TABLE 1 BD Max StaphSR assay performance compared with phenotypic methicillin (oxacillin and ceftiofloxacin) susceptibility results

Isolates (no. tested) ^a (n = 1,807)	Distribution of isolates by BD Max StaphSR ^b	
	MRSA	MSSA
MRSA (907)	904	3
MSSA (900)	8 ^c	892

^a Methicillin-resistant (MRSA) and -susceptible (MSSA) *S. aureus* clinical isolates defined by the oxacillin and/or ceftiofloxacin 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.

^c Six MSSA isolates were *mecA/C* positive using an in-house PCR screening assay, and genes were confirmed to be *mecA* on sequencing analysis. This would provide a corrected specificity of 99.8% (898/900).

between the BD Max StaphSR and phenotypic assays were repeated. Remaining discrepant results on repeat testing were evaluated further by using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) and in-house PCR assays for detection of *nuc* and/or *mecA/C* (21) and epidemiology typing (i.e., multilocus sequence typing [MLST], *spa* and *SCCmec* typing) (22).

Among the 1,807 *S. aureus* isolates included in the study, all but 2 (99.9%; 1,805/1,807) were correctly identified by the BD Max StaphSR assay as *S. aureus*. These two negative results (complete absence of amplification signals) were confirmed on a second attempt, while positive signals were recorded from the internal control Cy5.5 channel (sample processing control) and bacterial identification confirmed by MALDI-TOF and the presence of *nuc*. Among the MRSA subset (i.e., oxacillin- and/or ceftiofloxacin-resistant results), 904 (99.7%) isolates were also genotypically characterized as MRSA by the BD Max StaphSR kit (Table 1). Three MRSA isolates were classified as MSSA by the assay. Although the system detected the presence of *mecA/C* and the *nuc* gene, the final MRSA-negative results provided by the BD Max StaphSR were due to negative results for the MREJ (complete absence of amplification signals), which was confirmed in a second attempt. These isolates were screened for *mecA/C* using a multiplex PCR assay and confirmed to harbor the *mecA* gene by sequencing analysis. These isolates were ST1 (t922), ST772 (t657), and ST8 (t008) and harbored *SCCmec* types V, V, and IV, respectively.

A total of 892 (99.1%) MSSA isolates had BD Max StaphSR results in agreement with the methicillin phenotype (Table 1). Eight MSSA isolates characterized as MRSA by the system had results as follows: 6 isolates with confirmed susceptible oxacillin MIC results by broth microdilution (MIC, ≤ 0.25 to 1 $\mu\text{g/ml}$) and oxacillin (16 to 24 mm)/ceftiofloxacin (22 to 29 mm) disk tests had *mecA/C*-positive results by PCR, which were confirmed to be *mecA* by sequence (see Table S2 in the supplemental material). The remaining 2 MSSA strains characterized by the system as MRSA were negative for *mecA/C* by PCR.

A total of 64 (7.1%) MSSA isolates showed results compatible with a dropout genotype (i.e., *mecA/C* negative and MREJ region positive). These putative dropout mutants were distributed among 51 institutions in 32 states in all nine U.S. Census regions (Table 2). Higher rates of dropout mutants among the MSSA population were observed in the East South Central (13.0%) and East North Central (11.0%) regions. The Mountain region had the

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

U.S. Census region	No. of isolates	Mutants ^a	
		No.	%
1. New England	100	4	4.0
2. Mid-Atlantic	100	7	7.0
3. East North Central	100	11	11.0
4. West North Central	100	5	5.0
5. South Atlantic	100	9	9.0
6. East South Central	100	13	13.0
7. West South Central	100	6	6.0
8. Mountain	100	2	2.0
9. Pacific	100	7	7.0
Total	900	64	7.1

^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).

lowest rate (2.0%), while the remaining regions had rates between 4.0% and 9.0%.

BD Max StaphSR showed high sensitivity (99.7%) for the detection of MRSA compared with the phenotypic methicillin (oxacillin and/or ceftiofloxacin) results. Three MRSA isolates showed MSSA results by the BD Max StaphSR, which were due to nonreactive signals for the MREJ region. One isolate belonged to clonal complex (CC) 8 (ST8-MRSA-IV), while the other two isolates were associated with CC1 (ST1-MRSA-V and ST772-MRSA-V). ST772 is a single-locus variant of ST1 (23), and a previous study reported that the assay did not recognize the MREJ region of 6.8% of tested isolates, which included those belonging to CC93, CC6, or CC1 (ST772) (24).

Nevertheless, these results suggest a low prevalence of MREJ regions among isolates in the United States that are not recognized by the primers and probes utilized by the systems. The isolates included in this study were collected from 146 medical centers across different geographic locations (nine U.S. Census regions). In addition, isolates were recovered from multiple specimen types and exhibited distinct antimicrobial susceptibility profiles. These broad selection criteria were intentionally applied to provide maximum strain variability, and the results indicate the ability of the system to correctly identify *S. aureus* and MRSA among diverse collections of organisms. However, several studies have documented the overwhelming presence of USA300 (CC8) and USA100 (CC5) carrying *SCCmec* types IV and II, respectively, in the United States (22, 25–27), while the MRSA population in Europe, Latin America, and Asia-Pacific countries seem to be more heterogeneous (22, 25, 28). Therefore, validation prior to clinical use in regions other than the United States seems prudent.

A total of eight MSSA strains were assigned as MRSA by BD Max StaphSR (99.1% specificity). However, six out of eight isolates in fact carried the *mecA* gene, which would provide a corrected specificity rate of 99.8%. Other studies performed in the United States and Europe have reported sensitivity and specificity rates of $\geq 94.3\%$ and $\geq 97.7\%$, respectively (29, 30). However, it is important to mention that these studies evaluated the performance of the BD Max StaphSR from nasal swab samples or directly from blood specimens. Moreover, an overall rate of dropout mutants at 7.1% was documented, with higher rates in the East South Central and East North Central regions. When applying

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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