AAC
Journals.ASM.org

DNA Microarray Profiling of a Diverse Collection of Nosocomial Methicillin-Resistant *Staphylococcus aureus* Isolates Assigns the Majority to the Correct Sequence Type and Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Type and Results in the Subsequent Identification and Characterization of Novel SCC*mec*-SCC_{M1} Composite Islands

Anna C. Shore,[a,c] Orla M. Brennan,[a] Emily C. Deasy,[a] Angela S. Rossney,[b] Peter M. Kinnevey,[a] Ralf Ehricht,[d] Stefan Monecke,[d,e] and David C. Coleman[a]

Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College, Dublin, Ireland[a]; National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland[b]; Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin, Ireland[c]; Alere Technologies GmbH, Jena, Germany[d]; and Institute for Medical Microbiology and Hygiene, Faculty of Medicine "Carl Gustav Carus," Technical University of Dresden, Dresden, Germany[e]

One hundred seventy-five isolates representative of methicillin-resistant *Staphylococcus aureus* (MRSA) clones that predominated in Irish hospitals between 1971 and 2004 and that previously underwent multilocus sequence typing (MLST) and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were characterized by *spa* typing (175 isolates) and DNA microarray profiling (107 isolates). The isolates belonged to 26 sequence type (ST)-SCC*mec* types and subtypes and 35 *spa* types. The array assigned all isolates to the correct MLST clonal complex (CC), and 94% (100/107) were assigned an ST, with 98% (98/100) correlating with MLST. The array assigned all isolates to the correct SCC*mec* type, but subtyping of only some SCC*mec* elements was possible. Additional SCC*mec*/SCC genes or DNA sequence variation not detected by SCC*mec* typing was detected by array profiling, including the SCC-fusidic acid resistance determinant Q6GD50/*fusC*. Novel SCC*mec*/SCC composite islands (CIs) were detected among CC8 isolates and comprised SCC*mec* IIA-IIE, IVE, IVF, or IVg and a *ccrAB4*-SCC element with 99% DNA sequence identity to SCC_{M1} from ST8/t024-MRSA, SCC*mec* VIII, and SCC-CI in *Staphylococcus epidermidis*. The array showed that the majority of isolates harbored one or more superantigen (94%; 100/107) and immune evasion cluster (91%; 97/107) genes. Apart from fusidic acid and trimethoprim resistance, the correlation between isolate antimicrobial resistance phenotype and the presence of specific resistance genes was ≥97%. Array profiling allowed high-throughput, accurate assignment of MRSA to CCs/STs and SCC*mec* types and provided further evidence of the diversity of SCC*mec*/SCC. In most cases, array profiling can accurately predict the resistance phenotype of an isolate.

Staphylococcus aureus is a versatile pathogen responsible for a wide range of infections, reflecting its ability to express an extensive array of virulence factors and antimicrobial resistance genes. Methicillin-resistant *S. aureus* (MRSA) was first reported in 1961 in the United Kingdom, and subsequently a variety of different MRSA clones that exhibited resistance to numerous antimicrobial agents emerged (6, 22). Different epidemic and pandemic clones have spread worldwide since the 1970s (6). Today, MRSA strains are a major nosocomial problem worldwide and have also emerged as a significant cause of infections among otherwise healthy individuals in the community and among animals (10, 55).

While many virulence-associated genes in *S. aureus* are part of the core genome (such as those encoding microbial surface components recognizing adhesive matrix molecules [MSCRAMMs] and the polysaccharide intercellular adhesion [*ica*] locus), many are encoded on mobile genetic elements (MGEs) that can be horizontally transferred between *S. aureus* isolates and from coagulase-negative staphylococci (CoNS) (29). Many different types of MGEs encoding virulence genes have been identified in *S. aureus*, including bacteriophages, pathogenicity islands, plasmids, staphylococcal cassette chromosome (SCC) and SCC-like elements, and genomic islands (3, 32).

Antimicrobial resistance in *S. aureus* can be due to spontaneous chromosomal mutations, but resistance is more commonly due to the acquisition of MGEs, including plasmids, transposons, and SCC elements harboring antimicrobial resistance genes (32). SCC elements include SCC*mec*, which harbors the methicillin resistance gene *mecA*. Two divergent *mecA* alleles have been described in MRSA to date (20, 46). A search of GenBank revealed an additional 32 variants that differ at the nucleotide sequence level and 28 protein variants. Eleven different SCC*mec* types, as well as numerous subtypes, have been identified in MRSA, and each type has a unique combination of cassette chromosome recombinase (*ccr*) genes which are involved in the integration and excision of

Received 18 June 2012 **Returned for modification** 13 July 2012
Accepted 30 July 2012
Published ahead of print 6 August 2012
Address correspondence to Anna C. Shore, anna.shore@dental.tcd.ie.
Supplemental material for this article may be found at http://aac.asm.org/.

doi:10.1128/AAC.01247-12

the element, as well as *mec* complex genes, including *mecA* and, when present, the *mec* regulatory genes *mecI* and *mecR1* (15, 20, 28, 46). Each different SCC*mec* type has been assigned a Roman-numeral name in the order in which it was identified (20). SCC*mec* subtypes differ from SCC*mec* types mainly in the joining or "J" regions, i.e., the regions outside the *ccr* and *mec* gene complexes (20). Subtypes are designated by the use of either lower- or uppercase alphabetic suffixes after the relevant Roman-numeral SCC*mec*-type name (20). Lowercase alphabetic suffixes (e.g., IVa, IVb, or IVc) are used to indicate DNA sequence variation in the J regions of SCC*mec* subtypes, while uppercase alphabetic suffixes (e.g., IVA, IIA, IA) indicate variation due to the presence or absence of mobile genetic elements (20). SCC elements without *mecA* but with *ccr* genes and sometimes with additional antimicrobial resistance or virulence-associated determinants, as well as composite islands (CIs) consisting of a combination of two or more SCC, SCC-like, or SCC*mec* elements, have also been described in *S. aureus* and CoNS (2, 11, 18, 20, 21, 25, 30, 37, 47). Evidence suggests that CoNS are reservoirs for SCC*mec* in *S. aureus*. Due to the diversity and frequency of identification of novel SCC and SCC*mec* types and subtypes, some reported novel elements do not follow any standardized or internationally agreed rules of nomenclature. While it is now recommended that nomenclature of novel SCC*mec* types and SCC types and subtypes should be undertaken in conjunction with the International Working Group on the Classification of SCC elements, this does not always happen, and there are ambiguities in the classification of SCC and SCC*mec* elements in the published literature. A binary system is currently in development to simplify this confusing SCC*mec* subtype nomenclature (20).

Effective and informative molecular typing plays an important role in monitoring the emergence, spread, and evolution of different MRSA clones. Multilocus sequence typing (MLST) is commonly used for MRSA typing, and while it has provided invaluable insights into its evolution, it lacks the discriminatory ability required for local or outbreak investigations and is relatively expensive and labor-intensive, as it involves amplification and sequencing of internal fragments of seven housekeeping genes (40). In contrast, *spa* typing involves DNA sequencing of a variable number tandem repeat (VNTR) region of just one gene (the *S. aureus* protein A *spa* gene), provides greater discrimination than MLST, and is widely used internationally for local and global epidemiological studies (16). Accurate identification of the type and subtype of the SCC*mec* element harbored by a MRSA isolate enhances understanding of the genetic relatedness of MRSA isolates. SCC*mec* typing commonly involves the use of several multiplex PCR assays to identify the *ccr* and *mec* complex genes which are used to define the SCC*mec* type and to identify the main characteristics of the J regions for SCC*mec* subtype determination and which can harbor integrated transposons and/or plasmids with additional antimicrobial resistance determinants. In the case of novel SCC*mec* elements, complete nucleotide sequencing is commonly carried out. The combination of MLST and SCC*mec* typing data is used to describe MRSA clones (14), and for the purpose of the present study, clones are defined as MRSA isolates with the same sequence type (ST) and the same or very closely related SCC*mec* type. Strains are defined as isolates not just with the same ST and SCC*mec* type but also with additional shared traits, such as specific combinations of virulence or antimicrobial resistance genes.

In recent years, major advances in high-throughput whole-genome sequencing technologies have resulted in the development of more in-depth typing methods, including single-nucleotide polymorphism (SNP) analysis of whole-genome sequences (17) and different microarray platforms (29). A recent study demonstrated the potential of SNP analysis of whole-genome sequences for investigating evolutionary relatedness and for epidemiological tracking of MRSA (17). However, this approach is currently expensive and time-consuming, and data analysis is too complicated for routine typing. DNA microarray systems have been developed based on the whole-genome sequences that are available for *S. aureus* and allow simultaneous hybridization of an isolate's genome against the entire gene content of multiple *S. aureus* genomes. While these DNA microarray systems provide a large amount of information about the isolate's genome, their use is restricted mainly to specialized research laboratories, as data analysis is complicated (29, 50, 52, 56). In contrast, several groups have recently developed more focused DNA microarrays (12, 35, 36, 43), with one system in particular offering simultaneous high-throughput genotyping of *S. aureus* isolates by assigning isolates to an MLST clonal complex (CC) or ST and to an SCC*mec* type and detection of species-specific markers, accessory gene regulator (*agr*) alleles, capsule types, MSCRAMMs, and a range of clinically relevant antimicrobial resistance and virulence-associated genes (34–36).

Like in many other countries, MRSA has been a major problem in Irish hospitals since the 1970s (4, 7, 19, 45). Previous studies of nosocomial MRSA isolates recovered in Irish hospitals over a 33-year period using MLST and detailed SCC*mec* typing revealed that the predominant MRSA clone was replaced after a certain period of years (45). Dominant clones identified included ST250-MRSA-I/I-*pls* in the 1970s and the early 1980s, the ST239-MRSA-IIIHg/IIIHg-p1258/Tn*554* clone in the 1980s, and the ST8-MRSA-IIA-IIE, ST36-MRSA-II, and ST22-MRSA-IV clones in the 1990s and early 2000s. The ST22-MRSA-IV clone has continued to predominate in Irish hospitals to the present day (45).

The purpose of the current study was to perform an in-depth molecular characterization of the predominant MRSA clones recovered from patients in Irish hospitals between 1971 and 2004 using *spa* typing and a DNA microarray system. The DNA microarray was investigated to (i) determine its value for genotyping MRSA isolates representative of major pandemic clones that have been previously investigated by MLST and detailed SCC*mec* typing, (ii) identify the range of virulence and antimicrobial resistance genes harbored by these MRSA clones, and (iii) study the correlation between antimicrobial resistance phenotype and the presence of antimicrobial resistance genes.

MATERIALS AND METHODS

MRSA isolates. A total of 175 MRSA isolates, representative of the most prevalent antibiogram-resistogram (AR) and pulsed-field gel electrophoresis (PFGE) types of MRSA isolates recovered among patients in Irish hospitals at different time periods between 1971 and 2004, were investigated (45).

Molecular typing. SCC*mec* typing of all isolates was undertaken previously using individual PCR assays to detect *mec* complexes A and B and *ccr* complexes 1, 2, and 3 and analysis of the joining (J) regions by a previously described multiplex PCR method (38, 45). For all ST8 isolates previously identified as harboring novel SCC*mec* II and IV variant elements, in-depth molecular characterization of the SCC*mec* elements was performed using long-range PCR amplification and sequencing (45). As

part of the present study, all isolates harboring SCC*mec* IV were subtyped using a multiplex PCR method (33) which detects the SCC*mec* IV subtypes IVA, IVa, IVb, IVc, IVd, IVg, and IVh. Previously described *S. aureus* reference strains were used as positive controls for SCC*mec* IV subtyping (48). One hundred thirty of the 175 isolates were previously typed by multilocus sequence typing (MLST) (45). MLST was performed as described previously on the remaining 45 isolates as part of the present study (13, 49). All 175 isolates underwent *spa* typing as described previously (49).

DNA microarray analysis. One hundred seven of the 175 MRSA isolates investigated underwent DNA microarray analysis. These included (where possible) three isolates representative of each AR type, sequence type (ST), and SCC*mec* type/subtype combination previously identified among the 175 isolates during different time periods between 1971 and 2004 (45). The StaphyType kit (Alere Technologies, Jena, Germany) was used for DNA microarray analysis according to the manufacturer's instructions, which have been described in detail elsewhere (35, 36). The StaphyType kit consists of a DNA microarray chip adhered to each well of a microtiter strip; each chip consists of 334 *S. aureus* target sequences, including species-specific, antimicrobial resistance, and virulence-associated genes, genes involved in attachment, adhesion, and biofilm formation, as well as markers used for typing. Data generated by StaphyType arrays were analyzed for the presence or absence of these genes using Arraymate software (Alere Technologies), which can assign *S. aureus* isolates to STs and/or clonal complexes (CCs) by comparing each isolate's DNA microarray results to those of a diverse reference collection of previously characterized strains in the Arraymate database (36).

PCR to confirm the absence of *ccrAB2* genes. For five isolates that were previously identified as harboring *ccrAB2* and a class B *mec* complex by SCC*mec* typing PCR, the *ccrAB2* genes were not detected using the DNA microarray. PCR detection of *ccrAB2* in these isolates was repeated using a previously described multiplex *ccr* PCR assay (26) and previously described reference strains (49).

PCR amplification of the *S. epidermidis ccrAB4* gene. The presence of *S. epidermidis ccrAB4* was investigated by PCR in all isolates that yielded positive or ambiguous signals for *ccrA4* and/or *ccrB4* genes using the DNA microarray (which has primers and probes specific for *S. aureus ccrA4* and *ccrB4*) using previously described conditions and primers specific for the *S. epidermidis ccrAB4* genes (49). Since the majority of isolates that were positive for these *ccrAB4* genes exhibited the ST8-MRSA-IIA-IIE or IVE/IVF and *spa* type t190 genotype, all isolates belonging to these genotypes that underwent *spa* typing but not DNA microarray analysis were also investigated by PCR for the presence of the *S. epidermidis ccrAB4* genes.

Whole-genome sequencing. The whole genome of one MRSA isolate (AR13.1/3330.2) representative of the most frequently occurring ST-SCC*mec* type identified as harboring the *S. epidermidis ccrAB4* genes was sequenced to further investigate the location and adjacent sequences of these genes. 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 40×. 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 (42) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (1). Contigs identified as containing SCC-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 the SCC-related contigs in the whole-genome sequence of AR13.1/3330.2 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, United Kingdom) followed by amplimer sequencing by Geneservice (SourceBioscience, Guinness Enterprise Centre, Dublin). Data were analyzed, and overlapping sequences were assembled using the BLAST, Bionumerics, and DNA Strider software packages.

Confirmation of the genetic organization and location of *ccrAB4*. Having determined the location and genetic organization of the *ccrAB4* region of an SCC element adjacent to SCC*mec* in AR13.1/3330.2 using whole-genome sequencing, the genetic organization of this element was confirmed by PCR and amplimer sequencing using four overlapping primer pairs to amplify from the SCC*mec* element to the end of the *ccrAB4*-carrying element of AR13.1/3330.2. The primers used are listed in Table S1 in the supplemental material. These PCR assays were performed using chromosomal template DNA with GoTaq DNA polymerase (Promega Corporation, Madison, WI). PCR products were visualized by agarose gel electrophoresis, and the sizes of amplicons obtained were compared to the expected sizes of amplicons based on the whole-genome sequence (see Table S1).

The presence of the *ccrAB4*-carrying SCC element and its location adjacent to SCC*mec* were investigated in one isolate representative of each ST and SCC*mec* type combination identified as harboring the *S. epidermidis ccrAB4* genes, including ST8-MRSA-IIA (AR13/0132), ST8-MRSA-IIB (AR05/1345), ST8-MRSA-IIC (AR14/0246), ST8-MRSA-IID (AR13/3698), ST8-MRSA-IVE (AR43/3330.1), ST8-MRSA-IVF (AR43/3246), and ST94-MRSA-IVg (M03/0169.2), using GoTaq DNA polymerase (Promega). The primers described above for AR13.1/3330.2 were used for these PCR amplifications, except that primers J1_IVc_F2, J1_IVb_F2, and J1_IVg_F1 were used instead of primer J1_IIE_F3 for isolates harboring SCC*mec* types IVE, IVF, and IVg, respectively (see Table S1 in the supplemental material). PCR products were visualized by agarose gel electrophoresis, and the sizes of amplicons were compared to those obtained with template DNA from AR13.1/3330.2 and to the expected size of amplicons based on the whole-genome sequence of this isolate.

PCR detection of antimicrobial resistance determinants. Phenotypic resistance to selected antimicrobial agents where corresponding antimicrobial resistance genes were included on the DNA microarray was determined by disk diffusion susceptibility testing as described previously (41). Isolates that exhibited phenotypic resistance to particular antimicrobial agents for which associated resistance genes were not detected by the DNA microarray were further investigated using PCR assays and previously described primers and thermal cycling conditions specific for additional resistance genes. These investigations included PCR detection of the fusidic acid resistance gene *fusD* (5), the trimethoprim resistance genes *dfrD* (9), *dfrG* (44), and *dfrK* (24), the tetracycline resistance gene *tet*(L) and *tet*(O) (53), and PCR DNA amplification and sequencing to detect further mutations in *fusA* encoding elongation factor G (EF-G) (5). *Escherichia coli* pBS2187*dfrK* (23) and *S. aureus* CM.S2 (44) were used as positive controls for *dfrK* and *dfrG*, respectively. Positive controls for *fusD* and *dfrD* were not available for use in the present study. Following PCR amplification of the *fusA* gene, PCR products were purified using the Genelute PCR cleanup kit (Sigma-Aldrich, Tallaght, Dublin, Ireland), sequenced commercially by Geneservice, and analyzed using DNA Strider and Bionumerics software using the *fusA* gene consensus sequence of MRSA reference strain N315 (GenBank accession number NC_002745) as a reference sequence.

Nucleotide sequence accession number. The nucleotide sequence of the *ccrAB4*-carrying element from MRSA isolate AR13.1/3330.2 was deposited in the GenBank database under accession number HE858191.

RESULTS

***spa* typing.** The 175 MRSA isolates recovered in Irish hospitals between 1971 and 2004 were previously assigned to 26 ST-SCC*mec* types and subtypes (Table 1) (45, 49). In the present study, these 175 isolates were assigned to 35 *spa* types (Table 1). The *spa* type t190 predominated (41%; 73/175) and accounted for 99% (73/74) of ST8-MRSA-IIA-IIE and ST8-MRSA-IVE/IVF isolates (Table 1). Single *spa* types also accounted for the majority of isolates within four other clones: t008 in ST250-MRSA-I/I-*pls* (95%; 18/

TABLE 1 MLST CCs, STs, SCC*mec* types, and *spa* types of 175 MRSA isolates representative of different antibiogram-resistogram, ST, and SCC*mec* type combinations recovered from patients in Irish hospitals between 1971 and 2004 and the corresponding CCs/STs and SCC*mec* types deduced from DNA microarray profiles of 107 representative isolates

CC	MLST and SCC*mec* typing assignment for 175 isolates tested[b]	*spa* types for 175 isolates tested[c]	DNA microarray[a]		
			CC/ST-SCC*mec* type assignment by microarray for 107 isolates tested[d]	SCC*mec*-associated genes detected by microarray[e]	SCC*mec* type/subtype assignment following manual inspection of microarray profiles
8	ST250-MRSA-I (10)	t008 (10)	ST250-MRSA-I (4)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB1*, *dcs*, *pls*	I
8	ST250-MRSA-I-*pls* (9)	t008 (8), t121 (1)	ST250-MRSA-I (6)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB1*, *dcs*	I-*pls*
8	ST239-MRSA-III and SCC*Hg* (9)	t037 (8), t138 (1)	ST239-MRSA-III (8)	*mecA*, *ugpQ*, Δ*mecR1*(5 Δ*mecR1* negative), *mecR1*, *mecI*, *xylR*, *ccrAB3*, *ccrC*, *merA/B*, *erm*(A), *tet*(K) (1 negative)	III and SCC*Hg*
8	ST239-MRSA- III and SCC*Hg* -p1258/Tn*554* (4)	t037 (4)	ST239-MRSA-III (4)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB3*, *ccrC*, *merA/B*, *erm*(A), *tet*(K)	III and SCC*Hg*
8	ST247-MRSA-Ia (3)	t3503 (2), t052 (1)	ST247-MRSA-I (3)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB1*, *dcs*, *pls*, *aadD*	Ia
8	ST8-MRSA-IIA and *ccrAB4* (9)	t190 (9)	ST8-MRSA-II and CI (4)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *ccrB4*, *dcs* (1 *dcs* negative), *aadD*, *erm*(A)	IIA or IIB and *ccrAB4*
8	ST8-MRSA-IIB and *ccrAB4* (1)	t190 (1)	ST8-MRSA-II and CI (1)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *ccrB4*, *dcs*, *aadD*, *erm*(A)	IIA or IIB and *ccrAB4*
8	ST8-MRSA-IIC and *ccrAB4* (9)	t190 (9)	ST8-MRSA-II and CI (6)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *ccrAB2*, *ccrB4*, *dcs* (1 *dcs* negative), *aadD*, *erm*(A)	IIC and *ccrB4*
8	ST8-MRSA-IIC (1)	t190 (1)	ST8-MRSA-II (1)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *ccrAB2*, *dcs*, *aadD*, *erm*(A)	IIC
8	ST8-MRSA-IID and *ccrAB4* (24)	t190 (23), t2196 (1)	ST8-MRSA-II and CI (12)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *ccrB4*, *dcs* (4 *dcs* negative), *erm*(A)	IID and *ccrAB4*
8	ST8-MRSA-IID (3)	t190 (3)	ST8-MRSA-II (3)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *dcs*, *erm*(A)	IID
8	ST8-MRSA-IIE and *ccrAB4* (6)	t190 (6)	ST8-MRSA-II and CI (5)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *ccrAB2*, *ccrB4*, *dcs*, *erm*(A)	IIE and *ccrAB4*
8	ST8-MRSA-IIE (1)	t190 (1)	ST8-MRSA-II and CI (1)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *ccrAB2*, *erm*(A)	IIE
8	ST8-MRSA-IVE and *ccrAB4* (17)	t190 (17)	ST8-MRSA-IV and CI (2)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *ccrAB4* (1 *ccrA4* negative)	IVE or IVF and *ccrAB4*

(Continued on following page)

TABLE 1 (Continued)

CC	MLST and SCC*mec* typing assignment for 175 isolates tested[b]	*spa* types for 175 isolates tested[c]	DNA microarray[a]		
			CC/ST-SCC*mec* type assignment by microarray for 107 isolates tested[d]	SCC*mec*-associated genes detected by microarray[e]	SCC*mec* type/subtype assignment following manual inspection of microarray profiles
			CC8-MRSA-VI (1)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB4*	VI
8	ST8-MRSA-IVF and *ccrAB4* (3)	t190 (3)	CC8-MRSA-VI (3)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB4 (1 ccrA4* negative*)*	VI
8	ST94-MRSA-IVg and *ccrAB4* (1)	t4691 (1)	ST8-MRSA-IV and CI (1)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *ccrB4*, *dcs*	IV and *ccrAB4*
8	ST609-MRSA-IVA (1)	t064 (1)	CC8-MRSA-IV (1)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs*, *aadD*	IVA
22	ST22-MRSA-IVh (22)	t032 (9), t022 (2), t3505 (1), t1467 (1), t531 (1), t3506 (1), t3504 (1), t790 (1), t515 (1), t3501 (1), t1802 (1), t2945 (1), t4253 (1)	ST22-MRSA-IV (11)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs*	IV
22	ST22-MRSA-IVa (5)	t005 (3), t902 (1), t4902 (1)	ST22-MRSA-IV (2)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs*	IV
5	ST5-MRSA-II (17)	t045 (17)	ST5-MRSA-II (14)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *dcs* (2 *dcs* negative), *kdp*, *aadD*, *erm*(A)	II
5	ST5-MRSA-IV nonsubtypeable (1)	t001 (1)	CC5-MRSA-atypical SCC*mec* element (1)	*mecA*, *ugpQ*, Δ*mecR1*, *dcs*	Class B *mec* and *dcs*, no *ccr* genes detected
5	ST496-MRSA-II (1)	t002 (1)	ST5-MRSA-II (1)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *dcs*, *kdp*, *aadD*, *erm*(A)	II
30	ST36-MRSA-II (12)	t018 (9), t012 (3)	ST36/39-MRSA-II (6)	*mecA*, *ugpQ*, Δ*mecR1*, *mecR1*, *mecI*, *xylR*, *ccrAB2*, *dcs*, *kdp*, *aadD*, *erm*(A)	II
30	ST30-MRSA-IV nonsubtypeable (4)	t5093 (1), t012 (2), t021 (1)	ST30-MRSA-IV (4)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs* (2 *dcs* negative), *Q6GD50* (*fusC*)	IV and Q6GD50/*fusC*
45	ST45-MRSA-IVa (1)	t727 (1)	ST45-MRSA-IV (1)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs*	IV
12	ST12-MRSA-IVc (1)	t160 (1)	CC12-MRSA-IV (1)	*mecA*, *ugpQ*, Δ*mecR1*, *ccrAB2*, *dcs*	IV

[a] The StaphyType kit (Alere Technologies) was used for DNA microarray analysis. CI, composite island.
[b] Numbers in parenthesis refer to the numbers of isolates with the particular MLST and SCC*mec* type assignment indicated.
[c] Numbers in parenthesis refer to the numbers of isolates with the particular *spa* type assignment indicated.
[d] Numbers in parenthesis refer to the numbers of isolates with the MLST and SCC*mec* type assignment indicated as deduced from DNA microarray data.
[e] It is not possible to localize the antimicrobial resistance determinants *aadD*, *tet*(K), *erm*(A), *merA*, and *merB* to within the SCC*mec* elements using the array, but those that were detected and are commonly associated with a particular SCC*mec* type or subtype are listed.

19); t045 in ST5-MRSA-II (100%; 17/17); t037 in ST239-MRSA-III/III-p1258/Tn*554* (92%; 12/13), and t018 in ST36-MRSA-II (75%; 9/12) (Table 1). In contrast, the 27 ST22-MRSA-IV isolates exhibited 16 *spa* types, with *spa* type t032 predominating (33%; 9/27), followed by t005 (11%, 3/27) and t022 (7%, 2/27), with all other ST22 *spa* types being represented by single isolates (Table 1). In no instance was the same *spa* type identified among isolates belonging to different CCs, while only one *spa* type (t012) was detected among isolates belonging to different STs, ST36 and ST30, but to the same CC, CC30 (Table 1).

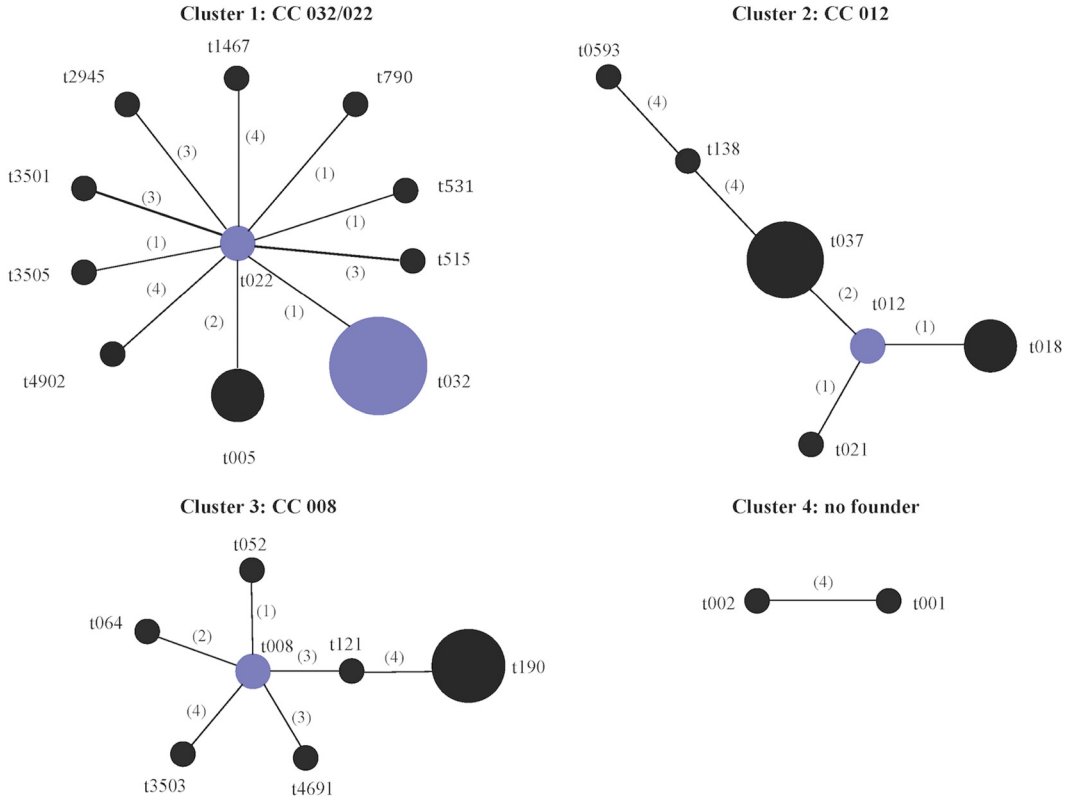

FIG 1 Based Upon Repeat Pattern (BURP) analysis of the *spa* types identified among 175 MRSA isolates representative of the different antibiogram-resistogram, sequence type, and SCC*mec* type combinations recovered from patients in Irish hospitals between 1971 and 2004. Twenty-six of the 35 *spa* types were grouped into four cluster groups using the BURP algorithm. *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value of ≤4, where cost accounts for the number of steps of evolution between *spa* types. A low cost value indicates close evolutionary relatedness between two *spa* types. The following six *spa* types were defined as singletons by BURP (t045, t160, t902, t3504, t3506, and t4253), as they could not be clustered with any other *spa* type, i.e., cost value of ≥5. The *spa* types t727, t1802, and t2196 were excluded, as they consisted of four repeat units only. Cost values are shown in parentheses. Group founders and cofounders (*spa* types with the second-highest group founder score) are shown in blue and are determined based on the *spa* type that shares the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. No founder was assigned to cluster 4.

Analysis of the 35 *spa* types represented among the study isolates using the Based Upon Repeat Pattern (BURP) algorithm assigned 26 of the 35 *spa* types to four clusters (Fig. 1). Cluster 1 (*spa*-CC 032/022) consisted of 11 of the 16 *spa* types identified among ST22-MRSA-IV isolates. Cluster 2 (*spa*-CC 012) consisted of all *spa* types exhibited by CC30 isolates and the CC8/ST239-MRSA-III *spa* types t037 and t138. This was not unexpected, because the ST239 genotype arose as a result of a large chromosomal replacement between ST8 and ST30 isolates encompassing the *spa* locus (39). Cluster 3 (*spa*-CC 008) consisted of all except one of the remaining *spa* types identified among the CC8 isolates, while cluster 4 consisted of two of three *spa* types found among CC5 isolates. The remaining *spa* types were not assigned to any cluster, because they consisted of four repeats only, which is considered to be too few data to deduce evolutionary history, or they differed from all other *spa* types by a cost of ≥5 (where cost indicates the steps of evolution between two *spa* types).

Correlation between DNA microarray analysis and MLST. The DNA microarray assigned the majority of the MRSA isolates investigated to the correct ST (92%; 98/107) (Table 1). Seven isolates were not assigned to any ST but were assigned to the correct CC (7%; 7/107), while two isolates were assigned to incorrect STs (2%; 2/107); but these incorrect STs were within the correct CCs. One isolate with ST496 (which is in CC5) was assigned to ST5, and one isolate belonging to ST94 (which is in CC8) was assigned to ST8 (Table 1). ST496 is a double-locus variant of ST5, while ST94 is a single-locus variant of ST8.

Correlation between SCC*mec* typing and SCC*mec* types deduced by DNA microarray analysis. The majority of MRSA isolates subjected to DNA microarray analysis were assigned to the correct SCC*mec* type (i.e., to SCC*mec* types I, II, III, or IV) (95%; 102/107) compared to results obtained previously by conventional SCC*mec* typing (Table 1). Table S2 in the supplemental material provides an overview of the main characteristics of the SCC*mec* types and subtypes investigated in the present study. Four ST8/t190 isolates that were previously assigned by SCC*mec* typing to SCC*mec* types IVE or IVF (J1 regions IVc and IVb, respectively, as well as class B *mec* and *ccrAB2* and lacking *dcs*) with an adjacent *ccrAB4* were assigned to SCC*mec* VI by the DNA microarray (Table 1). In these isolates, the class B *mec* complex and *ccrAB4* genes were detected using the DNA microarray, but *ccrAB2* was not detected (Table 1). One MRSA isolate belonging to CC5 with *spa* type t001, which was previously found to harbor SCC*mec* IV by SCC*mec* typing, was designated as an atypical SCC*mec* element by the DNA microarray (Table 1). Manual inspection of the SCC*mec*-associated DNA microarray signals of this isolate re-

vealed the presence of a class B *mec* complex and *dcs* but the absence of *ccrAB2*. These five isolates with anomalous SCC*mec* types, as deduced from their DNA microarray profiles because they lacked *ccrAB2*, were retyped using conventional SCC*mec* typing by PCR, which confirmed the absence of *ccrAB2* and suggested that these isolates may have lost the *ccr* genes. Lastly, manual inspection of the DNA microarray profiles indicated that while the eight ST239-MRSA-III*Hg* isolates yielded signals for *mecR1*, the probe for the truncated *mecR1* gene (Δ*mecR1*) did not yield a signal for five of these eight isolates (Table 1).

The DNA microarray did not automatically assign SCC*mec* subtypes to isolates, but manual inspection of the DNA microarray profiles allowed subtyping of SCC*mec* types I, II, and III*Hg* (Table 1). The differentiation of SCC*mec* subtypes Ia and I-*pls* is based on the presence of plasmid pUB110 carrying the aminoglycoside resistance gene *aadD* in SCC*mec* Ia and the absence of *pls* in SCC*mec* I-*pls* (Table 1). The SCC*mec* II subtypes IIA, IIB, IIC, IID, and IIE all lack *kdp*, which is normally associated with SCC*mec* II elements (Table 1). While the SCC*mec* subtypes IIA and IIB cannot be further differentiated using the array, SCC*mec* IIC lacks *xylR* and *mecI* due to the presence of a variant *mec* complex termed class A.3 *mec*. SCC*mec* subtype IID is characterized by the absence of *aadD*, while *xylR*, *mecI*, and *aadD* are absent in SCC*mec* IIE (Table 1). SCC*mec* type III*Hg* was identified by the presence of the mercury resistance genes *merA* and *merB* and by *ccrC* (Table 1). While the DNA microarray was unable to subtype the majority of SCC*mec* IV elements, recognition of SCC*mec* IVA was possible due to the presence of the pUB110-encoding gene *aadD* (Table 1). The array detected the *fusC* gene (designated Q6GD50 on the array) indicative of the presence of the SCC*fus* element (18) in the four ST30 isolates, but additional *ccr* genes (*ccrAB1*) normally associated with the presence of this SCC element were not detected. The *dcs* gene, located between *mecA* and *orfX* and normally found in SCC*mec* II and IV elements, was not detected in eight isolates harboring SCC*mec* II elements (ST8 and ST5) and eight isolates with SCC*mec* IV elements (ST8 and ST30), although this has been reported previously for the ST8 SCC*mec* IVE and IVF elements (45).

The DNA microarray also allowed the identification of the presence of *S. epidermidis ccrAB4* in ST8-MRSA isolates (Table 1). The presence of *S. epidermidis ccrAB4* has been reported previously in ST8 isolates (49). The array yielded positive or ambiguous signals for *ccrA4* and/or *ccrB4* in 36 CC8-MRSA isolates, including ST8-MRSA-IIA-IIE isolates (29/33), ST8-MRSA-IVE-IVF isolates (6/6), and the single ST94-MRSA-IVg isolate investigated using the array. The presence of *S. epidermidis ccrAB4* was confirmed in these isolates by PCR, indicating the possible presence of an SCC element in addition to SCC*mec* in these isolates. The presence of *S. epidermidis ccrAB4* was also investigated by PCR in the ST8-MRSA-IIA-IIE ($n = 21$) and ST8-MRSA-IVE-IVF ($n = 14$) isolates from the present study that did not undergo DNA microarray analysis; the presence of this gene was confirmed in 95% (20/21) and 100% (14/14) of these isolates, respectively.

Detection of a novel SCC*mec*-SCC composite island in ST8-MRSA-IIE isolate AR13.1/3330.2. Whole-genome sequencing was performed on one isolate (AR13.1/3330.2) representative of isolates harboring the *S. epidermidis ccrAB4* gene to determine the genetic organization of the region surrounding *ccrAB4*. Whole-genome sequencing of AR13.1/3330.2 yielded 344 contigs ranging in size from ca. 113 bp to 137 kb. The SCC-associated DNA sequences were identified in three contigs (13 kb, 11 kb, and 764 bp in size), and the gaps between these contigs were closed using long-range PCR amplification and sequencing with primers based on the surrounding contigs. The order of the contigs was confirmed by PCR. This analysis revealed the presence of an approximately 14-kb SCC element downstream and immediately adjacent to SCC*mec* IIE in AR13.1/3330.2 (Fig. 2a). This SCC element exhibited 99% DNA sequence identity to SCC_{M1} (Fig. 2b) previously identified in the Danish ST8-MRSA isolate M1 (*spa* type t024) (2).

In MRSA M1, the SCC_{M1} element exists as part of a composite island and is located directly adjacent to *orfX*, upstream of a truncated arginine catabolic mobile element (ACME) and SCC*mec* IVa (Fig. 2b). In contrast, in AR13/3330.2, the SCC*mec* IIE element was located adjacent to *orfX*, whereas SCC_{M1} was located downstream of SCC*mec* IIE (Fig. 2a). No ACME-*arc* genes were detected in AR13.1/3330.2, either in the whole-genome sequence or by DNA microarray analysis. The SCC_{M1} element comprises 18 ORFs that have been described previously (2), most significantly those encoding *ccrAB4*, a truncated copy of *ccrA* and a gene encoding a copper-transporting ATPase (*copA*) (Fig. 2a and b). SCC_{M1} is similar in its genetic organization to the part of SCC-CI element in *S. epidermidis* ATCC 12228 outside SCC*pbp* (99% DNA sequence identity) but significantly smaller in size and lacking the mercury and cadmium resistance genes (Fig. 2c). SCC_{M1} also shows 99% DNA sequence identity to a region downstream of *mecA* in SCC*mec* VIII (Fig. 2d). The composite island (CI) detected in AR13.1/3330.2 was designated SCC*mec* IIE-SCC_{M1} (Fig. 2a).

Confirmation of the presence of SCC_{M1} in representative CC8 isolates harboring the *S. epidermidis ccrAB4* genes. The presence of the SCC_{M1} element and its location downstream of SCC*mec* was investigated by PCR in seven additional isolates representative of other ST and SCC*mec* type combinations identified as harboring the *S. epidermidis ccrAB4*. Five of the seven isolates, including those representative of ST8-MRSA-IIA, ST8-MRSA-IIB, ST8-MRSA-IIC, ST8-MRSA-IID, and ST94-MRSA-IVg, yielded amplicons of the expected size for SCC_{M1}, and its location was downstream of SCC*mec*, as found in isolate AR13.1/3330.2 (Fig. 2a). These SCC*mec*-SCC CIs were assigned the following designations: SCC*mec* IIA-SCC_{M1}, SCC*mec* IIB-SCC_{M1}, SCC*mec* IIC-SCC_{M1}, SCC*mec* IID-SCC_{M1}, and SCC*mec* IVg-SCC_{M1}. For the remaining two isolates (ST8-MRSA-IVE and ST8-MRSA-IVF), the primer pairs located within the SCC_{M1} element (primer pairs 2, 3, and 4; see Table S1 in the supplemental material) yielded amplicons of the expected size, but those amplifying from the respective SCC*mec* elements to within the SCC_{M1} element (primer pairs 5 and 6; see Table S1 in the supplemental material) failed to yield any amplicons. These findings indicate that while the SCC_{M1} element is present in these ST8-MRSA isolates harboring SCC*mec* IVE and IVF, further investigations are required to determine the genetic organization of the SCC*mec* and SCC_{M1} elements in these isolates.

Additional typing markers and antimicrobial resistance, virulence-associated, and adhesion genes detected using the DNA microarray. The *agr* capsule and immune evasion cluster (IEC) types, as well as the antimicrobial resistance, virulence-associated, MSCRAMM, adhesion, and biofilm formation genes identified among the 107 MRSA isolates representative of the different ST and SCC*mec* type combinations investigated by DNA microarray

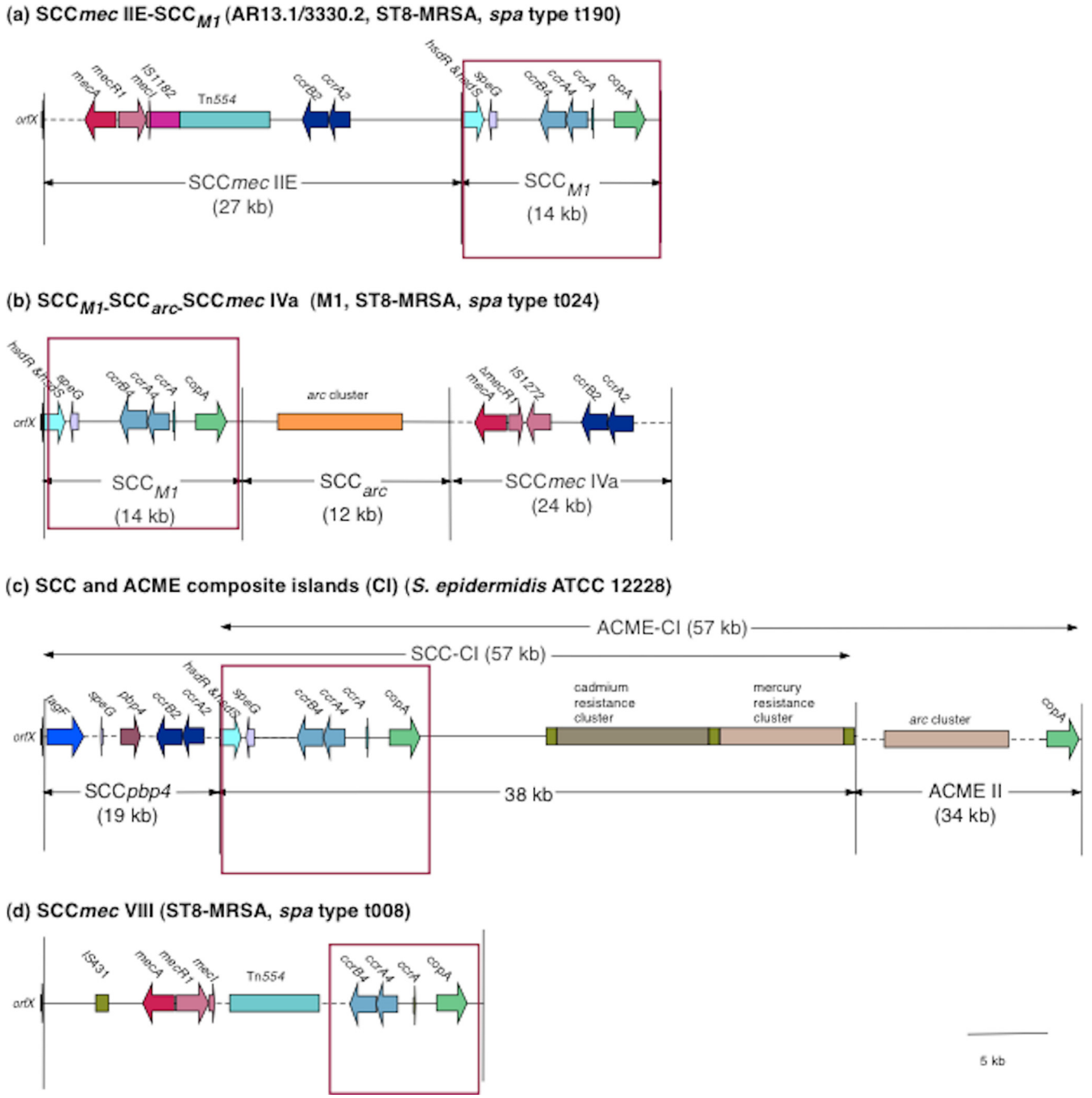

FIG 2 Schematic diagram showing the genomic organization of the novel SCC*mec*-SCC composite island (CI) SCC*mec* IIE-SCC_{M1} identified in the ST8-MRSA *spa* type t190 isolate AR13.1/3330.2 (GenBank accession number HE858191) (a), the SCC_{M1}-SCC_{arc}-SCC*mec* element previously reported in ST8-MRSA *spa* type t024 isolate M1 (HM030720) (b), the ACME- and SCC-CIs previously reported in *S. epidermidis* ATCC 12228 (NC004461) (c), and SCC*mec* VIII previously described in ST8-MRSA *spa* type t008 isolate C10682 (FJ390057) (d). The structure of the novel SCC*mec* IIE-SCC_{M1} element was determined by high-throughput whole-genome sequencing of AR13.1/3330.2 and was confirmed using primers spanning the SCC*mec*/SCC region. The areas surrounded by the red square indicate the regions of high similarity between SCC_{M1} identified in isolate AR13.1/3330.2 and the other SCC*mec*/SCC elements.

analysis in the present study, are shown in Table 2. The majority of isolates belonged to *agr* type I (75%; 80/107) and included all CC8 and CC22 isolates ($n = 66$ and 13, respectively). All CC5 ($n = 16$) and the single CC12 isolate belonged to *agr* type II, while isolates within *agr* III were CC30 ($n = 10$). The single CC45 isolate belonged to *agr* type IV. Most isolates belonged to capsule type 5 (78%; 83/107), with the remainder belonging to capsule type 8 (22%; 24/107). Various combinations of IEC genes were detected in the majority of isolates (91%; 97/107), with the most frequently occurring IEC type being type D (39%; 42/107). IEC type D was detected in the CC8 lineages ST239-MRSA-III, ST8-MRSA-IIA–IIE, and ST609-MRSA-IVA and consisted of the enterotoxin A

TABLE 2 *agr*, capsule and IEC types, antimicrobial resistance, virulence-associated, MSCRAMM, adhesion, and biofilm formation genes identified using the DNA microarray among isolates representative of each antibiogram-resistogram, ST, and SCC*mec* type combination recovered from patients in Irish hospitals between 1971 and 2004[a]

	Additional typing marker			Antimicrobial resistance genes		Virulence-associated genes		MSCRAMM, adhesion, and biofilm genes	
CC/ST-SCC*mec* type (*n*)	*agr*	*cap*	IEC (*n*)[b]	Always present	Variably present (% isolates positive)	Always present	Variably present (% isolates positive)	Always present	Variably present (% isolates positive)
CC8/ST250-MRSA-I/I-*pls* (10)	I	5	B (10)	*blaZ*, *merA/B*, *erm*(A), *sdrM*, *qacA*, *fosB*	*tet*(K) (70), *aacA-aphD* (90), *aphA3* and *sat* (40), *dfrS1* (40)	*sak*, *chp*, *scn*	*seb* (90), *sek* and *seq* (90)	*bbp*, *clfA*, *clfb*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC8/ST239-MRSA-III and SCC*Hg*/III and SCC*Hg*-p1258/Tn554 (12)	I	8	D (12)	*blaZ*, *merA/B*, *erm*(A), *tet*(M), *sdrM*, *fosB*	*tet*(K) (92), *qacA* (58), *qacC* (8), *aacA-aphD* (83), *aadD* (8), *aphA3* and *sat* (50), *dfrS1* (25), *mupA* (8), *cat*-pC194 (pMC524) (8)	*sea*, *sak*, *scn*	*sek* and *seq* (83)	*clfA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	*bbp* (58)
CC8/ST247-MRSA-Ia (3)	I	5	A (1), B (2)	*blaZ*, *merA/B* *tet*(M), *sdrM*, *qacA*, *aacA-aphD*, *aadD*, *fosB*	*erm*(A) (67)	*sak*, *chp*, *scn*	*sea* (33)	*clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC8/ST8-MRSA-IIA-IIE (33)	I	5	D (29), NT (1),[c] Neg (3)	*blaZ*, *erm*(A), *aacA-aphD*, *sdrM*, *fosB*, *merA/B*	*erm*(C) (6), *tet*(K) (3), *qacA* (97), *qacC* (3), *aadD* (36), *aphA3* and *sat* (85), *dfrS1* (3), *far1/fusB* (3), *mupA* (12)	.....	*sea* (91), *sak* (91), *scn* (88)	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fnbA*, *fnbB*, *sdrD*, *vwb*, *icaA*, *icaC*, *icaD*	*fib* (97), *map* (97), *sdrC* (3)
CC8/ST8-MRSA-IVE/F (6)	I	5	E (5), Neg (1)	*blaZ*, *erm*(A), *sdrM*, *aphA3* and *sat*, *fosB*	*tet*(M) (33), *qacA* (17)	.....	*sak* (83), *scn* (83)	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	*sdrD* (83)
CC8/ST94-MRSA-IVg (1)	I	5	Neg (1)	*blaZ*, *tet*(M), *sdrM*, *aacA-aphD*, *aphA3* and *sat*, *fosB*	.....	.....	.....	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC8/ST609-MRSA-IVA (1)	I	5	D (1)	*blaZ*, *erm*(C), *tet*(M), *sdrM*, *aacA-aphD*, *aadD*, *dfrS1*, *fosB*	.....	*sea*, *seb*, *sek* and *seq*, *sak*, *scn*	.....	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC icaD*	.....
CC22/ST22-MRSA-IVh/IVa (13)	I	5	B (8) Neg (5)	*blaZ*	*erm*(C) (46), *qacC* (8)	*egc*	*sec* and *sel* (85), *sak* (62), *chp* (62), *scn* (62)	*clfA*, *clfB*, *cna*, *ebpS*, *eno*, *fib*, *fnbA*, *sdrC*, *vwb*, *sasG*, *icaA*, *icaD*	*fnbB* (15), *icaC* (85), *bbp* (85), *ebh* (15), *map* (92), *sdrD* (85)
CC5/ST5-MRSA-II (14)	II	5	F (12) B (1) A (1)	*erm*(A), *sdrM*, *aadD*, *fosB*	*blaZ* (86), *qacA* (21), *merA/B* (21)	*tst*, *egc*,[c] *sak*, *chp*, *scn*	*sea* (7), *sep* (86), *sed*, *j* and *r* (71)	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....

(Continued on following page)

TABLE 2 (Continued)

CC/ST-SCC*mec* type (*n*)	Additional typing marker			Antimicrobial resistance genes		Virulence-associated genes		MSCRAMM, adhesion, and biofilm genes	
	agr	*cap*	IEC (*n*)[b]	Always present	Variably present (% isolates positive)	Always present	Variably present (% isolates positive)	Always present	Variably present (% isolates positive)
CC5/ST5-MRSA-IV nonsubtypeable (1)	II	5	B (1)	*merA* and *B*, *blaZ*, *erm*(A), *sdrM*, *fosB*	.....	*egc*, *sak*, *chp*, *scn*	.....	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *map*, *sdrC*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC5/ST496-MRSA-II (1)	II	5	B (1)	*erm*(A), *erm*(C), *aadD*, *sdrM*, *fosB*	.....	*egc*, *sak*, *chp*, *scn*	.....	*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC30/ST36-MRSA-II (6)	III	8	A (6)	*blaZ*, *erm*(A), *sdrM*, *aadD*, *fosB*	.....	*sea*, *egc*, *sak*, *chp*, *scn*	*tst* (67)	*bbp*, *clfA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fnbA*, *fib*, *map*, *sdrC*, *sdrD*, *vwb*, *icaA*, *icaC*, *icaD*	.....
CC30/ST30-MRSA-IV nonsubtypeable (4)	III	8	B (4)	*blaZ*, Q6GD50/*fusC*, *fosB*	*sdrM* (75)	*tst*, *egc*, *sak*, *chp*, *scn*	.....	*clfA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *map*, *sdrC*, *sdrD*, *vwb*, *icaA*, *icaC*, *icaD*	*bbp* (75)
CC45/ST45-MRSA-IVa (1)	IV	8	B (1)	*blaZ*, *merA* and *B*, *erm*(C), *far1*/*fusB*, *sdrM*	.....	*egc*, *sak*, *chp*, *scn*	.....	*bbp*, *clfA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb*, *sasG*, *icaA*, *icaC*, *icaD*	.....
CC12/ST12-MRSA-IVc (1)	II	8	G (1)	*blaZ*, *sdrM*, *fosB*	.....	*sep*, *seb*, *sec* (M14), *sak*, *scn*	.....	*clfA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *vwb*, *icaA*, *icaC*, *icaD*	.....

[a] The StaphyType kit (Alere Technologies) was used for DNA microarray analysis. *n*, number of isolates; *agr*, genes encoding accessory gene regulators; *cap*, genes encoding capsular proteins; CC, clonal complex; ST, sequence type; MSCRAMM, microbial surface components recognizing adhesive matrix molecules; ....., no genes detected.
[b] Immune evasion cluster (IEC) type as defined by van Wamel et al. (54): A = *sea*, *sak*, *chp*, and *scn*; B = *sak*, *chp*, and *scn*; C = *chp* and *scn*; D = *sea*, *sak*, and *scn;* E = *sak* and *scn*; F = *sep*, *sak*, *chp*, and *scn*; G = *sep*, *sak*, and *scn*; NT, novel IEC type consisting of *sak* and *sea* only; Neg (negative), no IEC genes detected.
[c] *seg* and *sei* were not detected in one and two ST5-MRSA-II isolates, respectively, found to carry the enterotoxin gene cluster (*egc*) genes seg/*sei*, *sem*, *sen*, *seo*, and *seu*.

gene *sea* and the genes encoding staphylokinase (*sak*) and the staphylococcal complement inhibitor (*scn*). Other IEC types represented included IEC types A ($n = 8$ isolates), B ($n = 28$ isolates), E ($n = 5$ isolates), F ($n = 12$ isolates), and G ($n = 1$ isolate).

The main virulence-associated genes identified among the MRSA isolates representative of the different STs and CCs are shown in Table 2. Ninety-four percent of isolates carried one or more superantigen genes with enterotoxin A (*sea*) (48%; 51/107) predominating, followed by *egc* (37%; 40/107), *tst* (21%; 22/107), and *sek* and *seq* (19%; 20/107). All isolates lacked the PVL locus genes *lukF-PV* and *lukS-PV*; the exfoliative toxin genes *etA*, *etB*, and *etD*; and ACME. All isolates harbored the MSCRAMM, adhesion, and biofilm genes *fnbA* (fibronectin binding protein A), *clfA* and *clfB* (clumping factor A and B), *ebpS* (elastin binding protein), *eno* (laminin binding protein), *icaA* (intracellular adhesion protein A), *icaD* (intracellular adhesion protein A), and *vwb* (van Willebrand factor binding protein). The majority of isolates also harbored *fib* (fibrinogen binding protein) (98%; 105/107) and *map* (major histocompatibility compound class II analog protein) (97%; 104/107).

The most common antimicrobial resistance genes (apart from *mecA*, which was detected in all isolates tested) were *blaZ* (beta-lactamase resistance gene) (97%; 104/107), *sdrM* (encoding a multidrug efflux pump conferring resistance to norofloxacin, acriflavine, and possibly ethidium bromide [57]) (87%; 93/107), *fosB* (fosfomycin resistance gene) (87%; 93/107), *erm*(A) (resistance to macrolides, lincosamides, and streptogramins) (79%; 85/107), *merA* and *merB* (mercury resistance genes) (59%; 63/107),

qacA (resistance to quaternary ammonium compounds, ethidium bromide) (52%; 56/107), and *aacA-aphD* (resistance to amikacin, gentamicin, kanamycin, and tobramycin) (53%; 57/107). CC8 isolates harbored the highest number of resistance determinants, with ST8-MRSA-IIA-IIE and ST239-MRSA-III carrying genes encoding resistance to 10 classes of antimicrobials, whereas ST22-MRSA-IV carried resistance to three classes of antimicrobials.

Correlation between antimicrobial resistance phenotype and genotype. The correlation between antimicrobial agent resistance phenotype (determined by disk diffusion) and genotype for the 107 MRSA isolates (determined by DNA microarray profiling) are shown in Table 3 for antimicrobial resistance genes yielding positive results only, i.e., all isolates yielded negative results for the genes encoding resistance to antimicrobials in the following antimicrobial classes, and results are not included in Table 3: chloramphenicol (*cfr* and *fexA*, encoding chloramphenical and florfenicol resistance); glycopeptides (*vanA*, *vanB*, and *vanZ*, encoding vancomycin and/or teicoplanin resistance); macrolides, lincosamides, or streptogramins (MLS) [*lnu*(A), *mef*(A), *mph*(C), *msr*(A), *vat*(A), *vat*(B), *vga*(A), *vgb*(A), encoding resistance to a range of MLS antimicrobial compounds].

Eighty-three of the 107 MRSA isolates exhibited both phenotypic and genotypic resistance to aminoglycosides. The three aminoglycoside resistance genes detected by the DNA microarray are *aacA-aphD*, encoding resistance to amikacin, gentamicin, kanamycin, and tobramycin; *aadD*, encoding resistance to amikacin, kanamycin, neomycin, and tobramycin; and *aphA3*, encoding resistance to kanamycin and neomycin. The level of resistance to amikacin encoded by *aacA-aphD* is low (27) and may be difficult to detect by disk diffusion. Hence, many isolates carrying *aacA-aphD* or *aadD* exhibited resistance in the intermediate category or were phenotypically susceptible to amikacin (Table 3). The relative frequencies of occurrence of the various aminoglycoside resistance gene combinations was as follows: *aacA-aphD* and *aphA3*, 31% (26/83); *aadD*, 24% (20/83); *aacA-aphD*, 17% (14/83); *aacA-aphD*, *aadD*, and *aphA3*, 16% (13/83); and *aphA3*, 7% (6/83). The phenotypic resistance profiles for each isolate harboring these gene combinations are shown in Table 3. In general (aside from the problem of amikacin resistance with isolates carrying *aacA-aphD* and/or *aadD*), correlation between phenotypic and genotypic expression of resistance was excellent (99%), with just one isolate carrying *aacA-aphD*, *aadD*, and *aphA3* exhibiting phenotypic susceptibility to gentamicin (Table 3).

For all other antimicrobials tested, apart from fusidic acid and trimethoprim, the correlation between phenotype and genotype was ≥97%. A 100% correlation between the antimicrobial resistance phenotype and the presence of a particular gene or genes was observed for high-level mupirocin resistance and *mupA*, ethidium bromide resistance and *qacA* and/or *qacC*, erythromycin resistance and *erm*(A) and/or *erm*(C), and chloramphenicol resistance and *cat* (Table 3). All beta-lactam-resistant isolates harbored *mecA*, while 97% (104/107) also harbored the beta-lactam resistance gene *blaZ*. One isolate that lacked *blaZ* exhibited intermediate resistance to ampicillin. All isolates harboring *tet*(K), *tet*(M), or both of these genes exhibited tetracycline resistance. Two isolates exhibited intermediate resistance to tetracycline, lacked *tet*(K) and *tet*(M) in their DNA microarray profile, and tested negative for *tet*(L) and *tet*(O) when investigated using conventional PCR assays. The mercury resistance genes *merA* and *merB* were detected in all but one isolate exhibiting resistance to mercuric chloride, but one isolate exhibiting susceptibility to mercuric chloride harbored *merA* and *merB*.

There were major discrepancies with fusidic acid and trimethoprim resistance. Only 29% of fusidic acid-resistant isolates (6/21) and 35% of trimethoprim-resistant isolates (9/26) were DNA microarray gene positive for genes encoding resistance to these agents (Table 3).

The fusidic acid resistance genes *far1/fusB* were detected in one ST45-MRSA-IV and one ST8-MRSA-IIB isolate, while the Q6GD50/*fusC* gene was detected in four ST30-MRSA-IV isolates. None of the remaining 15 fusidic acid-resistant isolates were found to harbor the alternative fusidic acid resistance gene *fusD* by *fusD*-specific PCRs (*fusD* is not detected by the DNA microarray). The *fusA* gene in each of these 15 fusidic acid-resistant isolates was amplified by PCR followed by amplimer sequencing, as mutations in *fusA* have previously been shown to be associated with fusidic acid resistance in *S. aureus*. Mutations in *fusA* were identified in all 15 isolates. Three different combinations of mutations were identified in these isolates, including (i) an amino acid substitution from leucine to lysine at position 461 in the protein sequence (L461K) in eight isolates (seven CC8 isolates: one ST8-MRSA-IID, one ST250-MRSA-I-*pls*, and five ST250-MRSA-I/I-*pls* isolates; one ST22-MRSA-IVh isolate), (ii) an amino acid substitution from leucine to serine at position 461 in the protein sequence (L461S) in five isolates (one ST5-MRSA-II, one ST239-MRSA-III, and three ST250-MRSA-I-*pls* isolates), and (iii) an amino acid substitution from alanine to valine and phenylalanine to leucine at positions 70 (A70V) and 406 (F406K), respectively, in the protein sequence of two isolates (two ST8-MRSA-IIC isolates).

Twenty-six isolates exhibited phenotypic resistance to trimethoprim, and only 35% (9/26) of these harbored the *dfrS1* gene, as determined by DNA microarray profiling (all were CC8 isolates as follows: ST609-MRSA-IVA [$n = 1$], ST8-MRSA-IIE [$n = 1$], ST239-MRSA-III [$n = 3$], and ST250-MRSA-I/I-*pls* [$n = 4$]). The *dfrS1* gene is the only trimethoprim resistance gene detected by the microarray. The remaining 17 trimethoprim-resistant isolates were investigated by a variety of specific PCR assays designed to detect other genes encoding trimethoprim resistance, including *dfrD*, *dfrG*, and *dfrK*. None of the 17 trimethoprim-resistant isolates harbored *dfrD* or *dfrK*, whereas three isolates harbored *dfrG* (all ST239-MRSA-III). The 14 isolates in which no *dfr* genes were identified belonged to ST8-MRSA-IIC ($n = 4$), ST8-MRSA-IIE ($n = 2$), ST8-MRSA-IVE ($n = 2$), ST8-MRSA-IVF ($n = 3$), ST5-MRSA-IV ($n = 1$), and ST239-MRSA-III ($n = 2$).

DISCUSSION

The application of *spa* typing and DNA microarray profiling for in-depth molecular characterization of a diverse collection of nosocomial MRSA isolates representative of clones that predominated in Irish hospitals between 1971 and 2004 revealed extensive genetic diversity among the 175 isolates investigated. A total of 35 *spa* types were identified among these 175 isolates that had previously been assigned to 13 STs representing six CCs by MLST and to 26 ST-SCC*mec* type combinations representative of pandemic and epidemic MRSA lineages by MLST and conventional SCC*mec* typing. Almost half of all *spa* types (46%; 16/35) were identified among isolates of the ST22-MRSA-IV clonal lineage, which represented only 15% of isolates investigated (27/175). In contrast, within the majority of other clonal lineages, isolates were assigned to one or two *spa* types only. In particular, the majority of ST8

TABLE 3 Correlation between antimicrobial resistance gene detection by array profiling and phenotypic detection of resistance by disk diffusion[f]

Gene(s)	Conferring resistance to:	No. of isolates with gene profile	Antimicrobial(s) tested (resistance profile obtained)[h]	No. of isolates resistant	Correlation between genotype and phenotype	
					No. of discrepancies	% correlation[g]
Aminoglycoside resistance genes			AMI, GEN, KAN, NEO, TOB	83	1	99 (106/107)
aacA-aphD	AMI, GEN, KAN, TOB	14	AMI, GEN, KAN, ..., TOB	5	0	
			....., GEN, KAN, ..., TOB	9[a]	0[a]	
aadD	AMI, KAN, NEO, TOB	20	AMI, ..., KAN, NEO, TOB	18	0	
			....., ..., KAN, NEO, TOB	2[a]	0[a]	
aphA3	KAN, NEO	6	....., ..., KAN, NEO, ...	6	0	
aacA-aphD and *aadD* and *aphA3*	AMI, GEN, KAN, NEO, TOB	13	AMI, GEN, KAN, NEO, TOB	12	0	
			....., ..., KAN, NEO, TOB	1	1	
aacA-aphD and *aphA3*	AMI, GEN, KAN, NEO, TOB	26	AMI, GEN, KAN, NEO, TOB	11	0	
			....., GEN, KAN, NEO, TOB	15[a]	0[a]	
aacA-aphD and *aadD*	AMI, GEN, KAN, NEO, TOB	4	AMI, GEN, KAN, NEO, TOB	4	0	
None		24	Susceptible to all ($n = 24$)	0	0	
Antiseptics, disinfectants, intercalating dye resistance genes			Ethidium bromide	58	0	100 (107/107)
qacA	QACs,[b] ethidium bromide, chlorhexidine	55	Ethidium bromide	55	0	
qacC	QACs,[b] ethidium bromide	2	Ethidium bromide	2	0	
qacA and *qacC*	QACs, ethidium bromide, chlorhexidine	1	Ethidium bromide	1	0	
None		49	Susceptible ($n = 49$)	0	0	
Beta-lactam (excluding methicillin) resistance gene			Ampicillin	107	3[c]	97 (104/107)
blaZ	Beta-lactams (not methicillin)	104	Ampicillin	104	0	
None		3	Susceptible ($n = 0$)	3[c]	3[c]	
Chloramphenicol resistance gene			Chloramphenicol	1	0	100 (107/107)
cat-pC194 (pMC524)	Chloramphenicol	1	Chloramphenicol	1	0	
None		106	Susceptible ($n = 106$)	0	0	
Fusidic acid resistance genes			Fusidic acid	21	15	86 (92/107)
far1 (fusB)	Fusidic acid	2	Fusidic acid	2	0	
Q6GD50 *(fusC)*	Fusidic acid	4	Fusidic acid	4	0	
None		101	Susceptible ($n = 86$)	15	15	
Heavy metal ions resistance genes			MER, PMA	63	2	98 (105/107)
merA	Mercury ions	0	Mercuric chloride	0	0	
merB	Organomercurial ions	0	Phenylmercuric acetate	0	0	
merA and *merB*	Mercury and organomercurial ions	63	MER, PMA	62	1	
None		44	Susceptible ($n = 43$)	1	1	
Macrolides, lincoamides, streptogramins (MLS) resistance genes			Erythromycin, (lincomycin)	93 (47)[d]	0	100 (107/107)
erm(A)	Erythromycin, Clindamycin	82	Erythromycin (lincomycin)	82 (46)[d]	0	
erm(C)	Erythromycin, Clindamycin	8	Erythromycin (lincomycin)	8 (1)[d]	0	
erm(A) and *erm*(C)		3	Erythromycin (lincomycin)	3 (0)[d]	0	
None		14	Susceptible ($n = 14$)	0	0	
Mupirocin resistance gene			Mupirocin (high level)	5	0	100 (107/107)
mupA-ileS2	Mupirocin (high level)	5	Mupirocin (high level)	5	0	
None		102	Susceptible ($n = 102$)	0	0	

(Continued on following page)

TABLE 3 (Continued)

Gene(s)	Conferring resistance to:	No. of isolates with gene profile	Antimicrobial(s) tested (resistance profile obtained)[h]	No. of isolates resistant	Correlation between genotype and phenotype	
					No. of discrepancies	% correlation[g]
Tetracycline resistance genes			Tetracycline	29[e]	2[e]	98 (105/107)[e]
tet(K)	Tetracycline	8	Tetracycline	8	0	
tet(M)	Tetracycline	8	Tetracycline	8	0	
tet(K) and *tet*(M)	Tetracycline	11	Tetracycline	11	0	
None		80	Susceptible ($n = 78$[e])	2[e]	2[e]	
Trimethoprim resistance gene			Trimethoprim	26	17	84 (90/107)
dfrS1	Trimethoprim	9	Trimethoprim	9	0	
None		98	Susceptible ($n = 81$)	17	17	

[a] Variations in expression of phenotypic resistance to amikacin are not considered to be discrepant results because the reduction in amikacin susceptibility encoded by *aacA-aphD* may be very slight (27).
[b] QAC, quaternary ammonium compound. *qacA* encodes resistance to QACs, divalent cations and intercalating dyes (such as ethidium bromide); *qacC* encodes resistance to QAC and intercalating dyes.
[c] One of the three ampicillin resistant isolates exhibited intermediate resistance to ampicillin.
[d] Numbers in square brackets indicate numbers of isolates exhibiting resistance to lincomycin.
[e] Two of 29 tetracycline-resistant isolates exhibited intermediate resistance, and no tetracycline resistance genes were detected.
[f] AMI, amikacin; GEN, gentamicin; KAN, kanamycin; MER, mercuric chloride; NEO, neomycin; PMA, phenylmercuric acetate; TOB, tobramycin.
[g] The numbers in parentheses refer to the total number of isolates for which the antimicrobial phenotype correlated with the genotype as determined by array profiling over the total number of isolates investigated.
[h] ....., no phenotypic resistance detected to the relevant aminoglycoside antimicrobial.

isolates (42% of total isolates investigated) were assigned to a single *spa* type, suggesting that the *spa* types of ST8 isolates may be more stable over time than those among ST22-MRSA-IV isolates. In support of this suggestion, Strommenger et al. previously reported that isolates within the different CC8 clones recovered at different time periods and from different geographic locations also exhibited the same *spa* types (51). A possible explanation for the greater variation in *spa* types among ST22-MRSA-IV isolates may lie in the higher number of repeat sequences in the VNTR region of the *spa* gene in this clone. The average number of repeats in the 16 *spa* types identified among ST22-MRSA-IV isolates in the present study was 13 (range, 4 to 17), with the most frequently occurring *spa* type (t032) carrying 16 repeats. In contrast, the *spa* type t190, to which all the ST8-MRSA-IIA-IIE and ST8-MRSA-IVE/IVF isolates were assigned, consists of only seven repeats.

DNA microarray profiling was found to be effective for genotyping MRSA isolates by assigning them to the correct CCs and/or STs and SCC*mec* types, indicating that this approach has the potential to replace MLST and SCC*mec* typing for genotyping and for investigating the evolutionary relatedness of MRSA. The advantages of using the DNA microarray for MLST and SCC*mec* typing include the fact that the DNA microarray also detects clinical relevant virulence and antimicrobial resistance genes and requires only a single multiplex PCR and a 2- to 3-h test procedure per isolate to generate data that require multiple multiplex PCRs and agarose gel electrophoresis to assign an SCC*mec* type/subtype and at least seven PCRs and subsequent sequencing to assign an ST by conventional methods. In a hospital setting, and particularly in an outbreak investigation, greater discrimination than that provided by ST and SCC*mec* type assignment is required to effectively type and track MRSA. While each clone included in the present study was found to have a characteristic combination of virulence and antimicrobial resistance genes, there were also differences observed in the carriage of these genes within the clones, e.g., the carriages of *sec*, *sel*, *erm*(C), and IEC were variable in ST22-MRSA-IV. Whether these differences could be used to provide enhanced discrimination and tracking of MRSA in hospitals either using the array as a standalone typing tool or in combination with other methods, such as *spa* typing, requires further investigation.

It is important to note that the array had difficulty differentiating between some sporadically occurring STs that are single-locus variants of more common STs and with subtyping of some SCC*mec* elements. While identification of some SCC*mec* I, II, and IV subtypes was possible following manual inspection of array profiles, in some cases this identification relied on the detection of the presence of a particular resistance determinant associated with a particular SCC*mec* type, such as *aadD* located on plasmid pUB110 in SCC*mec* types IA, IIA, IIB, IIC, and IVA. However, while this may aid in the identification of a particular SCC*mec* subtype, it is not possible to definitively localize this or any of the other possibly SCC*mec*-associated resistance determinants, i.e., *erm*(A), *tet*(K), and *mer*, which can be located on Tn*554* in SCC*mec* II and III, pT181 in SCC*mec* III, and p1258 in SCC*mec* III with SCCHg, respectively, to within the SCC*mec* element using the array, and results need to be confirmed by PCR. Array profiling facilitated the identification of possible DNA sequence variation in *mecR1* in some SCC*mec* III elements and in the *dcs* gene in some SCC*mec* II and IV elements that were not detected using conventional SCC*mec* typing PCR assays. In addition, the fusidic acid resistance determinant Q6GD50/*fusC* was detected in four ST30-MRSA-IV isolates using the array, indicating the possible presence of an SCC*fus* element in these isolates. Array profiling also indicated that five isolates harboring SCC*mec* IV may have lost *ccrAB2*, which was subsequently confirmed by PCR. Four of these isolates were assigned to ST8-MRSA-IVE/F, which also harbored a second set of *ccr* genes (*ccrAB4*) outside SCC*mec*, suggesting that these isolates may have lost *ccrAB2* genes because they were not essential for excision or integration of the SCC*mec* element due to the presence of alternative *ccr* genes. The ability to accurately identify SCC*mec* types is essential to the use of this

method for genotyping MRSA, and isolates such as these that may have lost *ccr* genes may be wrongly classified using SCC*mec* typing because of the presence of the second set of *ccr* genes outside the SCC*mec* element.

Several novel SCC*mec*-SCC-CIs were identified in the present study, each consisting of an SCC*mec* II or IV element (IIA-IIE, IVE, IVF, or IVg) and SCC_{M1} harboring *ccrAB4*. In *S. aureus*, the DNA sequence corresponding to the SCC_{M1} element has been identified only in MRSA belonging to CC8 and mainly to ST8 (2, 8, 58). CC8 is one of the largest MRSA lineages, consisting of a substantial number of MRSA clones with extensive diversity in SCC*mec* (14, 34, 45), and evidence suggests that the diversity in the SCC*mec* elements of MRSA isolates is due to horizontal transfer and recombination events between SCC*mec* and SCC elements from *S. aureus* isolates and CoNS. DNA sequence identity between SCC_{M1} in ST8-MRSA and the SCC-CI in *S. epidermidis* ATCC 12228 led Bartels et al. to speculate that the former may have evolved from the latter following recombination events between SCC*mec* and SCC-CI elements in MRSA and *S. epidermidis* (2). We previously reported that DNA sequence analysis of the SCC*mec* elements identified in ST8-MRSA isolates recovered in Ireland revealed the presence of SCC*mec* elements that may have been generated following recombination and genetic rearrangements between different SCC*mec* elements (45). The finding of the additional SCC_{M1} element in these ST8 isolates in the present study suggests that they have acquired the SCC_{M1} element either from another *S. aureus* or from CoNS. Only 8% (6/75) of the ST8/t190 or t2196 and ST94/t4691 isolates investigated in the present study did not carry the *ccrAB4* element, and while they may have lost SCC_{M1}, it is also possible that they never harbored it. Thus, these ST8 isolates carrying the SCC*mec* region only may be precursors of isolates harboring the SCC*mec*-SCC_{M1} elements.

Whether the acquisition of the SCC_{M1} element within the ST8 isolates occurred on one or multiple occasions remains to be determined. However, the presence of SCC_{M1} in association with three distinct groups of SCC*mec* elements (SCC*mec* IIA-IIE, SCC*mec* IVE/IVF, and SCC*mec* IVg) suggests at least three possible independent acquisitions of SCC_{M1}. Interestingly, the ST8-MRSA-IIA–IIE isolates which harbored the SCC*mec*-SCC_{M1} element predominated in Irish hospitals during the 1990s but since 2002 have been recovered only sporadically. This may be due in part to a fitness cost associated with harboring such a large genetic element. While the presence of an SCC element in addition to an SCC*mec* element in MRSA can complicate SCC*mec* typing, the presence of SCC elements, such as SCC_{M1}, in MSSA could also compromise the accuracy of rapid MRSA detection assays, such as the GeneXpert real-time PCR assay (49).

DNA microarray profiling allowed the detection of characteristic combinations of antimicrobial resistance and virulence genes among major MRSA clones. Only nine percent of isolates investigated lacked the bacteriophage-encoded IEC genes, and half of these belonged to ST22-MRSA-IV. Ninety-four percent of isolates carried one or more superantigen gene. The ST22-MRSA-IV isolates were the only isolates found to harbor *sec* and *sel*, which have been reported previously to be colocated on a pathogenicity island (32). The toxic shock toxin *tst* gene, which has also been found to be located on various pathogenicity islands (32), was identified in CC30 and CC5 isolates only. The enterotoxin genes *sek* and *seq* were identified only in the CC8 lineages ST239 and ST250 and have been reported to be colocated on various pathogenicity islands and bacteriophages, while *seb*, which has also been found to be located either on a pathogenicity island with *sek* and *seq* or on a plasmid, was also detected in ST250 isolates (32). Isolates belonging to ST22-MRSA-IV harbored the fewest antimicrobial resistance genes, with approximately half of the isolates harboring *blaZ* only. In contrast, isolates belonging to CC8 lineages (ST8-MRSA-IIA-E and ST239-MRSA-III/III-pI258/Tn*554*) harbored the greatest number of antimicrobial resistance genes (up to 17 in each case).

The present study revealed that, in most cases, the presence of a particular antimicrobial resistance gene correlated with phenotypic resistance to a specific type of antimicrobial agent(s), but absence of the gene was not always indicative of susceptibility. The latter was due in most cases either to the absence of additional genes encoding a particular resistance phenotype on the array (e.g., some trimethoprim-resistant isolates did not harbor the trimethoprim resistance gene *dfrS1* that can be detected using the DNA microarray) or to the fact that resistance was due to the presence of mutations in a specific gene which the DNA microarray does not detect (e.g., *fusA* gene mutations resulting in fusidic acid resistance). This highlights the fact that array profiling cannot replace antimicrobial susceptibility testing but can be used in conjunction with it to help identify the mechanism of resistance. The inclusion of alternative antimicrobial resistance genes on the array, such as additional tetracycline [*tet*(L) and *tet*(O)] and trimethoprim (*dfrG* and *dfrK*) resistance genes, would enhance the ability of the array to predict a resistance phenotype.

The presence of an aminoglycoside resistance gene(s) detected by array profiling could be used to accurately predict an isolate's phenotypic aminoglycoside resistance pattern. However, the phenotypic expression of aminoglycoside resistance could not always be used to infer which aminoglycoside resistance gene(s) was present. This was partly because of the fact that different aminoglycoside resistance genes encode resistance to the same antimicrobial agents and also because approximately half of the aminoglycoside-resistant isolates (52%; 43/83) carried two or more aminoglycoside resistance genes. For example, all isolates found to harbor a single aminoglycoside resistance gene exhibited resistance to the expected combinations of aminoglycosides, and all isolates exhibiting these phenotypes harbored the corresponding resistance genes. However, for isolates harboring two or three aminoglycoside resistance genes, the phenotype could be predicted from the aminoglycoside resistance genes detected, but the presence of a gene could not be reliably inferred from the phenotype. An advantage of the use of the array was confirmation of reduced susceptibility to amikacin in isolates carrying the *aacA-aphD* gene (27). This gene encodes low-level amikacin resistance that may not be detected using the standard 30-μg susceptibility testing disk. Among 14 isolates carrying *aacA-aphD* only, nine showed reduced amikacin susceptibility; the remainder were phenotypically susceptible. Inclusion on the array of genes encoding resistance to the aminocyclitol spectinomycin, including *spc* (carried on Tn*554*) and *aadA*, would be helpful to further differentiate among this MRSA population (31).

In conclusion, the present study showed that DNA microarray profiling can accurately assign isolates representative of major pandemic MRSA clones to the correct MLST and SCC*mec* types. Microarray profiling allows rapid, high-throughput genotyping and detection of clinically relevant staphylococcal virulence and antimicrobial resistance genes, and can, in most cases, be used to

predict the antimicrobial agent resistance phenotype of an isolate. The inclusion of additional antimicrobial resistance genes, including the recently described novel *mecA* gene (46), in future updated versions of the array would further enhance the usefulness of this system. The present study also provided further evidence of the diversity of SCC*mec* and SCC elements and the need for complete nucleotide sequencing to determine the genetic organization of these complex SCC*mec* elements and composite islands.

ACKNOWLEDGMENTS

This study was supported by the Microbiology Research Unit, Dublin Dental University Hospital.

We thank Grainne Brennan and the staff of the Irish National MRSA Reference Laboratory (NMRSARL) for antimicrobial susceptibility testing.

S. Monecke and R. Ehricht are both employees of Alere, the company that manufactures the StaphyType array kit.

REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Bartels MD, Hansen LH, Boye K, Sorensen SJ, Westh H.** 2011. An unexpected location of the arginine catabolic mobile element (ACME) in a USA300-related MRSA strain. PLoS One **6**:e16193. doi:10.1371/journal.pone.0016193.
3. **Carroll D, Kehoe MA, Cavanagh D, Coleman DC.** 1995. Novel organization of the site-specific integration and excision recombination functions of the *Staphylococcus aureus* serotype F virulence-converting phages phi 13 and phi 42. Mol. Microbiol. **16**:877–893.
4. **Carroll JD, et al.** 1989. A new methicillin- and gentamicin-resistant *Staphylococcus aureus* in Dublin: molecular genetic analysis. J. Med. Microbiol. **28**:15–23.
5. **Castanheira M, Watters AA, Mendes RE, Farrell DJ, Jones RN.** 2010. Occurrence and molecular characterization of fusidic acid resistance mechanisms among *Staphylococcus* spp. from European countries (2008). J. Antimicrob. Chemother. **65**:1353–1358.
6. **Chambers HF, Deleo FR.** 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol. **7**:629–641.
7. **Coleman DC, et al.** 1985. Susceptibility to antimicrobial agents and analysis of plasmids in gentamicin- and methicillin-resistant *Staphylococcus aureus* from Dublin hospitals. J. Med. Microbiol. **20**:157–167.
8. **Coombs GW, et al.** 2011. Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region. BMC Microbiol. **11**:215.
9. **Dale GE, Langen H, Page MG, Then RL, Stuber D.** 1995. Cloning and characterization of a novel, plasmid-encoded trimethoprim-resistant dihydrofolate reductase from *Staphylococcus haemolyticus* MUR313. Antimicrob. Agents Chemother. **39**:1920–1924.
10. **Deleo FR, Otto M, Kreiswirth BN, Chambers HF.** 2010. Community-associated meticillin-resistant *Staphylococcus aureus.* Lancet **375**:1557–1568.
11. **Diep BA, et al.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus.* Lancet **367**:731–739.
12. **El Garch F, et al.** 2009. StaphVar-DNA microarray analysis of accessory genome elements of community-acquired methicillin-resistant *Staphylococcus aureus.* J. Antimicrob. Chemother. **63**:877–885.
13. **Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus.* J. Clin. Microbiol. **38**:1008–1015.
14. **Enright MC, et al.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. U. S. A. **99**:7687–7692.
15. **García Álvarez L, et al.** 2011. MRSA harbouring a novel *mecA* homologue emerging in human and bovine populations in the UK and Denmark. Lancet Infect. Dis. **11**:595–603.
16. **Grundmann H, et al.** 2010. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med. **7**:e1000215. doi:10.1371/journal.pmed.1000215.
17. **Harris SR, et al.** 2010. Evolution of MRSA during hospital transmission and intercontinental spread. Science **327**:469–474.
18. **Holden MT, et al.** 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A. **101**:9786–9791.
19. **Humphreys H, et al.** 1989. Enterotoxin production by *Staphylococcus aureus* isolates from cases of septicaemia and from healthy carriers. J. Med. Microbiol. **28**:163–172.
20. **International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC).** 2009. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob. Agents Chemother. **53**:4961–4967.
21. **Ito T, et al.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **45**:1323–1336.
22. **Jevons MP.** 1961. Celbenin-resistant staphylococci. Br. Med. J. **i**:124–125.
23. **Kadlec K, Schwarz S.** 2009. Identification of a novel trimethoprim resistance gene, *dfrK*, in a methicillin-resistant *Staphylococcus aureus* ST398 strain and its physical linkage to the tetracycline resistance gene *tet*(L). Antimicrob. Agents Chemother. **53**:776–778.
24. **Kadlec K, Schwarz S.** 2010. Identification of the novel *dfrK*-carrying transposon Tn*559* in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. Antimicrob. Agents Chemother. **54**:3475–3477.
25. **Katayama Y, et al.** 2003. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome *mec* of methicillin-resistant *Staphylococcus aureus.* J. Bacteriol. **185**:2711–2722.
26. **Kondo Y, et al.** 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. **51**:264–274.
27. **Lelievre H, et al.** 1999. Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. J. Clin. Microbiol. **37**:3452–3457.
28. **Li S, et al.** 2011. Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **55**:3046–3050.
29. **Lindsay JA, Holden MT.** 2004. *Staphylococcus aureus*: superbug, super genome? Trends Microbiol. **12**:378–385.
30. **Luong TT, Ouyang S, Bush K, Lee CY.** 2002. Type 1 capsule genes of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. J. Bacteriol. **184**:3623–3629.
31. **Lyon BR, Skurray R.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol. Rev. **51**:88–134.
32. **Malachowa N, Deleo FR.** 2010. Mobile genetic elements of *Staphylococcus aureus.* Cell. Mol. Life Sci. **67**:3057–3071.
33. **Milheirico C, Oliveira DC, de Lencastre H.** 2007. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCC*mec* IV multiplex.' J. Antimicrob. Chemother. **60**:42–48.
34. **Monecke S, et al.** 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus.* PLoS One **6**:e17936. doi:10.1371/journal.pone.0017936.
35. **Monecke S, Jatzwauk L, Weber S, Slickers P, Ehricht R.** 2008. DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Clin. Microbiol. Infect. **14**:534–545.
36. **Monecke S, Slickers P, Ehricht R.** 2008. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol. Med. Microbiol. **53**:237–251.
37. **Mongkolrattanothai K, Boyle S, Murphy TV, Daum RS.** 2004. Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus.* Antimicrob. Agents Chemother. **48**:1823–1836.
38. **Oliveira DC, de Lencastre H.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **46**:2155–2161.
39. **Robinson DA, Enright MC.** 2004. Evolution of *Staphylococcus aureus* by large chromosomal replacements. J. Bacteriol. **186**:1060–1064.
40. **Robinson DA, Enright MC.** 2004. Multilocus sequence typing and the

evolution of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. **10**:92–97.
41. **Rossney AS, et al.** 2007. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. J. Clin. Microbiol. **45**:2554–2563.
42. **Rutherford K, et al.** 2000. Artemis: sequence visualization and annotation. Bioinformatics **16**:944–945.
43. **Saunders NA, Underwood A, Kearns AM, Hallas G.** 2004. A virulence-associated gene microarray: a tool for investigation of the evolution and pathogenic potential of *Staphylococcus aureus*. Microbiology **150**:3763–3771.
44. **Sekiguchi J, et al.** 2005. Cloning and characterization of a novel trimethoprim-resistant dihydrofolate reductase from a nosocomial isolate of *Staphylococcus aureus* CM.S2 (IMCJ1454). Antimicrob. Agents Chemother. **49**:3948–3951.
45. **Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC.** 2005. Seven novel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. Antimicrob. Agents Chemother. **49**:2070–2083.
46. **Shore AC, et al.** 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **55**:3765–3773.
47. **Shore AC, et al.** 2011. 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. Antimicrob. Agents Chemother. **55**:1896–1905.
48. **Shore AC, et al.** 2010. Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining *spa*, *dru*, and pulsed-field gel electrophoresis typing data. J. Clin. Microbiol. **48**:1839–1852.
49. **Shore AC, et al.** 2008. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis ccrAB4* in both methicillin-resistant *S. aureus* and MSSA. Antimicrob. Agents Chemother. **52**:4407–4419.
50. **Stegger M, Lindsay JA, Sorum M, Gould KA, Skov R.** 2010. Genetic diversity in CC398 methicillin-resistant *Staphylococcus aureus* isolates of different geographical origin. Clin. Microbiol. Infect. **16**:1017–1019.
51. **Strommenger B, et al.** 2006. Assignment of *Staphylococcus* isolates to groups by spa typing, SmaI macrorestriction analysis, and multilocus sequence typing. J. Clin. Microbiol. **44**:2533–2540.
52. **Sung JM, Lloyd DH, Lindsay JA.** 2008. *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multistrain microarray. Microbiology **154**:1949–1959.
53. **Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG.** 2000. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **45**:763–770.
54. **van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA.** 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J. Bacteriol. **188**:1310–1315.
55. **Weese JS.** 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J. **51**:233–244.
56. **Witney AA, et al.** 2005. Design, validation, and application of a seven-strain *Staphylococcus aureus* PCR product microarray for comparative genomics. App. Environ. Microbiol. **71**:7504–7514.
57. **Yamada Y, Hideka K, Shiota S, Kuroda T, Tsuchiya T.** 2006. Gene cloning and characterization of SdrM, a chromosomally-encoded multidrug efflux pump, from *Staphylococcus aureus*. Biolog. Pharma. **29**:554–556.
58. **Zhang K, McClure JA, Elsayed S, Conly JM.** 2009. Novel staphylococcal cassette chromosome *mec* type, tentatively designated type VIII, harboring class A *mec* and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **53**:531–540.