Reliability of the BD GeneOhm Methicillin-Resistant *Staphylococcus aureus* (MRSA) Assay in Detecting MRSA Isolates with a Variety of Genotypes from the United States and Taiwan[▽]

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The BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) assay is a molecular screening test for detection of MRSA in nasal colonization. This assay coamplifies the extremity of staphylococcal chromosome cassette *mec* (SCC*mec*) and adjacent chromosomal DNA at the SCC*mec* insertion site. Increasing reports of novel SCC*mec* types and the diverse genetic backgrounds of MRSA strains prompted us to test the accuracy of the BD GeneOhm MRSA kit with 914 MRSA isolates with a variety of SCC*mec* types harbored in 21 genetic backgrounds, as determined by the multilocus sequence type (ST). The BD GeneOhm MRSA assay was performed on colony lysates; purified genomic DNA (0.2 pg/μl and 0.2 ng/μl) was tested to confirm negative results from lysates. Of 914 MRSA isolates tested, 911 tested positive (detection rate, 99.7%). The SCC*mec* types carried by assay-positive isolates were I, II, III, IV, V, V_(SC2&5), VI, and VIII and SCC*mec* composite islands with *mec* class A and *ccr* complexes 2 and 4. One of the assay-negative isolates had a community-associated genotype: ST8, SCC*mec* type IV. However, this was an outlier among the 99.8% (434/435) ST8, SCC*mec* type IV-containing isolates that tested positive. The two other assay-negative isolates had a health care-associated genotype (ST5); both carried a distinct, uncommon, composite SCC*mec* type. In summary, the BD GeneOhm MRSA assay had a high rate of detection of MRSA isolates harboring common and uncommon SCC*mec* types from the United States and Taiwan.

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in the United States (16) has influenced many institutions and lawmakers to implement screening for MRSA colonization. In Illinois, screening for asymptomatic MRSA colonization has been mandated for all patients admitted to intensive care units (ICUs). The BD GeneOhm MRSA assay (BD Diagnostics GeneOhm, Québec City, Québec, Canada), approved for use for detection of MRSA colonization from nasal swabs, has been used by the University of Chicago Medical Center (UCMC) to identify patients who are colonized with MRSA upon admission to the ICU. However, since the initial validation of the assay (13), few studies have been done to validate the assay against genetically diverse MRSA isolates (1, 11, 20, 24). To date, no study has validated the BD GeneOhm MRSA assay with MRSA isolates circulating in the United States.

The BD GeneOhm MRSA assay uses a real-time PCR strategy (13) to detect the staphylococcal chromosome cassette *mec* (SCC*mec*) element. SCC*mec* harbors the *mecA* gene, which encodes a penicillin-binding protein (PBP 2a) with a low affinity for all available β-lactams. With rare exceptions, MRSA isolates carry the SCC*mec* integrated site specifically at the 3' end of the *orfX* gene in the chromosome. The primers and probes of the BD GeneOhm MRSA assay detect a proprietary sequence in the *orfX* gene adjacent to the SCC*mec* integration site and the SCC*mec* terminus, as described previously (13). Since coagulase-negative staphylococcus species are often me-

thicillin resistant and also carry SCC*mec* (12, 15, 19), the species-specific primer in *orfX* ensures that the assay will not mistake methicillin-resistant coagulase-negative *Staphylococcus* species for MRSA.

SCC*mec* elements are characterized by the *mec* class and *ccr* recombinase complex responsible for integration and excision of the element. They also have distinct inverted and direct repeats at the left and right termini (14). Eight SCC*mec* types (types I to VIII) have been delineated on the basis of the class of *mec* (class A, B, C1, C2, or D) and *ccr* gene complexes (*ccrAB* or *ccrC*) that are present (14). SCC*mec* types have been further stratified into subtypes due to variations in DNA sequence found in the 5' and 3' termini and the intervening regions between the *ccr* and *mec* gene complexes (14). Adding to the repertoire are composite SCC*mec* elements that contain two different *ccr* complexes. Attesting to the presumed ability of SCC*mec* elements to transfer between strains, a given SCC*mec* type can be found in the genomes of a variety of *S. aureus* genetic backgrounds, as defined by several methods, including multilocus sequence typing (MLST), *spa* typing, and pulsed-field gel electrophoresis (PFGE) typing (4). For instance, SCC*mec* type IV (SCC*mec* IV) elements with nearly identical DNA sequences have been found in MRSA strains MW2 (pulsotype USA400 and sequence type [ST] 1 [ST1]) (18) and FPR3757 (USA300 and ST8) (26) and the coagulase-negative species *Staphylococcus epidermidis* (9).

Although the primer and probe sets used in designing the BD GeneOhm MRSA assay were previously validated with a worldwide collection of MRSA isolates, methicillin-susceptible *S. aureus* (MSSA) isolates, and coagulase-negative staphylococci (13), the SCC*mec* types and genetic backgrounds of the isolates were not known then. Moreover, increasing numbers

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of reports have documented DNA sequence variation among *SCCmec* elements (1–3, 5, 9, 14, 22) that could affect detection with the BD GeneOhm MRSA assay. Indeed, Bartels et al. (1), Sissonen et al. (24), Francois et al. (11), and Rossney et al. (20) have reported on isolates in their collections from Denmark, Finland, Switzerland, and Ireland, respectively, that were not detected by this assay, despite the presence of an *SCCmec* element. We therefore sought to assess the reliability of the BD GeneOhm MRSA assay in detecting MRSA isolates that had a variety of *SCCmec* types and genetic backgrounds that we obtained from the United States. MRSA isolates from Taiwan that had genotypes that differed from those in our collection were also included.

MATERIALS AND METHODS

MRSA strains. The BD GeneOhm MRSA assay was tested with 914 MRSA culture isolates. Nine hundred six of these were stored in our collection as part of ongoing surveillance of MRSA in the years spanning 1995 to 2006, as described previously (5–8). Most were obtained in 2004 (44%) and 2005 (38.7%). Isolates used in this study were obtained from various geographic locations, including UCMC (which serves the Chicago, IL, metropolitan area and north-west Indiana) (78.9%), rural Alaska (8.6%), Texas (7.1%), Taipei, Taiwan (4.4%), and Pennsylvania (0.2%). Isolates from Alaska were from the Arctic Investigations Program, Centers for Disease Control and Prevention (8). Isolates from Taiwan were from the Tri-Services General Hospital, Taipei (5). All but one of the isolates from Dallas, TX, were from the University of Texas Southwestern Medical Center. To further expand the geographic range, additional isolates (NRS382 to NRS387) from six other states (Ohio, North Carolina, Mississippi, Connecticut, Louisiana, and Washington) in the United States were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). In addition, we included the *SCCmec* type VI typing strain from Herminia deLencastre from Portugal (0.1%). The MRSA isolates were obtained from a variety of sites of isolation. Of 688 isolates with a documented site of isolation, 33% were from skin or wounds, 20% were from abscesses, 16% were from the respiratory tract, 11% were from blood cultures, 9% were from nasal colonization, 3% were from bone or joint, 1% were from homografts, and 3% were from nonvascular devices (Foley or straight catheters [$n = 5$], drainage from Jackson Pratt devices [$n = 2$], wound drainage from gastrostomy tubes [$n = 2$]). Two percent of isolates were from vascular indwelling devices, and 2% were from peritoneal or other body fluids.

Isolates were selected from a variety of molecular types, as described in Results (Table 1). All isolates were subjected to the Staphaurex latex agglutination test (Remel, Lenexa, KS) to confirm the species identification and *mecA* PCR to confirm methicillin resistance. Isolates were stored in skim milk (Difco, BD, Sparks, MD) at -80°C . Antimicrobial susceptibility profiles were determined using the Vitek2 automated testing system (bioMérieux, Durham, NC). Isolates were studied with the approval of the University of Chicago Institutional Review Board.

Molecular typing. Typing of *SCCmec* elements was performed by PCR using type-specific primers, as described previously (5). *SCCmec* types I to VIII were assigned according to *ccr* complex and *mec* class, as specified by recently published guidelines for *SCCmec* nomenclature (14). The name of *SCCmec* V_T was recently changed to “*SCCmec* type V_(SC2&5)” (14). Control strains used for *SCCmec* typing, NCTC10442 (*SCCmec* I), N315 (*SCCmec* II), 85/2082 (*SCCmec* III), and WIS (*SCCmec* V), were kindly provided by Keiichi Hiramatsu and Teruyo Ito (Juntendo University, Japan). Strain MW2 (*SCCmec* type IV) was obtained from NARSA.

The genetic background of the isolates is reported as the ST, which was determined using MLST (10). This technique involves determining the DNA sequences of 7 housekeeping genes and comparing them with those deposited in an international database (www.saureus.mlst.net) hosted at Imperial College. The ancestral lineage (clonal cluster [CC]) of each ST was determined by eBURST analysis performed on the MLST web browser. The PCR primers for amplifying *mecA* were 5'-CTTTGCTAGAGTAGCACTCG-3' and 5'-GCTAGCCATTCC TTTATCTTG-3'. The PCR primers for amplifying *spa* were 5'-TAAAGACGA TCCTTCGGTGAGC-3' (nucleotides [nts] 945 to 966; GenBank accession number EF455822) and 5'-CAGCAGTAGTGCCGTTTGCTT-3' (nts 1319 to 1339; GenBank accession number EF455822) and were used according to the assay conditions reported previously (21).

TABLE 1. Prevalence of *SCCmec* types and results of BD GeneOhm MRSA assay

SCCmec type	ST	Total no. of isolates	% of total sample	Assay results ^e	
				No. of positive isolates	% positive isolates
I	ND ^f	1	0.1	1	100
II	All	247	27.0	247	100
	5	201	22.0	201	100
	5 slv ^g	2	0.2	2	100
	1	1	0.1	1	100
	8	2	0.2	2	100
	105	3	0.3	3	100
	231	18	2.0	18	100
	36	5	0.5	5	100
	45 slv	1	0.1	1	100
	89	1	0.1	1	100
	ND	13	1.4	13	100
III ^a	5	1	0.1	1	100
IV	All	638	69.8	637	99.8
	8	435	47.6	434	99.8
	8 slv	4	0.4	4	100
	1	117	12.8	117	100
	1 slv	1	0.1	1	100
	5	18	2.0	18	100
	5 slv	1	0.1	1	100
	59	25	2.7	25	100
	30	14	1.5	14	100
	474	1	0.1	1	100
	484	1	0.1	1	100
	535	1	0.1	1	100
	22	5	0.5	5	100
	508	1	0.1	1	100
	338 slv	1	0.1	1	100
	72	2	0.2	2	100
	ND	11	1.2	11	100
V _(SC2&5) ^b	59	15	1.6	15	100
V	All	17	1.9	17	100
	72	1	0.1	1	100
	87	1	0.1	1	100
VI	ND	1	0.1	1	100
VIII	All	2	0.2	2	100
	8	1	0.1	1	100
	5	1	0.1	1	100
Composite ^c	5	6	0.7	4	66.7
Untypeable ^d	5	1	0.1	1	100
Total		914	100	911	99.7

^a *SCCmec* types III and V_(SC2&5) are actually composite islands but are listed separately here to maintain historical consistency.

^b Previously called *SCCmec* V_T.

^c The features of all six isolates with composite *SCCmec* elements are detailed in Table 2.

^d Untypeable is defined as undetectable *ccr* genes or *mec* class using currently known typing primers.

^e “Assay” refers to the BD GeneOhm MRSA assay.

^f ND, not determined.

^g slv, single-locus variant.

TABLE 2. Characteristics of MRSA isolates with composite *SCCmec* types^a

Isolate	Molecular traits				Antibiotic susceptibility ^b						
	BD GeneOhm assay result	<i>ccr</i> alleles	<i>mec</i> class	ST	Ery	Cli	Cip	Gnt	Oxa	Rif	Van
788	+	2, 4	A	5	R	R	R	R	R	S	S
1205	+	2, 4	A	5	R	R	R	R	R	S	S
2411	+	2, 4	A	5	R	R	I	S	R	S	S
2754	+	2, 4	A	5	R	R	R	R	R	S	S
2582	—	2, 5	A	5	S	S	R	S	R	S	S
2915	—	2, 4	B	5	R	R	I	R	R	S	S

^a Although *SCCmec* types III and V_(5C2&5) are considered composite islands, data for the strain containing these elements are not included here but are shown in Table 1.

^b Ery, erythromycin; Cli, clindamycin; Cip, ciprofloxacin; Gnt, gentamicin; Oxa, oxacillin; Rif, rifampin; Van, vancomycin; R, resistant; S, susceptible; I, intermediate.

Performance of BD GeneOhm MRSA assay. Stored isolates were streaked onto tryptic soy agar (TSA) and incubated overnight at 37°C. To obtain DNA from crude cell lysates, several colonies from each plate were suspended in Tris (10 mM)-EDTA (0.5 mM), pH 8.0, to an optical density at 600 nm (OD₆₀₀) equal to a 0.5 McFarland standard (1×10^8 CFU/ml). A 50-μl aliquot of the cell suspension was transferred to a lysis tube supplied with the BD GeneOhm MRSA assay (version 3) kit and then processed according to the manufacturer's instructions. Briefly, the lysis tubes containing the cell suspension were vortexed for 5 min at high speed using a standard laboratory vortexing apparatus. To remove the cellular debris and unlysed cells, the samples were briefly centrifuged and heated at $95 \pm 2^\circ\text{C}$ for 2 min. The lysed samples were kept on ice until use. A 2.8-μl aliquot of each supernatant was added to 25 μl of reconstituted master mix in a SmartCycler tube (Cepheid, Sunnyvale, CA), and the tube was centrifuged for 5 s. The real-time PCR amplification and detection of products were performed on a SmartCycler instrument using the BD GeneOhm MRSA assay protocol provided by the manufacturer. In addition to the positive and negative controls provided in the assay kit, a fresh lysate from an MSSA isolate (strain RN4220) was included as an external negative control in each assay.

When lysates tested negative, the MRSA isolates were further tested by isolating genomic DNA (gDNA) using a DNeasy blood and tissue kit (Qiagen Sciences, MD), as recommended by the manufacturer, except that lysostaphin (50 μg/ml) was used in the resuspension buffer and the isolates were incubated at 37°C for 30 min. The concentration of genomic DNA was measured using a NanoDrop Technologies, Inc. (Rockland, DE), spectrophotometer; and dilutions of 10 ng/μl, 0.2 ng/μl, 2.0 pg/μl, and 0.2 pg/μl were prepared in order to test 9.1×10^6 , 1.8×10^5 , 1.8×10^3 , and 1.8×10^2 copies of gDNA per reaction mixture, respectively. The lysates from each negative sample were tested for the presence of *mecA*. Since coagulase-negative species can also harbor *mecA*, isolates were confirmed to be *S. aureus* by PCR detection of *spa*, the *S. aureus*-defining gene encoding protein A.

RESULTS

MRSA sample subjected to BD GeneOhm MRSA assay. A sample of 914 cultured MRSA isolates was chosen for testing the BD GeneOhm MRSA assay. This sample included every combination of *SCCmec* type and genetic background that was available in our collection. The percentage of each *SCCmec* type and the variety of genetic backgrounds (according to ST) harboring each *SCCmec* type that were tested with the BD GeneOhm MRSA assay are shown in Table 1. This consisted of 21 STs distributed over 8 *SCCmec* types, a nontypeable isolate, and composite *SCCmec* types. For example, there were isolates from 9 different STs that carried *SCCmec* type II and isolates from 15 different STs that carried *SCCmec* type IV. Among the 914 MRSA isolates that we tested, there were 8 different previously characterized *SCCmec* types [*SCCmec* types I, II, III, IV, V, V_(5C2&5), VI, and VIII] and 6 *SCCmec* types presumed to be composite islands since more than one *ccr* complex was detected. One isolate was defined as untypeable since the primers used to detect *ccr* complex allotypes 1 to

5 did not amplify a product. The two most prevalent *SCCmec* types were types IV (69.8%) and II (27%). Eighty-one percent of the *SCCmec* type II-containing isolates were ST5, and 68% of the *SCCmec* type IV-containing isolates were ST8. The predominance of these types reflects the fact that they are the most prevalent *SCCmec* types present among community-associated (CA) MRSA and health care-associated (HA) MRSA strains in the United States (7, 17). *SCCmec* type VIII contains *ccr* complex 4 and *mec* class A (28). The two *SCCmec* type V-containing isolates were from patients at UCMC; each had an unusual ST for the UCMC geographic area (ST72 and ST87). The two *SCCmec* type VIII-containing isolates were also from two patients at UCMC and had ST5 and ST8 genetic backgrounds. Isolates from Taiwan included the isolate containing *SCCmec* V_T (5), which was recently reclassified V_(5C2&5) (14).

The characteristics of the six strains presumed to contain composite *SCCmec* elements are shown in Table 2. There were three distinct types of the *ccr* complex and *mec* class combinations: four of the six tested positive for *ccr* complexes 2 and 4 with *mec* class A, one had *ccr* complexes 2 and 5 with *mec* class A, and one had *ccr* complexes 2 and 4 with *mec* class B. No epidemiologic link between the four isolates with the same *ccr* and *mec* class profile (*ccr* complexes 2 and 4 with *mec* class A) could be established with respect to date or place of isolation.

Results obtained with BD GeneOhm MRSA assay. Lysates from 914 MRSA isolates were subjected to the BD GeneOhm MRSA assay; 99.7% tested positive (Table 1). The percent from each *SCCmec* type testing positive, stratified by ST, is shown in Table 1. All but one *SCCmec* type IV-containing isolate and all isolates containing *SCCmec* types I, II, III, V, V_(5C2&5), VI, and VIII tested positive. Additionally, the untypeable isolate and 4/6 (67%) isolates with probable composite *SCCmec* elements (isolates 788, 1205, 2411, and 2754) tested positive in the assay; all four had *ccr* complexes 2 and 4 and *mec* class A (Table 2).

BD GeneOhm MRSA assay-negative MRSA isolates. All three assay-negative isolates were obtained from patients at UCMC. Features of these three isolates (isolates 2582, 2790, and 2915) are shown in Table 3. The lysates from all three isolates were *mecA* and *spa* positive by PCR, confirming the MRSA genotype and the DNA integrity of the lysates. The assay was repeated with fresh lysates of these three assay-negative isolates at least 6 times. For one isolate (isolate 2790), 6 of 7 (86%) of the replicate assays tested negative; on one

TABLE 3. Features of the three BD GeneOhm MRSA assay-negative isolates

Pt ^d	Isolate ^a	Date of isolation (mo/day/yr)	SCCmec type	ST	CA or HA ^c	Site of isolation details
1	2790	5/19/2005	IV	8	CA	Conjunctival exudates, eye
2	2582	4/7/2005	Composite (2A&5) ^b	5	CA	Fluid abdomen, pelvis
3	2915	6/17/2005	Composite (2B&4) ^b	5	HA	Biopsy culture, groin

^a The index isolate from each patient.

^b See Table 2 for details.

^c CA, defined as an isolate obtained within 3 days of a hospital admission; HA, defined as an isolate obtained >3 days after admission.

^d Pt, patient. All patients were inpatients.

occasion, this isolate tested positive. When the SCCmec types and STs were evaluated, it is clear that the isolates were unrelated. Two of the three assay-negative isolates (isolates 2915 and 2582) had a probable composite SCCmec type (Table 2), and one isolate (isolate 2790) carried SCCmec type IV (Table 2). Although the two isolates with probable composite SCCmec elements were ST5, the compositions of their SCCmec elements differed from each other; whereas isolate 2915 contained *ccr* complexes 2 and 4 and *mec* class B, isolate 2582 carried *ccr* complexes 2 and 5 and *mec* class A (Table 2). Although composite SCCmec types comprised 6/914 (0.7%) isolates in the total sample, 33% (2 of 6) of the isolates with composite elements (0.2% of all MRSA isolates tested) tested negative. This difference in detection of MRSA between isolates with composite and noncomposite elements was significant ($P < 0.001$, Fisher's exact test). This suggests that composite SCCmec types are more likely to test negative in the BD GeneOhm MRSA assay.

To substantiate that the negative assay results obtained using colony lysates were due to the DNA sequence of the isolates and were not an artifact of the lysate preparation, purified gDNA was assayed using concentrations of 10 ng/μl, 0.2 ng/μl, 2 pg/μl, and 0.2 pg/μl. The result was consistently negative for MSSA control strain RN4220 and the three assay-negative MRSA isolates when DNA concentrations spanning from 0.2 pg/μl to 0.2 ng/μl were used. However, with 10 ng/μl of gDNA, a false-positive result was consistently obtained using gDNA from the MSSA strain and the three MRSA assay-negative strains. Thus, to avoid false-positive results, gDNA concentrations between 0.2 ng/μl and 0.2 pg/μl were evaluated in this study.

DISCUSSION

Molecular screening tests have the advantage of rapidly identifying MRSA carriage prior to admission into health care institutions. The BD GeneOhm MRSA assay is one such assay in widespread use in the United States, despite the lack of reported data that validate the reliability of the test for detection of circulating strains of MRSA. In our sample of 914 culture-proven MRSA isolates, of which 96% were from the United States, we found that the BD GeneOhm MRSA assay correctly identified a variety of MRSA strains with different genotypes and SCCmec types with an overall detection rate of 99.7% (99.6% among the U.S. isolates). Three MRSA isolates (0.3%), all from our medical center, tested negative with the assay, despite the fact that they were MRSA by Vitek testing and *mecA* PCR. The assay detected isolates from a wide variety

of SCCmec types [types I, II, III, IV, V, V_(5C2&5), VI, and VIII, composite SCCmec types, and an untypeable isolate]. Not included in our strain collection was any isolate containing *ccr* complex 5 and *mec* class C1 (originally described by Berglund et al. from Sweden [2] and named SCCmec VII according to the revised nomenclature [14]). However, the prototype isolate from Sweden containing SCCmec type VII tested positive with the BD GeneOhm assay when it was performed by the manufacturer (BD Diagnostics, Québec City, Québec, Canada). Although a limitation of our study is that most of the isolates studied were ST8 and ST5 isolates that carried type IV and II SCCmec, respectively, the diversity of our sample was enhanced by including every isolate with an unusual sporadic and singleton genotype that we identified during prospective molecular surveillance of culture-proven MRSA isolates. A more diverse collection would be difficult to find since the genotypic distribution of isolates that are naturally circulating in the United States is limited to primarily SCCmec IV and SCCmec II in ST8 and ST5 genetic backgrounds, respectively (7, 25). In fact, compared with similar studies that tested the performance of the BD GeneOhm MRSA assay on MRSA isolates (1, 11, 20, 24), our study is the largest performed to date and the only study performed in the United States.

The SCCmec types of the three BD GeneOhm MRSA assay-negative index isolates were distinct from each other, and therefore, the isolates do not represent a single clone. Two of the three assay-negative isolates had different composite SCCmec elements, but they both had the genetic background ST5, which is associated with HA MRSA in the United States. The third negative isolate has genetic markers associated with CA MRSA; it carries SCCmec type IV in an ST8 genetic background. Thus, a BD GeneOhm MRSA-negative result is not restricted to isolates with an HA or CA MRSA genotype. Moreover, these data show that even a common SCCmec type (type IV) is capable of evolving polymorphisms that render it undetectable by this assay.

The failure to detect three isolates with the BD GeneOhm MRSA assay can most likely be explained by polymorphisms occurring in the SCCmec extremity of these isolates that rendered the primers or probe unable to hybridize to the target DNA. It is also possible that polymorphisms in *orfX* were responsible. In Copenhagen, Denmark, a high false-negative rate (12.6%) (1) was reported for the BD GeneOhm MRSA assay; an MRSA clone with genetic background belonging to ST8 and *spa* type t024 carried an SCCmec extremity sequence that was not recognized by the primers supplied in the kit. A region-specific assay which greatly decreased the incidence of false-negative results was designed and implemented (1). In

the United States, the ST8, USA300 MRSA isolates usually have *spa* type t008. Failure to detect MRSA isolates by this assay was also reported in Finland (24), Switzerland (11), and Ireland (20). These examples underscore the need to perform ongoing evaluation of the assay in regions where it is used. It is worthwhile mentioning that in contrast to the experience of Rossney et al. (20), we found that all isolates carrying SCCmec type V, including the type V variant found in Asia [V_(SC2&5)] (5), tested positive under all conditions. For the prototype strain of SCCmec type V, Rossney et al. (20) reported a negative result for colony lysates but a positive result for isolated genomic DNA, suggesting that the lysis procedure rather than the probe detection was at question.

Since we did not test clinical MSSA isolates, we did not address the possibility of detecting false-positive results for MSSA isolates with the BD GeneOhm assay. However, others have found that deletions within the SCCmec II cassette (27) or integration of SCC elements that lack *mecA* (23) could result in detection of an SCC cassette in MSSA isolates using the BD GeneOhm assay.

In conclusion, in contrast to the situation in Denmark and Finland, the BD GeneOhm MRSA assay detected a high percentage (99.7%) of MRSA genotypes with various SCCmec types obtained from various regions in the United States and Taiwan. DNA sequence analysis of the extremities of SCCmec elements of our assay-negative isolates is ongoing and will likely provide insight into the basis of the assay-negative results.

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