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Seven Novel Variants of the Staphylococcal Chromosomal Cassette *mec* in Methicillin-Resistant *Staphylococcus aureus* Isolates from Ireland

Anna Shore,[1] Angela S. Rossney,[2] Conor T. Keane,[2] Mark C. Enright,[3] and David C. Coleman[1]*

Microbiology Research Unit, Department of Oral Surgery, Oral Medicine and Pathology, School of Dental Science, Trinity College, University of Dublin, Ireland[1]; National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland[2]; and Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom[3]

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Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered in Irish hospitals between 1971 and 2002 were characterized using multilocus sequence typing (MLST) ($n = 130$) and SCC*mec* typing ($n = 172$). Where atypical SCC*mec* typing results were obtained, PCR amplification of entire SCC*mec* elements, analysis of amplimer mobility, and nucleotide sequencing were undertaken. MLST revealed that 129/130 isolates had the same genotypes as internationally spread MRSA clones, including ST239, ST247, ST250, ST5, ST22, ST36, and ST8. A novel genotype, ST496, was identified in one isolate. Half of the isolates (86/172) had SCC*mec* type I, IA, II, III, or IV. The remaining 86 isolates harbored novel SCC*mec* variants in three distinct genetic backgrounds: (i) 74/86 had genotype ST8 and either one of five novel SCC*mec* II (IIA, IIB, IIC, IID, and IIE) or one of two novel SCC*mec* IV (IVE and IVF) variants; (ii) 3/86 had genotype ST239 and a novel SCC*mec* III variant; (iii) 9/86 had a novel SCC*mec* I variant associated with ST250. SCC*mec* IVE and IVF were similar to SCC*mec* IVc and IVb, respectively, but differed in the region downstream of *mecA*. The five SCC*mec* II variants were similar to SCC*mec* IVb in the region upstream of the *ccr* complex but otherwise were similar to SCC*mec* II, except for the following regions: SCC*mec* IIA and IID had a novel *mec* complex, A.4 (Δ*mecI*-IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec*); SCC*mec* IIC and IIE had a novel *mec* complex, A.3 (IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec*); SCC*mec* IID and IIE lacked pUB110; SCC*mec* IIC and IIE lacked a region of DNA between Tn*554* and the *mec* complex; and SCC*mec* IIB lacked Tn*554*. This study has demonstrated a hitherto-undescribed degree of diversity within SCC*mec*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in Irish hospitals in 1971 10 years after the initial report of MRSA in England (14, 20). In line with trends worldwide, the prevalence of MRSA increased in Ireland during the 1990s, with one Irish hospital reporting a fourfold increase in the number of patients with MRSA between 1989 and 1998 (42). The European Antimicrobial Resistance Surveillance System (EARSS) reported that in Ireland in 2002, 42% of *S. aureus* isolates from blood cultures were methicillin resistant, one of the highest prevalence rates in Europe (8).

Methicillin-susceptible *S. aureus* (MSSA) isolates have the potential to become methicillin resistant due to acquisition of a mobile staphylococcal chromosomal cassette (SCC) carrying the *mecA* gene, termed SCC*mec* (23). The *mecA* gene encodes an extra penicillin-binding protein (PBP) 2a or PBP 2′ that has decreased affinity for β-lactam antibiotics, allowing cell wall synthesis to continue despite inactivation of native PBPs (12, 35). To date, five types of SCC*mec* element (SCC*mec* I, II, III, IV, and V) and a small number of variants have been characterized (16–19, 25, 31, 32). Each SCC*mec* element integrates at the same site ($attB_{scc}$) at the 3′ end of an open reading frame (ORF) of unknown function, designated *orfX* (17).

* Corresponding author. Mailing address: Microbiology Research Unit, Department of Oral Surgery, Oral Medicine and Pathology, School of Dental Science and Dublin Dental Hospital, Trinity College, University of Dublin, Dublin 2, Republic of Ireland. Phone: 353 1 6127276. Fax: 353 1 6127295. E-mail: dcoleman@dental.tcd.ie.

SCC*mec* consists of the *mec* gene and cassette chromosome recombinase (*ccr*) gene complexes. Five classes of *mec* gene complex (A to E), which vary in their genetic structure, have been described (22, 24). Each *mec* complex consists of an intact copy of *mecA*, a copy of IS*431mec* and, when present, complete or truncated *mec* regulatory genes *mecI* and *mecR1* (24). The *ccr* complex consists of the *ccr* genes *ccrA* and *ccrB* in combination (*ccrAB*) or *ccrC* alone, as well as adjacent ORFs (18, 23). The *ccrAB* and *ccrC* genes encode recombinases necessary for site- and orientation-specific integration and accurate excision of the SCC*mec* element. Five allotypes of the *ccr* gene complex have been identified (16, 18, 32). The rest of the SCC*mec* element outside the *ccr* and *mec* complex is known as the junkyard (J) region, because it contains genes that are nonessential components of SCC*mec* (19). The five SCC*mec* types described to date are defined on the basis of the class of *mec* gene complex and the type of *ccr* complex they possess, and variants of each type are defined by the J regions.

Variants of SCC*mec* types described include SCC*mec* IA, which differs from SCC*mec* I by the presence of an integrated plasmid, pUB110, downstream of the *mec* complex; SCC*mec* IIIA, which differs from SCC*mec* III by the absence of pT181 and its flanking IS*431* elements; and SCC*mec* IIIB, which lacks integrated copies of Tn*554*, pT181, and the *mer* operon with its associated insertion sequences (32). Variants of SCC*mec* IV include SCC*mec* IVa and SCC*mec* IVb as described by Ma and colleagues, which differ in their DNA sequences from the left

extremity to the *ccr* complex (L-C region) but which both carry the downstream constant region (*dcs*) (25). Both SCC*mec* IVc and IVd also differ in their L-C regions (SCC*mec* IVc carries an integrated copy of Tn*4001* flanked on either side by IS*256*) (19). SCC*mec* IV has a type 2 *ccr* complex and class B *mec*, but Oliveira and colleagues described an SCC*mec* IV element with type 4 *ccr* (25, 32). A type IVA SCC*mec* element has also been described that harbors the integrated plasmid pUB110 (31).

Multilocus sequence typing (MLST), SCC*mec* typing, and other molecular techniques have shown that the majority of nosocomial MRSA infections are caused by relatively few pandemic clones that have evolved by the introduction of SCC*mec* elements into five distinct epidemic MSSA lineages (33, 36). Using MLST and the program eBURST, the five pandemic MRSA lineages can be visualized as clonal complexes (CCs), which are groups of genotypes and MLST sequence types (STs) that share a recent common ancestor (11). CCs are named after the ST of the ancestral genotype. The CCs of the major nosocomial MRSA lineages are CC5, CC8, CC22, CC30, and CC45 (10). In all of these lineages, except possibly CC22, SCC*mec* has been acquired on multiple occasions. All major international nosocomial MRSA clones belong to one of these five CCs. The names originally assigned to the MRSA clones represent either a unique epidemiological characteristic or signify the geographic area from which they were first isolated. A more rational and unambiguous scheme has been proposed based on ST and SCC*mec* type (10, 37). Using this nomenclature, some of the more common MRSA clones are named as follows: CC5, ST5-MRSA-II (New York/Japan) and ST5-MRSA-IV (Pediatric); CC8, ST239-MRSA-IIIA (Brazilian), ST239-MRSA-III (Hungarian), ST247-MRSA-IA (Iberian), ST250-MRSA-I (Archaic), ST8-MRSA-II (Irish-1), and ST8-MRSA-IV (EMRSA-2, EMRSA-6); CC22, ST22-MRSA-IV (EMRSA-15); CC30, ST36-MRSA-II (EMRSA-16); CC45, ST45-MRSA-IV (Berlin) (10, 32, 33).

It has been suggested that genetic relatedness of particular MRSA isolates should be investigated by determining both the genotype of the MSSA isolate into which the SCC*mec* element was introduced and the type of SCC*mec* element it harbors (10). MLST has been shown to be the most powerful molecular technique for genotyping *S. aureus* isolates in long-term and global epidemiological studies (10, 33). The SCC*mec* type can be characterized using various PCR-based techniques that identify both the *ccr* and *mec* gene type or other sequences in the J regions specific to each SCC*mec* element (29, 31).

The objective of the present study was the molecular characterization of representative clinical MRSA isolates recovered in Irish hospitals between 1971 and 2002, examining their genetic relatedness to each other and to internationally recognized MRSA clones using MLST and SCC*mec* element analysis. The results of this investigation identified seven novel SCC*mec* variants and two new *mec* classes, which were further characterized.

MATERIALS AND METHODS

Bacterial isolates. A total of 172 MRSA isolates representative of the most prevalent phenotypes recovered in Irish hospitals during eight study periods between 1971 and 2002 were investigated (Table 1). Isolates were chosen to include at least two isolates of each type or subtype recognized during each study period. Prior to 1988 (study periods A, B, and C), isolates were classified into four phenotypes (termed early MRSA and phenotypes I, II, and III) based on a combination of antimicrobial resistance patterns, bacteriophage typing, plasmid screening, location of resistance determinants, and Southern hybridization analysis of plasmid and chromosomal DNA (4, 5). Phenotype I and phenotype II isolates were previously identified during study period B (1976 to 1984), but only phenotype II isolates were available for the present study. Isolates recovered after 1988 (study periods D through H) were characterized by a combination of techniques that included bacteriophage typing, restriction fragment length polymorphism (RFLP) analysis of SmaI-digested total cellular DNA using pulsed field gel electrophoresis (PFGE), random amplified polymorphic DNA analysis using PCR (RAPD-PCR), plasmid screening, enterotoxin profiling, biotyping (pigment production and hydrolysis of Tween 80 and urea), and antibiogram-resistogram (AR) typing (using a panel of 22 antimicrobials [the panel was extended to 23 in 1998]) (39–42, 44). In study periods D and E, isolates were assigned an AR type on the basis of the pattern of resistance to the antimicrobials in the AR typing panel in conjunction with additional data obtained from the phenotypic and genotypic investigations described above (39–41). Forty-four AR types (AR01 to AR44) and a number of subtypes have been recognized to date.

Isolates recovered between 1971 and 1998 were from Dublin hospitals only. Isolates from 1999 were recovered from patients in hospitals that participated in the North/South Study of MRSA in Ireland, a 2-week prevalence study of MRSA in both the Republic of Ireland and the North of Ireland (3, 44). Isolates of each AR type and subtype from both the North and South of Ireland were included. Ninety-eight percent of Irish hospitals participated in the North/South Study. The isolates recovered in 2002 were from blood culture specimens from those Irish hospitals ($n = 23$) that participated in EARSS (43). This level of participation in EARSS represents a population cover rate of >90%. Details of study isolates are shown in Table 1.

Among isolates from 1999, a previously unfamiliar AR pattern, designated AR43, was predominant among isolates from the North of Ireland. AR43 isolates from six patients were recovered in mixed culture with MRSA isolates exhibiting other AR patterns (five AR13 and one AR14 pattern) (3, 44). Proportionally more isolates exhibiting AR patterns AR43, AR13, and AR14 were included in the present study to investigate this observation.

Bacterial storage and culture conditions. MRSA isolates were stored at −80°C in Protect bacterial preserver vials (Technical Services Consultants Ltd., Heywood, United Kingdom) and were routinely cultured onto trypticase soy agar (Oxoid Limited, Basingstoke, United Kingdom) prior to incubation overnight at 37°C.

DNA isolation and amplification. Chromosomal DNA was prepared from each isolate using the QIAGEN DNeasy kit system (QIAGEN, Crawley, West Sussex, United Kingdom) according to the manufacturer's instructions. DNA was amplified in a Thermo Hybaid Multiblock system thermal cycler (Thermo Hybaid, Ashford, Middlesex, United Kingdom).

MLST. MLST was performed on 130 isolates (Table 1) by PCR amplification of internal fragments of seven housekeeping genes by using a previously described procedure and primers (9). Sequencing of both DNA strands was performed using the ABI Big-Dye Fluorescent Terminator system and an ABI 3700 automated sequencer (Applied Biosystems, Warrington, United Kingdom) at the Genomics Facility at the University of Bath (Bath, United Kingdom). The alleles at each of the seven housekeeping loci were identified by comparing the sequences obtained from the test isolates with sequences held in the MLST database (http://www.saureus.mlst.net). This database was also used to identify the allelic profile and hence the ST of each isolate.

SCC*mec* typing. SCC*mec* elements from all 172 isolates included in this study were typed using two methods. Firstly, a previously reported method (29) described in the present study as the simplex method was used to amplify the cassette chromosome recombinase (*ccr*) and *mec* gene complexes (*ccr-mec* genes). The primers of Ito et al. and Robinson and Enright were used for amplification of the *ccr* and *mec* genes, respectively (17, 37). Secondly, the multiplex PCR method of Oliveira and de Lencastre, which utilizes primers designed specifically to distinguish different regions of each SCC*mec* type, was used as described previously (31).

Nucleotide sequencing of SCC*mec*. The entire nucleotide sequence of the SCC*mec* element from two isolates was determined. One isolate (AR13.1/3330.2) from study period G (1999) was chosen because it was representative of isolates with *ccr-mec* genes indicative of SCC*mec* II by the simplex PCR method but lacking one to three of the five amplimers typical of SCC*mec* II when analyzed by the multiplex PCR SCC*mec* typing method. The second isolate (AR43/3330.1) from study period G also was chosen because it was representative of isolates with *ccr-mec* genes typical of SCC*mec* IV by the simplex method

TABLE 1. Irish MRSA isolates investigated from each study period and typed by MLST and SCC*mec* typing

Isolate phenotype[a]	No. of isolates investigated in study period[b]:								No. of isolates typed by:	
	A (1971–75)	B (1976–84)	C (1985–87)	D (1989)	E (1992–93)	F (1998)	G (1999)	H (2002)	MLST	SCC*mec* typing
Early MRSA	1	—	—	—	—	—	—	—	1	1
Phenotype II	—	15	—	—	—	—	—	—	5	15
Phenotype III	—	—	1	—	—	—	—	—	1	1
AR01	—	—	—	4	—	—	—	—	3	4
AR02	—	—	—	3	—	—	—	—	3	3
AR05	—	—	—	1	—	—	—	—	1	1
AR06	—	—	—	1	4	4	10	8	11	27
AR07.0/07.2	—	—	—	2	2	2	9	2	7	17
AR07.3/07.4	—	—	—	1	3	3	2	—	9	9
AR09	—	—	—	2	—	—	—	—	2	2
AR11	—	—	—	—	4	—	—	—	1	4
AR13	—	—	—	—	4	4	15	3	26	26
AR14	—	—	—	—	4	4	11	3	22	22
AR15	—	—	—	—	2	—	—	—	1	2
AR22	—	—	—	—	2	—	—	—	1	2
AR23	—	—	—	—	1	—	—	—	1	1
AR43	—	—	—	—	—	—	20	—	20	20
AR44	—	—	—	—	—	—	—	2	2	2
ARNT (urea +ve)[c]	—	—	—	1	2	—	—	3	6	6
New01[d]	—	—	—	—	—	—	1	—	1	1
New02[d]	—	—	—	—	—	—	1	—	1	1
New03[d]	—	—	—	—	—	—	2	2	4	4
Unf01[e]	—	—	—	—	—	—	—	1	1	1
Total in this study	1	15	1	15	28	17	71	24	130	172
Total in original study	20	350	67	104	321	220	714	398	NA[f]	NA

[a] The most prevalent phenotypes during each study period are underlined. —, isolates exhibiting this phenotype were not recovered during the study period.
[b] Isolates from groups A to F were from Dublin; isolates in group G were from the North and South of Ireland; isolates in group H were from the South of Ireland. References for the original study with each group are as follows: group A (reference 5); B (5); C (4); D (39, 42); E (39, 42); F (42); G (3, 44); H (38, 43).
[c] No AR type could be assigned. PFGE analysis showed that most urease-positive isolates exhibiting the AR06 pattern yielded PFGE patterns unlike the patterns obtained from other AR06 isolates. Hence, assigning urease-positive AR06 isolates to AR type 06 was considered incorrect, and these isolates were assigned to no type (NT) pending PFGE, MLST, and SCC*mec* analysis.
[d] These isolates were not assigned an AR type because they produced unfamiliar AR patterns; the AR type is pending on the results of plasmid screening, enterotoxin profiling, PFGE, phage typing, biotyping, MLST, and SCC*mec* typing.
[e] This isolate yielded an unfamiliar AR pattern and was not assigned an AR type, pending the outcome of MLST and SCC*mec* typing results in this study.
[f] NA, not applicable.

but with one of the two amplimers characteristic of SCC*mec* IV missing when isolates were investigated by the multiplex method.

Overlapping primers were designed to amplify the entire SCC*mec* element from both isolates using previously published SCC*mec* II and SCC*mec* IVa nucleotide sequences (17, 25) obtained from the Entrez-PubMed Nucleotide database website (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=nucleotide) (accession numbers D86934 [SCC*mec* II] and AB063172 [SCC*mec* IVa]). The primers used to amplify and sequence the SCC*mec* elements are listed in Table 2.

DNA fragments for sequencing and cloning were obtained by PCR amplification of chromosomal DNA using either *Pfu* DNA polymerase (Promega) for primer pairs dcs F and orfX R (Table 2) or the Expand Long Template PCR system (Roche) for all other primer sets (Table 2) according to the manufacturers' instructions. When each primer pair was used to amplify DNA from isolate AR13.1/3330.2, template DNA from at least one isolate exhibiting SCC*mec* II by both SCC*mec* typing methods was included as a control. Similarly, for AR43/3330.2 template DNA from at least one control isolate exhibiting SCC*mec* IV by both methods was included. Amplimers were purified using either the GenElute PCR Clean-Up kit (Sigma-Aldrich) or Wizard SV Gel and PCR Clean-Up system (Promega) prior to direct sequencing (Lark Technologies, Essex, United Kingdom) using primer walking or cloning into pBluescript II KS(−) phagemid and sequencing. Ligations of PCR products to pBluescript were facilitated by the presence of appropriate restriction endonuclease cleavage sites within the primers (Table 2). Transformation of competent *Escherichia coli* DH5α prepared using $CaCl_2$ and identification of recombinants using blue-white selection were performed according to the methods of Sambrook and Russell (45). DNA was sequenced using an Applied Biosystems 373A DNA sequencer (Foster City, Calif.) and dye-labeled terminators. Analysis of chromatograms and sequences were carried out using the 373A Data Analysis software program version 1.2.0 (Applied Biosystems) and DNA Strider 1.3f11 software (CEA/Saclay, Gif-sur-Yvette, France), respectively. Homology searches were performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/index.shtml). The CLUSTAL W sequence alignment computer program was used for alignments of nucleotide sequences (13).

Investigation of variant SCC*mec* elements. The SCC*mec* elements harbored by all isolates identified as having SCC*mec* types II and IV *ccr-mec* genes by the simplex method but with unusual SCC*mec* multiplex patterns were investigated by PCR amplification. The primers used for amplification and sequencing of SCC*mec* from isolate AR13.1/3330.2 (representative of isolates with SCC*mec* II *ccr-mec* genes by the simplex method but lacking one to three of the five SCC*mec* II multiplex amplimers) were used on all atypical SCC*mec* II isolates (Table 2). Similarly, the primers for amplification and sequencing of the SCC*mec* element of isolate AR43/3330.1 (representative of isolates with SCCmec IV *ccr-mec* genes by the simplex method but lacking one SCC*mec* IV multiplex amplimer) were used on all atypical SCC*mec* IV isolates (Table 2). PCR products were separated by electrophoresis in 1% (wt/vol) agarose gels, and the sizes of amplimers were compared to those obtained by amplification using the same primers on template DNA from AR13.1/3330.2 and AR43/3330.1 and to previously published SCC*mec* element sequences. Amplimers that showed variation from expected sizes were also sequenced.

Nucleotide sequence accession numbers. Nucleotide sequences were submitted to the GenBank database under accession numbers AJ810120 (SCC*mec* IIE from MRSA isolate AR13.1/3330.2), AJ810121 (SCC*mec* IVE from MRSA isolate AR43/3330.2), AJ810122 (partial *mec* complex A.4), and AJ810123 (partial SCC*mec* IIB).

RESULTS

MLST. Of the 172 MRSA isolates investigated in this study, 130 were examined by MLST (Table 1). These 130 isolates

TABLE 2. Primers used in this study for SCC*mec* element amplification and sequencing

Isolate	Primer pair	Nucleotide sequence[g]	Restriction site	Nucleotide coordinates	SCC*mec* region amplified
AR13.1/3330.2	IRLII F	CCC*TCGAGGG***CTCTGCGTATCAGTTAATGA**	XhoI	4684–4703[a]	L-C[c]
	ccrA R	ATTT*GCGGCCGC***GCTTCGATAGCCTGTTTCTG**	NotI	25490–25471[a]	
	ccrA F	ACG*CGTCGAC***CAAGTCATAGGCTATTTACG**	SalI	25417–25436[a]	C-M[d] (*ccr*A2-Tn*554*)
	Tn554 R	ACG*CGTCGAC***AAGCTATCCACGTTCAATCTCAAC**	SalI	32442–32419[a]	
	Tn554 F	ATTT*GCGGCCGC***CTTTAAAGGGTTTCGGAATA**	NotI	32365–32385[a]	C-M[d] (Tn*554-mecR1*)
	mecR1 R	CCC*TCGAGGG***CAATGCCTAAACCTAATCG**	XhoI	43754–43736[a]	
	mecR1 F	ATTT*GCGGCCGC***CGATTAGGTTTAGGCATTG**	NotI	43736–43754[a]	M-I[e] (*mecR1-ugpQ*)
	ugpQ R	CCCT*CGAGGG***GCTTCTGCAGGATCTTGG**	XhoI	47868–47851[a]	
	ugpQ F	CCCT*CGAGGG***CCAAGATCCTGCAGAAGC**	XhoI	47851–47878[a]	I-R[f] (*ugpQ-dcs*)
	dcs R	ATTT*GCGGCCGC***CGGTCATGGCTATGATTTAG**	NotI	56373–56354[a]	
	dcs F	ATTT*GCGGCCGC***GTCAATGAGATCATCTACAT**	NotI	56109–56128[a]	I-R[f] (*dcs*-right SCC*mec* junction)
	orfX R	ATTT*GCGGCCGC***CCCCAAGGGCAAAGCGAC**	NotI	57826–57810[a]	
AR43/3330.1	IRLII F	CCC*TCGAGGG***CTCTGCGTATCAGTTAATGA**	XhoI	4684–4703[a]	L-C[c]
	ccrA R	ATTT*GCGGCCGC***GCTTCGATAGCCTGTTTCTG**	NotI	25490–25471[a]	
	ccrA F	ATTT*GCGGCCGC***CAAGTCATAGGCTATTTACG**	NotI	9894–9913[b]	C-M[d] (*ccr*A2-*mecA*)
	mecA R	ACG*CGTCGAC***CCTATCTCATATGCTG**	SalI	18160–18145[b]	
	mecA F	ATTT*GCGGCCGC***GATTGGGATCATAGCGTCAT**	NotI	18021–18040[b]	M-I[e] (*mecA*-IS*431mec*)
	ISmec R	ACG*CGTCGAC***ACGGTGATCTTGCTCAATGA**	SalI	22780–22761[b]	
	ISmec F	CCC*TCGAGGG***CCTGACTGTCATTGTAC**	XhoI	22724–22740[b]	I-R[f] (IS*431mec*-right SCC*mec* junction)
	orfX R	ATTT*GCGGCCGC***CCCCAAGGGCAAAGCGAC**	NotI	25395–25379[b]	

[a] Nucleotide coordinates from SCC*mec* type II, accession number D86934.
[b] Nucleotide coordinates form SCC*mec* type IVa, accession number AB063172.
[c] L-C, the region from the left chromosome-SCC*mec* junction to the beginning of the *ccr* complex.
[d] C-M, the region from the *ccr* complex to the *mec* complex.
[e] M-I, the region from the *mec* complex region to IS*431mec*.
[f] I-R, the region from IS*431mec* to the right extremity of SCC*mec*.
[g] Text in bold indicates primer sequences, text in italics indicates restriction sites added to primer sequences, and plain text indicates extra bases added to aid with cloning.

included at least one isolate from each of the most common phenotypes identified among MRSA recovered between 1971 and 2002 from Irish hospitals (Table 1, study periods A to H). Multiple isolates were included for the predominant phenotypes during each study period (Table 1), except for study period B (1976 to 1984), for which isolates from only one of the two predominant phenotypes were available. MLST identified 10 STs belonging to each of the five major CCs described to date and one singleton (ST12) among the MRSA isolates investigated (Table 3). Isolates recovered prior to 1985 exhibited ST250, while the STs of the two most prevalent strains in 1989 were ST250 and ST239 (Tables 1 and 3). ST8 predominated in the 1990s, but by the late 1990s the prevalence of isolates with ST36 and ST22 increased (Tables 1 and 3). By 2002, ST22 had become the major ST. All except one ST were identical to those previously described. The exception was a double locus variant of ST5 (ST496), which differed from ST5 at both the *arcC* and *yqiL* alleles. ST5 has alleles 1 and 10 of *arcC* and *yqiL*, respectively, whereas ST496 had a new allele, 63, of *arcC* and allele 28 of *yqiL*. The *arcC* alleles 63 and 1 differ at a single nucleotide site, as do alleles 10 and 28 of *yqiL*. This novel ST was obtained from one isolate from 2002 exhibiting AR type AR07.2.

SCC*mec* typing. Using both the simplex and multiplex SCC*mec* typing methods 50% (86/172) of isolates carried SCC*mec* type I, IA, II, III, or IV (Table 3). SCC*mec* I was identified in 10 isolates (5.8%), SCC*mec* II was identified in 30 isolates (17.4%), SCC*mec* III was found in nine isolates (5.2%), and SCC*mec* IV was identified in 34 isolates (19.8%). Three isolates (1.7%) were found to harbor SCC*mec* I by the simplex method, but the multiplex method showed that they carried the previously described variant element SCC*mec* Ia (Table 3).

Identification of variant SCC*mec* types. Eighty-six isolates (50%) harbored two apparently different SCC*mec* elements when tested by both SCC*mec* typing methods, or the SCC*mec* type could not be inferred because the multiplex patterns produced did not exactly match those of previously described SCC*mec* elements (Table 3; Fig. 1).

Nine isolates were found to carry SCC*mec* I by the simplex method but failed to yield the 495-bp band characteristic of SCC*mec* I elements by the multiplex method, which results from amplification of a region downstream of the gene encoding a plasmin-sensitive surface protein (*pls*) found in SCC*mec* I (Table 3). Furthermore, the multiplex pattern obtained was indistinguishable from SCC*mec* IV (Fig. 1A, lanes 2 to 5). This pattern was designated SCC*mec* I *–pls*.

Three isolates were identified by the simplex method as carrying SCC*mec* III but their multiplex pattern, although similar to that of SCC*mec* III, was missing a band at 243 bp (Table 3; Fig. 1A, lanes 7 to 9). This band is the product of amplification of the locus between pI258 and Tn*554* and is found in all SCC*mec* III elements characterized to date. This pattern was designated SCC*mec* III –pI258/Tn*554*.

All 20 AR43 isolates were classified as SCC*mec* type IV by the simplex method but gave a multiplex PCR pattern from which the SCC*mec* type could not be inferred because only the multiplex *mecA* control amplimer found in all SCC*mec* types was obtained (Table 3; Fig. 1B, lanes 7 to 9). This multiplex pattern could be interpreted as a variant SCC*mec* IV multiplex pattern missing the 342-bp amplimer, which results from the amplification of *dcs* found in SCC*mec* IV. This pattern was called SCC*mec* IV *–dcs*.

The remaining 54 isolates belonged to phenotypes AR05, AR13, AR14, New01, and New03 (Table 1) and were identi-

TABLE 3. MLST, SCC*mec* type, and variant SCC*mec* elements of MRSA recovered in Ireland, 1971 to 2002

CC	ST	AR type or phenotype[a]	SCC*mec* type based on:			Variant SCC*mec* elements
			Simplex PCR	Multiplex PCR[b]	SCC*mec* name[c]	
8	239	AR01, AR15, AR23, AR44	III	III		
	239	PhIII, AR09	III	III − pI258/Tn*554*		
	250	Early MRSA (n = 1), PhII (n = 4)	I	I (n = 10)		
	250	PhII (n = 1), AR02 (n = 3)	I	IV or I − *pls* (n = 9)		
	247	AR22, New02	I	Ia		
	8	AR05	II	II − *kdp*	IIB	SCC*mec* IVb L-C region and lacks Tn*554*[f]
	8	AR13 (n = 6), AR14 (n = 3)	II	II − *kdp*	IIA	SCC*mec* IVb L-C region; class A.4 *mec*[d,f]
	8	AR14 (n = 10)	II	II − *kdp* & *mec*I	IIC	SCC*mec* IVb L-C region; class A.3 *mec*[d]; lacks ORFs between Tn*554* and *mec* complex[f]
	8	AR13 (n = 19), AR14 (n = 3), New01, New03	II	II − *kdp* & pUB110	IID	SCC*mec* IVb L-C region; class A.4 *mec*[d]; lacks pUB110 and second copy of IS*431*[f]
	8	AR13 (n = 1), AR14 (n = 6)	II	IV or II − *kdp*, *mecI* & pUB110	IIE	SCC*mec* IVb L-C region; class A.3 *mec*[d]; lacks pUB110, second copy of IS*431*, and ORFs between Tn*554* and *mec* complex[f]
	8	AR43 (n = 17)	IV	IV − *dcs*	IVE	SCC*mec* IVc variant, lacks *dcs*; has an unusual sequence in I-R region
	8	AR43 (n = 3)	IV	IV − *dcs*	IVF	SCC*mec* IVb variant, lacks *dcs*; has an unusual sequence in I-R region
5	5	AR07.3/07.4, AR11	II	II		
	5	Unf01	IV	IV		
	496	AR07.2 (n = 1)	II	II		
30	36	AR07.0/07.2 (n = 6)	II	II		
	30	ARNT (n = 4)	IV	IV		
22	22	AR06	IV	IV		
45	45	ARNT (n = 1)	IV	IV		
S[e]	12	ARNT (n = 1)	IV	IV		

[a] Where more than one ST/SCC*mec* type was identified within a phenotype (e.g., AR13 or AR07.0/07.2), the numbers represented by each ST or SCC*mec* type are shown in parentheses after the AR type or phenotype. For isolates identified as having genotype ST250, the numbers of isolates analyzed by MLST differed from the numbers subjected to SCC*mec* typing (TABLE 1), and more than one SCC*mec* type was identified within this genotype; therefore, the numbers of isolates identified with each ST and SCC*mec* type are indicated in parentheses after the phenotype and SCC*mec* type, respectively. Otherwise, numbers of isolates are as indicated in TABLE 1.

[b] Some patterns obtained with the multiplex PCR SCC*mec* typing method lacked amplimers corresponding to the amplification of the determinants indicated.

[c] SCC*mec* name assigned during the present study.

[d] These two variants of *mec* complex class A (designated A.3 and A.4) were recognized during the present study (Fig. 2 and 3).

[e] Singleton, no ancestral genotype has been assigned.

[f] SCC*mec* II variant.

fied as SCC*mec* II by the simplex method but gave variant SCC*mec* II multiplex patterns that lacked one to three of the five amplimers that would identify them as harboring SCC*mec* II (Table 3). Ten isolates lacked a 284-bp band corresponding to the amplification product from a region of the *kdp* operon found in SCC*mec* II (Table 3). This pattern was designated SCC*mec* II *–kdp* (Fig. 1B, lane 2). Ten isolates lacked both the 284-bp band (*kdp* operon) and a 209-bp amplimer (Table 3). The latter results from amplification of part of the *mecI* gene in SCC*mec* II. This pattern was termed SCC*mec* II *–kdp* & *mecI* (Fig. 1B, lane 3). Twenty-seven isolates lacked the 284-bp band (*kdp* operon) and the 381-bp amplimer (Table 3). This 381-bp amplimer results from amplification of the left junction between IS*431mec* and pUB110 found in SCC*mec* II. This pattern was called SCC*mec* II *–kdp* & pUB110 (Fig. 1B, lanes 4). Seven isolates produced a multiplex PCR pattern indistinguishable from the pattern of SCC*mec* IV (Table 3). This pattern could also be interpreted as a SCC*mec* II pattern missing the 284-bp (*kdp* operon), 209-bp (*mecI* gene), and 381-bp (pUB110) amplimers. This pattern was designated SCC*mec* II *–kdp*, *mecI* & pUB110 (Fig. 1B, lane 5).

Investigation of the SCC*mec* elements harbored by ST8 isolates with unusual SCC*mec* II and SCC*mec* IV multiplex patterns. Since all ST8 isolates (n = 74) exhibited variant multiplex patterns (Table 3), we decided to further investigate these SCC*mec* elements. Firstly, the entire SCC*mec* element from two ST8 isolates, AR 13.1/3330.2 and AR43/3330.1, with variant SCC*mec* II and SCC*mec* IV multiplex patterns, respectively, were sequenced. Isolate AR13.1/3330.2 was representative of isolates with SCC*mec* II by the simplex method but with SCC*mec* multiplex patterns lacking one to three of the amplimers typical of an SCC*mec* II element. AR 13.1/3330.2 exhibited multiplex pattern SCC*mec* II *–kdp*, *mecI* & pUB110 (i.e., it lacked all three bands, *kdp*, *mecI*, and pUB110). Isolate AR43/3330.1 was representative of isolates with SCC*mec* IV by the simplex method but with an SCC*mec* multiplex pattern lacking the 342-bp *dcs* amplimer (SCC*mec* IV *–dcs*). The genomic organizations of these two variant SCC*mec* elements based on their nucleotide sequence are shown schematically in Fig. 2. The genomic structures of the SCC*mec* elements of the remaining 72 ST8 isolates were also determined.

Novel variant of SCC*mec* II identified in AR13.1/3330.2. The SCC*mec* element in AR13.1/3330.2 consisted of a ~27-kb sequence containing a combination of regions previously identified in SCC*mec* II and IV and a novel *mec* complex (Fig. 2). Amplification of the L-C region yielded an amplimer of ~5.6 kb, which is similar in size to the expected amplimer for SCC*mec* IVb (Table 4). The nucleotide sequence of this 5.6-kb amplimer was found to have 99.7% similarity to the L-C region

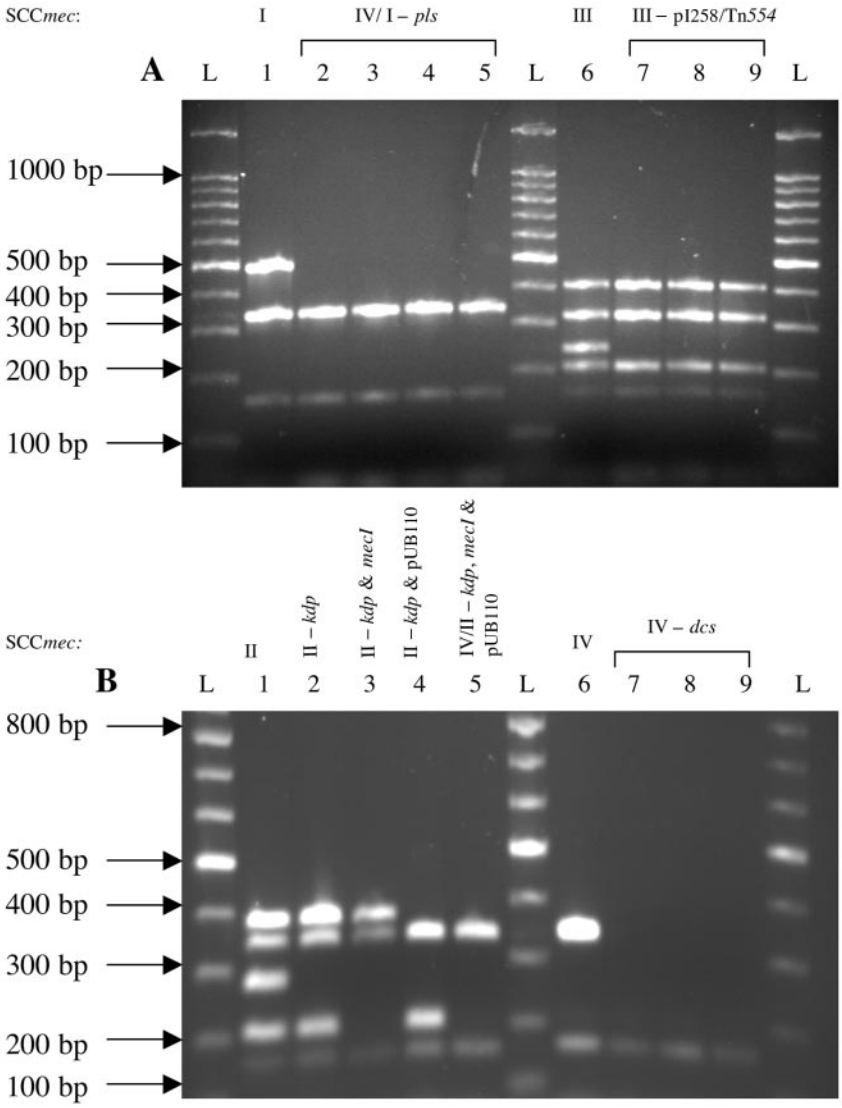

FIG. 1. SCC*mec* multiplex patterns and variant multiplex patterns found in Irish MRSA isolates. (A) Lane 1, SCC*mec* type I multiplex pattern; lanes 2 to 5, SCC*mec* IV multiplex pattern produced by AR02 isolates and six phenotype II isolates with SCC*mec* type I *ccr-mec* genes by the simplex method; lane 6, SCC*mec* type III multiplex pattern; lanes 7 to 9, the variant SCC*mec* III multiplex pattern (III –pI258/Tn*554*) characterized by the absence of the pI258/Tn*554* 243-bp amplimer, produced by AR09 and phenotype III isolates with SCC*mec* type III *ccr-mec* genes by the simplex method. (B) Lane 1, SCC*mec* type II multiplex pattern; lanes 2 to 5, the SCC*mec* multiplex patterns produced by isolates with phenotypes AR05, AR13, AR14, New01, and New03 with type II *ccr-mec* genes by the simplex method. These multiplex patterns appear to be related to the type II SCC*mec* multiplex pattern but lack the *kdp* amplimer (284 bp) (II –*kdp*, lane 2), or lack the *kdp* and *mecI* (209-bp) amplimers (II –*kdp* & *mecI*, lane 3), or lack the *kdp* and pUB110 (381-bp) amplimers (II –*kdp* & pUB110, lane 4), or lack the *kdp*, *mecI*, and pUB110 amplimers (II –*kdp*, *mecI* & pUB110, lane 5). This last pattern is the same as SCC*mec* IV. Lane 6, SCC*mec* type IV multiplex pattern; lanes 7 to 9, multiplex pattern produced by isolates with the AR43 phenotype (IV –*dcs*), which consists of the *mecA* control amplimer only. Lane L, 100-bp DNA ladders used as molecular size reference markers.

of SCC*mec* IVb and less than 10% similarity to the L-C regions of SCC*mec* types I, II, III, IVa, and IVc.

Analysis of the sequence of the *ccr* complex identified type 2 *ccr* genes (*ccrA2* and *ccrB2*) with closest similarity to those of SCC*mec* II and SCC*mec* IV elements (Table 5). Amplification and sequencing of the region between the *ccr* and *mec* complexes (C-M) in AR13.1/3330.2 using two primer pairs yielded a 7-kb and 6.5-kb product (Table 4) and confirmed the presence of a truncated integrated transposon, Tn*554* (Table 5). Five of the six ORFs (*tnpA*, *tnpB*, *tnpC*, *spc*, and *ermA*) previously identified within Tn*554* of SCC*mec* II were present in the SCC*mec* element of AR13.1/3330.2, and each ORF showed 100% homology at the amino acid level with those of SCC*mec* II (Table 5). However, Tn*554* of SCC*mec* of AR13.1/3330.2 was truncated by ~1,000 bp, resulting in the absence of an ORF of unknown function in Tn*554*. The six ORFs spanning a ~5-kb region encoding hypothetical proteins usually found between Tn*554* and the *mec* complex in SCC*mec* II were also absent (Fig. 2). Instead of these ORFs, a 1,865-bp IS*1182* sequence was present. A 253-bp truncated version of *mecI* (Δ*mecI*) was identified (*mecI* gene is usually 372 bp) (Fig. 2). Sequences of *mecR1* and *mecA* from AR13.1/3330.2 showed

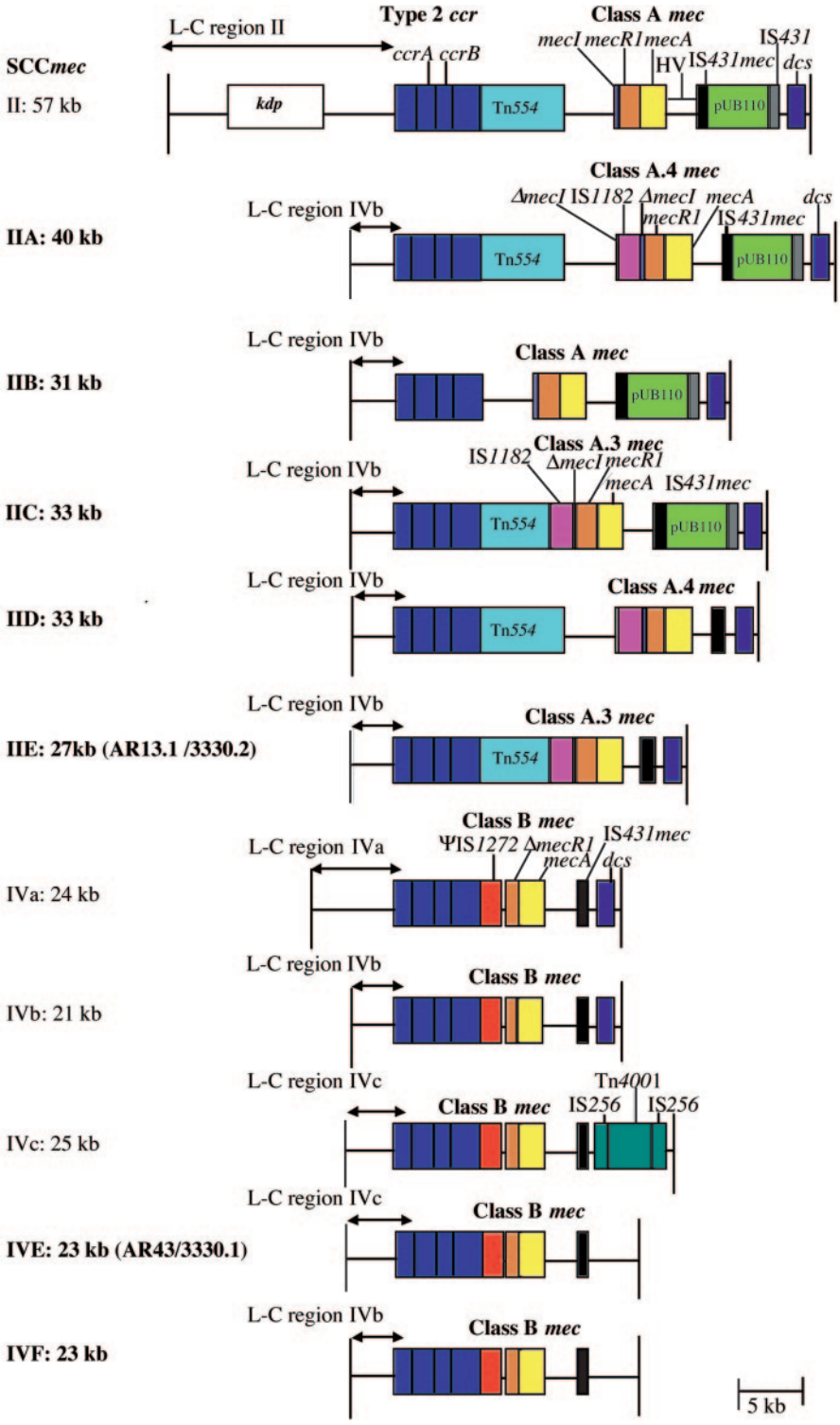

FIG. 2. Schematic diagram showing the genomic organization of SCC*mec* II, IIA, IIB, IIC, IID, IIE, IVa, IVb, IVc, IVE, and IVF elements. SCC*mec* IIA, IIB, IIC, IID, IIE, IVE, and IVF are novel variants that were identified in the present study. SCC*mec* II, IVa, IVb, and IVc were identified previously (19, 25), and the organization was determined based on the nucleotide sequences in the GenBank database under accession numbers D86934, AB063172, AB063173, and AB096217, respectively. The structure of SCC*mec* IIE and IVE were determined by sequencing of the entire SCC*mec* element of Irish MRSA isolates AR13.1/3330.2 (IIE; accession number AJ810120) and AR43/3330.1 (IVE; accession number AJ810121). The structures of SCC*mec* IIA, IIB, IIC, IID, and IVF were determined using the primers shown in Table 3 by observing the mobility of amplimers in agarose gels and in some cases by sequencing.

TABLE 4. Correlation between SCC*mec* multiplex types and amplimer sizes obtained using overlapping primer pairs to amplify the novel SCC*mec* element variants and comparison to those expected from typical SCC*mec* II and SCC*mec* IV elements

SCC*mec* multiplex type	SCC*mec* element	Amplimer size[a] (kb) with primer pair									Reference
		L-C, IRLII F-ccrA R	C-M, ccrA F-Tn*554* R	C-M, Tn*554* F-*mecR1* R	M-I, mecR1 F-ugpQ R	I-R, ugpQ F-dcs R	I-R, dcs F-orfX R	C-M, ccrA F-mecA R	M-I, mecA F-ISmec R	I-R, ISmec F-orfX R	
II	II	20	7	11	4.1	8.5	1.7	20	4.7	8	17
II − *kdp*	IIA	5.6	7	13*	4.1	8.5*	1.7	22	4.7	8	This study
II − *kdp*	IIB	5.6	7	No amplimer[c]	4.1	8.5	1.7	13	4.7	8	This study
II − *kdp* & *mecI*	IIC	5.6	7	6.5	4.1	8.5	1.7	14	4.7	8	This study
II − *kdp* & pUB110	IID	5.6	7	13	4.1	2.2	1.7	22	3.7	2.7	This study
II − *kdp*, *mecI* & pUB110	IIE[b]	5.6*	7*	6.5*	4.1*	2.2*	1.7*	14	3.7	2.7	This study
IV	IVa	8.9	No amplimer[d]	No amplimer[d]	4.1	3.2	1.7	8.5	4.7	2.7	25
IV	IVb	5.6	No amplimer[d]	No amplimer[d]	4.1	3.2	1.7	8.5	4.7	2.7	25
IV	IVc	6.1	No amplimer[d]	No amplimer[d]	4.1	No amplimer[e]	No amplimer[e]	8.5	4.7	5.3	19
IV − *dcs*	IVE[b]	6.1*	No amplimer[d]	No amplimer[d]	4.1	No amplimer[e]	No amplimer[e]	8.5*	4.7*	4.7*	This study
IV − *dcs*	IVF	5.6*	No amplimer[d]	No amplimer[d]	4.1	No amplimer[e]	No amplimer[e]	8.5	4.7	4.7*	This study

[a] Amplimer sizes for SCC*mec* IIA, IIB, IIC, IID, IIE, IVE, and IVF were calculated either by sequencing (indicated by *) or based on electrophoretic mobility in agarose gels. Amplimer sizes for SCC*mec* II, IVa, IVb, and IVc were calculated from sequences in the GenBank database, accession numbers D86934, AB063172, AB063173, and AB096217, respectively. All amplimer sizes are approximate. L-C, C-M, M-I, and I-R indicate the region of the SCC*mec* element amplified.
[b] SCC*mec* elements IIE and IVE were sequenced in full from isolates AR13.1/3330.2 and AR43/3330.1, respectively.
[c] No amplimer due to no Tn*554;* primers ccrA F-mecR1 R were used instead, resulting in an 11.5-kb amplimer, which was sequenced.
[d] No amplimer due to no Tn*554* in SCC*mec* IVa, IVb, IVc, IVE, and IVF.
[e] No amplimer due to no *dcs* region in SCC*mec* IVc, IVE, and IVF.

100% homology to sequences of other SCC*mec* elements (Table 5).

Amplification of the region across IS*431mec* to the *dcs* region downstream of the *mec* complex yielded a product of 2.2 kb, which is smaller than that of any previously described SCC*mec* element including SCC*mec* II and IVb (Table 4). Sequence data from this product showed that the hypervariable (HV) region (located between *mecA* and IS*431mec*) of AR 13.1/3330.2 is 997 bp shorter than that of SCC*mec* II and SCC*mec* IVb. The IS*431mec* region was 100% homologous to that of other SCC*mec* elements but lacked all DNA sequences associated with the integrated plasmid pUB110 or the second IS*431* sequence found in SCC*mec* II (Fig. 2).

The extreme right of the SCC*mec* element of AR13.1/3330.2 from IS*431* to the right chromosomal-SCC*mec* junction (I-R region) showed 100% homology with the same region of SCC*mec* II (Table 5). The 15-bp direct repeat sequence characteristic of SCC*mec* elements was identified at the right extremity of SCC*mec* (DR-R) and a 26-bp inverted repeat sequence (IR-R) that was identical to that of SCC*mec* IVb and SCC*mec* II was also found. All SCC*mec* elements identified to date have been found to be integrated at exactly the same nucleotide position of *orfX*. This ORF was identified outside the IR-R region, and SCC*mec* of AR13.1/3330.2 was integrated at the same nucleotide position in *orfX* as other SCC*mec* elements.

The novel genomic structure of the *mec* complex of the SCC*mec* element of AR13.1/3330.2 (IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec*) was more similar to the class A *mec* complex of MRSA (*mecI*-*mecR1*-*mecA*-IS*431mec*) found in SCC*mec* II and SCC*mec* III than to the class B *mec* complex (IS*1272*-Δ*mecR1*-*mecA*-IS*431m*ec) of SCC*mec* I and IV (Fig. 3). Due to its similarity to the class A *mec* complexes, this novel *mec* complex was designated class A.3 *mec* (Fig. 3).

Novel variant of SCC*mec* IV identified in AR43/3330.1. Isolate AR43/3330.1 carried a 23-kb SCC*mec* element, which is within the size range (20 to 25 kb) of previously described SCC*mec* IV elements, but sequence data indicated that it is distinctly different. The genomic structure of this SCC*mec* element showed most similarity to SCC*mec* IVc (Fig. 2). Amplification of the L-C region of AR43/3330.1 yielded an amplimer of ca 6.1 kb (Table 4), and sequencing revealed that it had 100% similarity with the L-C region of SCC*mec* IVc (Table 6). Amplification of the region from the *ccr* complex to IS*431mec* yielded amplimers that showed almost 100% sequence identity with the same regions of SCC*mec* IVc (Table 6). A class B *mec* complex was identified (Fig. 2), and an I-R region different from any previously described SCC*mec* element was determined. An amplimer of 4.7 kb was obtained following amplification of this region (Table 4). Sequence analysis identified IS*431mec*, but a ~4.4-kb region was identified between it and the end of *orfX*. Searches of the GenBank database showed that ~1.2 kb of this sequence had 100% homology to a sequence of the right extremity of the SCC*mec* element of two other MRSA isolates (15). This sequence in the GenBank database consists of *orfX* and the extreme right region of the SCC*mec* element, including the direct repeat (DR-R) and inverted repeat (IR-R) regions. The rest of the sequence between IS*431mec* and IR-R identified in AR43/3330.1 was found to have no homology to any sequences in the GenBank database.

Determination of the genomic organization of the SCC*mec* elements of the remaining ST8 isolates with unusual SCC*mec* II and SCC*mec* IV multiplex patterns. In total, 74 isolates had the ST8 genotype and gave variant SCC*mec* II and IV multiplex patterns (Table 3). In addition to determining the nucleotide structure of the SCC*mec* elements of two of these isolates as described above, the SCC*mec* elements of the remaining 72 isolates were characterized by analyzing the mobility of amplimers on agarose gels obtained with the overlapping primers used to amplify and sequence the SCC*mec* from isolates AR13.1/3330.2 and AR43/3330.1. In some cases, sequencing was performed on amplimers that differed in size from that expected from previously described SCC*mec* elements. These results are summarized in Table 3 and Table 4. This analysis identified five variant SCC*mec* elements, in addition to the two identified above by complete nucleotide sequencing. Of the

TABLE 5. Identities between ORFs of the novel SCC*mec* II variant element of Irish MRSA isolate AR13.1/3330.2 and the conventional SCC*mec* IVb and II elements

SCC*mec* element and ORF	Location[a]	Homology to ORFs of SCC*mec* IVb[b]			Homology to ORFs of SCC*mec* II[b]		
		ORF	% Homology[c]	Gene product	ORF	% Homology[c]	Gene product
IIE01	210–1145 (complement)	CM001	83.7	Hypothetical protein	—	—	—
IIE02	1123–1893 (complement)	CM002	100	Hypothetical protein	—	—	—
IIE03	2010–3440	M001	99.6	Hypothetical protein	—	—	—
IIE04	3560–5350	M002	99.7	Conserved hypothetical protein	N031	80.3	Conserved hypothetical protein
IIE05 (*ccr*A2)	5584–6933	M004	99.1	Cassette chromosome recombinase A2	N034	96.7	Cassette chromosome recombinase A2
IIE06 (*ccr*B2)	6955–8583	M005	97.8	Cassette chromosome recombinase B2	N037	98	Cassette chromosome recombinase B2
IIE07	9101–9451	M006	88.9	Conserved hypothetical protein	N041	100	Conserved hypothetical protein
IIE08	9538–9849	M007	89.4	Conserved hypothetical protein	N042	100	Conserved hypothetical protein
IIE09	9867–10373	M008	91.7	Conserved hypothetical protein	N043	99.4	Conserved hypothetical protein
IIE10	10394–10711	—[d]	—	—	N044	100	Pseudogene
IIE11 (*tnpA*)	10830–11915	—	—	—	N045	100	Transposase A
IIE12 (*tnpB*)	11912–13804	—	—	—	N046	100	Transposase B
IIE13 (*tnpC*)	13811–14188	—	—	—	N047	100	Transposase C
IIE14 (*spc*)	14339–15121	—	—	—	N048	100	*O*-Nucleotidyltransferase
IIE15 (*ermA*)	15247–15978 (complement)	—	—	—	CN026	100	rRNA adenine *N*-6-methyltransferase
IIE16 (*tnp* IS*1182*)	16499–18103	—	—	—	—	—	—
IIE17 (Δ*mecI*)	18207–18459 (complement)	—	—	—	CN036	67.7	Methicillin resistance protein MecI
IIE18 (*mecR1*)	18459–20216 (complement)	CM003	55.6	Truncated signal transducer protein MecR1	CN037	100	Signal transducer protein MecRI
IIE19 (*mecA*)	20316–22322	M010	100	Penicillin binding protein 2a	N058	100	Penicillin binding protein 2a
IIE20	22368–22796 (complement)	CM004	100	Conserved hypothetical protein	CN038	100	Conserved hypothetical protein
IIE21 (*ugpQ*)	22893–23636	CM006	100	Glycerophosphoryldiester phosphodiesterase	N039	100	Glycerophosphoryldiester phosphodiesterase
IIE22 (*tnp* IS*431mec*)	23901–24575	M011	100	Transposase for IS*431mec*	N062	100	Transposase for IS*431mec*
IIE23	24607–24846 (complement)	CM007	100	Conserved hypothetical protein	CN049	100	Conserved hypothetical protein
IIE24	25261–26556 (complement)	CM008	40.7	Truncated conserved hypothetical protein	CN050	100	Truncated conserved hypothetical protein

[a] The nucleotide positions were determined based on the sequences for SCC*mec* IIE deposited in the GenBank database under accession number AJ8101208.
[b] ORFs and gene products from SCC*mec* II and SCC*mec* IVb, based on nucleotide sequences in the GenBank database, accession number D86934 and AB063173, respectively (17, 25).
[c] Based on amino acid identity.
[d] —, no significant homology.

seven variants recognized, five SCC*mec* II variants (designated SCC*mec* IIA, IIB, IIC, IID, and IIE) were identified among the 54 isolates with variant SCC*mec* II multiplex patterns, and two novel SCC*mec* IV variants (designated SCC*mec* IVE and IVF) were found among the 20 isolates with variant SCC*mec* IV multiplex patterns (Table 3). A schematic representation of all seven SCC*mec* variants is shown in Fig. 2.

Additional novel variants of SCC*mec* II. Significant size variation was observed in the mobility of amplimers obtained from variant SCC*mec* II elements compared to the sizes expected from isolates with typical SCC*mec* II when the following three regions were amplified: the L-C region, Tn*554* to the *mec* complex (part of C-M region), and across pUB110 (part of I-R region) (Table 4). The 54 SCC*mec* II variants yielded the 5.6-kb fragment characteristic of the L-C region of SCC*mec* IVb (Table 4; Fig. 2). No amplimer obtained following amplification of the region from Tn*554* to *mecR1* in the SCC*mec* II variants matched the size expected from a typical SCC*mec* II element (Table 4). All but one isolate with the multiplex pattern SCC*mec* II *–kdp* ($n = 9$) and all isolates with the multiplex pattern SCC*mec* II *–kdp* & pUB110 ($n = 27$) produced amplimers of ca. 13 kb. This 13-kb region was sequenced from one isolate, and it was found that IS*1182* was inserted within the *mecI* gene, near the 3′ end, and that *mecI* carried a 16-bp deletion (Fig. 3). This new *mec* class A complex consisting of Δ*mecI*-IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec* was designated *mec* class A.4 (Fig. 3).

The Tn*554*-*mecR1* region of one isolate with the multiplex pattern SCC*mec* II *–kdp* could not be amplified with the same primers used on the other SCC*mec* II variants. Instead, a primer pair was used to amplify the DNA region from the *ccr* complex to *mecR1*, resulting in a ca. 11.5-kb amplimer (Table 4). Sequencing data revealed that this isolate retained only a few bases of the transposon Tn*554* normally found in SCC*mec* II and that all Tn*554* ORFs were absent. However, the six ORFs usually found between Tn*554* and the *mec* complex of SCC*mec* II were all present. This isolate also harbored a class A *mec* complex. Amplification of the same region in isolates with the multiplex pattern SCC*mec* II *–kdp* & *mecI* ($n = 10$) and SCC*mec* II *–kdp*, *mecI* & pUB110/IV ($n = 7$) yielded an amplimer of the same size as the amplimer obtained and sequenced from isolate AR13.1/3330.2 (Table 4) in which the region between Tn*554* and the *mec* complex was absent but which carried a *mec* class A.3 *mec* complex. All isolates with

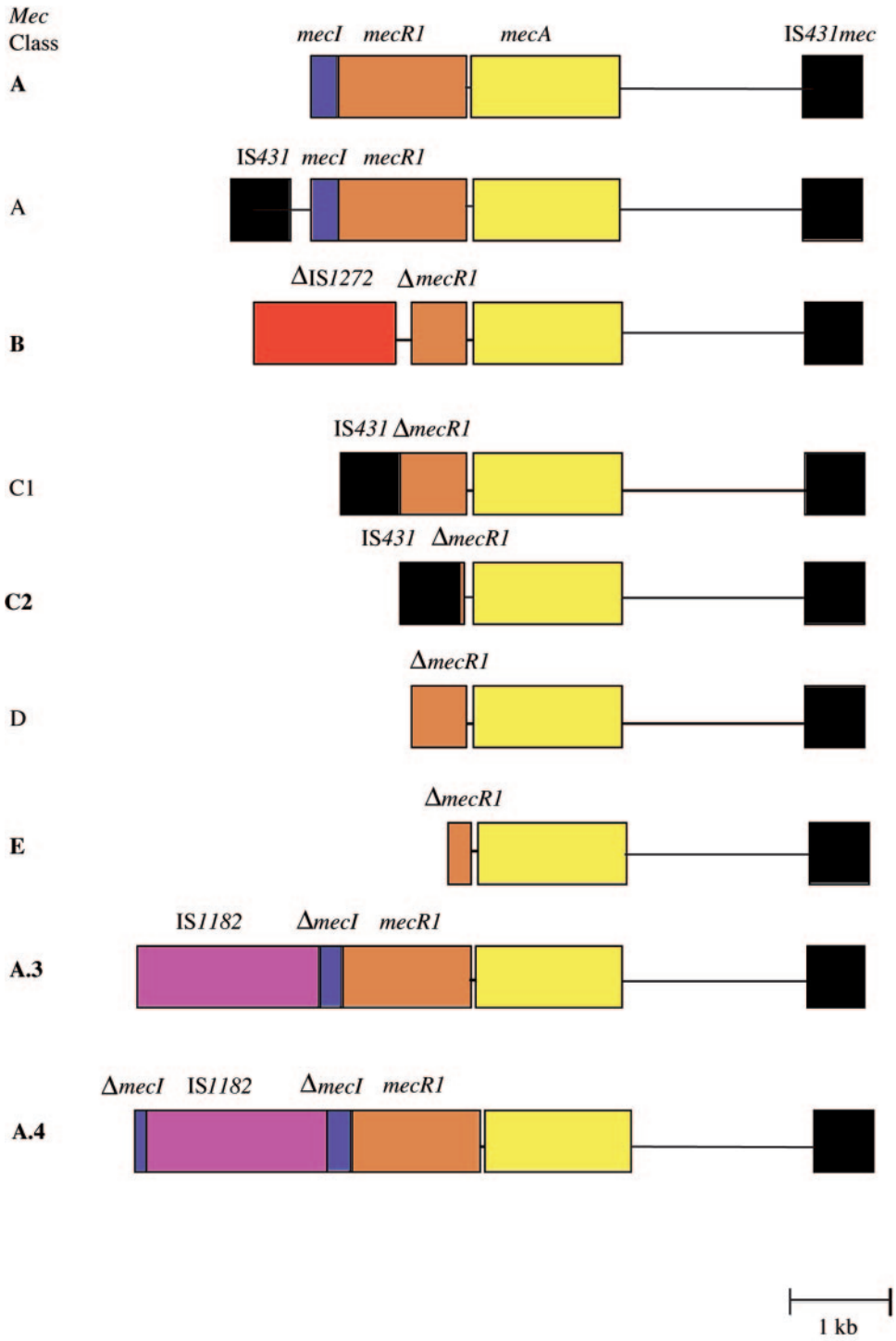

FIG. 3. Schematic diagram of the genetic organization of the *mec* complexes of staphylococci. The illustrations of *mec* complexes A, B, C1, C2, and D are based on studies by Katayama and coworkers (22), and the illustration of *mec* complex E is based on studies by Lim and coworkers (22, 24). The *mec* classes A, B, C2, and E indicated in bold have been previously identified in MRSA (18, 22, 24). The novel *mec* classes A.3 and A.4 were identified in the present study of Irish MRSA isolates. Class A.3 *mec* differs from class A *mec* in that it has a copy of *mecI* that is truncated at the 3′ end and an upstream copy of IS*1182* (IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec*). Δ*mecI* is only 253 bp, whereas intact *mecI* is 372 bp. In class A.4, *mec* IS*1182* is present within *mecI* and there is a 16-bp deletion of *mecI* immediately upstream of IS*1182* (Δ*mecI*-IS*1182*-Δ*mecI*-*mecR1*-*mecA*-IS*431mec*).

the SCC*mec* II –*kdp* (n = 10) and SCC*mec* II –*kdp* & *mecI* (n = 10) harbored the integrated plasmid pUB110 and the second copy of IS*431* found in SCC*mec*. Isolates with multiplex patterns SCC*mec* II –*kdp* & pUB110 (n = 27) and SCC*mec* II –*kdp*, *mecI* & pUB110/IV (n = 7) gave the same size amplimer as that obtained from isolate AR13.1/3330.2 for amplification of the region across pUB110 (Table 4), indicating the absence of pUB110 and the second IS*431* sequence.

TABLE 6. Identities between ORFs of the novel SCC*mec* IV variant element IVE of Irish MRSA isolate AR43/3330.1 and the conventional SCC*mec* IVc element

SCC*mec* element and ORF	Location[a]	Homology to ORFs of SCC*mec* IVc[b]		
		ORF	% Homology[c]	Gene product
IVE01	66–866 (complement)	CR007	100	Hypothetical protein
IVE02	896–2050 (complement)	CR008	100	Hypothetical protein
IVE03	2505–3551	R002	100	Hypothetical protein
IVE04	3744–4040	R003	100	Hypothetical protein
IVE05	4040–5833	R004	100	Hypothetical protein
IVE06 (*ccrA2*)	6067–7416	R005	99.1	Cassette chromosome recombinase A2
IVE07 (*ccrB2*)	7438–9051	R006	99.4	Cassette chromosome recombinase B2
IVE08	9573–9923	R007	100	Hypothetical protein
IVE09	10010–10321	R008	100	Hypothetical protein
IVE10	10333–10842	R009	100	Hypothetical protein
IVE11	10978–12159	R010	100	Hypothetical protein
IVE12	12146–12502	R011	100	Hypothetical protein
IVE13	12493–12822 (complement)	CR009	100	Putative transposase of IS*1272*
IVE14	12725–13711 (complement)	CR010 (Δ*mecR1*)	100	Truncated signal transducer protein MecR1
IVE15	13811–15817	R012 (*mecA*)	100	Penicillin binding protein 2a
IVE16	16388–17131 (complement)	CR011	100	Hypothetical protein
IVE17	18432–19106	R013	100	Transposase of IS*431mec*
IVE18	20286–20792	—[d]	—	Hypothetical protein
IVE19	20995–21576	—	—	Hypothetical protein
IVE20	22117–22590	—	—	Hypothetical protein

[a] The nucleotide positions were determined based on the sequences for SCC*mec* IVE deposited in the GenBank database under accession number AJ810121.
[b] ORFs and gene products from SCC*mec* IVc nucleotide sequence in the GenBank database, accession number AB096217 (19).
[c] Based on amino acid identity.
[d] —, no homology.

Additional novel variants of SCC*mec* IV. All 20 isolates with the AR43 phenotype which were found to carry SCC*mec* IV by the simplex method but gave the multiplex pattern SCC*mec* IV *–dcs* produced the expected size products for a type IVc SCC*mec* element when the primers used with isolate AR43/3330.1 were used, but differences were observed following amplification of the L-C and I-R regions (Table 4). Seventeen of the isolates had the same L-C region as SCC*mec* IVc (6.1-kb amplimer), but three isolates yielded a 5.6-kb product (Table 4). Sequencing of this amplimer confirmed it contained the SCC*mec* IVb L-C region. As with isolate AR43/3330.1, an amplimer that was larger than expected was obtained when the I-R region was amplified in all AR43 isolates (Table 4). This 4.7-kb region from an AR43 isolate with an L-C region similar to SCC*mec* IVb was sequenced and was 100% identical to that of isolate AR43/3330.1.

DISCUSSION

MLST and SCC*mec* element analysis of MRSA isolates recovered in Irish hospitals between 1971 and 2002 showed that clones representative of each of the five major clonal complexes and eight of the major pandemic lineages have been present in Ireland at some time over the past 30 years (Tables 1 and 3). This study also confirmed that there have been major changes in the dominant clonal types. The genotypes of the earliest MRSA during the 1970s and early 1980s were ST250-MRSA-I or its novel variant, ST250-MRSA-I *–pls*, followed in the mid- to late 1980s by a new variant, ST239-MRSA-III –pI258 & Tn*554*. In 1989, ST239-MRSA-III predominated, but during the 1990s this was displaced by ST8-MRSA-II, exhibiting six of the nine novel SCC*mec* variants identified in this study. In 2002, the dominant clone was ST22-MRSA-IV.

Previous studies have proposed that MRSA clones should be defined on the basis of their genetic background as identified by MLST and their SCC*mec* type, but a striking finding of the present study was the extent of variation within SCC*mec*. If clones among the Irish MRSA population are defined on the basis of all SCC*mec* variants recognized, the population includes 20 clonal types consisting of 10 previously recognized clonal types, 9 with previously described genetic backgrounds but with novel SCC*mec* variants (including two new variants of class A *mec*) and one with a previously unreported genetic background (a double locus variant of ST5 in association with SCC*mec* II) (Table 3). The greatest variation was seen among isolates with the ST8 genotype. For example, among the 54 isolates exhibiting SCC*mec* IV by the simplex method, only those with the ST8 genotype (20/54) had variant SCC*mec* elements. In fact, 86% of isolates (74/86) with novel SCC*mec* variants had the ST8 genotype, and all carried previously unreported variants of SCC*mec* II (IIA to IIE) or IV (IVE and IVF).

Because both SCC*mec* I and IV have a *mec* complex with IS*1272* inserted at the same junction point, it has been suggested that recombination has occurred between SCC*mec* I and other sequences to generate SCC*mec* type IV (25). The novel ST8 SCC*mec* variants identified in this study resemble rearrangements of SCC*mec* II and SCC*mec* IV. SCC*mec* variants IIA to IIE have an L-C region that is almost identical to that of SCC*mec* IVb, but the rest of each element has closest identity with SCC*mec* II, including a very similar genomic structure (Fig. 2).

SCC*mec* IIA to IIE appear to be closely related. SCC*mec* IIB was found in isolates from 1989, making it the earliest SCC*mec* II variant identified in this study. It harbors the original class A *mec* complex, but its similarity to SCC*mec* IVb in the L-C region make its provenance unclear. It is likely that SCC*mec* IIB was generated following loss of most of Tn*554*,

because it retains only ~40 bp of this element. Acquisition of IS*1182* by a class A *mec* complex may have given rise to the novel variants of class A *mec*, A.3 and A.4, seen in SCC*mec* IIC and IIE and SCC*mec* IIA and IID, respectively (Fig. 2 and 3). Since SCC*mec* IIC and IIA were present in isolates in 1993 and SCC*mec* IIE and IID were not found in isolates until 1998, it is likely that the latter derived from the former following loss of pUB110.

The presence of IS*1182* within *mecI*, although a novel finding, is not surprising. Mutations and deletions in *mecI* are common in MRSA (22), sometimes in association with insertion sequences. Class B and class C *mec* complexes have *mec*I and part of *mec*R1 deleted and insertions of IS*1272* or IS*431* flanking the truncated end of *mec*R1 (22). IS*1182* has been identified in *S. aureus* flanking the staphylococcal composite transposon Tn*5405*, which encodes aminoglycoside resistance determinants (6). Immediately adjacent to the insertion site of IS*1182* at the 3′ end of *mec*I in the class A.4 *mec* complex, a 16-bp deletion in *mecI* gene has occurred with no flanking target site duplications. Insertion sequences capable of promoting various types of genome rearrangements, including deletions, have been reported previously (26).

Other workers have reported possible rearrangements in the L-C regions of other SCC*mec* elements. Based on a multiplex SCC*mec* typing method, Wielders et al. (47) reported a SCC*mec* I element carrying the *kdp* region (normally part of L-C region of SCC*mec* II only) and also an SCC*mec* IV element with *pls* (normally part of L-C region of SCC*mec* I only) (47). Various different SCC*mec* IV elements have been described with diverse L-C regions (19, 25). The integrated plasmid pUB110 normally found in SCC*mec* II encodes resistance to kanamycin, tobramycin, and bleomycin (17). In the present study all isolates with SCC*mec* IID and IIE were resistant to kanamycin and tobramycin (42–44), although they lacked pUB110 in their SCC*mec* elements, indicating that resistance determinants for these aminoglycosides were present elsewhere on the genome and that pUB110 may have been lost. Loss or gain of plasmids such as pUB110 in SCC*mec* has been documented previously; SCC*mec* IA is believed to have evolved from SCC*mec* I by acquisition of pUB110 or vice versa (32). SCC*mec* IVA has been shown to harbor pUB110 (31), and Wielders et al. (47) described pUB110 associated with SCC*mec* III and SCC*mec* IV (47).

SCC*mec* IIA to IIE (40 to 27 kb) are smaller than previously described SCC*mec* II elements (57 kb), and SCC*mec* IIE, with a size of 27 kb, is more similar in size to SCC*mec* IV (20 to 25 kb) or SCC*mec* V (28 kb) (Fig. 2). It has been suggested that due to their relatively small size, SCC*mec* IV and SCC*mec* V are highly competitive mobile genetic elements that are more readily transferred between staphylococci than the larger SCC*mec* elements and, once acquired by the host, do not compromise its fitness (18, 25). This could also be true for the SCC*mec* II variants described in the present study, where the smaller size resulted from loss of regions that are not required for maintenance of methicillin resistance or precise excision and integration of SCC*mec*.

Like SCC*mec* IIA to IIE, the origin of the two ST8-MRSA-IV variants IVE and IVF identified here is unclear. SCC*mec* IVE is almost identical to SCC*mec* IVc, except in the region downstream of the *mec* complex, suggesting that SCC*mec* IVE may have arisen by recombination between an SCC*mec* IVc element and another SCC*mec* sequence. SCC*mec* IVF has the same L-C region as the ST8 SCC*mec* II variants, but the rest of the element is similar to SCC*mec* IVE (Fig. 2). SCC*mec* IVF may have evolved by recombination between a SCC*mec* II variant and SCC*mec* IVE, which donated the rest of the element including the unusual downstream region. Alternatively, SCC*mec* IVF may have evolved by recombination between SCC*mec* IVb and SCC*mec* IVE. Recombination events in the *dcs* region appear to be common, because other researchers have reported the absence of *dcs* amplimers following multiplex PCR of SCC*mec* elements in various genetic backgrounds (ST8 and ST30) (2, 36). In one case, a *dcs* amplimer was reported in SCC*mec* III, although it does not usually carry the *dcs* sequence (47).

Two other SCC*mec* variants were identified in the present study. ST250-MRSA-I –*pls* appears to be a variant of the archaic clone, ST250-MRSA-I. The *pls* gene of SCC*mec* I is located within the L-C region, which is not thought to be an important part of SCC*mec* (16). The variant ST239-MRSA-III –pI258 & Tn*554* may have evolved from ST239-MRSA-III by loss of the integrated plasmid pI258 or transposon Tn*554*, or both. Further work is under way to characterize these novel variants of SCC*mec* I and SCC*mec* III.

The novel variants identified in the present study yielded multiplex PCR patterns similar to those reported in other studies, with the exception of the patterns designated in this study as SCC*mec* II –*kdp* & pUB110 and SCC*mec* III –pI258 & Tn554 (1, 2, 36, 46). However, this is the only study to date that has fully characterized the SCC*mec* elements with some of these unusual SCC*mec* multiplex patterns. Three SCC*mec* variants have been identified previously in the ST8 genetic background: SCC*mec* II –*kdp* and SCC*mec* II –*kdp*, *mecI* & pUB110 were identified in two isolates described as Irish-1 from the United Kingdom and one AR14 phenotype from Ireland, and SCC*mec* IV –*dcs* was determined in three isolates (two phenotype AR43 from Ireland and one isolate from Australia) (36). Some of the isolates investigated in that study came from the Irish MRSA collections used in the present study. The similarity between isolates with phenotype AR43 (carrying SCC*mec* IVE or IVF) and isolates described as EMRSA-Irish 2 in Australia has been noted previously (21, 27). Irish isolates with phenotypes AR13 and AR14 resemble United Kingdom isolates described as Irish-1 (44). Due to the large number of these ST8 variants identified in this present study among Irish isolates, it is possible that these ST8 variant clones evolved in Ireland and spread to the United Kingdom and Australia.

Some of the multiplex patterns reported in the present study have been identified previously in distinctly different genetic backgrounds, including the multiplex pattern SCC*mec* II –*kdp* & *mec*I in two Japanese isolates with ST5 and ST89 genotypes (2), the SCC*mec* IV –*dcs* multiplex pattern in ST30 isolates from Greece (1), and the SCC*mec*I –*pls* multiplex pattern from one ST1 isolate from the United States (46). These patterns may occur if the variant SCC*mec* elements were acquired by isolates with different genetic backgrounds. Alternatively, the similar multiplex patterns in genotypes other than ST8 may be due to different mutations, deletions, or insertions in these highly variable regions. The fact that the multiplex patterns all

lack the same amplimer does not necessarily mean that the same SCC*mec* element is present. Further investigation into these SCC*mec* elements is required.

It has been recommended that the multiplex SCC*mec* typing method should be used with MLST for routine epidemiological typing of MRSA (10). The present study has shown that if the multiplex method had been used alone as the sole SCC*mec* typing method, half the isolates investigated would have been misclassified as harboring an erroneous SCC*mec* element or would have yielded SCC*mec* multiplex patterns that could not be interpreted as belonging to any of the currently described SCC*mec* types. Combining results of the multiplex and simplex SCC*mec* typing methods allowed correct classification of some of the unusual multiplex patterns. However, neither the simplex or multiplex methods recognized a number of SCC*mec* elements recently described in other studies, including SCC*mec* V and the SCC*mec* IV variants SCC*mec* IVa, IVb, IVc, or IVd (18, 19, 25). PCR amplification and sequencing of the entire SCC*mec* element was necessary for the complete characterization of the SCC*mec* elements harbored by isolates with ambiguous multiplex patterns. Such detailed investigations are unsuitable for routine typing purposes. Furthermore, if SCC*mec* is to be used for routine epidemiological typing, some method of standardizing nomenclature for the plethora of new and variant SCC*mec* types is needed. Equally important is the question of the epidemiological significance of variant SCC*mec* elements. As SCC*mec* typing is applied to larger collections of isolates, the epidemiological significance of these variants may become apparent. However, it is clear from this and other studies that SCC*mec* can evolve rapidly, and simple PCR methods for characterizing SCC*mec* elements may need to be updated regularly to include important new variants.

All MRSA clonal types identified in this present study belong to one of the five major lineages of nosocomial MRSA, with the exception of the previously described ST12-MRSA-IV, which is a relatively minor MRSA clone. Within CC5, the two major pandemic clones, ST5-MRSA-II (New York/Japan clone) and ST5-MRSA-IV (Pediatric clone), were identified among Irish MRSA isolates (Table 3). A novel genotype, ST496, was identified in one Irish isolate from 2002. This genotype differs from ST5 by single nucleotide substitutions in both the *arcC* and *yqiL* alleles and most likely arose from ST5 by point mutation. ST5-MRSA-II has been a common clonal type among Irish MRSA isolates since 1992 to 1993 but never predominated.

It is interesting that over the years in Ireland, some MRSA clones have spread and predominated but others that cause major problems in other countries have not dominated following their introduction into Ireland. For example, ST247-MRSA-IA has been identified as the dominant MRSA clone in many hospitals in Europe, especially in Spain and Portugal (7, 30), but never predominated in Irish hospitals. Similarly, some strains of ST239-MRSA-III, such as phenotype AR01, caused major problems in Irish hospitals but other isolates did not spread (e.g., ST239-MRSA-III, phenotype AR23) or were contained within a particular unit within one hospital (e.g., ST239-MRSA-III, phenotype AR44) (38, 40). In contrast, the prevalence of ST22-MRSA-IV (phenotype AR06) has increased and, by 2002, had become the predominant clone among Irish MRSA blood culture isolates investigated under the EARSS project. Interestingly, ST22-MRSA-IV is the most prevalent epidemic strain in the United Kingdom, where it is often referred to as EMRSA-15 (28).

In conclusion, this analysis of MLST and SCC*mec* elements has provided data fundamental to understanding the epidemiology and evolutionary history of MRSA in Ireland. It has revealed a hitherto-unsuspected degree of diversity within SCC*mec* and has raised many questions which need to be addressed.

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REFERENCES

1. **Aires de Sousa, M., C. Bartzavali, I. Spiliopoulou, I. S. Sanches, M. I. Crisostomo, and H. de Lencastre.** 2003. Two international methicillin-resistant *Staphylococcus aureus* clones endemic in a university hospital in Patras, Greece. J. Clin. Microbiol. **41:**2027–2032.
2. **Aires de Sousa, M., and H. de Lencastre.** 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J. Clin. Microbiol. **41:** 3806–3815.
3. **Anonymous.** 1999. North/South study of MRSA in Ireland 1999. Irish Department of Health and Children. [Online.] http://www.dohc.ie/publications/study_of_mrsa_in_ireland_1999.html.
4. **Carroll, J. D., H. M. Pomeroy, R. J. Russell, J. P. Arbuthnott, C. T. Keane, O. M. McCormick, and D. C. Coleman.** 1989. A new methicillin- and gentamicin-resistant *Staphylococcus aureus* in Dublin: molecular genetic analysis. J. Med. Microbiol. **28:**15–23.
5. **Coleman, D. C., H. Pomeroy, J. K. Estridge, C. T. Keane, M. T. Cafferkey, R. Hone, and T. J. Foster.** 1985. Susceptibility to antimicrobial agents and analysis of plasmids in gentamicin- and methicillin-resistant *Staphylococcus aureus* from Dublin hospitals. J. Med. Microbiol. **20:**157–167.
6. **Debrise, A., K. G. Dyke, and N. El Solh.** 1996. Characterisation of a *Staphylococcus aureus* transposon, Tn*5405*, located within Tn*5404* and carrying the aminoglycoside resistance genes, *aphA-3* and *aadE*. Plasmid **35:**174–188.
7. **Dominguez, M. A., H. de Lencastre, J. Linares, and A. Tomasz.** 1994. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. J. Clin. Microbiol. **32:**2081–2087.
8. **EARSS.** 2002. EARSS annual report 2002. [Online.] http://www.ndsc.ie/Publications/AntimicrobialResistance-EARSSReports/.
9. **Enright, M. C., P. J. Day, C. E. Davies, S. J. Peacock, and B. G. Peacock.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
10. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methcillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
11. **Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:** 3307–3316.
12. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:**513–516.
13. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene **73:**237–244.
14. **Hone, R., and C. T. Keane.** 1974. Characteristics of methicillin resistant *Staphylococcus aureus*. Ir. J. Med. Sci. **143:**145–154.
15. **Huletsky, A., R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. **42:** 1875–1884.
16. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of the three types of staph-

ylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:** 1323–1336.
17. **Ito, T., Y. Katayama, and K. Hiramatsu.** 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. **43:**1449–1458.
18. **Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu.** 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. **48:**2637–2651.
19. **Ito, T., K. Okuma, X. X. Ma, H. Yuzawa, and K. Hiramatsu.** 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist. Update **6:**41–52.
20. **Jevons, M. P.** 1961. Celbenin-resistant staphylococci. Br. Med. J. **i:**124–125.
21. **Johnson, H., Z. Johnson, M. Burd, D. Doyle, G. Glynn, H. Humphreys, P. McDonald, B. McDonnell, E. Mitchell, and A. Rossney.** 2000. 9th Int. Symp. Staphylococci Staphylococcal Infect., abstract no. 182.
22. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2001. Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS*431*-mediated mecI deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus*. Antimicrob. Agents Chemother. **45:**1955–1963.
23. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2000. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **44:**1549–1555.
24. **Lim, T. T., F. N. Chong, F. G. O'Brien, and W. B. Grubb.** 2003. Are all community methicillin-resistant *Staphylococcus aureus* related? A comparison of their *mec* regions. Pathology **35:**336–343.
25. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:** 1147–1152.
26. **Mahillon, J., C. Leonard, and M. Chandler.** 1999. IS elements as constituents of bacterial genomes. Res. Microbiol. **150:**675–687.
27. **McGechie, D., and D. Daley.** 2000. 9th Int. Symp. Staphylococci Staphylococcal Infect., abstract no. 13.
28. **Moore, P. C., and J. A. Lindsay.** 2002. Molecular characterisation of the dominant UK methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16. J. Med. Microbiol. **51:**516–521.
29. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, and K. Hiramatsu.** 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. **40:**4289–4294.
30. **Oliveira, D., I. Santos-Sanches, R. Mato, M. Tamayo, G. Ribeiro, D. Costa, and H. de Lencastre.** 1998. Virtually all methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the largest Portuguese teaching hospital are caused by two internationally spread multiresistant strains: the "Iberian" and the "Brazilian" clones of MRSA. Clin. Microbiol. Infect. **4:**373–384.
31. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
32. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
33. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. Lancet Infect. Dis. **2:**180–189.
34. **Reference deleted.**
35. **Reynolds, P. E., and D. G. J. Brown.** 1985. Penicillin-binding proteins of beta-lactam resistant strains of *Staphylococcus aureus*: effect of growth conditions. FEBS Lett. **192:**28–32.
36. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.
37. **Robinson, D. A., and M. C. Enright.** 2004. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. **10:**92–97.
38. **Rossney, A.** 2003. A new strain of MRSA imported from Singapore. Epi-Insight **4:**1.
39. **Rossney, A. S.** 1995. Methicillin-resistant *Staphylococcus aureus* in four Dublin hospitals (1988–1994). University of Dublin, Trinity College, Dublin, Ireland.
40. **Rossney, A. S., D. C. Coleman, and C. T. Keane.** 1994. Evaluation of an antibiogram-resistogram typing scheme for methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **41:**441–447.
41. **Rossney, A. S., D. C. Coleman, and C. T. Keane.** 1994. Antibiogram-resistogram typing scheme for methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **41:**430–440.
42. **Rossney, A. S., and C. T. Keane.** 2002. Strain variation in the MRSA population over a 10-year period in one Dublin hospital. Eur. J. Clin. Microbiol. Infect. Dis. **21:**123–126.
43. **Rossney, A. S., M. J. Lawrence, P. M. Morgan, M. M. Fitzgibbon, and B. O' Connell.** 2004. 11th Int. Symp. Staphylococci Staphylococcal Infect., abstract no. ME24.
44. **Rossney, A. S., P. McDonald, H. Humphreys, G. M. Glynn, and C. T. Keane.** 2003. Antimicrobial resistance and epidemiological typing of methicillin-resistant *Staphylococcus aureus* in Ireland (North and South), 1999. Eur. J. Clin. Microbiol. Infect. Dis. **22:**379–381.
45. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
46. **Shukla, S. K., S. V. Ramaswamy, J. Conradt, M. E. Stemper, R. Reich, K. D. Reed, and E. A. Graviss.** 2004. Novel polymorphisms in *mec* genes and a new *mec* complex type in methicillin-resistant *Staphylococcus aureus* isolates obtained in rural Wisconsin. Antimicrob. Agents Chemother. **48:**3080–3085.
47. **Wielders, C., A. Box, J. Verhoef, and A. Fluit.** 2003. Abstr. 103rd Gen. Meet. Am. Soc. Microbiol. 2003, abstr. A-068.