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Detection of Staphylococcal Cassette Chromosome *mec*-Associated DNA Segments in Multiresistant Methicillin-Susceptible *Staphylococcus aureus* (MSSA) and Identification of *Staphylococcus epidermidis ccrAB4* in both Methicillin-Resistant *S. aureus* and MSSA▽

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Methicillin-susceptible *Staphylococcus aureus* (MSSA) can arise from methicillin-resistant S. *aureus* (MRSA) following partial or complete excision of staphylococcal cassette chromosome *mec* (SCC*mec*). This study investigated whether multiresistant MSSA isolates from Irish hospitals, where MRSA has been endemic for decades, harbor SCC*mec* DNA. Twenty-five multiresistant MSSA isolates recovered between 2002 and 2006 were tested for SCC*mec* DNA by PCR and were genotyped by multilocus sequence typing and *spa* typing. All isolates lacked *mecA*. Three isolates (12%) harbored SCC*mec* DNA; two of these (genotype ST8/t190) harbored a 26-kb SCC*mec* IID (II.3.1.2) remnant that lacked part of *mecI* and all of *mecR1*, *mecA*, and IS*431*; the third isolate (ST8/t3209) harbored the SCC*mec* region from *dcs* to *orfX*. All three isolates were detected as MRSA using the BD GeneOhm and Cepheid's Xpert MRSA real-time PCR assays. Six isolates (ST8/t190, $n = 4$; ST5/t088, $n = 2$), including both isolates with the SCC*mec* IID remnant, harbored *ccrAB4* with 100% identity to *ccrAB4* from the *Staphylococcus epidermidis* composite island SCC-CI. This *ccrAB4* gene was also identified in 23 MRSA isolates representative of ST8/t190-MRSA with variant SCC*mec* II subtypes IIA to IIE, which predominated previously in Irish hospitals. *ccrAB4* was located 5,549 bp upstream of the left SCC*mec* junction in both the MRSA and MSSA isolates with SCC*mec* elements and remnants and 5,549 bp upstream of *orfX* in the four MSSA isolates with *ccrAB4* only on an SCC-CI homologous region. This is the first description of a large SCC*mec* remnant with *ccr* and partial *mec* genes in MSSA and of the *S. epidermidis* SCC-CI and *ccrAB4* genes in *S. aureus*.

Staphylococcus aureus is a significant human pathogen that can cause a wide variety of diseases, due in part to its ability to acquire and express an extensive array of virulence factors and antimicrobial resistance determinants. Mobile genetic elements are involved in the dissemination of virulence and resistance genes in *S. aureus* and include plasmids, bacteriophages, pathogenicity islands, transposons, and chromosomal cassettes (3, 4, 18, 24, 26, 30, 38, 45, 63, 64).

Following the introduction of methicillin into clinical use, methicillin-resistant *S. aureus* (MRSA) has emerged as a major nosocomial problem worldwide. Today MRSA continues to be a significant burden in hospitals but has also emerged as a problem in the community (22). Methicillin resistance in *S. aureus* is encoded by the *mecA* gene, which is located within a mobile staphylococcal cassette chromosome (SCC) element known as SCC*mec* (22). MRSA can emerge from methicillin-susceptible *S. aureus* (MSSA) upon site-specific integration of SCC*mec* into the *orfX* locus in the chromosome of a susceptible isolate. SCC*mec* consists of three regions: a *mec* complex carrying *mecA* and, if present, its regulatory genes *mecI* and *mecR1*, a *ccr* complex carrying cassette chromosome recombinase (*ccr*) genes, and a series of variable "junkyard" or J regions (30). To date, six types of SCC*mec* have been recognized in *S. aureus*, with three additional new types and numerous variants also recently being reported (14, 21, 48, 61). SCC*mec* has been found in other staphylococcal species, including *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* (19, 20, 62, 65). At least five non-*mec*-containing SCC elements have also been described, two in MSSA (SCC*cap1* and SCC476), one in *S. epidermidis* (SCC-CI with SCC*pbp4*), and one in *S. hominis* (SCC_{12263}) (31, 35, 41). The complexity and evolutionary history of SCC demonstrates the versatility with which *S. aureus* can acquire, disseminate, modify, and delete resistance determinants (14, 34, 43, 44).

MRSA has been endemic in Irish hospitals for many years. In 2003, the rate of methicillin resistance among *S. aureus* isolates recovered from blood culture was 42%, placing Ireland among the countries with the highest rates of MRSA in Europe

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(42). Monitoring of the epidemiological types that comprise the Irish MRSA population by antibiogram-resistogram (AR) typing and chromosomal DNA macrorestriction analysis using pulsed-field gel electrophoresis (PFGE) has shown that the MRSA population changed between 1999 and 2003, with a non-multiantibiotic-resistant strain exhibiting the AR type and PFGE group (AR-PFG) type 06-01 (ST22-MRSA-IV), displacing the previously predominant multiantibiotic-resistant strains AR-PFG type 13-00 or 14-00 (13-00 and 14-00 isolates exhibit the genotype ST8-MRSA-II) (54). In the present study, multiantibiotic resistance is defined as resistance to two or more classes of non-beta-lactam antibiotics (11). Isolates exhibiting AR-PFG 13-00 or 14-00 are resistant to the aminoglycoside antimicrobial agent gentamicin and accounted for 50% of the MRSA population in 1999 (55). Although largely replaced by AR-PFG 06-01 in acutely ill patients with invasive disease (for example, among patients with bacteremia), older strains may persist among chronically ill patients and in long-stay care units. The importance of the long-term persistence of MRSA in patients with osteomyelitis has been noted in both human and veterinary medicine (1, 58).

In some strains of MRSA or in some specific circumstances (e.g., during the absence of antibiotic selective pressure), SCC*mec* may be unstable and can be excised (8, 11). Furthermore, exposure of MRSA to the glycopeptide antibiotic vancomycin can also lead to *mecA* excision, and Noto et al. (43) reported that *S. aureus* may compensate for the fitness cost incurred by developing reduced susceptibility to vancomycin by deleting all or a portion of SCC*mec*. It has also been reported that when SCC*mec* is excised from multiantibiotic-resistant MRSA, the resulting MSSA strain may carry a larger number of resistance determinants than would usually be found in MSSA (11).

While there are vast amounts of data in the literature concerning the structure of the numerous SCC*mec* elements that have been identified in MRSA, there is only very limited information on segments of SCC*mec* in multiresistant MSSA isolates following partial excision or integration of SCC*mec* (6, 11, 13, 25, 57). The purpose of the present study was to investigate whether multiresistant MSSA isolates recovered from patients in Irish hospitals carried segments of SCC*mec* and to compare their genotypes to each other's and to those of multiresistant MRSA isolates previously recovered in Ireland using multilocus sequence typing (MLST) and *spa* typing. In selecting the MSSA isolates for analysis, gentamicin resistance was used as a surrogate marker for multiantibiotic resistance because evidence from a previous study indicated that in Ireland, multiantibiotic-resistant MRSA strains were also gentamicin resistant (53). In the present study, isolates carrying SCC*mec*- and/or SCC-specific DNA were subsequently tested using two commercially available real-time PCR assays for the rapid detection of MRSA from clinical specimens. The assays chosen were the BD GeneOhm MRSA and Cepheid's Xpert MRSA assays, both of which target the region from SCC*mec* to *orfX*.

MATERIALS AND METHODS

Isolates. All gentamicin-resistant MSSA (GrMSSA) isolates recovered between 2004 and 2006 ($n = 19$) from patients attending a large (936-bed) tertiary-referral Dublin hospital (hospital 1), together with GrMSSA isolates ($n = 6$) from five other Irish hospitals, referred to the Irish National MRSA Reference Laboratory for investigation of methicillin resistance, were included in the study (Table 1). One isolate per patient was investigated except in one instance, where two isolates with different antibiograms were recovered from one patient (Table 1). All isolates were identified as MSSA, typed by AR typing, and screened for the presence or absence of the *mecA* gene by PCR as described previously (56). Isolates were stored at −80°C in Protect bacterial preserver vials (Technical Services Consultants Ltd., Heywood, United Kingdom) and cultured on tryptone soya agar (Oxoid Ltd., Basingstoke, United Kingdom) prior to incubation overnight at 37°C.

During the course of the present study, PCR used for SCC*mec* typing and nucleotide sequence analysis of resulting amplimers showed that some GrMSSA isolates harbored segments of DNA with sequences similar to regions of SCC*mec* elements in MRSA isolates recovered in Ireland between 1989 and 2002. All 54 of these earlier isolates exhibited MLST sequence type 8 (ST8) and variant subtypes of SCC*mec* II (59). Isolates representative of each of the variant SCC*mec* II subtypes identified during that study ($n = 23$) were *spa* typed, and all exhibited the same *spa* type (t190). These 23 MRSA isolates recovered between 1989 and 2002 were subsequently investigated for carriage of *ccrAB4* because the earlier study was undertaken before amplification of *ccrAB4* formed part of any SCC*mec* typing scheme.

Chemicals, enzymes, and oligonucleotides. All chemicals used were of analytical grade or molecular biology grade and were purchased from the Sigma-Aldrich Chemical Co. (Tallaght, Dublin, Ireland). Enzymes were purchased from the Promega Corporation (Madison, WI) or Roche Diagnostics Ltd. (Lewes, East Sussex, United Kingdom) and were used according to the manufacturer's instructions. DNA molecular weight markers were purchased from Promega. Oligonucleotide primers were custom synthesized by the Sigma-Aldrich Company Ltd. (Haverhill, United Kingdom).

Molecular characterization. All isolates were investigated by SCC*mec* typing PCR, *spa* typing, and MLST. Genomic DNA was extracted using the DNeasy kit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions. PCRs were performed using GoTaq Flexi DNA polymerase (Promega) according to the manufacturer's instructions using the published protocols for each method described below. Amplifications were performed in a Thermo-Hybaid Multiblock system thermal cycler (Thermo-Hybaid, Ashford, Middlesex, United Kingdom). PCR products were visualized by conventional agarose gel electrophoresis and purified with the GenElute PCR cleanup kit unless otherwise indicated in the appropriate sections below (Sigma-Aldrich). Sequencing was performed commercially by Cogenics (Essex, United Kingdom) using an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

SCC*mec* typing PCRs. Three multiplex SCC*mec* typing PCRs were performed with template DNA from each isolate to determine if they harbored segments of SCC*mec* elements I to VI. Two of these methods were described previously by Kondo et al. (32), where they were named MPCR-1 and MPCR-2, respectively, and the third method was described previously by Oliveira and de Lencastre (47). The first multiplex PCR (MPCR-2), referred to here as PCR scheme 1, amplifies the class A, B, and C *mec* complexes (32). The second multiplex SCC*mec* typing method (MPCR-1), referred to here as PCR scheme 2, detects the presence of the *ccr* complex genes *ccrAB1*, *ccrAB2*, *ccrAB3*, *ccrAB4*, and *ccrC* (32). The third multiplex SCC*mec* typing method, referred to here as PCR scheme 3, amplifies partial nucleotide sequences in the *mec* complex (*mecI* gene only) and in the junkyard regions of SCC*mec* I to IV, including subtypes IA (I.1.1.3), IIIA (III.1), and IIIB (III.1.1.3) (47). In each of these multiplex PCR schemes, amplification of the *mecA* gene was used as an internal control.

The following *S. aureus* control strains and clinical isolates were used as positive controls for SCC*mec* typing PCR as indicated: phenotype II 43.2 (SCC*mec* I, *ccrAB1*) (59), CA05 (SCC*mec* IV, class B *mec*, *ccrAB2*) (36), WIS (class C *mec*) (29), 07.4/0237 (SCC*mec* II) (59), JCSC 4744 (IVA) (47), M00/0005.2 (*ccrAB4*), and E0898 (SCC*mec* III, class A *mec*, *ccrAB3 ccrC*).

All SCC*mec* typing amplimers were sequenced to confirm their identities using the PCR primers yielding those amplimers. Analysis of chromatograms and sequences was carried out using the TraceViewer software program (version 1.1.3; CodonCode Corporation, Massachusetts) and DNA Strider 1.3f11 software (CEA/Saclay, Gif-sur-Yvette, France), respectively. Homology searches were performed using BLAST software (http://ncbi.nih.gov/BLAST).

MLST. MLST was performed as described previously (15). PCR products were purified using the Qiaquick 96 PCR purification kit (Qiagen). Analysis of chromatograms and sequences was performed using the BioNumerics software package (version 5.0; Applied Maths, Ghent, Belgium). The alleles at each of the seven housekeeping loci were identified by comparison with sequences held in the MLST database (http://saureus.mlst.net). The allelic profile and hence the ST of each isolate was also determined using this database. The stringent group

TABLE 1. Patients' MRSA histories and phenotypic and genotypic characteristics of the 25 MSSA isolates investigated

Isolate[a]	MRSA history[b]	Resistance pattern		*spa* cluster[f]	*spa* type	MLST CC	ST	Amplimer(s) obtained with SCC*mec* typing PCR[g]		
		Aminoglycosides/ aminocyclitol[c]	Other antimicrobials[d]					PCR scheme 1 (*mec*)	PCR scheme 2 (*ccr*)	PCR scheme 3[i]
M06/0075	GrMRSA	GEN, KAN, NEO, SPC, STR, TOB	AMP, CAD, CIP, ERY, ETBR, LIN, MC, PMA	1	t190	8	ST8	Negative	*ccrAB4* and *ccrAB2*	*mecI* and *dcs*
M06/0179	GrMRSA	GEN, KAN, NEO, SPC, STR, TOB	CIP, ERY, LIN, MUP[e]	1	t190	8	ST8	Negative	*ccrAB4* and *ccrAB2*	*mecI* and *dcs*
M05/0146	GrMRSA	GEN, KAN, NEO, SPC, STR, TOB	AMP, CAD, CIP, ERY, ETBR, MC, MUP,[e] PMA, TMP	1	t190	8	ST8	Negative	*ccrAB4*	Negative
M02/0021	GrMRSA	AMI,[e] GEN, KAN, NEO, SPC, STR, TOB	AMP, CAD, CIP, ERY, ETBR, MC, MUP,[e] PMA	1	t190	8	ST8	Negative	*ccrAB4*	Negative
M04/0269	GrMRSA	GEN, KAN, NEO, SPC, STR, TOB	AMP, CAD, CIP, ERY, ETBR, MC, PMA	1	t3209	8	ST8	Negative	Negative	*dcs*
M06/0324	None	GEN, KAN, TOB	AMP, CIP	2	t2658	8	ST8	Negative	Negative	Negative
M06/0329	None	GEN, KAN, TOB	AMP, CIP	2	t2658	8	ST8	Negative	Negative	Negative
M05/0065	None	GEN, KAN, TOB	AMP, CAD, CIP,[e] MC, TMP	2	t2658	8	ST8	Negative	Negative	Negative
M05/0330	None	GEN, KAN, NEO, STR, TOB	AMP, SUL, TET, TMP	2	t064	8	ST8	Negative	Negative	Negative
M06/0004	GsMRSA	GEN, KAN, TOB	AMP, ERY, FUS, LIN, MUP	Singleton	t088	5	ST5	Negative	*ccrAB4*	Negative
M06/0288	Not known	GEN, KAN, NEO, TOB	ERY, FUS, LIN, MUP	Singleton	t088	5	ST5	Negative	*ccrAB4*	Negative
M05/0083	Not known	GEN, KAN, TOB	AMP, CAD, CIP, ERY, LIN, MUP	3	t379	22	ST22	Negative	Negative	Negative
M05/0077	None	GEN, KAN, TOB	AMP	3	t005	22	ST22	Negative	Negative	Negative
M04/0261	None	GEN, KAN, TOB	AMP, TMP	3	t005	22	ST22	Negative	Negative	Negative
M05/0232	None	GEN, KAN, TOB	AMP, CAD, TMP	3	t891	22	ST854	Negative	Negative	Negative
M06/0378	None	GEN, KAN, TOB	AMP, ERY, LIN, MUP, TET	4	t127	1	ST1	Negative	Negative	Negative
M04/0262	None	GEN, KAN, TOB	AMP, CAD,[e] TET	4	t1383	1	ST1135[h]	Negative	Negative	Negative
M05/0084	GsMRSA	AMI,[e] GEN, KAN, NEO, TOB	AMP, CAD, LIN, MUP	Singleton	t3500[h]	45	ST1096[h]	Negative	Negative	Negative
M05/0167	GsMRSA	GEN, KAN, TOB	AMP, ETBR, LIN, MUP	Singleton	t3500[h]	45	ST1096[h]	Negative	Negative	Negative
M05/0045	GsMRSA	GEN, KAN, TOB	AMP	Singleton	t2078	101	ST101	Negative	Negative	Negative
M05/0080	GsMRSA	GEN, KAN, TOB	AMP TMP	Singleton	t2078	101	ST101	Negative	Negative	Negative
M05/0223	None	GEN, KAN, TOB	AMP, CAD, FUS, MUP	Singleton	t021	30	ST30	Negative	Negative	Negative
M04/0260	None	GEN, KAN, TOB	ERY	5	t084	15	ST582	Negative	Negative	Negative
M05/0133	None	GEN, KAN, TOB	ERY	5	t491	15	ST582	Negative	Negative	Negative
M06/0392	None	GEN, KAN, TOB	AMP, CAD, TET	Singleton	t164	20	ST1134[h]	Negative	Negative	Negative

[a] One isolate per patient was included except in one instance, where two isolates with different antibiograms (M06/0179 and M06/0075) were recovered from one patient. Isolates shown with bold and underlined typeface yielded amplimers by SCC*mec* typing PCR.

[b] Not known, data on patient's MRSA history were not available.

[c] Aminoglycosides/aminocyclitols tested were as follows: AMI, amikacin; GEN, gentamicin; KAN, kanamycin; NEO, neomycin; SPC, spectinomycin; STR, streptomycin; TOB, tobramycin.

[d] Other antimicrobials tested included the following: AMP, ampicillin; CAD, cadmium acetate; CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; ETBR, ethidium bromide; FUS, fusidic acid; LIN, lincomycin; MC, mercuric chloride; MUP, mupirocin; PMA, phenyl mercuric acetate; RIF, rifampin; SUL, sulfonamide; TET, tetracycline; TMP, trimethoprim; VAN, vancomycin.

[e] Intermediately susceptible to the antimicrobial indicated.

[f] The BURP algorithm was applied to all isolates for the determination of clusters of related isolates based on the *spa* types identified (40).

[g] Sequencing was performed to confirm the identities of all amplimers obtained by SCC*mec* typing PCR. Negative, no PCR amplimers were obtained.

[h] New STs or *spa* types identified in the present study.

[i] See reference 47.

TABLE 2. Primers used in the present study

Primer application	Primer pair(s)	Nucleotide sequence (5′–3′)	Nucleotide coordinates	SCC*mec* region amplified	Reference
Amplification and sequencing of the SCC*mec* IID remnant	IRLII F	CTCTGCGTATCAGTTAATGA	4684–4703[a]	Left chromosomal/SCC*mec* junction to *ccrA2*	59
	ccrA R	GCTTCGATAGCCTGTTTCTG	25490–25471[a]		59
	ccrA2 F5	AACTTATCGAGATATTAGCC	25265–25284[a]	*ccrA2* to Tn*554*	This study
	Tn554 R	AAGCTATCCACGTTCAATCTCAAC	32442–32419[a]		59
	N044 F	AAATAGTATAATGCTCGGTC	30481–30500[a]	N044 to Tn*554*	This study
	TN554 R6	TGGAGACATATTAGACACAA	36440–36421[a]		This study
	Tn554 F7	GCGATAAAGGACAGTGACTT	36191–36210[a]	Tn*554* to *mecI*	This study
	mecI R2	AGGAAACAATCAAGTCGTTG	42507–42488[a]		This study
	mecI P2	ATCAAGACTTGCATTCAGGC	42428–42447[a]	*mecI* to *dcs*	47
	dcs R1	AGACGAAGATAAGAAAGAAC	56433–56414[a]		This study
	dcs F	GTCAATGAGATCATCTACAT	56109–56128[a]	*dcs* to right chromosomal/SCC*mec* junction	59
	orfX R	CCCAAGGGCAAAGCGAC	57826–57810[a]		59
Amplification of *ccrAB4* from SCC-CI in *S. epidermidis* from historic Irish nosocomial ST8-t190 MRSA isolates	α4.3	AGCGTATGAATCAAAA	27155–27170[b]	*ccrA4* to *ccrB4* of *S. epidermidis* SCC-CI	This study
	β4.3	CGATGACAAATTAAAAT	27907–27891[b]		This study
Determination of the location of *ccrAB4* in GrMSSA isolates with the SCC*mec* IID remnant and in MRSA isolates	ccrB4 F	TTTCGTCCATTACCTACATC	27075–27056[b]	*ccrB4* to left chromosomal/SCC*mec* IID remnant junction	This study
	LCIVb R	TGAGGAGTTTAACAAGTTAT	160–141[c]		This study
Determination of the location of *ccrAB4* in GrMSSA isolates with *ccrAB4* only	ccrB4 F	TTTCGTCCATTACCTACATC	27075–27056[b]	*ccrB4* to *orfX*	This study
	orfX R	CCCAAGGGCAAGCGAC	57826–57810[a]		59
Determination of the location of *dcs* in the GrMSSA isolate with *dcs* only	dcs F	GTCAATGAGATCATCTACAT	56109–56128[a]	*dcs* to *orfX*	59
	orfX R	CCCAAGGGCAAGCGAC	57826–57810[a]		59

[a] Nucleotide coordinates from the type II SCC*mec* type element, accession number D86934.
[b] Nucleotide coordinates from the *Staphylococcus epidermidis* ATCC 12228 composite island SCC-CI, accession number BK001539.
[c] Nucleotide coordinates from the SCC*mec* IID remnant, accession number AM983545.

definition for clonal complex (CC) determination was used, where isolates with specific STs are assigned to a particular CC if they are related to at least one other ST in that CC at six out of the seven MLST loci used (16). Isolates that do not share alleles at six of the seven MLST loci with any other ST in the MLST database are deemed singletons (16).

***spa* typing.** The primers and thermal cycling conditions recommended by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet) were used for *spa* typing (http://www.seqnet.org/). The Ridom StaphType software program, version 1.3 (Ridom Gmbh, Wurzburg, Germany), was used for *spa* sequence analysis, assignment of *spa* types, and BURP (based upon related patterns) *spa* clonal complex determination. The default parameters for cluster definition were used for BURP. These include the exclusion of *spa* types that are shorter than five repeats because they are deemed too short for the deduction of evolutionary history and the clustering of *spa* types only if the cost (i.e., the steps of evolution between two different *spa* types) is less than or equal to four (40).

Nucleotide sequencing of SCC*mec* remnant in isolate M06/0075. The entire nucleotide sequence of the SCC*mec* remnant from one MSSA isolate, M06/0075, was determined. This isolate was selected because, along with another isolate recovered from the same patient (M06/0179), it yielded the greatest number of SCC*mec* typing amplimers. Sequencing was undertaken to determine if these amplimers were part of a larger remnant of SCC*mec*. The element was amplified and sequenced from the left chromosomal SCC*mec* junction to *orfX* using a combination of previously described and newly designed overlapping primers based on the published SCC*mec* II nucleotide sequence (http://www.ncbi.nlm.nih.gov/sites/entrez; accession number D86934). The SCC*mec* II nucleotide sequence was chosen because three out of the four amplimers produced by GrMSSA isolate M06/0075 indicated the presence of an SCC*mec* remnant with

closest similarity to the type II SCC*mec* element (i.e., *dcs*, *mecI*, and *ccrAB2*). The primers used are listed in Table 2.

DNA fragments for sequencing were obtained by PCR amplification of chromosomal DNA using either the Expand high-fidelity PCR system (Roche) for the primer pair dcs F and orfX R or the Expand long-template PCR system (Roche) for all other primer sets according to the manufacturer's instructions. Amplified DNA was separated by agarose gel electrophoresis using at least 5-μl volumes of each PCR product. Amplimers were purified using either the GenElute PCR cleanup kit (Sigma-Aldrich) or the Qiaex II gel extraction kit (Qiagen) prior to direct sequencing using primer walking. Analysis of chromatograms and sequences and homology searches were performed as described above for sequencing of the SCC*mec* typing amplimers.

Investigation of MRSA isolates for presence of *ccrAB4*. Twenty-three MRSA isolates with the same MLST/*spa* type genetic background and similar SCC*mec* regions to those of the GrMSSA isolate M06/0075 were investigated to see whether they harbored *ccrAB4*. The *ccrAB4* gene was amplified using the primers α4.3 and β4.3 (Table 2). This primer pair was designed to be specific for the *ccrAB4* gene from *S. epidermidis* because the *ccrAB4* gene identified in the GrMSSA isolates in this study had 100% homology with *ccrAB4* from the *S. epidermidis* composite island SCC-CI. PCRs were performed using GoTaq Flexi DNA polymerase (Promega) according to the manufacturer's instructions. Amplifications were performed in a Thermo-Hybaid Multiblock system thermal cycler (Thermo-Hybaid, Ashford, Middlesex, United Kingdom) with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 2 min, annealing at 48°C for 1 min, and elongation at 72°C for 2 min. A final elongation step was performed for 2 min at 72°C. The MRSA isolate HDE288, which harbors SCC*mec* VI with a different *ccrAB4* allotype from that found in *S. epidermidis*, and *S. epidermidis* ATCC 12228, harboring SCC-CI with *ccrAB4*, were used as negative and positive controls, respectively, for this PCR assay (41, 48). Isolates harboring *ccrAB4* genes identical to those in *S. epidermidis* yield an 800-bp product using this primer pair, and isolates harboring *ccrAB4* from *S. aureus* should yield no product. PCR products were visualized by agarose gel electrophoresis and purified using the GenElute PCR cleanup kit (Sigma-Aldrich). The identities of amplimers from isolates representative of each variant SCC*mec* II subtype (IIA [II.3.1.1], IIB [II.3.2.1], IIC [II.3.3.1], IID [II.3.1.2], and IIE [II.3.3.2]) among these MRSA isolates were confirmed by sequencing.

Determination of location of *ccrAB4*. Two primers, ccrB4 F and LCIVb R, based on the *S. epidermidis ccrAB4* gene and the left extremity of the SCC*mec* IID remnant, respectively, were designed to investigate the location of *ccrAB4* in the two GrMSSA isolates with the SCC*mec* IID remnant (M06/0075 and M06/0179) recognized in the present study and in five MRSA isolates representative of each SCC*mec* II variant subtype IIA to IIE that were also found to harbor *ccrAB4*. A second primer pair, ccrB4 F and orfX R, based on the *S. epidermidis ccrAB4* gene sequence and *orfX*, respectively, was selected to locate *ccrAB4* in the four GrMSSA isolates that yielded *ccrAB4* only (M02/0021, M05/0146, M06/0004, and M06/0288). Primer sequences are shown in Table 2. DNA fragments were obtained by PCR amplification of chromosomal DNA using the Expand long-template PCR system (Roche) according to the manufacturer's instructions. Amplified DNA was separated by agarose gel electrophoresis using 5-μl volumes of each PCR product.

Determination of location of *dcs*. The Primer pair dcs F and orfX R (Table 2) was used to determine the location of *dcs* in the GrMSSA isolate (M04/0269) that yielded the *dcs* amplimer only by SCC*mec* typing PCR. DNA fragments were obtained by PCR amplification of chromosomal DNA as described above.

Investigation of GrMSSA isolates with SCC*mec*/SCC-associated DNA using real-time PCR MRSA rapid detection assays. The seven GrMSSA isolates harboring SCC*mec*/SCC-associated DNA identified in the present study were tested with two commercially available real-time PCR assays for the rapid detection of MRSA in clinical specimens to determine if such isolates would be detected as MRSA. Isolates were tested with the BD GeneOhm MRSA assay (BD Diagnostics, Ste Foy, Quebec, Canada) using a Smart Cycler II thermal cycler (Cepheid, Sunnyvale, CA) and with the Xpert MRSA assay using a GeneXpert DX system (version 1.2) real-time PCR platform (Cepheid) according to the manufacturer's instructions as described previously (52). *S. aureus* ATCC 25923 (MSSA), *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 43300 (MRSA) and *S. epidermidis* ATCC 12228 were used as control strains with both systems.

Nucleotide sequence accession numbers. The nucleotide sequence of the SCC*mec* IID remnant was submitted to the GenBank database under accession number AM983545.

RESULTS

The purpose of the present study was to investigate whether MSSA isolates from patients in an environment where MRSA has been endemic for many years harbor segments or remnants of SCC*mec*. Twenty-five gentamicin-resistant MSSA isolates were investigated by AR typing, SCC*mec* typing PCR, and genotyping using MLST and *spa* typing (Table 1). The majority of isolates (21/25; 84%) were multiantibiotic resistant (i.e., resistant to two or more classes of non-beta-lactam antibiotics [Table 1]). All lacked the *mecA* gene.

SCC*mec* typing PCR. Template DNA from all 25 isolates was tested for the presence of *mec* complex types A, B, and C (PCR scheme 1), *ccr* genes 1 to 5 (PCR scheme 2), and the various junkyard regions (including *mecI*) of SCC*mec* I to IV (PCR scheme 3). All isolates were *mecA* negative by PCR schemes 1 to 3 and failed to yield any *mec* complex amplimers when PCR scheme 1 was used, but 7/25 (28%) isolates yielded at least one SCC*mec* amplimer each when PCR scheme 2 or 3 was used (Table 1). These seven isolates belonged to the following three MLST/*spa* genotypes: ST5/t088 ($n = 2$), ST8/t3209 ($n = 1$), and ST8/t190 ($n = 4$) (Table 1).

Investigation of SCC*mec* amplimers generated from seven GrMSSA isolates. Two isolates, M06/0075 and M06/0179 (recovered from the same patient after an interval of 4 months), each yielded four amplimers: two amplimers of 1,287 bp and 937 bp were obtained using PCR scheme 2, while PCR scheme 3 yielded a 342-bp and a 209-bp amplimer (Table 1). The 1,287-bp amplimer was identical in size to an amplimer usually obtained with PCR scheme 2 from MRSA template DNA carrying the *ccrAB4* gene (Table 1). Nucleotide sequence analysis of this 1,287-bp amplimer showed 88% and 91% homology with *ccrAB4* from the MRSA SCC*mec* element $SCCmec_{NI}$ (14) and SCC*mec* VI (48), respectively, but showed 100% homology with *ccrAB4* from the SCC composite island SCC-CI, found in *S. epidermidis* ATCC 12228 (41). The 937-bp amplimer was identical in size to an amplimer usually obtained with PCR scheme 2 from MRSA template DNA carrying the MRSA *ccrAB2* gene and exhibited 100% sequence homology with *ccrAB2*. The 342-bp amplimer obtained with PCR scheme 3 was identical in size to an amplimer usually obtained with MRSA template DNA using PCR scheme 3 corresponding to the *dcs* region located downstream of *mecA* in SCC*mec* types I, II, and IV in MRSA isolates. Sequence analysis showed that this amplimer exhibited 100% sequence homology with *dcs* from MRSA. The 209-bp amplimer obtained with PCR scheme 3 was identical in size to an amplimer usually obtained with MRSA template DNA using PCR scheme 3 corresponding to the *mec* complex gene *mecI* and exhibited 100% sequence homology with *mecI*.

Four isolates (M05/0146, M02/0021, M06/0004, and M06/0288) each yielded one 1,287-bp amplimer by PCR scheme 2. This amplimer exhibited 100% sequence homology with *ccrAB4* from SCC-CI in *S. epidermidis* ATCC 12228.

One isolate (M04/0269) yielded one 342-bp amplimer by PCR scheme 3 (Table 3). This amplimer exhibited 100% sequence homology with the *dcs* region.

Genotyping. MLST grouped the 25 isolates into nine clonal complexes, with 13/25 (52%) isolates belonging to either CC8 ($n = 9$) or CC22 ($n = 4$) (Table 1). The seven GrMSSA

TABLE 3. Summary of findings from the present study and from previous studies reporting SCC*mec* fragments in methicillin-susceptible *S. aureus*

No. of MSSA isolates with SCC*mec* regions (source)[a]	Method used to detect SCC*mec* segments (reference)	SCC*mec* segments found (n^b)	MLST/*spa* genotype (n^b)	Further analysis of SCC*mec*-associated sequences undertaken	Reference
7 (four Irish hospitals)	PCR to detect *mec* complexes A, B, and C (32)	*ccrAB2*[c] (2);	ST8/t190 (4)		Present study
	PCR to detect *ccrAB1* to *–4*, *ccrC* (32)	*ccrAB4*[c] (6)	ST8/t3209 (1)	Yes[e]	
	Multiplex PCR (47)	*mecI*[c] (2); d*cs*[c] (3)	ST5/t088 (2)		
2 (i.v. drug users)	PCR to detect *ccrAB1* (28)	*ccrAB1*[c]	Not done	No	6
	Multiplex PCR (47)	*dcs*[c]			
26 (regions worldwide)	Real-time PCR (IDI-MRSA assay) to amplify the right SCC*mec*/*orfX* junction	Right SCC*mec*/*orfX* junction[d]	Not done	No	25
	PCR detection of *mecA* (39)				
6 (French hospitals)	PCR detection of *ccrAB1*, *ccrAB2*, and *ccrAB3* (36)	IS*431*-p*UB110*, *dcs*	ST8/t008 (1)	No	13
	Multiplex PCR (47)	IS*431*-p*UB110*-IS*431-dcs*			
	PCR detection of HVR-IS*431*-p*UB110*-IS*431-dcs-orfX*				
1 (nasal swab, HCW)	Real-time PCR (Genotype MRSA Direct) to amplify right SCC*mec*/*orfX* junction	Right SCC*mec*/*orfX* junction[c]	t498	No	57
	PCR to confirm the right SCC*mec*/*orfX* junction (7)				
	PCR detection of *mecA* (50)				
	PCR to detect *ccrAB1*, *ccrAB2*, and *ccrAB3* (46)				
169 (60 French hospitals)	Real-time PCR (IDI-MRSA assay) to amplify the right SCC*mec*/*orfX* junction	Right SCC*mec*/*orfX* junction	ST8/t008 (isolate 1)	No	11
	PCR to investigate HVR-IS*431*-p*UB110*-IS*431-dcs-orfX* (13) (2 isolates only investigated)	*dcs* only (isolate 1)	ST8/t024 (isolate 2)		
	PCR to detect *ccrAB1*, *ccrAB2*, and *ccrAB3* (28) (14 isolates investigated)	HVR-IS*431*-p*UB110*-IS*431-dcs* (isolate 2)			

[a] HCW, health care worker; i.v., intravenous.
[b] *n*, no. of isolates.
[c] Identity of amplimer(s) confirmed by DNA sequencing.
[d] IDI-MRSA assay amplimer identity was confirmed by conventional endpoint PCR.
[e] A 26-kb SCC*mec* remnant was sequenced from one ST8/t190 isolate, and *ccrAB4* was found to be located 5,549 bp upstream of SCC*mec*.

isolates that yielded amplimers by SCC*mec* typing PCR belonged to either ST8 ($n = 5$) or ST5 ($n = 2$) (Table 1). Among the isolates that did not yield SCC*mec* typing amplimers, the majority (14/18) belonged to a range of STs other than ST8 and ST5 (Table 1). Four isolates that did not yield SCC*mec* amplimers belonged to ST8 but had different *spa* types (t2658 and t064) from those of the five ST8 isolates that yielded SCC*mec*/SCC amplimers. The latter exhibited *spa* types t190 and t3209. BURP analysis assigned the *spa* types of these nine ST8 isolates into two clusters, one consisting of t190 and t3209 isolates and the other consisting of t2658 and t064 isolates. The remaining 16 isolates investigated fell into three additional *spa* clusters and five singleton groups. Two isolates exhibited a novel ST and *spa* type (Table 1). Two isolates with *spa* types t164 and t1383, respectively, exhibited two different novel MLST types.

Characterization of a novel SCC*mec* remnant in GrMSSA M06/0075. The GrMSSA isolate M06/0075 (genotype ST8/t190) was selected for more-detailed analysis because it was one of the two isolates that yielded the greatest number of amplimers with the SCC*mec* PCR typing schemes (Table 1). Analysis of the entire nucleotide sequence of the SCC element from the left chromosomal SCC junction to *orfX* revealed that this GrMSSA isolate harbored a large portion of an SCC*mec* element consisting of a ca. 26-kb contiguous sequence with a genomic organization similar to that of a variant SCC*mec* II element, SCC*mec* IID (II.3.1.2), previously identified among ST8 MRSA isolates from Ireland (59) (Fig. 1).

Although SCC*mec* IID (II.3.1.2) has not been fully sequenced, its genomic organization was determined previously by PCR amplification and comparison to the expected size for amplimers from fully and partially sequenced SCC*mec* II variant elements SCC*mec* IIA (II.3.1.1) and IIE (II.3.3.2), respectively (shown in Fig. 1) (59). The variant SCC*mec* elements IIA to IIE and IVE/IVF were identified and characterized previously by this laboratory (59). SCC*mec* IID (II.3.1.2) is similar in its genetic organization to SCC*mec* IIE (II.3.3.2), which has been sequenced in full (59). SCC*mec* IID (II. 3.1.2) and

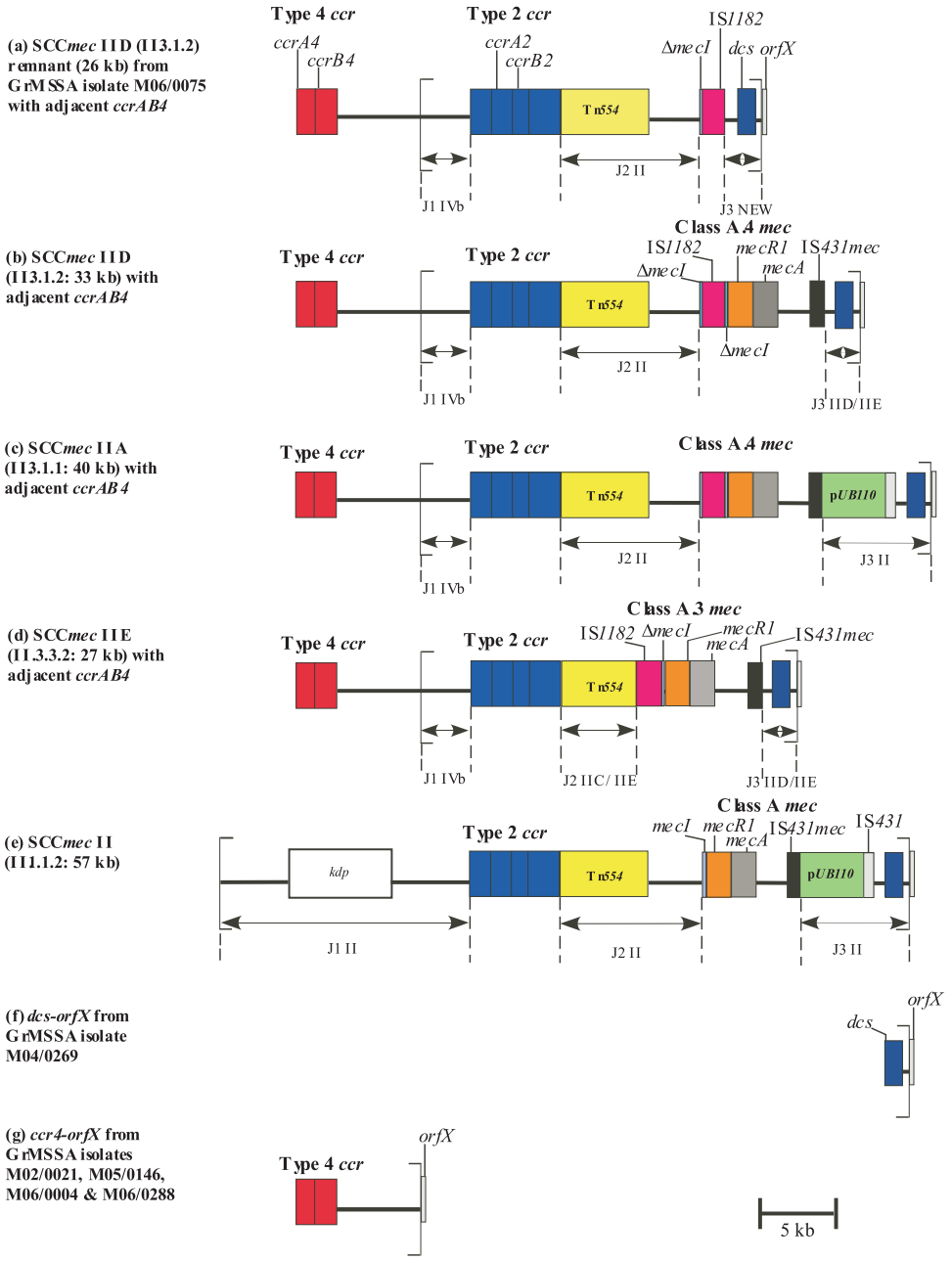

FIG. 1. Schematic diagram showing the genetic organization of the novel SCC*mec* IID (II.3.1.2) remnant from GrMSSA isolate M06/0075 determined in the present study (a) and the corresponding organization of SCC*mec* elements IID (II.3.1.2) (b), IIA (II.3.1.1) (c) IIE (II.3.3.2), (d), and II (II.1.1.2) (e), determined previously (28, 59). The structure of the SCC*mec* remnant from M06/0075 was determined by sequencing of the entire element (accession number AM983545) and was found to have closest similarity to SCC*mec* IID (II.3.1.2). The left and right extremities of the SCC*mec* elements/remnant are indicated with square brackets. Panels a to d also show the location of *ccrAB4* identified in the present study, 5,549 bp upstream of the left chromosomal/SCC*mec* junction region of the SCC*mec* IID remnant and SCC*mec* elements IID (II.3.1.2), IIA (II.3.1.1), and IIE (II.3.3.2), respectively. Panel f shows the location of *dcs* upstream of *orfX*, identified in the one GrMSSA isolate (M04/0269) that yielded *dcs* only by SCC*mec* typing PCR. Panel g shows the location of *ccrAB4* relative to *orfX*, a structure identified in the four GrMSSA isolates (M02/0021, M05/0146, M06/0004, and M06/0288) that yielded *ccrAB4* only by SCC*mec* typing PCR. The *ccrAB4* gene identified in the GrMSSA and MRSA isolates in the present study demonstrated 100% homology with *ccrAB4* previously identified in the composite island SCC-CI in *S. epidermidis* strain ATCC 12228 (41). The DNA sequence between *ccrAB4* and the SCC*mec* IID remnant in M06/0075 and between *ccrAB4* and *orfX* in M02/0021 in each case was 98.5% homologous with the DNA region between *ccrAB4* and SCC*pbp4* in SCC-CI from *S. epidermidis* (accession number BK001539, bases 26100 to 24570 and bases 23009 to 18990) except that a ca. 1.5-kb contiguous DNA sequence in this region of SCC-CI (bases 24569 to 23010) was absent in M06/0075 and in M02/0021. This SCC-CI region encodes a spermidine acetyltransferase, a truncated transposase, and the type I restriction proteins HsdS and HsdR, as well as nine other hypothetical proteins (41). All of these open reading frames were also present in the DNA sequences from M06/0075 and M02/0021 referred to above, apart from the partial DNA sequence of one hypothetical protein and the complete sequence of another.

SCC*mec* IIE (II.3.3.2) differ in the J2 and *mec* complex regions, but the J2 and *mec* complex regions of SCC*mec* IID (II.3.1.2) are identical to the corresponding regions of SCC*mec* IIA (II.3.1.1), which have also been sequenced (Fig. 1) (59). Therefore, although the SCC*mec* remnant of M06/0075 was most similar in genomic organization to SCC*mec* IID (II.3.1.2), its nucleotide sequence was compared with the nucleotide sequences of the J1, J3, and *ccr* complex regions of SCC*mec* IIE (II.3.3.2) and with those of the J2 and *mec* complex regions of SCC*mec* IIA (II.3.1.1). The J1 and *ccr* complex regions of M06/0075 shared 99.5% homology with the corresponding region of SCC*mec* IIE (II.3.3.2) (Fig. 1). The J2 region shared 99% homology with the J2 region of SCC*mec* IIA (II.3.1.1). Combining of data from the J1, J2, and *ccr* complex regions indicated that the SCC*mec* remnant of M06/0075 most closely resembles a type IID (II.3.1.2) SCC*mec* element (Fig. 1).

The major difference between SCC*mec* IID (II.3.1.2) and the SCC*mec* remnant in M06/0075 was in the *mec* complex and J3 regions. The *mec* complex of M06/0075 consisted of IS*1182* inserted within the *mecI* gene near the 3′ end at the exact nucleotide position and resulting in the same 16-bp deletion within *mecI* as previously identified in the class A.4 *mec* complex found in SCC*mec* IIA (II.3.1.1) and SCC*mec* IID (II.3.1.2) (59). There were five base pair differences between IS*1182* from class A.4 *mec* and M06/0075, including the final base of IS*1182*, which was absent in M06/0075. In addition, the remainder of the *mecI* gene (located immediately downstream of IS*1182* in class A.4 *mec*) and the rest of the *mec* complex, including the *mecR1*, *mecA*, and IS*431* genes, were absent in M06/0075 (Fig. 1). The region immediately adjacent to IS*1182* in M06/0075 shared 99.5% homology with the region extending from ca. 500 bp downstream of IS*431* in SCC*mec* IIE (II.3.3.2) to *orfX* at the extreme right chromosomal SCC*mec* junction. This region of both SCC*mec* IIE (II.3.3.2) and SCC*mec* IID (II.3.1.2) consists of a noncoding region normally found between IS*431* and *dcs* and the *dcs* region that has been identified previously in SCC*mec* I, II, and IV. The *orfX* gene was identified immediately adjacent to the SCC*mec* remnant in M06/0075. Furthermore, this SCC*mec* remnant was integrated at the same nucleotide position within *orfX* as other SCC/SCC*mec* elements. The 15-bp direct repeat sequence found at the right extremity of all SCC*mec* (DR-R) elements identified to date was also identified in M06/0075, as was the 26-bp inverted repeat sequence (IR-R), which had 100% similarity to the IR-R sequences of SCC*mec* II (II.1.1.2), IIE (II.3.3.2), and IVb (IV.2.1.1). Sequencing of the entire SCC*mec* remnant from the left chromosomal SCC*mec* junction to *orfX* failed to locate the *ccrAB4* gene within this element.

Investigation of ST8/t190 MRSA isolates for carriage of *ccrAB4* outside SCC*mec*. Nosocomial MRSA isolates ($n = 23$) with the same MLST and *spa* type as and similar SCC*mec* sequences to those of GrMSSA isolate M06/0075 were investigated to determine if they also harbored *ccrAB4* with 100% homology to *ccrAB4* of SCC-CI from *S. epidermidis*. These included isolates representative of each variant SCC*mec* II subtype previously identified among ST8/t190 MRSA isolates recovered from Irish hospitals between 1989 and 2002 (59). When these isolates were originally SCC*mec* typed, amplification of *ccrAB4* was not part of any published SCC*mec* typing scheme (59). All 23 isolates yielded amplimers of the expected size (800 bp) using the primer pair α4.3 and β4.3, which are specific for *ccrAB4* from SCC-CI from *S. epidermidis* (Table 2). For each set of PCR experiments, *S. aureus* HDE288, which harbors SCC*mec ccrAB4* (48), failed to yield any amplimers, whereas template DNA from *S. epidermidis* ATCC 12228, harboring SCC-CI *ccrAB4* (41), yielded the expected 800-bp amplimer, indicating that the primers α4.3 and β4.3 are specific for *ccrAB4* from SCC-CI in *S. epidermidis*. The identities of the amplimers from five MRSA isolates representative of each variant SCC*mec* subtype IIA to IIE (AR13/0132 [IIA, 3.1.1], AR05/0.1345 [IIB, II.3.2.1], AR14/0246 [IIC, II.3.3.1], AR13/3698 [IID, II.3.1.2], and AR13.1/3330.2 [IIE, II.3.3.2])(59) was confirmed by DNA sequencing and in each case showed 100% homology with *ccrAB4* in SCC-CI from *S. epidermidis*. The genomic organization of the SCC*mec* elements harbored by these MRSA isolates has been fully determined previously and showed that these isolates do not harbor *ccrAB4* within SCC*mec* (59). These results demonstrate that, like the SCC*mec* IID (II.3.1.2) remnant in the GrMSSA isolate M06/0075, *ccrAB4* is present in these ST8/t190 MRSA isolates with variant SCC*mec* elements IIA to IIE but it is not located within SCC*mec*.

Determination of location of *ccrAB4*. In total, six GrMSSA isolates harbored *ccrAB4* with 100% homology with that of the SCC-CI element from *S. epidermidis*. These included the two ST8/t190 GrMSSA isolates which also harbored the SCC*mec* IID remnant and the four GrMSSA isolates (genotypes ST8/t190 [$n = 2$] and ST5/t088 [$n = 2$]) that yielded only the *ccrAB*4 amplimer (Table 1). In addition, the 23 ST8-t190 MRSA isolates with SCC*mec* IIA to IIE investigated were also found to harbor this *ccrAB4* gene. PCR amplification of the region between the left extremity of the SCC*mec* IID remnant and the *ccrAB4* gene in the two GrMSSA isolates M06/0075 and M06/0179 and from the left extremity of SCC*mec* elements IIA to IIE to *ccrAB4* in the five MRSA isolates representative of each Irish variant SCC*mec* II subtype resulted in each isolate yielding a ca. 7-kb amplimer. DNA sequence analysis of this amplimer from one representative GrMSSA isolate, M06/0075, confirmed that *ccrAB4* was located 5,549 bp upstream of the left SCC*mec* IID remnant junction (Fig. 1). The region between *ccrAB4* and the SCC*mec* IID remnant in M06/0075 exhibited 98.5% DNA sequence homology to the corresponding DNA sequence located between *ccrAB4* and SCC*pbp4* in SCC-CI from *S. epidermidis* (accession number BK001539, bases 26100 to 24570 and bases 23009 to 18990), except that a ca. 1.5-kb contiguous DNA sequence in this region of SCC-CI (bases 24569 to 23010) was absent in M06/0075. The region located between *ccrAB4* and SCC*pbp4* in SCC-CI encodes nine hypothetical proteins, as well as a spermidine acetyltransferase, a truncated transposase, and the type I restriction proteins HsdS and HsdR (41). All of these open reading frames were also present in the DNA sequence identified between *ccrAB4* and the SCC*mec* IID remnant apart from the partial DNA sequence of one hypothetical protein and the complete sequence of another. All direct-repeat and inverted repeat (IR) sequences previously described at the left SCC*pbp4*/SCC-CI junction region in *S. epidermidis* (41) were also identified at the junction region of the DNA sequence located between *ccrAB4* and the SCC*mec* IID remnant in M06/0075. The DNA sequence of the SCC*mec* IID remnant J1 region was found immediately after the SCC-CI and SCC*pbp4* IR se-

quence and was found to be 100% homologous to the DNA sequence that normally lies immediately after the left IR sequence of SCC*mec* IID. The left IR of SCC*mec* was absent. Figure 1 shows a schematic representation of the location of *ccrAB4* in relation to SCC*mec* in some of the variant subtypes investigated, including SCC*mec* IIA, IID, and IIE. PCR amplification of the region between *ccrAB4* and *orfX* in the four GrMSSA isolates that yielded *ccrAB4* only by SCC*mec* typing PCR (Table 1) also yielded a 7-kb amplimer. DNA sequence analysis of the 7-kb amplimer from one representative isolate, M02/0021, revealed that the *ccrAB4* gene was located 5,549 bp upstream of *orfX* (Fig. 1) and that the region between *ccrAB4* and *orfX* was identical to that identified between *ccrAB4* and the SCC*mec* IID remnant in GrMSSA isolates M06/0075.

Determination of location of *dcs*. Amplification of the region from *dcs* to *orfX* in GrMSSA isolate M04/0269, which yielded *dcs* only, identified that *dcs* was located ca. 1.7-kb upstream of *orfX* (Fig. 1).

Investigation of GrMSSA isolates with SCC*mec*/SCC-associated DNA using real-time PCR rapid MRSA detection assays. Three of the seven GrMSSA isolates with SCC*mec*/SCC DNA yielded positive results by both the BD GeneOhm and Xpert MRSA assays. These included both isolates with *ccrAB4* and the SCC*mec* IID remnant (M06/0075 and M06/0179) and the one isolate with the region from *dcs* to *orfX* (M04/0269). The remaining four GrMSSA isolates that harbored the *ccrAB4* to *orfX* region tested negative in both assays.

Clinical details of patients with GrMSSA isolates yielding amplimers by SCC*mec* typing PCR. Clinical details of the patients from whom the five genotype ST8 isolates yielding SCC*mec* amplimers were recovered are as follows: M06/0075 and M06/0179 were recovered within 4 months of each other (M06/0075 was the first of the two isolates to be recovered) from a 31-year-old male with a 9-year history of chronic GrMRSA causing osteomyelitis of the left hip; M05/0146 (*ccrAB4* only) was cultured from an 82-year-old female with a fractured femur that was slow to heal; this patient had a 9-month history of GrMRSA; M02/0021 was from a patient for whom no clinical details were available, but GrMRSA was also recovered at the same time as the GrMSSA isolate; M04/0269 came from a 7-year-old female with cystic fibrosis from whom GrMRSA was subsequently isolated (Table 1). AR typing of all isolates except M06/0179 suggested AR type AR13 or AR14 (the inferred genotype of AR13 and AR14 MRSA is ST8-MRSA-II) (59). M06/0179 yielded a pattern that could not be assigned an AR type because it was susceptible to ampicillin.

The two patients yielding ST5 isolates were resident in long-stay care institutions. One patient (M06/0288) had a history of chronic leg ulcers but no known history of MRSA (Table 1). No clinical details are available on the other patient (M06/0004). AR types could not be reliably inferred from the AR pattern obtained with these isolates. None of the remaining GrMSSA isolates from patients with a history of MRSA yielded SCC*mec* typing amplimers, but in each case, MRSA isolates recovered from these patients were all susceptible to gentamicin (Table 1).

DISCUSSION

A small number of previous studies have identified MSSA isolates harboring segments of SCC*mec*-associated DNA, but characterization of these segments was limited to DNA sequencing of amplimers obtained by SCC*mec* typing PCR (e.g., *ccrAB1*, *dcs*, and IS*431*-pUB110) or to amplification of a region usually found downstream of *mecA* in MRSA (e.g., the SCC*mec* right-junction region to *orfX* and/or the hypervariable region usually found immediately downstream of *mecA* to *orfX*). Details of previous studies are summarized in Table 3. In the present study, in addition to DNA sequence analysis of the four SCC*mec* typing PCR amplimers found in 7 of the 25 GrMSSA isolates investigated (28%), the entire SCC*mec* remnant in 1 GrMSSA isolate was sequenced. This 26-kb SCC*mec* remnant exhibited very high similarity to the type IID SCC*mec* element of MRSA, except that regions of the *mec* complex structure, including part of *mecI* and the complete *mecR1*, *mecA*, and IS*431* genes, were absent (Fig. 1). The present study is the first report of such a large SCC*mec* remnant in MSSA, and although only part of the class A.4 *mec* complex was identified within the remnant (including truncated *mecI* and IS*1182* genes), this is also the first definitive report of *mec* complex genes in MSSA.

The GrMSSA isolates with the SCC*mec* IID remnant (i.e., M06/0075 and M06/0179) appear to be closely related to ST8 MRSA isolates that exhibited variant SCC*mec* II elements (IIA [II.3.1.1], IIB [II.3.2.1], IIC [II.3.3.1], IID [II.3.1.2], and IIE [II.3.3.2]) that were predominant in the Irish nosocomial MRSA population during the 1990s and continue to constitute part of the MRSA population in Ireland (59). They share the same MLST and *spa* type (ST8/t190), and similar antibiograms. Comparative sequence analysis of SCC*mec* IID and the SCC*mec* IID remnant identified in the present study revealed that they are almost identical except that part of the *mec* complex, including *mecA*, and a section of the J3 region are not found in the SCC*mec* IID remnant (Fig. 1). The patient from whom isolates M06/0075 and M06/0179 were recovered had a 9-year history of MRSA prior to culture of these GrMSSA isolates, suggesting that they may have derived from MRSA by partial loss of SCC*mec*. Unfortunately, none of the patient's MRSA isolates were available for investigation. Prolonged antibiotic therapy during the patient's protracted osteomyelitis infection, sublethal concentrations of antimicrobials at the site of infection due to poor penetration of antimicrobial agents into bone, and/or continued survival of the isolate in bone may have provided suboptimal growth conditions that favored the partial loss of SCC*mec*. Other researchers have suggested that MSSA can arise from MRSA both in vivo and in vitro by a spontaneous loss of *mecA* (8, 9, 11–13, 17, 23, 33, 43). Loss of *mecA* has been reported to occur under stressful conditions in vitro, such as long-term storage in antibiotic-free medium, nutrient starvation, elevated temperatures, or UV irradiation (17, 27, 49). In addition, Noto et al. have shown that exposure of MRSA isolates to vancomycin in vitro can result in a loss of *mecA* or portions of SCC*mec* (43). Daskalaki et al. reported a series of clonally related isolates recovered from a patient with bacteremia and found evidence that a GrMSSA isolate and a gentamicin-susceptible (GsMRSA) isolate were derived from a GrMRSA isolate by deletion of *mecA* and the gentamicin resistance genes *aacA-aphD*, respectively (8).

Although genotyping showed that 9 CCs were represented among the 25 GrMSSA isolates investigated in the present study, the 7 isolates that yielded amplimers by SCC*mec* typing

PCR belonged to only 2 CCs, CC8 ($n = 5$) and CC5 ($n = 2$). *spa* typing divided the nine CC8 isolates included in the study into two clusters; all five ST8 isolates yielding SCC*mec* typing PCR amplimers formed one cluster. All patients from whom these five ST8 isolates were recovered had a history of GrMRSA, and the AR pattern obtained with four of these five isolates suggested AR13 (ST8). None of the remaining 20 patients from whom the remaining 20 GrMSSA isolates were recovered had a history of GrMRSA. The other two GrMSSA isolates that yielded SCC*mec* amplimers belonged to CC5 (ST5), and both exhibited the same *spa* type (t088). Both isolates came from patients in long-stay care in geographically remote institutions. Hence, it is unlikely, although possible, that these isolates are epidemiologically related. However, it is interesting to note that the *spa* type t088 is relatively uncommon in the Ridom SpaServer database (frequency, 0.08%). MRSA with the genotype ST5-MRSA-II have been present in Ireland since 1989 but never predominated (59). Neither of these patients had a history of GrMRSA, but one did have GsMRSA.

In the present study, although three widely used multiplex SCC*mec* typing PCR schemes indicated that seven GrMSSA isolates harbored SCC*mec*-associated DNA, subsequent amplimer sequencing showed that only three isolates carried SCC*mec*-specific DNA (two with the SCC*mec* IID [II.3.1.2] remnant and one with the SCC*mec*-specific *dcs* region amplimer). All three isolates belonged to ST8, and BURP analysis of *spa* typing results placed all three isolates in the same *spa* cluster (shown in Table 1). Donnio et al. also reported an ST8 MSSA isolate with the *dcs* region only, but their isolate had a different *spa* type (t008 rather than t3209 as in the present study) (Table 1) (11). PCR amplification of the *dcs* to *orfx* region of M04/0269 revealed that *dcs* was located ca. 1.7 kb upstream of *orfX*. This is the same location as the *dcs* to *orfX* region in previously described SCC*mec* elements (59). Further investigations of isolate M04/0269 are required in order to identify the extent of SCC*mec* DNA upstream of *dcs* in this GrMSSA isolate. The remaining four GrMSSA isolates yielded a single amplimer by SCC*mec* typing PCR, corresponding to *ccrAB4.* This *ccrAB4* allele was also found in the two isolates carrying the SCC*mec* IID (II.3.1.2) remnant and in the 23 MRSA isolates harboring SCC*mec* II variant elements. In total, *ccrAB4* was identified in two distinct genetic backgrounds, ST8/t190 (MSSA and MRSA) and ST5/t088 (MSSA only). *ccrAB4* shared 100% DNA sequence homology with the *ccrAB4* gene identified in SCC-CI of *S. epidermidis* ATCC 12228 but was not located within a SCC*mec* remnant/element. *ccrAB4* homologues have been reported previously in MRSA in SCC*mec* VI and SCCmec_{N1}, but those *ccrAB4* genes share only 91% and 88% similarity, respectively, to *ccrAB4* of SCC-CI in *S. epidermidis* (14, 48).

SCC-CI is a 57-kb non-*mecA*-containing SCC element inserted into *orfX* in the genome of *S. epidermidis* ATCC 12228 (41). Nested within SCC-CI is a 19-kb SCC*pbp4* element, which harbors *ccrAB2* and genes encoding penicillin binding protein 4 (*pbp4*) and teichoic acid synthesis (*tagF*) (41). The rest of the SCC-CI element, which harbors *ccrAB4*, lies upstream of the left junction of SCC*pbp4*. In the present study, *ccrAB4* was located 5,549 bp upstream of the left extremity of the SCC*mec* IID remnant/SCC*mec* element junction in the two GrMSSA isolates with the SCC*mec* IID remnant and in the same position in the MRSA isolates and 5,549 bp upstream of *orfX* in the four GrMSSA isolates with *ccrAB4* only. This ca. 5.5-kb DNA sequence exhibited 98.5% sequence identity to the region located between *ccrAB4* and SCC*pbp4* in SCC-CI of *S. epidermidis* except for the absence of a ca. 1.5-kb region encoding hypothetical proteins. Further investigations are under way to completely characterize the element(s) harboring *ccrAB4* in these *S. aureus* isolates, but data from the present study indicate that *ccrAB4* is located on an SCC-CI-like element that lies immediately adjacent to either a SCC*mec* element/remnant (in MRSA and MSSA) or *orfX* (in MSSA). Investigations of the DNA region upstream of *ccrAB4* in these *S. aureus* isolates will be of particular interest since this region in SCC-CI harbors genes encoding resistance to cadmium and mercury and also harbors three IS*431* elements. Insertion sequence elements have been reported to promote genomic rearrangements (37), and any additional insertion sequence elements that may be present in these *S. aureus* isolates may have played a role in the deletion and rearrangement of regions of SCC in these isolates.

The identification of DNA in *S. aureus* with extensive homology to the *S. epidermidis* SCC-CI element provides further evidence for horizontal transfer of SCC*mec*/SCC DNA between *S. aureus* and coagulase-negative staphylococci (CoNS) (19, 41, 65). It has been suggested that the direction of transfer is from CoNS to *S. aureus* by an as yet unknown mechanism and that CoNS act as a reservoir for SCC*mec*/SCC DNA (2, 18, 20, 28, 60, 66). However, it is interesting to note that in the present study, the *ccrAB4* gene with 100% homology to that found in SCC-CI from *S. epidermidis* was identified in 29 *S. aureus* isolates (23 MRSA isolates and 6 MSSA isolates) belonging to two distinct genetic lineages (ST8/t190 [$n = 27$] and ST5/t088 [$n = 2$]), but previous studies have identified this *ccrAB4* gene in only one *S. epidermidis* strain (41). While this evidence may suggest that the *ccrAB4* gene is more common among or may even have originated in *S. aureus*, it may merely reflect the fact that the majority of previous studies of SCC*mec* in *S. aureus* and CoNS have not involved investigation of *ccrAB4* (19, 20). With the incorporation of detection of *ccrAB4* into an SCC*mec* typing scheme (32) and with the description of primers specific for *ccrAB4* from SCC-CI, it will be interesting to see the extent to which this *ccrAB4* allotype will be detected in various staphylococcal species in future studies. Further work involving *S. epidermidis* isolates and *S. aureus* isolates representing the different *S. aureus* genetic backgrounds from Ireland and other countries is required to determine the prevalence, significance, and origin of *ccrAB4* among *S. aureus* and *S. epidermidis* isolates.

Donnio et al. (11) investigated 247 multiresistant MSSA isolates from 60 French hospitals using the IDI-MRSA real-time PCR assay to target the right junction region between SCC*mec* and *orfX* and found that 169/247 isolates (68%) were positive, suggesting carriage of portions of SCC*mec*. These isolates belonged to more than 30 *spa* types, suggesting that partial loss of SCC*mec* was not restricted to specific *S. aureus* lineages, although isolates with the genotypes ST8 and ST5 predominated. In the present study, SCC*mec*/SCC-associated DNA was detected in only 28% of isolates but these isolates also exhibited either the ST8 or ST5 genotypes (Table 1). It is

important to highlight the fact that although Donnio et al. (11) used PCR to detect the *ccr* genes (*ccrAB1*, *ccrAB2*, *ccrAB3*, and *ccrC*), only 2 of the 169 IDI-MRSA assay-positive isolates were investigated further by PCR amplification of the region downstream of *mecA* to confirm the presence of SCC*mec* DNA sequences. It has been reported that the IDI-MRSA assay is not specific for SCC*mec* but also detects SCC sequences (10). Interestingly, a study of a genetically diverse collection of 42 MSSA isolates mainly from patients with bacteremia or skin infections or from healthy carriers reported that no isolate was found to harbor the *ccrAB*, *ccrC*, or *mecA* fragments when investigated by PCR and Southern hybridization analysis (44).

The presence of SCC- and SCC*mec*-associated DNA in MSSA has practical implications for the rapid detection of MRSA from clinical specimens and for tracking and genotyping of MRSA by SCC*mec* typing. There are now a number of commercially available assays that use real-time PCR to detect SCC-associated sequences directly from clinical specimens for the rapid detection of MRSA. They include the BD GeneOhm MRSA assay (10, 25, 51) and Cepheid's Xpert MRSA assay (52). During development of the IDI-MRSA assay, the forerunner of the BD GeneOhm MRSA assay, which was the first FDA-approved commercial system, Huletsky et al. found that 4.6% (26/569) of MSSA isolates investigated were positive, probably due to the presence of either SCC*mec* or SCC sequences (25). Another study using the IDI-MRSA assay to detect MRSA from broth enrichment culture found that 26% (77/298) of positive specimens carried MSSA (10). In the present study, the three GrMSSA isolates with SCC*mec*-specific DNA tested positive with both the BD GeneOhm and Xpert MRSA assays. Interestingly, Donnio et al. found that one of the MSSA isolates detected by the IDI-MRSA assay also harbored the SCC*mec dcs* region only (11). In contrast, the four GrMSSA isolates with the SCC-CI-associated *ccrAB4* gene tested negative with both assays. These findings suggest that kit-positive MRSA culture-negative specimens should not be dismissed lightly as false-positive kit results without taking the patient's prior MRSA history into account. The clinical significance of such positive results in patients with a history of MRSA is difficult to interpret.

SCC*mec* typing together with MLST is recommended for comparison of MRSA strains and is commonly used for routine epidemiological typing of MRSA (5). The presence of a second *ccr* gene (*ccrAB4*) outside of SCC*mec* in MRSA isolates could result in the misinterpretation of typing results for isolates carrying *ccrAB4* in addition to another *ccr* allotype within SCC*mec*, as was found in the present study. When this occurs, it is important to determine which *ccr* gene type is part of the SCC*mec* element and which is located outside of SCC*mec*.

In conclusion, this study has found that 28% of GrMSSA isolates investigated carry remnants of SCC or SCC*mec* and that some of these GrMSSA isolates may have been derived from a previously predominant MRSA clone in Irish hospitals. The study provides the first detailed description of a 26-kb SCC*mec* remnant with *ccr* and partial *mec* complex genes in an MSSA isolate. It is also the first report of *S. epidermidis* SCC-CI DNA, including SCC-CI *ccrAB4* genes, in MRSA and MSSA isolates, with a location outside of, but immediately adjacent to, SCC*mec* or *orfX*. The presence of SCC*mec*-associated DNA in MSSA should be considered when real-time PCR assays are used for the rapid detection of MRSA from clinical specimens. In addition, MRSA isolates with additional *ccr* genes located outside of SCC*mec* may pose problems for the interpretation of SCC*mec* typing results.

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