

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, Ireland¹; National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland²; and Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom³

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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 amplicon 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*-IS1182- Δ *mecI*-*mecR1*-*mecA*-IS431*mec*); SCC*mec* IIC and IIE had a novel *mec* complex, A.3 (IS1182- Δ *mecI*-*mecR1*-*mecA*-IS431*mec*); SCC*mec* IID and IIE lacked pUB110; SCC*mec* IIC and IIE lacked a region of DNA between Tn554 and the *mec* complex; and SCC*mec* IIB lacked Tn554. 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_{sc}*) at the 3' end of an open reading frame (ORF) of unknown function, designated *orfX* (17).

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 IS431*mec* 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 IS431 elements; and SCC*mec* IIIB, which lacks integrated copies of Tn554, 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

* 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.

extremity to the *ccr* complex (L-C region) but which both carry the downstream constant region (*dcs*) (25). Both SCCmec IVc and IVd also differ in their L-C regions (SCCmec IVc carries an integrated copy of Tn4001 flanked on either side by IS256) (19). SCCmec IV has a type 2 *ccr* complex and class B *mec*, but Oliveira and colleagues described an SCCmec IV element with type 4 *ccr* (25, 32). A type IVA SCCmec element has also been described that harbors the integrated plasmid pUB110 (31).

Multilocus sequence typing (MLST), SCCmec 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 SCCmec 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, SCCmec 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 SCCmec 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, EMSA-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 SCCmec element was introduced and the type of SCCmec 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 SCCmec 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 SCCmec 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 SCCmec element analysis. The results of this investigation identified seven novel SCCmec 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.

SCCmec typing. SCCmec 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 SCCmec type, was used as described previously (31).

Nucleotide sequencing of SCCmec. The entire nucleotide sequence of the SCCmec 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 SCCmec II by the simplex PCR method but lacking one to three of the five amplimers typical of SCCmec II when analyzed by the multiplex PCR SCCmec 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 SCCmec IV by the simplex method

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

^e This isolate yielded an unfamiliar AR pattern and was not assigned an AR type, pending the outcome of MLST and SCCmec typing results in this study.

^f NA, not applicable.

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

Overlapping primers were designed to amplify the entire SCCmec element from both isolates using previously published SCCmec II and SCCmec 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 [SCCmec II] and AB063172 [SCCmec IVa]). The primers used to amplify and sequence the SCCmec 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 des 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 SCCmec II by both SCCmec typing methods was included as a control. Similarly, for AR43/3330.2 template DNA from at least one control isolate exhibiting SCCmec 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* DH5 α prepared using CaCl₂ 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 SCCmec elements. The SCCmec elements harbored by all isolates identified as having SCCmec types II and IV *ccr-mec* genes by the simplex method but with unusual SCCmec multiplex patterns were investigated by PCR amplification. The primers used for amplification and sequencing of SCCmec from isolate AR13.1/3330.2 (representative of isolates with SCCmec II *ccr-mec* genes by the simplex method but lacking one to three of the five SCCmec II multiplex amplimers) were used on all atypical SCCmec II isolates (Table 2). Similarly, the primers for amplification and sequencing of the SCCmec element of isolate AR43/3330.1 (representative of isolates with SCCmec IV *ccr-mec* genes by the simplex method but lacking one SCCmec IV multiplex amplimer) were used on all atypical SCCmec 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 SCCmec 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 (SCCmec IIE from MRSA isolate AR13.1/3330.2), AJ810121 (SCCmec IVE from MRSA isolate AR43/3330.2), AJ810122 (partial *mec* complex A.4), and AJ810123 (partial SCCmec 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 SCCmec element amplification and sequencing

Isolate	Primer pair	Nucleotide sequence ^g	Restriction site	Nucleotide coordinates	SCCmec region amplified
AR13.1/3330.2	IRLII F	CCCTCGAGGGCTCTGCGTATCAGTTAATGA	XhoI	4684–4703 ^a	L-C ^c
	ccrA R	ATTTGCGGCCGCGCTTCGATAGCCTGTTTCTG	NotI	25490–25471 ^a	
	ccrA F	ACGCGTCCGACCAAGTCATAGGCTATTACG	Sall	25417–25436 ^a	C-M ^d (<i>ccrA2-Tn554</i>)
	Tn554 R	ACGCGTCCGACAAGTATCCACGTTCAATCTCAAC	Sall	32442–32419 ^a	
	Tn554 F	ATTTGCGGCCGCTTTAAAGGGTTTCGGAATA	NotI	32365–32385 ^a	C-M ^d (<i>Tn554-mecR1</i>)
	mecR1 R	CCCTCGAGGGCAATGCCTAAACCTAATCG	XhoI	43754–43736 ^a	
	mecR1 F	ATTTGCGGCCGCGGATTAGGTTTAGGCATTG	NotI	43736–43754 ^a	M-I ^e (<i>mecR1-ugpQ</i>)
	ugpQ R	CCCTCGAGGGGCTTCTGCAGGATCTTGG	XhoI	47868–47851 ^a	
	ugpQ F	CCCTCGAGGGCCAAAGATCCTGCAGAAGC	XhoI	47851–47878 ^a	I-R ^f (<i>ugpQ-dcs</i>)
	dcs R	ATTTGCGGCCGCGGTCATGGCTATGATTAG	NotI	56373–56354 ^a	
	dcs F	ATTTGCGGCCGCGTCAATGAGATCATCTACAT	NotI	56109–56128 ^a	I-R ^f (<i>dcs</i> -right SCCmec junction)
orfX R	ATTTGCGGCCGCCCAAGGGCAAAGCGAC	NotI	57826–57810 ^a		
AR43/3330.1	IRLII F	CCCTCGAGGGCTCTGCGTATCAGTTAATGA	XhoI	4684–4703 ^a	L-C ^c
	ccrA R	ATTTGCGGCCGCGCTTCGATAGCCTGTTTCTG	NotI	25490–25471 ^a	
	ccrA F	ATTTGCGGCCGCCAAGTCATAGGCTATTACG	NotI	9894–9913 ^b	C-M ^d (<i>ccrA2-mecA</i>)
	mecA R	ACGCGTCCGACCCTATCTCATATGCTG	Sall	18160–18145 ^b	
	mecA F	ATTTGCGGCCGCGATTGGGATCATAGCGTCAAT	NotI	18021–18040 ^b	M-I ^e (<i>mecA-IS431mec</i>)
	ISmec R	ACGCGTCCGACACGGTGATCTTGCTCAATGA	Sall	22780–22761 ^b	
	ISmec F	CCCTCGAGGGCCTGACTGTCATTGTAC	XhoI	22724–22740 ^b	I-R ^f (<i>IS431mec</i> -right SCCmec junction)
	orfX R	ATTTGCGGCCGCCCAAGGGCAAAGCGAC	NotI	25395–25379 ^b	

^a Nucleotide coordinates from SCCmec type II, accession number D86934.

^b Nucleotide coordinates from SCCmec type IVa, accession number AB063172.

^c L-C, the region from the left chromosome-SCCmec 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 IS431*mec*.

^f I-R, the region from IS431*mec* to the right extremity of SCCmec.

^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.

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

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

Nine isolates were found to carry SCCmec I by the simplex method but failed to yield the 495-bp band characteristic of SCCmec 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 SCCmec I (Table 3). Furthermore, the multiplex pattern obtained was indistinguishable from SCCmec IV (Fig. 1A, lanes 2 to 5). This pattern was designated SCCmec I-*pls*.

Three isolates were identified by the simplex method as carrying SCCmec III but their multiplex pattern, although similar to that of SCCmec 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 pl258 and Tn554 and is found in all SCCmec III elements characterized to date. This pattern was designated SCCmec III -pl258/Tn554.

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

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

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

CC	ST	AR type or phenotype ^a	SCCmec type based on:			Variant SCCmec elements
			Simplex PCR	Multiplex PCR ^b	SCCmec name ^c	
8	239	AR01, AR15, AR23, AR44	III	III		
	239	PhIII, AR09	III	III – pI258/Tn554		
	250	Early MRSA (<i>n</i> = 1), PhII (<i>n</i> = 4)	I	I (<i>n</i> = 10)		
	250	PhII (<i>n</i> = 1), AR02 (<i>n</i> = 3)	I	IV or I – <i>pls</i> (<i>n</i> = 9)		
	247	AR22, New02	I	Ia		
	8	AR05	II	II – <i>kdp</i>	IIB	SCCmec IVb L-C region and lacks Tn554 ^f
	8	AR13 (<i>n</i> = 6), AR14 (<i>n</i> = 3)	II	II – <i>kdp</i>	IIA	SCCmec IVb L-C region; class A.4 <i>mec</i> ^{d,f}
	8	AR14 (<i>n</i> = 10)	II	II – <i>kdp</i> & <i>mecI</i>	IIC	SCCmec IVb L-C region; class A.3 <i>mec</i> ^d ; lacks ORFs between Tn554 and <i>mec</i> complex ^f
	8	AR13 (<i>n</i> = 19), AR14 (<i>n</i> = 3), New01, New03	II	II – <i>kdp</i> & pUB110	IID	SCCmec IVb L-C region; class A.4 <i>mec</i> ^d ; lacks pUB110 and second copy of IS431 ^f
	8	AR13 (<i>n</i> = 1), AR14 (<i>n</i> = 6)	II	IV or II – <i>kdp</i> , <i>mecI</i> & pUB110	III	SCCmec IVb L-C region; class A.3 <i>mec</i> ^d ; lacks pUB110, second copy of IS431, and ORFs between Tn554 and <i>mec</i> complex ^f
	8	AR43 (<i>n</i> = 17)	IV	IV – <i>dcs</i>	IVE	SCCmec IVc variant, lacks <i>dcs</i> ; has an unusual sequence in I-R region
	8	AR43 (<i>n</i> = 3)	IV	IV – <i>dcs</i>	IVF	SCCmec IVb variant, lacks <i>dcs</i> ; has an unusual sequence in I-R region
	5	5	AR07.3/07.4, AR11	II	II	
5		Unf01	IV	IV		
496	36	AR07.2 (<i>n</i> = 1)	II	II		
	36	AR07.0/07.2 (<i>n</i> = 6)	II	II		
30	30	ARNT (<i>n</i> = 4)	IV	IV		
	22	AR06	IV	IV		
45	45	ARNT (<i>n</i> = 1)	IV	IV		
S ^e	12	ARNT (<i>n</i> = 1)	IV	IV		

^a Where more than one ST/SCCmec type was identified within a phenotype (e.g., AR13 or AR07.0/07.2), the numbers represented by each ST or SCCmec 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 SCCmec typing (TABLE 1), and more than one SCCmec type was identified within this genotype; therefore, the numbers of isolates identified with each ST and SCCmec type are indicated in parentheses after the phenotype and SCCmec type, respectively. Otherwise, numbers of isolates are as indicated in TABLE 1.

^b Some patterns obtained with the multiplex PCR SCCmec typing method lacked amplimers corresponding to the amplification of the determinants indicated.

^c SCCmec 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 SCCmec II variant.

fied as SCCmec II by the simplex method but gave variant SCCmec II multiplex patterns that lacked one to three of the five amplimers that would identify them as harboring SCCmec II (Table 3). Ten isolates lacked a 284-bp band corresponding to the amplification product from a region of the *kdp* operon found in SCCmec II (Table 3). This pattern was designated SCCmec 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 SCCmec II. This pattern was termed SCCmec 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 IS431*mec* and pUB110 found in SCCmec II. This pattern was called SCCmec II –*kdp* & pUB110 (Fig. 1B, lanes 4). Seven isolates produced a multiplex PCR pattern indistinguishable from the pattern of SCCmec IV (Table 3). This pattern could also be interpreted as a SCCmec II pattern missing the 284-bp (*kdp* operon), 209-bp (*mecI* gene), and 381-bp (pUB110) amplimers. This pattern was designated SCCmec II –*kdp*, *mecI* & pUB110 (Fig. 1B, lane 5).

Investigation of the SCCmec elements harbored by ST8 isolates with unusual SCCmec II and SCCmec IV multiplex patterns. Since all ST8 isolates (*n* = 74) exhibited variant multiplex patterns (Table 3), we decided to further investigate these

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

Novel variant of SCCmec II identified in AR13.1/3330.2. The SCCmec element in AR13.1/3330.2 consisted of a ~27-kb sequence containing a combination of regions previously identified in SCCmec 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 SCCmec IVb (Table 4). The nucleotide sequence of this 5.6-kb amplimer was found to have 99.7% similarity to the L-C region

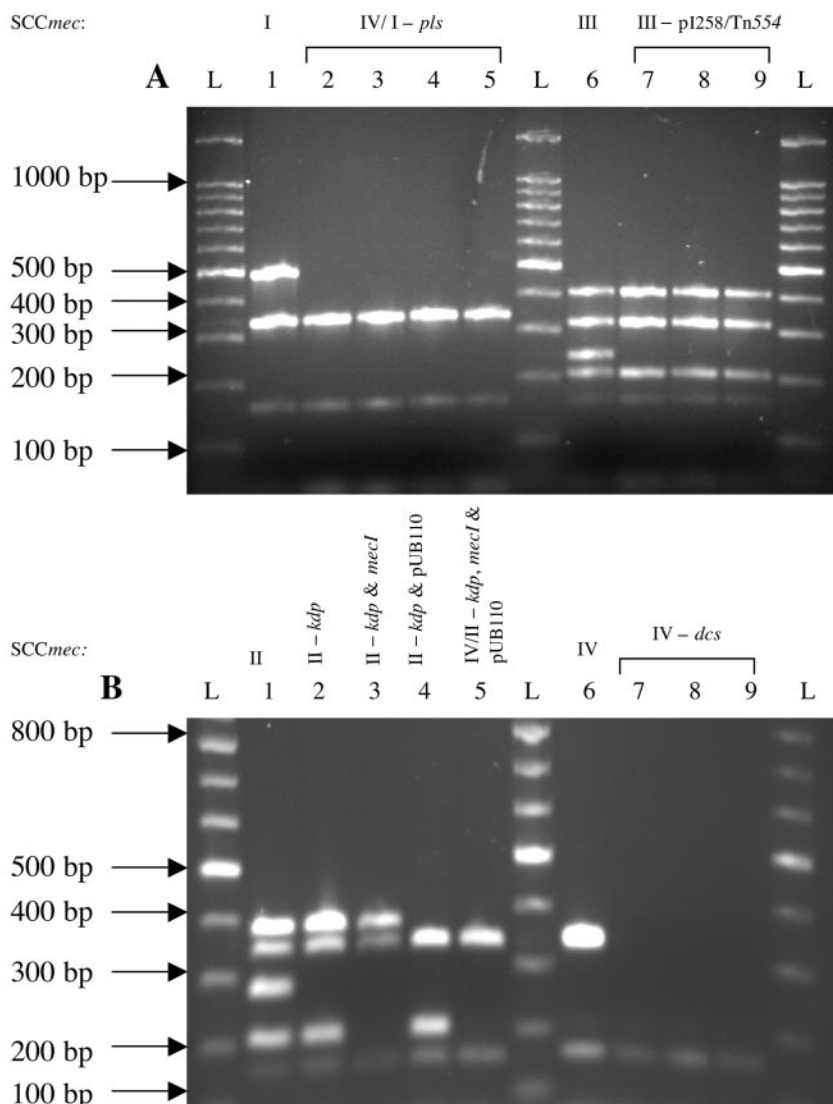


FIG. 1. SCCmec multiplex patterns and variant multiplex patterns found in Irish MRSA isolates. (A) Lane 1, SCCmec type I multiplex pattern; lanes 2 to 5, SCCmec IV multiplex pattern produced by AR02 isolates and six phenotype II isolates with SCCmec type I *ccr-mec* genes by the simplex method; lane 6, SCCmec type III multiplex pattern; lanes 7 to 9, the variant SCCmec III multiplex pattern (III -pI258/Tn554) characterized by the absence of the pI258/Tn554 243-bp amplicon, produced by AR09 and phenotype III isolates with SCCmec type III *ccr-mec* genes by the simplex method. (B) Lane 1, SCCmec type II multiplex pattern; lanes 2 to 5, the SCCmec 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 SCCmec multiplex pattern but lack the *kdp* amplicon (284 bp) (II -*kdp*, lane 2), or lack the *kdp* and *mecI* (209-bp) amplicons (II -*kdp* & *mecI*, lane 3), or lack the *kdp* and pUB110 (381-bp) amplicons (II -*kdp* & pUB110, lane 4), or lack the *kdp*, *mecI*, and pUB110 amplicons (II -*kdp*, *mecI* & pUB110, lane 5). This last pattern is the same as SCCmec IV. Lane 6, SCCmec 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 amplicon only. Lane L, 100-bp DNA ladders used as molecular size reference markers.

of SCCmec IVb and less than 10% similarity to the L-C regions of SCCmec 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 SCCmec II and SCCmec 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, Tn554 (Table 5). Five of the six ORFs (*tnpA*, *tnpB*, *tnpC*, *spc*, and *ermA*) previously identified within Tn554 of SCCmec II were present in the

SCCmec element of AR13.1/3330.2, and each ORF showed 100% homology at the amino acid level with those of SCCmec II (Table 5). However, Tn554 of SCCmec of AR13.1/3330.2 was truncated by ~1,000 bp, resulting in the absence of an ORF of unknown function in Tn554. The six ORFs spanning a ~5-kb region encoding hypothetical proteins usually found between Tn554 and the *mec* complex in SCCmec II were also absent (Fig. 2). Instead of these ORFs, a 1,865-bp IS1182 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

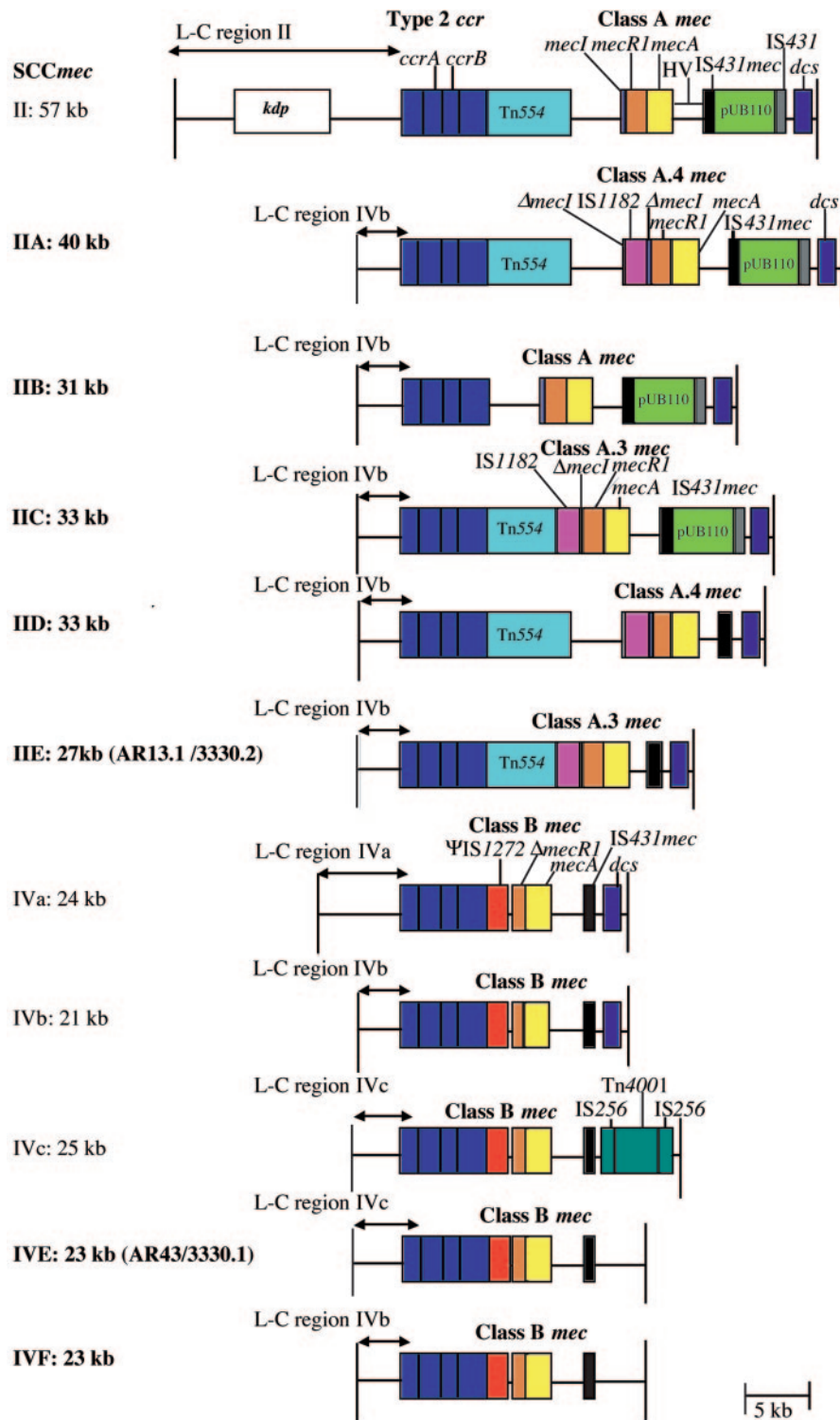


FIG. 2. Schematic diagram showing the genomic organization of SCCmec II, IIA, IIB, IIC, IID, IIE, IVa, IVb, IVc, IVE, and IVF elements. SCCmec IIA, IIB, IIC, IID, IIE, IVE, and IVF are novel variants that were identified in the present study. SCCmec II, IVa, IVb, and IVc were identified previously (19, 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 SCCmec IIE and IVE were determined by sequencing of the entire SCCmec element of Irish MRSA isolates AR13.1/3330.2 (IIE; accession number AJ810120) and AR43/3330.1 (IVE; accession number AJ810121). The structures of SCCmec 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 SCCmec multiplex types and amplicon sizes obtained using overlapping primer pairs to amplify the novel SCCmec element variants and comparison to those expected from typical SCCmec II and SCCmec IV elements

SCCmec multiplex type	SCCmec element	Amplicon size ^a (kb) with primer pair										Reference
		L-C, IRLII F-ccrA R	C-M, ccrA F-Tn554 R	C-M, Tn554 F-mecR1 R	M-I, mecR1 F-ugpQ R	I-R, ug pQ 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 – <i>kdp</i>	IIA	5.6	7	13*	4.1	8.5*	1.7	22	4.7	8	This study	
II – <i>kdp</i>	IIB	5.6	7	No amplicon ^c	4.1	8.5	1.7	13	4.7	8	This study	
II – <i>kdp</i> & <i>mecI</i>	IIC	5.6	7	6.5	4.1	8.5	1.7	14	4.7	8	This study	
II – <i>kdp</i> & pUB110	IID	5.6	7	13	4.1	2.2	1.7	22	3.7	2.7	This study	
II – <i>kdp</i> , <i>mecI</i> & 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 amplicon ^d	No amplicon ^d	4.1	3.2	1.7	8.5	4.7	2.7	25	
IV	IVb	5.6	No amplicon ^d	No amplicon ^d	4.1	3.2	1.7	8.5	4.7	2.7	25	
IV	IVc	6.1	No amplicon ^d	No amplicon ^d	4.1	No amplicon ^e	No amplicon ^e	8.5	4.7	5.3	19	
IV – <i>dcs</i>	IVE ^b	6.1*	No amplicon ^d	No amplicon ^d	4.1	No amplicon ^e	No amplicon ^e	8.5*	4.7*	4.7*	This study	
IV – <i>dcs</i>	IVF	5.6*	No amplicon ^d	No amplicon ^d	4.1	No amplicon ^e	No amplicon ^e	8.5	4.7	4.7*	This study	

^a Amplicon sizes for SCCmec IIA, IIB, IIC, IID, IIE, IVE, and IVF were calculated either by sequencing (indicated by *) or based on electrophoretic mobility in agarose gels. Amplicon sizes for SCCmec II, IVa, IVb, and IVc were calculated from sequences in the GenBank database, accession numbers D86934, AB063172, AB063173, and AB096217, respectively. All amplicon sizes are approximate. L-C, C-M, M-I, and I-R indicate the region of the SCCmec element amplified.

^b SCCmec elements IIE and IVE were sequenced in full from isolates AR13.1/3330.2 and AR43/3330.1, respectively.

^c No amplicon due to no Tn554; primers ccrA F-mecR1 R were used instead, resulting in an 11.5-kb amplicon, which was sequenced.

^d No amplicon due to no Tn554 in SCCmec IVa, IVb, IVc, IVE, and IVF.

^e No amplicon due to no *dcs* region in SCCmec IVc, IVE, and IVF.

100% homology to sequences of other SCCmec elements (Table 5).

Amplification of the region across IS431mec 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 SCCmec element including SCCmec II and IVb (Table 4). Sequence data from this product showed that the hypervariable (HV) region (located between *mecA* and IS431mec) of AR 13.1/3330.2 is 997 bp shorter than that of SCCmec II and SCCmec IVb. The IS431mec region was 100% homologous to that of other SCCmec elements but lacked all DNA sequences associated with the integrated plasmid pUB110 or the second IS431 sequence found in SCCmec II (Fig. 2).

The extreme right of the SCCmec element of AR13.1/3330.2 from IS431 to the right chromosomal-SCCmec junction (I-R region) showed 100% homology with the same region of SCCmec II (Table 5). The 15-bp direct repeat sequence characteristic of SCCmec elements was identified at the right extremity of SCCmec (DR-R) and a 26-bp inverted repeat sequence (IR-R) that was identical to that of SCCmec IVb and SCCmec II was also found. All SCCmec 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 SCCmec of AR13.1/3330.2 was integrated at the same nucleotide position in *orfX* as other SCCmec elements.

The novel genomic structure of the *mec* complex of the SCCmec element of AR13.1/3330.2 (IS1182- Δ mecI-mecR1-mecA-IS431mec) was more similar to the class A *mec* complex of MRSA (*mecI-mecR1-mecA-IS431mec*) found in SCCmec II and SCCmec III than to the class B *mec* complex (IS1272- Δ mecR1-mecA-IS431mec) of SCCmec 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 SCCmec IV identified in AR43/3330.1. Isolate AR43/3330.1 carried a 23-kb SCCmec element, which is within the size range (20 to 25 kb) of previously described SCCmec IV elements, but sequence data indicated that it is distinctly different. The genomic structure of this SCCmec el-

ement showed most similarity to SCCmec IVc (Fig. 2). Amplification of the L-C region of AR43/3330.1 yielded an amplicon of ca 6.1 kb (Table 4), and sequencing revealed that it had 100% similarity with the L-C region of SCCmec IVc (Table 6). Amplification of the region from the *ccr* complex to IS431mec yielded amplicons that showed almost 100% sequence identity with the same regions of SCCmec IVc (Table 6). A class B *mec* complex was identified (Fig. 2), and an I-R region different from any previously described SCCmec element was determined. An amplicon of 4.7 kb was obtained following amplification of this region (Table 4). Sequence analysis identified IS431mec, 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 SCCmec element of two other MRSA isolates (15). This sequence in the GenBank database consists of *orfX* and the extreme right region of the SCCmec element, including the direct repeat (DR-R) and inverted repeat (IR-R) regions. The rest of the sequence between IS431mec 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 SCCmec elements of the remaining ST8 isolates with unusual SCCmec II and SCCmec IV multiplex patterns. In total, 74 isolates had the ST8 genotype and gave variant SCCmec II and IV multiplex patterns (Table 3). In addition to determining the nucleotide structure of the SCCmec elements of two of these isolates as described above, the SCCmec elements of the remaining 72 isolates were characterized by analyzing the mobility of amplicons on agarose gels obtained with the overlapping primers used to amplify and sequence the SCCmec from isolates AR13.1/3330.2 and AR43/3330.1. In some cases, sequencing was performed on amplicons that differed in size from that expected from previously described SCCmec elements. These results are summarized in Table 3 and Table 4. This analysis identified five variant SCCmec elements, in addition to the two identified above by complete nucleotide sequencing. Of the

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

SCCmec element and ORF	Location ^a	Homology to ORFs of SCCmec IVb ^b			Homology to ORFs of SCCmec 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 (<i>ccrA2</i>)	5584–6933	M004	99.1	Cassette chromosome recombinase A2	N034	96.7	Cassette chromosome recombinase A2
IIE06 (<i>ccrB2</i>)	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 (<i>tpA</i>)	10830–11915	—	—	—	N045	100	Transposase A
IIE12 (<i>tpB</i>)	11912–13804	—	—	—	N046	100	Transposase B
IIE13 (<i>tpC</i>)	13811–14188	—	—	—	N047	100	Transposase C
IIE14 (<i>spc</i>)	14339–15121	—	—	—	N048	100	O-Nucleotidyltransferase
IIE15 (<i>ermA</i>)	15247–15978 (complement)	—	—	—	CN026	100	rRNA adenine N-6-methyltransferase
IIE16 (<i>tnp IS1182</i>)	16499–18103	—	—	—	—	—	—
IIE17 (Δ <i>mecI</i>)	18207–18459 (complement)	—	—	—	CN036	67.7	Methicillin resistance protein MecI
IIE18 (<i>mecR1</i>)	18459–20216 (complement)	CM003	55.6	Truncated signal transducer protein MecR1	CN037	100	Signal transducer protein MecRI
IIE19 (<i>mecA</i>)	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 (<i>ugpQ</i>)	22893–23636	CM006	100	Glycerophosphoryldiester phosphodiesterase	N039	100	Glycerophosphoryldiester phosphodiesterase
IIE22 (<i>tnp IS431mec</i>)	23901–24575	M011	100	Transposase for IS431mec	N062	100	Transposase for IS431mec
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 SCCmec IIE deposited in the GenBank database under accession number AJ8101208.

^b ORFs and gene products from SCCmec II and SCCmec 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 SCCmec II variants (designated SCCmec IIA, IIB, IIC, IID, and IIE) were identified among the 54 isolates with variant SCCmec II multiplex patterns, and two novel SCCmec IV variants (designated SCCmec IVE and IVF) were found among the 20 isolates with variant SCCmec IV multiplex patterns (Table 3). A schematic representation of all seven SCCmec variants is shown in Fig. 2.

Additional novel variants of SCCmec II. Significant size variation was observed in the mobility of amplimers obtained from variant SCCmec II elements compared to the sizes expected from isolates with typical SCCmec II when the following three regions were amplified: the L-C region, Tn554 to the *mec* complex (part of C-M region), and across pUB110 (part of I-R region) (Table 4). The 54 SCCmec II variants yielded the 5.6-kb fragment characteristic of the L-C region of SCCmec IVb (Table 4; Fig. 2). No amplimer obtained following amplification of the region from Tn554 to *mecR1* in the SCCmec II variants matched the size expected from a typical SCCmec II element (Table 4). All but one isolate with the multiplex pattern SCCmec II *-kdp* ($n = 9$) and all isolates with the multiplex pattern SCCmec 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 IS1182 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*-IS1182- Δ *mecI*-*mecR1*-*mecA*-IS431*mec* was designated *mec* class A.4 (Fig. 3).

The Tn554-*mecR1* region of one isolate with the multiplex pattern SCCmec II *-kdp* could not be amplified with the same primers used on the other SCCmec 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 Tn554 normally found in SCCmec II and that all Tn554 ORFs were absent. However, the six ORFs usually found between Tn554 and the *mec* complex of SCCmec II were all present. This isolate also harbored a class A *mec* complex. Amplification of the same region in isolates with the multiplex pattern SCCmec II *-kdp* & *mecI* ($n = 10$) and SCCmec 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 Tn554 and the *mec* complex was absent but which carried a *mec* class A.3 *mec* complex. All isolates with

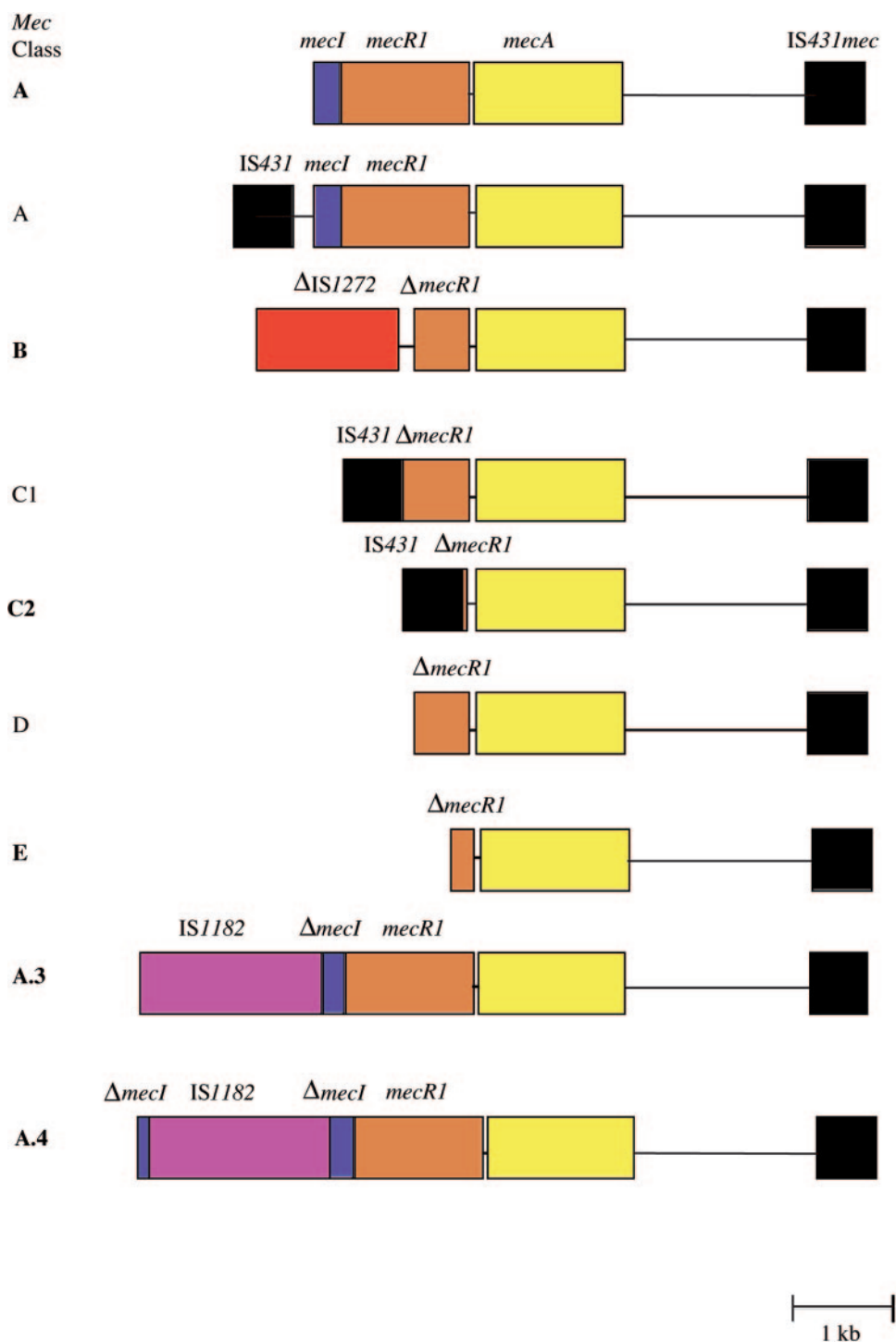


FIG. 3. Schematic diagram of the genetic organization of the *mec* complexes of staphylococci. The illustrations of *mec* complexes A, B, C1, C2, and D are based on studies by Katayama and coworkers (22), and the illustration of *mec* complex E is based on studies by Lim and coworkers (22, 24). The *mec* 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 *IS1182* (*IS1182-ΔmecI-mecRI-mecA-IS431mec*). Δ *mecI* is only 253 bp, whereas intact *mecI* is 372 bp. In class A.4, *mec IS1182* is present within *mecI* and there is a 16-bp deletion of *mecI* immediately upstream of *IS1182* (Δ *mecI-IS1182-ΔmecI-mecRI-mecA-IS431mec*).

the SCCmec II -*kdp* (*n* = 10) and SCCmec II -*kdp* & *mecI* (*n* = 10) harbored the integrated plasmid pUB110 and the second copy of *IS431* found in SCCmec. Isolates with multiplex patterns SCCmec II -*kdp* & pUB110 (*n* = 27) and SCCmec II

-*kdp*, *mecI* & pUB110/IV (*n* = 7) gave the same size amplicon 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 *IS431* sequence.

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

SCCmec element and ORF	Location ^a	Homology to ORFs of SCCmec 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 (<i>ccrA2</i>)	6067–7416	R005	99.1	Cassette chromosome recombinase A2
IVE07 (<i>ccrB2</i>)	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 IS1272
IVE14	12725–13711 (complement)	CR010 (Δ <i>mecR1</i>)	100	Truncated signal transducer protein MecR1
IVE15	13811–15817	R012 (<i>mecA</i>)	100	Penicillin binding protein 2a
IVE16	16388–17131 (complement)	CR011	100	Hypothetical protein
IVE17	18432–19106	R013	100	Transposase of IS431 <i>mec</i>
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 SCCmec IVE deposited in the GenBank database under accession number AJ810121.

^b ORFs and gene products from SCCmec IVc nucleotide sequence in the GenBank database, accession number AB096217 (19).

^c Based on amino acid identity.

^d —, no homology.

Additional novel variants of SCCmec IV. All 20 isolates with the AR43 phenotype which were found to carry SCCmec IV by the simplex method but gave the multiplex pattern SCCmec IV *-dcs* produced the expected size products for a type IVc SCCmec 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 SCCmec IVc (6.1-kb amplicon), but three isolates yielded a 5.6-kb product (Table 4). Sequencing of this amplicon confirmed it contained the SCCmec IVb L-C region. As with isolate AR43/3330.1, an amplicon 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 SCCmec IVb was sequenced and was 100% identical to that of isolate AR43/3330.1.

DISCUSSION

MLST and SCCmec 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 & Tn554*. In 1989, ST239-MRSA-III predominated, but during the 1990s this was displaced by ST8-MRSA-II, exhibiting six of the nine novel SCCmec 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 SCCmec type, but a striking finding of the present study was the extent of variation within SCCmec. If clones among the Irish MRSA population are defined on the basis of all SCCmec variants recognized, the population includes 20 clonal types consisting of 10 previously recognized clonal types, 9 with previously described genetic backgrounds but with novel SCCmec 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 SCCmec II) (Table 3). The greatest variation was seen among isolates with the ST8 genotype. For example, among the 54 isolates exhibiting SCCmec IV by the simplex method, only those with the ST8 genotype (20/54) had variant SCCmec elements. In fact, 86% of isolates (74/86) with novel SCCmec variants had the ST8 genotype, and all carried previously unreported variants of SCCmec II (IIA to IIE) or IV (IVE and IVF).

Because both SCCmec I and IV have a *mec* complex with IS1272 inserted at the same junction point, it has been suggested that recombination has occurred between SCCmec I and other sequences to generate SCCmec type IV (25). The novel ST8 SCCmec variants identified in this study resemble rearrangements of SCCmec II and SCCmec IV. SCCmec variants IIA to IIE have an L-C region that is almost identical to that of SCCmec IVb, but the rest of each element has closest identity with SCCmec II, including a very similar genomic structure (Fig. 2).

SCCmec IIA to IIE appear to be closely related. SCCmec IIB was found in isolates from 1989, making it the earliest SCCmec II variant identified in this study. It harbors the original class A *mec* complex, but its similarity to SCCmec IVb in the L-C region make its provenance unclear. It is likely that SCCmec IIB was generated following loss of most of Tn554,

because it retains only ~40 bp of this element. Acquisition of IS1182 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 SCCmec IIC and IIE and SCCmec IIA and IID, respectively (Fig. 2 and 3). Since SCCmec IIC and IIA were present in isolates in 1993 and SCCmec 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 IS1182 within *mecl*, although a novel finding, is not surprising. Mutations and deletions in *mecl* are common in MRSA (22), sometimes in association with insertion sequences. Class B and class C *mec* complexes have *mecl* and part of *meclR1* deleted and insertions of IS1272 or IS431 flanking the truncated end of *meclR1* (22). IS1182 has been identified in *S. aureus* flanking the staphylococcal composite transposon Tn5405, which encodes aminoglycoside resistance determinants (6). Immediately adjacent to the insertion site of IS1182 at the 3' end of *mecl* in the class A.4 *mec* complex, a 16-bp deletion in *mecl* 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 SCCmec elements. Based on a multiplex SCCmec typing method, Wienders et al. (47) reported a SCCmec I element carrying the *kdp* region (normally part of L-C region of SCCmec II only) and also an SCCmec IV element with *pls* (normally part of L-C region of SCCmec I only) (47). Various different SCCmec IV elements have been described with diverse L-C regions (19, 25). The integrated plasmid pUB110 normally found in SCCmec II encodes resistance to kanamycin, tobramycin, and bleomycin (17). In the present study all isolates with SCCmec IID and IIE were resistant to kanamycin and tobramycin (42–44), although they lacked pUB110 in their SCCmec 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 SCCmec has been documented previously; SCCmec IA is believed to have evolved from SCCmec I by acquisition of pUB110 or vice versa (32). SCCmec IVA has been shown to harbor pUB110 (31), and Wienders et al. (47) described pUB110 associated with SCCmec III and SCCmec IV (47).

SCCmec IIA to IIE (40 to 27 kb) are smaller than previously described SCCmec II elements (57 kb), and SCCmec IIE, with a size of 27 kb, is more similar in size to SCCmec IV (20 to 25 kb) or SCCmec V (28 kb) (Fig. 2). It has been suggested that due to their relatively small size, SCCmec IV and SCCmec V are highly competitive mobile genetic elements that are more readily transferred between staphylococci than the larger SCCmec elements and, once acquired by the host, do not compromise its fitness (18, 25). This could also be true for the SCCmec 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 SCCmec.

Like SCCmec IIA to IIE, the origin of the two ST8-MRSA-IV variants IVE and IVF identified here is unclear. SCCmec IVE is almost identical to SCCmec IVc, except in the region downstream of the *mec* complex, suggesting that SCCmec IVE

may have arisen by recombination between an SCCmec IVc element and another SCCmec sequence. SCCmec IVF has the same L-C region as the ST8 SCCmec II variants, but the rest of the element is similar to SCCmec IVE (Fig. 2). SCCmec IVF may have evolved by recombination between a SCCmec II variant and SCCmec IVE, which donated the rest of the element including the unusual downstream region. Alternatively, SCCmec IVF may have evolved by recombination between SCCmec IVb and SCCmec 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 SCCmec elements in various genetic backgrounds (ST8 and ST30) (2, 36). In one case, a *dcs* amplimer was reported in SCCmec III, although it does not usually carry the *dcs* sequence (47).

Two other SCCmec 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 SCCmec I is located within the L-C region, which is not thought to be an important part of SCCmec (16). The variant ST239-MRSA-III *-pI258* & Tn554 may have evolved from ST239-MRSA-III by loss of the integrated plasmid pI258 or transposon Tn554, or both. Further work is under way to characterize these novel variants of SCCmec I and SCCmec 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 SCCmec II *-kdp* & pUB110 and SCCmec III *-pI258* & Tn554 (1, 2, 36, 46). However, this is the only study to date that has fully characterized the SCCmec elements with some of these unusual SCCmec multiplex patterns. Three SCCmec variants have been identified previously in the ST8 genetic background: SCCmec II *-kdp* and SCCmec II *-kdp, mecl* & pUB110 were identified in two isolates described as Irish-1 from the United Kingdom and one AR14 phenotype from Ireland, and SCCmec 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 SCCmec 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 SCCmec II *-kdp* & *mecl* in two Japanese isolates with ST5 and ST89 genotypes (2), the SCCmec IV *-dcs* multiplex pattern in ST30 isolates from Greece (1), and the SCCmec I *-pls* multiplex pattern from one ST1 isolate from the United States (46). These patterns may occur if the variant SCCmec 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 amplicon does not necessarily mean that the same *SCCmec* element is present. Further investigation into these *SCCmec* elements is required.

It has been recommended that the multiplex *SCCmec* 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 *SCCmec* typing method, half the isolates investigated would have been misclassified as harboring an erroneous *SCCmec* element or would have yielded *SCCmec* multiplex patterns that could not be interpreted as belonging to any of the currently described *SCCmec* types. Combining results of the multiplex and simplex *SCCmec* typing methods allowed correct classification of some of the unusual multiplex patterns. However, neither the simplex or multiplex methods recognized a number of *SCCmec* elements recently described in other studies, including *SCCmec V* and the *SCCmec IV* variants *SCCmec IVa*, *IVb*, *IVc*, or *IVd* (18, 19, 25). PCR amplification and sequencing of the entire *SCCmec* element was necessary for the complete characterization of the *SCCmec* elements harbored by isolates with ambiguous multiplex patterns. Such detailed investigations are unsuitable for routine typing purposes. Furthermore, if *SCCmec* is to be used for routine epidemiological typing, some method of standardizing nomenclature for the plethora of new and variant *SCCmec* types is needed. Equally important is the question of the epidemiological significance of variant *SCCmec* elements. As *SCCmec* 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 *SCCmec* can evolve rapidly, and simple PCR methods for characterizing *SCCmec* 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 *SCCmec* 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 *SCCmec* and has raised many questions which need to be addressed.

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