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Performance of BD MAX™ StaphSR against a Contemporary and Diverse Collection of *Staphylococcus aureus* Clinical Isolates from the Nine USA Census Regions: Prevalence Analysis of Dropout Mutants

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been endemic in healthcare facilities in the United States and has emerged as a community-associated pathogen. Rapid and accurate detection of colonized individuals is critical to infection control practices. This study evaluated the performance of the BD MAX™ StaphSR assay (BD Diagnostics, Quebec, Canada) against MRSA and methicillin-susceptible *S. aureus* (MSSA).

Methods: 900 MSSA and 907 MRSA recovered from a variety of clinical specimens in 146 USA sites were included. The methicillin phenotype was defined by the oxacillin and/or cefoxitin susceptibility results obtained by broth microdilution and/or disk diffusion methods (CLSI M02-A12, M07-A10 and M100-S25). Isolates were tested on the BD MAX System according to manufacturer's instructions. Isolates showing discrepant results were screened for *nuc* (identification also confirmed by MALDI-TOF), and *mecA* and *mecC* by PCR and sequencing.

Results: Among 1,807 *S. aureus*, all but two (99.9%; 1,805/1,807) isolates were assigned as *S. aureus.* Among the MRSA subset, 99.7% (904/907) of isolates were characterized as MRSA by the BD MAX StaphSR assay. The three isolates classified as MSSA were due to negative results for the SCC*mec* right-extremity junction (MREJ) region. A total of 99.1% (892/900) of MSSA isolates had BD MAX results in agreement with the methicillin susceptible phenotype. Six MSSA had the *mecA* gene confirmed by PCR and sequencing confirming their identification as MRSA by the BD MAX StaphSR assay. The remaining two MSSA isolates were negative for *mecA* or *mecC.* Overall, 7.1% (64/900) of MSSA showed results compatible with a dropout genotype (i.e. *mecA/C*-negative and MREJ region-positive) and the highest rates of dropout mutants were observed in the East South Central (13.0%) and East North Central (11.0%) regions.

Conclusions: High agreement rates were observed between methicillin susceptibility phenotypes and BD MAX StaphSR genotypic results. BD MAX StaphSR correctly identified six MSSA (classified as susceptible by all phenotypic tests used) that in fact carried the *mecA* gene. Additionally, a high rate (7.1% overall) of dropout mutants were detected, emphasizing the importance for accurate classification of MRSA.

INTRODUCTION

Screening for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers has become an important tool for early detection and to help prevent MRSA spread. Early generations of molecular assays targeting the *mecA* gene could provide false-positive results due to co-presence of methicillin-resistant staphylococci other than *S. aureus* (i.e. CoNS). Performance evaluations of second generation assays targeting the staphylococcal cassette chromosome (SCC*mec*) – *orfX* right-extremity junction (MREJ) region reported the presence of *S. aureus* carrying a genetic element that lacked the *mecA* (so-called drop-out mutant), again resulting in false-positive reports.

Newer multiplex approaches targeting *mecA/mecC* and junction region sequences (i.e. BD MAX StaphSR and BD MAX MRSA*XT* assays) have been developed to minimize the likelihood of false-positive results, therefore minimizing unnecessary isolation precaution. However, a false-positive reaction can still occur in the presence of mixed populations of methicillin-resistant CoNS and a drop-out *S. aureus* mutant. This study aimed (i) to evaluate the performance of the BD MAX StaphSR assay; (ii) to determine the relative percentage rate of "*mecA/mecC* drop-out" mutants among methicillin-susceptible *S. aureus* (MSSA) collected from USA hospitals; (iii) to determine the relative percentage rate of MRSA with unrecognized MREJ region sequences; and (iv) to compare the results obtained by the BD MAX StaphSR with those from the BD MAX MRSA*XT* assay.

MATERIALS AND METHODS

Organism collection: A total of 907 MRSA and 900 MSSA were included (at least 100 MRSA and 100 MSSA from each USA Census region). Isolates were collected from 146 USA hospitals during the 2013 SENTRY Antimicrobial Surveillance Program (see **Table 1**). Diversity within this collection was provided by selecting isolates from multiple medical centers within each USA Census region and selection of isolates displaying distinct antimicrobial susceptibility profiles. Isolates were also recovered from multiple clinical specimen types (>30 types).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing for oxacillin and cefoxitin was performed by disk diffusion and broth microdilution, according to the Clinical and Laboratory Standards Institute (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 methods.

Additional experiments and definitions: Isolates were simultaneously subjected to the BD MAX StaphSR assay according to the manufacturer's instructions. A subset of MRSA (n=91) and MSSA (n=94) isolates were also subjected to the BD MAX MRSA*XT* assay according to the manufacturer's instructions. MSSA isolates tested by the BD MAX MRSA*XT* assay included all 64 *mecA/mecC* drop-out initially detected by the BD MAX StaphSR assay. The BD MAX StaphSR assay targets the *nuc* and *mecA/C* genes, and the MREJ region. Drop-out mutants were defined as reactive for the targeted *nuc* gene (*S. aureus*) and MREJ region, while *mecA/C*-negative by the BD MAX StaphSR assay. Isolates showing discrepant results regarding bacterial identification or the methicillin (oxacillin) status between the BD MAX StaphSR and phenotypic assays were repeated. Remaining discrepant results upon repeat testing were evaluated further using MALDI-TOF (bacterial identification confirmation) and *in-house* PCR assays for detection of *nuc* and/or *mecA/C*. This reaction used the following primers: mecA/C-F – TCACCAGGTTCAACYCAAAA and mecA/C-R – CCTGAATCWGCTAATAATATTTC. This PCR assay was performed using a multiplex approach, including primers targeting the *tuf* gene as an internal control.

RESULTS

- Among the 1,807 *S. aureus*, all but two (99.9%; 1,805/1,807) were correctly identified by the BD MAX StaphSR assay as *S. aureus.* These two negative results repeated on a second attempt, while identification was confirmed by MALDI-TOF and presence of *nuc*.
- Among the MRSA subset (i.e. oxacillin and/or cefoxitin resistant results), 99.7% (904/907) of isolates were genotypically characterized as MRSA (**Table 2**). Three MRSA isolates were classified as MSSA by the BD MAX StaphSR assay.
- These three false-negative results were due to negative reactions for the MREJ, while the system detected the presence of *mecA/C* and the *nuc* gene. These isolates were screened for *mecA/C* using a multiplex PCR assay and confirmed to harbor the *mecA* gene by sequencing analysis.
- A total of 99.1% (892/900) of MSSA isolates had BD MAX results in agreement with the methicillin phenotype (**Table 2**). Eight MSSA isolates were classified as MRSA by the system; however, six isolates had *mecA*-positive results confirmed by PCR and sequencing.
- A total of 7.1% (64/900) MSSA showed results compatible with a drop-out genotype (i.e. *mecA/C*-negative and MREJ region-positive). These putative drop-out mutants were distributed among 51 institutions in 32 states in all nine USA Census regions (**Table 3**).
- All results obtained by the BD MAX MRSA*XT* for MRSA detection were in agreement with those obtained previously by the BD MAX StaphSR assay.

Table 1. Distribution of MSSA and MRSA clinical isolates included in the study.

Population[a]	USA Census Region	Number of states selected	Number of sites	Isolate sample number
MSSA	1. New England	5	7	100
	2. Mid-Atlantic	3	23	100
	3. East North Central	5	26	100
	4. West North Central	7	15	100
	5. South Atlantic	7	24	100
	6. East South Central	4	9	100
	7. West South Central	4	14	100
	8. Mountain	5	9	100
	9. Pacific	5	18	100
	Total	**45**	**135**	**900**
MRSA	1. New England	4	6	101
	2. Mid-Atlantic	3	23	100
	3. East North Central	5	27	100
	4. West North Central	7	15	101
	5. South Atlantic	7	24	100
	6. East South Central	4	9	103
	7. West South Central	4	14	101
	8. Mountain	5	9	100
	9. Pacific	5	18	101
	Total	**44**	**145**	**907**

a. Methicillin-susceptible (MSSA) and -resistant (MRSA) *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 (M02-A12, M07-A10 and M100-S25).

Table 2. BD MAX StaphSR assay performance when compared with phenotypic methicillin (oxacillin and cefoxitin) susceptibility results.

	BD MAX StaphSR[b]	
Isolates (number tested)[a]	MRSA	MSSA
MRSA (907)	904	3
MSSA (900)	8[c]	892
Total (1,807)		

a. Methicillin-susceptible (MSSA) and -resistant (MRSA) *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 (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* upon sequencing analysis. This would provide a corrected specificity rate of 99.8% (898/900).

Table 3. Distribution of drop-out mutants among MSSA clinical isolates included in the study.

USA Census Region (No. of isolates)	Number of mutants[a]	% of mutants
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 drop-out mutants were defined as those isolates with a negative signal from the ROX channel (*mecA/C*-negative) and a reactive signal from the FAM channel (MREJ region-positive).

CONCLUSIONS

- BD MAX StaphSR showed high sensitivity (99.7%) for the detection of MRSA when compared with the phenotypic methicillin (oxacillin and/or cefoxitin) results.
- Three MRSA showed MSSA results by the BD MAX StaphSR, which were due to non-reactive signals for the MREJ region. These results suggest a low prevalence (0.3%) of MREJ regions among isolates in the USA that are not recognized by the primers and probes utilized by the system.
- 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%.
- Moreover, an overall rate of drop-out mutants at 7.1% was documented, with higher rates in the East South Central and East North Central regions, emphasizing the importance to correctly identify MRSA isolates and minimize the number of false-positive results.
- Although a small number of isolates were tested by the BD MAX™ MRSA*XT* assay, which targets identical sequences for MRSA detection as MAX StaphSR (i.e. MREJ and *mecA/C* genes), results from both assays were in agreement. This finding shows that BD MAX™ MRSA*XT* assay reliably detects MRSA and also recognizes *mecA/C* dropout mutants, minimizing the risk of false-positives.

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