Emergence of Sequence Type 779 Methicillin-Resistant *Staphylococcus aureus* Harboring a Novel Pseudo Staphylococcal Cassette Chromosome *mec* (SCC*mec*)-SCC-SCC<sup>CRISPR</sup> 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 *crr* 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.
gene complex (A to E) have been reported to date in staphylococci (7) (www.sccmec.org). The SCCmec-carried cer genes are necessary for precise integration and excision of the SCCmec element, and three genes (cerA, cerB, and cerC) have been described. Novel cer 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 cer genes into consideration. Each cer complex consists of either the cerA and cerB genes together or cerC and an associated open reading frame (ORF), previously termed cerAA (13), which is located directly upstream of cerC and exhibits between 35 and 41% DNA sequence similarity to cer genes cerA, cerB, and cerC. Eight types of the cer gene complex have been reported to date in MRSA, each with a different combination of cerA and cerB alleles or cerC (7) (www.sccmec.org). Numerous allelic variants of each of the cer allototypes 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 cerA4 and cerB4 allototypes have been reported without designated allelic prefixes and 10 alleles of the cerC1 allototype (cerC1 to cerC10) 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 SCCmec 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 2000 and 2006 (Table 1). M06/0171 exhibited type t878, but its SCCmec-t878 MRSA isolate M06/0171 was recovered in 2006 in an Irish pediatric hospital and was initially identified as part of an investigation of its SCCmec element. The database of isolates submitted to the Irish National MRSA Reference Laboratory (NMRSARL) was subsequently examined for other Molecular typing. All isolates underwent direct repeat unit (dru) typing, while M06/0171 was also subjected to multilocus sequence typing (MLST) and SCCmec typing, all as described previously (24, 28–30). SCCmec 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 cer complexes (31); and (iii) the joining or “J” regions (32). An additional simplex PCR using alternative ccrAB4 primers described previously by Ruppre 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 335 S. aureus gene sequences and alleles, including species-specific, antimicrobial resistance genes; virulence-associated genes; and typing markers and SCCmec-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 SCCmec element. High-throughput de novo sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, United Kingdom) using the Illumina genome analyzer system (Illumina, San Diego, CA). 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 SCCmec-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://prodigalornal.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 SCCmec-SCC-SCCmec 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{	extit{mec}}-SCC{	extit{CrrS}}-SCC{	extit{CRISPR}} element recovered in Irish hospitals between 2006 and 2011

<table>
<thead>
<tr>
<th>Hospital no.</th>
<th>Isolate no.</th>
<th>Yr of isolation</th>
<th>Age</th>
<th>Clinical details (sex)</th>
<th>Antimicrobial resistance pattern&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Antimicrobial resistance genes&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Virulence-associated genes&lt;sup&gt;d&lt;/sup&gt;</th>
<th>DNA microarray analysis&lt;sup&gt;e&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>H1</td>
<td>M00/0171</td>
<td>2006</td>
<td>3 y</td>
<td>Burn unit (female)</td>
<td>AMP, COP, FUS, MUP, NEO, TOB</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;18a&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;, aid&lt;sub&gt;D&lt;/sub&gt;, mupA&lt;sub&gt;A&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H2</td>
<td>E4233</td>
<td>2009</td>
<td>45 y</td>
<td>BSI (female)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;18a&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H3</td>
<td>M11/0114</td>
<td>2011</td>
<td>5 d</td>
<td>Screening sample, baby of patient from whom M11/0118 was recovered (N/A)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;18a&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H3</td>
<td>M11/0118</td>
<td>2011</td>
<td>30 y</td>
<td>Screening sample, mother of baby from whom M11/0114 was recovered (female)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;18a&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H4</td>
<td>E4449</td>
<td>2010</td>
<td>39 y</td>
<td>BSI (male)</td>
<td>AMP, COP, CAD&lt;sup&gt;d&lt;/sup&gt;, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H4</td>
<td>E2998</td>
<td>2006</td>
<td>54 y</td>
<td>BSI (male)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H5</td>
<td>E4550</td>
<td>2010</td>
<td>55 y</td>
<td>BSI (female)</td>
<td>AMP, COP, CAD&lt;sup&gt;d&lt;/sup&gt;, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
</tr>
<tr>
<td>H6</td>
<td>M11/0208</td>
<td>2011</td>
<td>18 y</td>
<td>Dermatology clinic (male)</td>
<td>AMP, COP, CAD&lt;sup&gt;d&lt;/sup&gt;, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<td>M08/0422</td>
<td>2008</td>
<td>24 y</td>
<td>Screening sample (female)</td>
<td>AMP, COP, CAD, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<td>H8</td>
<td>M07/0307</td>
<td>2007</td>
<td>Stillborn</td>
<td>Stillborn baby postmortem (N/A)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<td>H9</td>
<td>M09/0295</td>
<td>2009</td>
<td>41 y</td>
<td>Screening sample (male)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<td>H10</td>
<td>E4709</td>
<td>2010</td>
<td>54 y</td>
<td>BSI (female)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11y&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<tr>
<td>H11</td>
<td>M09/0302</td>
<td>2009</td>
<td>46 y</td>
<td>Screening sample (male)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;10a&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<td>H12</td>
<td>E4217</td>
<td>2009</td>
<td>59 y</td>
<td>BSI (male)</td>
<td>AMP, COP, FUS</td>
<td>mecA, ugpQ, ccrAA, ccrC&lt;sup&gt;f&lt;/sup&gt;, ccrA&lt;sub&gt;B&lt;/sub&gt;, ccrB&lt;sub&gt;4&lt;/sub&gt;, tdr&lt;sub&gt;11b&lt;/sub&gt;</td>
<td>blaZ, fnf&lt;sub&gt;C&lt;/sub&gt;, sdr&lt;sub&gt;M&lt;/sub&gt;</td>
<td>seh, sak, chp, scn, etD, adnB, cffB, sdrD</td>
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<sup>a</sup>Age of patient; y, years; d, days.

<sup>b</sup>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 (Van) [25].

<sup>c</sup>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].

<sup>d</sup>These isolates exhibited intermediate resistance to cadmium acetate.

<sup>e</sup>SCCM<textit{mec}>, antimicrobial resistance and virulence-associated genes were detected using the StaphType DNA microarray kit (Aere, Germany) [34]. ccrA<sub>A</sub> is a known ccrC-linked gene with 35 to 41% DNA sequence homology to other ccr genes.

<sup>f</sup>The following MSCRAMM, adhesion, and biofilm formation genes were detected in all 14 ST779/1878 MRSA isolates by DNA microarray analysis: icaA, icaC, icaD, bblp, cfbA, ebb, ebbS, eno, fbi, fmbA, fmbB, sdr<sub>C</sub>, vwb, and sa<sub>G</sub>.

<sup>g</sup>Antibodies or negative DNA microarray signals were obtained for the genes and isolates indicated. The presence of seh, cffB, and sdrD was confirmed in all 14 MRSA isolates by PCR.

<sup>h</sup>N/A, information not available.

<sup>i</sup>BSI, bloodstream infection.
was detected by DNA microarray analysis only (Table 1). The ccrAB4 gene was detected in M06/0171 following SCCmec 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 SCCmec 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 SCCmec-SCC-SCC-CCCRISPR 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 SCCmec-associated DNA sequences. A novel composite SCC element, which we termed a pseudo SCCmec-SCC-SCC-CCCRISPR 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 SCCmec element located immediately downstream of orfX and flanked by DR-1 and DR-2. It consisted of 15 ORFs and was termed a pseudo SCCmec 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 SCCmec X in MRSA isolate JCSC6945 (GenBank accession number AB505630). However, the mec complex genes were transcribed divergently from those in SCCmec 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 ΔmecR1 and IS431 (17-bp deletion in M06/0171), suggesting that two separate insertions of IS431 had occurred in these two SCCmec elements. This mec complex consists of mecA, a 17-bp ΔmecR1, and flanking IS431 sequences (Fig. 1). The presence of flanking IS431 sequences as well as the DNA sequence identity to the class C1-like mec complex of SCCmec 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 ΔmecR1 in the class C1 mec complex has a different truncation site resulting in a different ΔmecR1 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 SCCmec element. The fusC gene exhibited 100% amino acid sequence identity to fusC previously identified in SCCmec in methicillin-
FIG 1 Schematic diagram showing the genetic organization of the novel composite pseudo SCCmec-SCC-SCC\textsubscript{CRISPR} element harbored by the ST779/t878 MRSA isolate M06/0171 (GenBank accession number HE980450). The 51-kb composite pseudo SCCmec-SCC-SCC\textsubscript{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 \textit{mecA}, \textit{fusC}, and \textit{copp} are shown in red, purple, and green, respectively. The \textit{ccrAB} and \textit{ccrC} genes are shown in blue, the \textit{ccrAA} gene is shown in yellow, and the clustered regularly interspersed short palindromic repeats (CRISPRs) and the genes encoding CRISPR-associated proteins (\textit{cas9/csn1}, \textit{cas1}, \textit{cas2}, and \textit{orf_142}) are shown in pink. The direction of transcription for each ORF is indicated.

susceptible \textit{S. aureus} (MSSA) isolate MSSA476 (YP_042173) (12). Two ORFs associated with copper resistance, which we have designated \textit{coppB} and \textit{coppC}, were located downstream of \textit{fusC}. The \textit{coppB} gene exhibited 99% amino acid sequence similarity to an annotated ORF encoding a copper-exporting ATPase in \textit{Staphylococcus epidermidis} strain VCU120 (EHR82803), and the \textit{coppC} gene exhibited 100% amino acid sequence identity to an unannotated copper transport gene previously identified in an SCC\textit{mec} X element in the MRSA strain JCSC6945 (BAK53188) (8) (Fig. 1).

The second SCC region, located immediately downstream from the pseudo SCC\textit{mec} element and flanked by direct repeats DR-2 and DR-3, consisted of a 17.5-kb SCC element with 13 ORFs, including \textit{ccrAB} (Fig. 1). The \textit{ccrA} gene exhibited 93% amino acid sequence identity to \textit{ccrA} harbored by the \textit{S. aureus} strain CHE482 (ABL75417), and the \textit{ccrB} gene exhibited 98% amino acid sequence identity to \textit{ccrB} harbored by the \textit{Staphylococcus haemolyticus} strain MCS13 (BAJ53095). We have designated the \textit{ccrA} and \textit{ccrB} genes as allele 6 in each case, considering that five alleles of the \textit{ccrA} and \textit{ccrB} genes have already been described in \textit{S. aureus} and CoNS (15, 19, 20, 42). We recommend assigning each of these previously described \textit{ccrA} and \textit{ccrB} 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 \textit{ccrC} gene with 95% amino acid sequence identity to \textit{ccrC} harbored by the \textit{S. aureus} strain UMCG-M4 (ADCT79473), \textit{S. aureus} strain S0385 (YP_005732860), and \textit{Staphylococcus pseudintermedius} strain AVDL-32616 (ACT82830). We have designated this as allele \textit{ccrC} and allele \textit{ccrC} (Fig. 1), considering that alleles \textit{ccrC} to -10 of the \textit{ccrC} allele 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 (\textit{cas9/csn1}, \textit{cas1}, \textit{cas2}, and \textit{orf_142}) (Fig. 1). However, the \textit{cas} genes exhibited the highest amino acid sequence similarity (46 to 70%) to those in \textit{Staphylococcus lugdunensis} (NZ_AEQA01000016). The CRISPR region consists of clustered regularly interspersed 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 \textit{S. haemolyticus} with 4/12 repeats exhibiting between 93% and 100% DNA sequence identity.

**Confirmation of the presence of the pseudo SCCmec-SCC-SCC\textsubscript{CRISPR} element in other ST779/t878 MRSA isolates.**

The presence of the novel pseudo SCCmec-SCC-SCC\textsubscript{CRISPR} element identified in M06/0171 was confirmed in the 13 additional ST779/t878 MRSA isolates by PCR using previously described primers to amplify \textit{ccrAB} and \textit{ccrC} variable regions (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 \textit{ccrAB} and \textit{ccrC} revealed that the 13 additional ST779/t878 MRSA isolates harbored \textit{ccrAB} and \textit{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 SCCmec 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 SCCmec with a novel \textit{mec} complex, a fusidic acid resistance gene (\textit{fusC}), and two copper resistance genes but lacking \textit{ccr} genes. The second domain was an SCC with a novel \textit{ccrAB} allele, whereas the third element was an SCC with a novel \textit{ccrC} allele and a CRISPR region. Comparative sequence analysis of the novel pseudo SCCmec-SCC-SCC\textsubscript{CRISPR} element suggested that this CI may have originated in bacterial species and genera other than \textit{S. aureus} and \textit{Staphylococcus}, respectively. First, for some of the ORFs identified...
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 SCCmec diversity and indicate that genetic transfer between S. aureus, CoNS, and possibly other bacterial genera contributes to the emergence of novel SCCmec/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 pseud SCCmec 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 SCCmec typing both using DNA microarray profiling and using conventional SCCmec typing PCRs. The distant amino acid sequence similarity (93 to 95%) of ccrA, ccrB, and ccrC to their closest counterparts resulted in negative and/or ambiguous results by DNA microarray analysis for ccrAB and ccrC genes among the 14 ST779 MRSA isolates as well as no amplifiers for ccrAB by using the multiplex SCCmec 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 SCCmec typing PCRs to detect all ccr alleles identified to date would enhance detection of this and other recently described SCCmec elements (4, 8). The abundance of SCCmec elements in S. aureus and other staphylococci and the diversity evident within SCCmec elements constitute a challenge for SCCmec 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 pseud SCCmec-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 SCCmec II, and in several S. lugdunensis isolates (43, 48, 49), and in each case the CRISPR region was located downstream of the SCCmec element. CRISPR has also been detected previously in a novel SCCmec 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 mepillin resistance, such as copper or fusidic acid resistance or resistance 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 ST779/1878 MRSA with the novel pseudo SCCmec-SCC-SCC_Craspe element is warranted. SCCmec 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 SCCmec-SCC-SCC_Craspe 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 SCCmec elements in MRSA.

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REFERENCES


