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Emergence of Sequence Type 779 Methicillin-Resistant *Staphylococcus aureus* Harboring a Novel Pseudo Staphylococcal Cassette Chromosome *mec* (SCC*mec*)-SCC-SCC$_{CRISPR}$ Composite Element in Irish Hospitals

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infection in Irish hospitals for 4 decades, and replacement of predominant MRSA clones has occurred several times. An MRSA isolate recovered in 2006 as part of a larger study of sporadic MRSA exhibited a rare *spa* (t878) and multilocus sequence (ST779) type and was nontypeable by PCR- and DNA microarray-based staphylococcal cassette chromosome *mec* (SCC*mec*) element typing. Whole-genome sequencing revealed the presence of a novel 51-kb composite island (CI) element with three distinct domains, each flanked by direct repeat and inverted repeat sequences, including (i) a pseudo SCC*mec* element (16.3 kb) carrying *mecA* with a novel *mec* class region, a fusidic acid resistance gene (*fusC*), and two copper resistance genes (*copB* and *copC*) but lacking *ccr* genes; (ii) an SCC element (17.5 kb) carrying a novel *ccrAB4* allele; and (iii) an SCC element (17.4 kb) carrying a novel *ccrC* allele and a clustered regularly interspaced short palindromic repeat (CRISPR) region. The novel CI was subsequently identified by PCR in an additional 13 t878/ST779 MRSA isolates, six from bloodstream infections, recovered between 2006 and 2011 in 11 hospitals. Analysis of open reading frames (ORFs) carried by the CI showed amino acid sequence similarity of 44 to 100% to ORFs from *S. aureus* and coagulase-negative staphylococci (CoNS). These findings provide further evidence of genetic transfer between *S. aureus* and CoNS and show how this contributes to the emergence of novel SCC*mec* elements and MRSA strains. Ongoing surveillance of this MRSA strain is warranted and will require updating of currently used SCC*mec* typing methods.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant problem in hospitals and communities worldwide, and awareness of MRSA in animals and reports of its zoonotic spread have increased in recent years (1, 2). The success of MRSA is in part due to its ability to adapt rapidly to changing environments through the acquisition of mobile genetic elements (MGE) that harbor antimicrobial resistance determinants or virulence-associated genes which form part of the accessory genome (1). Resistance to methicillin and β-lactam antibiotics in staphylococci is determined by penicillin binding protein 2a (PBP2a) encoded by the methicillin resistance gene *mecA* (3). In MRSA, two distinct *mecA* gene types have been described and are carried on a large MGE termed the staphylococcal cassette chromosome *mec* (SCC*mec*) (4, 5). Both gene types were originally termed *mecA*; however, the second gene has recently been renamed *mecC* based on its significant divergence from the classical *mecA* gene type (5). Numerous alleles of the *mecA* gene type have also been described (5, 6).

The SCC*mec* element is highly variable, with extensive diversity identified in this cassette in different staphylococcal species, including the 11 SCC*mec* types and numerous subtypes from MRSA (4, 7, 8). Considerable indirect evidence has been reported for the horizontal transfer of SCC*mec* DNA between *S. aureus* and coagulase-negative staphylococci (CoNS), and SCC*mec* is more diverse and abundant among CoNS (9). While the mechanism(s) of transfer is unknown, similar SCC*mec* elements have been found in CoNS and *S. aureus*, in some cases from the same patient (10). CoNS may constitute a potentially significant reservoir for antibiotic resistance genes in *S. aureus* and may have a significant impact on the emergence of novel MRSA strains (11). SCC*mec* inserts into the 3′ end of the chromosomally located *orfX* gene and is characterized by the presence of flanking imperfect direct repeat (DR) sequences that are generated at both ends of the element following insertion into *orfX*. SCC*mec* elements harbor two fundamental components, the *mec* gene complex and the cassette chromosome recombinase (*ccr*) gene complex, and each SCC*mec* element is characterized by a unique combination of these genes. SCC elements harboring *ccr* genes but without *mecA* and SCC-like elements without *ccr* and *mec* genes have also been reported within *orfX* and flanked by DRs in staphylococci and often harbor additional virulence or antimicrobial resistance genes (9, 12).

The *mec* gene complex consists of *mecA* and, when present, the *mec* regulatory genes *mecR1* and *mecI* (7). Five classes of the *mec*

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gene complex (A to E) have been reported to date in staphylococci (7) (www.sccmec.org). The SCC*mec*-carried *ccr* genes are necessary for precise integration and excision of the SCC*mec* element, and three genes (*ccrA*, *ccrB*, and *ccrC*) have been described. Novel *ccr* genes and any subsequent subtypes are assigned new designations based upon guidelines published in 2009 (7), which take the sequence similarity of any previously published or forthcoming novel *ccr* genes into consideration. Each *ccr* complex consists of either the *ccrA* and *ccrB* genes together or *ccrC* and an associated open reading frame (ORF), previously termed *ccrAA* (13), which is located directly upstream of *ccrC* and exhibits between 35 and 41% DNA sequence similarity to *ccr* genes *ccrA*, *ccrB*, and *ccrC*. Eight types of the *ccr* gene complex have been reported to date in MRSA, each with a different combination of *ccrA* and *ccrB* allotypes or *ccrC* (7) (www.sccmec.org). Numerous allelic variants of each of the *ccr* allotypes have been reported based upon this criterion; however, the nomenclature is complicated, as not all variants have been assigned allelic numbers. For example, in recent years, five alleles of the *ccrA4* and *ccrB4* allotypes have been reported without designated allelic prefixes and 10 alleles of the *ccrC1* allotype (*ccrC1* to *ccrC10*) have been assigned in both MRSA and CoNS (9, 13–20).

MRSA has now been endemic in Ireland for over 3 decades, and clonal replacement has occurred on several occasions during this period (21–24). Over the last decade, MRSA isolates exhibiting sequence type 22 (ST22) and harboring SCC*mec* type IV (ST22-MRSA-IV) have predominated, accounting for approximately 80% of MRSA isolates recovered from patients in Irish hospitals (24). In the present study, we report the detailed molecular characterization of human clinical MRSA isolates recovered in Irish hospitals between 2006 and 2011 which exhibited a rare ST (ST779) and *spa* type (t878). Whole-genome sequencing of a representative isolate revealed a novel composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element carrying a clustered randomly interspersed palindromic repeat (CRISPR) region that encodes a prokaryotic defense mechanism against foreign DNA. The novel element was subsequently identified in all 14 of the ST779 isolates investigated.

MATERIALS AND METHODS

Bacterial isolates. MRSA isolate M06/0171 was recovered in 2006 in an Irish pediatric hospital and was initially identified as part of an investigation into 58 sporadically occurring MRSA isolates recovered in Irish hospitals between 2000 and 2006 (Table 1). M06/0171 exhibited *spa* type t878, but its SCC*mec* type could not be determined by conventional SCC*mec* typing PCRs or by DNA microarray profiling. Whole-genome sequencing of M06/0171 was undertaken to determine the genetic organization of its SCC*mec* element. The database of isolates submitted to the Irish National MRSA Reference Laboratory (NMRSARL) was subsequently examined for other *spa* type t878 isolates. Between 2006 and 2011, a total of 4,320 MRSA isolates were investigated by the NMRSARL, and approximately 80% were characterized as non-multiantibiotic-resistant phenotype AR06, indicative of ST22-MRSA-IV (22), the pandemic strain currently circulating in Irish hospitals. Half of the non-ST22-MRSA-IV isolates were *spa* typed during this time period, and 13 additional *spa* type t878 MRSA isolates were identified among the 431 MRSA isolates that were *spa* typed (Table 1). These isolates were investigated by DNA microarray profiling and detailed SCC*mec* analysis.

All isolates were identified as *S. aureus* using the tube coagulase test, and methicillin resistance was detected using 10-μg and 30-μg cefoxitin disks (Oxoid Ltd., Basingstoke, United Kingdom).

AR typing. All isolates were subjected to antibiogram-resistogram (AR) typing as described previously (25).

Copper resistance. All isolates were tested for susceptibility to copper sulfate (Sigma-Aldrich Chemical Company, Tallaght, Dublin, Ireland). One isolate, M06/0171, was tested using 0.125, 0.250, 0.5, 1, 2, 4, 8, and 16 mM concentrations and the Clinical and Laboratory Standards Institute (CLSI) agar plate dilution methodology (26). All 14 MRSA isolates were tested for copper sulfate resistance using the CLSI disk diffusion methodology using 4 mM copper sulfate antibiotic disks. The copper-susceptible *S. aureus* reference strain RN4220 (27) and the copper-resistant MRSA strain MRSA252 (12) were used as controls.

Molecular typing. All isolates underwent direct repeat unit (*dru*) typing, while M06/0171 was also subjected to multilocus sequence typing (MLST) and SCC*mec* typing, all as described previously (24, 28–30). SCC*mec* typing involved the use of previously described multiplex PCRs to detect (i) the class A, B, and C *mec* complexes (31); (ii) the type 1 to 5 *ccr* complexes (31); and (iii) the joining or "J" regions (32). An additional simplex PCR using alternative *ccrAB4* primers described previously by Ruppe et al. (33) was undertaken for the detection of additional *ccrAB4* alleles that are not detected using the *ccrAB4* primers described by Kondo et al. (31). Previously described MRSA reference strains were used as positive controls for these PCR assays (29). PCRs were performed using GoTaq Flexi DNA polymerase (Promega Corporation, Madison, WI) according to the manufacturer's instructions. PCR amplifications were performed in a G-storm GS1 thermocycler (Applied Biosystems, Foster City, CA). PCR products were visualized by conventional agarose gel electrophoresis and purified with the GenElute PCR cleanup kit (Sigma-Aldrich). Sequencing was performed commercially by Geneservice Limited (Source Bioscience, Guinness Enterprise Centre, Dublin, Ireland) using an ABI 3730xl Sanger sequencing platform.

DNA microarray analysis using the StaphyType kit. The StaphyType kit detects 333 *S. aureus* gene sequences and alleles, including species-specific, antimicrobial resistance genes; virulence-associated genes; and typing markers and SCC*mec*-associated gene sequences and can assign *S. aureus* isolates to an MLST sequence type (ST) and/or clonal complex (CC) (34, 35). Array procedures were performed according to the manufacturer's instructions.

Whole-genome sequencing of MRSA isolate M06/0171. The whole-genome sequence of one MRSA isolate, M06/0171, was determined in order to investigate the genetic organization of a possible novel SCC*mec* element. High-throughput *de novo* sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, United Kingdom) using the Illumina genome analyzer system (Illumina HiSeq 2000 platform; Illumina, Essex, United Kingdom). The average coverage across the genome was 111×. The reads were assembled into contigs using a Velvet *de novo* genome assembler (version 1.0.15; Illumina). Contigs were analyzed using the Artemis DNA sequence viewer and annotation tool (36) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (37). Any contig gaps identified between SCC*mec*-associated sequences were closed by primer walking using PCR with primers based on the surrounding contigs and GoTaq Flexi DNA polymerase (Promega) followed by amplimer sequencing and analysis using BioNumerics software version 5.1 (Applied Maths, Ghent, Belgium) and Artemis. Open reading frames (ORFs) were predicted using Artemis and prodigal (http://prodigal.ornl.gov/), and all ORFs were analyzed using the BLAST software package. Open reading frames were aligned with the best-fit matches in GenBank, and the locations of start codons, stop codons, and potential ribosomal binding sites were checked for consistency.

Confirmation of the genetic organization and location of the novel composite element in M06/0171. The genetic organization of the novel composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element in M06/0171 determined from the whole-genome sequence was confirmed using eight overlapping primer pairs to amplify the entire element (see Table S1 in the supplemental material). These PCR assays were performed by amplifying chromosomal DNA using the Expand long-template PCR system (Roche

TABLE 1 Epidemiological, clinical, phenotypic, and genotypic characteristics of the 14 ST779 and *spa* type t878 MRSA isolates harboring the novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element recovered in Irish hospitals between 2006 and 2011

Hospital no.	Isolate no.	Yr of isolation	Age[a]	Clinical details (sex)	Antimicrobial resistance pattern[b,c]	*dru* type	DNA microarray analysis[e]: SCC*mec* genes[e]	DNA microarray analysis[e]: Antimicrobial resistance genes[e]	DNA microarray analysis[e]: Virulence-associated genes[e,f]
H1	M06/0171	2006	3 y	Burn unit (female)	AMP, COP, FUS, MUP, NEO, TOB	dt8af	*mecA*, *ugpQ*, *ccrAA*, *ccrC*,[g] *ccrA4*, *ccrB4*	*blaZ*, *fusC*, *sdrM*, *aadD*, *mupA*	*seb*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*
H2	E4233	2009	45 y	BSI[i] (female)	AMP, COP, FUS	dt8af	*mecA*, *ugpQ*, *ccrB4*[g]	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*,[g] *sdrD*[g]
H3	M11/0114	2011	5 d	Screening sample, baby of patient from whom M11/0118 was recovered (N/A)[h]	AMP, COP, FUS	dt8af	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrB4*[g]	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*,[g] *sdrD*[g]
H3	M11/0118	2011	30 y	Screening sample, mother of baby from whom M11/0114 was recovered (female)	AMP, COP, FUS	dt8af	*mecA*, *ugpQ*, *ccrAA*, *ccrC*, *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*[g]
H4	E4449	2010	39 y	BSI (male)	AMP, COP, CAD,[d] FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrC*,[g] *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*
H4	E2998	2006	54 y	BSI (male)	AMP, COP, FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*, *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*
H5	E4550	2010	55 y	BSI (female)	AMP, COP, CAD,[d] FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrC*,[g] *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*, *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*
H6	M11/0208	2011	18 y	Dermatology clinic (male)	AMP, COP, CAD,[d] FUS	dt11y	*mecA*, *ugpQ*, *ccrB4*[g]	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *sdrD*,[g] *clfB*[g]
H7	M08/0422	2008	24 y	Screening sample (female)	AMP, COP, CAD, FUS	dt11y	*mecA*, *ugpQ*	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*,[g] *sdrD*
H8	M07/0307	2007	Stillborn	Stillborn baby postmortem (N/A)[h]	AMP, COP, FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*, *ccrC*,[g] *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*,[g] *sdrD*
H9	M09/0295	2009	41 y	Screening sample (male)	AMP, COP, FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrB4*[g]	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *sdrD*,[g] *clfB*[g]
H10	E4709	2010	54 y	BSI (female)	AMP, COP, FUS	dt11y	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrC*, *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sed*, *sej*, *ser*, *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*
H11	M09/0302	2009	46 y	Screening sample (male)	AMP, COP, FUS	dt10aj	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrB4*[g]	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sak*, *chp*, *scn*, *etD*, *edinB*, *sdrD*,[g] *clfB*[g]
H12	E4217	2009	59 y	BSI (male)	AMP, COP, FUS	dt11bm	*mecA*, *ugpQ*, *ccrAA*,[g] *ccrC*, *ccrB4*	*blaZ*, *fusC*, *sdrM*	*seb*,[g] *sak*, *chp*, *scn*, *etD*, *edinB*, *clfB*, *sdrD*

[a] Age of patient; y, years; d, days.

[b] Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including amikacin, ampicillin (AMP), cadmium acetate (CAD), chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid (FUS), gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin (MUP), neomycin (NEO), phenyl mercuric acetate, rifampin, spectinomycin, streptomycin, sulfonamide, tetracycline, tobramycin (TOB), trimethoprim, and vancomycin (25).

[c] Isolate M06/0171 was tested for susceptibility to copper sulfate (COP) by the CLSI agar plate dilution methodology (26). Copper resistance in the remaining 13 ST779 MRSA isolates was confirmed by the CLSI disk diffusion methodology (26).

[d] These isolates exhibited intermediate resistance to cadmium acetate.

[e] SCC*mec*, antimicrobial resistance and virulence-associated genes were detected using the StaphyType DNA microarray kit (Alere, Germany) (34). *ccrAA* is a known *ccrC*-linked gene with 35 to 41% DNA sequence homology to other *ccr* genes.

[f] The following MSCRAMM, adhesion, and biofilm formation genes were detected in all 14 ST779/t878 MRSA isolates by DNA microarray analysis: *icaA*, *icaC*, *icaD*, *bbp*, *clfA*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *sdrC*, *vwb*, and *sasG*.

[g] Ambiguous or negative DNA microarray signals were obtained for the genes and isolates indicated. The presence of *seb*, *clfB*, and *sdrD* was confirmed in all 14 MRSA isolates by PCR.

[h] N/A, information not available.

[i] BSI, bloodstream infection.

Diagnostics GmdH, Lewes, East Sussex, United Kingdom). PCR products were visualized by agarose gel electrophoresis, and the sizes of the amplimers obtained were compared to the expected size of the amplimers based on the whole-genome sequence.

PCRs to confirm the presence of the pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element in additional t878 MRSA isolates and to confirm ambiguous DNA microarray results. The presence of the novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element was investigated in the remaining 13 t878 MRSA isolates using previously described primers to amplify *ccrAB4* and *ccrC* (31, 33) and novel primers to detect the CRISPR region and the novel *mec* complex of M06/0171 (see Table S1 in the supplemental material). Amplimers obtained from all 13 isolates using *ccrAB4*-specific and *ccrC*-specific primers and amplimers obtained using CRISPR primers for 5/13 isolates (isolates M09/0295, M08/0422, M11/0208, M09/0302, and E4449) were sequenced and compared to the corresponding sequences of M06/0171 using BioNumerics and Artemis. The online tool CRISPRfinder (38) (http://crispr.u-psud.fr/Server/) was used for CRISPR sequence analysis. The presence of the genes encoding clumping factor B (*clfB*), serine aspartate repeat protein D (*sdrD*), and staphylococcal enterotoxin B (*seb*) was confirmed by PCR (see Table S1) due to ambiguous DNA microarray results.

Nucleotide sequence accession number. The nucleotide sequence of the novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element harbored by M06/0171 has been deposited in GenBank under accession number HE980450.

RESULTS

Phenotypic and genotypic characteristics of isolates. Fourteen *spa* type t878 MRSA isolates recovered from separate patients in 12 different Irish hospitals between 2006 and 2011 were investigated (Table 1). These represented 0.32% (14/4,320) of all MRSA isolates submitted to the Irish NMRSARL between 2006 and 2011 and 3.2% (14/431) of non-AR06 isolates (indicative of ST22-MRSA-IV, the predominant MRSA clone in Irish hospitals since 2002) *spa* typed by the Irish NMRSARL during the same period. All isolates exhibited resistance to ampicillin and fusidic acid. M06/0171 was also resistant to mupirocin, neomycin, and tobramycin and was copper resistant with a copper MIC of 4 mM as determined by agar dilution. The remaining 13 isolates were also resistant to copper as determined by disk diffusion (Table 1). Four isolates exhibited resistance to cadmium (Table 1). The isolates exhibited four *dru* types, were assigned to ST779, and belonged to *agr* type III and capsule type 5 (Table 1). All isolates harbored the beta-lactamase resistance gene *blaZ*, the fusidic acid resistance gene *fusC*, and the multidrug-efflux pump gene *sdrM*. The mupirocin and aminoglycoside resistance genes, *mupA* and *aadD*, respectively, were detected in M06/0171 only (Table 1). All isolates harbored the exfoliative toxin gene *etD*; the epidermal cell differentiation inhibitor gene *edinB*; the enterotoxin gene *seb*; the immune evasion cluster (IEC) genes *sak*, *chp*, and *scn* (IEC type B) (39); and genes for microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), adhesion, and biofilm formation. The enterotoxin genes *sed*, *sej*, and *ser* were detected in six isolates (Table 1).

SCC*mec* typing. SCC*mec* typing PCRs were performed on isolate M06/0171 only, while SCC*mec* analysis of the other 13 t878 isolates was performed by DNA microarray profiling (Table 1). Isolate M06/0171 was found to harbor *mecA* by SCC*mec* typing PCR and DNA microarray profiling, but no *mec* regulatory genes were detected by either method. The *mec* complex-associated gene *ugpQ* was detected in M06/0171 using the DNA microarray (Table 1). The *ccrC* gene was detected in M06/0171 following SCC*mec* typing PCR but was ambiguous by DNA microarray, and *ccrAA* was detected by DNA microarray analysis only (Table 1). The *ccrAB4* gene was detected in M06/0171 following SCC*mec* typing PCR using the primers designed by Ruppe et al. and the DNA microarray (Table 1) but was not detected using the primers described by Kondo et al. (31, 33).

For the remaining 13 t878 isolates, the DNA microarray detected the following SCC*mec* genes: *mecA* (13/13 isolates), *ugpQ* (13/13 isolates), *ccrC* (7/13 isolates, including four yielding ambiguous signals), *ccrB4* (12/13 isolates, including five yielding ambiguous signals), and *ccrAA* (10/13 isolates, including seven yielding ambiguous signals) (Table 1).

Identification of a novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element in MRSA isolate M06/0171. Whole-genome sequencing of the ST779/t878 MRSA isolate M06/0171 yielded 89 contigs ranging in size from 216 bp to 226 kb, and 25 of these were >40 kb. Six contigs were identified with SCC*mec*-associated DNA sequences. A novel composite SCC element, which we termed a pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element, was identified. The novel element was ca. 51 kb in size, consisted of 43 ORFs (see Table S2 in the supplemental material), was located at the 3′ end of the *orfX* gene, and was flanked by imperfect direct repeat (DR) and inverted repeat (IR) sequences (Fig. 1, DR-1 and DR-4 and IR-1 and IR-6). Two additional DRs and four additional IRs were identified within the element (Fig. 1, DR-2 and DR-3 and IR-2, IR-3, IR-4, and IR-5) demarcating a three-domain composite element (Fig. 1).

The first SCC region of the novel element consisted of a 16.3-kb pseudo SCC*mec* element located immediately downstream of *orfX* and flanked by DR-1 and DR-2. It consisted of 15 ORFs and was termed a pseudo SCC*mec* element because while a *mec* complex was identified in this 16.3-kb region, there were no *ccr* genes (Fig. 1). The *mec* complex genes exhibited 100% DNA sequence identity to the class C1-like *mec* complex previously identified in SCC*mec* X in MRSA isolate JCSC6945 (GenBank accession number AB505630). However, the *mec* complex genes were transcribed divergently from those in SCC*mec* X (8) but in the same direction as all other *mec* regions described to date (Fig. 1). Additionally, variation was exhibited within the intergenic region between Δ*mecR1* and IS*431* (17-bp deletion in M06/0171), suggesting that two separate insertions of IS*431* had occurred in these two SCC*mec* elements. This *mec* complex consists of *mecA*, a 17-bp Δ*mecR1*, and flanking IS*431* sequences (Fig. 1). The presence of flanking IS*431* sequences as well as the DNA sequence identity to the class C1-like *mec* complex of SCC*mec* X indicated that this *mec* complex should be assigned to class C *mec*. To date, three subtypes of the class C *mec* complex have been reported, class C1 (40), class C2 (41), and class C1-like (8). The Δ*mecR1* in the class C1 *mec* complex has a different truncation site resulting in a different Δ*mecR1* length (73 bp), indicating a separate genetic event from that of the class C1-like *mec* complex; therefore, we propose that the class C1-like *mec* complex be renamed class C3 *mec*. Since the novel *mec* complex in M06/0171 has the same genetic organization as that of the class C3 *mec* complex but (i) is transcribed divergently and (ii) exhibits variation within the intergenic region, we propose that the novel subtype of the class C *mec* complex identified in the present study in M06/0171 be designated class C4 *mec* complex.

In addition to the *mec* complex, genes encoding fusidic acid (*fusC*) and copper resistance were also identified within the pseudo SCC*mec* element. The *fusC* gene exhibited 100% amino acid sequence identity to *fusC* previously identified in SCC$_{fus}$ in methicillin-

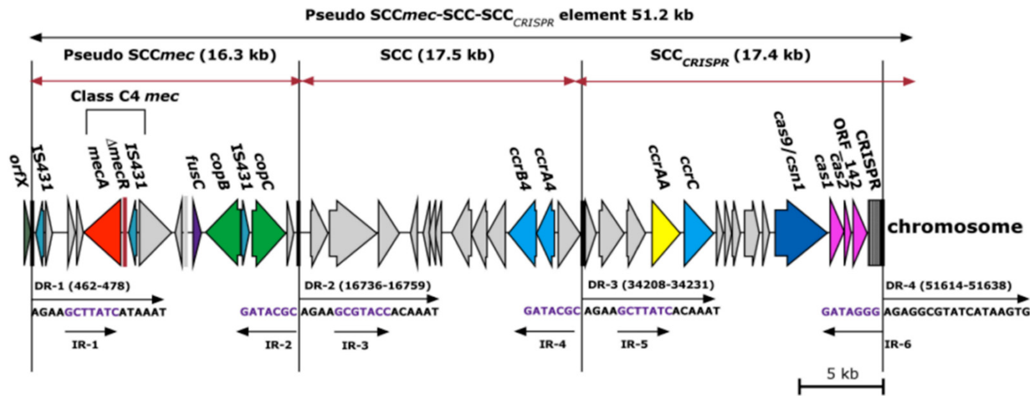

FIG 1 Schematic diagram showing the genetic organization of the novel composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element harbored by the ST779/t878 MRSA isolate M06/0171 (GenBank accession number HE980450). The 51-kb composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element, as well as each of the individual SCC elements of this composite island, is flanked by direct repeat (DR) and inverted repeat (IR) sequences. The methicillin, fusidic acid, and copper resistance genes *mecA*, *fusC*, and *copB*/*copC* are shown in red, purple, and green, respectively. The *ccrAB4* and *ccrC* genes are shown in blue, the *ccrAA* gene is shown in yellow, and the clustered regularly interspaced short palindromic repeats (CRISPRs) and the genes encoding CRISPR-associated proteins (*cas9/csn1*, *cas1*, *cas2*, and ORF_142) are shown in pink. The direction of transcription for each ORF is indicated.

susceptible *S. aureus* (MSSA) isolate MSSA*476* (YP_042173) (12). Two ORFs associated with copper resistance, which we have designated *copB* and *copC*, were located downstream of *fusC*. The *copB* gene exhibited 99% amino acid sequence similarity to an annotated ORF encoding a copper-exporting ATPase in *Staphylococcus epidermidis* strain VCU120 (EHR82803), and the *copC* gene exhibited 100% amino acid sequence identity to an unannotated copper transport gene previously identified in an SCC*mec* X element in the MRSA strain JCSC6945 (BAK53188) (8) (Fig. 1).

The second SCC region, located immediately downstream from the pseudo SCC*mec* element and flanked by direct repeats DR-2 and DR-3, consisted of a 17.5-kb SCC element with 13 ORFs, including *ccrAB4* (Fig. 1). The *ccrA4* gene exhibited 93% amino acid sequence identity to *ccrA4* harbored by the *S. aureus* strain CHE482 (ABL75417), and the *ccrB4* gene exhibited 98% amino acid sequence identity to *ccrB4* harbored by the *Staphylococcus haemolyticus* strain MCS13 (BAJ53095). We have designated the *ccrA4* and *ccrB4* genes as allele 6 in each case, considering that five alleles of the *ccrA4* and *ccrB4* genes have already been described in *S. aureus* and CoNS (15, 19, 20, 42). We recommend assigning each of these previously described *ccrA4* and *ccrB4* alleles an allelic number 1 to 5 in order of publication.

The third SCC region, located immediately downstream of the SCC element and flanked by DR-3 and DR-4, consisted of a 17.4-kb SCC element with 14 ORFs (Fig. 1). This SCC region harbored a *ccrC1* gene with 95% amino acid sequence identity to *ccrC1* harbored by *S. aureus* strain UMCG-M4 (ADC79473), *S. aureus* strain S0385 (YP_005732860), and *Staphylococcus pseudintermedius* strain AVDL-32616 (ACT82836). We have designated this as allotype *ccrC1* and allele *ccrC11*, considering that alleles *ccrC1* to *-10* of the *ccrC1* allotype have been previously reported (18). The final SCC region also carried a clustered regularly interspersed short palindromic repeat (CRISPR) region and four CRISPR-associated genes (*cas9/csn1*, *cas1*, *cas2*, and ORF_142) (Fig. 1). However, the *cas* genes exhibited the highest amino acid sequence similarity (46 to 70%) to those in *Staphylococcus lugdunensis* (NZ_AEQA01000016). The CRISPR region consists of clustered regularly interspaced short palindromic repeats that are generally segments of DNA captured from viral or plasmid sequences and are located between the conserved direct repeat sequences of the CRISPR region (43). Analysis of the DNA sequences of the variable interspersed sequences in this CRISPR region using the online tool CRISPRfinder revealed the most probable origins of each individual variable interspersed sequence (Table 2). Twelve interspersed repeats were identified, and the most common similarity detected was that to *S. haemolyticus* with 4/12 repeats exhibiting between 93% and 100% DNA sequence identity.

Confirmation of the presence of the pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element in other ST779/t878 MRSA isolates. The presence of the novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element identified in M06/0171 was confirmed in the 13 additional ST779/t878 MRSA isolates by PCR using previously described primers to amplify *ccrAB4* and *ccrC* and novel primers to amplify CRISPR and the *mec* complex (see Table S1 in the supplemental material). All isolates yielded amplimers of the expected size compared to M06/0171. Sequencing of the amplimers obtained for *ccrAB4* and *ccrC* revealed that the 13 additional ST779/t878 MRSA isolates harbored *ccrAB4* and *ccrC* genes identical to each other and to those of M06/0171. Sequencing of amplimers obtained following amplification of the CRISPR region in 5/13 isolates revealed that they harbored CRISPR regions identical to each other and to that of M06/0171.

DISCUSSION

The present study reports the emergence of ST779/t878 MRSA harboring a novel 51-kb pseudo SCC*mec* composite island (CI) in Ireland. In-depth molecular analysis revealed that the novel CI consisted of three distinct and unique domains, each demarcated by direct repeat sequences. The first domain was a pseudo SCC*mec* with a novel *mec* complex, a fusidic acid resistance gene (*fusC*), and two copper resistance genes but lacking *ccr* genes. The second domain was an SCC with a novel *ccrAB4* allele, whereas the third element was an SCC with a novel *ccrC* allele and a CRISPR region. Comparative sequence analysis of the novel pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element suggested that this CI may have originated in bacterial species and genera other than *S. aureus* and *Staphylococcus*, respectively. First, for some of the ORFs identified

TABLE 2 Highest probable matches for the 13 variable interspersed DNA sequences in the clustered regularly interspaced short palindromic repeat (CRISPR) region in ST779/t878 MRSA isolates[a] generated using the online CRISPRfinder software tool

CRISPR repeat no.	BLASTn result[b]	GenBank accession no.	% DNA sequence similarity	% query coverage
1	*Phenylobacterium zucineum* HLK1 plasmid	CP000748	100	60
2	*Geobacillus thermoleovorans*	CP003125	100	86
3	*Megamonas hypermegale*	FP929048	100	66
4	*Bacteroides xylanisolvens* XB1A	FP929033	100	100
5	*Staphylococcus haemolyticus*	AP006716	100	93
6	*S. haemolyticus*	AP006716	100	93
7	*Shewanella piezotolerans*	CP000472	100	80
8	*S. haemolyticus*	AP006716	93	100
9	*Escherichia blattae*	CP001560	100	56
10	*Methylophaga* sp.	CP003380	100	70
11	*S. epidermidis* plasmid	GQ900454	100	86
12	*S. haemolyticus*	AP006716	100	93

[a] The CRISPR regions of six isolates were sequenced (isolates M06/0171, M09/0295, M08/0422, M11/0208, M09/0302, and E4449).
[b] The BLASTn algorithm was used to search for similar sequences in GenBank compared to each of the variable interspersed repeats in the CRISPR region in the novel SCC$_{CRISPR}$ element.

within the CI the highest amino acid identity was to ORFs from non-*S. aureus* staphylococcal species. In addition, the interspersed sequences of the CRISPR region located within the CI exhibited the highest DNA sequence identity to CoNS and to other genera. Taken together, these data provide further evidence for SCC*mec* diversity and indicate that genetic transfer between *S. aureus*, CoNS, and possibly other bacterial genera contributes to the emergence of novel SCC*mec*/SCC elements and CIs and ultimately to the emergence of novel MRSA strains.

While SCC elements lacking *mecA* have been reported previously (29), to the best of our knowledge there have been only two previous reports of SCC elements harboring *mecA* but lacking *ccr* genes in MRSA (44, 45). The presence of *ccr* genes on the adjacent SCC elements in ST779 MRSA suggests that even though the pseudo SCC*mec* lacks *ccr* genes, it and possibly the entire CI may be mobilized using the *ccr* genes on either of the adjacent SCC elements. This type of mechanism has been suggested previously as a means of mobilization of the arginine catabolic mobile element (ACME) (46).

The present study highlights the difficulties associated with SCC*mec* typing both using DNA microarray profiling and using conventional SCC*mec* typing PCRs. The distant amino acid sequence similarity (93 to 95%) of *ccrA4*, *ccrB4*, and *ccrC* to their closest counterparts resulted in negative and/or ambiguous results by DNA microarray analysis for *ccrAB4* and *ccrC* genes among the 14 ST779 MRSA isolates as well as no amplimers for *ccrAB4* using the multiplex SCC*mec* typing PCR of Kondo et al. (31). It was only by using *ccrAB4* primers and PCRs that detect additional alleles that the *ccrAB4* allele of the novel CI was detected. Updating currently used multiplex SCC*mec* typing PCRs to detect all *ccr* alleles identified to date would enhance detection of this and other recently described SCC*mec* elements (4, 8). The abundance of SCC*mec* elements in *S. aureus* and other staphylococci and the diversity evident within SCC*mec* elements constitute a challenge for SCC*mec* nomenclature, for which guidelines have been published by the IWG-SCC (5, 7), but these are not always adhered to.

An unusual feature within the pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ was the presence of the CRISPR/*cas* region. The CRISPR region is a recently described class of repetitive DNA element, and it and the CRISPR-associated genes (*cas*) are involved in the protection of the bacterial genome against foreign invading DNA, i.e., viral and plasmid DNA (43, 47). The CRISPR region has been identified in approximately 40% of prokaryotes and 90% of archaea, and multiple distinct CRISPR loci have been located on prokaryotic genomes (47). The CRISPR region consists of multiple short nucleotide repeat sequences, varying from 21 to 37 bp in length, separated by unique variable spacer sequences which originate from phage DNA and provide a record of genetic encounters (38). The *cas* genes are involved in cleavage of the CRISPR RNA precursor in each repeat, and the resulting cleaved products act as leaders for other *cas* gene protein products, guiding them to foreign invading DNA (47). It remains unclear how segments of foreign invading DNA are incorporated into the CRISPR region (47). The relatedness of the *cas* genes can vary within a bacterial species as well as between different species, and particular CRISPR/*cas* loci are associated with particular strains within a species (47). Interestingly, all ST779 MRSA isolates investigated in the present study appeared to harbor the same CRISPR region. The CRISPR/*cas* region is uncommon among staphylococci, though it has been detected in one CC75/ST1850-MRSA-IVa isolate, an *S. epidermidis* isolate harboring SCC*mec* II, and in several *S. lugdunensis* isolates (43, 48, 49), and in each case the CRISPR region was located downstream of the SCC*mec* element. CRISPR has also been detected previously in a novel SCC*mec* V subtype harbored by four ST398 MRSA isolates (50). The same *cas* region gene organization detected in the ST779 MRSA isolate M06/0171 in the present study is present in *S. lugdunensis* strain M23590, i.e., *cas9*, *cas1*, *cas2*, and a *cas*-associated gene (ORF_142). However, they exhibit low amino acid sequence similarity, and the CRISPR region is upstream of the *cas* genes in the *S. lugdunensis* strain.

Comparison of the CRISPR region identified in the ST779 MRSA isolates with available staphylococcal CRISPR region sequences in GenBank revealed that the ST779 MRSA CRISPR region has a significantly higher number of variable spacer regions than those described previously (12 compared with 2 to 6 spacer regions). Additionally, each spacer sequence was unique to the staphylococcal strain in which it was reported. The genetic diversity of the CRISPR spacer sequences has been reported previously, and it has been suggested that CRISPR loci have potential for typing of strains and microbial populations. However, the use of a

given CRISPR locus for typing and epidemiological analysis has to be critically assessed due to its rarity in staphylococci and the various rates of polymorphisms within this region (47). The role of the CRISPR/*cas* locus in ST779 MRSA requires further investigation, to determine which of the *cas* genes are responsible for acquiring additional variable spacer regions and which of the *cas* genes are responsible for spacer lead targeted defense against foreign DNA.

Whether ST779 MRSA will become a more widespread MRSA clone remains to be determined, but it is possible that the novel composite element harbored by this clone may confer advantageous attributes in addition to methicillin resistance, such as copper or fusidic acid resistance or resistance or immunity to foreign invading DNA encoded by CRISPR. Several other isolates exhibiting CC779/ST779 or closely related STs have been reported previously, indicating their sporadic presence in Australia (WA-MRSA-100) Canada, Germany, Thailand, the United Arab Emirates, and the United Kingdom (http://saureus.mlst.net/) (13, 51). Ongoing surveillance of ST779/t878 MRSA with the novel pseudo SCC*mec*-SCC-SCC_{CRISPR} element is warranted. SCC*mec* typing methods will need to be updated to ensure successful detection and monitoring of this and other emerging MRSA strains. The identification of a novel pseudo SCC*mec*-SCC-SCC_{CRISPR} element exhibiting sequence similarity to non-*S. aureus* staphylococci as well as to other genera further indicates the potential role that other organisms may play in the emergence of novel SCC*mec* elements in MRSA.

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