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Characterization of a Novel Arginine Catabolic Mobile Element (ACME) and Staphylococcal Chromosomal Cassette *mec* Composite Island with Significant Homology to *Staphylococcus epidermidis* ACME type II in Methicillin-Resistant *Staphylococcus aureus* Genotype ST22-MRSA-IV

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ABSTRACT

The arginine catabolic mobile element (ACME) is prevalent among ST8-MRSA-IVa (USA300) isolates and evidence suggests that ACME enhances the ability of ST8-MRSA-IVa to grow and survive on its host. ACME has been identified in a small number of isolates belonging to other MRSA clones but is widespread among coagulase-negative staphylococci (CoNS). This study reports the first description of ACME in two distinct strains of the pandemic ST22-MRSA-IV clone. A total of 238 MRSA isolates recovered in Ireland between 1971 and 2008 were investigated for ACME using a DNA microarray. Twenty-three isolates (9.7%) were ACME-positive, all were either MRSA genotype ST8-MRSA-IVa (7/23, 30%) or ST22-MRSA-IV (16/23, 70%). Whole-genome sequencing and comprehensive molecular characterization revealed the presence of a novel 46-kb ACME and SCC*mec* composite island (ACME/SCC*mec*-CI) in ST22-MRSA-IVh isolates (*n* = 15). This ACME/SCC*mec*-CI consists of a 12-kb DNA region previously identified in ACME type II in *S. epidermidis* ATCC 12228, a truncated copy of the J1 region of SCC*mec* I and a complete SCC*mec* IVh element. The composite island has a novel genetic organization with ACME located within *orfX* and SCC*mec* located downstream of ACME. One *pvl*-positive ST22-MRSA-IVa isolate carried ACME located downstream of SCC*mec* IVa as previously described in ST8-MRSA-IVa. These results suggest that ACME has been acquired by ST22-MRSA-IV on two independent occasions. At least one of these instances may have involved horizontal transfer and recombination events between MRSA and CoNS. The presence of ACME may enhance dissemination of ST22-MRSA-IV, an already successful MRSA clone.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of infection among patients in hospitals and in the community worldwide. The success of MRSA is partly due to its ability to adapt rapidly and to survive mainly through the acquisition and expression of exogenous genes encoded by plasmids, bacteriophages and other mobile genetic elements from other *S. aureus* strains and from coagulase-negative staphylococci (CoNS) (2, 9, 11, 20).

ST8-MRSA-IVa (also known as USA300) is the predominant community-acquired (CA)-MRSA strain in the USA where its incidence is also increasing in healthcare settings but this clone has also been recovered in many other countries worldwide (28). The extensive spread and success of ST8-MRSA-IVa has been partially attributed to the presence of a mobile genetic element termed the arginine catabolic mobile element (ACME) which is thought to play an important role in its growth and survival (3). While most ST8-MRSA-IVa isolates identified to date contain ACME (8, 15, 28), it has only been identified in a small number of other MRSA genotypes including ST5-MRSA-II (4, 8), ST59-MRSA-IVa (4), ST97-MRSA-V (5), ST1-MRSA-IVa (5), ST5-MRSA-IV (6) and ST239-MRSA-III (7) and among just two ST8-methicillin-susceptible *S. aureus* isolates (8). ACME has also been identified among CoNS including *S. epidermidis*, *S. haemolyticus* and *S. capitis* where it appears to be more prevalent and to have a more diverse genetic organization than in *S. aureus* (1, 3, 13, 21).

The ACME elements described in detail to date range in size from 31 kb in ST8-MRSA-IVa to 34 kb in *S. epidermidis* (3). They are integrated downstream of the staphylococcal chromosomal cassette (SCC) harboring the methicillin resistance gene *mecA* (SCC*mec*) and use the same attachment site for integration within *orfX* as SCC*mec*. Similar to SCC*mec* elements, ACME is flanked by repeat sequences, and SCC*mec*-encoded cassette chromosome recombinase (*ccr*) genes catalyze integration and excision of ACME from the staphylococcal chromosome (3).

ACME exists as a composite island with SCC*mec* IVa in ST8-MRSA-IVa and within the staphylococcal composite island SCC-CI in *S. epidermidis* strain ATCC 12228 (3).

The two main gene clusters identified in ACME include the *arc* genes (*arcA*, *B*, *C* and *D*) and the oligopeptide permease operon (*opp*) genes (*opp-3A, B, C, D* and *E*) (3). Both *arc* and *opp* are homologs of genes that are recognized bacterial virulence factors (3). The ACME-*arc* genes encode a complete arginine deiminase pathway that converts L-arginine to carbon dioxide, ATP and ammonia. Arginine deiminase is a virulence factor in the human pathogen *Streptococcus pyogenes* (3). All *S. aureus* isolates carry an *arc* operon on the chromosome. Diep *et al*. (2006) speculated that the presence of a second ACME-encoded *arc* operon in ST8-MRSA-IVa may enhance the ability of ST8-MRSA-IVa to grow and survive within its host. Similar to the *arc* operon, all *S. aureus* isolates have native *opp* operons (*opp-1* and *opp-2*) which are also found in many other bacterial species and encode ABC transporter systems (3). Disruption of *opp-1* and *-2* has been shown to result in significant growth defects and attenuated virulence in *S. aureus* (3). The precise function of ACME has not yet been determined but studies using animal models have shown that while ACME does not directly enhance virulence in ST8-MRSA-IVa, it does improve its fitness and ability to colonize skin and mucous membranes (4, 19).

Three ACME allotypes have been described to date in staphylococci. Type I ACME habors both the *arc* and *opp-3* gene clusters and has been identified in MRSA and *S. epidermidis* (1, 3, 13). Type II ACME habors the *arc* genes but lacks *opp-3* while type III harbors *opp-3* but lacks the *arc* genes (3, 13). To date, types II and III ACME have only been identified in *S. epidermidis* and variants of ACME I, II and III have also been identified in *S. epidermidis* (1, 13).

MRSA have been endemic in Irish hospitals for more than three decades and different MRSA clones have emerged, spread and predominated during different time periods (e.g. ST250-MRSA-I/I-*pls*, ST239-MRSA-III/III-p*1258*/Tn*554*, ST8-MRSA-IIA–E and ST22-MRSA-IV in the 1970s, 1980s, 1990s and 2000s, respectively) (24). Since 2002, isolates belonging to the pandemic MRSA clone ST22-MRSA-IV have predominated and now account for more than 80% of MRSA isolates recovered from patients in Irish hospitals (26). CA-MRSA isolates harboring the Panton-Valentine leukocidin genes *lukF-PV* and *lukS-PV* have also been recovered in Ireland and belong predominantly to the ST8-MRSA-IV and ST30-MRSA-IV genotypes but *pvl*-positive ST22-MRSA-IV, ST80-MRSA-IV and ST154-MRSA-IV isolates have also been recognized (22).

The purpose of the present study was to investigate hospital-acquired (HA)- and CA-MRSA isolates from Ireland for the presence of ACME. The results of this investigation identified, for the first time, ACME in ST22-MRSA-IV. Comprehensive molecular characterization identified a novel ACME and SCC*mec* composite island (SCC*mec*-CI) with DNA sequence identity with regions of ACME type II previously only described in *S. epidermidis*, the J1 region of SCC*mec* type I and a SCC*mec* type IVh element together with a novel genomic organization of ACME and SCC*mec*.

MATERIALS AND METHODS

MRSA isolates. A total of 238 MRSA isolates recovered in Ireland between 1971 and 2008 were investigated for the presence of ACME. Isolate details are shown in Table 1. Isolates representative of diverse genetic backgrounds were selected and comprised (i) 107 MRSA isolates representative of each different antibiogram-resistogram (AR) type, multilocus sequence type (MLST) and SCC*mec* type combination identified among MRSA isolates recovered from patients in Irish hospitals between 1971 and 2002 (24), (ii) 25 MRSA isolates representative of the MLST and SCC*mec* type combinations identified among *pvl*-positive MRSA isolates recovered from patients in Ireland between 1999 and 2005 (22) and (iii) 106 MRSA isolates recovered from patients and environmental sites in four wards of a 700-bed acute hospital in Dublin in 2007 and 2008 (26).

All isolates were previously typed by AR typing, pulsed-field gel electrophoresis (PFGE) and SCC*mec* typing as described elsewhere (22, 24, 26). Isolates carrying the SCC*mec* IV element recovered in 2007 and 2008 had previously undergone SCC*mec* IV subtyping for subtypes IVa, IVb, IVc, IVd, IVE, IVF, IVg and IVh (Table 1) (26). All *pvl*-positive SCC*mec* IV isolates had previously been subtyped for SCC*mec* IVa–IVF only (22) but any non-subtypeable isolates were subtyped as part of the present study using the method of Milheirico *et al.* (2007) (12) that also recognizes SCC*mec* IV subtypes IVg and IVh (Table 1). Isolates recovered between 1971 and 2002 had not previously undergone SCC*mec* IV subtyping but as part of the present study they were also subtyped using the method of Milheirico *et al.* (2007) (12) (Table 1). All *pvl*-positive isolates and isolates recovered between 1971 and 2002 had previously undergone MLST (22, 24). Isolates recovered in 2007 and 2008 previously underwent typing by sequencing the SCC*mec*-associated direct repeat unit (*dru*) and the staphylococcal protein A (*spa*) gene (26). One isolate representative of each *spa* type underwent MLST and the sequence type (ST) of all other isolates belonging to the same *spa* type was inferred from this ST (26).

DNA microarray analysis. All isolates were investigated using a DNA microarray system, the StaphyType Kit (Alere Technologies GmbH, Jena, Germany). The StaphyType Kit consists of individual DNA microarrays mounted in 8-well microtiter strips which detect 334 *S. aureus* gene sequences and alleles including species-specific, antimicrobial resistance and virulence-associated genes, and typing markers. Virulence genes investigated include the ACME-*arcA*, *arcB*, *arcC* and *arcD* genes (hereafter referred to as ACME-*arc*). The ArrayMate software (Alere Technologies) which was used to analyze data generated by the microarray system can assign isolates to inferred MLST STs and/or clonal complexes (CCs) by comparing the DNA microarray results of the test isolates to microarray profiles from a collection of reference strains held in the ArrayMate database that have been previously typed by MLST (16, 17). Genomic DNA was extracted from all isolates by enzymatic lysis and the Qiagen DNeasy kit (Qiagen, Crawley, West Sussex, UK) as described previously (16). The DNA microarray procedures have been described previously and were performed according to the manufacturer's instructions (14, 16).

Molecular typing of ACME-*arc*-positive MRSA isolates. All ACME-*arc*-positive MRSA isolates that had not previously been typed by *spa* and *dru* typing were subjected to *spa* and *dru* typing as described previously (26, 27). Any ACME-*arc*-positive isolate that had not previously undergone MLST analysis has its ST assigned using the DNA microarray.

ACME-*arcA* PCR and sequencing. Previously described primers (3) and conditions (5) were used to confirm the presence of the ACME-*arcA* gene in all ACME-*arc*-positive isolates detected by microarray analysis. DNA fragments were obtained by PCR amplification of chromosomal DNA using GoTaq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions. PCR products were visualized by agarose gel electrophoresis.

Whole genome sequencing and PCR to close gaps between contigs. The whole genome of one MRSA isolate representative of the predominant *spa*, *dru* and SCC*mec* type combination of ST22-MRSA-IV isolates identified as harboring ACME-*arc* genes (M08/0126) was sequenced to determine the location and genetic organization of ACME. High-throughput de novo sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, UK) using the Illumina Genome Analyzer System (Illumina, Essex, UK). Contigs were analyzed using the Artemis DNA sequence viewer and annotation tool (23) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Contigs that were identified as containing ACME or SCC*mec*-associated DNA sequences were aligned using the BioNumerics (version 5.1) (Applied Maths, Ghent, Belgium) and DNA Strider (version 1.3f11) (CEA Saclay, Gif-sur-Yvette, France) software packages. Any gaps identified between ACME and SCC*mec*-related contigs in the whole genome sequence of M08/0126 were closed by primer walking using PCR with primers based on the surrounding contigs and the Expand long-template PCR system (Roche Diagnostics Ltd, West Sussex, UK) followed by amplimer sequencing. Data were analysed and overlapping sequences were assembled using the BLAST, Bionumerics and DNA Strider software packages.

Confirmation of the genetic organization and location of ACME and SCC*mec*. Having determined the genetic organization of ACME and SCC*mec* in the ST22-MRSA-IVh isolate M08/0126 using the whole genome sequence, the inferred genetic organization was confirmed by designing three overlapping primer pairs based on this sequence to amplify the ACME and SCC*mec* region of M08/0126 extending from (i) *orfX* to ACME-*arcA* (Table 2, primers orfX F1 and arcA R1), (ii) ACME-*arcA* to the region with homology to the J1 region of SCC*mec* I (Table 2, primers arcA F1 and ΔCE010 R1) and (iii) the SCC*mec* I J1 region to SCC*mec* IVh (Table 2, primers ΔCE010 F1 and J3IVh R1). These PCR assays were performed by amplifying chromosomal DNA using the Expand long-template PCR system (Roche

Diagnostics Ltd.). PCR products were visualized by agarose gel electrophoresis and the sizes of amplicons obtained were compared to the expected size of amplicons based on the whole genome sequence (Table 2).

Confirmation of the genetic organization of ACME and SCC*mec* in all ACME-*arc*-positive ST22-MRSA-IVh isolates was performed using primer pairs designed to amplify the junction regions between *orfX* and ACME (Table 2, primers orfX F1 and ACMEII R1) and the region with homology to J1 of SCC*mec* I and SCC*mec* IVh (Table 2, ACME/SCCmec I F1 and J3IVh R1). PCR assays were performed using GoTaq DNA polymerase (Promega). PCR products were visualized by agarose gel electrophoresis and the sizes of amplicons were compared to those obtained with template DNA from the whole genome sequenced isolate M08/0126 and to the expected size of amplicons based on the whole genome sequence of this isolate (Table 2). Since all isolates yielded amplicons of the same size using these PCR assays, the PCR products for the whole genome sequenced isolate (M08/0126) and one other isolate (M08/0119) were sequenced commercially by Geneservice (Source BioScience plc, Dublin, Ireland) to confirm the DNA sequence of these junction regions.

Confirmation of the location of ACME in all ACME-*arc*-positive ST8-MRSA-IVa isolates was performed using PCR with primers C and IVa R1 (Table 2) to amplify from the J1 region of SCC*mec* IVa to ACME I based on the previously published whole-genome sequence of the ST8-MRSA-IVa isolate FPR3757 (Genbank accession number NC007793). Template DNA from one ACME-*arc*-positive ST22-MRSA-IVa isolate (E1401) which failed to yield any amplicons using the primers based on the ACME/SCC*mec* region of the whole genome sequenced ST22-MRSA-IVh isolate M08/0126 was also investigated using the ST8-MRSA-IVa/USA300-specific primers. PCR assays were performed as described above and the sizes of amplicons were compared to the expected size of amplicons based on the whole genome sequence of ST8-MRSA-IVa isolate FPR3757 (Table 2). The PCR products obtained for one

ST8-MRSA-IVa isolate (ML224) and the ST22-MRSA-IVa isolate (E1401) were sequenced by Geneservice (Source BioScience plc, Dublin, Ireland) to confirm the DNA sequence of this junction region in these isolates.

Nucleotide sequence accession numbers. The nucleotide sequence of the ACME/SCC*mec* composite island (SCC*mec*-CI) of ST22-MRSA-IVh has been deposited in GenBank under accession number FR753166.

RESULTS

Identification of ACME-*arc*-positive MRSA isolates. Twenty-three of the 238 isolates investigated (9.7%) were found to be positive for ACME-*arc* genes (Table 3). Using MLST, SCC*mec* typing and the DNA microarray, the ACME-*arc*-positive isolates were assigned to two distinct genotypes, ST8-MRSA-IVa (7/23, 30%) and ST22-MRSA-IV (16/23, 70%) (Table 3). The DNA microarray results for the ACME-*arc*-positive isolates are shown in Table 4.

ACME-*arc*-positive ST8-MRSA-IVa isolates. The seven ACME-*arc*-positive ST8 isolates were *pvl*-positive, harbored SCC*mec* IVa, exhibited four pulsed-field types (PFTs), belonged to *spa* type t008 (6/7 isolates) or t4306 (1/7 isolates) and to *dru* type dt9g (6/7 isolates) or dt9z (1/7 isolates). Each of these *spa or dru* type pairs differ in single repeats only (Table 4).

The DNA microarray assigned these isolates to the ST8-MRSA-IV clone and, in addition to detecting *mecA* and the ACME-*arc* genes, showed they all harbored the beta-lactam resistance gene *blaZ*, the erythromycin resistance genes *msr*(A) and *mph*(C), the fosfomycin resistance gene *fosB*, enterotoxins K and Q (*sek* and *seq*, respectively), the *pvl* genes *lukF-PV* and *lukS-PV* and the immune evasion cluster (IEC) genes *sak*, *chp* and *scn* (Table 4). The DNA microarray differentiated these seven isolates into three microarray groups designated ST8-MRSA-IV (a)–(c) (Table 4). Microarray group ST8-MRSA-IV (a) contained five isolates harboring the aminoglycoside and streptothricin genes *aphA3* and *sat,* respectively. The single isolate in microarray group ST8-MRSA-IV (b) also harbored *aphA3* and *sat*, but in addition, carried the multidrug resistance gene *cfr* and the chloramphenicol resistance gene *fexA*. This is the first ST8-MRSA-IVa (USA300) isolate reported to carry *cfr*; detailed localization of *cfr* and *fexA* to a novel conjugative plasmid (pCSFS7) in this isolate has been described in a recent study (25). The single isolate in microarray group ST8-MRSA-IV (c) lacked *aphA3*, *sat, cfr* and *fexA* (Table 4).

ACME-*arc*-positive ST22-MRSA-IV isolates. Fifteen of the 16 ACME-*arc*-positive ST22-MRSA-IV isolates (94%) were *pvl*-negative, harbored SCC*mec* IVh, exhibited PFT 01154, *spa* type t3185 and *dru* type dt10o (Table 3). One ST22-MRSA-IV isolate was *pvl*-positive, harbored SCC*mec* IVa (22) and exhibited a different PFT (01003), *spa* (t2480) and *dru* (dt10am) type to the other ACME-*arc*-positive ST22-MRSA-IV isolates (Table 3).

The DNA microarray assigned these 16 ACME-*arc*-positive isolates to the ST22-MRSA-IV clone and, in addition to detecting *mecA* and the ACME-*arc* genes, showed they all carried the macrolide, lincosamide and streptogramin B resistance gene *erm*(C) and the enterotoxin gene cluster (*egc*) consisting of *seg*, *sei*, *sem*, *sen, seo* and *seu/y* (Table 4). Fifteen of the sixteen ST22-MRSA-IV isolates harbored the beta-lactam resistance gene *blaZ.* These isolates were differentiated into four microarray groups designated ST22-MRSA-IV (a)–(d) (Table 4). Microarray group ST22-MRSA-IV (a) consisted of the single *pvl*-positive ST22-MRSA-IVa isolate. This was the only ST22-MRSA-IV isolate that harbored the *pvl* genes but lacked the IEC genes *sak*, *chp* and *scn*. Microarray group ST22-MRSA-IV (b) contained 12 ST22-MRSA-IVh isolates characterized by the presence of genes encoding resistance to lincomycin (*lnu*(A)), aminoglycosides (*aacA-aphD* and *aadD*) and mupirocin (*mupA*), as well as the IEC genes. Microarray groups ST22-MRSA-IV (c) and (d) consisted of one and two isolates, respectively. Isolates in both groups harbored the IEC genes but lacked the antimicrobial resistance genes *lnu*(A), *aacA-aphD*, *aadD* and *mupA*, characteristic of microarray group ST22-MRSA-IV (b) isolates. In addition, microarray group ST22-MRSA-IV (c) isolates lacked the *blaZ* gene (Table 4).

Confirmation of ACME-*arcA* in isolates identified as ACME-*arc*-positive by DNA microarray. The presence of ACME in the 23 ACME-*arc*-positive isolates was confirmed by PCR using previously described primers specific for the ACME-*arcA* region. All isolates yielded amplicons of the expected size corresponding to the amplification of an internal segment of ACME-*arcA*.

Novel ACME and SCC*mec* composite island in ST22-MRSA-IVh isolate M08/0126. Whole genome sequencing of the ST22-MRSA-IVh isolate M08/0126 yielded 272 contigs ranging in size from ca. 200 bp to 200 kb. ACME and/or SCC*mec*-associated DNA sequences were identified in five contigs ranging in size from 1.4 kb to 73 kb. The gaps between ACME and SCC*mec*-related contigs were closed using long-range PCR amplification and sequencing with primers based on the surrounding contigs. This analysis revealed the presence of a novel ca. 46-kb ACME and SCC*mec* composite island (ACME/SCC*mec*-CI) in M08/0126 consisting of a 12-kb DNA region previously identified in ACME type II in *S. epidermidis* ATCC 12228, a truncated copy of the J1 region of SCC*mec* I and a complete SCC*mec* IVh element (Fig. (1a)). Because the present study focused on the novel 46-kb ACME/SCC*mec*-CI element, DNA regions outside of the SCC*mec* and/or ACME sequences of any of these or the other contigs are not discussed further here.

In all MRSA and methicillin-resistant *S. epidermidis* (MRSE) isolates reported to harbor SCC*mec* and ACME, SCC*mec* has always been found to be located within *orfX* with ACME downstream of SCC*mec* (Fig. 1(b) and (c)). While the ACME/SCC*mec*-CI was integrated at the same nucleotide position within *orfX* in M08/0126 as in all other MRSA/MRSE isolates described to date, the ACME element was located within *orfX* and the SCC*mec* element was located downstream of ACME (Fig. 1(a)).

The ACME-CI was flanked by 18-bp direct repeat (DR) sequences, one abutting the *orfX*/ACME junction within the ACME-CI (DR-1, Fig. 1(a)) and the other in the chromosomal region immediately adjacent to the right terminus of SCC*mec* IVh (DR-2, Fig. 1(a)). Identical 7-bp inverted repeat (IR) sequences were also identified within DR-1 (IR-1, Fig. 1(a)) and immediately preceding DR-2 (IR-2, Fig. 1(a)). Two additional 18-bp DRs and three additional 7-bp IRs were also identified within the ACME-CI. The two additional DRs were identified in the DNA sequence between ACME and the region with homology to the J1 region of SCC*mec* I (Fig.

1(a), DR-3; 13/18 nucleotides identical to DRs-1 and -2) and within SCC*mec* IVh adjacent to the J1 SCC*mec* I/SCC*mec* IVh junction (Fig. 1(a). The three additional IRs were identified within DR-3 (Fig. 1) and DR-4 (Fig. 1(a) and at the J1 SCC*mec* I/SCC*mec* IVh junction region within J1 SCC*mec* I (Fig. 1(a). The ACME region in M08/0126 consisted of a ca. 12-kb DNA sequence and included the ACME-*arc* genes in the same order and orientation as previously described for ACME (Fig.1(a)). The ACME-*arc* genes (including *argR*) exhibited ≥99.8% DNA sequence identity with those of the ST8-MRSA-IVa (USA300) isolate FPR3757 and *S. epidermidis* ATCC 12228 (Fig. 1(b) and (c)). The ACME-*arc* genes were surrounded by a ca. 5.8-kb DNA sequence (2.7 kb downstream and 3.1 kb upstream) with 99% DNA sequence identity with the region surrounding ACME-*arc* in *S. epidermidis* ATCC 12228 (Fig. 1). A complete IS*431* element was identified immediately adjacent to the ACME region (Fig. 1(a)). A ca. 9.5-kb region consisting of several open reading frames (ORFs) in the same order and orientation as previously only found in the J1 region of SCC*mec* I (Genbank accession number AB033763) was identified ca. 1.5 kb upstream of IS*431* in the ACME-CI (Fig. 1(a)). This region included all ORFs previously identified within the J1 region of SCC*mec* I extending from the SCC*mec* I/chromosomal junction to within ORF CE010. ORF CE010 contains a Shine-Dalgarno repeat and is similar to the gene encoding the *S. aureus* plasmin sensitive surface protein (*pls*). All ORFs within this region of ACME-CI, except for the one ORF with homology with CE010, exhibited 99–100% DNA sequence identity with the corresponding ORFs from SCC*mec* I. The CE010 region in SCC*mec* I consists of a 5097-bp DNA sequence while in ACME-CI it was 3451 bp and exhibited 50% DNA sequence identity with CE010; no other significant homology was found with any sequence in the Genbank database. A complete SCC*mec* IVh element with a class A *mec* complex and type 2 *ccr* genes was identified adjacent to this SCC*mec* I region in ACME-CI (Fig. 1).

Confirmation of the presence of ACME-CI in other ST22-MRSA-IVh isolates. The presence of the ACME-CI in the remaining 14 ACME-*arc* positive ST22-MRSA-IVh isolates was confirmed by PCR using primers designed to amplify the *orfX*/ACME and the J1 SCC*mec* I/SCC*mec* IVh junction regions in ACME-CI (Table 2). All isolates yielded amplicons of the expected size using both primer pairs. Sequencing of the amplicons from M08/0126 and one other isolate (M08/1119) confirmed that these junction regions were identical to the corresponding region determined by whole genome sequencing of M08/0126. PCR amplification using these two primers pairs was also attempted on template DNA from the ACME-*arc*-positive ST22-MRSA-IVa isolate E1401 (Table 2) but no amplicons were obtained.

Determination of the location of ACME in ST8-MRSA-IVa and ST22-MRSA-IVa isolates. Investigation of the location of ACME in the seven ACME-*arc*-positive ST8-MRSA-IVa isolates was performed using PCR and primers to amplify from the J1 region of SCC*mec* IVa to within ACME type I based on the previously published whole genome sequence of the ACME-positive ST8-MRSA-IVa (USA300) isolate FPR3757. In addition, PCR using this SCC*mec* IVa/ACME primer pair was also performed on ST22-MRSA-IVa isolate E1401 because this isolate failed to yield any amplicons using ACME-CI specific primers and, unlike the other ACME-positive ST22-MRSA-IV isolates, E1401 harbored SCC*mec* IVa which is the same SCC*mec* type identified in the ACME-positive ST8 isolates recognized in the present study. All isolates yielded amplicons of the expected size indicating the presence of ACME adjacent to SCC*mec* in these isolates, similar to the location described previously for ST8-MRSA-IVa (USA300). Sequencing of the amplicons from one ST8-MRSA-IVa isolate (ML224) and the ST22-MRSA-IVa isolate (E1401) revealed that the SCC*mec* IVa/ACME junction regions in these two isolates exhibited 100% DNA sequence identity with each other and with that of the ST8-MRSA-IVa (USA300) isolate FPR3757.

DISCUSSION

Although ACME is widespread among ST8-MRSA-IVa (USA300) isolates, it has only been identified in a small number of isolates belonging to other MRSA clones. This report is, to the best of our knowledge, the first description of ACME with significant DNA sequence identity with ACME type II from *S. epidermidis* in the pandemic ST22-MRSA-IV clone. The emergence of an ACME-positive variant of ST22-MRSA-IV may have the potential to enhance the growth and survival of this already successful MRSA clone. This is especially worrying because like ST8-MRSA-IVa (USA300), one of the ACME-positive ST22-MRSA-IV isolates was also *pvl*-positive. While ACME does not directly act as a virulence factor or contribute directly to the ability of staphylococci to cause disease, evidence suggests that it contributes to bacterial growth, survival, transmission and colonization within the host (3, 4, 13, 19).

In the present study, ACME was identified in two distinct strains of ST22-MRSA-IV; one strain (represented by a single isolate) was *pvl*-positive while the other (represented by 15 isolates) was *pvl*-negative. The *pvl*-positive strain was recovered in 2004 from a 69 year-old Irish male with CA-MRSA bacteremia; it had a distinct *spa* (t2480), *dru* (dt10am), PFT (01003) and SCC*mec* type (IVa). Isolates of the *pvl*-negative strain (*spa* t3185, *dru* dt10o, PFT 01154 and SCC*mec* IVh) were recovered from one patient and a variety of environmental sites in one ward in a Dublin hospital during a four-week period in 2008. Isolates of the latter strain were differentiated into three subgroups using the DNA microarray; the largest group (represented by 12 isolates) harbored genes encoding lincomycin, aminoglycoside and high-level mupirocin resistance, and an IEC-encoding lysogenic bacteriophage. The combination of ACME and high-level mupirocin resistance in ST22-MRSA-IV is an important finding because while ACME may enhance host-tissue colonization, high-level mupirocin resistance may also play a role in successful host colonization by resisting nasal decolonization by mupirocin therapy, a major strategy in preventing the spread of MRSA.

The ACME-positive ST22-MRSA-IVh isolates harbored a 46-kb ACME/SCC*mec*-composite island (CI) with several novel characteristics. Firstly, this ACME/SCC*mec*-CI consisted of a 12-kb ACME region with >99% DNA sequence identity with part of ACME II previously only described in *S. epidermidis*, a 9.5-kb DNA region with significant homology to part of the J1 region of SCC*mec* I and a SCC*mec* IVh element. The presence of a DNA sequence with significant identity to the J1 region of SCC*mec* I was a surprising finding since SCC*mec* types I and Ia have not been identified among Irish MRSA isolates since 1999. MRSA strains that predominated in Irish hospitals in the 1970s and 1980s harbored SCC*mec* I and strains harboring SCC*mec* Ia were recovered sporadically between 1989 and 1999 (24). It is interesting to speculate that both ACME and the SCC*mec* I J1 region of the ST22-MRSA-IVh ACME/SCC*mec*-CI may have originated in CoNS and that recombination and horizontal transfer of DNA occurred between CoNS and a ST22-MRSA-IVh isolate resulting in this novel ACME-SCC*mec*-CI. While the precise order of these events and the direction of transfer are unknown, the higher prevalence and diversity of ACME and SCC*mec* among CoNS and the fact that the ACME II allotype has not previously been identified in MRSA suggests that it may have originated in CoNS with subsequent transfer to *S. aureus* (13). However, confirmation of this hypothesis requires detailed molecular characterization of ACME and SCC*mec* in CoNS. Other studies have also found evidence to suggest interspecies transfer of DNA, including ACME, SCC*mec*, SCC-CI and antimicrobial resistance genes between *S. aureus* and CoNS (13, 18, 25, 27).

A unique and striking feature of the novel ACME/SCC*mec*-CI reported in the present study is the location of ACME and SCC*mec* relative to *orfX*. In contrast to previously described ACME/SCC-CIs in which ACME is located downstream of SCC*mec* (3), SCC*mec* was located downstream of ACME in the ST22-MRSA-IVh isolates. This suggests that ACME may have integrated into the chromosome of these isolates prior to the integration of SCC*mec*. Future analysis of ST22-MSSA isolates for the presence of ACME may help to further clarify this. The IS*431* element identified adjacent to the ACME region of ST22-MRSA-IVh may have played a role in the emergence of this novel ACME/SCC*mec* element, as insertion sequence elements have been shown previously to promote genetic rearrangements (10).

In the ACME-positive *pvl*-positive ST22-MRSA-IVa isolate reported in the present study, ACME was located downstream of SCC*mec* IVa as previously described in ST8-MRSA-IVa (USA300) isolates (3). The same ACME and SCC*mec* IVa genetic organization was also found among ACME-positive *pvl*-positive ST8-MRSA-IVa isolates recovered in Ireland between 2003 and 2005, suggesting that such isolates may be a more likely source of ACME in the *pvl*-positive ST22-MRSA-IVa isolate rather than the ST22-MRSA-IVh isolates with the novel ACME-SCC*mec*-CI first recognised in 2008. However, CoNS may also have been the source of the ACME element in the earlier isolate. More detailed studies of ACME and SCC*mec* in *S. aureus* and CoNS are required to clarify this.

In conclusion, the finding of ACME in two distinct strains of ST22-MRSA-IV and the significant differences identified in the genetic organization of ACME in both strains suggests that ACME has been acquired by ST22-MRSA-IV on at least two independent occasions and that this acquisition may have involved interspecies transfer of ACME between CoNS and *S. aureus*. This finding also suggests that ACME has the potential to spread widely not only among ST8-MRSA-IV and ST22-MRSA-IV isolates but also among other MRSA clones. To date, the horizontal transfer of ACME appears to be a relatively rare event, because although it is widespread among ST8-MRSA-IVa it has only been identified among a small number of isolates belonging to other MRSA clones (4, 5, 6, 7, 8), and only 10% of MRSA isolates in the present study were found to harbor ACME. While the reasons for this are still unclear, it may be that acquisition of a large mobile genetic element such as ACME, which previously have been reported to be >30-kb in size, can compromise the fitness of the host. However, ACME identified in the ST22-MRSA-IVh isolates in the present study was the smallest ACME described to date (ca. 14 kb from DR-1 to DR-3) and therefore, may be a more competitive mobile genetic element and may not adversely affect bacterial fitness. The presence of ACME may provide a selective advantage to ST22-MRSA-IV isolates and may therefore enhance further dissemination of this already successful MRSA clone**.** Confirmation of this suggestion requires ongoing surveillance of ST22-MRSA-IV and other MRSA strains to determine if ACME-positive ST22-MRSA-IV isolates become widespread and if this ACME element successfully spreads among other MRSA strains. This study provides further evidence of the ongoing and rapid evolution of MRSA and of the importance of CoNS as a potential reservoir of virulence-associated and antimicrobial resistance genes in *S. aureus.*

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TABLE 1. MRSA isolates investigated for the presence of ACME-*arc* genes by DNA microarray analysis[a]

MRSA Source / Description	Time period (Years)	Reference	MRSA genotype	Number investigated	Number ACME-*arc*-positive
Irish hospitals	1971–2002	(24)	ST250-MRSA-I/I - *pls*	10	0
			ST239-MRSA- III/III - Tn*554*/p*I258*	12	0
			ST247-MRSA-Ia	3	0
			ST8-MRSA-IIA/IIB/IIC/IID/IIE	33	0
			ST8-MRSA-IVE/IVF	6	0
			ST22-MRSA-IVh	11	0
			ST22-MRSA-IVa	2	0
			ST36-MRSA-II	6	0
			ST5-MRSA-II	14	0
			ST30-MRSA-IV non-subtypeable	4	0
			ST45-MRSA-IVa	1	0
			ST496-MRSA-II	1	0
			ST12-MRSA-IVc	1	0
			ST609-MRSA-VA	1	0
			ST94-MRSA-IVg	1	0
			ST5-MRSA-IV non-subtypeable	1	0
pvl-positive CA-MRSA	1999–2005	(22)	ST8-MRSA-IVa	8	7
			ST22-MRSA-IVa	1	1
			ST22-MRSA-IVh	1	0
			ST30-MRSA-IVc	4	0
			ST30-MRSA-IV non-subtyeable	7	0
			ST154-MRSA-IVg	1	0
			ST80-MRSA-IVc	2	0
			ST5-MRSA-IVa	1	0
Four wards in one Irish hospital	2007–2008	(26)	ST22-MRSA-IVh	100	15
			ST22-MRSA-IVa	1	0
			ST8-MRSA-IIE	2	0
			ST8-MRSA-II novel subtypes	1	0
			ST87-MRSA-IVb	1	0
			ST36-MRSA-II	1	0
			Total	**238**	**23 (9.7%)**

[a] The StaphyType Kit DNA microarray (Alere Technologies) was used (16).

TABLE 2. Primers designed and used in the present study.

Primer application	Primer pair	Nucleotide sequence (5'-3')	Nucleotide coordinates	Region amplified	Product size
Long-range PCR to confirm the genetic organization of the ACME/SCC*mec* region in ST22-MRSA-IVh M08/0126	orfX F1 arcA R1	CATTCAGCAAAATGACATTC AATGGTACAAGGACCCATTC	377-396[a] 8264-8245[a]	*orfX* to ACME-*arcA*	7887 bp
	arcA F1 ΔCE010 R1	GTTTGAGCAAATTTGTCATG ATCAGGACTACCTGGTTCCA	8088-8107[a] 16735-16716[a]	*arcA* to SCC*mec* I J1-like region	8647 bp
	ΔCE010 F1 J3IVh R1	CATATGAAACTAAACGCGTA ACCAAGCTATCATAGGATGT	16683-16702[a] 24967-24948[a]	SCC*mec* I J1-like region to J3 region of SCC*mec* IVh	8284 bp
Confirmation of genetic organization of ACME and SCC*mec* in all ACME-*arc*-positive ST22-MRSA-IVh isolates	orfX F1 ACMEII R1	CATTCAGCAAAATGACATTC GAGACTGCTTCTTTGCTCAC	377-396[a] 1320-1301[a]	*orfX* to ACME	943 bp
	ACME/SCC*mec*I F1 J3IVh R1	AGTTACTGCTAATGGAACGG ACCAAGCTATCATAGGATGT	23808-23827[a] 24967-24948[a]	SCC*mec* I J1-like region to J3 region of SCC*mec* IVh	1159 bp
Confirmation of location of ACME in all ACME-*arc*-positive ST8-MRSA-IVa isolates and ST22-MRSA-IVa isolate E1401	IVa R1 Primer C	CACGTTATGGAGGTGCTCTG CCTCCTTCACTTAGCACTG	57628-57647[b] 58123-58092[b]	J1 region of SCC*mec* IVa to ACME I	495 bp

[a] Nucleotide coordinates based on the nucleotide sequence of the *orfX*/ACME/SCC*mec* region of ST22-MRSA-IVh isolate M08/0126 (GenBank accession number FR753166).

[b] Nucleotide coordinates based on the nucleotide sequence of the ST8-MRSA-IVa (USA300) strain FPR3757 (Genbank accession number NC007793).

TABLE 3. Details of 23 ACME-*arc*-positive MRSA isolates

MRSA description / source	**Isolate no.**	**Year of isolation**	**Isolate source**	**Antimicrobial resistance pattern[a]**	**ST[b]**	**SCC*mec***	***spa***	***dru***	**PFT**	**DNA microarray group[c]**
pvl-positive	ML224	2003	Skin abscess	AMP, CAD, CIP, ERY, KAN, NEO	8	IVa	t008	dt9g	99023	ST8-MRSA-IV (a)
	M05/0199	2005	Pneumonia (USA travel)	AMP, CIP, ERY, KAN, NEO	8	IVa	t008	dt9g	99025	ST8-MRSA-IV (a)
	M05/0259	2005	Face infection	AMP, ERY, KAN, NEO	8	IVa	t008	dt9g	99023	ST8-MRSA-IV (a)
	M05/0028	2005	Buttock abscess	AMI, AMP, ERY, KAN, NEO	8	IVa	t008	dt9g	99023	ST8-MRSA-IV (a)
	M04/0266	2005	Inguinal lymphadenitis	AMI, AMP, ERY, KAN, NEO	8	IVa	t4306	dt9g	99017	ST8-MRSA-IV (a)
	M05/0060	2005	Skin scalp abscess	AMI, AMP, CHL, ERY, KAN, LIN, NEO	8	IVa	t008	dt9g	99024	ST8-MRSA-IV (b)
	M05/0100	2005	Staff screening	AMP, CIP, ERY	8	IVa	t008	dt9z	99023	ST8-MRSA-IV (c)
	E1401	2003	Blood/Bacteremia	AMP, ERY	22	IVa	t2480	dt10am	01003	ST22-MRSA-IV (a)
Four-ward study	M08/0119	2008	Nasal	AMP, CAD, CIP, ERY, KAN, LIN, MUP, TOB	22	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0121	2008	Air	AMP, CAD, CIP, ERY, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0122	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0123	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0124	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0126	2008	Mattress	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0129	2008	Bathroom floor	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0131	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0132	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0133	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0135	2008	Mattress	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0161	2008	Bathroom floor	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (b)
	M08/0128	2008	Air	AMP, CAD, CIP, ERY, GEN, KAN, LIN, MUP, TOB	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (c)
	M08/0134	2008	Air	AMP, CAD, CIP, ERY	22	IVh	t3185	dt10o	01154	ST22-MRSA-IV (d)
	M08/0136	2008	Mattress	AMP, CAD, CIP, ERY	22[b]	IVh	t3185	dt10o	01154	ST22-MRSA-IV (d)

[a] Antimicrobials tested included AMI, amikacin; AMP, ampicillin; CAD, cadmium acetate; CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; ethidium bromide; fusidic acid; GEN, gentamicin; KAN, kanamycin; LIN, lincomycin; mercuric chloride; MUP, mupirocin; NEO, neomycin; phenyl mercuric acetate; rifampicin; SPC, spectinomycin; STR, streptomycin; sulphonamide; tetracycline; TOB, tobramycin; trimethoprim; vancomycin.

[b] ST, Sequence type assigned by DNA microarray analysis using the StaphyType Kit (Alere Technologies). Other STs were also determined by MLST.

[c] Isolates within each ST-SCC*mec* type (ST8-MRSA-IV or ST22-MRSA-IV) were assigned to DNA microarray groups based on the presence of a unique combination of virulence and/or antimicrobial resistance genes. DNA microarray results are shown in Table 4.

TABLE 4. DNA microarray[a] hybridization profiles of the ACME-*arc*-positive ST8-MRSA-IVa, ST22-MRSA-IVh and ST22-MRSA-IVa MRSA isolates identified in the present study.

	Genotype	**ST8-MRSA-IVa**			**ST22-MRSA-IVa**	**ST22-MRSA-IVh**		
	DNA Microarray Group[b]	**ST8-MRSA-IV** **(a)**	**(b)**	**(c)**	**ST22-MRSA-IV** **(a)**	**ST22-MRSA-IV** **(b)**	**(c)**	**(d)**
	No. of isolates	**5**	**1**	**1**	**1**	**12**	**1**	**2**
DNA Microarray								
Gene class	**Genes**							
Species markers	*katA, coa, nuc, spa*	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
***agr* group**	*agr* group I	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
SCC*mec*-associated markers	*mecA*	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
	delta mecR	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
	mecR/I	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	ccrA/B-1	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Q9XB68-dcs	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
	ccrA/B-2	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
	kdp-SCC locus	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	ccrA/B-3	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	ccrC, ccrA/B-4	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Antimicrobial resistance genes	*blaZ/I/R1*	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	Neg	**Pos**
	erm(A), *erm*(B)	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	erm(C)	Neg	Neg	Neg	**Pos**	**Pos**	**Pos**	**Pos**
	lnu(A)	Neg	Neg	Neg	Neg	**Pos**	Neg	Neg
	msr(A), *mph*(C)	**Pos**	**Pos**	**Pos**	Neg	Neg	Neg	Neg
	aacA-aphD	Neg	Neg	Neg	Neg	**Pos**	Neg	Neg
	aadD	Neg	Neg	Neg	Neg	**Pos**	Neg	Neg
	aphA3/sat	**Pos**	**Pos**	Neg	Neg	Neg	Neg	Neg
	dfrS1	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	far1	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	mupA	Neg	Neg	Neg	Neg	**Pos**	Neg	Neg
	tet(K)	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	tet(M)	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	cat	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	cfr	Neg	**Pos**	Neg	Neg	Neg	Neg	Neg
	fexA	Neg	**Pos**	Neg	Neg	Neg	Neg	Neg
	fosB	**Pos**	**Pos**	**Pos**	Neg	Neg	Neg	Neg
Virulence-associated genes	*tst1*	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	sea, seb, see, seh	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	sec/l, sed/j/r	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	seg/i/m/n/o/u	Neg	Neg	Neg	**Pos**	**Pos[c]**	**Pos**	**Pos**
	sek/q	**Pos**	**Pos**	**Pos**	Neg	Neg	Neg	Neg
	lukF/S-PV	**Pos**	**Pos**	**Pos**	**Pos**	Neg	Neg	Neg
	sak/chp/scn	**Pos**	**Pos**	**Pos**	Neg	**Pos**	**Pos**	**Pos**

	Genotype	**ST8-MRSA-IVa**			**ST22-MRSA-IVa**	**ST22-MRSA-IVh**		
	DNA Microarray Group[b]	**ST8-MRSA-IV**			**ST22-MRSA-IV**	**ST22-MRSA-IV**		
		(a)	**(b)**	**(c)**	**(a)**	**(b)**	**(c)**	**(d)**
	No. of isolates	**5**	**1**	**1**	**1**	**12**	**1**	**2**
DNA Microarray								
Gene class	**Genes**							
	etA/B/C	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	edinA/B/C	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	arcA/B/C/D	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
Capsule type	capsule type 5	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**	**Pos**
	capsule type 8	Neg	Neg	Neg	Neg	Neg	Neg	Neg

[a] The StaphyType Kit (Alere Technologies) was used for DNA microarray analysis. Full datasets are available upon request.

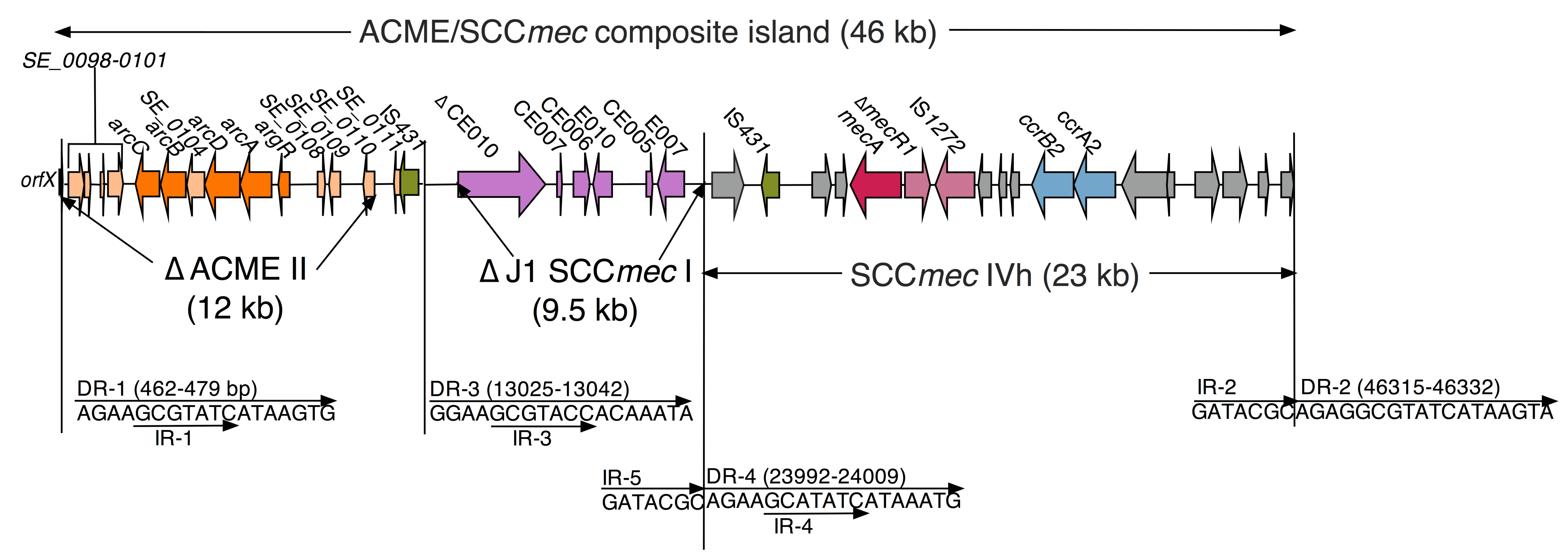
[b] Isolates within each ST-SCC*mec* type were assigned to DNA microarray groups based on the presence of a unique combination of virulence and/or antimicrobial resistance genes. The ST8-MRSA-IV and ST22-MRSA-IV isolates were differentiated into three group and four groups, respectively (shown in Table 3).

[c] One isolate M08/0122 in the microarray group ST22-MRSA-IV (b) was positive for the enterotoxin gene cluster genes *seg*, *sem*, *sen, seo* and *seu/y* but lacked *sei*.

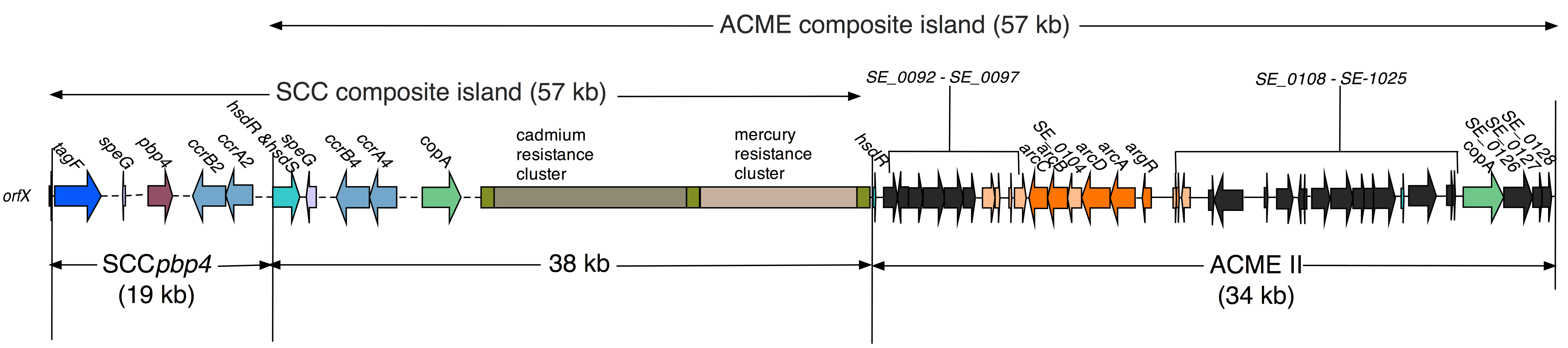
Figure legend

FIG. 1. Schematic diagram showing the genetic organization of (a) the novel ACME/SCC*mec* composite island (CI) identified in the present study in ST22-MRSA-IVh isolate M08/0126 (Genbank accession number FR753166), (b) the ACME-CI previously reported in *S. epidermidis* ATCC 12228 (NC004461) and (c) the ACME and SCC*mec* elements previously identified in the ST8-MRSA-IVa (USA300) isolate FPR3757 (FPR3757). The structure of the novel ACME-CI was determined by high-throughput whole-genome sequencing of M08/0126 and was confirmed using primers spanning the ACME/SCC*mec* region. DR, direct repeat sequence; IR, inverted repeat sequence.

(a) M08/0126 (ST22-MRSA-IVh)



(b) *S. epidermidis* ATCC 12228



(c) *S. aureus* FPR3757 (ST8-MRSA-IVa/USA300)