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Characterization of *Staphylococcus aureus* Isolates with a Partial or Complete Absence of Staphylococcal Cassette Chromosome Elements[▽]

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Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) by single-locus PCR assays that target the extremity of the staphylococcal cassette chromosome-*mec* (SCC*mec*) and part of the adjacent *S. aureus*-specific open reading frame gene (*orfX*) is a significant diagnostic advancement, since it provides real-time detection directly from screening specimens. However, isolates harboring *mecA* deletions within SCC*mec* may result in false-positive identification of MRSA in these assays. We characterized 24 methicillin-susceptible *S. aureus* (MSSA) isolates that tested positive in one such assay to investigate this phenomenon. Seven isolates resembled USA100 and carried SCC*mec* II elements with *mecA* deletions that spanned 20 to 46 kbp. The *mecA* excisions in USA100-resembling isolates appeared to be linked with IS*431* transposable elements present in SCC*mec* II. For 17 isolates that resembled USA400 and/or MSSA476, the identity and possible excision of SCC elements could not be confirmed. The downstream common sequence (*dcs*) shared by SCC*mec* I, II, and IV elements was detected in these isolates. Sequence analysis of the chromosomal regions flanking the missing SCC element revealed an intact SCC integration site, a duplicate *dcs*, and the enterotoxin gene cluster downstream of *orfX*. An annealing sequence for one of the SCC*mec*-specific primers (mecii574) in the single-locus PCR assay was identified in the duplicate *dcs*. In the absence of SCC, a 176-bp amplicon can be generated from this mecii574 annealing sequence to yield a false-positive result. In conclusion, partial SCC*mec* II excisions via IS*431* elements in strains that resembled USA100 and the presence of a duplicate mecii574 annealing sequence in strains that resembled USA400/MSSA476 were identified as causes for false-positive results in a single-locus PCR assay that targets the SCC*mec*/*orfX* junction.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen associated with significant morbidity, mortality, and hospitalization costs (3, 8, 9, 19, 41). Since 2003, more than 60% of *S. aureus* infections were caused by MRSA in intensive care units of hospitals belonging to the U.S. National Nosocomial Infection Surveillance system (20). In the last decade, MRSA has also emerged in community settings, causing skin and soft tissue infections and other deep-seated infections in individuals without conventional health care-associated risk factors for MRSA (10). The resistance to β-lactam antibiotics in MRSA is mediated by an altered penicillin-binding protein (PBP2a) encoded by the *mecA* gene (13). This resistance determinant resides on a mobile genetic element termed the staphylococcal cassette chromosome-*mec* (SCC*mec*) that integrates downstream of a *S. aureus*-specific open reading frame (*orfX*) (11, 14). Eight major SCC*mec* types ranging from 22 to 64 kbp in size have been described to date (1, 16-18, 26, 34, 44).

A single-locus PCR assay, utilizing a *S. aureus*-specific *orfX* gene primer (Xsau325) and a combination of SCC*mec*-specific primers that anneal at the extremity of SCC*mec* to amplify the SCC*mec*/*orfX* junction, was first proposed by Huletsky et al. for MRSA detection (15). Since *mecA* resides on SCC*mec*, detection of the SCC*mec*/*orfX* junction is considered a surrogate for the detection of MRSA. There are now a number of commercially available assays that identify MRSA based on the site-specific integration of SCC*mec* at *orfX* (36, 38, 43). Shortly after the introduction of these tests, there were reports of assay-positive specimens that only contained methicillin-susceptible strains of *S. aureus* (2, 4, 12, 32, 35-38, 40, 43). Although investigators mentioned SCC homologues and partial SCC*mec* deletions as plausible reasons for discrepant results, few studies have determined why these "false-positive" test results occurred. In an effort to determine possible causes, we characterized 24 methicillin-susceptible *S. aureus* (MSSA) isolates, collected from 10 healthcare institutions in the United States and Canada, which produced "false-positive" results in one such single-locus PCR assay.

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MATERIALS AND METHODS

Bacterial isolates. Twenty-four *Staphylococcus aureus* that were identified as MRSA by the BD GeneOhm MRSA assay (version 3; BD Diagnostics, Quebec, Quebec, Canada) originating from hospitals in six states (Illinois, Maryland, Massachusetts, Ohio, Indiana, and North Carolina) and four Canadian hospitals in two provinces (Ontario and Quebec) were examined. The *S. aureus* isolates were grown on Columbia agar supplemented with 5% sheep blood (Oxoid,

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TABLE 1. Primers used in this study

Test and primer	Sequence (5′–3′)	Description	Source or reference
MRSA/PVL detection			
16S-F	AGA GTT TGA TCA TGG CTC AG	16S rRNA	25
16S-R	GGA CTA CCA GGG TAT CTA AT		
mecA-F	AAA ATC GAT GGT AAA GGT TGG C	*mecA*	25
mecA-R	AGT TCT GCA GTA CCG GAT TTG C		
nuc-F	GCG ATT GAT GGT GAT ACG GTT	*nuc*	25
nuc-R	AGC CAA GCC TTG ACG AAC TAA AGC		
luk-F	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A	*lukS/F* (PVL)	23
luk-R	GCA TCA AST GTA TTG GAT AGC AAA AGC		
SCC typing			
KDP F1	AAT CAT CTG CCA TTG GTG ATG C	Locus B (SCC*mec* II)	33
KDP R1	CGA ATG AAG TGA AAG AAA GTG G		
DCS F2	CAT CCT ATG ATA GCT TGG TC	Locus D (SCC*mec* II)	33
DCS R1	CTA AAT CAT AGC CAT GAC CG		
IS431 P4	CAG GTC TCT TCA GAT CTA CG	Locus G (SCC*mec* II)	33
pUB110 R1	GAG CCA TAA ACA CCA ATA GCC		
ccr typing			
α1	AAC CTA TAT CAT CAA TCA GTA CGT	*ccrA1* (SCC*mec* I)	22
α2	TAA AGG CAT CAA TGC ACA AAC ACT	*ccrA2* (SCC*mec* II)	22
βc	ATT GCC TTG ATA ATA GCC ITC T	*ccrB1* (SCC*mec* I) and *ccrB2* (SCC*mec* II)	22
spa typing			
932-F	GCC AAA GCG CTA ACC TTT TA	Protein A (*spa*)	B. Kreiswirth, unpublished data
1740-R	TCC AGC TAA TAA CGC TGC AC		
J1 PCR			
LIR2-F	CGA ATT AAA CAC GCT TGC TC	J1 (SCC*mec* II)	This study
LIR2-R	CAG AAG TCT ACA GGT CTT GC		
Long-range PCR			
3.0-R	CTC AGA CAG CAA TTT CCC G	Downstream of SCC*mec*	This study
4.2-R	CCC TAA AAA TAC CTG TAG GGG		
Detection of MGE targets			
MW756-F	TGG TTA GCT ATG AAT GTA GTT GC	Hypothetical protein (*ν*Sa3), unique to MW2	45
MW756-R	GTC CAT CCT CTG TAA TTT TGC		
sel2-F	ATC ATA CAG TCT TAT CTA ACG GCG	*sel2* (*ν*Sa3)	This study
sel2-R	TCT GGA AGA CCG TAT CCT GTG		
SAS0898-F	GAA GTA GCA GAT ATA TTG GGC G	Intergenic region of *SAS0898/0899* (ϕSa4)	This study
SAS0899-R	ATC ACT GGC AAG TCC TAC C		
SAS0922-F	GAG GGA TAA ACT AAT GAA AGA ACG	Hypothetical phage protein (ϕSa4), unique to MSSA476	This study
SAS0922-R	TCA TAG AAC CTA AAC GTC CCG		
SCC*mec*/*orfX* junction detection			
Xsau325	GGA TCA AAC GGC CTG CAC A	*orfX*	15
mecii574	GTC AAA AAT CAT GAA CCT CAT TAC TTA TG	SCC*mec* I, II, IV	15
SCC*mec* II gene elements detection and sequencing			
kdpB2	TAA ACT GTG TCA CAC GAT CCA T	*kdpB*	22
II-48600R	TCT TAG ATA GTA GCC AAC GGC	J3 of SCC*mec* II	This study

Nepean, Ontario, Canada) for 16 to 18 h at 35°C. Suspension cultures were prepared by inoculating a single bacterial colony into 5 ml of BD BBL brain heart infusion broth (Becton Dickinson, Sparks, MD) and grown for 16 to 18 h at 35°C with agitation. The *S. aureus* control strains for molecular analyses included MRSA strain N315 (SCC*mec* II) (17), USA100 MRSA and USA400 MRSA (27), and MSSA476 (an MSSA strain that resembles the USA400 SmaI pulsed-field type) (7).

Determination of methicillin susceptibility. Methicillin susceptibility was determined by oxacillin broth microdilution testing in accordance with Clinical and Laboratory Standards Institute guidelines (5, 6). Methicillin susceptibility was further confirmed by PCR detection of the *mecA* determinant (25) (see below).

PCR template preparation. DNA templates were prepared by suspending *S. aureus* colonies in 0.5 ml of neutralizing buffer (30 mM Tris [pH 8.4], 2 mM EDTA [pH 9.0]) that contains 50 μl of 0.1-mm glass beads (Scientific Industries, Bohemia, NY) and heating at 100°C for 2 min. The lysates were vortexed for 2 min and clarified by centrifugation at 20,800 × *g* for 5 s. Templates for long-range PCR were prepared with a High-Pure PCR template purification kit (Roche Diagnostics, Laval, Quebec, Canada).

Primer synthesis and PCR amplification. Custom oligonucleotide primers were purchased from Invitrogen (Burlington, Ontario, Canada) (Table 1). PCR amplifications were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA). Unless otherwise specified, monoplex and multiplex PCR assays for amplicons <1.5 kbp were performed in 25-μl reactions containing 1.25 U of AmpliTaq DNA polymerase, 0.1 mM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$ (Roche Diagnostics), and 0.5 μM concentrations (each) of forward and reverse primers with the following thermocycling parameters: 94°C for 2 min, 30 cycles of 94°C for 1 s, 55°C for 15 s, and 72°C for 7 min. Long-range PCRs were performed in 50-μl reactions using an Expand Long Template PCR system (Roche Diagnostics) with the following parameters: 92°C for 2 min; 10 cycles of 92°C for 10 s, 50°C for 15 s, and 68°C for 10 min; followed by 20 cycles of 92°C for 10 s, 50°C for 15 s, and 68°C for 10 min (with an additional 20-s extension time incorporated per subsequent cycle), and then 68°C for 7 min. PCR amplification products were resolved on 0.5× Tris-borate EDTA agarose gels containing 0.5 μg of ethidium bromide/ml for digital photography under UV illumination.

MRSA PVL multiplex PCR. Detection of 16S rRNA, *mecA*, *lukS/F*, and *nuc* genes was performed as a multiplex PCR assay with the following concentrations of primer pairs: 0.5 μM *luk*, 0.4 μM *mecA*, 0.3 μM 16S rRNA (internal control), and 0.2 μM *nuc* (23, 25).

SCC*mec* type determination. SCC*mec* types were determined by using a combination of SCC*mec* typing assays as described previously (22, 33). The J1 region of SCC*mec* II was detected by monoplex PCR using the LIR2 primers.

Detection of mobile genetic elements. The *S. aureus* pathogenicity island *ν*Sa3 present in MW2 (GenBank accession no. BA000033) and the bacteriophage

TABLE 2. Characteristics of *S. aureus* isolates

Isolate	Source	PCR targets downstream of *mecA*		PCR targets upstream of *mecA*			Molecular typing		νSa3 (*MW756*/*sel2*)	ϕSa4 (SAS0898-0899/ SAS0922)
		Locus D	Locus G	*ccr*	Locus B	J1 region[a]	PFGE	*spa*		
IDI2406	Hospital A (Canada)	+	−	*ccrAB2*	+	SCC*mec* II	USA100	TJMBMDMGMK	NA[b]	NA
IDI2407	Hospital A	+	−	*ccrAB2*	+	SCC*mec* II	USA100	TJMBMDMGMK	NA	NA
IDI2445	Hospital B (United States)	+	−	*ccrAB2*	+	SCC*mec* II	USA100	TJMBMDMGMK	NA	NA
IDI2515	Hospital C (Canada)	+	+	–	+	SCC*mec* II	USA100	TJMBMDMGMK	NA	NA
IDI2595	Hospital D (Canada)	+	−	*ccrAB2*	+	SCC*mec* II	USA100	TJMBMDMGMK	NA	NA
IDI2643	Hospital E (United States)	+	−	–	+	SCC*mec* II	USA100	TJMBMDMGMK	NA	NA
IDI2683	Unknown	+	+	–	−	–	USA100	TMDMGMK	NA	NA
IDI2235	Hospital F (United States)	+	−	–	−	–	USA400	TJFKBPE	–/–	–/–
IDI2239	Hospital F	+	−	–	−	–	USA400	UJFKPE	–/–	–/–
IDI2247	Hospital F	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
IDI2250	Hospital F	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
IDI2253	Hospital F	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
IDI2256	Hospital F	+	−	–	−	–	USA400	TJFKBPE	–/–	–/–
IDI2491	Hospital G (United States)	+	−	–	−	–	USA400	UJFKPE	–/–	–/–
IDI2492	Hospital H (United States)	+	−	–	−	–	USA400	ULE	–/–	–/–
IDI2500	Hospital I (United States)	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
IDI2545	Hospital A	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
OTT004	Hospital J (Canada)	+	−	–	−	–	USA400	U4FKBPE	–/–	–/–
OTT009	Hospital J	+	−	–	−	–	USA400	UJFKAFK	–/–	+/+
OTT3498	Hospital J	+	−	–	−	–	USA400	UJFFLE	–/–	+/+
OTT3573	Hospital J	+	−	–	−	–	USA400	UJFFKBPE	–/–	–/–
OTT3574	Hospital J	+	−	–	−	–	USA400	U4FKBPE	–/–	–/–
OTT3596	Hospital J	+	−	–	−	–	USA400	UJGBGGJAGJ	–/–	–/–
OTT3598	Hospital J	+	−	–	−	–	USA400	UJFKBPE	–/–	–/–
USA400	Sunnybrook Health Sciences Centre	+	−	*ccrAB2*	−	SCC*mec* IVa	USA400	UJJFKBPE	+/+	–/–
MSSA476	PHRI	+	−	*ccrAB1*	−	–	USA400	UJJFKBPE	–/–	+/+

[a] J1 region, upstream of the *ccr* genes.
[b] NA, not applicable.

ϕSa4 associated with MSSA476 (GenBank accession no. BX571857) were detected by PCR (24). The gene targets *MW756* (nucleotides [nt] 824637 to 825009) and *sel2* (nt 828744 to 829329) were surrogates for νSa3. The intergenic region of *SAS0898*/*SAS0899* (nt 985159 to 985508) and *SAS0922* (nt 994863 to 995288) were surrogates for ϕSa4.

DNA sequence determination. Long-range PCR products were purified by using a High-Pure PCR product purification kit (Roche Diagnostics) for sequence determination at the TCAG sequencing facility (Hospital for Sick Children, Toronto, Ontario, Canada).

***spa* typing.** Strain typing based on the polymorphic X-region of the protein A gene (*spa*) was performed essentially as described previously (39), except that the primers 932-F and 1740-R were used for amplification and sequencing of the *spa* amplicon. The Kreiswirth *spa* nomenclature obtained by the *spa* typing tool (http://fortinbras.us/cgi-bin/spatyper/spaTyper.pl) was adopted for the present study.

PFGE. The Canadian standardized protocol for pulsed-field gel electrophoresis (PFGE) typing of MRSA was adopted for MSSA typing in the present study (28). SmaI-restricted DNA profiles were digitized and analyzed with BioNumerics v6.0 (Applied Maths, Austin, TX). XbaI (Roche Diagnostics)-digested *Salmonella* serotype Braenderup H9812 DNA was used as the reference standard for band size determination in BioNumerics. Alternatively, the Lambda Ladder PFG Marker (New England Biolabs, Pickering, Ontario, Canada) was used as the reference standard in Southern analyses.

Southern hybridization. Southern blot hybridization and detection were performed by using an ECL direct nucleic acid labeling and detection system (GE Healthcare, Piscataway, NJ). Briefly, SmaI-digested chromosomal DNA was resolved by PFGE and transferred onto Hybond-N^+ nylon membrane (GE Healthcare). DNA probe corresponding to the 342-bp locus D (33) was used for hybridization, and Kodak BioMax Light film (Rochester, NY) was used for signal detection.

RFLP. Restriction fragment length polymorphism (RFLP) of long-range PCR amplicons was used to assess the similarity between the amplified products. Restriction enzymes BclI, EcoRI, HincII, HindIII, and XbaI (Roche Diagnostics) were used according to the manufacturer's instructions.

RESULTS

Molecular and phenotypic characterization. The 24 isolates were determined to be *Staphylococcus aureus* by the presence of the *S. aureus*-specific *nuc* gene. Susceptibility to methicillin was determined by broth microdilution and confirmed by the absence of *mecA* with PCR. These methicillin-susceptible *S. aureus* (MSSA) did not carry the genes for PVL. The downstream common sequence (locus D, *dcs*) shared by SCC*mec* I, II, and IV was detected in all 24 MSSA isolates (Table 2). Locus B from the *kdp* gene cluster of SCC*mec* II was detected in isolates IDI2406, IDI2407, IDI2445, IDI2595, IDI2515, and IDI2643. In addition, isolates IDI2515 and IDI2683 were also positive for locus G (linearized pUB110 plasmid) that is typically present in SCC*mec* II. Apart from IDI2683, all SCC*mec* II isolates were positive for the SCC*mec* II-specific J1 target. The PCR target for the *ccr* gene complex of SCC*mec* II was detected in isolates IDI2406, IDI2407, IDI2445, and IDI2595.

Strain typing and genetic deletion determination. The PFGE and *spa* typing results are summarized in Table 2. The seven isolates with remnants of SCC*mec* II resembled USA100 MRSA in their pulsed-field profile and *spa* type; the remaining 17 isolates resembled USA400 MRSA and/or MSSA476 (Fig. 1). Southern blot hybridization with the locus D probe detected genetic deletions of approximately 18 to 45 kbp in the SCC*mec* II isolates that resembled USA100, and approximately 20 kbp in the USA400/MSSA476-resembling isolates (Fig. 1).

Mapping of deletion junction. In order to map the deletion junction, long-range PCR using upstream and downstream primers based on the positive PCR targets that flanked the missing *mecA* determinant was performed. Four SCC*mec* II deletion patterns were observed among the seven isolates that resembled USA100 (Fig. 2). Long-range PCR with primers α2 (*ccrAB2*) and II-48600R (pUB110) for isolates IDI2406,

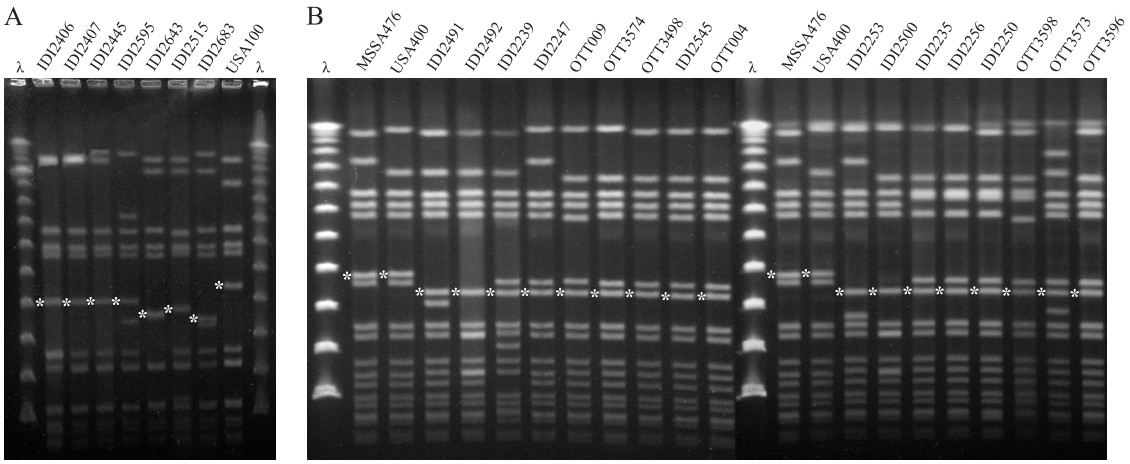

FIG. 1. PFGE of MSSA isolates that resembled USA100 (A) and USA400/MSSA476 (B) were resolved with the Lambda Ladder PFG Marker (λ) for Southern blotting. SmaI fragments that hybridized with the locus D probe are indicated with an asterisk.

IDI2407, IDI2445, and IDI2595 yielded an ~8-kbp product (pattern A). RFLP analyses using EcoRI, HindIII, HincII, or XbaI indicated the PCR product from IDI2595 is different from the other three isolates (data not shown). Sequence determination revealed nt 33837 to 54641 (20.8 kbp) of SCC*mec* II (GenBank accession no. D86934.2) were missing in IDI2406, IDI2407, and IDI2445, and nt 34927 to 54641 (19.7 kbp) were missing in IDI2595. For IDI2515 (pattern B) the primers kdpB2 (*kdpB*) and pUB110 R1 (locus G) were used to successfully amplify a 9-kbp product, and for IDI2643 (pattern C) the primers kdpB2 (*kdpB*) and DCS F2 (locus D) amplified an 8-kbp product. Deletions from nt 17486 to 49295 (31.8 kbp) and nt 16339 to 54641 (38.3 kbp) were identified for IDI2515 and IDI2643, respectively. For IDI2683 (pattern D), PCR results suggested all SCC*mec* II sequences upstream of the pUB110 plasmid were missing. As such, a chromosomal primer (3.0-R) upstream of the SCC integration site was designed for amplification with primer pUB110 R1. A 2.6-kbp product was obtained; sequence data confirmed that nt 3012 to 49295 (46.3 kbp) was absent in IDI2683.

For the 17 isolates that resembled USA400/MSSA476, no SCC remnant other than locus D was detected. Therefore, a chromosomal primer (4.2-R) that anneals at nt 65312 to 65292 in the MW2 genome (equivalent to nt 64019 to 63999 in MSSA476) was designed for long-range PCR in conjunction with the Xsau325 primer that anneals at nt. 34013 to 34031 of MW2 and MSSA476. A 7-kbp PCR product with identical RFLP patterns when digested with BclI, HincII, or XbaI (data not shown) was obtained. The sequence of the amplicons was identical to MSSA476 without SCC*far* (nt 34150 to 56985), MW2 without SCC*mec* IVa (nt 34150 to 58278), the SCC integration site of MSSA strain 15575 (GenBank accession no.

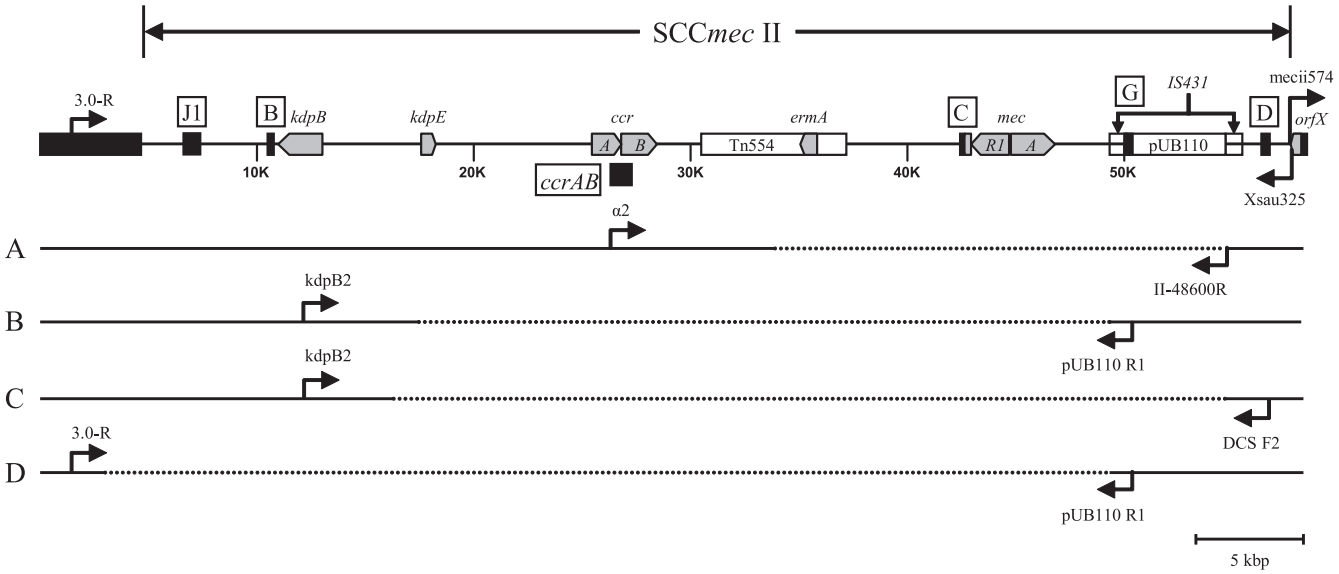

FIG. 2. Internal deletions of SCC*mec* II. The prototypical SCC*mec* II is illustrated with key genetic elements (gray chevron) and locations of PCR targets (black box) on the top. PCR primers (arrows) used in long-range PCRs to span the deleted region (segmented line) are as indicated for the four deletion patterns: A (IDI2406, IDI2407, IDI2445, and IDI2595), B (IDI2515), C (IDI2643), and D (IDI2683).

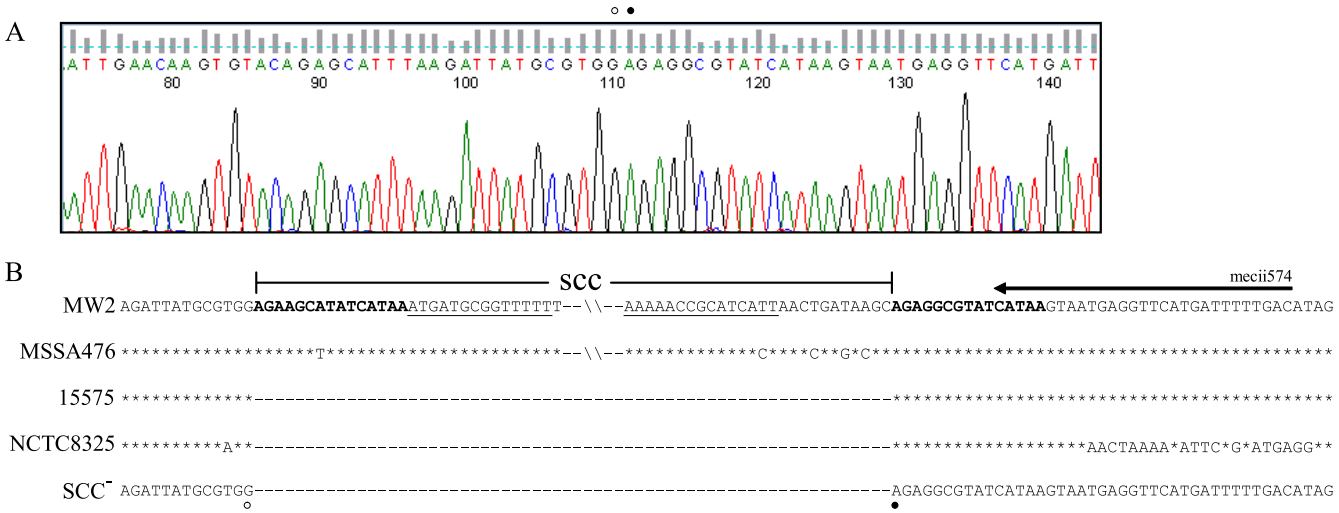

FIG. 3. SCC integration site in isolates that resembled USA400/MSSA476. (A) A representative chromatogram of the Xsau325/4.2-R amplicon sequenced with the Xsau325 primer. The SCC boundaries are denoted with open and closed circles. (B) Sequence alignment of the SCC borders delineated from the 17 isolates that resembled USA400/MSSA476 (SCC$^-$) with MW2, MSSA476, and *S. aureus* strain 15575, and NCTC8325 (GenBank accession no. CP000253) illustrating the missing SCC element that encompassed the direct repeat sequence (in boldface) at the left border to the inverted repeat sequence (underlined) on the right border. The secondary mecii574 annealing sequence is illustrated with an arrow. The open circle corresponds to nt 34149 (MW2 and MSSA476), 608 (15575), and 34151 (NCTC8325); the closed circle corresponds to nt 58279 (MW2), 56986 (MSSA476), 609 (15575), and 34152 (NTCT8325).

EU272079), MSSA strain NCTC8325, and highly homologous to several SCC*mec*-excisant strains generated *in vitro* (16, 31) (Fig. 3).

Detection of mobile genetic elements. In the absence of their SCC elements, detection of pathogenicity islands and bacteriophages that typically associate with MW2 and MSSA476 was performed to help identify the 17 USA400/MSSA476-resembling strains. The ϕSa4-associated genes were detected in isolates OTT009 and OTT3498. None of the gene targets present on either mobile genetic elements were detected in the remaining 15 isolates (Table 2).

DISCUSSION

Single-locus PCR assays based on the site-specific integration of SCC*mec* at *orfX* provide real-time identification of MRSA directly from specimens. There have been reports describing *S. aureus* isolates containing SCC*mec* "remnants" (without *mecA*), and misidentified as MRSA in single-locus PCR assays (15, 35, 36, 40). In most cases, these MSSA isolates were not fully characterized to elucidate the cause of the misidentification. In the report by Shore et al., the molecular characterization of MSSA isolates with residual SCC*mec* elements provided one explanation of the false-positive reactions in single-locus PCR assays, although this present study characterized only three MSSA isolates (40).

In the present study, we characterized 24 MSSA isolates, from geographically diverse regions of North America, which were detected as MRSA with a single-locus PCR assay, and identified two possible explanations for the discrepant results. Molecular typing and PCR analyses identified seven isolates that resembled USA100 (*spa*, TJMBMDMGMK and related) with remnants of SCC*mec* II. Internal deletions of SCC*mec* II terminated at IS*431* sequences (nt 49295 to 50086 and 54642 to 55432 in SCC*mec* II) at the 3′ border. IS*431* elements are ubiquitous in staphylococci and play a role in gene transfer (21, 42). Partial SCC*mec* II excisions at IS*431* sequences during *in vitro* exposure to vancomycin have been observed, possibly as a fitness compensation mechanism (30). Therefore, it is likely the excision events in SCC*mec* II isolates from the present study were mediated through IS*431*.

There were 17 isolates that resembled USA400/MSSA476 with undetermined SCC elements (*spa*, UJFKBPE and related). Characterization of these isolates led to the discovery of a second cause of false-positive results in the single-locus PCR assay. The annealing sequence for the SCC*mec*-specific primer (mecii574) is located in the *dcs* region (GenBank annotation *MW0025*) of SCC*mec* IVa in MW2. A homologue of *MW0025*, which also contains a mecii574 annealing sequence, exists adjacent to the SCC integration site in both MW2 and MSSA476 (GenBank annotations *MW0048* and *SAS0048*, respectively). The mecii574 sequence from within SCC*mec* IVa yields the intended 278-bp amplicon for positive MRSA identification in the single-locus PCR assay. However, the absence of an intact SCC*mec* IVa (or SCC*far*) positions the secondary mecii574 annealing sequence immediately downstream of the *orfX*-specific primer (Xsau325). In this configuration, a 176-bp amplicon can be generated and detected as a false-positive signal (Fig. 4).

The identity of the USA400/MSSA476-resembling isolates remains unclear. Sequence data identified the presence of the putative transposase and enterotoxin genes (*seh* and Δ*seo*) downstream of an intact SCC integration site. The enterotoxin gene cluster has been proposed to block the excision of SCC*mec* IVa in MW2 (29). Apart from two isolates that contained the MSSA476-associated bacteriophage (ϕSa4), mobile genetic elements associated with MW2 (νSa3) and MSSA476 were not detected in the USA400/MSSA476 isolates. Therefore, it is

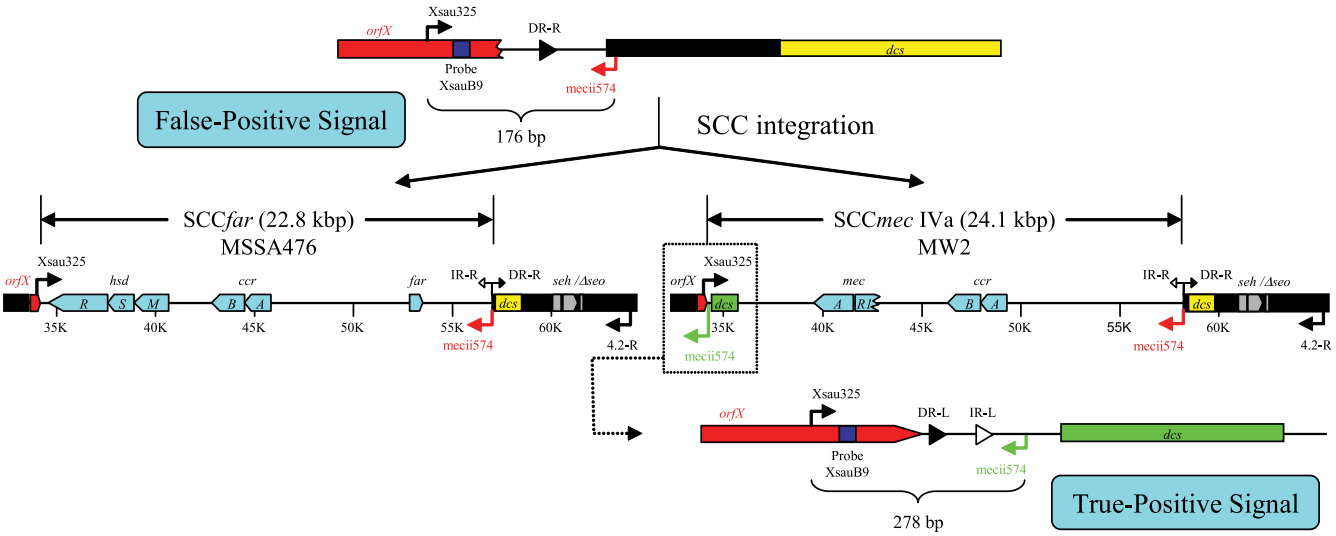

FIG. 4. Absence of SCC elements in USA400 or MSSA476 strains. In the absence of an SCC, the secondary mecii574 annealing sequence (in red) is located 176 bp downstream of the Xsau325 primer to yield a false-positive signal in single-locus PCR assay (top). The acquisition of SCC in MW2 and MSSA476 prohibits amplification between Xsau325 and the secondary mecii574 sequence (middle). However, the mecii574 sequence (in green) from within SCC*mec* IVa in MW2 does yield the intended 278-bp amplicon for positive MRSA identification (bottom).

plausible that the irreversible integration of SCC upstream of the enterotoxin gene cluster had never occurred, and these USA400/MSSA476-resembling isolates may be MSSA strains where the acquisitions of mobile genetic elements such as SCC, pathogenicity islands, and bacteriophages have not taken place to derive the contemporary MW2 and MSSA476 strains. A comprehensive surveillance and characterization of MSSA isolates that test positive in single-locus PCR assays would be beneficial in determining the frequency of false MRSA identification due to the presence of *dcs* at the SCC integration site and in elucidating the identity of these isolates.

In summary, we determined that the single-locus PCR assay for MRSA detection performed as it was designed, to detect a SCC*mec*/*orfX* junction. However, detection of the SCC*mec*/*orfX* junction by itself may not be sufficient to confirm the presence of an intact SCC*mec* element. Our investigation of SCC*mec* II excisant strains revealed that IS*431* elements may have been responsible for partial excisions in SCC*mec* type II that encompassed the *mecA* determinant. In addition, we determined that USA400/MSSA476-resembling isolates where SCC integration has not taken place would also yield a positive reaction in the single-locus PCR assay due to the presence of an additional mecii574 annealing sequence at the SCC integration site. Ongoing surveillance and molecular characterization of such isolates would be beneficial for future improvement in the performance of these MRSA screening tools.

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