Molecular Epidemiological Typing of Emerging Methicillin-Resistant *Staphylococcus aureus* Strains in the Community, Among Livestock and in Healthcare Facilities in Ireland, 2001-2015

A thesis submitted to the University of Dublin, Trinity College, in fulfilment of the requirements for the Doctor of Philosophy by

Gráinne I. Brennan

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Microbiology Research Unit,

Dublin Dental University Hospital,

University of Dublin,

Trinity College Dublin,

Ireland

Declaration

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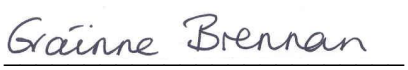

Gráinne Brennan

SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been endemic in Irish hospitals for four decades and displacement of predominant clones has occurred several times. Sequence type (ST) 22 carrying staphylococcal cassette chromosome (SCC) *mec* type IV has predominated in Irish healthcare facilities (HCF) since 2002. In recent years, an increase in sporadically-occurring non-ST22-MRSA-IV from bloodstream infections (BSIs) was observed indicating that strain displacement may occur again. Elsewhere, sporadically-occurring strains recognised in Ireland have emerged as predominant strains in HCFs.

The first part of this study undertook an in-depth molecular characterisation of 276 sporadically-occurring MRSA isolates from patients attending Irish hospitals, general practitioners (GP) and a veterinary laboratory from 2001-2015. All isolates underwent antimicrobial susceptibility testing (AST), *spa* typing and DNA microarray profiling to investigate the genetic backgrounds, SCC*mec*-associated genes and antimicrobial resistance and virulence genes. The 276 isolates were assigned to 86 *spa* types, 14 multilocus sequence typing (MLST) clonal complexes (CC), 11 STs and 18 combinations of SCC*mec* genes. Combining the CCs and SCC*mec* types resulted in 48 different type combinations. Community associated (CA), livestock associated (LA) and healthcare-associated (HCA) MRSA lineages were identified including CC1-MRSA-IV (22.5%, 62/276) & CC5-MRSA-V (8.3%, 23/276), CC398-MRSA-V (3.6%, 10/276) & CC130-MRSA-XI (0.4%, 1/276) and ST239-MRSA-III (1.8%, 5/276), respectively. Common virulence genes included the immune evasion cluster (IEC) (86.6%, 239/276), the enterotoxin gene cluster (*egc;* 46.7%, 129/276) and enterotoxin genes *sek*/*q* (13.0%, 36/276) and *seh* (26.8%, 74/276). The isolates harboured multiple resistance genes including those encoding resistance to beta-lactams (*blaZ/blaZ*$_{xi}$, 96%, 264/276), macrolides (*erm*(A), *erm*(B), *erm*(C), *lnu*(A) and/or *mph*(C), 59.8%, 165/276), aminoglycosides (*aacA-aphD, aadD* and/or *aphA3,* 43.2%, 119/276) and tetracycline (*tet*(K) and/or *tet*(M), 25.0%, 69/276), as well as the SCC*mec*/SCC-encoded fusidic acid resistance gene *fusC* (19.2%, 53/276).

Several nosocomial outbreaks caused by CA-MRSA lineages were investigated, including two by *pvl*-positive ST772-MRSA-IV, one by CC1-MRSA-IV and one by CC5-MRSA-V. These isolates harboured multiple resistance and virulence genes. In the case of one ST772-MRSA-IV outbreak, a healthcare worker (HCW) who had recently been hospitalised in India where this strain predominates subsequently transmitted the strain to babies in a neonatal intensive care unit in Ireland. This highlights the need for MRSA screening of HCWs and of the threat posed by the spread of *pvl*-positive CA-MRSA into hospitals.

Livestock-associated CC398 MRSA were also identified (n = 12) which along with an additional nine CC398 MRSA and 10 CC398 methicillin susceptible *S. aureus* (MSSA) were further investigated. The CC398 MRSA harboured SCC*mec* IVa or V_T, exhibited *spa* type t011 or t034 and carried different combinations of multiple resistance genes including those encoding resistance to erythromycin, tetracycline, spectinomycin and aminoglycosides. Four distinct incidents of CC398 MRSA were identified, including the transmission of CC398 from a veterinarian with recent travel to Belgium to a horse and between pigs (n = 9) and farm workers (n = 9) on two farms, one of which had been restocked with gilts from Germany. These findings have significant implications for human and animal health and the Irish agricultural industry.

The relatively high prevalence of the SCC*mec*/SCC-encoded *fusC* gene coupled with an increasing prevalence of fusidic acid resistant MRSA in Ireland was also investigated further. Seventeen different *spa*-CC-SCC*mec* type combinations were identified among 53 *fusC*-positive MRSA isolates. Similar SCC*mec-fusC* gene combinations were detected indicating the spread of these elements between different MRSA strains. Seven isolates had novel combinations of SCC*mec* and *fusC* or genetic backgrounds in which *fusC* had not previously been reported.

Whole-genome sequencing (WGS) of five isolates identified novel chimeric SCC*mec-fus* elements in four isolates while one isolate harboured a composite island consisting of SCC*mec* IV & SCC*fus*.

Detailed and accurate typing is essential for tracking the spread of MRSA, but traditional methods often lack the discriminatory power required for outbreaks. The next part of this study evaluated the usefulness of DNA microarray profiling for differentiating outbreak isolates including highly-clonal ST22-MRSA-IV and CA-MRSA strains. Twenty-five ST22-MRSA-IV isolates from five separate outbreaks underwent DNA microarray profiling, AST and *spa* typing. Microarray profiling differentiated 56% of isolates (14/25 from 3/5 outbreaks) that were grouped together based on AST and *spa* typing alone. However, for 36% of isolates (9/25 from 3/5 outbreaks) DNA microarray profiles were indistinguishable but differences in *spa* type and AST profiles were detected. Additionally, the key differences commonly detected among ST22-MRSA-IV were fusidic acid and cadmium resistance but *fusA* mutations and cadmium resistance genes are not included on the microarray panel. Among CA-MRSA lineages associated with outbreaks, although the DNA microarray successfully differentiated CC1-MRSA-IV strains, it was unable to further differentiate CC5-MRSA-V. In both cases however, the DNA microarray proved useful in providing additional information relating to the characterisation of these strains.

Advances in WGS have led to the use of this technology in nosocomial outbreaks but data interpretation and lack of standardised nomenclature has limited its widespread use. Ridom SeqSphere+ software is an automated system for the analysis of WGS data utilising a core-genome MLST (cgMLST) scheme involving 1,861 *S. aureus* genes. This part of this study investigated ST22-MRSA-IV isolates from patients and their hospital environment over a six-week period using cgMLST to identify transmission events and to compare the results to those obtained using previously published single nucleotide variation (SNV) analysis of the same WGS data and conventional molecular epidemiological (CME) typing data using *spa*, *dru* and PFGE typing. The 41 isolates were assigned to 16 distinct cgMLST clusters, nine of which involved ≥2 isolates suggesting multiple cross transmission events (CTEs). Seventeen isolates which had not been previously been assigned to a CTE were assigned to Cluster Types with other isolates using cgMLST. A pairwise comparison of isolates revealed no correlation between SNV and CME typing methods, but good agreement between cgMLST and CME typing.

Rapid detection of patients colonised with MRSA using chromogenic media can be used to limit the spread of MRSA. However, comparative studies with a diverse range of MRSA genotypes (including *mecC*-positive isolates) and levels of oxacillin resistance are lacking. This part of the study evaluated four MRSA chromogenic agars using a diverse collection of *S. aureus* isolates including strains expressing varying levels of oxacillin resistance and isolates from patient samples. While the limit of detection (LOD) for MRSA SelectII, ChromID MRSA and Colorex MRSA was 1.5×10^1 CFU/ml, MRSA Brilliance2 had a significantly higher LOD (1.5×10^4 CFU/ml). All media demonstrated ≥99% sensitivity for MRSA but only 85% to 73% (for MRSA SelectII) specificity when challenged with MSSA isolates. A high level of false positives (~50%) was obtained with all four media with *mec*-negative borderline-oxacillin resistant *S. aureus* (BORSA) isolates. Although the reduced specificity observed with MSSA and BORSA isolates is of concern, the high sensitivity of this media and its ability to recover almost all MRSA tested confirms the value of chromogenic agar in MRSA detection.

The rapid adaption and evolution of MRSA and the blurring of boundaries between HCA-, CA- and LA-MRSA is of concern. However, the enhanced resolution offered by WGS will undoubtedly provide improved strain tracking to inform the implementation of more effective infection prevention and control strategies. Furthermore, the ability of WGS to identify novel resistance mechanisms among MRSA isolates will inform the evidence base for the future development of antibiotic stewardship.

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Acknowledgements

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Abbreviations

ACME	Arginine catabolic mobile element
agr	Accessory gene regulator
AR	Antibiogram resistogram
BLAST	Basic local alignment search tool
BORSA	Borderline oxacillin resistance *S. aureus*
bp	Base pair
BSIs	Blood stream infections
BURP	Based upon repeating patterns
CA	Community associated
CBA	Columbia blood agar
CC	Clonal complex
ccr	*cassette chromosome recombinase*
CDC	Centre for Disease Control
CFU	Colony forming units
cgMLST	Core genome multilocus sequence typing
CHIP	Chemotaxis inhibitory protein
CI	Composite island
CLSI	Clinical and Laboratory Standards Institute
CME Typing	Conventional molecular epidemiological typing
CoNS	Coagulase negative staphylococci
CRIPSR	Clustered regularly interspaced short palindromic repeats
Ct	Cycle threshold
CTE	Cross transmision event
DNA	Deoxyribonucleic acid
DR	Direct repeat
EARS-Net	European Antimirobial Resistance Surveillance Network (formerly EARSS)
EARSS	European Antimirobial Resistance Surveillance System
ECDC	European Centre for Disease Control
EDTA	Ethylenediaminetetraacetic acid
EF-G	Elongation factor G
egc	*Enterotoxin gene cluster*
E-MRSA	Epidemic MRSA
ERI	Earliest recovered isolate
ET	Exfoliative toxins
et al.,	And others
EUCAST	European Committee on Antimicrobial Susceptibility Testing
Fig	Figure
g	Gram
GISA	Glycopeptide intermediate *Staphylococcus aureus*

GPs	General Practitioners
h	Hours
HCA	Healthcare associated
HCAI	Healthcare associated infection
hGISA	heterogenous Glycopeptide intermediate *Staphylococcus aureus*
HPSC	Health Protection Surveillance Centre
HRP	Horseradish peroxidase
hVISA	heterogenous Vancomycin intermediate *Staphylococcus aureus*
i.e.	That is
IEC	Immune evasion cluster
IR	Inverted repeat
IS	Insertion sequence
ISS	Insertion site sequence
IWG-SCC	International working group on the classification of staphylococcal cassette chromosome elements
J regions	Joining regions
kb	Kilobase pair
L	Litre
LA	Livestock associated
LoD	Limit of Detection
mec	Methicillin resistance gene complex
mg	Milligram
MGE	Mobile genetic element
MH	Mueller Hinton
MHS	Mueller Hinton agar containing 5% NaCl
MIC	Minimum inhibitory concentration
min	Minute
ml	Millilitre
MLS	Macrolide- lincosamide streptogramin B
MLST	Multilocus sequence typing
MRSA	Methicillin resistant *Staphylococcus aureus*
MSCRAMMS	Microbial surface components recognising adhesive matrix molecules
MSSA	Methicillin susceptible *Staphylococcus aureus*
MST	Minimum spanning tree
n	number
NaCl	Sodium chloride
ng	nanogram
NICU	Neonatal intensive care unit
NMRSARL	National MRSA Reference Laboratory
NT	No type

orf	*Opening reading frame*
PBP/ PBP2a	Penicillin binding protein/ penicillin binding protein 2a
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PVL	Panton Valentine leukocidin
RPM	Rotations per minute
s	Seconds
SAgs	Staphylococcal superantigens
SCC	Staphylococcal cassette chromosome
SCC-CI	Staphylococcal cassette chromosome- composite island
SCC*mec*	Staphylococcal cassette chromosome *mec*
SCIN	Staphylococcal complement C3 convertase inhibitor
SCV	Small colony variant
SeqNet	European Network of Laboratories for sequenced based typing of microbial pathogens
SSTI	Skin and soft tissue infection
SNV	Single nucleotide variants
spa	Staphylococcal protein A
SSSS	Staphylococcal scalded skin syndrome
ST	Sequence type
TAT	Turnaround time
TBE buffer	Tris borate EDA buffer
TE buffer	Tris EDTA buffer
TSST	Toxic shock syndrome toxin
UCD	University College Dublin
UK	United Kingdom
Unf	Unfamiliar
USA	United States of America
UV	Ultraviolet
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRE	Vancomycin resistant enterococci
VRSA	Vancomycin resistant *Staphylococcus aureus*
w/v	weight/volume
WGS	Whole genome sequencing
%	percentage
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
Δ	deletion (of part of a gene)
°C	Degrees celsius
α	alpha
β	beta

ϒ	gamma
Ψ	pseudo
µg	microgram
µl	microlitre

Publications

Some of the original work presented in this thesis has been published in referreed international publications as listed below. Offprints of the manuscripts are included at the end of this thesis.

Brennan GI, Abbott Y, Burns A, Leonard F, McManus BA, O'Connell B, Coleman DC and Shore AC. 2016. The Emergence and Spread of Multiple Livestock-Associated Clonal Complex 398 Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Strains among Animals and Humans in the Republic of Ireland, 2010-2014. *PLoS One* **17:** 11(2):e0149396.

Brennan GI, Herra C, Coleman DC, O'Connell B and Shore AC. 2016. Evaluation of commercial chromogenic media for the detection of meticillin-resistant *Staphylococcus aureus*. *J Hosp Infect* **92:** 287-92.

Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S and Coleman DC. 2014 Extensive genetic diversity identified among sporadic methicillin-resistant *Staphylococcus aureus* isolates recovered in Irish hospitals between 2000 and 2012.*Antimicrob Agents Chemother* **58:** 1907-17.

Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S and Coleman DC. 2014. Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing multiresistance. *J Clin Microbiol* **52:**859-70.

Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, Slickers P, Coleman DC. 2013. Emergence of sequence type 779 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo staphylococcal cassette chromosome *mec* (ΨSCC*mec*)-SCC-SCC*CRISPR* composite element in Irish hospitals. *Antimicrob Agents Chemother* **57:** 524-31.

Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B. 2012. Emergence of hospital- and community-associated Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. *J Clin Microbiol* **50:** 841-7.

Chapter 1

Introduction

1.1 Staphylococci

Staphylococci are Gram-positive, catalase positive facultative anaerobes which, when examined under the microscope, appear as grape-like clusters. To date, over 40 different species of staphylococci have been described which are commonly divided into two groups, coagulase-positive and coagulase-negative staphylococci, based on their ability or inability to produce the coagulase enzyme that activates prothrombin and converts fibrinogen into fibrin (Archer, 1998). Coagulase-negative staphylococci (CoNS) are frequently considered as commensals or contaminants on healthy human skin and mucous membranes with *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* the most common CoNS associated with human infections (Becker *et al.,* 2014).

1.1.1 Staphylococcus aureus

Staphylococcus aureus is a coagulase-positive staphylococcal species and forms part of the normal microbial flora of both humans and animals. Approximately 30% of healthy individuals carry *S. aureus* in their anterior nares, however other sites are also commonly colonised including the throat, axilla, perineum, gastrointestinal tract and groin (Fig. 1.1). Despite this however, *S. aureus* is also considered to be the most pathogenic of the staphylococci and has been associated with a wide range of infections such as skin and soft tissue infections (SSTIs), bloodstream infections (BSIs), food poisoning, endocarditis, osteomyelitis, septic arthritis and pneumonia (Fig. 1.1) (Wertheim & Melles, 2005; Deurenberg & Stobberingh, 2008). Furthermore, it is among the most common cause of healthcare-associated infections (HCAIs) worldwide and has also emerged as a significant cause of infections in the community and among animals.

1.2 Bacterial mechanisms of resistance to antimicrobial agents

Antimicrobial agents act by inhibiting cell wall synthesis, protein synthesis, nucleic acid synthesis or mediate disruption of cell wall integrity (McDermott *et al.,* 2003). It has

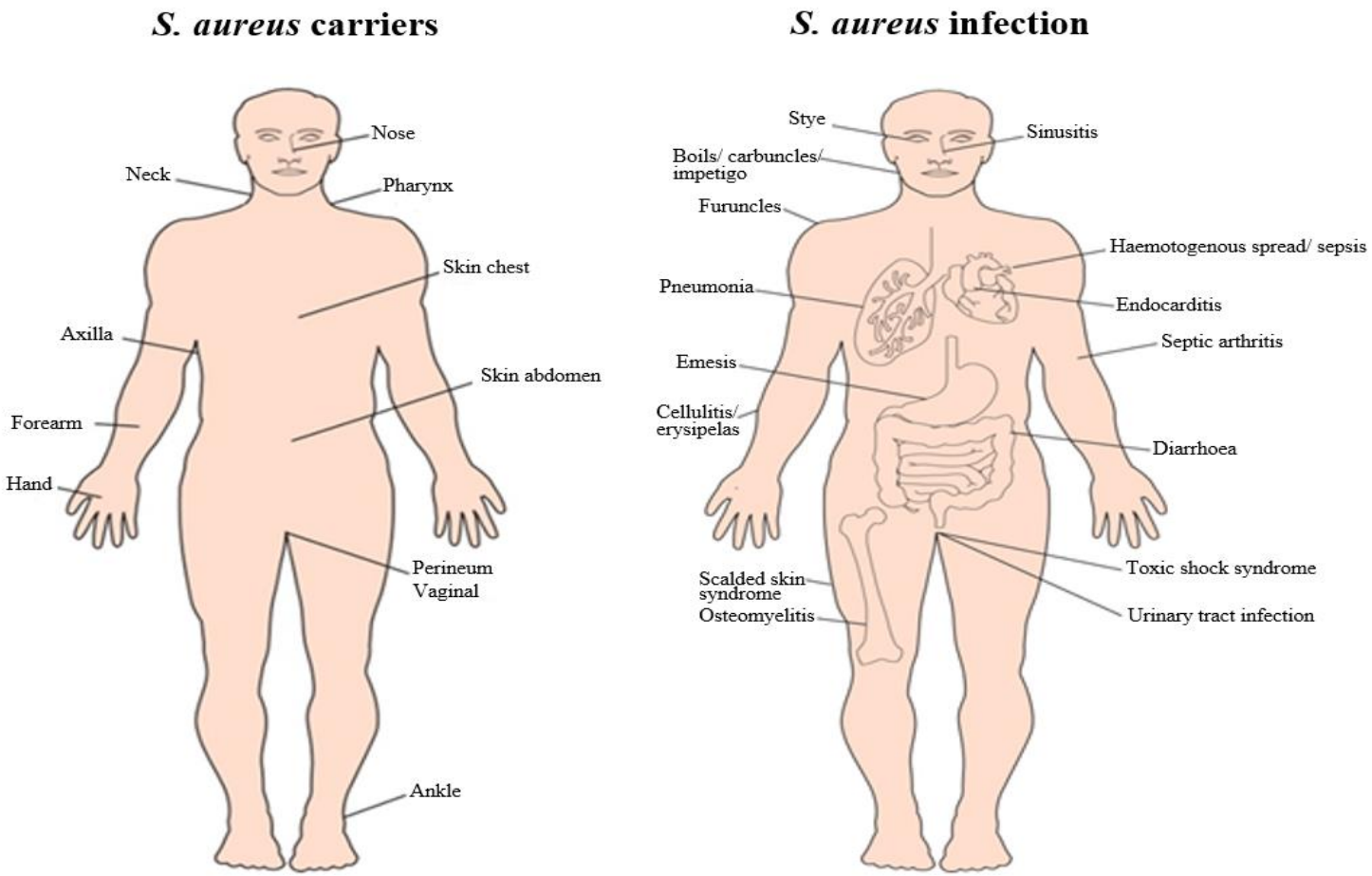


Figure 1.1 The diversity of sites of carriage and infection of *Staphylococcus aureus* in humans. Adapted from Wertheim *et al.,* 2005.

been shown that the introduction of new antibiotics into clinical practice is frequently followed by the development of bacterial resistance due to the potential selection of resistant strains (McDermott *et al.,* 2003; Schito, 2006; Holmes *et al.,* 2016). Antimicrobial resistance in bacteria develops through spontaneous mutations in the chromosome and/or by the acquisition of genes that lead to resistance through a variety of mechanisms:

(i) permeability of the cell wall changes restricting access of the antimicrobial agent to the target site;

(ii) bacterial cells acquire genes encoding efflux pumps which, when expressed actively, transport the antimicrobial agent out of the cell;

(iii) modification of the drug target site;

(iv) enzymatic modification or degradation of the drug;

(v) acquisition of alternative metabolic pathways to those inhibited by the drug and;

(vi) overproduction of the drug target site (McDermott *et al.,* 2003; Van Hoek *et al.,* 2011).

Antimicrobial resistance genes are often carried on mobile genetic elements (MGEs) such as plasmids, transposons, pathogenicity islands and staphylococcal cassette chromosome (SCC) elements, many of which have also be reported in CoNS and which can be transferred through horizontal gene transfer (Table 1.1) (Malachowa & Deleo, 2010). Furthermore, the acquisition of a single resistance gene may result in phenotypic resistance to multiple classes of antimicrobial agents (Table 1.1).

1.2.1 Staphylococcal cassette chromosome mec

Methicillin inhibits bacterial growth by binding to the enzymatic site of the penicillin binding proteins (PBP) that catalyse cross-linking or transpeptidation of the bacterial cell wall. This results in the weakening of the structure of the cell wall and the release of the cytoplasmic contents of the cell causing cell death. However, in MRSA the presence of an

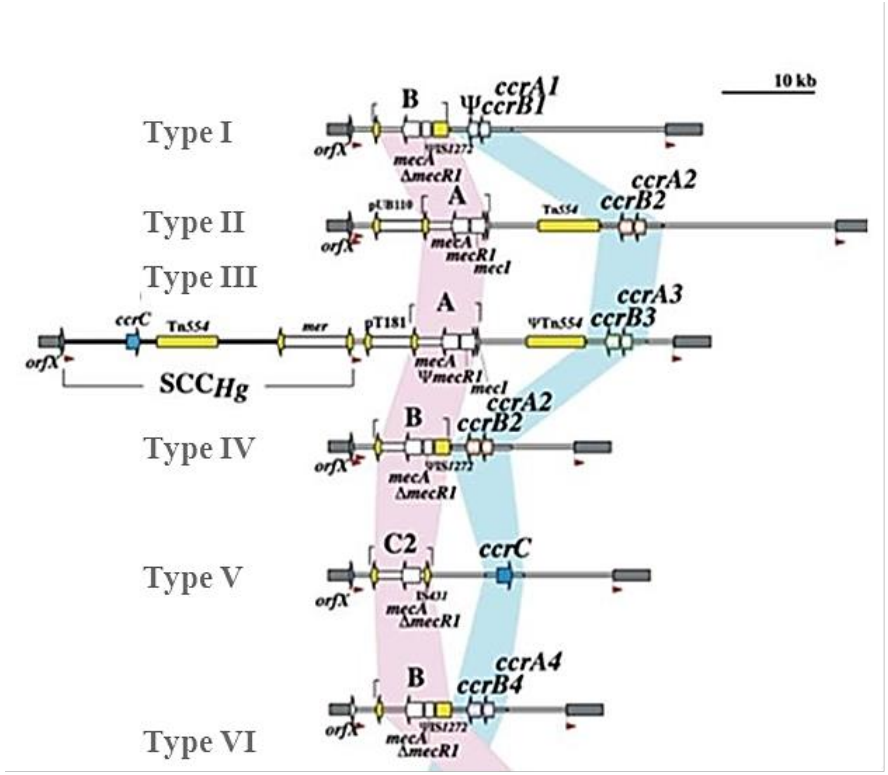
Table 1.1 Examples of acquired resistance genes identified in *Staphylococcus aureus*

Antimicrobial agent	Target	Resistance mechanism	Associated resistance genes
Methicillin, oxacillin and cefoxitin	Peptidoglycan biosynthesis i.e. PBPs	Alternative binding site, PBP2a; antibiotics only bound at levels in excess of therapeutic range	*mecA, mecC*
Vancomycin	D-ala-D-ala	Alternative subunit for peptidoglycan biosynthesis	*vanA, vanB*
Aminoglycosides	30S ribosomal subunit	Enzymatic inactivation of antimicrobial agent	*aadA-aphD, aadD, aphA3*
Fusidic acid	EF-G	Protection of the EF-G binding site	*fusB, fusC, fusD, fusF*
Linezolid	23S ribosomal subunit	Methylation of 23S rRNA	*cfr*
Macrolides and lincosamides	50S ribosomal subunit	Methylation of target site	*erm*(A), *erm*(B), *erm*(C)
		Enzymatic inactivation of the antimicrobial agent	*msr*(A), *mph*(C), *lun*(A)
Mupirocin	Isoleucyl tRNA synthetase	Alternative binding site	*ileS2, mupB*
Tetracycline	30S ribosomal subunit	Enzymatic modification of 30S ribosomal target site	*tet*(M), *tet*(O)
		Efflux pump	*tet*(K), *tet*(L)
Tigecycline	30S ribosomal subunit	Multidrug efflux pump	*mepA*
Spectinomycin	30S ribosomal subunit	Enzymatic inactivation of antimicrobial agent	*spc*
Fluoroquinolone	DNA synthesis	Alteration in target enzyme	*gyrA, gyrB*

altered PBP termed PBP2a, encoded for by the *mecA* or *mecC* gene, which has decreased affinity for β-lactam antibiotics and which permits cell wall synthesis to continue despite the inactivation of the native PBPs.

The *mecA* and *mecC* genes are carried on staphylococcal cassette chromosome (SCC) elements termed SCC*mec* which are MGEs that insert into the *S. aureus* chromosome at a specific site designated *orfX* and which can encode antibiotic resistance and/or virulence determinants (Shore & Coleman, 2013). The acquisition of SCC*mec* by *S. aureus* resulted in the emergence of MRSA and it has been shown that this has occurred on several different occasions in different *S. aureus* lineages (Deurenberg & Stobberingh, 2008; Holden *et al.,* 2013). SCC*mec* elements are characterised by the presence of a cassette chromosome recombinase (*ccr*) and *mec* gene complex, flanking inverted and direct repeat sequences and joining (J) regions outside the *mec* and *ccr* genes which can carry additional resistance determinants (Fig. 1.2) (IWG-SCC, 2009). The *ccr* complex encodes recombinase proteins that mediate site- and orientation-specific integration and excision of the SCC*mec* element into and out of the chromosome, while the *mec* complex consists of *mecA* or *mecC*, and the regulatory genes including *mecR1*, *mecR2* and *mecI.* Transcription of *mecA* is regulated by the sensor inducer encoded by *mecR1,* the repressor encoded by *mecI* and the anti-repressor encoded by *mecR2* (Arêde *et al.*, 2012). To date five *mec* and eight *ccr* complexes have been described and, based on distinct combinations of *mec* and *ccr* complexes, 12 SCC*mec* types have been reported to date among MRSA (Fig. 1.2) (IWG-SCC, 2009; Shore *et al.,* 2011a; Li *et al.,* 2013; Shore & Coleman, 2013; Wu *et al.,* 2015).

In addition to 12 SCC*mec* types, numerous subtypes have also been described in MRSA, and in particular in SCC*mec* IV where 12 diverse subtypes designated IVa-IVk and IVA have been recognised (Shore *et al.,* 2005; IWG-SCC, 2009; Milheirico *et al.,*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Type II
Type III
Type IV
Type V
Type VI
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orfX
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ccrA2
ccrB2
pUB110
Tn554
mecR1
mecI
ccrC
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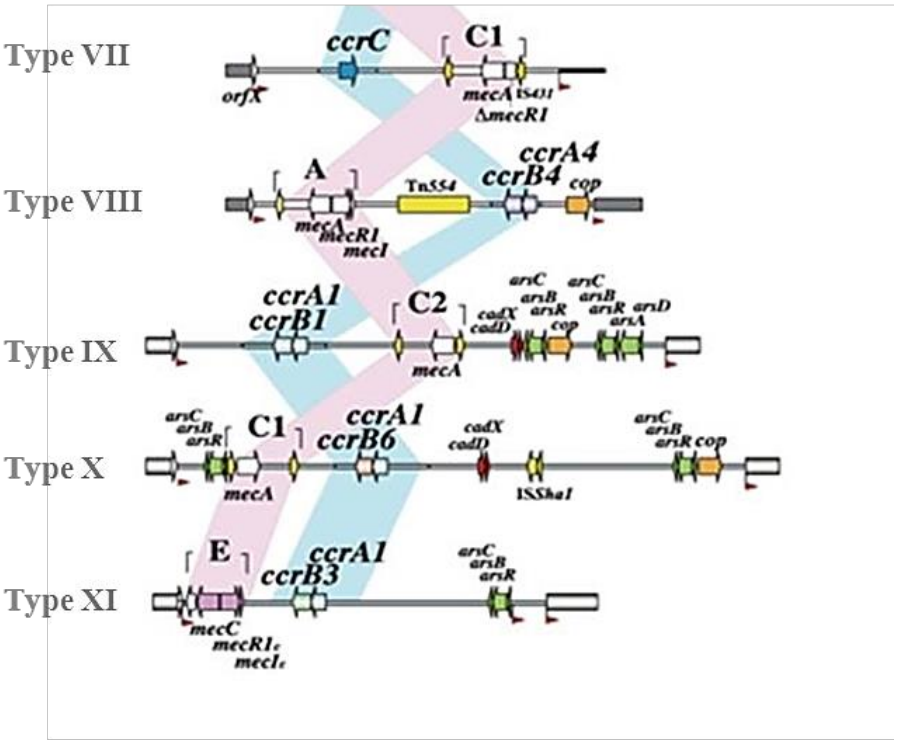
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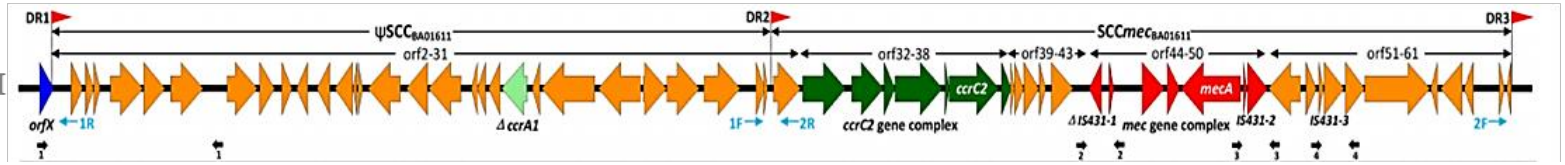
mecIε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DR2
DR3
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SCCmecBA01611
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orf32-38
orf39-43
orf44-50
orf51-61
orfX
1R
Δ ccrA1
1F
2R
ccrC2 gene complex
ccrC2
ΔIS431-1
mec gene complex
mecA
IS431-2
IS431-3
2F

Figure 1.2 A schematic diagram showing the genetic organisation of the 12 SCC*mec* types described to date in MRSA. SCC*mec* I, NCTC10442 (Genbank accession number AB033763); SCC*mec* II, N315 (D86934); SCC*mec* III, 85/2082 (AB037671); SCC*mec* IV, CA05 (AB063172); SCC*mec* V, WIS/WBG8318 (AB121219); SCC*mec* VI, HDE288 (AF411935); SCC*mec* VII, JCSC6082 (AB373032); SCC*mec* VIII, C10682 (FJ390057); SCC*mec* IX, JCSC6943 (AB505628); SCC*mec* X, JCSC6945 (AB505630); SCC*mec* XI, LGA251 (FR821779.1); SCC*mec* XII BA01611 (KR187111). Direct repeats that comprise integration site sequences of SCC are located at both extremities of SCC*mec*. The location of the five (A-E) classes of *mec*-gene complexes in each element is indicated by the pink belt in Types I-XI and in red in type XII. The locations of *ccr*-gene complexes are indicated by the blue belt (Types I-XI) or green (Type XII). Insertion sequences and transposons are indicated in yellow (Type I-XI). Representative genes related to heavy metal resistance and integrated plasmids located in the J regions are also indicated. Adapted from Hiramatsu *et al.,* 2013 (Types I-XI) and Wu *et al.,* 2015 (Type XII).

2007; Shore & Coleman, 2013). These harbour the same *ccr* and *mec* complex genes as one of the 12 SCC*mec* types, but exhibit sequence variation in the J regions (IWG-SCC, 2009).

1.2.1.1 SCC, SCC-like elements and ΨSCC/SCC*mec* elements, composite islands and chimeric elements

In the absence of *mecA* or *mecC,* a range of other SCC elements have also been described in *S. aureus* and other staphylococcal species, depending on the presence and/or absence of the *ccr* and/or *mec* complexes. These elements contain the common features of SCC*mec* in that they integrate into the *orfX* and are flanked by direct and inverted repeating units (Table 1.2).

SCC elements harbour the *ccr* complex but lack *mecA* or *mecC*. In addition they may also carry other antimicrobial resistance genes forming part of a larger composite island (CI) consisting of multiple SCC*mec*/SCC elements. The presence of a particular resistance gene is usually indicated in the name of the SCC element e.g. SCC*fus* harbours the fusidic acid resistance gene *fusC* (Holden *et al.,* 2004).

Pseudo (Ψ) SCC and SCC*mec* elements lack the *ccr/mec* and *ccr* genes, respectively. ΨSCC elements can be further divided into three groups (i) arginine catabolic mobile elements (ACME), (ii) SCC-like elements and (iii) SCC*mec* remnants that lack the *mecA* and *ccr* genes but have a similar structure to previously described SCC*mec* elements without the *ccr, mec* and intervening genes (Shore & Coleman, 2013). Chimeric elements consisting of a single fused SCC*mec* element that harbour multiple resistance encoding genes such as *mecA* and *fusC* have also been reported (Ellington *et al.,* 2015).

Table 1.2 Examples of SCC elements, pseudo (Ψ)SCC, Ψ SCC*mec* and chimeric elements identified in staphylococci (Adapted from Shore and Coleman, 2013 and Ellington *et al.,* 2015).

Element type (definition)	Examples (size)	Associated staphylococcal species	Characteristic genes			
			***ccr* type**	***mec* complex genes**	**Additional antibiotic resistance genes identified**	**Reference**
SCC (*ccr* but no *mec* complex)	SCC_{M1} (14 kb)	*S. aureus*	*ccrAB4*	None	None	Shore *et al.,* 2012; Bartels *et al.,* 2011
	SCC476 or SCC*fus* (23 kb)	*S. aureus*	*ccrAB1*	None	*fusC*	Holden *et al.,* 2004
	SCC*Hg* (32 kb)	*S. aureus*	*ccrC*	None	*merA* & *merB*	Ito *et al.,* 2001
	$SCC_{M06/0171}$ (18 kb)	*S. aureus*	*ccrAB4*	None	None	Kinnevey *et al.,* 2013
	SCC_{CRISPR} (17 kb)	*S. aureus*	*ccrC*	None	*CRISPR*	Kinnevey *et al.,* 2013
	SCC*cap1* (27 kb)	*S. hominis*	*Ψccr*	None	None	Luong *et al.,* 2002
	SCC*h1435* (22 kb)	*S. haemolyticus*	*ccrC*	None	None	Takeuchi *et al.,* 2005
	SCC_{pbp4} (19 kb)	*S. epidermidis*	*ccrAB2*	None	*pbp4* & *tagF*	Mongkolrattanothai *et al.,* 2004
	$SCC_{ATCC12228}$ (38 kb)	*S. epidermidis*	*ccrAB4*	None	*copA*, mercury & cadmium resistance gene clusters	Mongkolrattanothai *et al.,* 2004

Continued overleaf

Table 1.2 continued

Element type (definition)	Examples (size)	Associated staphylococcal species	Characteristic genes			
			***ccr* type**	***mec* complex genes**	**Additional antibiotic resistance genes identified**	**Reference**
	SCC_{12263} (ca. 21 kb)	*S. hominis*	*ccrAB1*	None	None	Katayama *et al.,* 2003
Pseudo (ψ) SCC*mec* (*mec* complex but no *ccr*)	ψSCC*mec* II.5 (28 kb)	*S. aureus*	None	*mecA* & *ΔmecR1*	*ant(4')*	Han *et al.,* 2009
	ψSCCmec_{16691} (11 kb)	*S. aureus*	None	*IS431*, *mecA*, *mecR1* and *mecI* (Class A *mec*)	None identified	Chen *et al.,* 2010b
	ψSCC$mec_{M06/0171}$ (16 kb)	*S. aureus*	None	*IS431*, *mecA* & *ΔmecR1* & *IS431* (class C4 *mec*)	*copB*, *copC* & *fusC*	Kinnevey *et al.,* 2013
	ψSCC*mec* (*h1435*)[b]	*S. haemolyticus*	None	*mecA* &*ΔmecR1*	*cadD, cadX, arsC, arsB* & *arsR*	Takeuchi *et al.,* 2005
Pseudo (ψ) SCC (Lack *ccr* and *mec* genes)	ACME, ΔACME or ψSCC*arc* (12–34 kb)	Various staphylococcal species	None	None	*arc* & opp operons (opp not present in ΔACME/ψSCCarc)	Diep *et al.,* 2006; Shore *et al.,* 2011b; Bartels *et al.,* 2011
	ψSCC*h1*, ψSCC*h2*, ψSCC*h3* & ψSCC*h4*[b]	*S. haemolyticus*	None	None	*kdp* (SCC*h1*)	Takeuchi *et al.,* 2005
	ψ$SCC_{M10/0061}$ (3 kb)	*S. aureus*	None	None	None	Shore *et al.,* 2011a
	SCC-like or IE25923 (5.9 kb)	*S. aureus*	None	None	None	Ito *et al.,* 2001; Ito *et al.,* 2003; Jansen *et al.,* 2006

Continued overleaf

Table 1.2 continued

Element type (definition)	Examples (size)	Associated staphylococcal species	Characteristic genes			
			ccr type	*mec* complex genes	Additional antibiotic resistance genes identified	Reference
	CC_{V14} (ca. 5 kb)	*S. aureus*	None	None	None	Arakere *et al.,* 2009
	CC6082 (5.5 kb)	*S. aureus*	None	None	None	Berglund *et al.,* 2008
	SCC*mec* II remnant (ψSCC_{ECT-R2}) (12 kb)	*S. aureus*	None	None	*ant(4')*	Lindqvist *et al.,* 2012
Composite islands (2 or more SCC, SCC*mec* or ψ elements)	ψ $SCC_{mec M06/0171}$, $SCC_{M06/0171}$ & SCC_{CRISPR} (51 kb)	*S. aureus*	*ccrAB4* & *ccrC*	*IS431*, *mecA* & *ΔmecR1* & *IS431* (class C4 *mec*)	CRISPR, *fusC, copB, copC*	Kinnevey *et al.,* 2013
	ΔACME II (ψSCC*arc*), ΔJ1 SCC*mec* I & SCC*mec* IVh (46 kb)	*S. aureus*	*ccrAB2*	*IS431*, *mecA*, *ΔmecR1* & *IS1272* (Class B *mec*)	*arc* operon	Shore *et al.,* 2011b
	SCC*mec* III & SCC*Hg* (67 kb)	*S. aureus*	*ccrAB3* & *ccrC*	*IS431*, *mecA*, *mecR1* and *mecI* (Class A *mec*)	*cadA, cadC, tet*(K), *merA* & *merB*	Ito *et al.,* 2001
	SCC*mec*IVa & SCC_{476} (45 kb)	*S. aureus*	*ccrAB2* & *ccrAB1*	*IS431*, *mecA*, *ΔmecR1* & *IS1272* (Class B *mec*)	*fusC*	Ellington *et al.,* 2015
	SCC_{149} & SCC*mec*IVa (42 kb)	*S. aureus*	*ccrAB2* & *ccrAB1*	*IS431*, *mecA*, *ΔmecR1* & *IS1272* (Class B *mec*)	*fusC*	Ellington *et al.,* 2015

Continued overleaf

Table 1.2 continued

Element type (definition)	Examples (size)	Associated staphylococcal species	Characteristic genes			
			***ccr* type**	***mec* complex genes**	**Additional antibiotic resistance genes identified**	**Reference**
	SCC_{M1} & SCC*mec* IIE (41 kb)	*S. aureus*	*ccrAB4* & *ccrAB2*	*IS431, mecA, mecR1*, *ΔmecI*, *IS1182* (Class A.3 *mec*)	*spc* & *erm*(A)	Shore *et al.,* 2012
	SCC*mec* XI & $\psi SCC_{M10/0061}$	*S. aureus*	*ccrA1B3*	*blaZ*, *mecC*, *mecR1*, *mecI* (Class E *mec*)	Arsenic resistance operon	Shore *et al.,* 2011a
SCC*mec* chimeric elements (SCC*mec* fused with additional resistance gene)	SCC*mec-fus* I-IV (18-25 kb)	*S. aureus*	*ccrAB2*	*IS431*, *mecA*, *ΔmecR1* & *IS1272* (Class B *mec*)	*fusC*	Ellington *et al.,* 2015

1.2.2 The emergence of antimicrobial resistance in S. aureus

Antimicrobial resistance in *S. aureus* was first described in the 1940s shortly after the introduction of penicillin into clinical practice (Fig. 1.3) with reports of penicillin resistance among *S. aureus* isolates due to the production of a β-lactamase, an enzyme that degrades the β-lactam ring of penicillin. Subsequently, penicillinase-resistant semi-synthetic β-lactam antibiotics such as methicillin and other derivatives including oxacillin, cefoxitin and flucloxacillin were developed (Moellering, 2012). However, within two years *S. aureus* once again developed resistance leading to the detection of methicillin-resistant *S. aureus* (MRSA) (Jevons, 1961).

Following the worldwide increase in the prevalence of MRSA in hospitals during the 1970s, alternative treatment options were required and glycopeptides such as vancomycin and teicoplanin were introduced in the 1970s and became the choice of treatment for serious MRSA infections (Levine, 2006). However in 1996 isolates exhibiting reduced susceptibility to vancomycin were identified in Japan and subsequently became known as vancomycin-intermediate *S. aureus* (VISA) (Hiramatsu *et al.,* 1997a) and have since been reported elsewhere including Ireland (Hiramatsu *et al.,* 1997b; Denis, 2002; El Solh *et al.,* 2003; Torun *et al.,* 2005; Robert *et al.,* 2006; Fitzgibbon *et al.,* 2007; Gomes *et al.,* 2015). Heterogeneous characteristics displayed by VISA have also been reported where the majority of a population exhibit a susceptible profile to vancomycin but a subpopulation exhibit a minimum inhibitory concentration (MIC) of 4-8 mg/L and are described as heterogenous VISA (hVISA) (Fitzgibbon *et al.,* 2007). While the mechanism of resistance among VISA and hVISA is still unclear, the thickening of the cell wall along with mutations in the ribosomal gene *rpoB* have been most frequently reported (Gardete & Tomasz, 2014). Although reports of VISA and hVISA populations were initially limited to

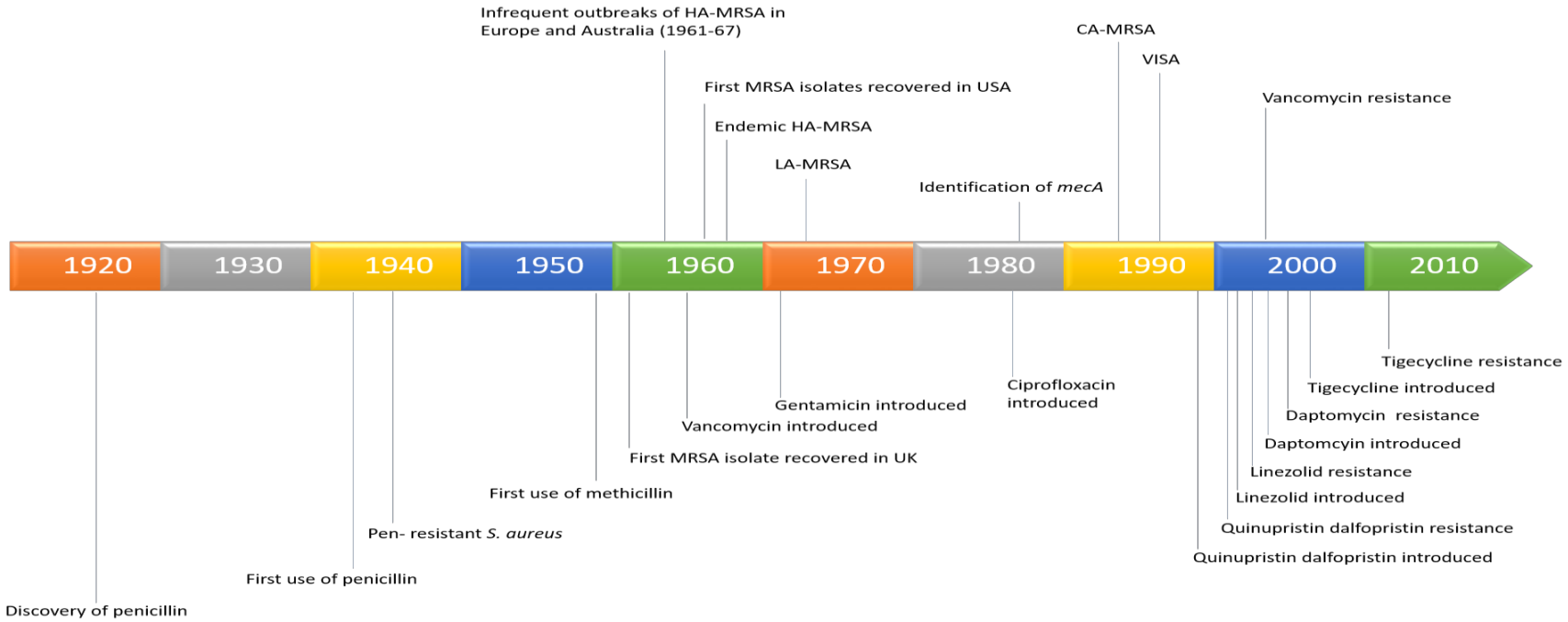


Figure 1.3 Timeline of the emergence of antimicrobial resistance in *Staphylococcus aureus* since the discovery of penicillin in the 1928 including the emergence of penicillin (pen) resistant *S. aureus,* methicillin resistant *S. aureus* (MRSA) in hospitals (hospital associated (HA)-MRSA), the community (community associated (CA)-MRSA), livestock associated (LA)-MRSA and the worldwide emergence of vancomycin-intermediate *S. aureus* (VISA) and the years in which other antimicrobial agents were introduced for clinical use and the subsequent reported resistance.

MRSA strains, reduced susceptibility has also recently been reported among methicillin-susceptible *S. aureus* (MSSA) strains (Panesso *et al.,* 2015).

Subsequent to reports of VISA and hVISA, the first vancomycin-resistant *S. aureus* (VRSA) was reported in the USA in 2002 (CDC, 2002) where genetic analysis identified the Tn*1546* plasmid which had previously been shown to harbour the *vanA* gene in *Enterococcus faecalis* (Weigel *et al.,* 2003). The isolate was recovered from a patient who had previously undergone vancomycin therapy and from whom a vancomycin-resistant *E. faecalis* (VRE) was also recovered (CDC, 2002). Since then, although reports of VRSA isolates remain rare, additional cases have been reported in the USA, India and Egypt (Weigel *et al.,* 2003; Tiwari & Sen, 2006; Finks *et al.,* 2009; El-banna *et al.,* 2015).

Alternative treatment options to glycopepitdes did not become available until the early 2000s with the introduction of linezolid, a protein synthesis inhibitor used to treat Gram-positive infections including MRSA, VISA and VRE (Diekema & Jones, 2001). As previously seen with other antibiotics, shortly after its introduction, resistance to linezolid was first reported in VRE (Prystowsky *et al.,* 2001) but subsequently emerged in MRSA (Tsiodras *et al.,* 2001). Resistance to linezolid in *S. aureus* arises due to mutations in the 23S rRNA or ribosomal protein (L proteins) or through the acquisition *cfr, cfr*(B) or *optrA,* and, while still relatively rare, it has been reported in strains from humans and animals (Kehrenberg & Schwarz, 2006; Kehrenberg *et al.*, 2009; Shore *et al.*, 2010; Li *et al.*, 2015; Wang *et al.*, 2015; Shore *et al.,* 2016). In addition, resistance to linezolid has also been recognised in CoNS and in particular in association with *S. epidermidis* causing outbreaks in Ireland (O'Connor *et al.,* 2015; Gabriel *et al.,* 2015).

A number of newer antimicrobial agents have also recently become available for the treatment of MRSA including daptomycin, tigecycline and quinupristin-dalfopristin.

However, resistance to all of these agents has subsequently been described among MRSA (Fig. 1.3). Resistance to all of these agents can be due to mutation or, additionally, in the case of quinupristin-dalfoprisitin, due to the acquisition of streptogramin A resistance genes (*vat* or *vga*) (Hershberger *et al.,* 2004; Bayer *et al.*, 2013; Herrera *et al*., 2016). Most recently ceftaroline, a novel class of cephalosporin, was licensed for use in the USA and Europe in 2010 and 2012, respectively. Despite its recent introduction and novel structure however, resistance to ceftaroline has already been reported among MRSA in Europe, the USA and Thailand due to mutations in the PBP2a protein encoded by *mecA* preventing binding of the antimicrobial agent to the target site (Mendes *et al.,* 2012; Long *et al.,* 2014; Alm *et al.,* 2014).

1.2.3 Fusidic acid resistance in Staphylococci

Fusidic acid is a tetracyclic triterpenoid derived from the fungus *Fusidium coccinum* that was first introduced into clinical use in the early 1960s where it acts by targeting elongation factor G (EF-G) (Godtfredsen, *et al.*, 1962). The crystal structure of EF-G from *Thermus thermophilus* was first described in 1994 (Czworkowski *et al.,* 1994). During elongation, after the formation of each peptide bond, EF-G binds to the ribosome and, under GTP hydrolysis, catalyses translocation. Following this, EF-G and the ribosome recycling factor bind to the post- termination complex to catalyse disassembly of the post-termination complex. EF-G has low intrinsic activity in GTP hydrolysis that is stimulated by the interaction with the ribosome due to large conformational changes occurring when it binds to the 70S subunit (Chen *et al.,* 2010a). The protein is a five-domain protein where domain I and II form a globular structure that is conserved in all other ribosomal GTPases. However, although domains III, IV and V in EF-G derived from *S. aureus* are highly similar to that from *T. thermophilus,* they are in a different orientation relative to domains I and II (Chen *et al.*, 2010a). Fusidic acid targets a pocket between domains I, II and III of

EF-G, locking EF-G in a conformation intermediate between GTP-bound and GDP-bound forms preventing its release from the ribosome and therefore blocking the next stage of protein synthesis (Martemyanov *et al.*, 2001; Besier *et al.*, 2003; Castanheira *et al.*, 2010; Chen *et al.,* 2010a).

Fusidic acid is available in intravenous, oral and topical preparations for the treatment of staphylococcal infections including MRSA, hVISA and VISA. When given systemically it is widely disseminated throughout the body including in areas such as bone, joint fluid, prostate and large abscesses (Collignon & Turnidge, 1999; Howden *et al*. 2004; Howden & Grayson, 2006). In addition to staphylococci, corynebacteria and Gram-positive anaerobes such as clostridia, *Peptococcus* and *Peptostreptococcus* species are susceptible to fusidic acid, but there is limited activity against enterococci and streptococci. Most Gram-negative bacteria are also resistant to fusidic acid with the exception of *Neisseria* and *Moraxella* species, *Legionella pneumophila* and *Bacteroides fragilis*. Some strains of *Mycobacterium tuberculosis* and *Mycobacterium leprae* have also shown borderline susceptibility (Collignon & Turnidge, 1999; Howden & Grayson, 2006).

Resistance to fusidic acid occurs through two main mechanisms (Table 1.3). Alterations in the drug binding site arise due to chromosomal mutations in the *fusA* gene, which encodes EF-G, or *rplF* (*fusE),* which encodes ribosome protein L6. To date over 30 *fusA* mutations have been described, however many of these have only been reported in laboratory mutants and, although the majority of these occur in domain III or amino acid positions 404-483, they have not been confirmed to cause resistance (Besier *et al.,* 2003; Lannergård *et al.,* 2009; Farrell *et al.,* 2011). Alterations in the binding site has been shown to increase the fusidic acid MIC by at least 32-fold. Among clinical isolates the substitution L461K which results in high-level fusidic acid resistance (fusidic acid MIC >256 mg/L) is the most frequently described mutation (Besier *et al.*, 2003). Mutations

Table 1.3 Mechanisms of fusidic acid resistance identified in *Staphylococcus aureus*

Resistance gene	Resistance mechanism	Location	Level of resistance	Reference
fusA	Mutations within the EF-G binding domain	Chromosomal EF-G gene fusA	>256 mg/L	Castanheira et al., 2010
fusB	EF-G protective protein	Plasmid pUB101 or chromosomal	4-32 mg/L	Castanheira et al., 2010
fusC	EF-G protective protein	SCC_{476},	4-8 mg/L	Holden et al., 2004
		$SCCmec_{N1}$,		Ender et al., 2007
		SCCmec-SCC-SCC_{crispr},		Kinnevey et al., 2013
		SCCmecIVa-SCC_{476}		Ellington et al., 2015
		SCCmecIVa-SCC_{149}		
		SCCmec-fus I		
		SCCmec-fus II		
		SCCmec-fus III		
		SCCmec-fus IV		
fusE	Mutation within the rplF gene encoding the ribosomal protein L6	Chromosomal	4-32 mg/L	Norström et al., 2007

L461S and L461F at the same site have also been reported along with substitutions at positions 457 (H457Y and H457Q) but these lead to lower MICs compared to L461K. Along with mutations in *fusA*, alterations in the L6 portion of *rplF* among small colony variant (SCV) *S. aureus* have also been reported suggesting an alternative site of action for fusidic acid and which has been termed *fusE.* These alterations arise due to multiple base pair deletions and cause isolates to exhibit MICs of 4-32 mg/L (Norström *et al.*, 2007; Lannergård *et al.*, 2009; Farrell *et al.,* 2011).

Along with chromosomal mutations that lead to fusidic acid resistance, acquired mechanisms of resistance which lead to the protection of the binding site by FusB-family proteins encoded for by the *fusB, fusC, fusD* and *fusF* genes have also been described (Table 1.3) (Nagaev *et al.,* 2001; Holden *et al.,* 2004; O'Neill & Chopra, 2006; O'Neill *et al.,* 2007; Lin *et al*., 2014; Chen *et al*., 2015). The *fusD* gene has only been described in *Staphylococcus saprophyticus* (O'Neill *et al.,* 2007) and *fusF* has only been reported in *Staphylococci cohnii* (Chen *et al.,* 2015). However, *fusB* and *fusC* has been reported among clinical *S. aureus* and CoNS isolates (Holden *et al.,* 2004; Ender *et al.,* 2007; Castanheira *et al.*, 2010a; Castanheira *et al.*, 2010b; Kinnevey *et al.*, 2013; Ellington *et al.*, 2015).

Among *S. aureus, fusB* has been reported to be carried on the pUB101 plasmid (O'Neill & Chopra, 2006), while *fusC* has been associated with a number of different SCC elements including composite islands and chimeric elements (Holden *et al.,* 2004; Kinnevey *et al.*, 2013; Ellington *et al.*, 2015). Additionally while detailed characterisation of *fusC*-positive MRSA isolates is limited, it has been reported in association with a number of MRSA genotypes including sequence types (STs) 1, 5, 45 and ST149 (Castanheira *et al.*, 2010; Ellington *et al.,* 2015; Baines *et al.,* 2016).

In Ireland an increase in fusidic acid resistance among MRSA recovered from blood stream infection (BSIs) has also been observed, however detailed characterisation of these isolates is lacking (NMRSARL, 2014). The increase of fusidic acid resistance among *S. aureus* in New Zealand has been linked with the emergence of three clones including ST5-MRSA, ST1-MSSA and ST1-MRSA all of which harbour *fusC* and which has been found to be invariably present within the SCC element with or without *mecA* in the form of chimeric elements of SCC*mec* IV. In the UK it has been suggested that the increase in fusidic acid resistance is due to the increase in the dissemination of community associated (CA)-MRSA and the uncontrolled use of topical fusidic acid in the community for the treatment of SSTIs (Ellington *et al.*, 2015).

1.3 *Staphylococcus aureus* virulence factors

The success of *S. aureus* and its ability to cause a wide range of infections is due to its ability to express a wide range of adhesins and virulence factors (Table 1.4), which include haemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, and immune-modulatory factors that facilitate cellular invasion, bacterial growth and reduction of immune system cells (Archer, 1998; Gordon & Lowy, 2008; Oogai *et al.,* 2011).

Genes encoding virulence factors associated with attachment and persistence are found within the core genome of *S. aureus*. Surface proteins, known as microbial surface components recognising adhesive matrix molecules (MSCRAMMs) mediate adherence to host tissues. MSCRAMMs, encoded for by *bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA* and *fnbB* which are located on the core genome, bind molecules such as elastin, laminin, bone sialoprotein, collagen, fibronectin and fibrinogen and are involved in establishing of *S. aureus* infection (Foster & Höök, 1998; Gordon & Lowy, 2008). The staphylococcal capsular polysaccharide aids in evasion of the host immune system (O'Riordan & Lee, 2004). It has also been reported that differences in the MSCRAMMs expressed by different

Table 1.4 The main virulence factors expressed by *Staphylococcus aureus* and the associated clinical presentations

Type of virulence factor	Selected factor	Biological effect	Genes	Examples of associated clinical conditions
Attachment	Fibronectin binding protein[a]	Attachment to fibronectin in host cell	*fnbpA, fnbpB*	Endocarditis, osteomyelitis, septic arthritis, prosthetic device and catheter related infections
	Collagen binding protein[a]	Attachment to collagen in host cell	*cna*	
	Fibrinogen binding protein[a]	Attachment to fibrinogen in host cell, protection against phagocytosis	*clfA, clfB*	
	Elastin binding protein[a]	Attachment to tissues abundant in elastin	*ebpS*	
Persistence	Polysaccharide intercellular adhesion	Biofilm accumulation	*ica* locus	Relapsing infections, cystic fibrosis
	Small colony variants	Persistence	*hemB* mutation	
	Arginine catabolic mobile element	Enhanced survival on skin	*arcA, arcB, arc, arcD*	
Evasion of immune system	Capsular polysaccharide	Inhibits chemotaxis and phagocytic engulfment	*capH, capI, capJ, capK*	Invasive skin infections
	Staphylococcal protein A	Inhibits antibody mediated clearance and interacts with platelets	*spa*	
	Immune evasion complex	Inhibits immune response	*sea, sak, chp, scn, sep*	
Destruction of	Panton Valentine leucocidin	Lysis of human leukocytes	*lukF-PV, lukS-PV*	Invasive skin infections and

Continued overleaf

Table 1.4 continued

Type of virulence factor	Selected factor	Biological effect	Genes	Examples of associated clinical conditions
host defences (tissues and cells)	Lipase	Hydrolyses lipids	*lip*	necrotising pneumonia (CA-MRSA strains associated with PVL), abscesses
	Protease	Hydrolyses proteins	*clp*	
	Hyaluronidase	Hydrolyses hyaluronic acid	*hysA*	
	α and β haemolysin	Damage to membranes of platelets and monocytes	*hla, hlb*	
	Enterotoxins	Stimulate T cells to release cytokines causing damage to hot tissue	*sea – seu*	Food poisoning
	Toxic shock syndrome	Stimulate T cells to release cytokines causing damage to hot tissue	*tst*	Toxic shock syndrome
	Exfoliative toxins	Bind to desmosomes and disrupt epidermal layers	*etA, etB, etD*	Scalded skin syndrome, bullous impetigo

[a] Collectively these virulence factors are termed microbial surface components recognising adhesive matrix molecules (MSCRAMMs).

S. aureus strains may be associated with different infections (Foster & Höök, 1998; Tung *et al.,* 2000; Menzies, 2003; McCarthy & Lindsay, 2013).

In contrast to the genes located on the core genome, genes encoding toxins and superantigens that are associated with damage and destruction of host cell tissues are carried on MGEs. Alpha haemolysin, encoded for by *hla*, is a cytolytic toxin which plays a role in the pathogenesis of *S. aureus* pneumonia and can cause septic shock in humans (Essmann *et al.,* 2003; Bubeck Wardenburg *et al.,* 2007). The toxin targets the membranes of platelets and monocytes causing cell death by necrosis or apoptosis and resulting in septic shock (Dinges *et al.,* 2000).

Staphylococcal superantigens (SAgs) carried on bacteriophages, genomic islands or pathogenicity islands cause increased production of T-lymphocytes and include staphylococcal enterotoxins and toxic shock syndrome toxin-1 (TSST-1), which can lead to food poisoning when ingested or toxic shock when expressed systemically. The exfoliative toxins ETA, ETB and ETD, encoded for by *etA, etB* and *etD* respectively, are the sole causative agents of staphylococcal scalded skin syndrome (SSSS) and bullous impetigo which primarily affects infants and young children (Bukowski *et al*., 2010). The ETA and ETB toxins are glutamate-specific serine proteases that cleave a single peptide bond in the desmoglein-1 preventing cell-to-cell adhesion in the upper epidermidis. However, while the mode of action of ETD is similar to that of ETA and ETB, its role in disease is not yet clear (Plano, 2004). Furthermore, the exfoliative toxin ETC has only been reported in *S. aureus* strains of animal origin including livestock and mice but has not yet been associated with infections in humans (Sato *et al.*, 1994; Bukowski *et al*., 2010).

The Panton-Valentine leukocidin (PVL) exotoxin is a pore-forming cytolytic toxin that targets leucocytes and is associated with severe SSTIs among otherwise healthy people

along with more serious infections such as necrotising pneumonia, fasciitis and septicaemia. The toxin is comprised of two synergistic bacteriophage-encoded proteins LukF-PV and LukS-PV that are encoded by *lukF-PV* and *lukS-PV* genes. The expression of PVL has been linked with CA-MRSA, however with the detection of PVL among MSSA and also in healthcare associated (HCA)-MRSA it is now widely accepted that PVL is not limited to CA-MRSA. There is also evidence to suggest that there is significantly higher morbidity and mortality associated with patients with PVL-positive MRSA infections compared to those with PVL-negative MRSA infections, however there is on-going debate surrounding these observations (Otto, 2013a).

The immune evasion cluster (IEC) proteins encoded for by *sak, chp, scn*, *sep* and *sea* are carried on β-haemolysin converting bacteriophages (van Wamel *et al.,* 2006; Malachowa & Deleo, 2010; Thammavongsa *et al.,* 2015) which integrate into the *S. aureus* chromosome within the *hlb* gene (Coleman *et al.*, 1989, Coleman *et al.,* 1991; Malachowa and Deleo, 2010; Thammavongsa *et al.*, 2015). The IEC proteins protect *S. aureus* from the host innate immune system. The chemotaxis inhibitory protein (CHIP), encoded by *chp*, inhibits human neutrophil chemotaxis and activation while a staphylococcal complement C3 convertase inhibitor (SCIN) encoded for by *scn* prevents neutrophil phagocytosis of *S. aureus.* The *sak* gene encodes a staphylokinase protein which converts plasminogen to plasmid inhibiting α-defensins and reduces the activity of immunoglobulin IgG. Enterotoxin A (*sea*) is associated with food borne illnesses causing gastroenteritis and toxic shock. It acts by binding to T cells and antigen presenting cells leading to proliferation of T cells and the release of cytokines. To date eight IEC types have been described each carrying different combinations of *sea, sak, sep, chp* and *scn* (Table 1.5) (van Wamel *et al.,* 2006; Price *et al.,* 2012). While the IEC plays a crucial role in colonisation of humans, it is not present among the majority of animal-associated *S. aureus*

Table 1.5 Immune evasion cluster (IEC) types described to date in *Staphylococcus aureus*[a]

IEC type	Genes present[b]
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*
H	*scn*

[a]Adadpted from van Wamel *et al.* (2006)

[b]*sea,* staphylooccal enterotoxin A; *sak,* staphylokinase; *chp*, chemotaxis inhibitory protein; *scn,* staphylococcal complement inhibitor; *sep,* staphylococcal enterotoxin P

strains (Verkaik *et al.,* 2011; Cuny *et al.,* 2015) and is thought to be the only MGE that is present in human lineages of *S. aureus* but absent in animal strains allowing these strains to produce β-haemolysin (Sung *et al.,* 2008).

The ACME was first described in the CA-MRSA strain USA300 in 2006 but since then has been described in a number of different lineages and also among CoNS (Shore *et al.,* 2011b; Urushibara *et al.,* 2012; Espedido *et al.,* 2012; Otto, 2013; Hellmark *et al.,* 2013; Fard-Mousavi *et al.,* 2015; Urushibara *et al.,* 2016). The ACME can contain one or two gene clusters, namely *arc* and/or *opp-3*. The *opp-3* cluster encodes an ABC transporter protein that is associated with peptide nutrient uptake, quorum sensing, cell adhesion and expression of virulence determinants. The *arc* cluster encodes an arginine deaminase pathway that converts L-arginine to carbon dioxide, ATP and ammonia (Pi *et al.,* 2009). While all *S. aureus* harbour native *arc* and *opp* operons, the presence of ACME has been shown to increase survival and growth in host cells. Additionally the deletion of ACME significantly reduced the pathogenicity of USA300 (Diep *et al.,* 2008).

1.4 Epidemiological typing of MRSA

Detailed typing of MRSA is essential in determining the relatedness of isolates and for tracking strains in both short and long-term epidemiological studies. The ideal epidemiological typing technique must satisfy a number of criteria which include a standardised, sensitive, specific and objective method, suitable for the rapid and reliable differentiation between unrelated isolates of a bacterial species (Stefani *et al.,* 2012).

While historically typing of MRSA was primarily based on phenotypic characteristics including the antimicrobial susceptibility profile of the isolate, molecular techniques are now more widely used. Pulsed-field gel electrophoresis (PFGE), which compares the banding patterns from whole-genome macro-restriction digestion using rare-

cutting restriction endonucleases such as *smaI* was previously considered to be the gold-standard for typing of MRSA, particularly in outbreak situations. However, this too has been replaced with DNA sequencing-based techniques such as staphylococcal protein A (*spa*) gene typing and multi-locus sequence typing (MLST) (Stefani *et al.,* 2012).

Multilocus sequence typing is based on the sequencing of the internal fragments of seven housekeeping genes. Sequencing of these genes results in allelic profiles and subsequent STs, which are then assigned to lineages that are designated clonal complexes (CCs). In contrast *spa* typing utilises the repeat regions in the *spa* gene where genetic events lead to DNA sequence variation in the repeat regions resulting in different *spa* types and is more discriminatory and less time-consuming than MLST. For both MLST and *spa* typing, online databases and standardised nomenclatures allow easy inter-laboratory comparison of data and tracking of strains (Mediavilla *et al.,* 2012). Direct repeat units (*dru*) adjacent to IS*431* within the SCC*mec* region of MRSA isolate, which are constant regardless of SCC*mec* type, have also been utilised in the discrimination of MRSA with particular usefulness in unrelated isolates (Goering *et al.,* 2008). Further discrimination of MRSA strains can be achieved by combining these methods with SCC*mec* typing and *dru* typing to discriminate distinct strains within a given ST/CC or *spa* type (IWG-SCC, 2009; Shore *et al.,* 2010).

More recently, advances in whole-genome sequencing (WGS) technology have led to the development of WGS as well as DNA microarrays based either on the entire *S. aureus* genomes or selected genes for both short and long-term epidemiological investigations of MRSA (Köser *et al.,* 2012; Harris *et al.,* 2013; Price *et al.,* 2014). While WGS has reduced in cost in recent years, it is still time consuming and beyond the capabilities of many diagnostic and reference laboratories, particularly in relation to the associated complex bioinformatic data analysis and interpretation.

1.4.1 Antibiogram-resistogram typing

Susceptibility testing using a panel of antimicrobial agents is commonly used in clinical microbiology laboratories to guide patient treatment (van Belkum *et al.,* 2007). The resulting antibiogram indicates the pattern of *in-vitro* resistance or susceptibility to the panel of antimicrobials tested (Singh *et al.,* 2006). Susceptibility patterns expressed as diameters of zones of inhibition have been reported to be useful as a method of antibiogram typing when used in conjunction with information generated by other methods (van Belkum *et al.,* 2007). Variations between strains may occur due to loss or gain of MGEs such as plasmids or transposons harbouring resistance determinants or mutations in genes targeted by antibiotics because of antibiotic selective pressures within the healthcare environment (Rossney *et al.,* 1994a). The inclusion of antimicrobials that are not in clinical use enhances the value of the antibiogram as a typing method by minimising variation due to selective pressure in the hospital environment. Combining antibiogram typing with the investigation of resistance to heavy metals (resistogram typing) to generate antibiogram-resistogram (AR) patterns can further enhance the value of susceptibility testing as a typing method and has previously been used as a frontline method for typing MRSA (Rossney *et al.,* 1994a; Rossney *et al.,* 1994b; Rossney *et al.,* 2007).

1.4.2 Multilocus sequence typing

Multilocus sequence typing has been used to characterise a range of bacterial species along with yeasts and fungi. For *S. aureus,* this method involves the sequencing of seven housekeeping genes that encode proteins which are essential for cell viability. These genes are variable but highly conserved, easily sequenced and have been identified within the genome of all isolates of a particular strain (Fig. 1.4). Each gene is assigned an allele number and genetic polymorphisms within the sequences of each of the seven selected housekeeping genes are considered distinct alleles. The alleles of each of the seven loci

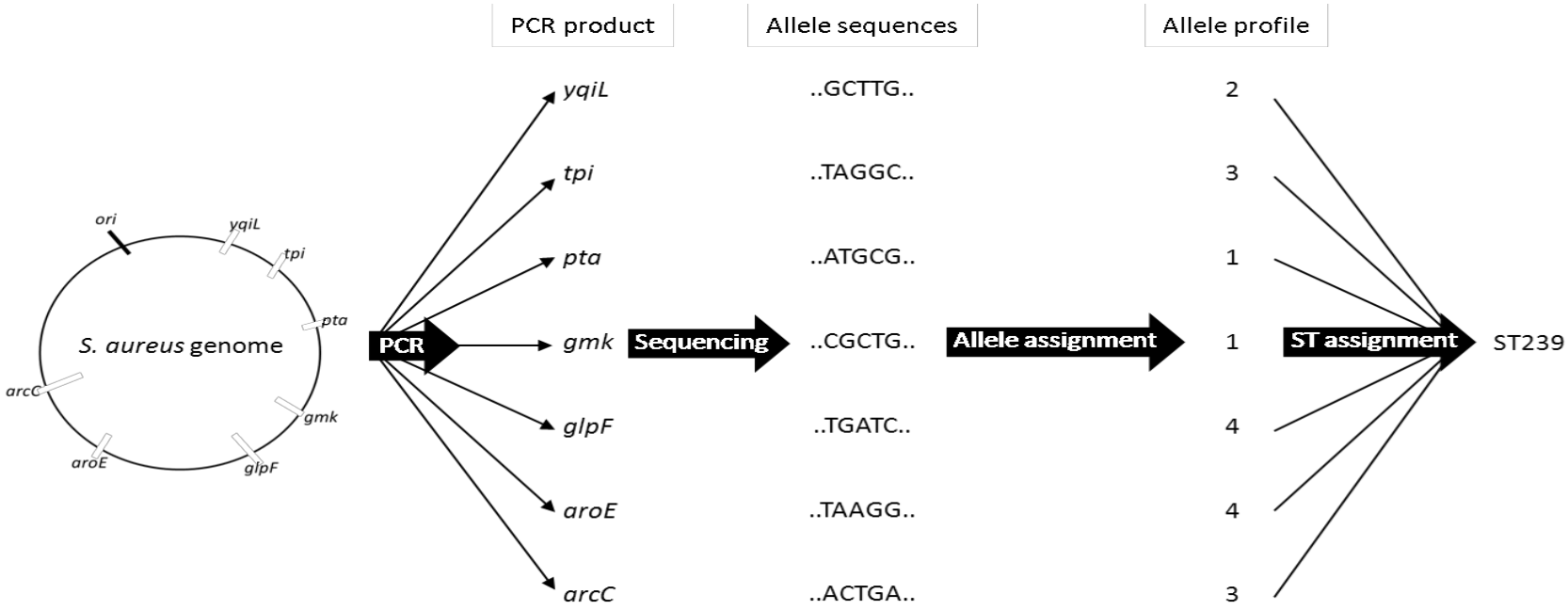


Figure 1.4 Multilocus sequence typing of *S. aureus* involves the sequencing of seven housekeeping genes (*arc, aroE, glpF, gmk, pta, tpi* and *yqiL*) located within the core genome of *S. aureus* (represented by the black circle) that are amplified using published primers and protocols which can be viewed on the MLST website (http://www.mlst.net). Once sequenced, amplimers are compared to known alleles for each of the genes that are also available on the MLST website. The combination of the seven alleles is used to assign a sequence type (ST) to the isolate. If 5/7 alleles are identical in two isolates, they are assigned to the same clonal complex.

provide a profile, which is allocated an ST. There are many potential alleles at each locus and so it is unlikely that identical alleles will occur by chance. Therefore, isolates with the same profile are considered members of the same clone (Enright *et al.,* 2000). Different STs can be assigned to CCs using a Based Upon Repeating Sequence Types (eBURST) algorithm that examines the evolutionary relatedness of isolates. MRSA isolates are assigned to the same CC if a minimum of five MLST alleles are identical to at least one other ST within the CC.

Variation within housekeeping genes accumulates slowly and so isolates with the same allelic profile may be related to a common ancestor that existed many years ago (Enright *et al.,* 2000). Therefore, MLST is used to investigate strain evolution but is not suitable for short term studies such as outbreaks. It is less discriminatory than PFGE (which measures variations that occur relatively quickly) and it is expensive, labour intensive and requires access to DNA sequencing facilities.

1.4.3 Staphylococcal protein A (spa) gene typing

Sequencing of the polymorphic X region of the *spa* gene is an alternative to MLST. The polymorphic X region consists of a variable number of 24 base pairs (bp) repeats located immediately upstream of the region encoding the C-terminal cell wall attachment sequence. The X region is flanked on either side by well-conserved regions that allow the use of PCR primers for amplification and DNA sequence typing (Fig. 1.5). The X region polymorphisms occur as a result of point mutations, deletions, duplications and insertions. Different repeat sequences are assigned an alpha-numeric code and the order of the specific repeats defines the *spa* type (Mellmann *et al.,* 2007). *spa* typing recognises mutations or repeat insertion/deletion events that can cause changes in the repeat sequence allowing the method to be used in both short-term and long-term epidemiological studies.

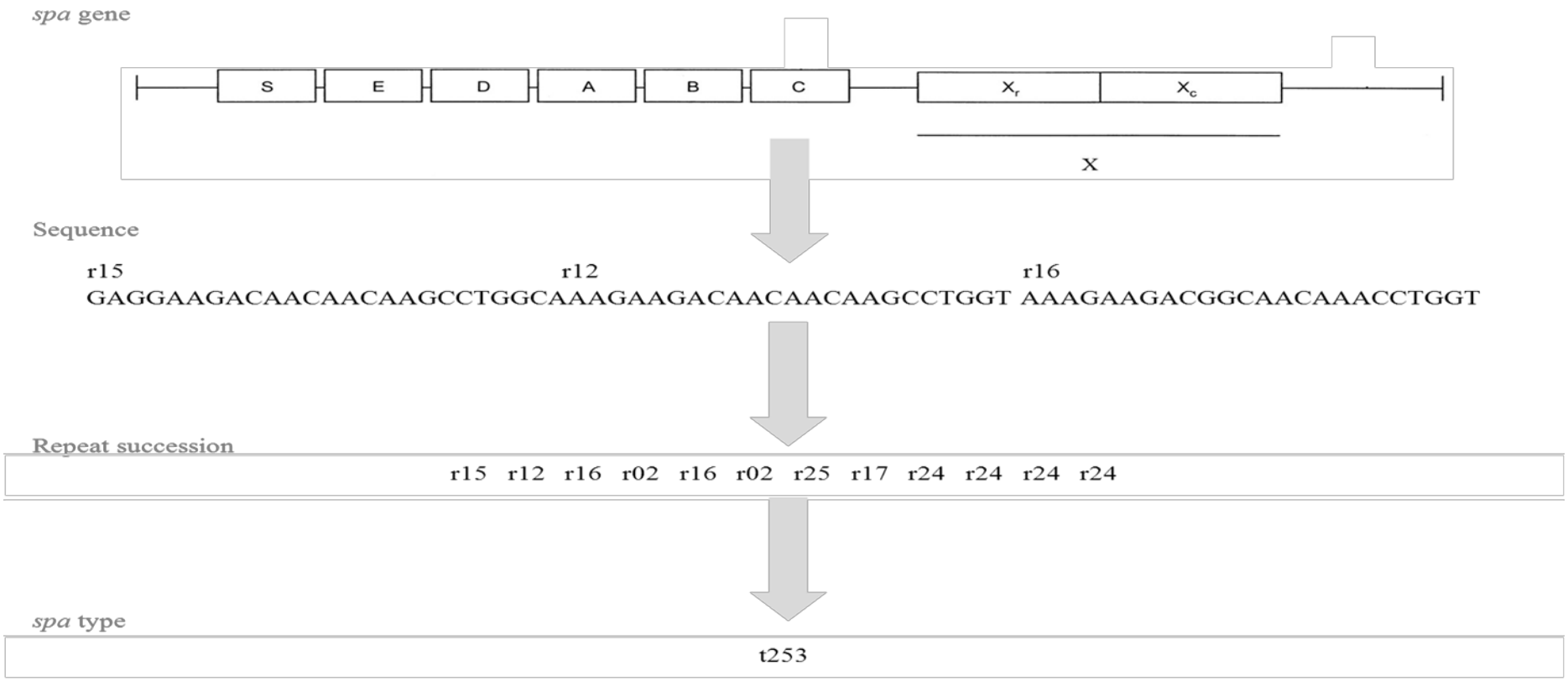


Figure 1.5 *spa* typing involves the amplification and sequencing of the polymorphic X region of the *spa* gene using a primers *spa* 1113F and *spa* 1514R as described by the European network of Laboratories for sequence based typing of Microbial pathogens (www.seqnet.org). *spa* types are assigned based on the variable quantity of repeating units within the X region and the variation within those units using the Ridom Staphtype software

(Ridom GmbH). Boxes indicate segments of the gene coding for the signal sequence (S), the immunoglobulin G-binding regions (A–D), a region homologous to A–D (E), and the variable X region (X), which includes the short sequence repeats (Xr) and the cell wall attachment sequence (Xc). Adapted from Shopsin *et al.,* 1999.

Shopsin *et al.,* (1999) and Koreen *et al.,* (2004) both suggested that recombination events involving the *spa* locus are rare making *spa* typing especially useful for long-term studies.

An internet based database has been established to collate data obtained by *spa* typing (http://www.ridom.de/spa-server). Laboratories submit data to the database and a curator is responsible for ensuring that all data submitted are of high quality. Where isolates exhibiting a particular *spa* type have been investigated by MLST and the MLST profile also submitted, these data are available in the database. Hence the MLST ST and/or CC can be inferred for future isolates with that particular *spa* type.

spa typing has many advantages over PFGE and MLST. It is rapid, reproducible and relatively easy to perform, and data are easily shared among laboratories (Hallin *et al.,* 2007a). Studies have shown that results are comparable between laboratories and that, although *spa* typing is more discriminatory than MLST, there is good agreement between MLST and *spa* typing (Koreen *et al.,* 2004) and good concordance between *spa* typing and PFGE (Shopsin *et al.,* 1999; Sabat *et al.,* 2006; Hallin *et al.,* 2007). However, as with all sequence-based techniques, the method is expensive and sequencing facilities may not be widely available in clinical laboratories (Malachowa *et al.,* 2005).

1.4.4 Staphylococcal Cassette Chromosome mec Typing

SCC*mec* typing is based on the molecular characterisation of the isolates *mec* complex, *ccr* type and portions of the intervening J regions, predominantly by PCR (Hallin *et al.,* 2007). It is now recommended for the international comparison of MRSA strains that *S. aureus* isolates should be described by their MLST ST and for MRSA, by their SCC*mec* type. For example, the most prevalent UK epidemic MRSA clone is EMRSA-15. This clone is designated ST22 by MLST and harbours SCC*mec* type IV and so is designated as ST22-MRSA-IV (Cookson *et al.,* 2007). Despite its use in international

nomenclature, limitations associated with SCC*mec* typing relate particularly to the constant requirement to update protocols in accordance with newly described SCC*mec* types and variants along with the multiple PCR reactions required to determine the SCC*mec* type and subtype (Turlej *et al.*, 2011).

1.4.5 DNA microarray profiling

The discriminatory power of a typing technique is essential when investigating outbreaks in nosocomial environments. While combining several different techniques such as *spa, dru* and PFGE can improve the discrimination of MRSA isolates, it is costly and time consuming as well as difficult in terms of data analysis to perform several different techniques in order to achieve the required discriminatory power (Shore *et al.,* 2010b). The availability of whole-genome sequences has led to the development of high-throughput DNA microarrays for typing of *S. aureus* isolates that detect the presence or absence of specific genes such as the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH). This DNA microarray involves the simultaneous detection of 336 *S. aureus* genes and alleles including species-specific markers (*spa, nuc, coa)*, accessory gene regulator (*agr)* alleles, antimicrobial resistance and virulence genes, toxins and capsular genes and MSCRAMMs. Software compares the array profile of a test isolate to a large database of previously investigated strains and assigns isolates to MLST CCs/STs and SCC*mec* types (Monecke & Ehricht, 2005; Monecke *et al.,* 2008).

This DNA microarray has been used previously to characterise *S. aureus* isolates (both MRSA and MSSA) throughout Europe, Australia and Africa (Feßler *et al.,* 2010; Coombs *et al.,* 2012; Shore *et al.,* 2012; Kinnevey *et al.,* 2014) and has been shown to reliably assign isolates to the correct CCs/STs and SCC*mec* types (Shore *et al.,* 2012) along with good inter laboratory comparability. It is less time consuming than conventional MLST, SCC*mec* typing and multiple PCRs for virulence and resistance genes while

providing more information about each isolate (Shore *et al.,* 2012). However, to date the usefulness of this technology for the discrimination of highly clonal strains in outbreak situations has not been investigated.

1.4.6 Whole-genome sequencing

Whole-genome sequencing involves randomly fragmenting an entire genome and sequencing each fragment, with the resulting multiple sequences assembled against known reference strains in order to characterise and compare isolates. Until relatively recently, WGS was primarily used for evolutionary studies of bacteria or for the detailed characterisation of a single isolate due to the time and financial constraints involved. More recently however, advances in high-throughput WGS technology and reductions in WGS costs have allowed more access to the technology, which has led to the application of this technology in short-term epidemiological investigations such as outbreaks involving numerous different organisms including MRSA (Cheung *et al.,* 2011; Lindsay, 2014; Price *et al.,* 2014; Schmid *et al.,* 2014; Croucher & Didelot, 2015; McGann *et al.,* 2016; Ugolotti *et al.,* 2016). Additionally, the usefulness of WGS to guide antimicrobial therapy by investigating known resistance determinants has also been explored (Price *et al.,* 2013a; Köser *et al.,* 2014; Ramana, 2014).

In relation to infection control procedures, WGS allows improved discrimination of isolates that are differentiated from each other based on single nucleotide variations (SNV) in the WGS data. This differentiation enables the identification of transmission events directly between patients particularly among highly clonal HCA-MRSA strains (Croucher & Didelot 2015; Kinnevey *et al.,* 2016). The application of WGS to infection control outbreak investigations has shown the spread of an outbreak strain into the community (Harris *et al.*, 2013). Other studies on a variety of bacterial species have already shown that WGS-based typing, based either on SNV or on gene-by-gene allelic profiling of the core

genome is the ultimate diagnostic tool for strain typing (Price *et al.*, 2013a; Price *et al.*, 2013b; Ruppitsch *et al.*, 2015; Kwong *et al.*, 2015).

While improved turnaround time (TAT) and reduced cost of the technology makes it attractive to clinical microbiology, to date many studies have been based on retrospective analysis since outstanding challenges surrounding WGS including sample preparation, library construction, bioinformatics and quality control resulting in the slow uptake of the technology in routine diagnostic areas (Ekblom & Wolf, 2014; Goldberg *et al.,* 2015; McGann *et al.,* 2016). Prior to the adoption of WGS technology must undergo rigorous validation protocols involving standardisation, optimisation, validation and where possible automation. Ongoing rapid developments in WGS instrumentation and data analysis software have hindered the publication of guidelines to assist in validation of the technology (Ekblom & Wolf, 2014). Significant financial investment is required to secure the necessary equipment for WGS, in addition to costs relating to data storage which also require consideration due to the vast amount of data generated (Ekblom & Wolf, 2014). Along with these however, data analysis and interlaboratory comparability of WGS data is perhaps the biggest challenge for laboratories utilising WGS technology coupled with the lack of standardised nomenclature to facilitate global exchange of data in a manner similar to the way in which MLST and SCC*mec* has been used for MRSA (Ruppitsch *et al.*, 2015).

Software permitting the automatic analysis of WGS data has been developed where genome-wide genes are analysed and isolates are typed based on a core-genome MLST (cgMLST) protocol resulting in higher discrimination and more accurate strain typing. However, to date, publications in relation to the utilisation of this software with specific MRSA clones are limited and the usefulness of this software for identifying MRSA transmission events, particularly for highly-clonal MRSA strains in an endemic setting,

where strain differentiation is particularly challenging, are lacking (Ruppitsch *et al.*, 2015; Shore *et al.*, 2016; Kinnevey *et al.*, 2016; Ugolotti *et al.*, 2016).

1.5 The evolution and epidemiology of MRSA

Since it was first reported in 1961 (Jevons, 1961) MRSA has become pandemic in hospitals throughout the world and is among the most common causes of HCAIs. Multiple independent acquisitions of SCC*mec* by different *S. aureus* strains has led to the emergence of numerous different lineages of MRSA, however only a limited number of clones are associated with HCA-MRSA, each with a specific genetic background and SCC*mec* type. This includes ST5-MRSA-II (CC5), ST8-MRSA-II and ST239-MRSA-III (CC8), ST22-MRSA-IV (CC22), ST36-MRSA-II (CC30) and ST45-MRSA-II (CC45) (Enright *et al.,* 2002; Deurenberg & Stobberingh, 2008; Lindsay, 2010; Stefani *et al.,* 2012; Holden *et al.,* 2013). Healthcare-associated MRSA of CC5 and CC8 are widespread throughout many countries including USA, South America, Canada, Africa, Asia and Australia (Chambers & Deleo, 2009; Monecke *et al.,* 2011; Stefani *et al.,* 2012). In Europe ST22-MRSA-IV is the predominant clone (Grundmann *et al.,* 2014).

The predominance of relatively few HCA-MRSA clones, suggests that some strains have specific attributes that enable them to survive and spread in the hospital environment (Holden *et al.,* 2013) and, once established within a healthcare setting, are difficult to eradicate. Circulating clones have been shown to vary over time between hospital and community settings. Phylogenetic studies have revealed the intercontinental spread of different clones in hospitals through North America, Europe, South America and Asia (Harris *et al.,* 2010). There is now also evidence that suggests that some of these strains have an increasing association with community-onset infection, indicating the export of CA-MRSA clones into the community (Mollaghan *et al.,* 2010; González-Domínguez *et al.,* 2012; Grundmann *et al.,* 2014).

Phylogenetic studies have indicated that the ST22-MRSA-IV lineage first emerged in the early 1990s in the UK from where it spread throughout the UK and Ireland, and now accounts for approximately 80% of MRSA bacteremias in Ireland (NMRSARL, 2014). The emergence of this strain coincided with the introduction of fluoroquinolone antibiotics into clinical use and currently resistance to fluoroquinolones is almost universal in ST22-MRSA-IV (Bal *et al.,* 2016).

Other studies on the evolution of the major HCA-MRSA clones have revealed strong evidence for the accumulation of a wide range of antibiotic resistance-associated mutations and MGEs associated with their emergence in hospital epidemics (McAdam *et al.,* 2012; Harris *et al.,* 2013). In addition, other genes have been identified that are implicated in the emergence of particular *S. aureus* strains. For the ST239-MRSA-III clone, the presence of a novel adhesion gene, *sasX,* on a large bacteriophage-like element has been implicated in the virulent phenotype of this clone in hospitals in Asia and the UK (Li *et al.,* 2012), although it does not account for prevalence in all locations (Edgeworth *et al.,* 2007; Holden *et al.,* 2010; Harris *et al.,* 2010).

Along with pandemic MRSA clones, other sporadically occurring clones have been recovered from single or only a few patients which appear to be less able to spread (Hallin *et al.,* 2008; Monecke *et al.*, 2011). While some studies have shown that the genetic backgrounds of these sporadic isolates is close to those of epidemic strains (Hallin *et al.*, 2008) others failed to identify links between epidemic behaviour and particular lineages (Blanc *et al.*, 2000; Albrecht *et al.*, 2011). Furthermore, numerous studies have shown that many MRSA clones which occur sporadically in one region may be predominant in other regions indicating the potential of these strains to emerge as predominant strains elsewhere (Monecke *et al.,* 2011) and highlighting the importance of characterising these strains regardless of their prevalence in any given geographic location.

1.5.1 Community-associated MRSA

While traditionally MRSA has been associated with hospitals, other healthcare facilities and patients who frequent these, since the 1990s there have been increasing reports of MRSA infections arising in the community among people lacking these risk factors. This increasing prevalence of CA-MRSA has been associated with colonisation of healthy individuals and a range of clinical diseases from SSTIs to severe and life-threatening necrotising pneumonia, amongst others. Furthermore, outbreaks of CA-MRSA have been reported in specific community settings and groups, including among those in prisons, crèches, gymnasia, and military bases and among Australian Aborigines and Native Americans (David & Daum, 2010).

Initially, differences observed in the molecular characteristics of CA- and HCA-MRSA including the presence of smaller SCC*mec* elements and specific toxins and virulence factors among CA-MRSA allowed these strains to be easily distinguished. However, many of these distinguishing factors no longer apply (David & Daum, 2010). Unlike HCA-MRSA which is limited to a few predominant clones, there are currently over 20 distinct lineages associated with CA-MRSA, five of which are globally disseminated and two of which (ST8-MRSA-IV and ST30-MRSA-IV) may be considered pandemic (Mediavilla *et al.,* 2012). Molecular studies have shown that CA-MRSA clones have arisen independently of HCA-MRSA by the horizontal gene transfer of SCC*mec* and PVL toxin genes (*pvl*) into MSSA strains (David & Daum, 2010).

While many theories have been proposed to account for the rapid global emergence of diverse CA-MRSA strains, it is unlikely that one single cause was responsible for the emergence of CA-MRSA. In addition mathematical models predict that these CA-MRSA strains will ultimately displace HCA-MRSA strains in healthcare settings (D'Agata *et al.*, 2009; Skov & Jensen, 2009). Furthermore, other studies have shown that infection

prevention and control strategies focused on infected individuals only are not capable of achieving control of CA-MRSA and suggest that decolonisation strategies targeting the paediatric population colonised with CA-MRSA have the potential of achieving disease elimination (Wang *et al.*, 2013).

1.5.2 Livestock-associated MRSA

First reported among pigs in the Netherlands with the recognition of CC398, livestock associated (LA)-MRSA has since been reported among a range of livestock and horses, as well as in humans in several European countries along with America and Australia, particularly in regions with high-density pig farming (van Cleef *et al.,* 2015). Genomic analyses have revealed that CC398 first emerged in humans and, through human-animal interactions, transferred to animals where it adapted to its new host through the loss and gain of several MGEs before reintroduction to humans. These strains often exhibit resistance to multiple classes of antimicrobial agents but lack many *S. aureus* virulence genes. Newly acquired MGEs involved in the human to animal switch include genes encoding resistance to tetracycline and trimethoprim as well as SCC*mec* (Lekkerkerk *et al.,* 2015), while those lost include the human specific bacteriophage-encoded IEC genes (McCarthy *et al.,* 2012). While CC398 MRSA is predominantly associated with colonisation in animals, serious infections in humans have been reported as well as spread to, and within, the healthcare system. Methicillin-susceptible *S. aureus* belonging to CC398 have also been reported from animals and humans and have been associated with a range of CA- and HCA- infections in humans, many without livestock contacts (van Cleef *et al.,* 2015).

While most LA-MRSA strains harbour *mecA,* since 2011, *mecC* MRSA has been reported in MRSA recovered from humans, livestock, wild animals and companion pets. To date these reports have been limited to Europe with the exception of a single isolate

recovered from a cat in Australia (Petersen *et al*., 2013a; Paterson *et al.*, 2014; Espinosa-Gongora *et al.*, 2015; Worthing *et al.*, 2016). Although the prevalence of the *mecC*-MRSA strains in many countries is low (Deplano *et al.,* 2014), an overall increase in frequency in 2010 and 2011 was reported in countries such as Denmark, where reporting of all new MRSA cases is mandatory (Petersen *et al.,* 2013). Unlike CC398, *mecC* MRSA are susceptible to most other classes of antibiotics and have been found to belong to lineages CC130, CC1943 and ST425, all of which have been associated previously with cattle and as such, suggested a zoonotic MRSA reservoir for human infections (Petersen *et al.,* 2013). In addition homologues of *mecC* have also been reported in *Staphylococcus xylosus, Staphylococcus stepanovicii* and *Staphylococcus scirui* suggesting that the origin of *mecC,* like *mecA,* is among CoNS (Paterson *et al.,* 2014).

Initially, the laboratory detection of *mecC* MRSA proved challenging for diagnostic laboratories with significant differences in the reliability of chromogenic agars from different manufacturers described (Skov *et al.,* 2014). Using automated systems, *mecA*-MRSA exhibit resistance to both oxacillin and cefoxitin, however *mecC*-MRSA frequently exhibit resistance only to cefoxitin due to a higher affinity for the PBP2a encoded by *mecC* in comparison to that encoded by *mecA* (Kim *et al.,* 2012). Initially molecular detection tests for MRSA also failed to detect *mecC*-MRSA (Shore *et al.,* 2011a) but modified PCR assays have now been reported using either *mec*-specific primers or *mec* universal primers (Pichon *et al.,* 2012; Stegger *et al.,* 2012). In order to address these potential shortcomings in conventional laboratory detection techniques, studies are required to validate their effectiveness and suitability in the correct detection and identification of *mecC*-MRSA isolates.

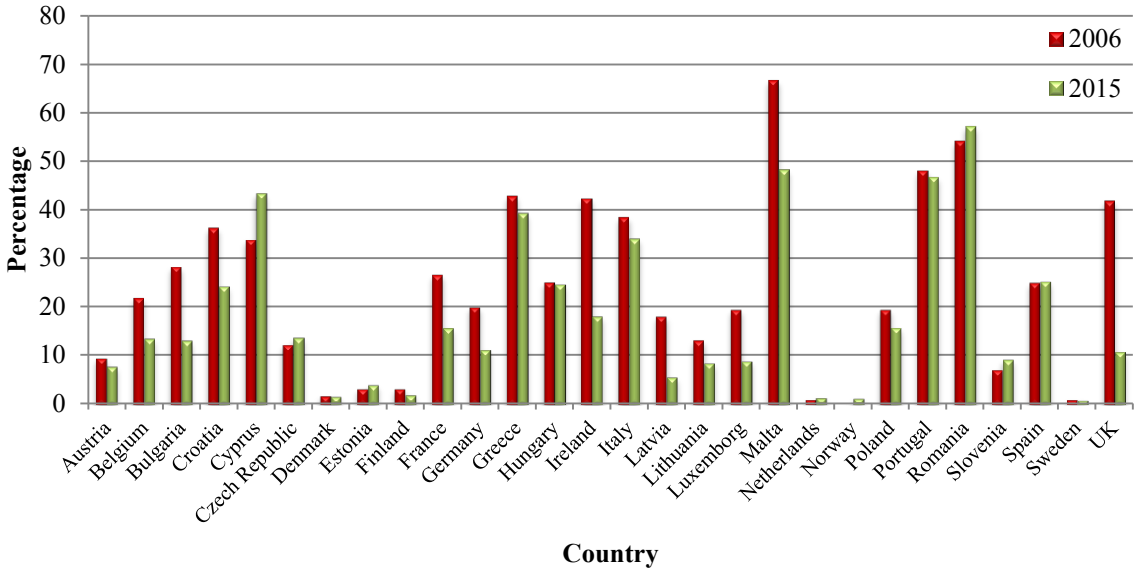
1.6 MRSA Surveillance

1.6.1 The European Antimicrobial Resistance Network

The European Antimicrobial Resistance Network (EARS-Net) is a European surveillance system for monitoring antimicrobial resistance. Formerly the European Antimicrobial Resistance Surveillance System (EARSS), it was established in 1998 with the aim of showing how international antimicrobial resistance data could inform decisions relating to antimicrobial stewardship and was coordinated through the Dutch National Institute for Public Health and the Environment (RIVM). In January 2010, EARSS was transferred to the European Centre for Disease Prevention and Control (ECDC) and, along with the transfer, came the name change. Currently all 28 European Union (EU) member countries along with Norway and Iceland submit data on clinically significant pathogens recovered from BSIs allowing the monitoring of the occurrence and spread of antimicrobial resistance throughout Europe. Along with *S. aureus* resistance in other clinically significant pathogens monitored include *Pseudomonas aeruginosa, Acinetobacter baumanii, Enterococcus faecalis, Kelbsiella pneumonia, Escherichia coli* and *Streptococcus pneumoniae*. The EARS-Net project is based on a network of representatives from member states collecting the data from their respective countries and submitting data to the ECDC. In addition the coordination committee collaborate with other European surveillance groups including the European surveillance of antimicrobial consumption committee and the healthcare associated infections surveillance network (ECDC, 2014). In Ireland the data collation is coordinated through the Health Protection Surveillance Centre (HPSC) and a network of surveillance scientists located in microbiology laboratories throughout the country.

Since inception of the EARSS programme, the proportion of *S. aureus* isolates recovered from BSIs that are MRSA has decreased throughout Europe (Fig. 1.6). Currently

(a)



(b)

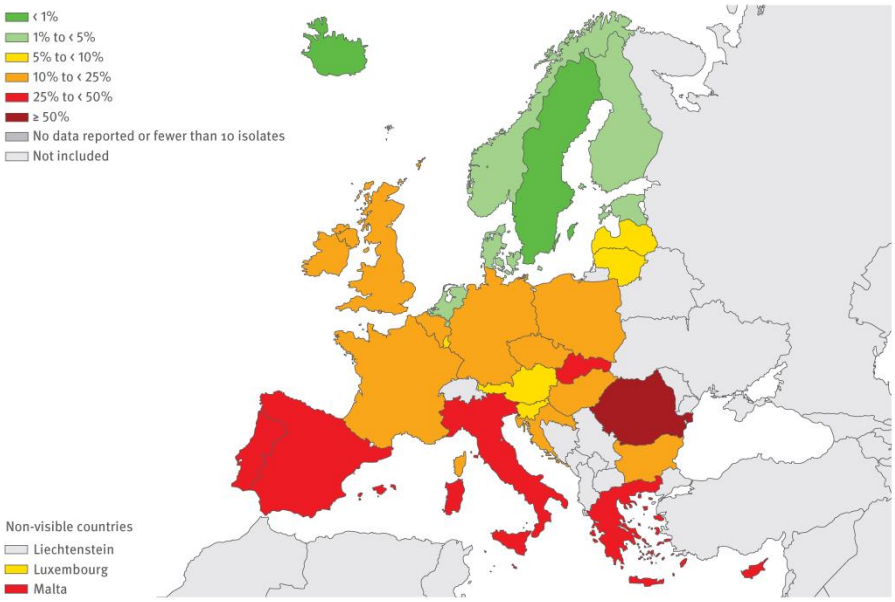


Figure 1.6 The prevalence of MRSA isolates recovered from bloodstream infections (BSIs) in Europe in 2015. (a) The percentage changes in the proportion of *S. aureus* isolates from BSIs recovered since the establishment of the European Surveillance System which were resistant to methicillin. (b) The percentage prevalence rates of MRSA among *S. aureus* BSIs in Europe in 2015. Data obtained from the ECDC interactive database (http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial_resistance/database/Pages/database.aspx)

only Romania reports an MRSA rate of >50% while Italy, Greece, Portugal and Austria all report rates of 25-50%. Scandinavian countries including Finland, Sweden and Norway along with Iceland report a prevalence rate of between 1-5%, with only the Netherlands reporting a rate of <1%. The remaining countries report between 5-25% of isolates as MRSA (Fig. 1.6) including Ireland with a rate of 18.4% (HPSC, 2016).

1.6.2 The Irish National MRSA Reference Laboratory

The Irish National MRSA Reference Laboratory (NMRSARL) was opened in January, 2002 in St. James's Hospital, Dublin, with the primary role of the laboratory being to assist clinical microbiology laboratories in the correct detection and identification of MRSA strains in Ireland. Isolates are submitted directly from patients attending community medical practitioners and hospitals from throughout Ireland. Additionally the laboratory provides laboratory support to the HPSC for the MRSA component of EARS-Net in Ireland. All MRSA isolates recovered from BSI in Ireland are submitted to the laboratory in order to monitor the current circulating strains of MRSA for the development of resistance against commonly used antimicrobial agents.

Isolates submitted to the laboratory are investigated for resistance against a range of antimicrobial agents including vancomycin, teicoplanin, fusidic acid and mupirocin along with newer antimicrobial agents including tigecycline, quinupristin-dalfopristin and ceftaroline (NMRSARL, 2014).

1.6.3 Epidemiological typing of MRSA in Ireland

Since the establishment of the NMRSARL, the laboratory has carried out epidemiological typing of MRSA recovered throughout the country using AR typing, PFGE and *spa* typing along with PCR for the detection of resistance and virulence genes

such as *mecA, mecC, nuc* and *pvl.* However, detailed genotyping using MLST and SCC*mec* typing is not routinely performed in the NMRSARL.

Like elsewhere in Europe and worldwide, the epidemiology of MRSA in Ireland has changed over time. Healthcare-associated MRSA was first recognised in Ireland in 1971 and since then, molecular typing has shown that different MRSA clones have predominated at different times including the ST250-MRSA-I/I-*pls* clone in the 1980s, the ST239-MRSA-III*Hg* in the early 1990s, ST5-MRSA-II, ST36-MRSA-II and ST8-MRSA-IIA-E in the late 1990s, and, since the early 2000s, the ST22-MRSA-IV (Rossney *et al.,* 2003; Shore *et al.,* 2005; Rossney *et al.,* 2006).

Currently, the ST22-MRSA-IV clone continues to predominate in Irish hospitals and accounts for 70-80% of all MRSA isolates recovered from BSIs annually and has been extensively studied (Fig. 1.7) (Rossney *et al.,* 2006; Mollaghan *et al.*, 2010; Shore *et al.*, 2010b; Grundmann *et al.*, 2010; McNicholas *et al.*, 2011; Creamer *et al.*, 2012; Grundmann *et al.*, 2014; Kinnevey *et al.*, 2016; Shore *et al.*, 2016). However, this strain is highly clonal leading to difficulties in differentiating strains and identifying transmission events. A combination of several different typing methods including PFGE, *spa* and *dru* typing has been shown to enhance differentiation of ST22-MRSA-IV isolates (Creamer *et al.,* 2012). However more recently, WGS has been used for the discrimination of ST22-MRSA-IV isolates in an endemic setting, identifying significantly more transmission events, including those between patients and patients and the hospital ward environment, than were identified using the more conventional typing methods (Creamer *et al.*, 2012; Kinnevey *et al.*, 2016). However, utilisation of multiple typing methods or WGS is costly and time consuming. In addition, interpretation of WGS can be difficult and a lack of standardised nomenclature prevents interlaboratory comparison (Bletz *et al.*, 2015).

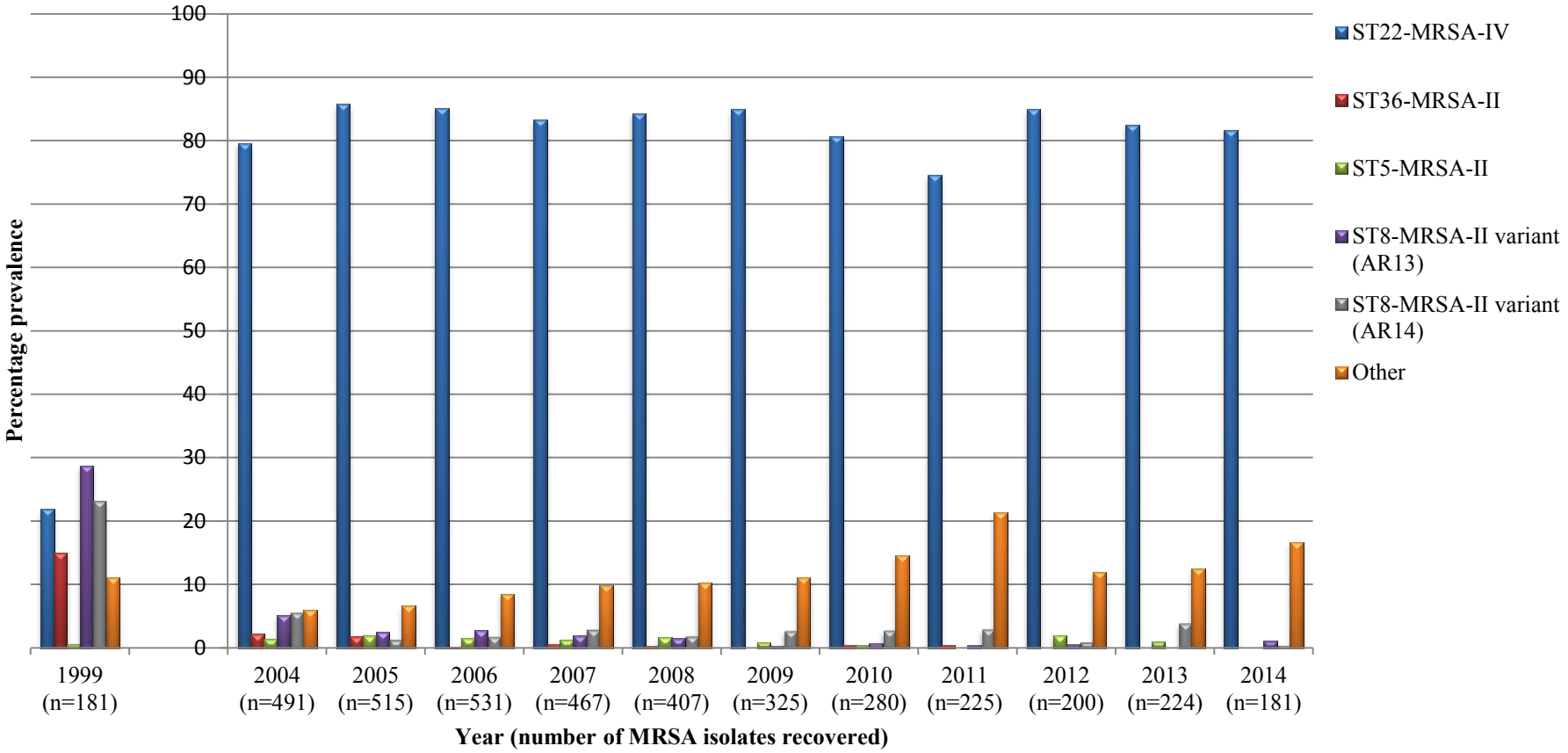


Figure 1.7 Epidemiological type distribution among MRSA isolates recovered from blood stream infections in Ireland (1999-2014). Isolates were assigned a ST-SCC*mec* type based on antibiogram-resistogram (AR) typing and *spa* typing. Where it was not possible to determine an ST-SCC*mec* type, isolates were assigned to an 'other' category. Adapted from NMRSARL 2015.

An increasing number of sporadically-occurring MRSA strains have also been recognised from BSIs in Irish hospitals. However, limitations in the current methodology (AR and *spa* typing) used in the NMRSARL has meant that detailed characterisation and differentiation of these strains is not possible and has led to these isolates to be collectively referred to as 'Other' (Fig. 1.7) (NMRSARL, 2014). Few studies have characterised sporadically- occurring MRSA strains in detail. Due to their potential to emerge and predominate as a cause of infections in the Irish healthcare setting further detailed molecular characterisation of these isolates is required so that appropriate infection prevention and control measures can be implemented to prevent further spread.

Throughout Europe and elsewhere, CA-MRSA has been increasing (Mediavilla *et al.,* 2012; Tong & Kearns, 2013), and while there has been no true prevalence study carried out on the rate of CA-MRSA in Ireland, there is evidence to suggest its increasing prevalence in Ireland based on studies of *pvl*-positive MRSA (NMRSARL 2014; Shore *et al.,* 2014). Over time the predominant clones of *pvl*-positive MRSA have also changed in Ireland where previously ST8-MRSA-IV and ST30-MRSA-IV were the predominant clones, ST772-MRSA-V, associated with the Indian subcontinent, emerged in 2010 and 2011. Additionally multiple importation incidences of different *pvl*-positive MRSA clones highlights the need for ongoing surveillance and detailed characterisation of these CA-MRSA (Shore *et al.,* 2014).

Despite the prevalence of CC398 MRSA in continental Europe and the sporadic reports of CC398 MRSA in the United Kingdom (including Northern Ireland) (Loeffler & Lloyd, 2010; Hartley, *et al.,* 2014; Hadjirin *et al.,* 2015), to date, LA-MRSA in Ireland has not been widely reported (Brennan *et al*., 2012; Burns *et al.*, 2014). Also despite the LA-MRSA connection, *mecC*-MRSA has only been recovered from two humans in Ireland (Shore *et al.,* 2011) with no known cases recovered from animals despite the increasing

reports of *mecC*-MRSA throughout Europe (Paterson *et al.,* 2012; Paterson *et al.,* 2014a; Espinosa-Gongora *et al.,* 2015). Ongoing surveillance and molecular characterisation of MRSA isolates is warranted to monitor this.

1.6.4 Surveillance of antimicrobial resistance in Ireland

The antimicrobial rates reported by the NMRSARL relate only to MRSA BSI isolates due to the comprehensive collection of these isolates received in the laboratory under the EARS-Net project. In contrast while isolates recovered from sites other than blood are investigated in the NMRSARL, due to the random selection of these isolates submitted to the reference laboratory from clinical laboratories no such prevalence data exists (NMRSARL, 2014). Currently ST22-MRSA-IV is the predominant strain circulating in Irish hospitals with a non-multiantibiotic resistance profile exhibiting resistance only to erythromycin, lincomycin and cadmium acetate, although increasing resistance has been noted against fusidic acid and mupirocin (NMRSARL, 2014). In contrast, non-ST22-MRSA-IV strains recovered from BSIs and other sites, often which are associated with CA-MRSA strains, have also been reported to exhibit greater resistance and harbour additional virulence genes (Shore *et al.,* 2014).

1.7 Aims of this study

1. It has previously been shown that displacement of the predominant MRSA clones circulating in Irish hospitals has occurred on several occasions since the early 1970s (Shore *et al.*, 2005). The current predominant strain ST22-MRSA-IV has been circulating since the early 2000s, however in recent years an increase in sporadically occurring non-ST22-MRSA-IV strains has been observed among isolates recovered from BSIs suggesting that possible strain displacement may occur again in the future. While extensive studies have characterised the ST22-MRSA-IV strains in Ireland, details of the genotypes and resistance and virulence genes harboured by sporadically occurring non-ST22-MRSA-IV strains is lacking along with information about MRSA recovered from non-BSIs, including those potentially from community and livestock sources. **The first aim of this study was to characterise sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and attending General Practitioners (GPs) in the community between 2001-2014 using *spa* typing and DNA microarray analysis to investigate their genotypes, resistance, virulence and SCC*mec* associated genes.**
2. Fusidic acid is used for the treatment of a wide range of skin and soft tissue infections along with systemic infections. Despite a decrease in the usage of the antibiotic, an increase in resistance has been reported in the UK and New Zealand. In particular resistance has been associated with the *fusC* gene, which has been reported to be carried on a variety of different SCC elements including SCC*mec* composite islands and chimeric elements in a range of different genetic backgrounds. **Following on from the in-depth molecular analysis of sporadically-occurring MRSA isolates recovered in Ireland described above, the second aim of this study was to investigate the phenotypic and genotypic characteristics of the *fusC*-positive**

MRSA isolates identified and to determine the genetic organisation of potential novel SCC*mec* elements harbouring *fusC*.

3. Detailed and accurate typing methods are essential for tracking the spread of MRSA, however traditional epidemiological typing techniques look at unspecified differences in the chromosome (PFGE) or a limited number of genes (MLST, *spa* typing) and therefore lack the discriminatory power required in outbreak situations. In addition, the highly-clonal ST22-MRSA-IV clone predominates and is endemic in Irish hospitals, making strain differentiation using conventional typing methods, very difficult. DNA microarray profiling examines 334 *S. aureus* genes and alleles including species-specific, antimicrobial resistance and virulence associated genes potentially improving discrimination of MRSA isolates in outbreaks. **The third aim of this study was to evaluate the usefulness of DNA microarray profiling for differentiating MRSA isolates in outbreak situations involving (i) the highly clonal and endemic HCA-MRSA clone ST22-MRSA-IV, and (ii) possible CA-MRSA strains causing outbreaks in the nosocomial environment.**

4. Advances in WGS sequencing have led to its utilisation in nosocomial outbreak investigations however data interpretation and lack of standardised nomenclature has limited its widespread use. Ridom SeqSphere^{+} software is an automated system for the analysis of WGS data utilising cgMLST nomenclature resulting in higher discrimination and more accurate strain typing. While studies evaluating the suitability of this software in outbreak situations have been carried out, there is no such study comparing the ability of cgMLST to identify cases of patient-to-patient transmission in an endemic setting over a defined period of time with that as determined using SNV analysis. **Therefore the fourth aim of this study was to investigate a collection of isolates recovered from a patient in a single ward and their environment over a six-week period using cgMLST to identify transmission events and to compare the**

results to those obtained using previously published SNV analysis of the same WGS data.

5. Active surveillance for MRSA and rapid detection of MRSA colonised patients has the potential to limit the spread of MRSA in hospital settings. However, changes in the epidemiology of MRSA have led to challenges for diagnostic laboratories for the detection of these strains. Traditional laboratory techniques for the detection of MRSA include chromogenic media, however there are few comparative studies evaluating each of the media types using different genotypes, borderline oxacillin resistant *S. aureus* (BORSA) isolates and *mecC*-MRSA isolates. **Therefore, the final aim of this project was to evaluate commercial chromogenic media to determine its appropriateness for the detection of MRSA including emerging strains of MRSA harbouring *mecC* and strains exhibiting varying levels of oxacillin resistance.**

Chapter 2

Materials and Methods

2.1 Bacterial isolates

2.1.1 Staphylococcus aureus isolates

A total of 562 MRSA, 45 MSSA and 20 BORSA were investigated in the present study. A summary of the isolates used for the different investigations in this study are described in Table 2.1, with further details provided in the individual chapters describing the use of the isolates. All isolates were submitted to the Irish NMRSARL between 2001 and 2015 from various sources including healthcare facilities, the environment within healthcare facilities, GPs and a veterinary laboratory.

2.2 General microbiological methods

2.2.1 Bacterial culture media, isolate storage and growth conditions

All *S. aureus* isolates were stored at -70°C on cryoprotective beads (Protect Beads, Technical Service Consultants Ltd., UK,) at the NMRSARL. Unless otherwise stated, all isolates were cultured by removing a single bead from the storage vial using a sterile inoculating wire and inoculated on to Columbia blood agar (CBA) (E & O Laboratories, Bonnybridge, Scotland) containing 7 % (v/v) defibrinated horse blood followed by overnight incubation for 18 hours (h) at 35°C in a static incubator (Memmert, Schwabach, Germany).

2.2.2 Chemical, enzymes, buffers and oligonucleotides

All chemicals used, unless otherwise indicated, were of analytical or molecular biology grade and were purchased from the Sigma-Aldrich Chemical Co. (Arklow, Wicklow, Ireland). Custom synthesised oligonucleotides were purchased from Sigma-Aldrich at a concentration of 100 µM and diluted to a concentration of 10 µM using molecular biology grade water (Sigma-Aldrich) and were stored at -20°C.

Table 2.1 *Staphylococcus aureus* isolates investigated in the present study

Number of isolates & phenotype	Source	Year(s) isolates recovered	Purpose of study	Chapter
276 MRSA	Patients in Irish hospitals and their environments and patients in the community	2001-2015	Characterisation of sporadically-occurring MRSA in Ireland	3
11 MRSA	Pigs (*n* = 9) and horses (*n* = 2) from Irish farms	2012-2013	Characterisation of CC398 MRSA isolates emerging in Ireland	3
3 MSSA	Patients in Irish hospital	2014-2014	Comparative molecular analysis of CC398 MRSA and MSSA isolates recovered from humans in Ireland	3
8 MSSA	Pigs from Irish farms	2010	Comparative molecular analysis of CC398 MRSA and MSSA isolates recovered from animals in Ireland	3
38 MRSA	Patients in Irish hospitals, their environment and patients in the community	2010-2014	Molecular characterisation of *pvl*-positive ST772-MRSA-V in Irish hospitals	3
53 MRSA	Patients in Irish hospitals	2001-2014	Phenotypic and genotypic characterisation of isolates harbouring *fusC*	4
5 MRSA	Patients in Irish hospitals	2008-2011	Determination of the genetic organisation of novel SCC*mec* elements harbouring *fusC*	4
25 MRSA	Patients in Irish hospitals involved in five outbreaks in three separate hospitals	2010, 2014	Evaluation of DNA microarray profiling for differentiating ST22-MRSA-IV isolates in outbreak situations	5

Continued overleaf

Table 2.1 continued

Number of isolates & phenotype	Source	Year(s) isolates recovered	Purpose of study	Chapter
18 MRSA	Patients in a neonatal ICU during an outbreak	2011-2012	Evaluation of DNA microarray profiling for differentiating non ST22-MRSA-IV isolates (CC5-MRSA-IV) during outbreak situations	5
51 MRSA	Patients in a general ward in a large teaching hospital and their environment during an outbreak	2013-2015	Evaluation of DNA microarray profiling for differentiating non ST22-MRSA-IV isolates (CC1-MRSA-IV) during outbreak situations	5
41 MRSA	Patients and their environments in a single ward in one hospital over a six-week period	2007	Evaluation of automated cgMLST scheme for the interpretation of WGS data for routine analysis of ST22-MRSA-IV isolates and for the identification of transmission events	5
34 MSSA, 171 MRSA and 20 BORSA	Patients in Irish hospitals	1971-2014	Evaluation of chromogenic media for the detection of MRSA in Ireland	6

Abbreviations: MRSA, methicillin resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus;* BORSA, borderline-oxacillin resistant *S. aureus*; CC, clonal complex; SCC*mec*; staphylococcal cassette chromosome *mec*; MLST, multilocus sequence typing; WGS, whole-genome sequencing; ICU, intensive care unit.

Enzymes, DNA molecular weight markers (100 bp and 1 kilobase (kb) and 6 X blue orange DNA loading dye were purchased from the Promega Corporation (Madison, Wisconsin, USA). The DNA loading dye was used at a working concentration of 1 X and was used to track migration of DNA samples in agarose gels during electrophoresis.

2.2.3 Water, buffers and solutions

An Elga Option 7 water purification system (Veolia Water, Celbridge, Ireland) was used to provide ultra- pure water for the preparation of buffers and agarose gels. Molecular biology grade water was purchased from Sigma-Aldrich and was used in all PCR reactions, DNA elutions and dilutions.

Tris (hydroxymethyl methylamine)/ethylenediaminetetraacetic acid (EDTA) buffer (TE) was prepared using 2.5 milliliter (ml) Tris-HCL (10 mM, pH 7.6), purchased from VWR International (Dublin, Ireland) and 0.5 ml EDTA (1 mM, pH 7.5), purchased from Sigma-Aldrich. Both reagents were added to 247 ml ultra-pure water to yield a final volume of 250 ml which was then aliquoted into 3 ml volumes in sterile bijoux and autoclaved prior to use.

Tris-borate/EDTA (TBE) buffer (Sigma-Aldrich) was used at a 0.5 X concentration as an electrophoresis buffer for agarose gel electrophoresis and for preparing agarose gels. This was prepared at a 10 X concentration and consisted of 0.45 M Trizma base, 0.45 M boric acid and 0.01 M EDTA (pH 8), which was then diluted in ultrapure water to a 0.5 X concentration.

2.2.4 Disposable laboratory consumables

Individually wrapped sterile disposable plastic pipettes (Greiner Bio One International GmbH, Kremsmünster, Austria) were used to measure and transfer liquid volumes between 1 and 25 ml. For volumes of between 1 µl and 1 ml, sterile plastic pipette

tips (Greiner Bio One International GmbH) were used with variable volume Gilson pipettes (Gilson Inc., Middleton, WI, USA). Sterile 1.5 ml polypropylene microfuge tubes (Eppendorf U.K. Ltd., Stevenage, U.K.) were used to hold volumes below 1.5 ml.

Filter pipette tips (Molecular BioProducts Inc., California, USA) and sterile 0.2 ml PCR tubes (Molecular BioProducts Inc.) were used for PCR experiments.

2.3 Initial phenotypic and genotypic analysis of isolates

The initial phenotypic and molecular investigation of all isolates was performed as part of the routine investigation of isolates at the NMRSARL, is outlined in Fig. 2.1 and is described below in more detail. Several American Type Culture Collection (ATCC) strains were used as reference strains to ensure the quality of individual tests and as positive or negative control strains (Table 2.2).

2.3.1 Identification of isolates as S. aureus

Identification of isolates as *S. aureus* was confirmed using the tube coagulase test to detect the presence of staphylocoagulase where a single colony from overnight growth on CBA was inoculated into 3 ml a nutrient broth (Oxoid, Basingstoke, UK) and incubated at 35°C for a minimum period of two hours (Rossney *et al.*, 1990). A 1 ml vial of normal human control plasma (Technoclone, Surrey, UK) was reconstituted using 1 ml of sterile water and to it two drops of heparin (CP Pharmaceuticals, Flintshire, UK) were added. Nine drops of the inoculated nutrient broth culture were added to one drop of the plasma-heparin preparation in a sterile tube which was incubated overnight (18 h) at 35°C. Following incubation the tubes were examined for the presence of a clot. *S. aureus* ATCC 25923 was used as a positive control and *S. epidermidis* ATCC 12228 was used as a negative control.

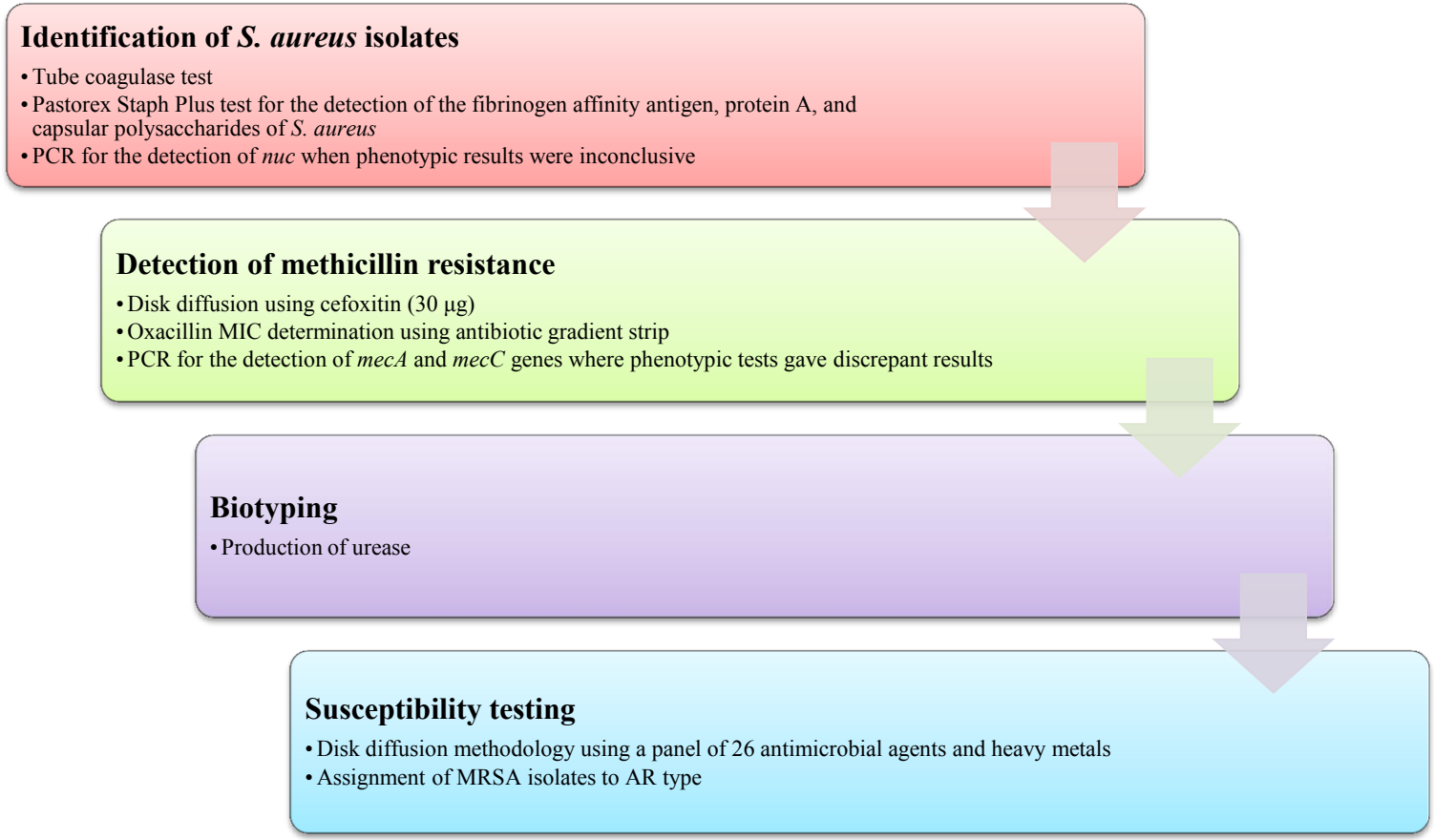


Figure 2.1 Flowchart showing the initial phenotypic and molecular tests used in the investigation of methicillin resistant *S. aureus* (MRSA) isolates at the National MRSA Reference Laboratory.

Abbreviations: MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; AR type, antibiogram-resistogram type.

Table 2.2 American Type Culture Collection reference strains used in the phenotypic investigation of isolates in the present study

Investigation	Reference Strain	Reference
Coagulase test	*S. aureus* ATCC 252923 (P)	N/A
	S. epidermidis ATCC 12228 (N)	N/A
Urease hydrolysis	*S. aureus* ATCC 10442 (P)	N/A
	Enterococcus faecalis ATCC 29212 (N)	N/A
Oxacillin MIC determination	*S. aureus* ATCC 29213 (methicillin susceptible)	N/A
	S. aureus ATCC 43300 (methicillin resistant)	N/A
Susceptibility testing[a]	*S. aureus* ATCC 252923 (methicillin susceptible)	CLSI, 2009 Rossney *et al.,* 2007
	S. aureus ATCC 29213 (methicillin susceptible)	EUCAST, 2013

[a]Susceptibility testing was carried out against a panel of 25 antimicrobial agents including amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, linezolid, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin, trimethoprim, vancomycin, Abbreviations: ATCC, American Type Culture Collection; (P), positive; (N), negative; N/A, not applicable; MIC, minimum inhibitory concentration.

Isolates were also investigated using the Pastorex™ Staph-Plus *S. aureus* identification kit (Bio-Rad, Steenvorde, France) which detects the fibrinogen affinity antigen, protein A, and capsular polysaccharides of *S. aureus* in accordance with the manufacturer's instructions.

Both methods were used for the confirmation of identification because the tube coagulase test alone may have yielded incorrect results due to the coagulase production by non-*S. aureus* staphylococci such as *S. intermedius* or production by non-staphylococcal species including *Pseudomonas aeruginosa* and *Serratia marcesens*. Furthermore, although each tube coagulase test was incubated for 24 h, isolates producing fibrinolysin may be positive after only 6 h incubation but negative after 24 h (Rossney *et al.*, 1990).

If an isolate was neither clumping factor nor staphylocoagulase positive, identification was based on the detection of the thermonuclease gene *nuc* using real time PCR as described in section 2.3.6 below.

2.3.2 Detection of methicillin resistance

Standardised methodology, in accordance with guidelines from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was used to confirm methicillin susceptibility. Briefly single colonies, grown overnight on CBA were suspended in saline to a turbidity equivalent to a 0.5 McFarland standard (1.5×10^8 colony forming units (CFU)/ml) measured using a CrystalSpec Nephelometer (Becton Dickinson, Dun Laoghaire, Ireland) and were lawned on Mueller Hinton (MH) agar (E & O Laboratories). Once dried, a cefoxitin 30 μg disk (Oxoid) was placed on the plate which was then incubated overnight at 35°C. The resulting zones of inhibition were measured using a ProtoZone Zone Reader (Synbiosis, Cambridge, U.K.) and interpreted as

susceptible or resistant using the interpretive criteria where a zone size of ≤ 21 mm was considered resistant (EUCAST, 2013).

The oxacillin MIC of each isolate was determined using an antibiotic gradient strip (E-test, Biomérieux, Marcy l'Etoile France) where a bacterial suspension, prepared as described above, was inoculated on to MH agar containing 5% (w/v) NaCl which was then incubated at room temperature for 10 minutes (min) in order to dry prior to the application of the gradient strip. Following incubation of 24 h at 35°C the MIC was determined to be the point above at which there was no visible growth.

Additionally, when isolates yielded discrepant phenotypic results, real-time PCR was used to investigate isolates for the presence of the *mecA* or *mecC* genes as described in section 2.3.36 below.

2.3.3 Biotyping

Isolates were investigated for the hydrolysis of urease using Christensen's urea media containing a urea base (Oxoid) with 2% (w/v) urea (Merck, Darmstadt, Germany). Urease production was used in assigning AR types to isolates (see section 2.3.5).

2.3.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates involved disk diffusion methodology as recommended by EUCAST (EUCAST, 2013) using an extensive panel of antimicrobial agents and heavy metals (Table 2.3). Briefly, a bacterial inoculum at a density equivalent to 0.5 McFarland turbidity standard was prepared overnight growth on CBA and the suspension was inoculated on to the MH agar plate using a rotary plater (Mast Group Ltd., Liverpool, U.K.). Antimicrobial discs were positioned as shown in Fig. 2.2 and following overnight incubation at 35°C, zone sizes were measured using a ProtoZone Zone Reader and determined as resistant, intermediate or susceptible. Isolates investigated prior to 2012

Table 2.3 Antimicrobial agents and interpretive criteria used for antimicrobial susceptibility testing

Antimicrobial agent	Disk concentration (mg/L)	Zone Size (mm)			Reference
		Resistant (≤)	Intermediate	Susceptible (≥)	
Amikacin	30	14	15-19	20	EUCAST, 2013[1]
Ampicillin	10	28	N/A	29	Rossney *et al.,* 2007
Cadmium acetate	130	10	11-15	16	Rossney *et al.,* 2007
Cefoxitin	30	21	N/A	22	EUCAST, 2013[1]
Chloramphenicol	30	12	13-17	18	EUCAST, 2013[1]
Ciprofloxacin	5	15	16-20	21	EUCAST, 2013[1]
Erythromycin	15	13	14-22	23	EUCAST, 2013[1]
Ethidium bromide	60	13	N/A	15	Rossney *et al.,* 2007
Fusidic acid	10	23	24-26	27	EUCAST, 2013[1]
Gentamicin	10	12	13-14	15	EUCAST, 2013[1]
Kanamycin	30	13	14-17	18	CLSI, 2013[1]
Lincomycin	2	14	15-16	17	Rossney *et al.,* 2007
Linezolid	10	18	N/A	19	EUCAST, 2013[1]
Mercuric chloride	10	13	N/A	15	Rossney *et al.,* 2007
Mupirocin	200	15	16-29	30	EUCAST, 2013[1]
Mupirocin	5	12	13-19	20	Rossney *et al.,* 2007
Neomycin	30	15	16-17	18	Rossney *et al.,* 2007
Phenyl mercuric acetate	10	24	25-28	29	Rossney *et al.,* 2007
Rifampicin	5	16	17-19	20	EUCAST, 2013[1]
Spectinomycin	500	13	14-19	20	Rossney *et al.,* 2007
Streptomycin	25	13	14-15	16	Rossney *et al.,* 2007
Sulphonamide	300	12	13-16	17	CLSI, 2013[1]
Tetracycline	30	14	15-18	19	EUCAST, 2013[1]
Tobramycin	10	17	N/A	19	EUCAST, 2013[1]

Continued overleaf

Table 2.3 continued

Antimicrobial agent	Disk concentration (mg/L)	Zone Size (mm)			Reference
		Resistant (≤)	Intermediate	Susceptible (≥)	
Trimethoprim	5	10	11-15	16	EUCAST, 2013[1]
Vancomycin	30	14	N/A	15	Rossney *et al.,* 2007

[1]Prior to 2013 resulting zone sizes were interpreted in accordance with Rossney *et al.,* 2007. Since 2013 zone sizes have been interpreted in accordance with EUCAST. Abbreviations: mg/L; milligrams per litre, mm; millimetres, N/A; not applicable.

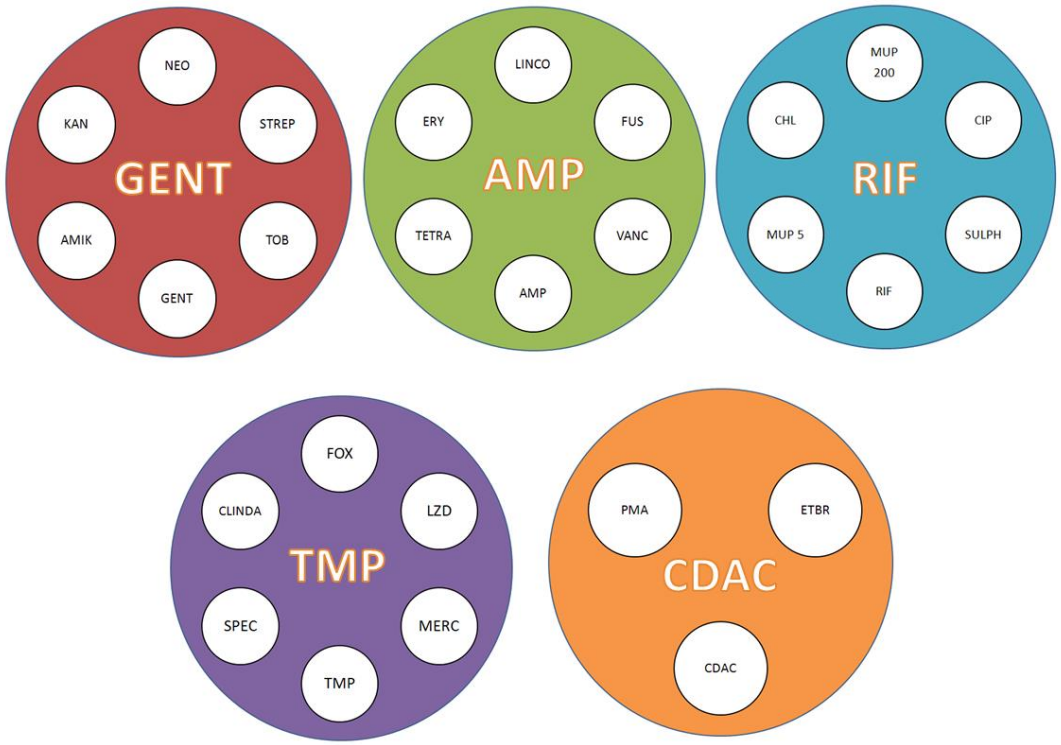


Figure 2.2 Schematic representation of the positioning of antimicrobial and heavy metal discs on Mueller Hinton agar plates used for susceptibility testing. Abbreviations: GENT, gentamicin; AMIK, amikacin; KAN, kanamycin; NEO, neomycin; STREP, streptomycin; TOB, tobramycin; AMP, ampicillin; TETRA, tetracycline; ERY, erythromycin; LINCO, lincomycin; FUS, fusidic acid; VANC, vancomycin; RIF, rifampicin; MUP5, mupirocin 5 µg/L; CHL, chloramphenicol; MUP200, mupirocin 200 µg/L; CIP, ciprofloxacin; SULPH, sulphonamides; TMP, trimethoprim; SPEC, spectinomycin; CLINDA, clindamycin; FOX, cefoxitin; LZD, linezolid; MERC, mercuric chloride; CDAC, cadmium acetate; PMA, phenyl mercuric acetate; ETBR, ethidium bromide.

were interpreted in accordance with Clinical Laboratory Standards Institute (CLSI) interpretative criteria (CLSI, 2013) while those recovered after 2012 were interpreted using EUCAST criteria (Table 2.3). Where there were no EUCAST or CLSI interpretive criteria available, criteria was developed in-house in the NMRSARL (Rossney *et al*., 2007; McManus *et al*., 2015) (Table 2.3).

2.3.5 Antibiogram-resistogram typing

Each MRSA isolate was assigned an AR type based on its susceptibility profile and the ability of the isolate to hydrolyse urease. The AR types were assigned chronologically and to date 46 AR type numbers have been assigned at the NMRSARL i.e. AR01- AR46 (Rossney *et al.*, 1994a, Rossney *et al.,* 1994b). Subtypes of AR types were identified based on slight variations to an existing pattern, including, for example, the loss of a plasmid carrying a resistance gene which resulted in the loss of phenotypic resistance to the corresponding antimicrobial agent. These subtypes were assigned by the addition of a third numeral to the AR type (e.g. AR06.1). The most frequently occurring AR types and subtypes recognised among MRSA recovered in Ireland are shown in Table 2.4.

Where an antimicrobial susceptibility profile of an isolate was unlike previously recognised AR types, such isolates were described as unfamiliar (Unf). Isolates described as 'NT' (no type) exhibit an AR pattern similar to previously recognised AR types but the isolate's ability to produce urease indicated that the isolates does not belong to this AR type. One such example includes AR06 where AR06 and variants of AR06, which are urease negative, are associated with approximately 80% of MRSA investigated as part of the EARS-Net project each year but when these isolates display an ability to hydrolyse urease they are reported as NT.

Table 2.4 Resistance patterns of the most commonly occurring antibiogram-resistogram (AR) types recognised among MRSA isolates recovered from patients in Irish hospitals between 2010 and 2013

AR type	Resistance Pattern
AR06.1	Ap
AR06.1c	ApCp
AR06.1cf	ApCdFd
AR06.3c	ApCdCp
AR06.3cf	ApCdCpFd
AR06.5c	ApCdCpEr
AR06.5cf	ApCdCpFd
AR06.6	ApEr
AR06.6c	ApCpEr
AR06.6cf	ApCpErFd
AR07.0	Ak*ApCdCpErKnLnNmSpTb
AR13.1mf	Ak*ApCdCpEbErFdGnKnLnMcMpNmPmaSmSpTb
AR14.4m	Ak*ApCdCpEbErGnKnMcMpNmPmaSmSpTbTp

Resistance profiles for commonly occurring AR types based on isolates investigated by the NMRSARL as part of the EARS-Net project between 2010 and 2013. AR were types determined using a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadmium acetate (Cd), chloramphenicol, ciprofloxacin (Cp), erythromycin (Er), ethidium bromide (Eb), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride (Mc), mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate (Pma), rifampicin, spectinomycin (Sp), streptomycin (Sm), sulphonamide, tetracycline, tobramycin (Tb), trimethoprim (Tp), vancomycin, Suffixes (lower case) after AR types indicate resistance to the following antimicrobials: c; ciprofloxacin, f; fusidic acid, m; mupirocin, *; may be moderate resistant or susceptible.

2.3.6 Genotypic analysis

For the detection of *nuc*, *pvl*, *mecA* and *mecC* genes using real-time PCR, DNA was extracted from isolates by suspending three to four colonies from an overnight growth on CBA at 35°C in 1 ml of TE and boiling it at 100°C for 15 min in a dry block heater (Grant Instruments Ltd., Cambridge, U.K.). Following this, the suspension was cooled slightly and then centrifuged at 12,000 x g for 5 min. The supernatant was removed and stored on ice until required.

Detection of *mecC* was carried out in a singleplex reaction while the detection of *mecA, nuc* and *pvl* was performed in a triplex reaction. For the triplex reaction, a reaction mix was prepared for each isolate containing 12.5 µl 2 X Quantifast Multiplex kit (Qiagen) together with 0.5 µl of each forward and reverse primer and probe (Life Technologies, California, USA) (Table 2.5) and yielding a final concentration of 0.5 µM and 0.2 µM of each primer and probe respectively. Each probe was labeled with a fluorescent dye to detect the accumulation of PCR products during the exponential phase of the reaction. The final volume was increased to 20 µl using 3 µl of molecular grade water. For a triplex reaction, the reaction mix was similar to that for the triplex reaction except that an additional 1.5 µl of molecular grade water was added to increase the final volume to 20 µl. For each isolate, a reaction mix was dispensed into a 96-well microtitre tray (Life Technologies) and 5 µl of DNA was added before the tray was sealed tightly with a disposable plastic cover (Life Technologies).

Real-time PCR was performed on the Applied Biosystems AB7500 Real Time PCR platform (Life Technologies) using the standard PCR mode. The cycle parameters included an initial denaturation stage for two min at 50°C followed by 40 cycles for 15 seconds (s) (denaturation), one min annealing and an extension stage at 60°C. The Applied Systems software was used to determine the threshold cycle (Ct) of each value.

Table 2.5 Primers used for real-time PCR detection of antimicrobial resistance and virulence genes[1]

Target gene(s)	Protein encoded	Primer name	Sequence 5'-3'	Expected amplimersize (bp)
lukF/S-PV	Panton Valentine leukocidin	pvl-F	GCTGGACAAAACTTCTTGGAATATC	105
		pvl-R	TTTTGCAGCGTTTTGTTTTCG	
		pvl^{NED}	CAGAATTTATTGGTGTCCTATC	
nuc	Thermonuclease	nuc-F	CAAAGCATCCTAAAAAAGGTGTAGAGA	86
		nuc-R	TTCAATTTTCTTTGCATTTTCTACCA	
		nuc^{FAM}	TGGTCCTGAAGCAAGTG	
mecA	PBP2a	mecA-F	TGCTAAAGTTCAAAAGAGTATTTATAACAACA	103
		mecA-R	TGTGCTTACAAGTGCTAATAATTCACC	
		mecA^{VIC}	CTCAGGTACTGCTATCC	
mecC		mecC-F	TTGGCTCCTAATGCTAATGCAA	64
		mecC-R	AGCAAGCAATAGAATCATCAGACAA	
		mecC^{NED}	CGGGCAAAAAATAT	

[1]Unpublished primers used for routine real-time PCR assays by the National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland.

Abbreviations: bp; base pair

2.4 In depth molecular analysis of isolates

2.4.1 Genomic DNA extraction from isolates

2.4.1.1 Matrix extraction method

For *spa* typing (section 2.4.3) genomic DNA was extracted from isolates using a 6% InstaGene matrix solution (BioRad, München, Germany) where 2-3 colonies of *S. aureus* grown overnight at 35°C on CBA were washed with 500 µl of sterile water and then centrifuged at 12,000 x g for 1 min. Once the supernatant was removed, the pellet was resuspended in 200 µl of matrix solution and incubated at 56°C for 20 min. Following this, the suspension was vortexed and heated for a further 8 min at 100°C followed by centrifugation at 12,000 x g for 3 min. The supernatant was removed and used immediately or stored at -20°C until required.

2.4.1.2 DNeasy blood and tissue kit extraction method

Genomic DNA for use with the DNA microarray (section 2.4.3) and for the detection of resistance genes using end-point PCR (section 2.4.2) was extracted using the lysis buffers and solutions provided with the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany) and the Qiagen (Crawley, West Sussex, U.K.) DNeasy blood and tissue kit. Following overnight incubation at 35°C on CBA, a single colony was subcultured on to CBA and incubated for a further 24 h at 35°C. All cells grown on the plate were removed and added to 200 µl lysis buffer A1 and 1.7 µl lysis enhancer (buffer A2) (provided with the *S. aureus* Genotyping Kit 2.0). The suspension was vortexed and then incubated at 37°C in a shaking incubator at 250 rpm for 60 min. Following lysis, 25 µl of proteinase K was added along with 200 µl of AL buffer (both of which are supplied with the DNeasy kit) and the samples were incubated at 70°C for 30 min. The DNA was eluted from the samples by passing each one through a mini-column containing a silica gel

membrane that binds DNA and allows cellular debris to wash through. After washing with buffers provided with the kit, the DNA was eluted using 50 µl nuclease free water (Sigma-Aldrich) with centrifugation at 14,000 x g for 1 min. The resulting DNA was concentrated and solvents removed by an evaporation step at 70°C for 30 min in a dry block heater (Grant Instruments) with the caps of the samples removed. DNA samples were run on a 0.8 % (w/v) agarose gel to test the quality of the DNA and ensure the absence of RNA. The concentration of the DNA (ng/µl) was determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, USA).

2.4.2 End-point PCR

Genomic DNA for use with end-point PCR was extracted as described in section 2.4.1.2. For PCRs with expected amplimer sizes of less than 5 kb in size GoTaq DNA polymerase (Promega) was used, while for those with expected amplimer sizes of greater than 5 kb the Expand long template PCR system (Roche Diagnostics Ltd.) was used. All PCRs were carried out in accordance with manufacturer's instructions using a Thermal Cycler (ThermoFisher Scientific Inc., Massachusetts, USA). Primers used in each section of the current study are described in the relevant chapters. All PCR products were visualised by 0.8 % (w/v) agarose gel electrophoresis and were purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich) or the PCR Purification Kit (Qiagen) as instructed by the manufacturers.

2.4.3 spa typing

spa typing was performed in accordance with the protocol described by SeqNet, The European Network of Laboratories for Sequenced Based Typing of Microbial Pathogens, using the primers spa1113f (5'-TAA AGA CGA TCC TTC GGT GAG-3' and spa1514r (5'-CAG CAG TAG TGC CGT TTG CTT-3'). Genomic DNA was extracted using a 6% InstaGene matrix solution (see section 2.4.1.1). The thermal cycling conditions consisted

of an initial denaturation (5 min at 80°C) followed by 35 cycles of denaturation (45 s at 94°C), annealing (45 s at 60 C) and extension (90 s at 72°C) with a final extension stage (10 min at 72°C). Following purification of the PCR amplimers to remove unincorporated dNTPs and primers using a QIAquick PCR Purification Kit (Qiagen), sequencing was performed commercially (see section 2.4.5) and the sequences were analysed using the StaphType software package version 1.5 (Ridom Gmbh, München, Germany) and *spa* types were assigned using the SpaServer website (http://spaserver2.ridom.de) .

2.4.4 DNA microarray profiling

DNA microarray analysis was performed using the StaphyType DNA microarray (Alere Technologies Gmbh) in accordance with the manufacturer's instructions. Following genomic DNA extraction and concentration determination (see section 2.4.1.2), samples were diluted to a 5 µl volume with water to a final concentration of 0.5-1.5 µg of DNA. A linear primer elongation reaction was performed using a PCR mastermix and primers supplied with the microarray kit and a thermal cycler (Thermofisher). This allows for the simultaneous amplification of targets and labeling with biotin-16-dUTP and uses one specific primer for each of the 336 targets included on the microarray (Fig. 2.3). A PCR mastermix was prepared using 4.9 µl labelling buffer (B1) and 0.1µl enzyme (B2) supplied with the *S. aureus* Genotyping Kit 2.0. To this a volume of 5 µl of DNA was added for a linear PCR reaction which consisted of an initial denaturation at 96°C for 5 min followed by 45 cycles of 96°C for 20 s, 50°C for 20 s and 72°C for 30 s.

The array chips were washed using 200 µl of nuclease free water followed by 100 µl of the hybridisation buffer (buffer C1 supplied with the StaphyType kit) and incubation at 55°C for 2 min. Once the C1 buffer was removed from the chip, a hybridisation mixture was added containing 5 µl of biotin-16-dUTP labelled PCR product and 5µl of buffer C1 and the chip was incubated at 55° C for one hour with shaking at 550 rpm.

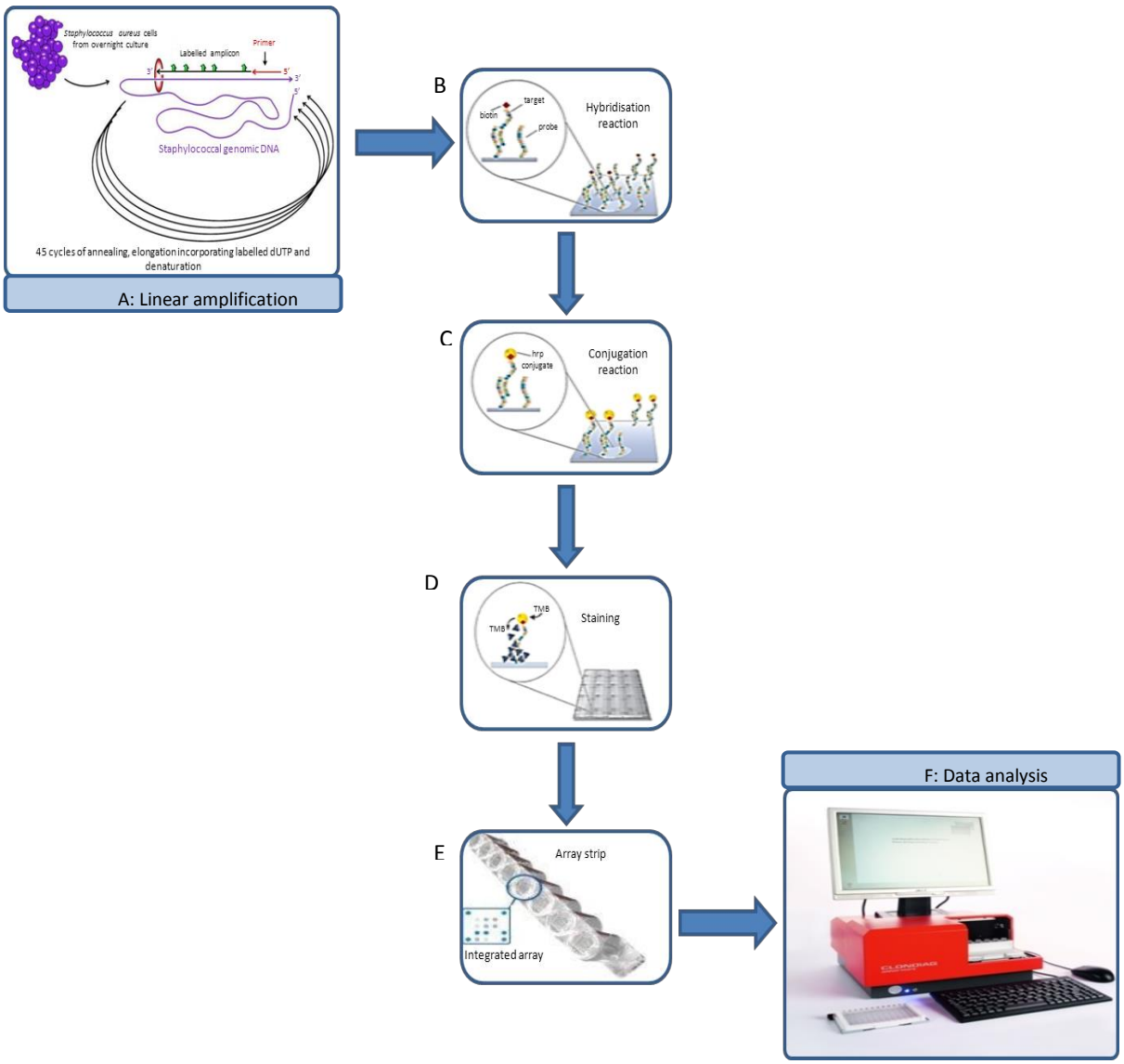


Figure 2.3 Schematic diagram illustrating the DNA microarray analysis process using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies) involving linear amplification of the genomic staphylococcal DNA using a single primer for all 336 gene targets and biotin labelling. The biotin labelled amplicons are hybridised to the array chips (B) followed by a conjugation reaction (C) with horseradish peroxidase conjugation (HRP-conjugate) with the final precipitation step with the tetramethylbenzidine (TMB) substrate (D). Each well on the 8-well array microarray strip contains a single DNA chip (E) and these images are analysed using the Arraymate reader, which records an image of each DNA microarray chip and the raw data is analysed and interpreted using IconoClust software (F).

Following removal of the hybridisation mixture, chips were washed three times using 200 µl of washing buffer (C2, supplied with DNA array kit) prior to labeling with a horseradish peroxidase (HRP) - conjugate of HRP-streptavidin (C3, *S. aureus* Genotyping Kit 2.0) with buffer C4 (conjugate buffer, *S. aureus* Genotyping Kit 2.0) in a ratio of 1:100. Chips were incubated at 30°C for 10 min with shaking at 550 rpm. A final washing step using 200 µl of washing buffer 2 (C5, *S. aureus* Genotyping Kit 2.0) was then carried out followed by the final staining of the HRP-conjugate using 100 µl of the HRP substrate supplied with the kit (Reagent D1, tetramethylbenzadine) at room temperature for 5 min. After removal of the D1 reagent chips were analysed using the ArrayMate reader and Iconoclust software (Alere Technologies GmbH) and signals were interpreted as positive, negative or ambiguous using a previously described algorithm which was based on the light transmitted through each of the individual spots on the microarray (Monecke *et. al.,* 2008). The specific genes targeted by the DNA microarray are listed in Table 2.6. Breakpoints were defined for each target based on the average values for control spots and species markers (*femA, gapA, katA, coa, spa, sak* and *sbi*). Where the value was less than 25% the target was assigned as negative, 25-33% yielded ambiguous results while >33% was assigned a positive result (Monecke *et al.,* 2008). The software also examined a database of previously investigated isolates and assigned each isolate an MLST ST and/or CC and for MRSA isolates only, to a SCC*mec* type.

2.4.5 Sequencing

Sequencing of PCR amplimers was performed commercially by Geneservice (Source Bioscience, Waterford, Ireland) using an ABI3730xl Sanger DNA analyser with an ABI BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems). Sequences were

Table 2.6 Genes targeted by the *S. aureus* Genotyping Kit 2.0 DNA microarray

Gene target	Description	Genomic Location
aacA-aphD	Gentamicin, kanaymicn and tobramycin resistance	Transposon
aadD	Amikacin, kanamycin, neomycin, and tobramycin resistance	Plasmid
aphA3	Kanamycin, neomycin resistance	Transposon
***arc*A/B/C/D**	Arginine/ ornithine genes	ACME cluster
***arg* I-IV**	Accessory gene regulatory genes alleles I-IV	Core genome
aur	Aureolysin secreted zinc-metalloproteinase	Core genome
bap	Surface protein involved in biofilm formation	Transposon
bbp	Bone sialoprotein-binding protein	Core genome
blaI	Beta-lactamase repressor	Plasmid
blaR	Beta-lactamase regulatory protein	Plasmid
blaZ	Beta-lactamase gene	Plasmid
blaZ-SCCmec XI	Beta-lactamase gene associated with SCC*mec* XI	Plasmid
cap1	Capsular polysaccharide, serotype 1	Genomic Island
cap5	Capsular polysaccharide, serotype 5	Genomic Island
cap8	Capsular polysaccharide, serotype 8	Genomic Island
cat	Chloramphenicol, florfenicol resistance	
***ccrA* 1-4**	Cassette chromosome recombinase A 1-4	SCC/ SCC*mec*
***ccrAA*-85-2082**	Cassette chromosome recombinase AA, marker for *ccr*C	SCC/ SCC*mec*
***ccrAA*-MRSA ZH47**	Cassette chromosome recombinase A alleles, specific for MRSAH47	SCC/ SCC*mec*
***ccrB* 1-4**	Cassette chromosome recombinase B 1-4	SCC/ SCC*mec*
cfr	Phenicols, lincosamides, oxazolidinones, streptogramin B	Plasmid
chp	Chemotaxis-inhibiting protein	*hlb* converting phage
clfA	Clumping factor A	Core genome
clfB	Clumping factor B	Core genome
cna	Collagen-binding adhesion	Genomic Island
coa	Staphylocoagulase	Core variable
delta_*mecR*	Truncated signal transducer protein MecR1	SCC*mec*
dfrS1	Trimethoprim resistance	Transposon
ebh	Cell wall associated fibronectin-binding protein	Core genome
ebpS	Cell surface elasting binding protein	Core genome
edinA/B/C	Epidermal Cell differentiation inhibitor precursor A-C	Pathogenicity Island
eno	Enolase phosphopuruvate hydratase laminin binding protein	Core genome
***erm*(A)**	Macrolide-lincosamide-streptogramin B resistance	Transposon
***erm*(B)**	Macrolide-lincosamide-streptogramin B	Plasmid

Continued overleaf

Table 2.6 continued

Gene target	Description	Genomic Location
	resistance	
***erm*(C)**	Macrolide-lincosamide-streptogramin B resistance	Plasmid
etA/B/D	Exfloiative toxin A/B/D	Pathogenicity Island
fexA	Chloramphenicol and florfenicol resistance	Transposon
fib	Fibrinogen binding protein	Core genome
fnbA	Fibronectin-binding protein A	Core variable
fnbB	Fibronectin binding protein B	Genomic Island
fosB	Fosfomycin resistance	Core variable
***fosB*-plasmid**	Fosfomycin-plamsid	Plasmid
fusB	Fusidic acid resistance	Plasmid
fusC	Fusidic acid resistance	SCC
gapA	Glyceraldehyde 3- phosphate dehydrogenase	Core genome
hI	Putative membrane protein	Core genome
hla	Haemolysin alpha toxin	Core genome
hlb	Haemolysin beta	Core genome
***hlb*-undisrupted**	Haemolysin beta without phage insertion	Core genome
hld	Haemolysin delta	Core genome
hlgA	Haemolysin gamma	Core genome
***hsdS*1 - 3**	Type 1 site-specific deoxyribnuclease subunit	Core genome
hysA1/2	Hyaluroniate lyase 1-2	Genomic Island
icaA	Intercellular adhesion protein A	Gene cluster
icaC	Intercellular adhesion protein C	Gene cluster
icaD	Intercellular adhesion protein D	Gene cluster
idsA	Transferrin-binding protein	Gene genome
ImrP	Putative transporter protein	Core genome
***lnu*(A)**	Lincosamide resistance protein	Plasmid
isaB	Immunodominant antigen B	Core variable
katA	Catalase A	Core genome
***kdp*A-E**	Potassium-translocating ATPase A-E	SCC*mec*
lukD, E, F, S, X, Y	Leukocidins	Genomic Island
lukF-PV*(P83), *lukM	F/S component of leukocidin from ruminants	
lukF-PV*, *lukS-PV	Panton-Valentine leukocidin	Prophage
map	Major histocompatibility complex class II analogue protein	Core variable
***mph*(C)**	Macrolide-phosphotransferase II	Core genome
mecA	Meticillin resistance	SCC*mec*
***mec*C**	B-lactam resistance novel *mecA* homologue	SCC*mec*
mecI	Meticillin-resistance regulatory protein	SCC*mec*
mecR1	Signal transducer protein MecR1	SCC*mec*

Continued overleaf

Table 2.6 continued

Gene target	Description	Genomic Location
mefA	Macrolide efflux protein A	Transposon
merA	Mercuric resistance Hg (II) reductase	SCC*mec*/pI258
merB	Mercuric resistance, alkylmercury lyase	SCC*mec*/pI258
mprF	Defensin resistance gene protein	Core variable
***msr* (A)**	Macrolide efflux pump-Erythromycin resistance	Core genome
mupA*/ *ileS2	Mupiriocin resistance	Plasmid
nuc	Thermostable nuclease	Core genome
Q2FXCO	Hypothetical protein	Core genome
Q2YUB3	Multidrug resistance protein	Core genome
Q7A4X2	Hypothetical protein	Core genome
Q9XB68-*dcs*	Hypothetical protein	SCC*mec*
qacA/C	Quaternary ammonium compounds, Ethidium Bromide	Plasmid
rrnD1	Domain 1 of 23S-RNA	Core variable
saeS	Histidine protein kinase	Core genome
sak	staphylokinase	*Hlb* converting phage
sarA	Staphylococcal accessory regulator A	Core genome
sasG	*S. aureus* surface protein G	Genomic Island
sat	Streptothricin acetyltransferase	
sbi	IgG binding protein	Prophage
scn	Staphylococcal complement inhibitor	*Hlb* converting phage
sdrC	Serine aspirate repeat protein C	Core genome
sdrD	Serine aspirate repeat protein D	Core genome
sdrM	Efflux pump	Core genome
sea	Enterotoxin A	*Hlb* converting phage
seb, sek, seq	Enterotoxin B, K and Q	Pathogenicity Island
sec, sel	Enterotoxin C and L	MBE gene cluster
sed, sej, ser	Enterotoxin D, J and R	Plasmid
see	Enterotoxin E	Pathogenicity Island
seg, sei, selm, seln, selo, selu,egc	Enterotoxin G, I, M, N, O, U= enterotoxin gene cluster	Pathogenicity Island
seh	Enterotoxin H	Transposon
setB	Staphylococcal exotoxin-like protein B	Core variable
setC	Staphylococcal exotoxin-like protein	Transposons
spa	Staphylococcal protein A	Core genome
splA	Serine protease A	Genomic Island
splB	Serine protease B	Genomic Island
splE	Serine proteaseE	Genomic Island
***ss*/01-11 alleles**	Staphylococcal superantigen-like proteins 1-11	Genomic Island
sspA	Extracellular serine protease	Core genome
sspB	Staphopain B protease	Core genome
sspP	Papin-like cysteine protease	Core genome
***tet*(K)**	Tetracycline efflux protein	Plasmid
***tet*(M)**	Tetracycline resistance ribosomal	Transposon

Continued overleaf

Table 2.6 continued

Gene target	Description	Genomic Location
	protection protein	
tst	Toxic shock syndrome toxin-1	Pathogenicity Island
ugpQ	Glycerophosphoryl diester phosphodiesterase	SCC*mec*
vanA/B/Z	Vancomycin/ teicoplain-A type resistance protein	Plasmid
***vat*(A)**	Virginiamycin A acetyltransferase	Plasmid
***vat*(B)**	Acetyltransferase inactivating streptogramin A	Plasmid
vgaA	Streptogramin A, and related compounds resistance	Plasmid
vgaB	Virginiamycin B hydrolase stretpogramin lyase	Plasmid
vraS	Sensor protein	Core genome
vwb	Van willebrand factor binding protein	Core genome
xylR	Xylose repressor associated with SCC*mec*	SCC*mec*

Linear amplification was performed on all genes targets with subsequent hybridisation to a DNA microarray *S. aureus* Genotyping Kit 2.0, (Alere Technologies GmbH) followed by analysis using a designated reader and IconoClust software (Alere Technologies GmbH). The location on of the gene targets are indicated and include the core genome, variable genome (i.e. mobile genetic elements including pathogenicity islands, SCC*mec* or SCC-like elements, plasmids, bacteriophages or transposons). Adapted from *S. aureus* Genotyping Kit 2.0 User Manual.

analysed using the StaphType software package version 1.5 (Ridom Gmbh) (http://spaserver2.ridom.de) or using BioNumerics software version 7.5 (Applied Maths, Ghent, Belgium) as stated in the relevant chapters.

Chapter 3

Molecular epidemiological typing of unusual and emerging MRSA strains in Ireland

3.1 Introduction

Molecular epidemiological typing of HCA-MRSA has shown that the majority of nosocomial MRSA isolates belong to a limited number of epidemic clones that emerged following the independent acquisition (at least 20 times) of SCC*mec* by successful MSSA clones belonging to five phylogenetically distinct lineages or CCs i.e. CC5, CC8, CC22, CC30 and CC45, which subsequently spread to different regions of the world (Monecke *et al.*, 2011). While the reasons for the success of some clones over others are largely unknown, different patient populations, antimicrobial prescribing practices and infection prevention and control procedures have all been suggested (Knight *et al.,* 2012). For example, the ability of the ST22-MRSA-IV clone to develop resistance either through the acquisition of resistance genes or chromosomal mutations, has been linked to the increasing predominance of this clone in the UK while a decrease in fluoroquinolone and cephalosporin usage in the UK have been coupled with a decline in the prevalence ST36-MRSA-II (Cook *et al.*, 2011; Knight *et al.*, 2012) . In contrast, changes in non-pathogen-specific infection prevention and control procedures and improved line care have not been shown to directly impact the rate of MRSA infections in hospitals. On the contrary, in many instances interventions including hand washing, education, hospital cleaning and behavioural changes have been associated with an increase in the rate of MSSA recovered over the same time (Knight *et al.*, 2012).

Many of these predominant MSSA lineages have acquired SCC*mec* on multiple occasions leading to different clones within each CC such as SCC*mec* IV within CC22 and SCC*mec* I-V in CC5. It has been suggested that the SCC*mec* type along with the loss or acquisition of other MGEs may be the reason for the emergence of epidemic MRSA strains of similar genetic background, independent of fitness cost (Hallin *et al.*, 2008).

In addition to the dominant lineages, other MRSA clones have also been reported but are often limited to specific geographical areas or have been recovered from only a few patients and as such are described as sporadic clones in other regions (Enright *et al.*, 2002; Aires De Sousa & De Lencastre, 2004; Monecke *et al.*, 2011). Similarities between these sporadic clones and CA-MRSA have been reported including genetic diversity, carriage of smaller SCC*mec* elements (SCC*mec* IV and V), susceptible antimicrobial profile and the presence of the *pvl* genes (Aires de Sousa & de Lencastre, 2003). Additionally, while these clones are categorised as sporadic in some areas, in other countries these strains may represent the predominant strains circulating including such examples as the CA-MRSA lineage CC88-MRSA-IV that is frequently reported in Australia but rarely reported in Europe or America (Monecke *et al.*, 2011). Other similar strains with limited geographical predominance include ST772-MRSA-V, which represents a recently emerged HCA epidemic clone in India and, where outside of India, strain is rarely reported except among patients with known epidemiological links to India (Nadig *et al.*, 2012). Another example includes the *pvl*-negative ST59-MRSA-IV that is rarely reported in Australia and Asia unlike its *pvl*-positive counterpart, which is frequently associated with infections in these regions (Monecke *et al.*, 2011).

Since the emergence of CA-MRSA, its link with PVL has widely been discussed with initial suggestions of this as a putative marker for CA-MRSA. However, it is now widely accepted that, although PVL plays a role in pathogenicity of some CA-MRSA strains, its presence in both MSSA and HCA-MRSA strains, and its absence in some CA-MRSA strains means that it is no longer an acceptable marker for CA-MRSA (Voyich *et al.*, 2006; Rossney *et al.*, 2007). Several *pvl*-positive CA-MRSA clones have replaced previously predominant HCA-MRSA clones in some regions including the ST8-MRSA-IV replacement of ST5-MRSA-II in the USA and ST772-MRSA-V replacement of ST239-

MRSA-III in India (DeLeo *et al.,* 2010; Mediavilla *et al.,* 2012; Nadig *et al.,* 2012; Otto, 2013a; Uhlemann *et al.,* 2014) or have led to nosocomial outbreaks throughout Europe and America (Linde *et al.*, 2005; Otter and French, 2011). In contrast, similarly detailed studies of *pvl*-negative strains circulating in hospitals or in the community have not been previously reported, with only limited information relating to specific strains available (Monecke *et al.*, 2011).

In addition to HCA-, CA- and sporadic MRSA, the emergence of novel MRSA strains among livestock and subsequent transmission of these strains to humans have led to further concerns (Catry *et al.,* 2010; Jamrozy *et al.,* 2012; Pletinckx *et al.,* 2013). These concerns arise primarily in countries with low rates of HCA-MRSA such as Denmark and the Netherlands, where LA-MRSA accounted for 39% of all new MRSA cases in 2011 and was mainly CC398-MRSA (Hetem *et al.,* 2013). Despite its widespread prevalence in mainland Europe, CC398 MRSA has only been reported sporadically in the UK (including Northern Ireland) among piglets, horses, turkeys, bovine bulk tank milk and retail pork, while the pig population in Ireland has remained free of CC398-MRSA (Loeffler *et al.*, 2009; Anon, 2013; Burns *et al.*, 2014; Hadjirin *et al.*, 2015; Hall *et al.,* 2015). More recently, a novel *mecC* gene carried on SCC*mec* XI has been described among MRSA from humans and animals in a number of European countries with a single isolate also reported from Australia, with several studies providing evidence for the zoonotic spread of these strains (Shore *et al.*, 2011a; García-Álvarez *et al.*, 2011; Petersen *et al.*, 2013; Paterson *et al.*, 2014a; Espinosa-Gongora *et al.*, 2015; Worthing *et al.*, 2016).

Many detailed investigations of the predominant HCA-MRSA clones prevalent in different regions of the world have shown that, over time, clonal replacement has occurred (Pérez-Roth *et al.,* 2004; Shore *et al.,* 2005; Amorim *et al.,* 2007; Conceição *et al.,* 2007; Albrecht *et al.,* 2011; Monecke *et al.,* 2011). Within Ireland a similar trend has been

observed where from 1971 to 2002, CC8/ST250-MRSA-I was replaced by CC8/ST239-MRSA-III, which in turn was replaced by ST5-MRSA-II, ST8-MRSA-IIA-E, ST8-MRSA-IVE/F and finally by ST22-MRSA-IV in 2002, which remains the predominant strain today (Fig. 1.7, Chapter 1) (Shore *et al.,* 2005; Rossney *et al.,* 2006; Shore *et al.,* 2010b; NMRSARL, 2014; Grundmann *et al.,* 2014). Prior to 1999, the ST22-MRSA-IV strain was only sporadically identified among isolates recovered from BSIs in Ireland, however it subsequently emerged and is now associated with approximately 80% of MRSA BSI isolates recovered each year (NMRSARL, 2014). Similarly, changes in the molecular epidemiology of *pvl*-positive MRSA recovered in Ireland have also been described where an increasing diversity in the clonal types of *pvl*-positive MRSA was observed between 2006 and 2011 along with increased virulence potential and multiresistance (Shore *et al.*, 2014). This study also highlighted the increasing prevalence of *pvl*-positive CA-MRSA in Ireland but *pvl*-negative CA-MRSA were not investigated.

Despite the predominance of ST22-MRSA-IV, sporadically-occurring MRSA strains, including *pvl*-negative and *pvl*-positive, recovered from BSIs in Ireland have been increasing in frequency in recent years from 12.1% in 2005 to 23.1% in 2011 and currently accounts for approximately 20-25% of MRSA BSIs each year (Fig. 1.7) (NMRSARL, 2014). Furthermore, additional *pvl*-negative sporadic MRSA strains are identified each year among non-BSI isolates submitted to the Irish NMRSARL from hospitalised patients and patients in the community. However, unlike *pvl*-positive strains, to date no detailed studies have been carried out to investigate these *pvl*-negative sporadically-occurring MRSA strains, or the possible emergence of any other strains within our hospitals and communities in Ireland (Shore *et al.*, 2014).

Due to the blurring of demarcation lines between HCA-, CA- and LA-MRSA, the increasing prevalence of sporadic MRSA and the potential of sporadic MRSA strains to

replace currently dominant HCA-MRSA clones, it is essential that populations of new and emerging MRSA strains are monitored and characterised in detail. In addition, sporadic MRSA strains may constitute a significant potential reservoir for virulence and resistance genes located on MGEs for other, more prevalent MRSA strains. Therefore, the aim of this part of the present study was to perform in-depth molecular typing of unusual and emerging *pvl*-negative MRSA strains in Ireland. This included *spa* typing and DNA microarray profiling to investigate the genetic backgrounds, SCC*mec*-associated genes, antimicrobial resistance genes and virulence-associated genes among a collection of sporadically-occurring MRSA isolates recovered from animals and patients in Irish hospitals and in the community between 2001 and 2015.

3.2 Materials and methods

3.2.1 Isolate selection and investigation

A total of 276 *pvl*-negative MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015 were investigated in order to further characterise unusual and emerging MRSA strains submitted to the NMRSARL. These included isolates recovered from patients in Irish hospitals and the community through GPs and were designated sporadic if they exhibited an unusual AR pattern which was different to those of the endemic ST22-MRSA-IV strain circulating in Irish hospitals. A full list of all isolates along with the associated clinical details of the patients from whom the isolates were recovered is listed in Appendix Table 1.

In addition to the above sporadically-occurring MRSA isolates, two additional groups of isolates were also investigated:

- Twelve CC398 MRSA isolates recovered from humans, including nine pig-farm workers, were recognised among the above collection of 276 isolates. An additional 11 MRSA and eight MSSA isolates recovered from animals and three MSSA isolates recovered from humans, all of which were CC398, were also investigated. Animal isolates were obtained from the Veterinary Microbiology and Parasitology Laboratory in the University College Dublin (UCD). These MRSA isolates were included in the current study as they were the first cases of CC398-MRSA recovered from animals in Ireland and were animals at the farms where CC398-MRSA had been recovered from farm workers. CC398-MSSA isolates were included in the study in order to compare MSSA and MRSA isolates of the same lineages to each other.

- 38 *pvl*-positive MRSA isolates which were associated with (i) two clusters in neonatal intensive care units (NICUs) (cluster 1, 11 isolates; cluster 2, 6 isolates) (ii) patients in the community and other hospitals (21 isolates). These isolates were included as they were identified as ST772, an emerging and unusual MRSA strain in Ireland.

Culture media, growth and storage conditions for all isolates are described in Chapter 2, section 2.1.1. All isolates were previously confirmed as *S. aureus* and underwent phenotypic susceptibility testing as detailed in Chapter 2, section 2.3.

3.2.2 Molecular investigation

3.2.2.1 Whole-genomic DNA isolation

Whole-genomic DNA for DNA microarray profiling was extracted from isolates using the lysis buffer and solutions supplied with the *S. aureus* Genotyping Kit 2.0 and the Qiagen DNeasy kit as described in Chapter 2, section 2.4.1.3.

3.2.2.2 DNA microarray profiling

DNA microarray profiling was carried out on all isolates using the *S. aureus* Genotyping Kit 2.0 to investigate the presence of antimicrobial resistance genes, virulence genes and SCC*mec* associated genes as described in Chapter 2 section 2.4.4. Additionally the DNA microarray was also used to assign isolates to MLST CC/ST and, for MRSA, to SCC*mec* types.

Isolates for which discrepancies were identified between their antimicrobial resistance phenotype and the presence or absence of antimicrobial resistance genes identified by DNA microarray profiling underwent PCRs to confirm the presence/absence of the antimicrobial resistance gene using the primers listed in Table 3.1, the PCR

Table 3.1 Primers used to confirm the presence or absence of specific antimicrobial agent resistance genes in isolates that yielded discrepant results between phenotypic susceptibility testing and the detection of resistance genes by DNA microarray profiling

Target gene	Primer name	Sequence (5′ - 3′)	Amplicon size (bp)	Reference
aacA-aphD	aacA-aphD- F	TATACAGAGCCTTGGGAAGATG	352	McManus *et al*., 2015
	aacA-aphD- R	CCTCGTGTAATTCATGTTCTGGCA		
merA	merA- F	TCAAACGTCAAGGCTGCAAG	333	McManus *et al*., 2015
	merA- R	CGTAACCTTCACCAATCCATCG		
merB	merB- F	ACTAGATAGAGCTTCGGTTACAGG	327	McManus *et al*., 2015
	merB- R	GAACCGGACCGCGTTGTAAG		
qacA	qacA- F	GATCGCACGGTCTATAAGGATT	324	McManus *et al*., 2015
	qacA- R	CGAGGCCAAATAAAGCAAATCC		
qacC	qacC- F	CTTAAATCTTCAGAAGGCTTTTTCAA	382	McManus *et al*., 2015
	qacC- R	CGAAACTACGCCGACTATGATTAA		
Iles2/mupA	ileS2F	ACGAAATAAGTGATACTCTAGGAG	453	McManus *et al*., 2015
	ileS2R	AGTCCATGTCAACCCAGTATCCT		

conditions listed in references indicated in Table 3.1 and as described in Chapter 2, section 2.4.2.

3.2.2.3 *spa* typing

All isolates were *spa* typed in accordance with the SeqNet protocol described in Chapter 2, section 2.4.5. *spa* types were clustered into different groups using Based Upon Repeating Pattern (BURP) analysis with Ridom StaphType software package (version 1.5) where the calculated cost between *spa* types was less than or equal to four and where *spa* types shorter than five repeats were excluded.

3.2.2.4 Detection of additional resistance genes

All CC398 *S. aureus* isolates underwent PCRs for (i) additional antimicrobial resistance genes not included on the DNA microarray but commonly associated with CC398 *S. aureus* isolates including those encoding resistance to tetracycline, spectinomycin trimethoprim and erythromycin, and, for CC398 MRSA (ii) SCC*mec* IV or V subtypes (depending on which SCC*mec* type was identified) (Table 3.2). All ST779 isolates were investigated for the presence of *ccrAB4* and *ccrC* using primers previously described by Ruppe *et al.,* and Kondo *et al.,* respectively (Table 3.2) (Kondo *et al.*, 2007; Ruppe *et al.*, 2009). All PCRs were performed as described in Chapter 2, section 2.4.2 and using primers shown in Table 3.2.

Table 3.2 Primers used for PCRs for additional antimicrobial resistance genes and SCC*mec* subtyping of CC398 and ST779 *S. aureus* isolates

Isolates that underwent PCR	Target	Target gene	Primer name	Sequence (5′ - 3′)	Amplicon size (bp)	Reference
CC398	MLS_B resistance	*erm*(T)	ermT- F	ATTGGTTCAGGGAAAGGTCA	536	Feßler *et al*., 2010
			ermT- R	GCTTGATAAAATTGGTTTTTGGA		
	Spectinomycin resistance	*spc*	spc- F	ACCAAATCAAGCGATTCAAA	561	Feßler *et al*., 2010
			spc- R	GTCACTGTTTGCCACATTCG		
	Tetracycline resistance	*tet*(L)	tetL- F	TCGTTAGCGTGCTGTCATTC	267	Ng *et al*., 2001
			tetL- R	GTATCCCACCAATGTAGCCG		
		tet(K)	tetK-F	TCGATAGGAACAGCAGTA	169	Ng et al. 2001
			tetK-R	CAGCAGATCCTACTCCTT		
		tet(M)	tetM-F	GTGGACAAAGGTACAACGAG	406	Ng et al. 2001
			tetM-R	CGGTAAAGTTCG TCACACAC		
	Trimethoprim resistance	*dfrD*	dfrD-F	CCCTGCTATTAAAGCACC	606	Dale et al.1995
			dfrD-R	CATGACCAGATAACTC		
		dfrG	dfrG- F	TGCTGCGATGGATAAGAA	315	Argudín et al. 2011
			dfrG- R	TGGGCAAATACCTCATTCC		
		dfrK	dfrK- F	CAAGAGATAAGGGGTTCAGC	214	Argudín et al. 2011
			dfrK- R	ACAGATACTTCGTTCCACTC		
	SCC*mec* IV subtyping	SCC*mec* IVa	J IVa F	ATAAGAGATCGAACAGAAGC	278	Milheirico et al., 2007; Ma et al., 2002
			J IVa R	TGAAGAAATCATGCCTATCG		
		SCC*mec* IVb/IVF	J IVb F	TTGCTCATTTCAGTCTTACC	336	Milheirico et al., 2007; Ma et al., 2002
			J IVb R	TTACTTCAGCTGCATTAAGC		
		SCC*mec* IVc/IVE	J IVc F	CCATTGCAAATTTCTCTTCC	483	Ma et al., 2006; Milheirico et al., 2007
			J IVc R	ATAGATTCTACTGCAAGTCC		
		SCC*mec* IVd	J IVd F	TCTCGACTGTTTGCAATAGG	575	Ma et al., 2006; Milheirico et al., 2007
			J IVd R	CAATCATCTAGTTGGATACG		
		SCC*mec* IVg	J IVg F	TGATAGTCAAAGTATGGTGG	792	Milheirico et al., 2007; Kinnevey et al., 2014
			J IVg R	GAATAATGCAAAGTGGAACG		
		SCC*mec* IVh	J IVh F	TTCCTCGTTTTTTCTGAACG	663	Milheirico et al., 2007; Ma et al., 2002
			J IVh R	CAAACACTGATATTGTGTCG		

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Table 3.2 continued

Isolates that underwent PCR	Target	Target gene	Primer name	Sequence (5′ - 3′)	Amplicon size (bp)	Reference
	SCC*mec* V subtyping	*ccrB2a*	ccrB2 F	CGAACGTAATAACATTGTCG	203	Kinnevey et al., 2014; Higuchi et al., 2008
			ccrB2 R	TTGGCWATTTTACGATAGCC		
		ccrC2	ccrC2-F2	ATAAGTTAAAAGCACGACTCA	257	Kinnevey et al., 2014; Higuchi et al., 2008
			ccrC2- R2	TTCAATCCTATTTTTCTTTGTG		
		ccrC8	ccrC8-F	GCATGGGTACTCAATCCA	562	Kinnevey et al., 2014; Higuchi et al., 2008
			ccrC8-R	GGTTGTAATGGCTTTGAGG		
ST779-t878	*ccr* type 4	*ccrAB4*	α4U	GCGACGAATCAAATGTCCTTACTG	1308	Ruppe *et al,* 2009
			β4U	ATCGCTCCAGTGTCTATACTTCGC		
	ccrC	*ccrC*	γR	CCTTTATAGACTGGATTATTCAAAATAT	518	Kondo *et al.,* 2007
			γF	CGTCTATTACAAGATGTTAAGGATAAT		

Abbreviations: bp, base pairs; MLS_B, macrolide, lincosamide and streptogramin B.

3.3 Results

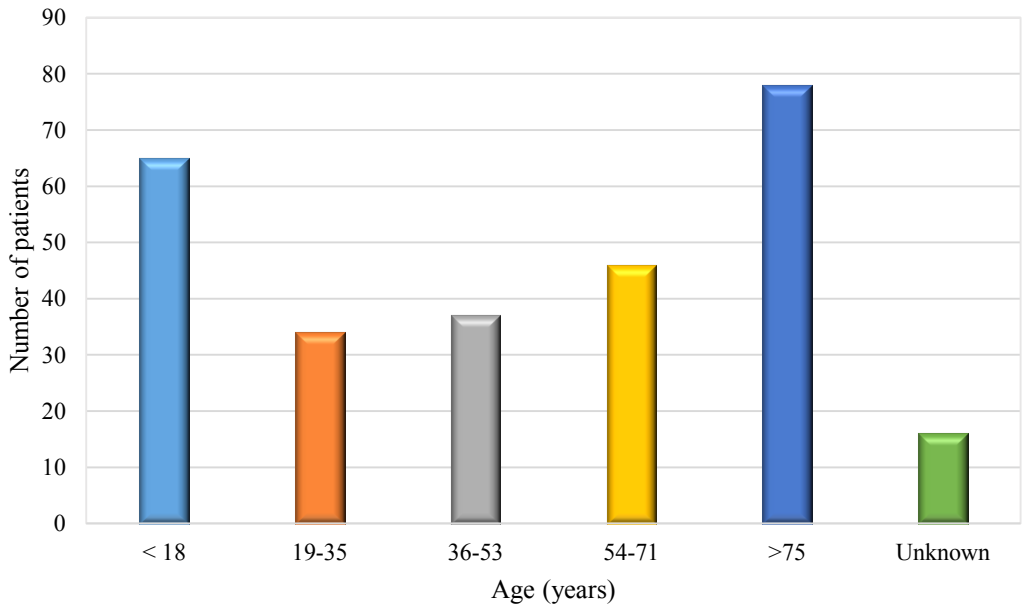
3.3.1 Patient population

Patients from which the 276 sporadically-occurring MRSA isolates were recovered from ranged in age from several days old to 95 years of age with a mean age of 46 years (Panel A, Fig. 3.1). Geographically, isolates were recovered throughout Ireland but 46.8% (132/276) were recovered in hospitals or GP clinics in Dublin. Clinical details of the patients were limited with none available for 15.9% (44/276) of isolates and a further 52.2% (144/276) recovered from screening swabs during outbreak investigations or as part of routine screening protocols in the hospitals concerned (Panel B, Fig. 3.1). Twenty percent of isolates (56/276) were recovered from BSIs and 73% of these (41/56) were recovered from patients > 50 years of age with the remaining patients being < 50 years and six < 25 years. Of the remaining isolates, 11.6% (32/276) were recovered from a range of infection sites including wounds, post-surgical sites, eyes, leg wounds and ulcers (Panel B, Fig. 3.1). Specific details relating to each patient and isolate are detailed in Appendix Table 1.

3.3.2 Phenotypic and genotypic diversity among sporadically-occurring MRSA

Details of the antimicrobial susceptibility and genotyping data of the 276 sporadically-occurring MRSA isolates recovered between 2001 and 2015 in Irish hospitals and the community as well as the virulence and antimicrobial resistance genes detected among these isolates are shown in Table 3.3 and Figs. 3.2-3.9, with further details described below.

Panel A



Panel B

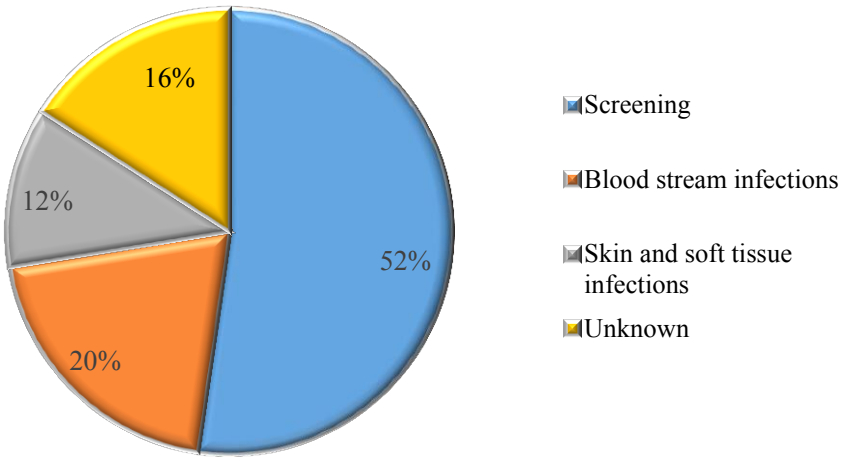


Figure 3.1 Clinical details of patients in Irish hospitals and in the community from whom the 276 sporadically-occurring MRSA isolates investigated in this study were recovered between 2001 and 2015. Panel A shows the age profile of the patients. The unknown category includes patients for whom date of birth was not available. Panel B shows the types of clinical samples from which the MRSA isolates were recovered. Screening samples were collected in accordance with specific hospital policies during either routine

screening or outbreak investigations which included nasal, throat, groin and pooled samples of all three sites. Skin and soft tissue infections isolates included recovered from wound infections, post-surgical sites, eyes, ears, leg wounds and ulcers.

Table 3.3 Multilocus sequence type clonal complexes and sequence types, SCC*mec* types, *spa* types, phenotypic resistance profiles and resistance and virulence genes detected among the sporadically-occurring MRSA isolates investigated in the present study

CC/ST (*n*)[a]	SCC*mec* type (n)[b]	*spa* type (n)	Phenotypic resistance (n)[c]	Resistance genes (n)[b]	Virulence genes (n)[b, d]
CC1 (78)	IV (62), IV-SCC*fus* (16)	t127 (62), t386 (1), t922 (1), t2246(1), t2279 (12), t16173 (1)	Ap, Cd (37), Eb (3), Er (63), Fd (18), Kn (57), Mp (45), Nm (57), Sp (4), St (56), Te (53), Tb (7), PMA (1)	*blaZ* (77), *erm*(A) (5), *erm*(C) (58), *aphA3 & sat* (57), *ileS2* (45), *tet*(K) (41), *qacA* (43), *lnu*(A) (1), *vga* (5), *aadD* (1), *fusB* (2), *fusC* (16), *qacC* (2), *sdrM* (76), *fosB* (5)	*sek/q* (15), *seh* (74), *egc* (7), *sea* (19), *sak* (75), *scn* (75), *chp* (1)
CC1-ST772 (2)	V	t657 (1), t1839 (1)	Ap, Cd (1), Cp (1), Er (1), Gn, Kn, Nm (1), Tb, Tp	*blaZ*, *mph*(C) (1), *msr*(A) (1), *aacA-aphD, aphA3& sat,* (1), *fosB, sdrM*	*sec/l, egc, scn*
CC5 (52)	I (2), I & *fusC* (1), II trun (1), IV (15), IV & *ccrB4* (7), IV-*fusC* (1), V (23), V & *fusC* (1), VI (1)	t002 (34), t045 (2), t067 (2), t088 (3), t105 (1), t12117 (1), t1340 (2), t1567 (1), t1594 (1), t1781 (1), t311 (1), t442 (1), t8892 (2)	Ak (3), Ap, Cd (17), Er (19), Eb (6), Fd (6), Gn (26), Kn (29), Mc (11), Mp (3), Nm (6), PMA(12), Sp (1), Su (1), Te (1), Tb (32), Tp (5)	*blaZ* (46), *aphA3* & *sat* (5), *aadD* (4), *fosB* (51), *aacA-aphD* (25), *ileS2* (4), *merA/B* (12), *qacA* (8), *fusC* (3), *msr*(A) (5), *qacC* (4), *mph(C)* (5), *erm*(C) (14), *dfrS1* (2), *sdrM* (51), *tet*(K) (1), *tet*(M) (1), *erm*(A) (2)	*egc* (47), *tst* (5), *sed/j/r* (32), *sec/l* (3), *sak* (51), *chp* (24), *scn* (51), *sea* (38), *seb* (2), *sep* (6), *etA* (1)
CC5-ST5 (4)	II (3), I (1)	t045 (1), t463 (1), t002 (1), t010 (1)	Ak (1), Ap, Cd (3), Cp (2), Er (3), Fd (3), Kn (2), Ln, Nm (2), Sp (1), Tb (2)	*blaZ*, *erm*(A) (3), *fosB*, *sdrM*, *aadD* (2), *erm*(C) (1)	*tst* (3), *sec/l* (1), *sed/j/r, egc, sak, sea* (2), *scn, chp* (3)
CC5-ST5/ST225 (3)	II	t002	Ak (2), Ap, Cp, Er, Gn (1), Ln, Kn (2), Mp (1), Nm (2), Sp,	*blaZ, erm*(A), *aadD* (2), *fosB*, *aacA-aphD* (1), *sdrM*, *qacC* (1), *ileS2* (1)	*sed/j/r* (2), *egc, sea* (1), *sak, chp, scn*

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Table 3.3 continued

CC/ST (*n*)[a]	SCC*mec* type (n)[b]	*spa* type (n)	Phenotypic resistance (n)[c]	Resistance genes (n)[b]	Virulence genes (n)[b, d]
			Tb (2)		
CC5-ST835 (2)	V & *ccrB4*	t1567	Ap, Cd (1), Tp	*fosB, sdrM*	*sed/j/r, egc, sea, sak, scn*
CC6 (3)	IV	t701 (1), t11019 (1), t304 (1)	Ap, Cd (2)	*blaZ, fosB, sdrM* (2)	*sak*, *scn*, *sea* (1)
CC8 (9)	IV (5), III-SCC$_{Hg}$ (1), IV & V & *fusC* (1), V (1) VI & *fusC* (1),	t008 (3), t037 (1), t064 (3), t11173 (1), t14362 (1)	Ap, Cd (3), Cp (2), Cl (1), Er (5), Fd (2), Gn (2), Kn (4), Ln (5), Mc (1), Nm (1), PMA (1), Rf (4), Sp (1),St (2), Su (4), Te (3), Tb (4) Tp (4)	*blaZ, erm*(A) (2), *aadD* (1), *tet*(M) (3), *cat* (1), *fosB, qacC* (1), *merA/B* (1), *aacA-aphD* (3), *erm*(C) (3), *fusC* (2), *dfrS1* (2), *sdrM*	*sek/q* (5), *seb* (4), *hlb* (4), *sak* (5), *chp* (1), *scn* (5), *sea* (2), ACME (1)
CC8-ST239 (4)	III (1), III-SCC*Hg* (3)	t037	Ak (2), Ap, Cd (3), Cp (1), Cl (2), Er (4), Gn (1), Kn, Ln, Mc (3), Nm, PMA (3), Sp (1), St, Su (2), Te, Tb	*blaZ, erm*(A), *aadD* (3), *tet*(M), *cat* (3), *fosB, qacC* (3), *merA/B* (3), *aacA-aphD* (1), *sdrM*, *aphA3 & sat* (1), *tet*(K) (3)	*sek/q*, *sea, sak, scn*
CC8-ST250 (2)	I	t1883 (1), t008 (1)	Ap, Er, Fd(1), Gn (1), Kn (1), Ln, Rf (1), Sp, St, Su, Tb (1)	*erm*(A), *aacA-aphD* (1), *fosB, sdrM,*	*sek/q* (1), *seb* (1), *sea* (1), *sak* (1), *chp* (1), *scn*
CC8-ST8 (9)	IV (6), IIA/B/D (2), V (1)	t008 (3), t451 (2), t1476 (1), t190 (2), t064 (1)	Ap, Cp (6), Tp (5), Ak (2), Cd (4), Cp (6), Er (6), Eb (2), Fd (1), Gn (3), Kn (7), Nm (6), PMA (3), Sp (2), Su (2), Te (4), Tb (5), Tp (5)	*blaZ, merA/B* (3), *erm*(A) (2), *msr*(A) (4), *mph*(C) (4), *aacA-aphD* (5), *aadD* (2), *tet*(M) (2), *tet*(K) (1), *aphA3* (5), *fusB* (1), *fosB*, *qacA* (3), *sdrM, dfrS1* (2)	*seb* (3), *sek/q* (6), *sed/j/r* (1), *sea* (4), *sak*, *chp* (5), *scn*, ACME (1)

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Table 3.3 continued

CC/ST (*n*)[a]	SCC*mec* type (n)[b]	*spa* type (n)	Phenotypic resistance (n)[c]	Resistance genes (n)[b]	Virulence genes (n)[b, d]
CC8-ST72 (1)	IV	t126	Ap	*blaZ, sdrM, fosB*	*egc, sak, chp, scn*
CC22 (29)	IV	t002 (1), t005 (1), t022 (1), t032 (67), t513(1), t100 (1), t14499 (1), t14500 (1), t1653 (1), t1802 (1), t223 (1), t2231 (1), t3387 (1), t3444 (1), t790 (1), t891 (1), t4559 (1), t4623 (1), t5121 (1), t515 (1), t5485 (1), t1084 (1), NT (1)	Ap, Cd (13), Cp (21), Er (17), Eb (2), Fd (7), Gn (4), Kn (4), Ln (15), Mp (3), Rf (1), Sp (1), Te (3), Tb (5), Tp (3)	*blaZ, erm*(C) (14), *erm*(A) (3), *aacA-aphD* (3), *ileS2* (3), *dfrS1*(3), *tet*(K) (3), *aphA3 & sat*(1), *vga* (1), *fosB* (1), *qacA* (1)	*egc*, *sec/l* (12), *sed/j/r* (1), *tst* (2), *sak* (22), *chp* (21), *scn* (22), ACME (1)
CC30 (7)	IV & *fusC* (4), IV (2), IV & *ccrC* & *fusC* (1)	t012 (2), t021 (2), t5730(1), t382(1), t018 (1)	Ap, Cd (6), Fd (5)	*blaZ, fusC* (5), *sdrM*, *fosB*	*tst* (6), *egc*, *sec/l* (1), *sak, chp* (6), *scn*
CC30-ST36/39 (1)	II	t007	Ak, Ap, Cd, Er, Gn, Kn, Ln, Nm, Sp, Tb	*blaZ, erm*(A), *aacA-aphD, aadD, fosB, qacA*	*tst, sec/l, egc*, *sak, scn*
CC45 (27)	IV (18), IV & *fusC* (8), V (1)	t015 (1), t026 (1), t065 (2), t2277 (2), t2642 (1), t383 (1), t727 (11), t728 (2), t4545 (4), t563 (1), t671	Ap, Cd (18), Cp (11), Cl (1), Er (10), Fd (19), Gn (1), Kn (2), Ln (9), Mp (1), Nm (2), PMA (1), Sp (3), Te (1),Tb (2), Tp (2)	*blaZ, merA/B* (8), *ileS2* (2), *erm*(C) (8), *tet*(M) (1), *fusC* (8), *aacA-aphD* (1), *qacC* (1), *erm*(A) (2), *sdrM* (26), *aadD* (1), *dfrS1*(1), *fosB* (1), *cat* (1), *fusB* (2)	*egc* (26), *tst* (1), *sec/l* (9), *sed/j/r* (4), *hlb* (1), *sak* (18), *chp* (18), *scn* (18)
CC59- ST59 (3)	V	t316 (2), t10127 (1)	Ap, Cd (2), Cp, Er (2), Fd, Mp	*blaZ, msr(A), fusB*, *ileS2, qacC* (1), *sdrM*	*seb, sek/q, sak, chp, scn*
CC59- ST87 (1)	IV	t216	Ap, Er, Kn, Nm	*blaZ, msr(A), mpb(B), aphA3 & sat, sdrM*	*sek/q*, *seb, sak, chp, scn*
CC80 (1)	IV	t8154	Ap, Cd	*blaZ, sdrM*	*seb, sek/q, sak, chp, scn*, *etD*, *edinB*
CC88 (5)	IV (4), V (1)	t10785 (1), t2622 (1), t786 (2), t5562 (1)	Ap, Cd (2), Cp (1), Er (3), Ln (3), Rf (1), Sp	*blaZ, dfrS1*(1), *sdrM*, *erm*(A) (3)	*sec/l* (4), *sak* (4), *scn, chp* (2)

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Table 3.3 continued

CC/ST (*n*)[a]	SCC*mec* type (n)[b]	*spa* type (n)	Phenotypic resistance (n)[c]	Resistance genes (n)[b]	Virulence genes (n)[b, d]
			(3), Te (1), Tp (1)		
CC97 (1)	V	t12025	Ap	*blaZ, sdrM*	*hlb*
CCT130 (1)	XI	t12399	Ap	$blaZ_{xi}$, *sdrM*	*hlb*
CC398 (12)	IV (2), V(10)	t011 (11), t034 (1)	Ap, Cl (2), Er (10), Gn (2), Kn (3), Ln (10), Nm (1), Sp (7), Te, Tb (3), Tp (4)	*blaZ, erm*(C) (1), *aacA-aphD* (2), *tet*(M), *fexA* (2), *tet*(K) (10), *erm*(B) (1), *aadD* (1), *erm*(A) (9), *sdrM* (10),	*sak* (1), *chp* (1), *scn* (1), *hlb* (11)
CC779 (6)	ΨSCC*mec* & *fusC*-SCC-SCC_{CRISPR}	t878 (5), t11021 (1)	Ap, Cd (1), Fd	*blaZ, fusC, sdrM*, *qacA* (1)	*etd/edinB*, *sed/j/r* (1), *sak*, *chp, scn*
CC779-ST779 (13)	ΨSCC*mec* & *fusC*-SCC-SCC_{CRISPR} (13),	t878	Ap, Cd (3), Fd	*blaZ, fusC, sdrM*	*etd/edinB*, *sed/j/r* (5), *seb* (4) *sak, chp*, *scn*

[a]CC/ST, multilocus sequence type clonal complex (CC)/ sequence type (ST) as assigned using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH.);

n, number of isolates. The number of isolates is only indicated where not all isolates exhibited the same phenotype or harboured the same gene.

[b]SCC*mec,* Staphylococcal Cassette Chromosome *mec* as determined using the *S. aureus* Genotyping Kit 2.

[c]Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including Ak; amikacin, Ap; ampicillin; Cd, cadmium acetate, Cl; chloramphenicol, Cp, ciprofloxacin; Er, erythromycin; Eb; ethidium bromide, Fd, fusidic acid; Gn; gentamicin, Kn; kanamycin, Ln, lincomycin; linezolid, Mc mercuric chloride, Mp; mupirocin, Nm; neomycin, PMA; phenyl mercuric acetate, PrI; propramide isoethionate, Rf; rifampicin, Sp; spectinomycin, St, streptomycin, Su; sulphonamide, Te; tetracycline, Tb; tobramycin, Tp, trimethoprim and vancomycin.

Continued overleaf

Table 3.3 continued

[d]The immune evasion complex (IEC) type of each isolate was determined based on the combination of the IEC genes detected as described by van Wamel *et al.,* 2006. IEC type A: *sea, sak, chp, scn*; IEC type B: *sak, chp, scn;* IEC type C: *chp, scn*; IEC type D: *sea, sak, scn*; IEC type E: *sak, scn*; IEC type F: *sep, sak, chp, scn*; IEC type G: *sep, sak, scn.*

[e]egc enterotoxin gene cluster including *seg, sei, sem, sen, seo* and *seu.*

3.3.2.1 Genotypes

The 276 MRSA isolates were assigned to 14 MLST CCs with CC1 predominating and accounting for 28.9% (80/276) of isolates followed by CC5 (22.1%, 61/276), CC22 (10.5%, 29/276), CC45 (9.8%, 27/276), CC8 (9.1%, 25/276) and CC779 (6.9%, 19/276) (Fig. 3.2 and Table 3.3). Clonal complexes 398, 30, 88, 59 and 6 were represented by 12 (4.3%), eight (2.9%), five (1.8%), four (1.5%) and three (1.1%) isolates, respectively, while the remaining CCs (CC80, CC97 and CC130) were each represented by one isolate (Fig. 3.2 and Table 3.3).

Fifty-seven of the 276 isolates, from five different CCs, were further differentiated into STs including ST772 (CC1, 2/276), ST5 (CC5, 7/276), ST835 (CC5, 2/276), ST72 (CC8, 1/276), ST239 (CC8, 4/276), ST250 (CC8, 2/276), ST8 (CC8, 9/276), ST36/39 (CC30, 1/276), ST59 (CC59, 3/276), ST87 (CC59, 1/276), ST398 (CC398, 12/276) and ST779 (13/276) (Fig. 3.2 and Table 3.3).

Eighteen different combinations of SCC*mec* genes were identified among the isolates (Fig. 3.3 and Table 3.3). SCC*mec* type IV and variants predominated accounting for 62.7% of isolates (187/276) followed by SCC*mec* V and variants (16.7%, 46/276). Other SCC*mec* types and variants identified included the CI ΨSCC*mec-fusC*-SCC-SCC$_{CRISPR}$ (6.9%, 19/276) and SCC*mec* I, II, III, VI and variants thereof (2.2%, (6/276), 2.9%, (8/276) and 2.5% (7/176), and 0.7% (2/276), respectively. SCC*mec* XI was recognised in a single isolate (0.4%).

SCC*mec* types combined with the CCs/STs resulted in 48 different type combinations with CC1-MRSA-IV (22.5%, 62/276) predominating followed by CC22-MRSA-IV (10.5%, 29/276), CC5-MRSA-V (8.3%, 23/276), CC1-MRSA-IV & SCC*fus* (5.8%, 16/276), CC45-MRSA-IV (6.5%, 18/276) and CC5-MRSA-IV (5.4%, 15/276) (Fig.

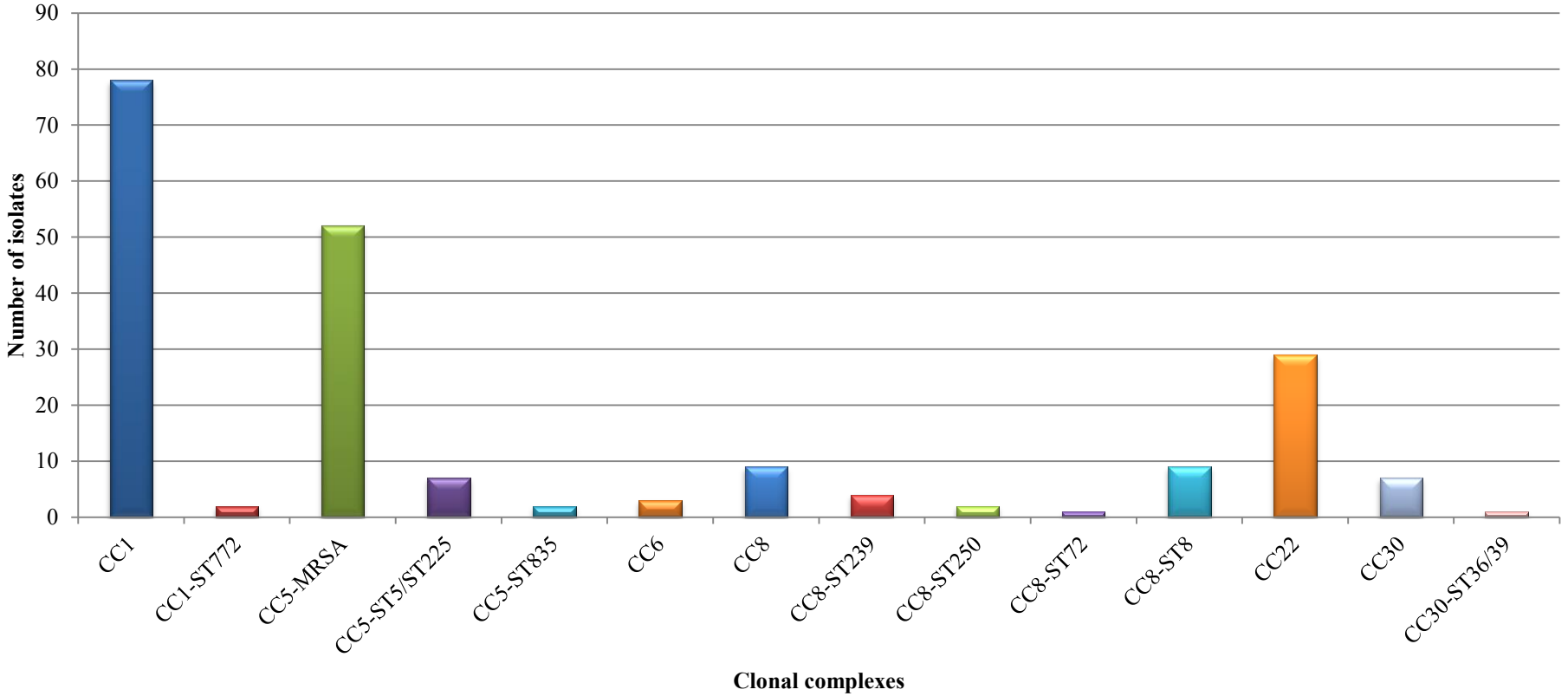

Figure 3.2 The MLST clonal complexes (CCs) identified among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015.

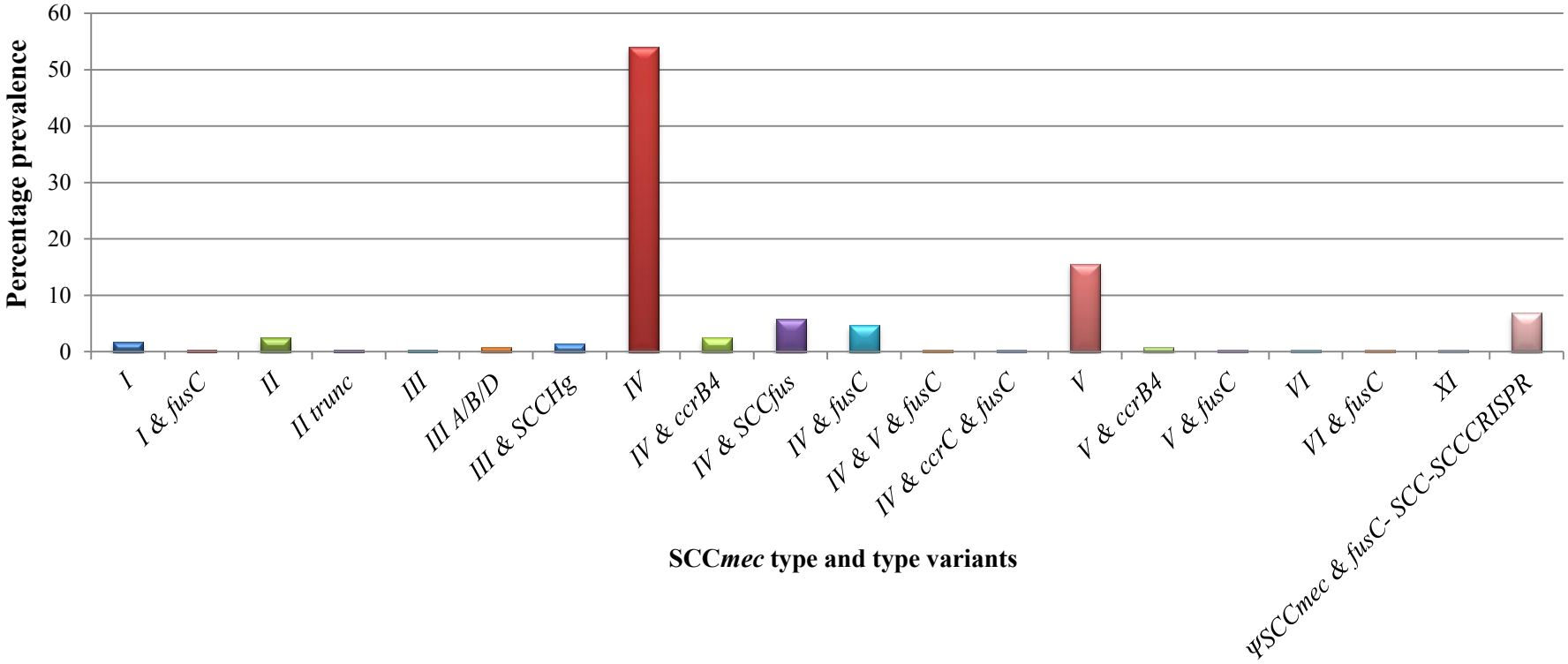

Figure 3.3 Percentage distribution of each SCC*mec* type and variant detected among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015.

Abbreviations: trunc, truncated.

3.4). The remaining CC/ST-SCC*mec* type combinations each accounted for <5% of the isolates (≤ 10 isolates each) (Fig. 3.4). Among the CC1-MRSA-IV and CC5-MRSA-V isolates 63.8% (51/80) and 29.5% (18/61), respectively were associated with outbreaks including a CC1-MRSA-IV outbreak which spanned a two-year period in a large teaching hospital. These outbreaks are discussed in more detail in Chapter 5.

Isolates within CC5 and CC8 exhibited the greatest diversity in relation to ST and SCC*mec*; with 11 and eight different SCC*mec* types and variants, respectively (Fig. 3.4 and Table 3.3). Among CC5 isolates the SCC*mec* types identified included SCC*mec* types I (4.9%, 3/61), type I & SCC*fusC* (1.6%, 1/61), type II (9.8%, 6/61), type II truncated due to a *ccrAB4* deletion (1.6%, 1/61), type IV (24.6%, 15/61), type IV & *ccrB4* (11.5%, 7/61), type IV & *fusC* (1.6%, 1/61), type V (37.8%, 23/61), type V & *fusC* (1.6%, 1/61), type V & *ccrB4* (3.3%, 2/61) and type VI (1.6%, 1/61) (Fig. 3.3 and Table 3.3). The 25 CC8 isolates harboured eight different SCC*mec* elements including SCC*mec* types I, IIA/B/D, III, III & SCC_{Hg}, IV, IV & V & *fusC*, V and VI & *fusC*. Within CC8-SCC*mec* specific clones identified included ST8-MRSA-IIA/B/D ST8-MRSA-V, ST239-MRSA-III/III_{Hg} and ST250-MRSA-I. In contrast to the diversity of the SCC*mec* elements within CC5 and CC8, only one SCC*mec* type, SCC*mec* IV, was identified among CC22 MRSA isolates (Fig. 3.4 and Table 3.3).

Fifty isolates (18.1%) from six CCs (CC1, CC5, CC8, CC30 and CC779) carried additional *ccr* genes. SCC*Hg* was carried along with SCC*mec* III by four CC8/ST239 isolates (Fig. 3.4 and Table 3.3). The SCC-associated fusidic acid resistance gene *fusC*, either with or without the SCC*fus ccrAB1* genes, was detected in 19.2% of isolates (53/276) and in association with isolates harbouring SCC*mec* I, IV or V among six CCs (CC1, CC5, CC8, CC30, CC45 and CC779) (Fig. 3.4 and Table 3.3). One isolate carrying

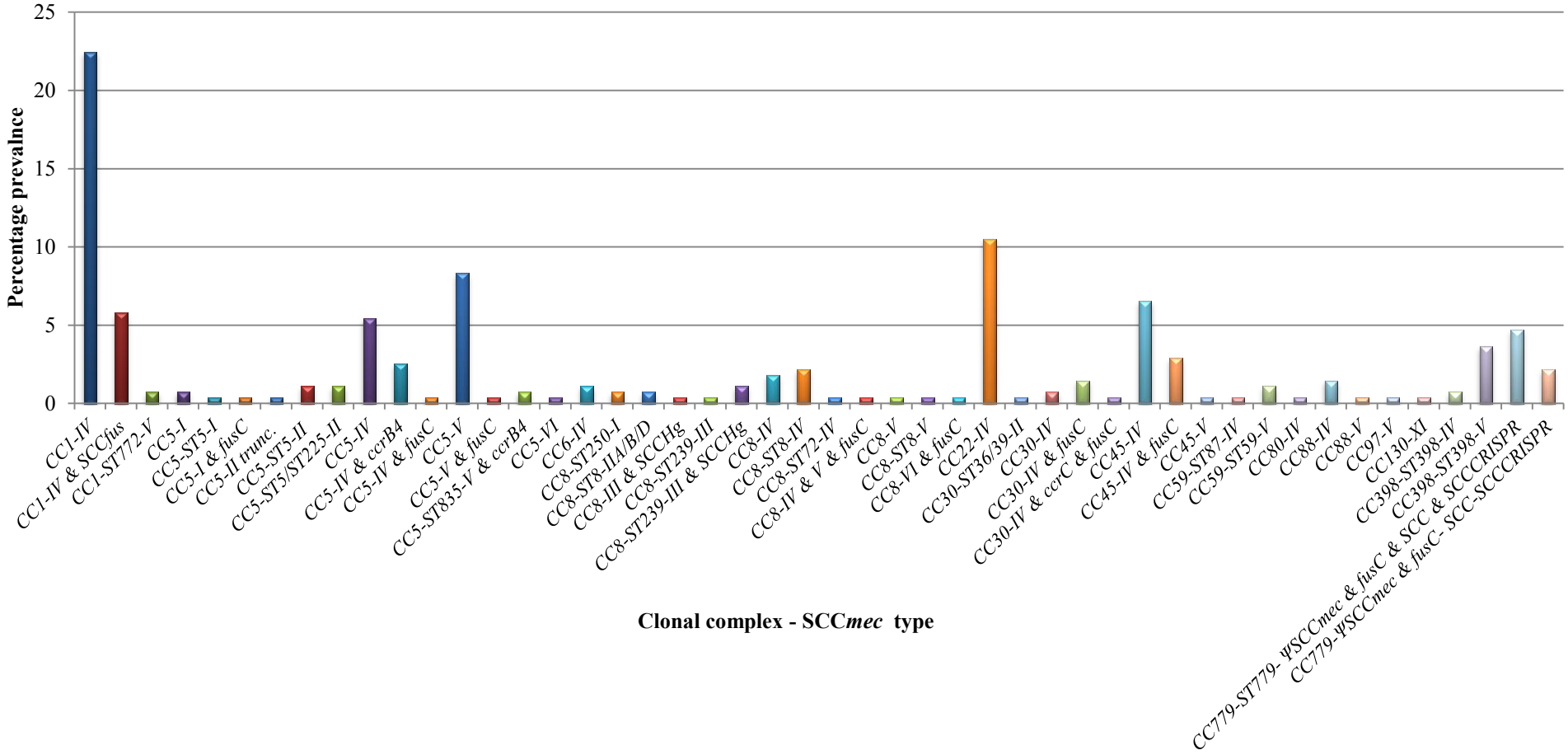

Figure 3.4 The percentage of each MLST sequence type (ST) and/or clonal complex (CC) and SCC*mec* type combination identified among 276 MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015. Abbreviations; trun; truncated

SCC*mec* IV and *fusC* also harboured an additional *ccrC* gene (CC30). Further characterisation of MRSA isolates carrying *fusC* is described in Chapter 4.

Eighty-six *spa* types were identified among the 276 isolates investigated, 56 (65.1%) of which were represented by only one isolate each. The most frequently identified *spa* types included t127 (CC1, 22.5%, 62/276), t002 (CC5, 12.7%, 35/276), t878 (ST779, 6.5%, 18/276), t2279 (CC1, 4.3%, 12/276) and t011 (CC398, 4.0%, 11/276) which accounted for 50.0% (138/276) of the isolates (Table 3.3). Isolates within CC22-MRSA-IV (n = 29) exhibited the greatest number of *spa* types with 21 different types identified (Table 3.3). Fifteen *spa* types were identified within CC5 (n = 62) while CC1 isolates (n = 82) exhibited eight *spa* types (Table 3.3). BURP analysis clustered 89% (246/276) of the isolates into 12 different *spa* clonal clusters (*spa*-CCs) and 27 singletons (Fig. 3.5). The remaining isolates (30/276, 11%) were excluded as their *spa* types consisted of less than five repeat successions. The largest *spa*-CC identified included 64 isolates (24%) representative of two *spa* types, t127 and t922, however neither *spa* type was recognised as the founder *spa* type (Cluster K, Fig. 3.5). Both t127 and t922 were assigned to CC1 by DNA microarray analysis and other isolates assigned to CC1 included those of *spa* types t386, t2246, t2279 and t16173, however each of these *spa* types were excluded from the BURP analysis due to short repeat successions.

3.3.2.2 Virulence-associated genes

The most frequently identified virulence genes among the MRSA isolates were the IEC genes *scn* and *sak* which were detected among 86.6% (239/276) and 85.1% (235/276) of isolates, respectively, (Fig. 3.6). Other IEC genes detected included *chp* (39.9%, 110/276), *sea* (27.5%, 76/276) and *sep* (2.2%, 6/276) (Fig. 3.6). Only CC97 (n = 1), CC130 (n = 1) and CC398 (n = 11/12) isolates were IEC-negative (Fig. 3.6). The IEC type

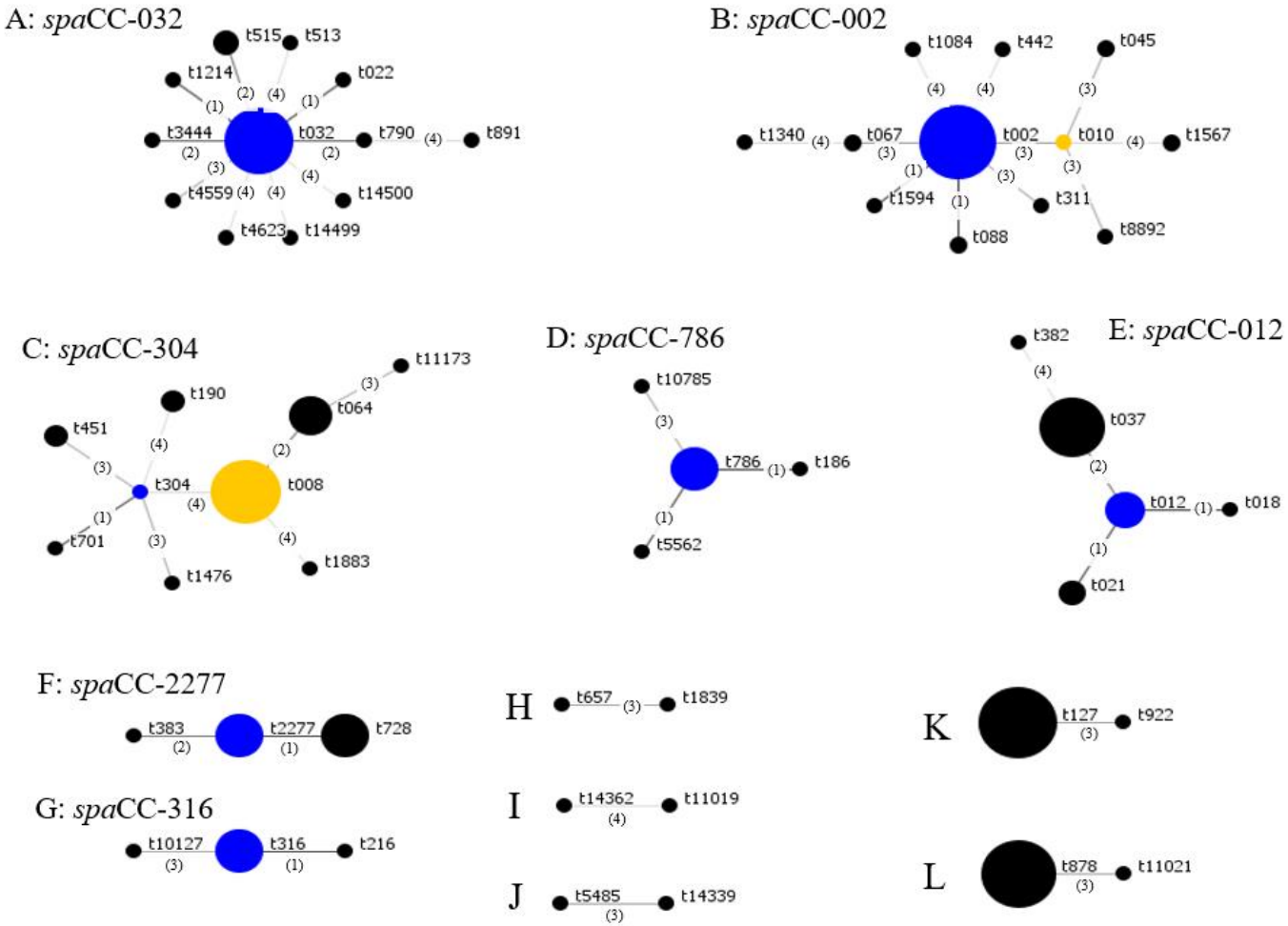
A: spaCC-032
t515
t513
t1214
t022
t3444
t032
t790
t891
t4559
t14500
t4623
t14499
B: spaCC-002
t1084
t442
t045
t1340
t067
t002
t010
t1567
t1594
t311
t088
t8892
C: spaCC-304
t11173
t190
t064
t451
t304
t008
t701
t1476
t1883
D: spaCC-786
t10785
t786
t186
t5562
E: spaCC-012
t382
t037
t012
t018
t021
F: spaCC-2277
t383
t2277
t728
G: spaCC-316
t10127
t316
t216
H
t657
t1839
I
t14362
t11019
J
t5485
t14339
K
t127
t922
L
t878
t11021

Figure 3.5 Clustering of 86 *spa* types identified among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015 using based upon repeating patterns (BURP) analysis with Ridom StaphType software package (version 1.5). *spa* types were clustered together where the cost between *spa* types, i.e. the number of genetic events between *spa* types, was less than or equal to four and where *spa* types shorter than five repeats were excluded (10 *spa* types, 30/276 isolates). Founder and co-founder *spa* types for each *spa* clonal complex (*spa*-CC) are shown as blue and yellow circles, respectively, and the cost between each *spa* type is shown in parenthesis. The size of each circle represents the number of isolates of each *spa* type in proportion to other *spa* types in the cluster. Fourteen percent of *spa* types recognised in 7% (18/276) of isolates clustered in *spa*-CC 032 (A) with *spa* type t032 identified as the *spa*-CC founder. A further 60 isolates (22%) with 12 different *spa* types (14%) clustered in *spa*-CC 002 (B). *spa*-CC 304 (C) included 20 isolates (7%) comprising of 11% of *spa* types (9/86). *spa*-CC 012 (D) included 12 isolates (4%) with five different *spa* types (6%). Five isolate (2%) of four different *spa* types (5%) were included in *spa*-CC 786 (E) while *spa*-CC 316 (F) included three different *spa* types (4%) from 1% (4/276) of isolates. Five isolates (2%) clustered in *spa*-CC 2277 (G) which included three *spa* types (4%). The remaining clusters (H-L) contained a total of 89 isolates and 10 different *spa* types. Among these one cluster (K) accounted for 64 isolates (24%) but only included two *spa* types (2%, t127 and t922). Ten percent of isolates (28/276) representative of 16 different *spa* types (t007, t011, t015, t065, t126, t563, t2231, t2480, t2622, t4545, t5121, t5730, t8154, t12025, t12117 and t12399) were defined as singletons while the remaining Among these *spa* types (t026, t386, t463, t727, t1781, t1802, t2246, t2279, t2642 and t16173) were excluded from the analysis due the short repeat succession recognised in the *spa* gene (<5).

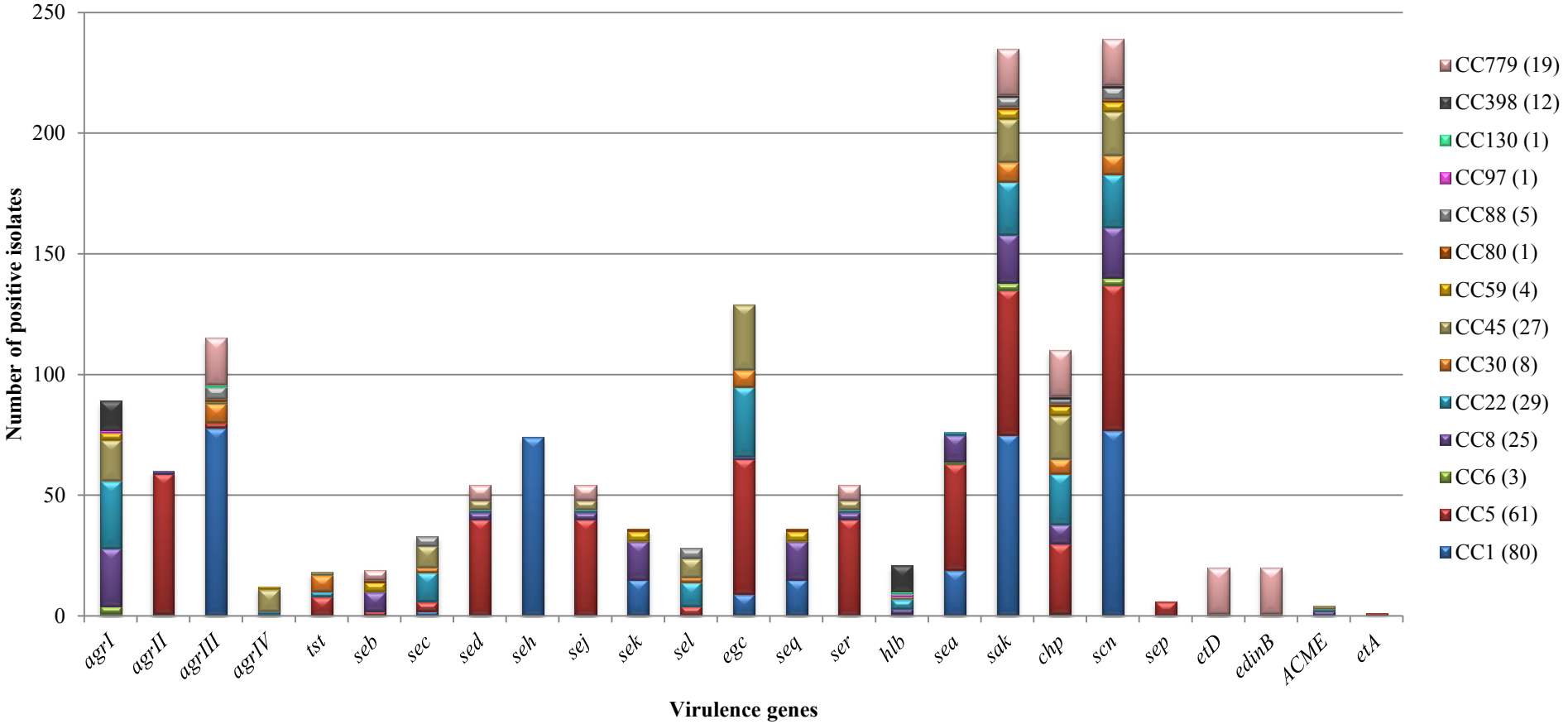

Figure 3.6 Virulence genes identified by DNA microarray profiling among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015. The total number of isolates associated with each MLST clonal complex (CC) is shown in parentheses.

B (*sak, chp* and *scn*), accounted for 35.9% (86/239) of isolates and was the most frequently recognised IEC type among IEC positive isolates, followed by IEC type E (*sak* and *scn*) (28.0%, 67/239), IEC type D (*sea, chp* and *scn*) (23.8%, 57/238) and IEC type A (*sea, sak, chp* and *scn*), (7.9%, 19/238). The IEC type C (*chp* and *scn*) was found in only a single isolate (M13/0590, CC88-t2622-MRSA-IV). The single IEC gene, *scn* (IEC H) was found in three isolates (1.3%, 3/238) while type F (*sep, sak, chp* and *scn*) and G (*sep, sak* and *scn*) were found in four (1.7%) and two (0.8%) isolates, respectively.

The accessory gene regulator (*agr*) alleles III and I were the most dominant *agr* types representing 41.7% (115/276) and 32.2% (89/276) of the isolates, respectively, from multiple CCs (Fig. 3.6). The *agr* type II was detected in 21.7% of isolates (60/276), predominantly from CC5 (96.7%, 58/60 isolates) while *agr* type IV accounted for only 4.3% of isolates (12/276) and nine of which were assigned to CC45 (Fig. 3.6).

The most common toxin genes detected were the enterotoxin gene cluster (*egc*) which was detected in 46.7% of isolates (129/276), predominantly belonging to CCs 5, 22 and 45 (Fig. 3.6). The enterotoxin genes *sek* and *seq* were harboured by 13.0% (36/276) of isolates, the majority of which belonged to either CC1 or CC8, while 6.9% of isolates (19/276) belonging to CC5, CC8, CC59 and CC80 harboured *seb* (Fig. 3.6). The enterotoxin genes *seh* was present in 26.8% of isolates (74/276) and all of which were assigned to CC1 while *sec* and *sel* were each harboured by 12.0% (33/276), predominantly represented by CC22 but also including CC1, CC5, CC30, CC45 and CC88 (Fig. 3.6). Various combinations of the enterotoxin genes *sed, sej* and *ser* were detected in 19.6% (54/276) of isolates, predominantly within CC5 (Fig. 3.6). The ACME-*arc* genes were detected in only 1.5% (4/276) of isolates, two of which were CC8-MRSA-IV/t008 while the remaining isolates were ST22-MRSA-IV/t14499 and CC45-MRSA-V/t563. Exfoliative toxin D (*etd*) was detected among 7.2% (20/276) of isolates, 19 of which were ST779-

MRSA- ΨSCC*mec-fusC*-SCC-SCC$_{CRISPR}$ while the remaining isolate was ST80-MRSA-IV. Exfoliative toxin A (*etA*) was detected in one CC5 isolate (0.4%), while *tst* was present in 6.5% (18/276) of the isolates belonging to CC1, CC5, CC22, CC30 and CC45 (Fig. 3.6).

3.3.2.3 Antimicrobial resistance genes and phenotypes

Details of the antimicrobial resistance genes detected among the 276 isolates are shown in Fig. 3.7 and Table 3.3. The beta-lactamase gene *blaZ/blaZ$_{XI}$,* was the most frequently detected antimicrobial resistance gene and was present in 96% (265/276) of isolates, followed closely by *sdrM* encoding a general efflux pump (87.3%, 241/276). The macrolide, lincosamide and streptogramin B (MLS$_B$) resistance genes *erm*(A), *erm*(B), *erm*(C)*, lnu*(A), and *mph*(C) were detected in various combinations in 59.8% (165/276) of isolates from all CCs except CC6, CC80, CC97 and CC130 but *erm*(B) was only detected in a single CC398 isolate and *lnu*(A) was only detected in a single CC1 isolate (Fig. 3.7). Tetracycline resistance genes *tet*(K) and *tet*(M) were detected in 21.3% (59/276) and 8.3% (23/276) of isolates, respectively and predominantly among those belonging to CC1 (69.5%, 41/59) and CC398 (52.2%, 12/23). The aminoglycoside resistance genes *aacA-aphD, aadD* and *aphA3* were harboured by 16.3% (45/276), 6.5% (18/276) and 25.7% (71/276) of isolates, respectively. The *aacA-aphD* genes were more frequently detected in CC5 (57.8%, 26/45) and CC8 (22.2%, 10/45) isolates while *aphA3* was more common among CC1 isolates (81.7%, 58/71). The *mupA/ileS2* gene encoding high-level mupirocin resistance was detected in 21.0% (58/276) of isolates, the majority of which were CC1 (CC1-MRSA-IV/t127) (77.5%, 45/58). The *fusB* and *fusC* genes were detected in 2.9% (8/276) and 19.2% (53/276) of isolates in four (CC1, CC8, CC45 and CC59) and six (CC1, CC5, CC8, CC30, CC45 and CC779) CCs, respectively (Fig. 3.7). The *qacA* and *qacC* genes encoding resistance to quaternary ammonium compounds were detected in 20.7% (57/276) and 5.4% (15/276) of isolates, respectively, while the mercury resistance genes

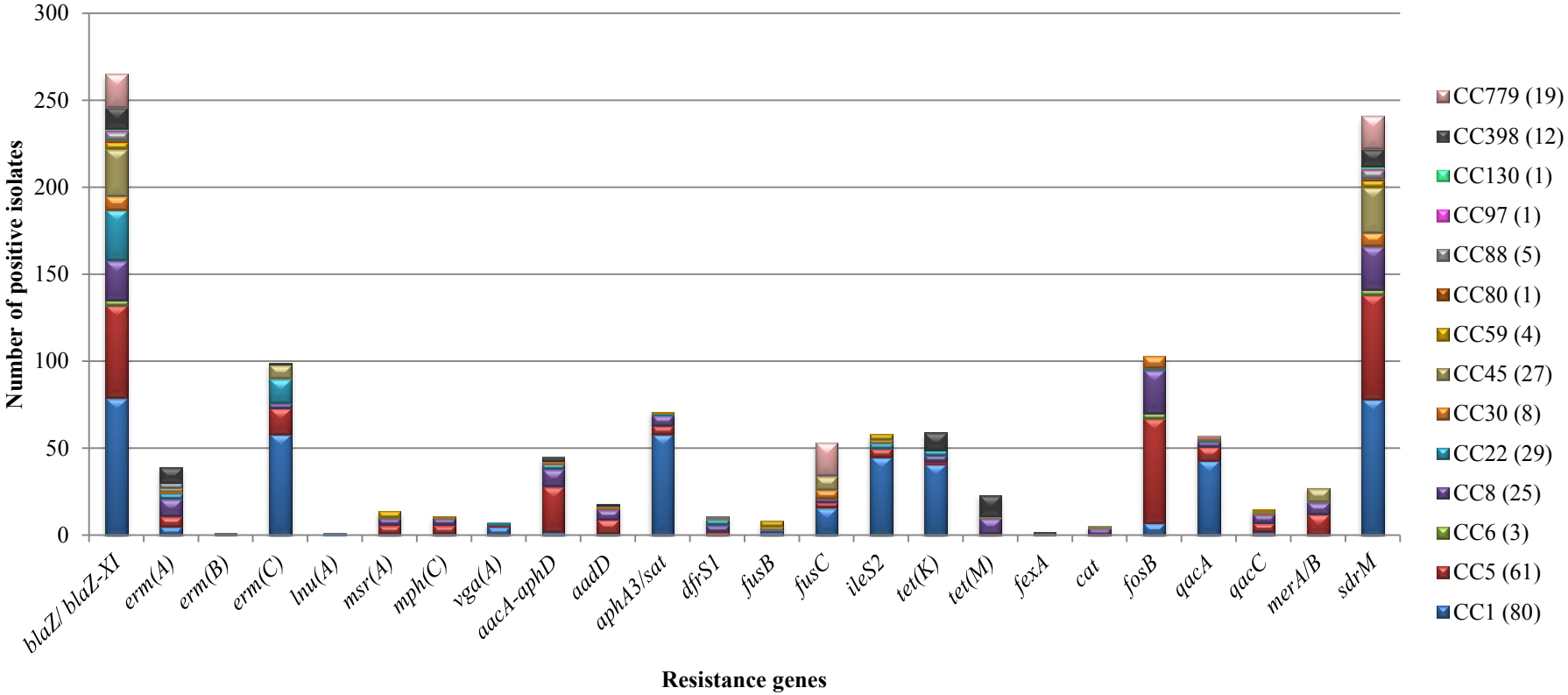

Figure 3.7 Antimicrobial resistance genes identified by DNA microarray profiling in MLST clonal complexes (CCs) among 276 sporadically occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015. The total number of isolates associated with each CC is shown in parentheses.

merA and *merB* was detected in 9.8% (27/276) of isolates. Chloramphenicol resistance, encoded by *fexA* and *cat,* was detected in 2.5% (7/276) of isolates with *fexA* limited to CC398 while *cat* was identified among CC8 and CC45 isolates. Resistance to trimethoprim encoded by *dfrS1* was detected in 4.0% (11/276) of isolates belonging to CC5 (*n* = 2), CC8 (*n* = 4), CC22 (*n* = 3), CC45 (*n* = 1) and CC88 (*n* = 1) (Fig. 3.7).

Three or more resistance genes that encoded resistance to clinically relevant classes of antimicrobial agents including aminoglycosides, macrolides-lincosamides, tetracycline, fusidic acid and mupirocin were detected among 97.8% (270/276) of isolates and included isolates from all CCs with the exception of CC6, CC80, ST97 and CC130 (Fig. 3.8). Overall, CC5, CC8 and CC1 isolates carried the greatest number of resistance genes encoding resistance to ten (CC5 and CC8) and nine (CC1) classes of agents including aminoglycosides, beta-lactams, trimethoprim, macrolides and lincosamides, fusidic acid and chloramphenicol (Fig. 3.8).

The phenotypic antimicrobial resistance profiles of the 276 MRSA isolates are shown in Fig. 3.9. All isolates exhibited phenotypic resistance to at least one antimicrobial agent tested with 94.9% (262/276) exhibiting resistance to two or more classes of antimicrobial agents i.e. exhibited multidrug resistance. For the majority of isolates and antimicrobial resistance genes detected, phenotypic resistance correlated with the presence of a corresponding resistance gene(s) present on the DNA microarray. In addition, phenotypic resistance was determined against several antimicrobial agents for which the mechanism of resistance is due to chromosomal mutations such as ciprofloxacin (22.5%, 62/276) and rifampicin (2.6%, 7/276) (Fig. 3.9). Discrepancies detected among the isolates in terms of the presence of a specific antimicrobial resistance gene by DNA microarray analysis and PCR despite a susceptible profile to the specific antimicrobial agent arose in relation to ethidium bromide and the *qac* genes (16%, 46/276), mercury and *merA* and

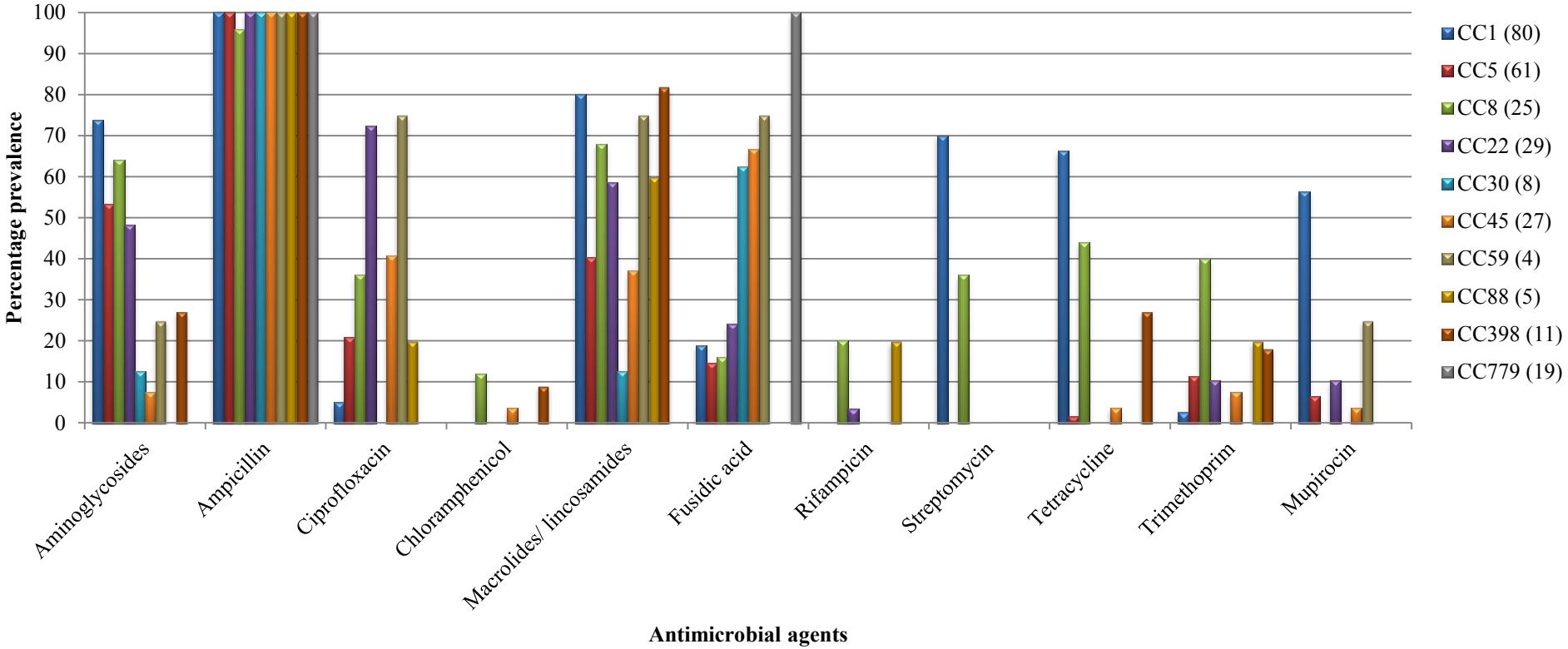

Figure 3.8 Percentage prevalence of resistance genes present in each clonal complex (CC) among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015. The total number of isolates associated with each MLST CC is shown in parentheses. Three additional CCs (CC6, CC80, CC97 and CC130) representing six isolates are excluded from the chart as each isolate lacked resistance genes encoding resistance to three or more different classes of clinically relevant antibiotics. Resistance genes detected encode resistance to ampicillin (*blaZ/blaZ*$_{XI}$), macrolides and lincosamides (*erm*(A), *erm*(B), *erm*(C), *lnu*(A), *mph*(C)), virginamycin (*vga),* aminoglycosides (*aac-aphD, aadD, aphA3*), trimethoprim (*dfrS1),* fusidic acid (*fusB/C*), mupirocin (*mupA*), chloramphenicol (*fexA*/*cat*).

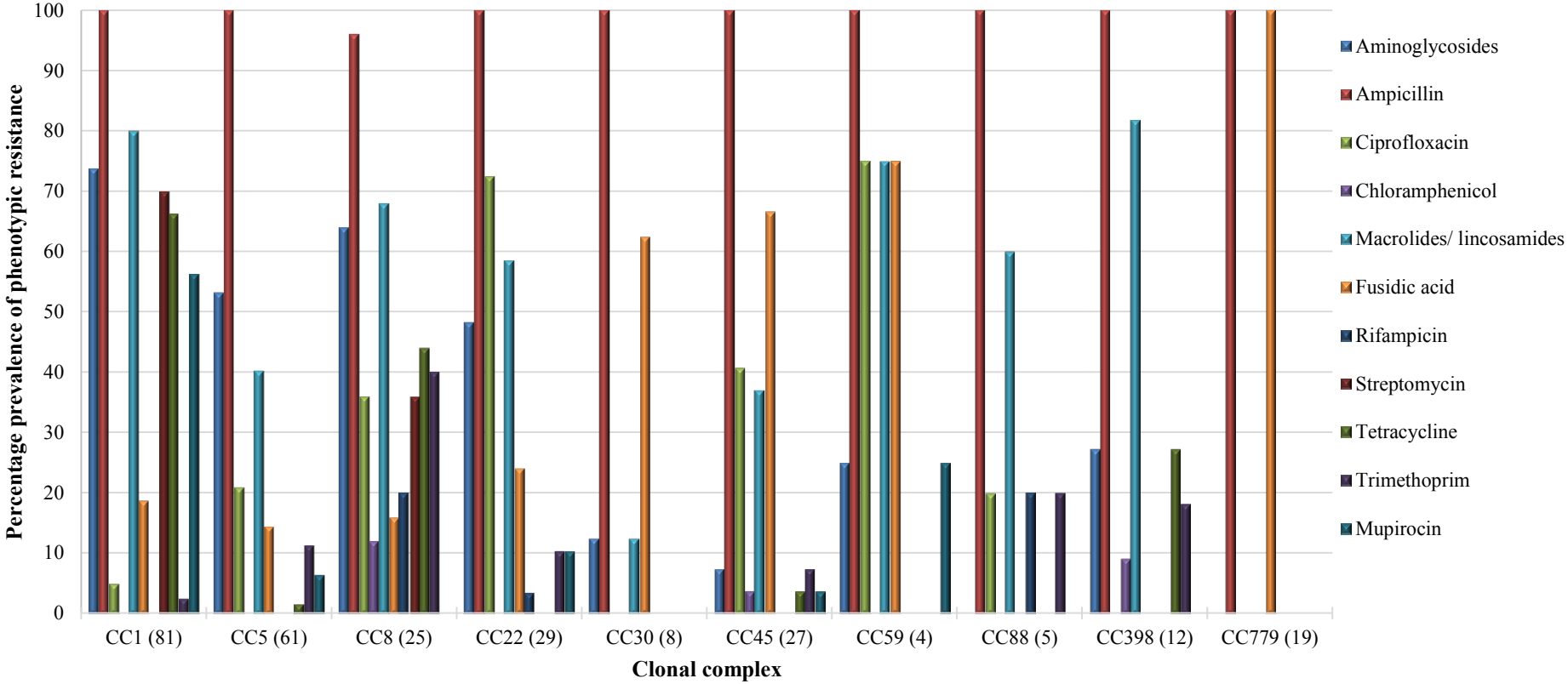

Figure 3.9 Percentage prevalence of phenotypic resistance exhibited by isolates in each clonal complex (CC) among 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015. The total number of isolates associated with each MLST CC is shown in parentheses. Three additional CC (CC6, CC80, CC97 and CC130) representing six isolates are excluded from the chart as they did not express phenotypic resistance to less than three different classes of clinically relevant antibiotics.

merB (2.5%, 7/246), mupirocin and *ileS2* (1.5%, 4/276), tetracycline and *tet*(K) (1.4%, 4/276) and gentamicin and *aacA-aphD* (0.7%, 2/276).

3.3.2.4 Molecular characterisation of ST779-MRSA-ΨSCC*mec* & *fusC*-SCC-SCC$_{CRIPSR}$ isolates

Nineteen sporadically-occurring MRSA isolates recovered between 2001 and 2015 were assigned to *spa* type t878 and t11210 and CC779-ST779. Isolates associated with CC779 are not widely characterised in the literature and so these isolates were further investigated.

All 19 ST779-MRSA-ΨSCC*mec* & *fusC*-SCC-SCC$_{CRIPSR}$ isolates were recovered from patients ranging in age from 1 day to 84 years and were from BSIs (n = 8) or from routine screening sites, with the exception of one isolate that was recovered during a post mortem of a stillborn baby (M07/0307) and one recovered from a patient attending a dermatology clinic (M11/0208) (Table 3.4). The 19 isolates harboured *blaZ*, *fusC* and *sdrM* and exhibited phenotypic resistance to ampicillin and fusidic acid (Table 3.4). In addition, 21.0% (4/19) of isolates also exhibited resistance to cadmium acetate. Virulence genes detected included the *etD* and *edinB*, clumping factor B (*clfB)* and IEC type B (*sak, chp* and *scn*) and six isolates (31.6%) also carried the enterotoxin genes *sed, sej* and *ser* and four harboured *seb* (Table 3.4).

All isolates harboured the SCC*mec*-associated genes *mecA* and *ugpQ* and gave negative and/or ambiguous results by DNA microarray analysis for the *ccrAA* and *ccrC* genes. Recently, another study from this laboratory identified a novel composite island among CC779 consisting of three domains including a Ψ chimeric SCC*mec* element which carried the *mecA* and *ugpQ* genes similar to class C *mec* along with *fusC* but lacked *ccr* genes, an additional SCC element carrying a novel *ccrAB4* allele and a second SCC

Table 3.4 Epidemiological, phenotypic and genotypic characteristics of ST779-MRSA-ΨSCC*mec*-SCC-SCC$_{CRIPSR}$ isolates recovered from patients in Irish hospitals between 2007 and 2013

Isolate no.	Year of isolation	Age[a]	Clinical details[b] (sex)	Antimicrobial resistance[c]	SCC*mec* genes[d]	Antimicrobial resistance genes[d]	Virulence-associated genes[d]
M09/0295	2001	49 y	Screening sample (male)	Ap, Fd	*mecA, ugpQ, ccrAA, ccrC, ccrB4*	*blaZ, fusC, sdrM*	*seb, sed, sej, ser, etD, edinB, clfB, sdrD,* IEC B
M07/0307	2007	Stillborn	Stillborn baby postmortem (unknown)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB, clfB, sdrD,* IEC B
E4298	2009	25 y	BSI (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB,* IEC B
M09/0302	2009	58 y	Screening sample (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB,* IEC B
E4217[d]	2009	59 y	BSI (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB, clfB, sdrD,* IEC B
E4233[d]	2009	45 y	BSI (female)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*seb, sed, sej, ser, etD, edinB, clfB, sdrD,* IEC B
E4709[d]	2010	54 y	BSI (female)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*sed, sej, ser, etD, edinB, clfB, sdrD,* IEC B
M11/0118	2011	30 y	Screening sample, mother of baby from whom M11/0114 was recovered (female)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*seb, etD, edinB, clfB, sdrD,* IEC B

Continued overleaf

Table 3.4 continued

Isolate no.	Year of isolation	Age[a]	Clinical details[b] (sex)	Antimicrobial resistance[c]	SCC*mec* genes[d]	Antimicrobial resistance genes[d]	Virulence-associated genes[d]
M11/0114[d]	2011	5 d	Screening sample, baby of patient from whom M11/0118 was recovered (unknown)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB, clfB, sdrD,* IEC B
E4433[d]	2010	84 y	BSI (unknown)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD*, *edinB*, *clfB*, IEC B
E4972[d]	2012	65 y	BSI (unknown)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD*, *edinB*, *clfB*, IEC B
M12/0167[e]	2012	75 y	Screening sample (female)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB, clfB,* IEC B
M13/0482[d]	2013	4 d	Screening sample (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD*, *edinB,* IEC B
M13/0483[d]	2013	1 d	Screening sample (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD*, *edinB*, *clfB*, IEC B
M13/0484[d]	2013	1 d	Screening sample (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD*, *edinB*, *clfB*, IEC B
M08/0422	2008	24 y	Screening sample (female)	Ap, Cd, Fd	*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*etD, edinB,* IEC B
E4449[d]	2010	39 y	BSI (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*sed, sej, ser, etD, edinB, clfB, sdrD,* IEC B
E4550[d]	2010	55 y	BSI (female)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*seb, sed, sej, ser, etD,edinB, clfB, sdrD,* IEC B

Continued overleaf

Table 3.4 continued

Isolate no.	Year of isolation	Age[a]	Clinical details[b] (sex)	Antimicrobial resistance[c]	SCC*mec* genes[d]	Antimicrobial resistance genes[d]	Virulence-associated genes[d]
M11/0208[d]	2011	18 y	Dermatology clinic (male)		*mecA, ugpQ, ccrAA, ccrC, ccrB4*		*sed, sej, ser, etD, edinB, sdrD, clfB,* IEC B

[a]d; days old, y; years of age.

[b]BSI; blood stream infection.

[c]Antimicrobial resistance was determined against a panel of 25 antimicrobial agents including amikacin, Ap; ampicillin (Ap), cadmium acetate (Cd), chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid (Fd), gentamicin, kanamycin, lincomycin, linezolid, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, propramide isoethionate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin, trimethoprim and vancomycin.

[c]SCC*mec,* antimicrobial resistance and virulence genes were determined using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH).

[d]*ccrAA, ccrAB4* and/or *ccrC* yielded negative or ambiguous results for these isolates using the DNA microarray and the presence of these genes was confirmed by PCR.

[e]All isolates with the exception of M12/0167 were assigned t878 while M12/0167 was assigned t11201 using Ridom StaphType software

N/A; not applicable

element which carried a novel *ccrC* allele has been described among isolates of this (Kinnevey *et al.,* 2013). Upon investigation by PCR the 18 isolates were found to harbour *the ccrAA, ccrB4* and *ccrC* genes (Table 3.4). Sequencing of the *ccrAB4* and *ccrC* amplimers from these 13/19 isolates showed genes identical to each other and those in isolate M06/0171, a ST779 MRSA isolate which has previously been shown to harbour this pseudo SCC*mec* element with *ccrA4*, *ccrB4*, and *ccrC* genes that exhibited 93 – 95% amino acid sequence similarity to previously reported MRSA *ccrA4, ccrB4* and *ccrC* (Kinnevey *et al.,* 2013).

3.3.2.5 Molecular epidemiology of CC398 *S. aureus* isolates recovered from animals and humans in Ireland

The 12 CC398-MRSA isolates characterised in sections 3.3.1.1-3.3.1.2 above were the first CC398-MRSA isolates recovered from humans in Ireland. To further investigate the emergence of this strain in Ireland an additional 11 MRSA isolates recovered from pigs on the farms where some of the human CC398-MRSA isolates (9/12) were recovered were also investigated. Additionally a further 11 MSSA comparator isolates recovered from humans (n = 3) and animals (n = 8) were also included in this section of the study (Table 3.5). All isolates were investigated by DNA microarray analysis and assigned to CC398.

3.3.2.5.1 CLONAL COMPLEX 398 MRSA

The 23 CC398 MRSA isolates were from five epidemiologically distinct incidents, two of which consisted of animal and human isolates (Incidents 2 & 3, Table 3.5). All MRSA isolates were assigned to *agr* type I and to two closely related *spa* types, t011 (82.6%, 19/23) and t034 (17.4%, 4/23). The MRSA isolates harboured either SCC*mec* types V_T (5C2 & 5 i.e. type 5 ***ccr*** genes (*ccrC1* allele 2), class C2 *mec* and class 5 *ccr* genes (*ccrC1* allele 8); 19/22, 87%, 20/23) or IVa (2B i.e. type 2 *ccr* genes (*ccrAB2*) and

Table 3.5 Epidemiological, phenotypic and genotypic characteristics of CC398 methicillin-resistant and methicillin-susceptible *S. aureus* (MRSA and MSSA) identified in Ireland from animals and humans

Methicillin resistance phenotype	Incident no.	Year	No. of isolates	Host[a]	Sample site/clinical presentation[a]	*spa* type[b]	SCC*mec* type	IEC type[c]	Antimicrobial resistance[d]	Antimicrobial resistance genes[c]
MRSA	1	2011	1	Human	Nursing home resident nasal swab	t011	IVa	Negative (*hlb*+)	Ap, Gn, Kn, Tb, Te, Tp	*blaZ, aacA-aphD, tet*(M)*, dfrK*
MRSA	2	2012	2	Horse & human	Horse umbilical abscess; veterinarian nasal swab	t011	IVa	B (*sak*, *chp* & *scn*)	Ap, Er, Gn, Ln, Kn, Tb, Te, Tp	*blaZ, erm*(C), *aacA-aphD*, *tet*(M), *dfrG* & *dfrK*
MRSA	3	2012 & 2013	18	Pig (*n* = 9) & human (*n* = 9)	Pig joint abscess (1); Pig nasal swab (1),	t034	Vt	Negative (*hlb*+)	Ap, Er, Ln, Sp, Te, Tp	*blaZ, erm*(A), *tet(*K), *tet(*M), *dfrG, spc*
					Pig nasal swab (1),	t034	Vt	Negative (*hlb*+)	Ap, Er, Ln, Sp, Te, Tp	*blaZ, erm*(A), *tet(*M), *dfrG, spc*
					Pig nasal swab-(1); Pig farmer nasal swab (8)	t011	Vt	Negative (*hlb*+)	Ap, Er, Ln, Sp, Te	*blaZ, erm*(A), *tet*(K), *tet*(M), *spc*
					Pig nasal swab (2)	t011	Vt	Negative (*hlb*+)	Ap, Ch, Er, Kn, Ln, Gn, Nm, Tb, Te, Tp	*blaZ, erm*(B), *aacA-aphD, aadD, tet*(K), *tet*(M), *fexA*

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Table 3.5 continued

Methicillin resistance phenotype	Incident no.	Year	No. of isolates	Host[a]	Sample site/clinical presentation[a]	*spa* type[b]	SCC*mec* type	IEC type[c]	Antimicrobial resistance[d]	Antimicrobial resistance genes[c]
					Pig farmer nasal swab (1)	t011	Vt	Negative (*hlb*+)	Ap, Ch, Er, Kn, Ln, Nm, Tb, Te, Tp	*blaZ, erm*(B), *aadD, tet*(K), *tet*(M), *fexA, tet*(L), *dfrG, dfrK*
					Pig nasal swab (1)	t011	Vt	Negative (*hlb*+)	Ap, Ch, Er, Ln, Nm, Tb, Te, Tp	*blaZ, erm*(A), *erm*(B), *aadD, tet*(M), *tet*(K) *fexA, tet*(L), *dfrG, dfrK*
					Pig nasal swab (1)	t011	Vt	Negative (*hlb*+)	Ap, Er, Gn, Kn, Ln, Nm, Sp, Tb, Te, Tp	*blaZ, erm*(A), *erm*(B), *aadD, tet*(M), *tet*(L), *dfrG, dfrK, spc*
					Pig nasal swab (1)	t011	Vt	Negative (*hlb*+)	Ap, Er, Kn, Ln, Nm, Sp, Te, Tb, Tp	*blaZ, erm*(A), *erm*(B), *aadD, tet*(K), *tet*(M), *dfrG, dfrK, tet*(L), *spc*
MRSA	4	2013	1	Human	Child skin abscess with family contact working with pigs	t034	Vt	Negative (*hlb*+)	Ap, Er, Ln, Sp, Te, Tp	*blaZ*, *erm*(A), *tet*(K), *tet(*M), *dfrG*, *spc*
MRSA	5	2014	1	Horse	Not known	t011	IV	Negative (*hlb*+	Ap, Gn, Kn, St, Tb, Te, Tp	*blaZ, aacA-aphD, tet*(M),

Continued overleaf

Table 3.5 continued

Methicillin resistance phenotype	Incident no.	Year	No. of isolates	Host[a]	Sample site/clinical presentation[a]	*spa* type[b]	SCC*mec* type	IEC type[c]	Antimicrobial resistance[d]	Antimicrobial resistance genes[c]
										dfrK
MSSA	6	2010	3	Pig	Nasal swabs	t108 (1) t108 (1) t4854 (1)	N/A N/A N/A	Negative Negative Negative	Ap, Cp, Sp, Te Ap, Cp, Er, Ln, Sp, Te Ap, Er, Ln, Sp, Te	*blaZ, tet*(M), *spc* *blaZ, erm*(C), *tet*(M), *spc* *blaZ, erm*(C), *tet*(M), *spc*
MSSA	7	2010	5	Pig	Nasal swabs	t034	N/A	Negative	Ap, Cp, Er, Ln, Sp, Te	*blaZ, erm*(A), *tet*(M*), spc*
MSSA	8	2014	1	Human	BSI	t571	N/A	C (*chp* & *sc*n)	Ap, Er	*blaZ*
MSSA	9	2014	1	Human	BSI	t011	N/A	D (*sea, sak* & *scn*)	None	None
MSSA	10	2016	1	Human	Recurrent abscesses in a 26 year old male living in rural Ireland	t034	N/A	B (*sak, chp, scn)*, *pvl*	Ap, Er, Ln, Sp	*blaZ, erm*(A), *spc*

[a]The number of isolates is only indicated when not all isolates within a given incident exhibited the same characteristic.

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Table 3.5 continued

[b]*spa* types were assigned to each isolate based on the repeat succession pattern using Ridom Staph Type software. Repeat successions recognised were t011; 08-16-02-25-34-24-25, t034; 08-16-02-25-02-25-34-24-25, t108; 08-16-02-25-24-25, t571; 08-16-02-25-02-25-34-25, t4854; 08-16-02-25-24.

[c]Antimicrobial resistance and virulence genes were detected using the *S. aureus* Genotyping Kit 2.0 (Alere, Germany) while *dfrG, dfrK, tet*(L) and *spc* were detected by PCR.

[d]Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 25 antimicrobial agents including amikacin, ampicillin (Ap), cadmium acetate; chloramphenicol, ciprofloxacin (Cp). erythromycin (Er), ethidium bromide, fusidic acid, gentamicin (Gn), kanamycin (Kn), lincomycin (Ln) linezolid, mercuric chloride, mupirocin, neomycin (Nm), phenyl mercuric acetate, propramide isothionate, rifampicin, spectinomycin (Sp), streptomycin (St), sulphonamide, tetracycline (Te), tobramycin (Tb), trimethoprim (Tp) and vancomycin.

Abbreviations: BSI, bloodstream infection; N/A, not applicable; IEC, immune evasion complex.

class B *mec*, 13%, 3/23). The majority lacked the IEC genes (91.3%, 21/23) and carried multiple genes encoding resistance to various combinations of antimicrobial agents (Table 3.5). The majority of isolates harboured one or more genes encoding resistance to beta lactams (100%, 23/23, *blaZ*), tetracycline (100%, 23/23, *tet*(M), *tet*(K), *tet*(L)) and MLS_B compounds (91.3%, 21/23: *erm*(A), *erm*(B), *erm*(C)), whilst genes encoding resistance to spectinomycin (65.2%, 15/23, *spc*), trimethoprim (47.8%, 11/23, *dfrG*, *dfrK*), aminoglycosides (39.1%, 9/23, *aacA-aphD*, *aadD*), and, less commonly chloramphenicol (17.4%, 4/23, *fexA*), were also detected (Table 3.5).

The first CC398 MRSA isolate identified in Ireland (Incident 1, Table 3.5) was recovered from an elderly patient in nursing home in 2011, was *spa* type t011 and harboured SCC*mec* IVa. Similar to the majority of other t011 isolates identified, this isolate lacked IEC genes but differed in the antimicrobial resistance genes that it harboured; although this isolate carried *blaZ*, *aacA-aphD*, *tet*(M) and *dfrK* it lacked additional trimethoprim (*dfrG*), tetracycline (*tet*(L) & *tet*(K)) and aminoglycoside (*aadD*) resistance genes detected in some other t011 isolates as well as *fexA*, *spc* and *erm* genes (Table 3.5).

Incident 2 involved two indistinguishable CC398-MRSA isolates recovered in 2012 from an umbilical abscess in a foal admitted to the UCD veterinary hospital and a nasal swab of one of the attending clinicians. Similar to the isolate in Incident 1, these isolates were assigned to *spa* type t011 and SCC*mec* IVa, but harboured IEC genes and additional resistance determinants (*erm*(C) & *dfrG*) (Table 3.5).

The majority of CC398-MRSA isolates belonged to Incident 3, which consisted of 18 isolates from two farms in 2012 and 2013. Farm 1 isolates were recovered from a pig joint abscess and from nasal swabs from other pigs which, prior to the recovery of these isolates,

was restocked with gilts from Germany as well as from farm workers (*n* = 4). Farm 2 isolates were recovered from farm workers (*n* = 5) on a farm which had been restocked with pigs from Farm 1. All isolates within Incident 3 harboured SCC*mec* V_T and lacked IEC genes. Three isolates, from a pig joint abscess and two pig nasal swabs, were *spa* type t034, carried *blaZ*, *erm*(A), *tet*(M), *dfrG* and *spc* and differed only in the absence of *tet*(K) in one of the pig nasal swab isolates (Table 3. 5). Interestingly, the CC398 MRSA isolate from Incident 4 was recovered from a child with a skin abscess with a family contact working with pigs and although no epidemiological link between the isolates was identified, it was indistinguishable from 2/3 t034 pig isolates in Incident 3 (Table 3.5).

The remaining isolates within Incident 3 (n=15) were *spa* type t011 and six different combinations of antimicrobial resistance genes were identified with many isolates harbouring multiple genes encoding resistance to MLS_B compounds, tetracycline and trimethoprim as well as aminoglycoside resistance genes, *fexA* and *spc* (Table 3.5). One isolate from a pig nasal swab was indistinguishable from 8/9 pig farmer nasal swabs and two pig nasal swabs were indistinguishable (Table 3.5).

The final CC398 MRSA isolate was recovered from a horse in 2014 (Incident 5) and, similar to the human isolate in Incident 1, it was assigned to *spa* type t011, SCC*mec* IVa, was IEC negative and harboured *blaZ*, *aacA-aphD*, *tet*(M) and *dfrK*. However, in contrast to the Incident 1 isolate, this isolate exhibited streptomycin resistance (Table 3.5).

3.3.2.5.2 Clonal Complex 398 MSSA

Five distinct incidents of CC398 MSSA, three from humans (two BSIs and one skin swab) and two from nasal carriage in pigs (eight isolates), were also investigated in the current study (Table 3.5). Isolates from animals and humans were distinguished from each other in terms of *spa* types, the presence or absence of IEC genes and antimicrobial

resistance genes and phenotype (Table 3.5). Each of the three isolates recovered from humans isolates were also distinct from each other; two were recovered from patients in two different hospitals in 2014 while the third was recovered from a patient attending a GP in the community, each exhibited different *spa* types (t034, t571 and t011), harboured different combinations of IEC genes and exhibited different antimicrobial resistance profiles with one susceptible to all antimicrobial agents tested. One isolate, recovered from a patient experiencing recurrent skin abscesses also harboured *pvl* (Table 3.5).

In contrast, the pig CC398 MSSA isolates were recovered from nasal swabs of pigs on two farms in 2010 and these isolates lacked IEC genes and harboured genes encoding resistance to multiple antimicrobial agents (Table 3.5). Each pig CC398 MSSA incident (Incident 6 and 7, Table 3.5) consisted of multiple isolates. In addition, each incident occurred on a different farm and isolates in Incident 6 were phenotypically and genotypically distinct from isolates in Incident 7 (Table 3.5). The three isolates within Incident 6 exhibited the same or very closely related *spa* types that differed by the absence of one repeat unit only (t108 & t4854) and harboured similar but variable resistance genes including *blaZ, tet*(M) and *spc* with two isolates also harbouring *erm*(C) and two exhibiting ciprofloxacin resistance (Table 3.5). Isolates within Incident 7 exhibited a different *spa* type (t034) to those in Incident 6 and although they harboured similar resistance genes and exhibited ciprofloxacin resistance, the Incident 7 isolates harboured *erm*(A) and not *erm*(C). These t034 MSSA isolates were similar to the t034 MRSA isolates but lacked *dfrG* and *tet*(K) (Table 3.5).

3.3.3 Molecular epidemiology of multidrug-resistant ST772-MRSA-V recovered in Ireland

DNA microarray profiling assigned 38 isolates, predominantly recovered from patients, with just one isolate from the hospital environment, in Irish hospitals and the community between 2009 and 2014 to CC1 and ST772 with SCC*mec* type V, the details of which are shown in Table 3.6. The majority of isolates (33/38, 86.9%) were assigned to *spa* type t657 with the remaining isolates assigned to *spa* types t345, t3786, t1839, t3798 and t8503.

All isolates exhibited phenotypic resistance to ampicillin and aminoglycosides (gentamicin, neomycin, tobramycin and kanamycin) and harboured the associated antibiotic resistance genes including *blaZ, aacA-aphD* and *aphA3*. One isolate (M13/0625) was susceptible to erythromycin while all others exhibited resistance and harboured *msr*(A) and *mph*(C). All isolates also harboured the *fosB* gene encoding resistance to fosfomycin along with the general efflux pump *sdrM.* The enterotoxin genes *sec* and *sel*, and *egc* were harboured by all isolates along with the IEC gene *scn*. Along with *scn* 33/38 isolates (86.8%) also harboured the additional IEC gene *sea* (Table 3.6). All except two isolates (94.7%, 36/38) harboured *pvl.* The remaining two isolates (M12/0155 and M13/0625) which lacked *pvl,* along with M11/0426, M11/0437 and M12/0006, also lacked *sea.* All isolates harboured genes encoding clumping factor, bone sialoprotein-binding protein, collagen binding adhesion, cell surface elastin binding protein and fibronectin binding protein.

Among the isolates, 44.7% (17/38) were associated with NICU clusters in two separate maternity hospitals (Table 3.6). Cluster 1 occurred over a nine-month period during which time 11 isolates were recovered. The initial isolate (M10/0338) was

Table 3.6 Epidemiological characteristics and antimicrobial resistance and virulence genes detected among 38 ST772-MRSA-V isolates recovered from patients and their environment in hospital and in the community in Ireland between 2009 and 2014

Isolate number (year of isolation)	MRSA-positive site	Ethnicity	Relevant clinical information	CA/HCA (age)[a]	*spa* type[b]	Antimicrobial resistance profile[c]	Antimicrobial resistance genes[d]	Virulence associated genes[d]
M10/0338 (2010)	Nasal, umbilicus, perineum	Irish	NICU cluster 1[e]	HCA (7 days)	t657	Ak (I), Ap, Cp, Er (I), Gn, Kn, Nm, Tb, Tp	*blaZ, msr*(A)[f], *mph*(C)[f], *aacA-aphD, aphA3 & sat, fosB, sdM*	*lukF/S*-PV[g], *sea*[f], *sec* & *sel*, *egc*[h] *scn, bbp, clfA, clfB, cna, ebh, ebpS, eno, fib, fnbA, fnbB, map, icaA, icaC, icaD, sdrC, sdrD, vwb, sasG*
M10/0342 (2010)	Nasal	Irish	NICU cluster 1	HCA (15 days)	t657	Ak (I), Ap, Cd, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0349 (2010)	Nasal, umbilicus	Irish	NICU cluster 1	HCA (20 days)	t657	Ak (I), Ap, Cd, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0082 (2011)	Nasal, umbilicus	Irish	NICU cluster 1	HCA (3 days)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0085 (2011)	Nasal, umbilicus	Irish	NICU cluster 1	HCA (24 days)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0107 (2011)	Umbilicus	Irish	NICU cluster 1	HCA (9 days)	t657	Ak (I), Ap, Cd, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0120 (2011)	Nasal, umbilicus	Irish	NICU cluster 1	HCA (9 days)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0092 (2011)	Nasal, axilla, groin	Indian	NICU cluster 1- Healthcare worker working in NICU, probable index case	HCA (29 y)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0097 (2011)	Unknown	Irish	NICU cluster 1- Healthcare worker working in NICU	HCA (35 y)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		

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Table 3.6 continued

Isolate number (year of isolation)	MRSA-positive site	Ethnicity	Relevant clinical information	CA/HCA (age)[a]	*spa* type[b]	Antimicrobial resistance profile[c]	Antimicrobial resistance genes[d]	Virulence associated genes[d]
M11/0167 (2011)	Nasal, throat	Indian	NICU cluster 1- Family contact of colonised NICU staff member, child of probable index case	HCA (16 months)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0093 (2011)	Environment	N/A	NICU cluster 1- Horizontal surface in NICU	N/A	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0035 (2011)	Unknown	Indian	Pustule on back	CA (9 y)	t657	Ap, Er (I), Gn, Kn, Nm, Tb, Tp		
M09/0243 (2009)	Unknown	Unknown	In patient with no previously positive MRSA screen	HA (82 y)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0045 (2010)	Unknown	Unknown	Unknown	CA (29 y)	t657	Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0203 (2010)	Eye	Unknown	Unknown	HA (96 y)	t657	Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0131 (2010)	Unknown	Indian	Unknown	CA (28 y)	t657	Ap, Cd, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0361 (2010)	Ear	Indian	Unknown	CA (18 months)	t657	Ak (I), Ap, Cd(I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M10/0033 (2010)	Unknown	Irish with history of travel to India	History of recurrent boils and abscesses	CA (22 y)	t345	Ak (I), Ap, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		

Continued overleaf

Table 3.6 continued

Isolate number (year of isolation)	MRSA-positive site	Ethnicity	Relevant clinical information	CA/HCA (age)[a]	*spa* type[b]	Antimicrobial resistance profile[c]	Antimicrobial resistance genes[d]	Virulence associated genes[d]
E4837 (2011)	Blood culture	N/A	Unknown	N/A (73 y)	t3798	Ap, Cd(I), Cp, Er (I), Gn(I), Kn, Nm, Tb, Tp		
E4944 (2011)	Blood culture	N/A	Unknown	N/A (3 y)	t657	Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0267 (2011)	Leg abscess	N/A	Ex-intravenous drug user	CA (34 y)	t657	Ak (I), Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0294 (2011)	Groin	Irish	NICU cluster 2[e]	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0295 (2011)	Blood culture	Irish	NICU cluster 2	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0299 (2011)	Eye	Irish	NICU cluster 2	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0415 (2011)	Environment	N/A	NICU cluster 2	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0426 (2011)	Environment	N/A	NICU cluster 2	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M11/0437 (2011)	Environment	N/A	NICU cluster 2	N/A	t657	Ak (I), Ap, Cd (I), Cp ,Er (I), Gn, Kn, Nm, Tb, Tp		
M12/0006 (2011)	Wrist swab	Indian	Severe cellulitis & abscesses on wrist. Partner of healthcare worker	CA	t657	Ap, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M12/0089 (2012)	Wound swab, episiotomy	Indian	Unknown	N/A (31 y)	t657	Ak (I), Ap, Cd, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		

Continued overleaf

Table 3.6 continued

Isolate number (year of isolation)	MRSA-positive site	Ethnicity	Relevant clinical information	CA/HCA (age)[a]	*spa* type[b]	Antimicrobial resistance profile[c]	Antimicrobial resistance genes[d]	Virulence associated genes[d]
M12/0130 (2012)	N/A	Indian	Family contact of M12/0089	N/A (31 y)	t657	Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M12/0155 (2012)	N/A	Indian	Family contact of M12/0089	N/A (56 y)	t657	Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M12/0112 (2012)	N/A	N/A	Abscess	N/A (24 y)	t657	Ak (I), Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M12/0186 (2012)	Wound swab	Indian	Healthcare worker in Ireland, recent travel to India	N/A (32 y)	t657	Ak (I), Ap, Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M13/0545 (2013)	N/A	Indian	Unknown	N/A (35 y)	t657	Ap, Cd (I), Cp, Er (I), Gn, Kn, Nm, Tb, Tp		
M13/0625 (2013)	N/A	Irish	Unknown	N/A (83 y)	t1839	Ap, Gn, Kn, Tb, Tp		
M14/0131 (2014)	Nasal swab	Indian	Family history of MRSA	N/A (38 y)	t3387	Ap, Cd (I), Cp, Er, Gn, Kn, Nm, Tb, Tp		
M14/0704 (2014)	N/A	Indian	Unknown	N/A (26 y)	t8503	Ak, Ap, Cp, Er, Gn, Kn, Nm, Te, Tb, Tp		
M14/0838 (2014)	N/A	Irish	Fourniers gangrene	N/A (54 y)	t657	Ak, Ap, Cd (I), Cp, Er, Gn, Kn, Nm, Tb, Tp		

[a]CA, community-associated; HCA, healthcare-associated; N/A, not available; y, years of age.

[b]*spa* types were assigned to each isolate based on the repeat succession pattern using Ridom Staph Type software. Repeat successions recognised were t345; 26-23-13-21-17-34-34-33-34, t657; 26-23-13-21-17-34-33-34, t1839; 26-23-13-21-17-34-34-34-33-34, t3387; 26-16-21-17-34-33-34, t3798; 26-23-34-21-17-34-34-33-34, t8503; 26-23-21-17-34-33-34.

Continued overleaf

Table 3.6 continued

[c]Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 25 antimicrobial agents including Ak; amikacin, Ap; ampicillin, Cd; cadmium acetate, chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, ethidium bromide, fusidic acid, gentamicin, Kn; kanamycin, lincomycin; linezolid, mercuric chloride, mupirocin, Nm; neomycin, phenyl mercuric acetate, propramide isoethionate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, Tb; tobramycin, Tp, trimethoprim and vancomycin, I; intermediate resistance.

[d]Antimicrobial resistance and virulence genes identified using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH).

[e]NICU, neonatal intensive care unit. Cluster 1 involved 11 isolates recovered as part of an outbreak investigation in a maternity hospital. Cluster 2 involved six isolates recovered as part of an outbreak investigation in a different maternity hospital.

[f]One isolate (M13/0625) lacked the *msr(A), mph*(C). All other isolates harboured the resistance genes listed.

[g]Two isolates (M12/0155 and M13/0625) lacked the *lukF/S-PV* genes and the *sea* genes. In addition M11/0426, M11/0437 and M12/0006 also lacked the *sea* gene. All other isolates harboured the virulence genes listed.

[h]*egc* enterotoxin gene cluster including *seg, sei, sem, sen, seo* and *seu*.

recovered in October 2010 and, in the following two-week period, a further two isolates (M10/0342 and M10/0349) were recovered from babies in the NICU (Table 3.6). Isolates were recovered from a nasal screening swab of the first baby and from nasal and umbilicus swabs of the second baby. At this time, extensive staff screening and screening of the parents with babies in the unit failed to identify a source and there were no further cases until March 2011 when nasal and umbilical screening swabs from two babies yielded ST772-MRSA-V isolates (M11/0082 and M11/0085) that were indistinguishable to the original isolates recovered (Table 3.6). In May a further two isolates (M11/0107 and M11/0120) were recovered from screening swabs of babies in the unit (Table 3.6). Once again, extensive staff screening was undertaken involving 148 staff members including medical, nursing, midwifery, maternity care assistants, household cleaning staff, radiology staff, social workers and biomedical engineers. In addition environmental screening of horizontal surfaces surrounding the cots of MRSA-positive babies and staff areas were also sampled. On this occasion ST772-MRSA-V was recovered from one environmental sample (M11/0093) along with two healthcare workers (M11/0092 and M11/0097), one of who was of Indian ethnicity (M11/0092) and who had recently returned from India where she had been hospitalised to give birth (Table 3.6). In this incidence both the healthcare worker and her child (M11/0167) were colonised with ST772-MRSA-V strains which were indistinguishable to each other and to all other isolates recovered in this cluster. Attempts to successfully decolonise the healthcare worker failed due to the throat colonisation of the child, who due to his young age, could not be decolonised.

The second cluster involving ST772-MRSA-V also occurred in a NICU in a different hospital (Table 3.6). Here MRSA was recovered from a groin swab, eye swab and blood culture sample from three separate babies along with three environmental

samples however in this incident no source was identified as the index case in the cluster. In addition two isolates (M11/0426 and M11/0437) recovered from environmental swabs during this investigation lacked the *sea* genes which were present in the other environmental isolate (M11/0415) along with those recovered from the patients in the unit (Table 3.6).

While the age of patients from which ST772-MRSA-IV were recovered ranged from several days old to 96 years, the median age of the patients was 34 years. Clinical details were unavailable for 26.3% (10/38) of patients; however for the remaining isolates three patients had systemic infection while the remaining isolates were recovered from nasal, wrist, groin, eye, ear and wound swabs (Table 3.6). Thirteen patients (34.2%) were Indian while an additional one patient (M10/0033) had recently travelled to India. Three isolates (M12/0089, M12/0130 and M12/0155) were recovered from one family and all three isolates exhibited indistinguishable *spa* types. Here, resistance profiles were also indistinguishable to each other with the exception of amikacin to which M12/0089 exhibited intermediate resistance and M12/0130 and M12/0155 were susceptible (Table 3.6). However M12/0155 lacked the *pvl* genes and the collocated *sea* genes. The only other *pvl*-negative isolate assigned to ST772 (M13/0625) was recovered from a sporadic case that was unrelated to any other cases in the current study. In one other case (M14/0131) there was a familial contact with MRSA however the isolate recovered from the family contact was not available for investigation (Table 3.6).

3.4 Discussion

This study is the first to characterise in detail *pvl*-negative sporadically-occurring MRSA from hospitals and communities in Ireland. Molecular epidemiological typing revealed that in addition to many well-known HCA-MRSA clones such as ST239-MRSA-III and ST8-MRSA-II, many of the strains identified here have been reported elsewhere as CA-MRSA e.g. CC1-MRSA-IV, CC5-MRSA-V, and LA-MRSA e.g. CC398-MRSA-IV/V_T and CC130-MRSA-XI. However, this is the first report of many of these strains in Ireland, and in some instances in healthcare facilities in Ireland, highlighting the threat that exists.

3.4.1 Identification and characterisation of unusual and emerging MRSA strains in Ireland

The present study revealed an extensive genetic diversity among sporadically-occurring MRSA isolates in Ireland with 48 different MLST CC/ST and SCC*mec* type combinations and 86 *spa* types identified among 276 MRSA isolates, predominantly recovered from patients, between 2001 and 2015. On the basis of MLST CC, the majority of the isolates investigated here were assigned to previously reported epidemic HCA- and CA-MRSA lineages including CC1, CC5, CC8, CC22, CC30 and CC45 which is similar to other studies (Aires de Sousa & de Lencastre, 2003; Hallin *et al.*, 2008).

Some of the sporadic MRSA strains described here have emerged as predominant MRSA strains in other regions. For example, the ST772-MRSA-V clone predominates in Indian hospitals while ST239-MRSA-III is the predominant clone in many hospitals in Asia and South America (Harris *et al.*, 2010; Menegotto *et al.,* 2012; Brennan *et al.,* 2012; Shambat *et al.*, 2012). Detailed molecular characterisation of these isolates allows

early identification of these emerging MRSA strains and ultimately allows infection control and prevention measures to be implemented to prevent further spread. However, while the presence of sporadically-occurring strains demonstrates the ease and perhaps the frequency of international spread of MRSA, the fact that they remain in relatively low numbers suggests that they struggle to compete in the same niche as established nosocomial clones (Nimmo *et al.,* 2013).

Among the isolates investigated 19 (6.9%, 19/276) were assigned to CC779 and all harboured a novel ΨSCC*mec-fusC*-SCC-SCC$_{CRISPR}$ element. The mobility of SCC*mec* is attributed to the presence of the *ccr* gene complex. While the ΨSCC*mec* element described here harbours the *mecA* gene, it lacks the *ccr* genes giving rise to the name ΨSCC*mec.* This ΨSCC*mec* element forms part of a larger CI which also harbours two adjacent SCC elements, one of which harboured *ccrAB4* while the other *ccrC*. The association of *ccr* and mobilisation of the SCC*mec* element suggests that, among the ST779, the mobility the CI was mediated by the SCC elements, a mechanism which has been previously described in isolates harbouring ACME (Goering *et al.*, 2007).

In the present study CC1-MRSA-IV, CC5-MRSA-IV, CC5-MRSA-V, CC22-MRSA-IV and CC45-MRSA-IV were the predominant clones but it is important to note that some of the CC1 (51/80, 63.8%) and CC5 (18/61, 29.5%) isolates were recovered during outbreaks and that these numbers are skewed in favour of these strains. Although, ST22-MRSA-IV is currently the most prevalent clone in Irish hospitals, 29 ST22-MRSA-IV isolates were identified in the present study of sporadically-occurring MRSA strains based on unusual antimicrobial resistance phenotypes including resistance to tetracycline and trimethoprim encoded for by the *tet*(K) and *dfrS1,* respectively.

Unlike CC8 and CC5 where there was significant diversity observed with regards to SCC*mec*, only SCC*mec* IV has been reported in ST22 worldwide. However, even within SCC*mec* IV significant diversity has been described with 12 different SCC*mec* IV subtypes described (Milheiriço *et al.*, 2007). For the purpose of the present study the subtypes within SCC*mec* IV were not investigated using conventional SCC*mec* typing methods and SCC*mec* subtyping is not currently possible using DNA microarray technology. However, the inability of the DNA microarray to subtype SCC*mec* IV and to detect a novel ΨSCC*mec-fus*-SCC-SCC$_{CRISPR}$ described above highlights a limitation of using DNA microarray analysis for the typing of MRSA and the need to ensure that conventional SCC*mec* typing schemes are updated in line with newly recognised types (Shore *et al.*, 2011; Li *et al.*, 2011; Wu *et al.*, 2015).

Only four isolates (1.5%) were found to harbour the ACME-*arc* gene, three of which were CC8-MRSA-IV/t008 while the remaining isolates were ST22-MRSA-IV/t14499 and CC45-MRSA-V/t563. The presence of ACME is thought to lead to improved fitness in CA-MRSA strains (Diep *et al.*, 2008). Although mainly reported previously in ST8-MRSA-IV isolates, ACME has also been detected in a number other CA and HCA lineages including ST1-IVa, ST5-II, ST5-IV, ST22-IVa, ST22-IVh, ST59-IVa, ST97-V and ST239-III (Goering *et al.*, 2007; Ellington *et al.*, 2008; Bartels *et al.*, 2011; Sabat *et al.*, 2013b; Takano *et al.*, 2013; Senok *et al.*, 2016). Additionally ACME has recently also been reported among LA-MRSA ST398 on a CI (Sabat *et al.*, 2015). Although variations within the ACME element are reported, it is not possible to determine the structure of the element using the DNA microarray and further WGS of the isolates is required to determine if the ACME element present in these isolates is similar to previously reported ST22-MRSA-IV isolates harbouring ACME in Ireland which

exhibited a novel CI structure with ACME located downstream of SCC*mec* (Shore *et al.*, 2011; Senok *et al.*, 2016).

Similar to other previously reported CC1-MRSA-IV isolates, the majority of CC1-MRSA-IV isolates identified here harboured the enterotoxin gene *seh* along with the enterotoxin genes *sek* and *seq*. While the SCC*fus* element has been widely reported in CC1, where *fusC* is located on a SCC element together with *ccrAB1*, the *fusC* gene was identified in eighteen isolates without the SCC*fus ccrAB1* genes indicating a possible chimeric element with *mecA* and *fusC* and which is described in further detail in Chapter 4. Despite the large number of CC1-MRSA-IV isolates identified in the present study (n=80) only six different *spa* types were recognised with 62 of the isolates being assigned to a single *spa* type, t127, highlighting the limited genetic diversity of this clone in Ireland and perhaps its recent introduction.

In contrast to the relative lack of diversity among spa and SCC*mec* types seen in CC1, nine different SCC*mec* types and variants were recognised among the 52 CC5-MRSA isolates and 13 different *spa* types. While HCA CC5-MRSA-IV has been recognised throughout the world and is considered pandemic, CA CC5-MRSA-V is less frequently reported with only sporadic incidences reported in Germany and Abu Dhabi (Monecke *et al.*, 2011).

Twenty-seven isolates were assigned to CC45 with the majority of these harbouring SCC*mec* IV (66.7%, 18/27) while other SCC*mec* elements included IV & fusC (8) and V (1). The CC45-MRSA-IV clone has been widely reported in Europe as a HCA-MRSA strain however in recent years the prevalence of this clone in hospitals has been declining (Albrecht *et al.,* 2011). In addition SCC*fus* and SCC*mec* IV chimeric elements harbouring

SCCmec IV along with *fusC* have also been reported in CC45 MRSA isolates, and are generally considered to be CA-MRSA strains (Ellington *et al.,* 2015).

Worryingly almost all (96%) of the sporadically-occurring MRSA isolates harboured multiple resistance genes and exhibited phenotypic resistance to multiple antimicrobial agents. This was most notable in CC1, CC5, CC8 and CC45 with varying combinations of aminoglycoside, MLS_B and tetracycline resistance. This is worrying, particularly for those clones that are considered to be CA-MRSA e.g. CC1, since CA-MRSA have been shown to be fitter and more virulent than HCA-MRSA but often lack multidrug resistance. However, these CA-MRSA clones exhibiting multidrug resistance represent a worrying development in the evolution of CA-MRSA which has also been noted elsewhere (DeLeo *et al.*, 2010; Shore *et al.*, 2014). The CC5 and CC8 isolates harboured as many as nineteen different resistance genes in comparison to CC97 and CC130 which each harboured only the *blaZ/blaZ*$_{XI}$ resistance gene and the general efflux pump gene *sdrM* suggesting that these strains may have emerged from a different ecological niche where there is less selective pressure in terms of antibiotics.

The fusidic acid resistance gene *fusC* was detected among 19.2% (53/276) of isolates belonging to a variety of lineages (CC1, CC8, CC30 and CC45). Elsewhere an increase in fusidic acid resistance among *S. aureus* has been associated with CC1-MSSA and CC5-MRSA (Williamson & Chen, 2015), however in this study a greater number of CC1 and CC45 strains most frequently harboured the *fusC* gene (16/276 and 8/276, respectively). The relatively high prevalence of *fusC*, which has been reported to be co-located with SCC*mec*, either as a composite or chimeric element, is worrying and has possible implications for the emergence of MRSA clones in areas of high fusidic acid

usage and the diversity among the SCC*mec* and genotypes harbouring the *fusC* gene requires further investigation and is the subject of Chapter 4.

The prompt initiation of antibiotic therapy to treat MRSA infections is essential in patient management in order to reduce morbidity, however, approximately 40% of all antibiotics prescribed in U.S. acute care hospitals are either unnecessary or inappropriate (Doron & Davidson, 2011). Along with the risk to the patient of adverse reactions to the antibiotic, the misuse of antibiotics has also contributed to the growing problem of antibiotic resistance and the increased use of one antibiotic can lead to changes in the epidemiology of MRSA (Lawes *et al.*, 2015). It has also been shown that the chosen antibiotic prescription strategy may have a larger impact on CA-MRSA selection in ICUs than in general wards. This is important since ICU patients are frequently transferred between hospitals and wards, thereby increasing the risk for intra- and inter-hospital dissemination of resistant strains (Kardaś-Słoma *et al.,* 2011). In Scotland, results of a National Antibiotic Stewardship programme resulted in an overall reduction in usage of antibiotics and suggested that removal of selective pressures to below a certain threshold may have played a role in the control of both HCA-MRSA and CA-MRSA (Lawes *et al.,* 2015). In England a similar reduction in the use of some antibiotics has also been reported (Ellington *et al.,* 2015). However, in Ireland the total antibiotic consumption rate has increased from 22.6 defined daily dose (DDD) in 2011 to 25.6 DDD per 1000 inhabitants per day in Quarter 1, 2015 (HPSC, 2015). While many hospitals have antibiotic stewardship programmes in place (National Clinical Effectiveness Committee, 2013), a national programme covering all hospitals and general practitioners should be considered in Ireland in order to ensure that the inappropriate use of antibiotics does not occur and that multiresistant MRSA strains such as those identified in this study, do not become more widespread.

With the exception of CC130, CC97 and the majority of CC398, all other isolates harboured two or more virulence genes including enterotoxins, exfoliative toxins, ACME, and IEC which are located on MGEs and can be transferred between strains. The lack of virulence genes, in particular IEC genes, in CC130, CC97 and the majority of CC398 isolates, together with the fact that these genotypes are predominantly considered to be animal associated, indicates a possible animal origin for these strains (Price *et al.*, 2012). In the present study an animal origin was also indicated for many of the CC398 based on epidemiological information (Table 3.5).

3.4.2 Emergence of CA-MRSA strains in Irish hospitals

In the present study, several multidrug-resistant CA-MRSA clones including *pvl*-positive ST772-MRSA-V and *pvl*-negative CC1-MRSA-IV and CC5-MRSA-V, were identified as part of outbreak incidences in several different hospitals. For one of the ST772-MRSA-V outbreaks, the source was traced to a staff member who had recently travelled to India where she had been hospitalised to give birth to her son, who, upon screening through the local occupational health department, was also found to carry the ST772-MRSA-V strain. Unfortunately it was not possible to identify the source in the case of the second ST772-MRSA-V outbreak or the CC1-MRSA-IV outbreak, the latter which resulted in a prolonged outbreak within the ward spanning over a two-year period. Further details of these outbreaks are described in Chapter 5.

The CC1-MRSA-IV and CC5-MRSA-V isolates harboured multiple resistance genes including those encoding resistance to fusidic acid, mupirocin, aminoglycosides and tetracycline which can result in patient management difficulties for clinical staff identifying suitable antimicrobial options for treatment of the MRSA carriage. Although a high percentage of these isolates (CC1, 65.4% and CC5, 29.5%) were recovered during

outbreak investigations in healthcare facilities, these strains have been recovered elsewhere in the country on a number of different occasions including in the community, and the emergence of these strains in healthcare facilities show the ease at which CA-MRSA strains have the potential to emerge and spread within healthcare facilities (NMRSARL, 2014).

Transmission of MRSA between healthcare workers and patients has been widely reported but current Irish MRSA guidelines recommend staff screening only during outbreak investigations (National Clinical Effectiveness Committee, 2013). It has been suggested that screening of staff in settings where MRSA is endemic should be considered or randomised periodic screening in order to identify asymptomatic healthcare staff who are MRSA carriers (Otter & French, 2010). However, as shown in the case of the ST772-MRSA-V incident, screening of persons who have recently travelled or have been hospitalised in areas of high MRSA prevalence should also be considered.

The spread of CA-MRSA into hospitals and the potential replacement of traditional HCA-MRSA strains with CA-MRSA strains have several important infection control and prevention implications. While stringent infection prevention and control practices are in place in healthcare facilities aimed at reducing the rate of MRSA transmission, no such protocols exist for many of the areas that are considered as high risk environments for CA-MRSA including sports teams, crèches and prisons. Furthermore, although *pvl*-positive CA-MRSA in Ireland has been extensively studied (Rossney *et al.,* 2007; Shore *et al.*, 2014), much of the information in Ireland regarding CA-MRSA is gathered on an *ad-hoc* basis with no true prevalence survey completed and limited data relating to *pvl*-negative lineages available (NMRSARL, 2014). Cross-transmission of CA-MRSA in both the hospital and the community has been well documented but an improved

understanding of the acquisition, colonisation and transmission of CA-MRSA is necessary in order to direct appropriate infection control and prevention procedures and minimise potential issues which may arise.

3.4.3 The emergence of LA-MRSA in Ireland

A number of different LA-MRSA clones were identified in the present study which is of concern, not only for patient safety but also because of the potential for hitherto undetected reservoirs of MRSA in Ireland. In Europe CC398 is the predominant LA-MRSA clone. Elsewhere CC6, ST9, ST59, ST97 and CC130 have also been associated with animals, many of which were also identified here. While limited clinical details were available for patients from whom these isolates were recovered, the presence of an intact *hlb* among ST97 and CC130 isolates and some CC398 isolates strongly suggests possible animal sources (Cuny *et al.,* 2015).

One CC130-MRSA-XI *mecC*-positive isolate was identified during the present study, representing the third SCC*mec* XI isolate reported in Ireland to date. This isolate exhibited *spa* type t12399 and, like the two previously reported *mecC* MRSA recovered from Ireland (Shore *et al.,* 2011), exhibited resistance only to ampicillin and lacked the *blaZ* gene but harboured the variant $blaZ_{XI}$. In addition, DNA microarray analysis revealed that the isolate harboured the untruncated β-haemolysin toxin gene *hlb* and lacked IEC genes. The isolate was recovered from an elderly man living in a rural area in Ireland, and although not currently working as a farmer, had had contact with others working with livestock.

3.4.3.1 The spread of LA-CC398 *S. aureus* in Ireland

The identification of CC398 MRSA among animals and humans in Ireland along with its importation and spread highlights a combination of inadequate biosecurity at country, farm and veterinary hospital level. Although the majority of CC398 MRSA isolates identified harboured multiple antimicrobial resistance genes, they lacked many of the virulence genes including toxic shock syndrome toxin, PVL and *hlb*-disrupting lysogenic prophages that encode the IEC genes. The combination of SCC*mec* types and *spa* types identified, together with epidemiological data confirms multiple incidences of importation and direct transmission between animals and the humans caring for them. The risk posed by contact with livestock needs to be considered when screening high-risk groups for MRSA on admission to Irish hospitals. Animal infections caused by MRSA are not notifiable in Ireland and neither is it mandatory to screen imported livestock for MRSA. However, in light of this study, these factors should be reconsidered.

This is the first report of CC398 MRSA in the Republic of Ireland. Although extensive studies of farm animals in Ireland are limited (Horgan *et al.,* 2011; van Cleef *et al.,* 2011; Burns *et al.,* 2014), the number of incidences identified here in a relatively short period of time gives cause for concern. During 2012 and 2013, three separate incidences, involving ten people, nine pigs and a horse were identified. While the sources of these infections were identified (a Belgian veterinarian and pigs imported from Germany), an additional isolate recovered from a patient in a rural nursing home in 2011 and for which the source was not determined suggests that there may be other, yet undetected sources of CC398-MRSA in Ireland. In addition, one isolate was recovered from a child who had worked with his father on a pig farm in the UK and which exhibited the same *spa* and SCC*mec* types as previously reported CC398-MRSAfrom the UK

(Hartley *et al.,* 2014), suggesting that CC398 MRSA may be more widespread than previously reported.

Interestingly, many of the previous reports of CC398 MRSA confirm an animal to human transmission route; however in the present study, human to animal transmission was evident in the case of the veterinarian who had recently travelled to Belgium, where CC398 is common among veterinary personnel and horses (van den Eede *et al.*, 2009; Denis *et al.*, 2009). Here, although the isolate harboured the multiresistance profile associated with the animal strains, it also harboured IEC genes, which are involved in evading the human immune system, indicating a human source and transmission due to inadequate infection control procedures in the veterinary hospital.

In the present study CC398 MSSA were also recovered from humans and pigs but there was no molecular epidemiological evidence of the zoonotic spread of these strains, indicating their independent emergence, with both harbouring traits typical of human and animal CC398 MSSA clades, respectively. The CC398 MSSA was found to have spread among pigs on two farms and these are potential precursors for the emergence of CC398 MRSA (Vandendriessche *et al.,* 2013). Among the CC398 MSSA only one isolate was found to harbour the *pvl* genes. The frequency with which *pvl* has been recognised among CC398 isolates remains rare, however cases have been reported in China and Europe where European isolates also had a connection with China (Yu *et al.,* 2008; Cuny *et al.,* 2015; Becker *et al.,* 2015; Koyama *et al.,* 2015).

Although the major threat described here relates to the transmission of CC398 MRSA from pigs to humans via direct or indirect contact, CC398 MRSA have also been reported in retail meat products, including pork (Hadjirin *et al.*, 2015), indicating another potential threat to public health and the Irish agricultural sector. Ongoing surveillance for

CC398-MRSA and -MSSA in Ireland and the characterisation of such strains is essential due to their potential impact on human and animal health along with the agricultural industry (Voss *et al.,* 2005; Salmenlinna *et al.,* 2010; McCarthy *et al.,* 2012; Senneville *et al.,* 2014). Early recognition of these strains in imported animals through enhanced and perhaps mandatory screening is essential in order to protect the livestock industry and in particular the health of those working in the industry from MRSA infection.

3.5 Conclusion

This study has highlighted a number of important issues regarding MRSA in Ireland including a high level of diversity among MRSA strains circulating in hospitals and communities, especially those harbouring SCC*mec* IV and SCC*mec* V. The prevalence of resistance and virulence genes identified among this diverse collection of isolates emphasises the reservoir of resistance and virulence genes that may enhance virulence and limit treatment options for MRSA into the future. The recognition of a number of prolonged outbreaks in hospital wards caused by known CA-MRSA strains highlights the importance of staff screening particularly for those who have been hospitalised in areas where MRSA is endemic. The identification of a number of LA-MRSA clones which, along with a lack of effective infection control and prevention measures, threaten not only the farmers and their animals, but also pose significant potential risks to the multi-million Euro agricultural and food industry in Ireland, as well as having the potential to spread within hospitals. The previous observation of recurrent strain displacement in Ireland (Shore *et al.,* 2005) suggests that such displacement is likely to occur again in the future. Any one of the many sporadic MRSA strains identified here has potential to displace the currently predominant ST22-MRSA-IV strain highlighting the need for effective and ongoing surveillance of the MRSA population in order to ensure that appropriate treatment options and control measures are in place to prevent emerging multiantibiotic strains becoming widespread and possibly epidemic.

Chapter 4

Phenotypic and genotypic analysis of MRSA harbouring *fusC*

4.1 Introduction

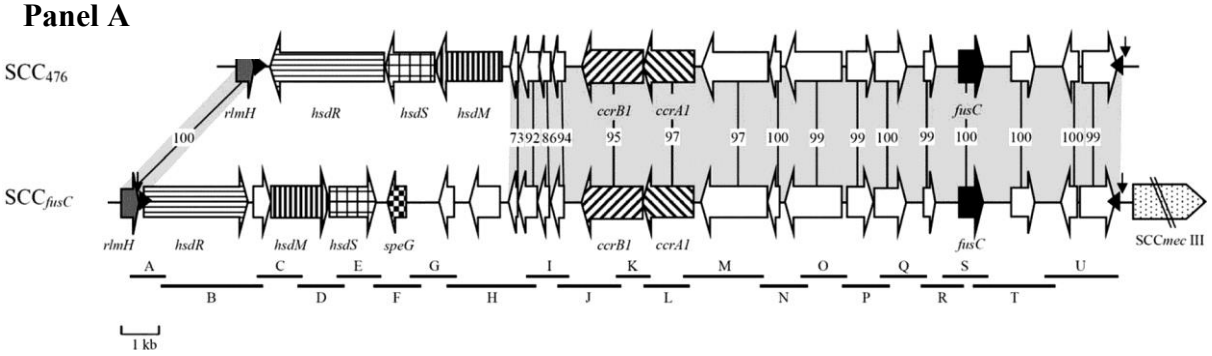
Fusidic acid is an antibiotic used in topical and intravenous forms for the treatment of staphylococcal infections including MRSA, hVISA and VISA by targeting EF-G. Resistance to fusidic acid emerges in *S. aureus* through mutations in the *fusA* gene which encodes EF-G, or through the acquisition of *fusB* or *fusC*, which encode proteins that protect the target region (Ellington *et al.,* 2015). The prevalence rate of fusidic acid resistance among *S. aureus* throughout the world varies from one region to another. While low rates of fusidic acid resistance among *S. aureus* have been reported in Australia and America (~5%), higher rates have been reported in Greece (49%), Kuwait (20%) and New Zealand (13%). In the case of the latter three countries, in each case, a single MRSA clone has been associated with fusidic acid resistance (Nimmo *et al.,* 2003; Zinn *et al.,* 2004; Howden & Grayson, 2006). Furthermore, fusidic acid resistance has also been described among CA-MRSA strains in Australia, New Zealand, Saudi Arabia and Europe (Nimmo & Coombs, 2008; Kinnevey *et al.,* 2014; Shore *et al.,* 2014; Ellington *et al.,* 2015; Baines *et al.,* 2016; Senok *et al.,* 2016).

An increase of fusidic acid resistant *S. aureus* in New Zealand has been closely linked to the emergence of three specific clones, ST1-MSSA, ST1-MRSA-IV and ST5-MRSA-IV. There, ST5-MRSA-IV has emerged as a predominant HCA- and CA-MRSA strain with fusidic acid resistance rates as high as 89% among isolates of this lineage (Baines *et al.,* 2016). In all three clones, the mechanism of fusidic acid resistance has been associated with the presence of *fusC* (Williamson *et al.,* 2014). Similarly in the UK, fusidic acid resistance has also been reported in MRSA isolates belonging to ST1 and ST5, but also in ST45 and ST149 (CC5) and, in all cases, mediated by *fusC* (Ellington *et al.,* 2015). Furthermore *fusC*-mediated resistance has also been reported among the HCA-

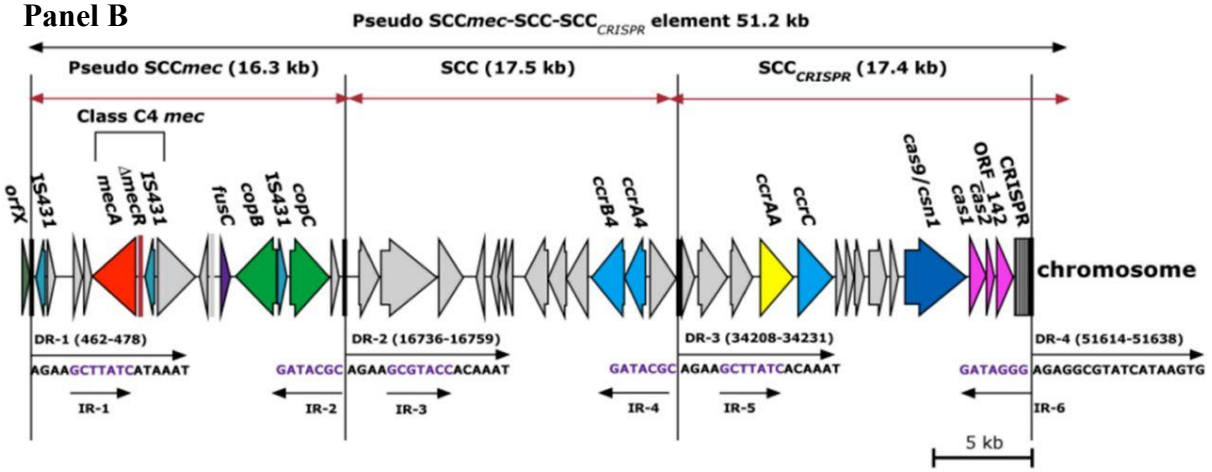
MRSA clones ST239-MRSA-III, ST238-MRSA-III and ST5-MRSA-II in Taiwan (Chen *et al.,* 2015).

Although *fusC* has been reported in association with a limited number of *S. aureus* genetic backgrounds, it has been shown that SCC*mec*/SCC-mediated horizontal gene transfer is the predominant mechanism of *fusC* dissemination among *S. aureus*. The *fusC* gene has been located either (i) within a SCC element, either alone or adjacent to a SCC*mec* element where it forms part of a CI, or (ii) within a SCC*mec* element, where it is termed a chimeric element due to the presence of *mecA* and *fusC* within a single SCC*mec* element (Fig. 4.1). The *fusC* gene was first recognised on a SCC element (SCC_{476}) in ST1-MSSA which, although lacking the *mec* gene complex, harboured the *ccr* genes and integrated into the *S. aureus* at the same chromosomal *orfX* site as SCC*mec* elements (Fig. 4.1, Panel A) (Holden *et al.,* 2004). Following this, another SCC-like element, termed SCC*fus,* which differed from SCC_{476} due to the presence of an additional resistance gene *speG*, which encodes polyamine resistance, was reported in ST239-MRSA within SCC*mec* III (Fig. 4.1, Panel A) (Lin *et al.,* 2014). Subsequently *fusC* was found as part of a CI in ST779-MRSA with a ΨSCC*mec* element and an additional SCC element harbouring *crispR*, which is involved in the protection of the bacterial genome against foreign DNA (Fig. 4.1 panel B) (Kinnevey *et al.,* 2013; Lin *et al.,* 2014). Most recently, another CI has been identified in the UK in association with ST149-MRSA where a novel SCC_{149} element harbouring *fusC* was identified adjacent to SCC*mec* IVa (Fig. 4.1, Panel C) (Ellington *et al.,* 2015). Chimeric elements of SCC*mec-fusC* have also been recognised in the UK and New Zealand among ST1-, ST5- and ST45-MRSA isolates harbouring SCC*mec* IV (Fig. 4.1, Panel D and E) (Ellington *et al.,* 2015; Baines *et al.,* 2016). In New Zealand, the emergence of these chimeric SCC*mec-fusC* elements

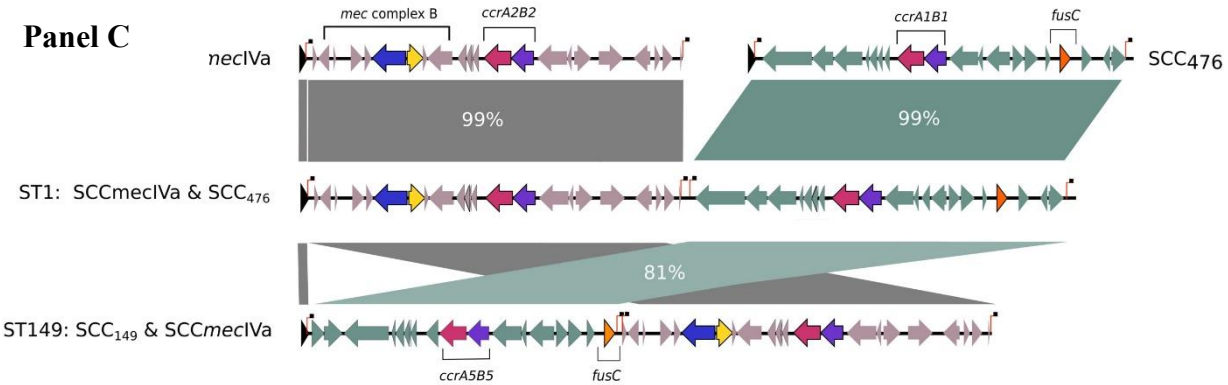
Panel A



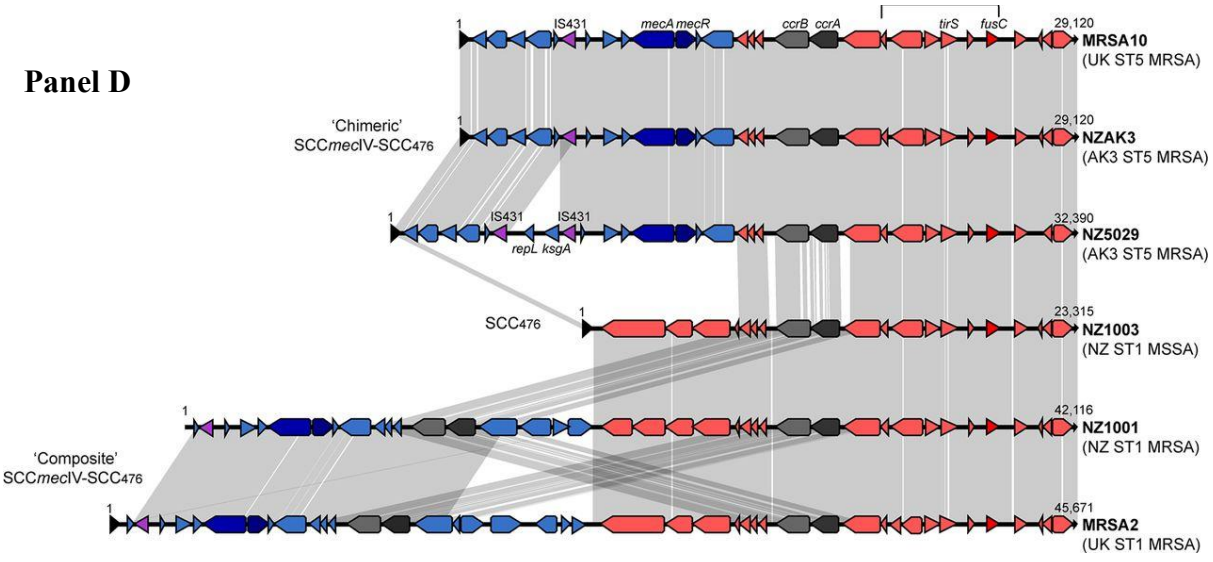
Panel B



Panel C



Panel D



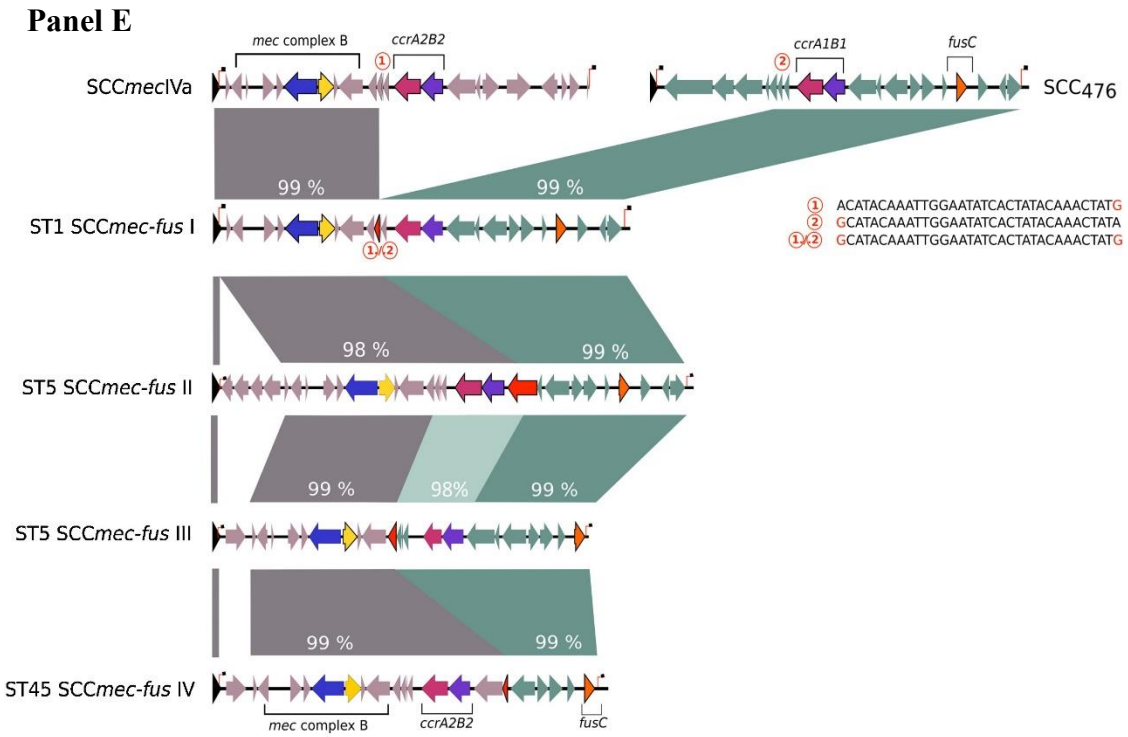


Figure 4.1 Genetic organisation of previously reported *S. aureus* SCC and SCC*mec* elements harbouring *fusC*.

Panel A: Genetic organisation of SCC*fus* (GenBank accession no. KF527883) detected as part of a composite island in ST239-MRSA-III compared with that of SCC_{476} (GenBank accession no. NC_002953.3) identified in ST1-MSSA. The patterned arrows represent the putative open reading frames. The genes are drawn according to their sequences and function. *hsdR*, *hsdM*, and *hsdS* encode the R, M, and S subunits of the type I restriction-modification system. *speG* encodes spermidine acetyltransferase. White arrows represent other genes encoding hypothetical proteins. The integration site sequences (ISS) are indicated by the vertical arrows. The inverted repeat sequences are indicated by the thin horizontal arrows. The homologous regions between the two SCCs are shown by shading, and the numbers in the shading indicate the percentages of homology between the corresponding sequences. The horizontal bars at the bottom represent the PCR products used to confirm the presence of the SCC*fusC* element. The identities of *hsdR*, *hsdM*, and *hsdS* between the two elements were below 45%. Adapted from Lin *et al.,* 2014.

Panel B: Genetic organisation of the composite island ΨSCC*mec*-SCC-SCC$_{CRISPR}$ harboured by the ST779/t878 MRSA isolate M06/0171 (GenBank accession number HE980450). The 51-kb composite island (CI), as well as each of the individual SCC elements of this CI, are flanked by direct repeat (DR) and inverted repeat (IR) sequences. The methicillin, fusidic acid, and copper resistance genes *mecA*, *fusC*, and *copB/copC* are shown in red, purple, and green, respectively. The *ccrAB4* and *ccrC* genes are shown in blue, the *ccrAA* gene is shown in yellow, and the clustered regularly interspaced short palindromic repeats (CRISPRs) and the genes encoding CRISPR-associated proteins (*cas9/csn1*, *cas1*, *cas2*, and ORF_142) are shown in pink. The direction of transcription for each ORF is indicated. Adapted from Kinnevey *et al.,* 2013.

Panel C: Genetic organisation of the SCC elements that encode *fusC* identified adjacent to a second SCC element (SCC*mec* IV, in grey) in CIs in ST1 and ST149 (and its single-locus variant ST2942). The direct repeat sequences (DRscc) that delimit each SCC are shown as vertical red lines topped by a black square. Adapted from Ellington *et al.,* 2015.

Panel D: Genetic organisation of the SCC$_{476}$ element in ST1 MSSA (represented by strain NZ1003), the chimeric SCC*mec* IV-SCC$_{476}$ element in ST5 MRSA (strain NZAK3), and the composite SCC*mec*-SCC$_{476}$ element in ST1 MRSA (strain NZ1001). The transposon-containing region within the chimeric SCC*mec*IV-SCC$_{476}$ element in strain NZ5029 is illustrated along with SCC*mec* elements from MRSA2 and MRSA10. Blue or purple arrows indicate sequences present within SCC*mec* regions. The direction of the arrows indicates the direction of transcription for open reading frames. Only coding sequences of >200 bp are shown. Gray shaded areas represent regions that share >99% nucleotide sequence identity. Adapted from Baines *et al.,* 2016.

Panel E: Genetic organisation of SCC*mec-fus* chimeric elements which were formed from portions of SCCmec (in grey) and SCC$_{476}$-like cassettes (in teal) and were flanked by direct repeat sequences (DRscc). Percentage DNA sequence homologies to the corresponding portion of SCC*mec* IVa or SCC476 are shown above each SCC*mec-fus*. The change in sequence homology from SCC*mec* to SCC476 (denoted and detailed by red numbers in red circles) occurred within coding sequences (shown as red arrow heads). Adapted from Ellington *et al.,* 2015.

has been associated with an increase in the usage of topical fusidic acid (Baines *et al.,* 2016). In contrast, in the UK, these *fus* elements have emerged in MRSA at a time of decreasing usage of fusidic acid and instead have been linked to the increasing dissemination of CA-MRSA (Ellington *et al.,* 2015).

In Ireland, an increase in fusidic acid resistance among MRSA from BSIs from 16% to 48% has been reported in recent years (Fig. 4.2) and while in 2014, 81.2% (72/88) were associated with the main HCA-MRSA clone in Ireland, ST22-MRSA-IV, non ST22-MRSA-IV isolates accounted for 18% (16/88) (NMRSARL, 2014). The predominant mechanism of fusidic acid resistance among ST22-MRSA-IV isolates has previously been shown to due to mutations in *fusA* resulting in high-level fusidic acid resistance (Brennan, 2013). However, the mechanism of fusidic acid resistance among non-ST22-MRSA-IV isolates has not been investigated, apart from t878/ST779-MRSA isolates harbouring ΨSCC*mec*-SCC-SCC$_{CRISPR}$ with *fusC* (Kinnevey *et al.,* 2013). In Chapter 3 of the present study, DNA microarray profiling of sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and the community between 2001 and 2014 identified *fusC* among 19.2% (53/276) of isolates. The *fusC* gene was identified among MRSA isolates belonging to CCs 1, 5, 45 and 779, which have been reported previously, but additional isolates were also assigned to CC8 and CC30 which have not been previously reported to harbour *fusC* (Table 4.1).Furthermore, in the present study, *fusC* was found in association with SCC*mec* elements that have not have previously been reported to harbour *fusC* including SCC*mec* I, IV & V and VI (Table 4.1).

Due to the increasing prevalence and diversity of *fusC* encoded fusidic acid resistance in MRSA, the aim of this part of the present study was to further explore the phenotypic and genotypic characteristics *fusC*-positive MRSA and to determine the genetic organisation of any potentially novel SCC*mec*/SCC*fus* elements identified.

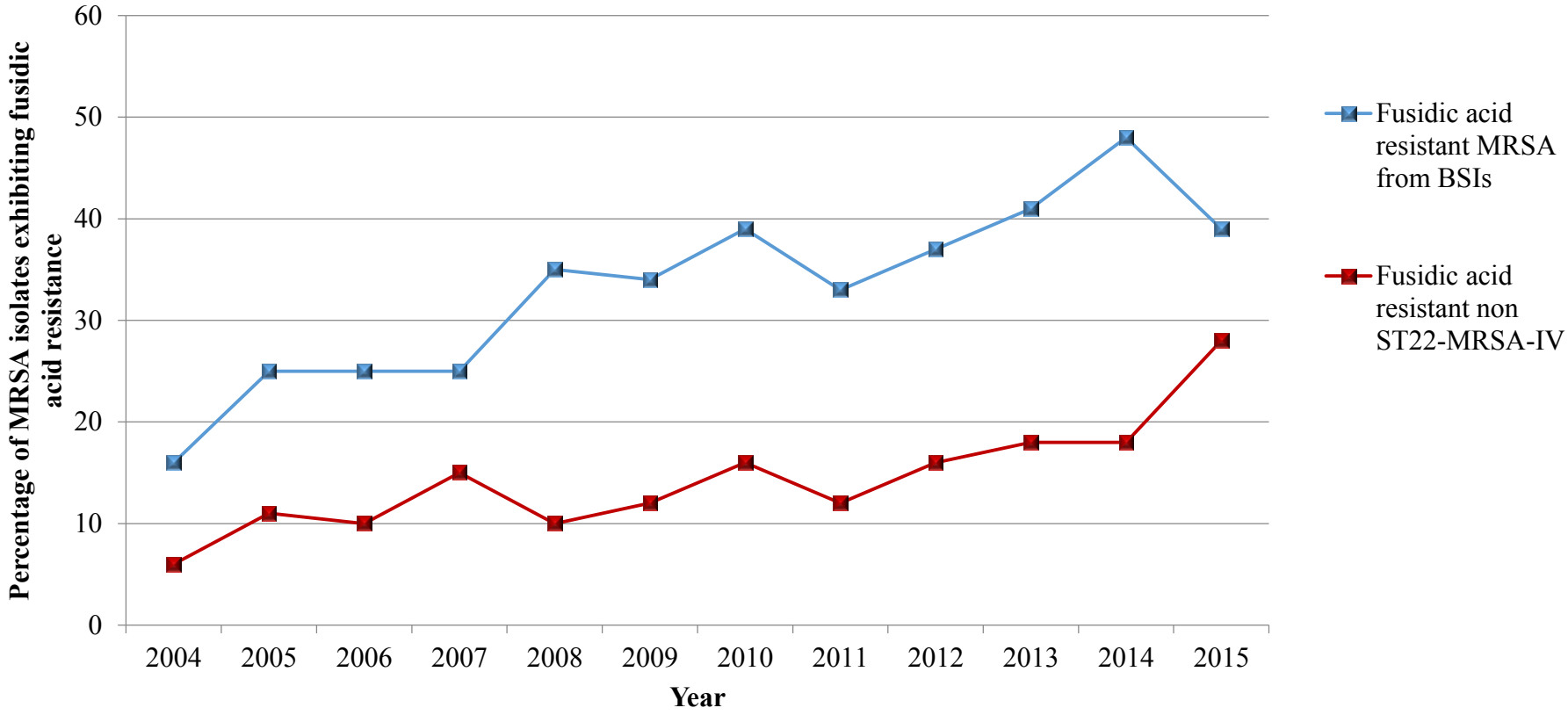


Figure 4.2 The percentage of MRSA isolates recovered from bloodstream infections (BSIs) in Ireland between 2004 and 2014 that exhibited fusidic acid resistance. The data is based on the MRSA isolates from BSIs investigated in the National MRSA Reference Laboratory as part of the European Antimicrobial Resistance Sureveillance Network. The blue line indicates the percentage of all MRSA isolates investigated that exhibited fusidic acid resistance each year while the red line indicates the percentage of MRSA isolates that were fusidic acid resistant but which were not identified as ST22-MRSA-IV. Adapted from NMRSARL, 2015.

Table 4.1 Genotypes and SCC*mec* genes of 53 MRSA isolates found to harbour *fusC* among a collection of 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and the community between 2001 and 2015[a]

Putative SCC*mec*-*fusC* element type[b]	CC-*spa* type (*n*)[c, d]	*mec* complex[d]	*ccr* genes[d]	SCC*mec* & *fusC* type designation	Resistance genes[d]	Virulence genes[d, e]	Fusidic acid MIC (mg/L)[f]	Comment/ Reference[g]
Composite islands	CC1-t127 (6)[c]	B	*AB1, AB2*	IV & SCC*fus*	*blaZ* (5), *fusC, erm*(A) (3), *sdrM, erm*(C) (2), *qacC* (1)	*seh* (3), *sek/q* (3), *egc* (4), IEC D	4.0-8.0	Ellington *et. al.,* 2015
	CC1-t2279 (9)	B	*AB1, AB2*	IV & SCC*fus*	*blaZ, fusC, fosB* (1), *sdrM*	*seh* (8), *sek/q* (6), IEC D (8)	4.0-16.0	Ellington *et. al.,* 2015
	CC1-t16173 (1)	B	*AB1, AB2*	IV & SCC*fus*	*blaZ, erm*(C), *fusC, sdrM*	*seh, sek/q, egc,* IEC D	4.0	Ellington *et. al.,* 2015
	CC8-t14362 (1)[c]		*AB1, AA, C*	IV & V & *fusC*	*blaZ, fusC, fosB, sdrM,*	*sed*		Novel genetic background and novel SCC*mec*-*fusC* combination
	CC30-t382 (1)[c]	B	*AB2, C*	IV, *ccrC* & *fusC*	*blaZ, fusC, sdrM, fosB*	*tst, sec/l,* IEC B	4.0	Novel SCC*mec*-*fusC* combination
	CC779-t878	C	*AB4, C*	ΨSCC*mec*-	*blaZ, fusC, erm*(C)	*seb* (4),	4.0-16.0	Kinnevey *et al.,*

Continued overleaf

Table 4.1 continued

Putative SCC*mec*-*fusC* element type[b]	CC-*spa* type (*n*)[c, d]	*mec* complex[d]	*ccr* genes[d]	SCC*mec* & *fusC* type designation	Resistance genes[d]	Virulence genes[d, e]	Fusidic acid MIC (mg/L)[f]	Comment/ Reference[g]
	(18)			SCC-SCC$_{CRISPR}$	(1), *fosB* (2), *sdrM*	*sed/j/r* (6), *sek/q* (1), IEC B (16)		2013
	CC779-t11021(1)	C	*AB4, C*	ΨSCC*mec*-SCC-SCC$_{CRISPR}$	*blaZ, fusC, sdrM, qac,*	*etD/edinB,* IEC B	4.0	Kinnevey *et al.,* 2013
Chimeric elements	CC5-t105 (1)	B	*AB2*	IV & *fusC*	*blaZ, fusC, fosB, sdrM*	*tst, sec/l, egc,* IEC D		Ellington *et. al.,* 2015
	CC5-t002 (1)[c]	B	*AB1*	I & *fusC*	*blaZ, aadD, fusC, fosB, qacC, sdrM*	*tst, sec/,l, sed/j/r, egc,* IEC B	4.0	Novel SCC*mec*-*fusC* combination
	CC5-t311(1)[c]	C	*AA, C*	V & *fusC*	*blaZ, fusC, erm*(C), *sdrM, fosB*	*seb,* IEC E		Novel SCC*mec*-*fusC* combination
	CC8-t008(1)[c]	B	*AB4*	VI & *fusC*	*blaZ, fusC, fosB, sdrM,*	IEC E	4.0	Novel genetic background and novel SCC*mec*-*fusC* combination

Continued overleaf

Table 4.1 continued

Putative SCC*mec*-*fusC* element type[b]	CC-*spa* type (*n*)[c, d]	*mec* complex[d]	*ccr* genes[d]	SCC*mec* & *fusC* type designation	Resistance genes[d]	Virulence genes[d, e]	Fusidic acid MIC (mg/L)[f]	Comment/ Reference[g]
	CC30-t012 (2) [c]	B	*AB2*	IV & *fusC*	*blaZ, fusC, sdrM, fosB*	*tst, egc,* IEC B	4.0	Previously reported SCC*mec*-*fusC* combination but novel genotype
	CC30-t021(2)	B	*AB2*	IV & *fusC*	*blaZ, fusC, sdrM, fosB*	*tst, egc,* IEC B	4.0	Previously reported SCC*mec*-*fusC* combination but novel genotype
	CC45-t383(1)	B	*AB2*	IV & *fusC*	*blaZ, fusC, sdrM*	*sec, sed/j/r, egc,* IEC B		Ellington *et. al.,* 2015
	CC45-t728 (1)	B	*AB2*	IV & *fusC*	*blaZ, fusC, sdrM*	*sec/l, sed/j/r, egc,* IEC B	4.0	Ellington *et al.,* 2015
	CC45-t2277(2)	B	*AB2*	IV & *fusC*	*blaZ, fusC, sdrM*	*sec/l, sed/j/r, egc,* IEC B		Ellington *et. al.,* 2015

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Table 4.1 continued

Putative SCC*mec*-*fusC* element type[b]	CC-*spa* type (*n*)[c, d]	*mec* complex[d]	*ccr* genes[d]	SCC*mec* & *fusC* type designation	Resistance genes[d]	Virulence genes[d, e]	Fusidic acid MIC (mg/L)[f]	Comment/ Reference[g]
	CC45-t4545(4)	B	*AB2*	IV & *fusC*	*blaZ, erm*(C) (1), *fusC, sdrM*	*egc,* IEC B	4.0	Ellington *et. al.,* 2015

[a] Isolates were investigated as part of a larger collection of 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and their environments and from the community as detailed in Chapter 3.

[b]Composite islands (CIs) consist of two or more distinct SCC/SCC*mec* elements separated by direct and inverted repeats. In this instance the element was designated a putative CI if *mec* and *ccr* genes indicative of a particular SCC*mec* type were detected in addition to *fusC* and *ccrAB1* genes, indicative of the presence of SCC*fus*. Chimeric elements consist of single fused element, flanked by direct and inverted repeats. In this instance, the element was designated as putative chimeric element if *mec* and *ccr* genes indicative of a particular SCC*mec* type were detected in addition *fusC* but without the SCC*fus*-related *ccrAB1* genes.

[c]One isolate representative of CC1-t127-MRSA-IV & *fusC* (E3258), CC5-t002-MRSA-I & *fusC* (M11/0141), CC5-t311-MRSA-VI & *fusC* (M11/0260), CC8-t008-MRSA-VI & *fusC* (M08/0515) and CC30-t012-MRSA-IV & *fusC* (M10/0343) were selected for whole-genome sequencing based on unusual combinations of SCC*mec* and *fusC* and/or genetic backgrounds that had not previously associated with *fusC*. The schematic structure of elements identified in each isolate investigated by whole-genome sequencing is shown in Fig. 4.6. Two isolates assigned to CC30-t382-SCC*mec* IV, *ccrC* & *fusC* and CC8-t14362-SCC*mec* IV & V & *fusC*, both of which harboured novel combinations of SCC*mec-fusC*, were not included for WGS due to resources that were available at the time of selection.

Continued overleaf

Table 4.1 continued

[d]Multilocus sequence type clonal complexes (CCs), SCC*mec*, resistance (including *fusC*) and virulence-associated genes were detected using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH) (Chapter 3). *spa* typing was performed in accordance with the SeqNet protocol and *spa* types were assigned to each isolate based on the repeat succession patterns using Ridom Staph Type software (Chapter 3).

[e]The immune evasion complex (IEC) type of each isolate was determined based on the combination of the IEC genes detected as described by van Wamel *et al.,* 2006. IEC type B, *sak, chp, scn;* IEC type D, *sea, sak, scn*; IEC type E, *sak, scn*. The enterotoxin gene cluster (*egc*) includes *seg, sei, sem, sen, seo* and *seu*.

[f]The fusidic acid minimum inhibitory concentration (MIC) of each isolate was determined using gradient strips in accordance with EUCAST interpretive criteria (EUCAST, 2013) and the range of the fusidic acid MICs for isolates belonging to each CC-*spa* and SCCmec-*fusC* combination is shown in milligram per litre (mg/L)).

[g]Where the combination of SCC*mec-fusC* genes in association with a particular genotype have been reported previously, the relevant reference is provided. Where the combination of SCC*mec-fusC* genes identified represented either a novel combination of SCC*mec-fusC* or a previously reported combination of SCC*mec-fusC* in a previously unreported genetic background, then this is indicated.

4.2 Materials and methods

4.2.1 Isolates

Fifty-three MRSA isolates identified as harbouring *fusC* following DNA microarray profiling of sporadically-occurring MRSA isolates recovered from patients in hospitals and communities in Ireland between 2011 in 2015 were included in this part of the present study (Table 4.1). In Chapter 3, these isolates were assigned to MLST-CCs, and SCC*mec,* virulence and antimicrobial resistance genes were detected by DNA microarray profiling. The isolates also underwent antimicrobial susceptibility testing to 25 antimicrobial agents, including fusidic acid, and *spa* typing described in Chapter 3.

4.2.2 Fusidic acid MIC determination

The fusidic acid MIC of each isolate was determined in accordance with EUCAST methodology (EUCAST, 2013). Briefly, isolates were recovered from -70°C storage by overnight incubation on CBA at 35°C. An inoculum equivalent to 0.5 McFarland turbidity standard was prepared in saline and the bacterial suspension was lawned onto MH agar by dipping a cotton tipped applicator swab into the inoculum suspension. Excess liquid was removed from the swab by turning the swab against the inside of the tube. The inoculum was spread evenly over the plate using a rotary plater. A fusidic acid gradient strip (E-test, Biomérieux) was placed on the lawned plate and then examined to ensure that all air bubbles behind the strip were removed. Inverted plates were incubated overnight at 35°C and resulting growth was examined to look for the presence of colonies within the elliptical zone of inhibition. Isolates exhibiting a fusidic acid MIC of >1.0 mg/L were defined as fusidic acid resistant (Fig. 4.3) (EUCAST, 2013).

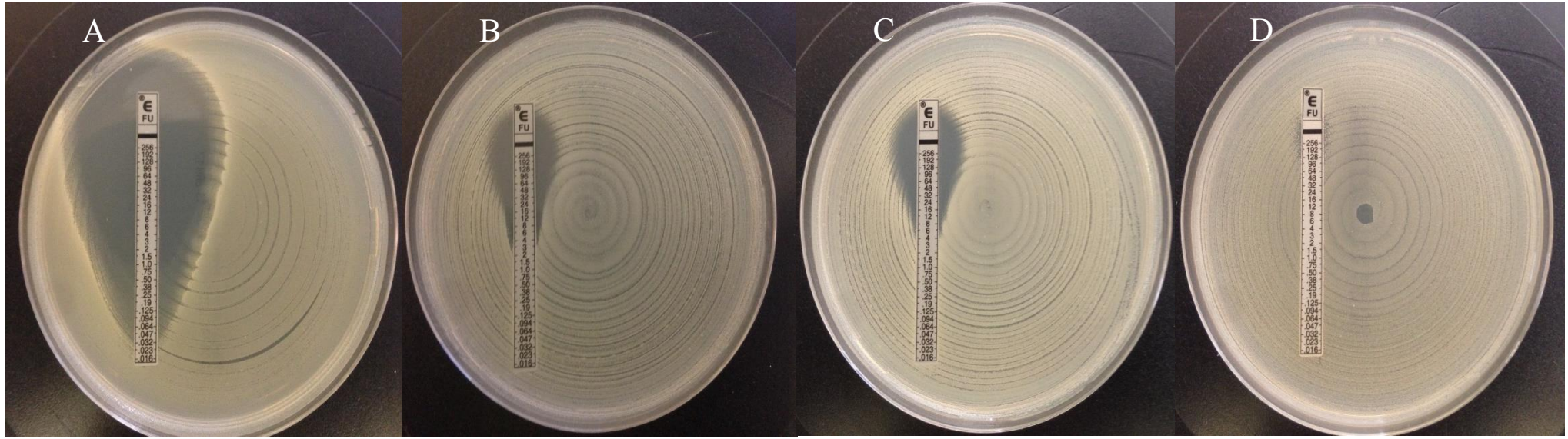


Figure 4.3. Fusidic acid minimum inhibitory concentrations (MIC) determination using fusidic acid gradient strips (E-test, BioMérieux). The fusidic acid MIC was determined in accordance with EUCAST methodology and interpretive criteria (EUCAST, 2013) where plates were examined to identify colonies within the elliptical zone of inhibition. Isolates exhibiting a fusidic acid MIC breakpoint of >1.0 mg/L were defined as resistant. Panel A: A fusidic acid susceptible *S. aureus* control isolate ATCC29213 with a fusidic acid MIC of 0.064 mg/L. Panels B and C: Low-level fusidic acid resistance detected among isolates investigated with MICs of 4.0 mg/L and 12.0 mg/L, respectively. Panel D: A MRSA isolate (not included in the current study) exhibiting high-level fusidic acid resistance where the MIC is >256 mg/L.

4.2.3 Whole-genome sequencing

Five of the *fusC*-positive MRSA isolates were selected for WGS because they either harboured novel combinations of *fusC* and SCC*mec* genes [CC5-t002-SCC*mec* I & *fusC* (M11/0141), CC5-t311-SCC*mec* V & *fusC* (M11/0260) and CC1-t127-SCC*mec* IV & SCC*fus* (E3258)] or *fusC* in novel genetic backgrounds [CC8-t008-SCC*mec* VI & *fusC* (M08/0515) and CC30-t021-SCC*mec* IV & *fusC* (M10/0343)] (Table 4.1). An additional two isolates assigned to CC30-t382-SCC*mec* IV, *ccrC* & *fusC* and CC8-t14362-SCC*mec* IV & V & *fusC*, both of which harboured novel combinations of SCC*mec-fusC*, did not undergo WGS due to resource constraints at the time of selection (Table 4.1). The isolates that were selected for WGS were chosen to represent as diverse a range as possible of genotypes and SCC*mec-fusC* combinations identified in the present study.

High-throughput *de-novo* sequencing was performed commercially by Geneservice Ltd. using the Illumina Genome Analyser system (Illumina, HiSeq 2000 platform, Illumina, Essex, UK) with fluorophore detection (Fig. 4.4). Libraries were generated by bridge amplification creating clonal clusters of library molecules directly on the surface of the sequencing flow cell (Fig. 4.4, Panel A). The nucleotides used during sequencing were labelled with different fluorophores which were pushed through flow cell lanes and allowed to anneal to the library clusters where a single nucleotides anneals at a time (Fig. 4.4, Panel B- C) (Shin *et al.,* 2014). After annealing, excess nucleotides were washed away and the fluorophore emissions from each cluster were imaged. An enzymatic reaction cleaved the fluorophore followed by the next round of nucleotide annealing (Fig. 4.4, Panel D). Each nucleotide was coded by a different colour emission and the resulting data was converted into nucleotide sequence (Shin *et al.,* 2014).

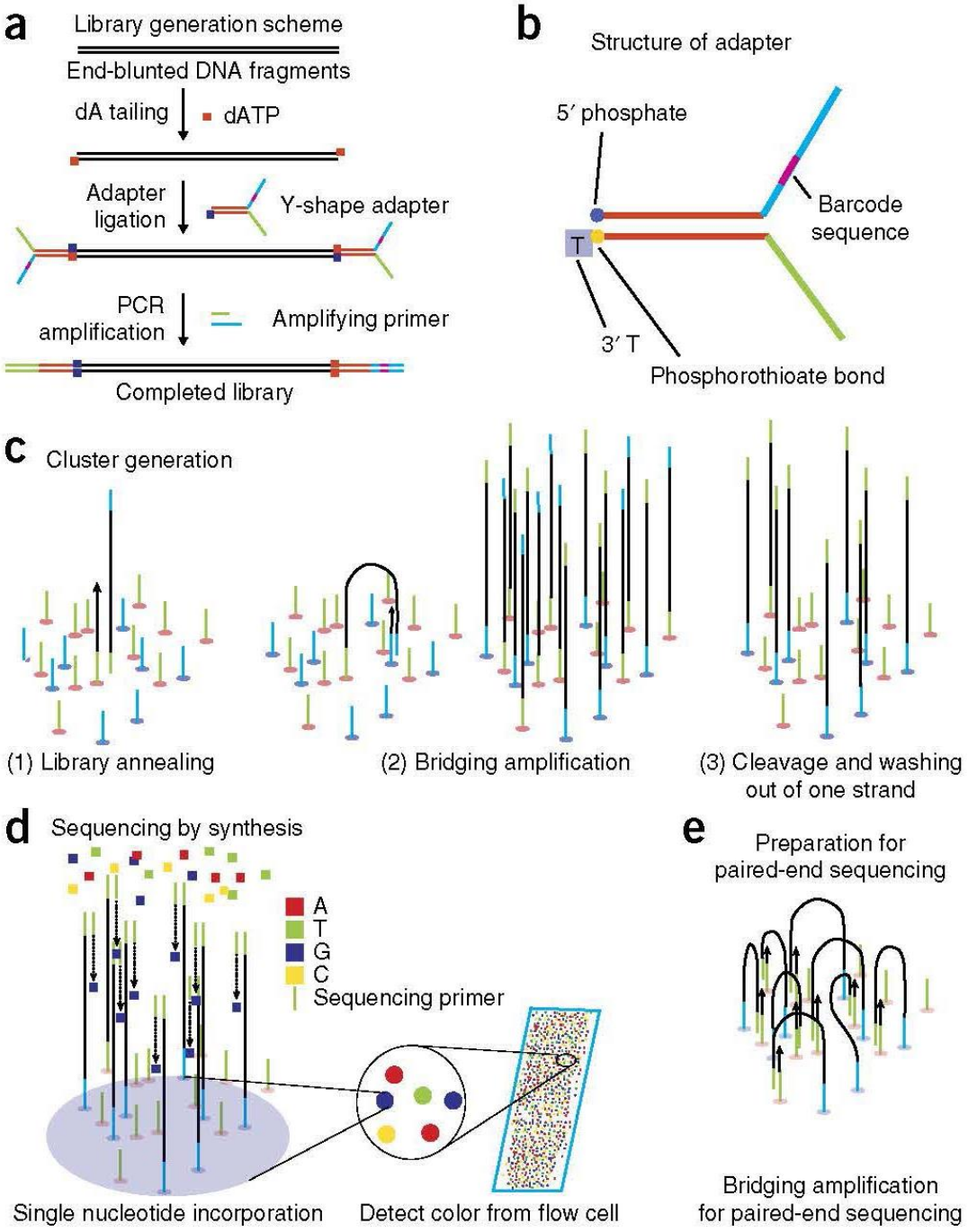


Figure 4.4 Overview of whole-genome sequencing using Illumina technology. (a) The library is composed of fragments of double-stranded DNA that can be recognised on the sequencer. DNA to be sequenced is fragmented to 200-600 base pairs and flanked by adapter sequences. A 3′ dA tail is added using DNA polymerase without 3′-5′ exonuclease activity. dA tailing prevents concatemerisation of DNA fragments and allows use of a dT-tailed adapter which reduces dimer formation. A Y-shaped adapter is then ligated using A-T base pairing and the correctly ligated libraries are amplified. (b) The 5′ end of the Y-

shaped adapter contains a phosphate group and the 3′ end contains dT. Phosphorothionate bonds provide resistance to nuclease. (c) Cluster generation on the surface of the flow cell. (1) Denatured libraries are annealed to the short oligonucleotides on the surface of the flow cell. The distance between DNA molecules is long enough to prevent overlapping. (2) Bridging amplification generates clusters. (3) One strand of the ds-DNA library is cleaved and washed out for unidirectional sequencing. (d) Primers for inserts are annealed for the sequencing of the insert DNA. In each sequencing cycle, protected and fluorescently labelled A, T, G and C bases are applied. After the addition of each nucleotide, the sequencing reaction is stopped and the image is taken. As the newly added nucleotides with each cluster are identical, the signal is high enough to be detected by a light sensor. After the image is taken the protection group and the fluorescent molecules are removed. (e) When the first strand sequencing reaction is finished, the synthesised strand is removed and the process is repeated for the opposite strand. Adapted from Shin *et al.,* 2014.

Analysis and assembly of the whole-genome sequencing data was performed using the Artemis viewer software (http://www.sanger.ac.uk/resources/software/artemis). The assembled genomes were annotated against previously published *S. aureus* and SCC*mec* elements including MSSA476 (Genbank accession number BX571858) and SCC*mec* I, NCTC10442 (AB033763); SCC*mec* II, N315 (D86934), SCC*mec* III, 85/2082 (AB037671), SCC*mec* IV, CA05 (AB063172), SCC*mec* V, WIS/WBG8318 (AB121219), SCC*mec* VI, HDE288 (AF411935), SCC*mec* VII, JCSC6082 (AB373032), SCC*mec* VIII, C10682 (FJ390057) and SCC*mec* XI, LGA251 (FR821779.1) using the BioNumerics genome analysis tool (GAT) (version 7.5, Applied Maths). Contigs that contained SCC-related DNA sequences were identified and analysed using BioNumerics software version 7.5. Gaps between contigs were confirmed by PCR using the primers listed in Table 4.2. The genetic organisation of each element was confirmed by PCR along the entire SCC*mec*-*fusC* element using the primers listed in Table 4.2 where the sizes of the amplimers obtained were compared to the expected size of the amplimers based on the whole-genome sequence. A schematic diagram of each element was prepared using Lucidchart software (www.lucidchart.com).

Table 4.2 Primers used for PCR to confirm contig gap closures and the genetic organisation of the SCC*mec-fusC* elements identified by whole-genome sequencing

Isolate no. (CC-*spa* type-SCC*mec* & *fusC*)	Target region	Primer name	Nucleotide sequence (5′-3′)	Expected product size (bp)
M11/0141 (CC5-t002-MRSA-I & *fusC)*	*orfX-mecA*	M11/0141-1	acgtttaggcccatacacca	5842
		M11/0141-2	atcttggggtggttacaacg	
	mecA-ccrB1	M11/0141-3	acgttgtaaccaccccaaga	6730
		M11/0141-4	gctgcgagtaacaccacaaa	
	ccrB1-orf1	M11/0141-5	tcgcagaacatacctttgagc	6142
		M11/0141-6	tgtggagcaacatactgtgga	
	orf1- chromosome	M11/0141-7	gcgaactctgttgtatggttga	5168
		M11/0141-8	ttacttcgctagacttacccag	
M08/0515 (CC8-t008-MRSA-VI & *fusC)*	*orfX-mecA*	M08/0515-1	aaatgatgcgggttgtgtta	6000
		M08/0515-2	tcaggttacggacaaggtga	
	mecA- orf2	M08/0515-3	cccaatttgtctgccagttt	5820
		M08/0515-4	gaacacccgaaaacctcaaa	
	orf2- orf3	M08/0515-5	gtgccttagggttgagtgga	5758
		M08/0515-6	aacaacgtaccgacccagtc	
	orf3- orf4	M08/0515-7	gactgggtcggtacgttgtt	5304
		M08/0515-8	agcttggcgacctttcataa	
	orf4- orf5	M08/0515-9	ccgtggctaatccagtagga	5318
		M08/0515-10	gggatttgcagattcgaaga	
	orf5- orf6	M08/0515-11	ccgcgtgcagtattgaatta	5101
		M08/0515-12	ggggcacatttgataattgg	
	orf6- orf7	M08/0515-13	cccttgaccttcagcgatag	5272
		M08/0515-14	cggtggttaagcaatcccta	
	orf7- chromosome	M08/0515-15	tggtgcttgggaaagaaaag	1579

Continued overleaf

Table 4.2 continued

Isolate no. (CC-*spa* type-SCC*mec* & *fusC*)	Target region	Primer name	Nucleotide sequence (5′-3′)	Expected product size (bp)
		M08/0515-16	ccaccatttaacaccctcca	
M10/0343 (CC30-t012-MRSA-IV & *fusC)*	*orfX-ugpQ*	M10/0343-1	acgtttaggcccatacacca	5183
		M10/0343-2	tgtggcatgatttcttctgc	
	ugpQ-mecRI	M10/0343-3	ctgcaggatcttgtttggt	4147
		M10/0343-4	gcaacaatacgcttgtttcg	
	mecRI-ccrB2	M10/0343-5	aaggcattccgacaaattga	5094
		M10/0343-6	catggcaaaggaacgaatct	
	orf8- orf9	M10/0343-7	acgggattttgggattgtgc	5483
		M10/0343-8	aaccgtcgtgcaaatgtacc	
	orf9- fusC	M10/0343-9	acatttgcacgacggttcaa	6477
		M10/0343-10	ggagaggtagacaaggcagg	
E3258 (CC1-t127-MRSA-IV & *fusC*)	*orfX- ugpQ*	E3258-1	ttggaagcaagccatagcaga	3419
		E3258-2	tgctagtccattcggtccagta	
	ugpQ-IS272	E3258-3	cagcacgtgcaaaccaactt	4198
		E3258-4	tgggtttcactcggatgtctg	
	IS272-ccrB2	E3258-5	accctggcttaagttgtcca	3143
		E3258-6	acgcgtagtaacacacgtca	
	ccrB2- orf10	E3258-7	ttggagctcaggtctgttca	3705
		E3258-8	agtacacgccaggattacca	
	orf10- orf11	E3258-9	cgacgtcctacttcacgttg	4761
		E3258-10	tcatacacctggtcacacgc	
	orf11- chromosome	E3258-11	gcgtgtgaccaggtgtatga	5973
		E3258-12	aagggcgtattggatggcaa	
	Chromosome- *orf12*	E3258-13	tgccatccaatacgcccttt	3116

Continued overleaf

Table 4.2 continued

Isolate no. (CC-*spa* type-SCC*mec* & *fusC*)	Target region	Primer name	Nucleotide sequence (5′-3′)	Expected product size (bp)
		E3258-14	gcctatgctaagcgaccagt	
	orf12- ccrA1	E3258-15	gtgtaagatgcgcaagtggg	3010
		E3258-16	cgcaagccttatcaagtgcg	
	ccrA1- chromosome	E3258-17	tcggctaatgccttgatggt	3932
		E3258-18	tggaaaagcccaacctagcg	
	Chromosome- *orf13*(after SCC*mec*)	E3258-19	gctggtcgagcaccttatga	3818
		E3258-20	agtgtcttgcgtccgtttttc	
M11/0260 (CC5-t311-MRSA-V & *fusC*)	Contig break 1	M11/0260-1	tcatggcatggcgaaaatcc	305
		M11/0260-2	atgtcccaagctccattttgt	
	Contig break 2	M11/0260-3	cgttgagcaagtgatggaaa	616
		M11/0260-4	aaatggcgtattgatgagacg	
	orfX- ccrC1	M11/0260-5	tcgtcattggcggatcaaac	6971
		M11/0260-6	tccgactgctctgacgtact	
	ccrC1-mecA	M11/0260-7	tgaagtcgtcgagcgtgtaa	5474
		M11/0260-8	ttggccaatacaggaacagca	
	mecA- orf14	M11/0260-9	acgattgtgacacgatagcca	4703
		M11/0260-10	tgctcccaattcttcacccc	
	orf14- ccrC1	M11/0260-11	tttgacagacggtggggtga	4502
		M11/0260-12	gacgtttcattgccggacg	
	ccrC1-orf15 (after SCC*mec*)	M11/0260-13	agagtgacaaaagtggaggagt	6036
		M11/0260-14	agcgtatcgattaagagtgaggt	

Abbreviations: bp; base pairs

4.3 Results

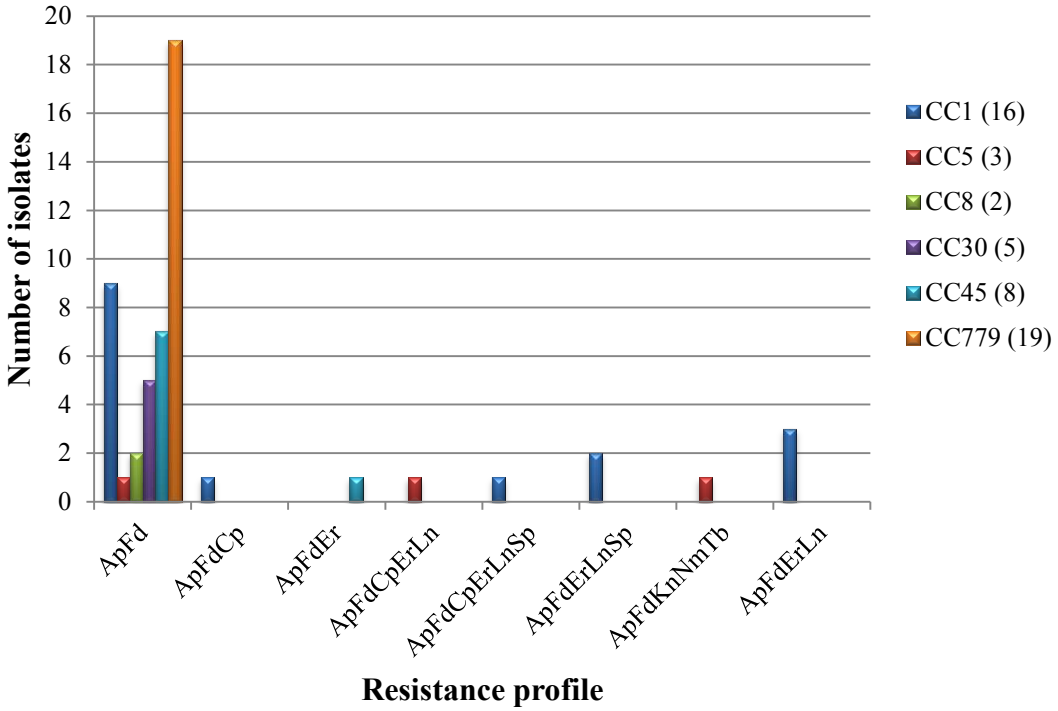
4.3.1 Phenotypic and genotypic characteristics of fusC-positive MRSA

The fusidic acid MICs of the 53 isolates ranged from 4-16 mg/L and all harboured *fusC* and *mecA*. Forty three isolates (81.1%) exhibited resistance to just one other antimicrobial agent tested, ampicillin, 42 (79.3%) of which carried *blaZ* (Fig. 4.5). Eight isolates (15.1%) also exhibited resistance to erythromycin which was associated with the presence of *erm*(A) (37.5%, 3/8) and *erm*(C) (62.5%, 5/8). Three isolates exhibited resistance to spectinomycin (5.7%) and one was resistant to kanamycin, neomycin and tobramycin and harboured *aadD* (1.9%) (Fig. 4.5). Thirteen isolates (24.5%) also harboured the fosfomycin resistance gene *fosB* (Fig. 4.5).

DNA microarray analysis assigned the isolates to six CCs; CCs 1 (30.2%, 16/53), 5 (5.7%, 3/53), 8 (3.8%, 2/53), 30 (9.4%, 5/53), 45 (13.5%, 8/53) and 779 (35.8%, 19/53) (Table 4.1). Two *spa* types were recognised amongst the isolates in CC779 (t878 (18/19) and t11021 (1/19)), CC5 (t002 (2/3) and t311 (1/3)) and CC8 (t008 (1/2) and t14362 (1/2) while three *spa* types were recognised in CC1 (t127 (6/16), t2279 (9/16) and t16173 (1/16)) and CC30 (t012 (2/5), t021 (2/5) and t382 (1/5)). The seven isolates within CC45 were assigned to four *spa* types (t383 (1 isolate), t728 (1 isolate), t2277 (2 isolates), t4545 (4 isolates)).

Overall eight SCC*mec* type combinations were identified among the 53 isolates (Table 4.1). Additional *ccrAB1* genes were identified in 30.1% (16/53) of isolates, all of which were CC1 and were indicative of the presence the previously described CI involving SCC*mec* IV and a SCC*fus* element (Table 4.1). Furthermore, two additional putative CIs were recognised, in the form of SCC*mec* IV & V & *fusC* and SCC*mec* IV, *ccrC* and *fusC*

A Phenotypic resistance



B Genotypic resistance

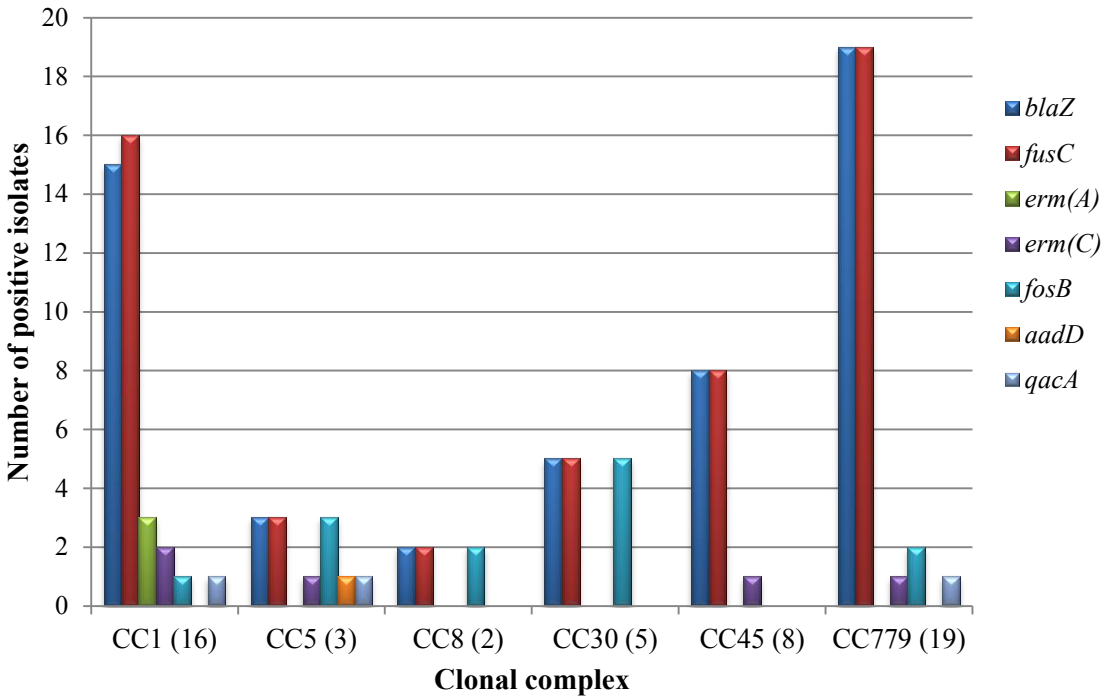


Figure 4.5 Phenotypic and genotypic resistance profiles detected among the 53 fusidic acid (Fd) resistant MRSA isolates. All isolates harboured the *mecA* gene and exhibited reduced susceptibility to cefoxitin. Additional phenotypic resistance patterns (panel A)

were determined in accordance with EUCAST interpretive criteria against a panel of 25 antimicrobial agents including amikacin, ampicillin (Ap), cadmium acetate, cefoxitin, chloramphenicol, ciprofloxacin (Cp), erythromycin (Er), ethidium bromide, gentamicin, kanamycin (Kn), lincomycin (Ln), linezolid, mercuric chloride, mupirocin, neomycin (Nm), phenyl mercuric acetate, rifampicin, spectinomycin (Sp), streptomycin, sulphonamide, tetracycline, tobramycin (Tb), trimethoprim and vancomycin

Resistance genes (panel B) were detected by the *S. aureus* Genotyping Kit 2.0. (Alere Technologies GmbH). The clonal complex (CC) for each isolate was assigned using the DNA microarray *S. aureus* Genotyping Kit 2.0 and the total number of isolates in each CC is shown in parentheses.

(each 1.9%, 1/53) because *mec* and *ccr* genes indicative of a particular SCC*mec* type were detected in addition to *fusC* and *ccr* genes other than *ccrAB1*.

Similarly, putative chimeric elements were recognised where *mec*A and *ccr* genes were detected indicative of a particular SCC*mec* type in addition to *fusC* but without any other *ccr* related genes. These included ΨSCC*mec-fus*-SCC-SCC$_{CRISPR}$ (35.8%, 19/53), SCC*mec* IV & *fusC* (24.5%, 13/53) and one isolate (1.9%) was assigned to each of the following combinations: SCC*mec* V & *fusC,* SCC*mec* I & *fusC* and SCC*mec* VI & *fusC* (Table 4.1).

Seventeen different CC, *spa* and SCC*mec* type combinations were identified with CC779-t878-ΨSCC*mec-fusC*-SCC$_{CRISPR}$ predominating (34.0%, 18/53), followed by CC1-t2279-SCC*mec* IV & SCC*fus* (17.0%, 9/53) and CC1-t127-SCC*mec* IV and SCC*fus* (11/.3%, 6/53) (Table 4.1). In addition to CC1, CC5, CC45 and CC779, all of which have previously been found to carry *fusC* (Kinnevey *et al.,* 2013; Williamson *et al.,* 2014; Ellington *et al.,* 2015; Baines *et al.,* 2016), *fusC* was also detected in CC30. Furthermore, although previously reported in ST239-MRSA-III, *fusC* was recognised in two different CC8 clones in the current study (t008 and t14362) (Table 4.1).

4.3.2 Novel SCCmec-fusC elements identified by whole-genome sequencing

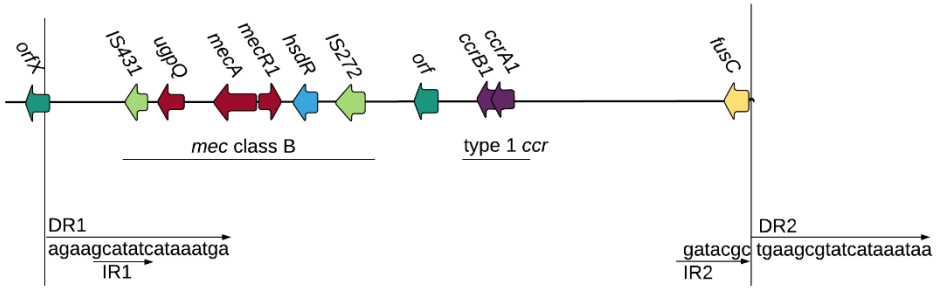
Whole-genome sequencing revealed the presence of five distinct and novel SCC*mec-fusC* elements in each of the five isolates investigated and a schematic representation of each of these elements is shown in Fig. 4.6. In each of these novel elements, *fusC* was located within or adjacent to SCC*mec* and, the SCC*mec-fusC* elements were integrated at *orfX* and were flanked on each end by direct and inverted repeat sequences (DRs and IRs). With the exception of isolate M11/0260 (CC5-MRSA-V & *fusC*) which harboured a class C *mec* complex, all other isolates harboured the class B *mec* complex but with different

combinations of *ccr* genes. In each of these isolates, *fusC* exhibited 100% DNA sequence homology to that previously identified in SCC*fus* in $MSSA_{476}$.

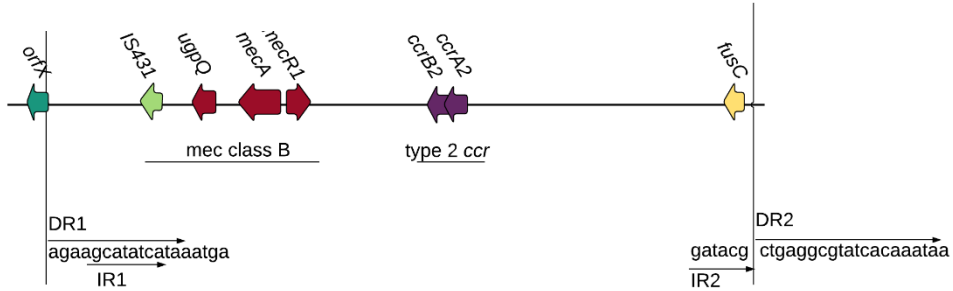
Four of the SCC*mec-fusC* elements identified were chimeric elements consisting of a single SCC*mec* element, flanked by DR and IRs, consisting of a SCC*mec* element, either SCC*mec* VI, I, IV or V and *fusC* (Fig. 4.6 (A) - (C) and (E), respectively). The remaining isolate harboured a composite element comprised of two distinct elements, SCC*mec* IV (*ccrAB2* and class B *mec*) and SCC*fus* (*ccrAB1* and *fusC*), each flanked by DRs and IRs (Fig. 4.6 (D)).

(A) CC8-t008-SCC*mec* VI & *fusC* 38.3kb (M08/0515)

(B) CC5-t002-SCC*mec* I & *fusC* 22.1kb (11/0141)



(C) CC30-t012-SCC*mec* VI & *fusC* 23.1kb (M10/0343)



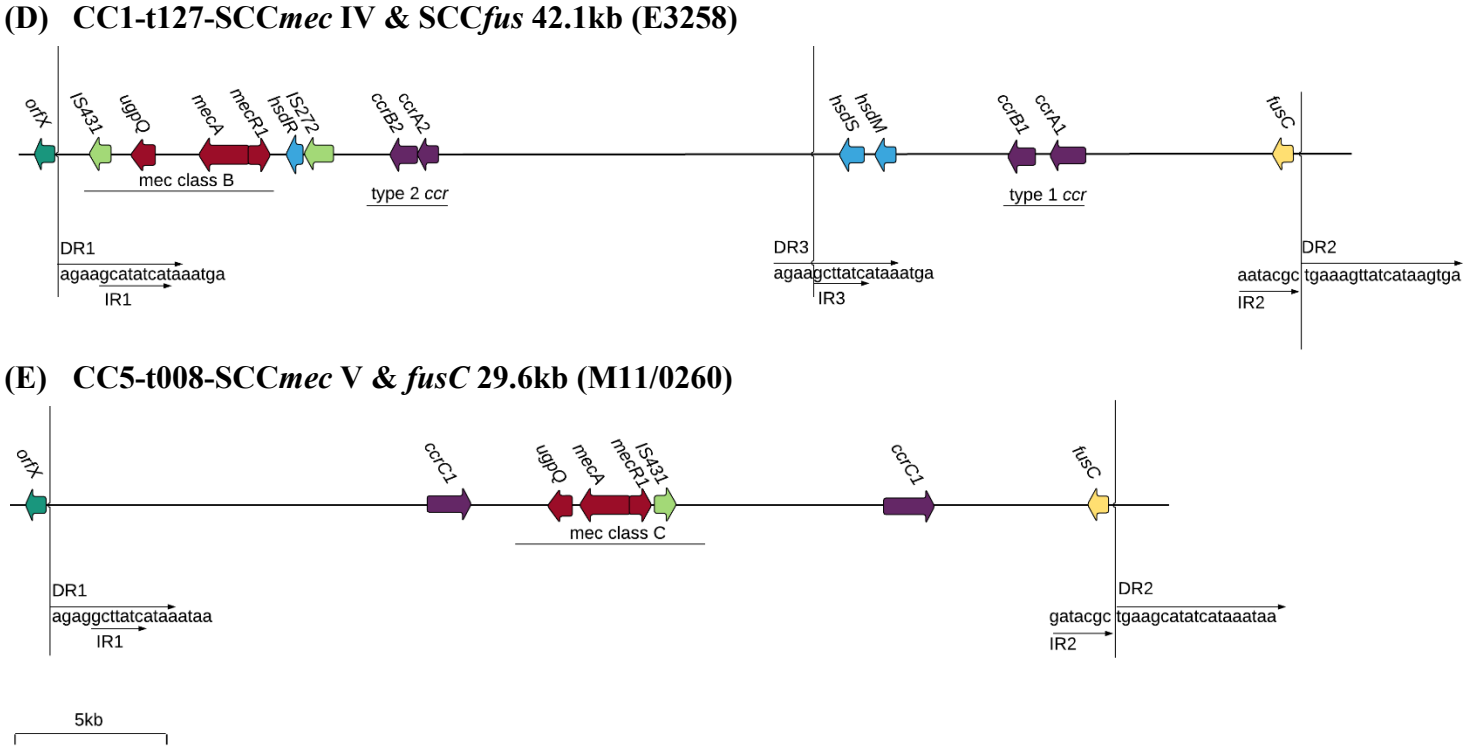


Figure 4.6 Schematic representation of the genetic organisation of the novel SCC*mec*-f*usC* elements identified among the five fusidic acid resistant MRSA isolates in the present study as determined by whole-genome sequencing. The genetic organisation of the SCC*mec*-f*usC* elements were confirmed by PCR using primers spanning the entire SCC*mec*/*fusC* region (Table 4.2). Isolate numbers are indicated in parenthesis. The *mec* and *ccr* gene complexes are shown in red and purple, respectively, while *fusC* is shown in yellow. Abbreviations: DR, direct repeat; IR, inverted repeat; kb, kilobase.

4.4 Discussion

Antimicrobial resistance is a major concern worldwide and MRSA is one of the most problematic pathogens (WHO, 2014). Resistance against all classes of antimicrobial agents has been described in MRSA with the majority due to the acquisition of resistance genes carried on MGEs (Deleo *et al.,* 2009). In the present study a relatively high rate of detection of *fusC* was identified among genetically diverse MRSA isolates recovered throughout Ireland (Chapter 3) which was coupled with the recent observation of an increasing prevalence of fusidic acid resistance among MRSA in Ireland (Fig. 4.2), (NMRSARL, 2014). Of the six CCs identified here among *fusC* MRSA, all are commonly associated with CA-MRSA including CC1-MRSA-IV, CC5-MRSA-I, CC5-MRSA-V and CC30-MRSA-IV (Monecke *et al.,* 2011). Additionally HCA-MRSA genotypes were identified including CC5-MRSA-IV and CC45-MRSA-IV (Monecke *et al.,* 2011), although *fusC* was not identified in the most common HCA-MRSA clone in Irish hospitals, ST22-MRSA-IV.

Extensive genetic diversity was observed among these *fusC*-positive MRSA isolates in relation to their genetic backgrounds and the SCC*mec-fusC* elements that they harboured. A total of 17 different combinations of CC-*spa*-SCC*mec* type combinations were identified among the 53 isolates. Although *fusC* was frequently associated with the SCC*fus* element in the current study accounting for 30.2% of *fusC*-positive MRSA isolates (16/53), it was limited to CC1. While previously published *fusC* elements including CC1-SCC*mec* IV & SCC*fus* and CC779-ΨSCC*mec-fus*-SCC-SCC_{CRISPR} were detected in the current study, 17.0% (9/53) of isolates appeared to harbour possible novel and complex SCC*mec-fusC* CIs and chimeric elements (Table 4.1). Whole-genome sequencing of five

of these isolates confirmed the presence of novel and distinct chimeric *fusC* and SCC*mec* elements in four isolates and a CI of SCC*mec* & SCC*fus* in one isolate (Fig. 4.3).

The result of this study suggests that the spread of SCC*mec-fusC* elements has occurred between different MRSA strains. While 7/9 SCC*mec*/*fusC* gene combinations identified here were unique to a particular CC, the combination of SCC*mec* IV and *fusC* was detected in 13 isolates belonging to four different CCs and seven *spa* types indicating that the successful spread of a chimeric element has occurred between different MRSA lineages. The evidence from this study also suggests that, in some instances, different strains within the same CCs have acquired different SCC*mec-fusC* elements i.e. isolates within CCs 8, 5, 30 and 45 harboured two, three, two and four different combinations of SCC*mec*/*fusC* genes, respectively, indicating that a selective pressure exists promoting the spread of these elements.

The earliest SCC*mec-fus* chimeric element described in the current study was recovered in 2008 (CC8-MRSA-IV & *fusC*). In a previous study investigating MRSA in healthcare facilities in Ireland between 1971 and 2007, CC30 harbouring *fusC* were described suggesting that *fusC*-positive MRSA have been circulating in Irish hospitals for several years (Shore *et al.,* 2012). While the source of these elements in these MRSA isolates is unknown, previous studies have revealed that SCC*mec* originated in CoNS (Shore & Coleman, 2013). In fact, CoNS appear to be a reservoir for resistance genes in *S. aureus* (Otto, 2013b). Therefore, it is plausible that CoNS are a reservoir for *fusC* (Castanheira *et al.,* 2010a; Castanheira *et al.,* 2010b). Methicillin-susceptible *S. aureus* could also be a source of *fusC* in MRSA. However, direct evidence supporting either of these suggestions is lacking and analysis of fusidic acid resistance rates and detailed comparative analysis of the SCC*mec-fusC* elements in MRSA, MSSA and CoNS is required.

While the precise route of transmission of SCC*mec* and SCC in *S. aureus* is unknown, it has been shown that the excision and site- and orientation-specific integration of SCC*mec* into and out of the *S. aureus* genome is due to recombinases encoded by *ccr* genes (Shore & Coleman, 2013). The presence of *fusC* on SCC elements with *ccr* genes but in the absence of *mec* both here and in previous studies suggests that the SCC element emerged as a system to facilitate the horizontal gene transfer of various resistance and virulence genes (Monecke *et al.*, 2016). In the present study *fusC* was found to be present within the SCC*mec* element or on an additional SCC element adjacent to SCC*mec* and to date this is the only known mechanism of *fusC* dissemination (Baines *et al.,* 2016).

The success of HCA-MRSA clones has been attributed to the spread, stability and selection of MGEs along with clonal adaptation of strains to the healthcare environment, particularly through the rapid adaptation of clones to antibiotic exposure (Lindsay *et al.,* 2012). The SCC*mec* IV element is the most widespread SCC*mec* element among MRSA and has been described in multiple HCA- and CA-MRSA CCs including CCs 1, 5, 8, 22, 30, 45, 80, 88 and 93 (Chambers & Deleo, 2009; Monecke *et al.,* 2011). Interestingly, in the present study *fusC* was most frequently associated with SCC*mec* IV, accounting for 54.7% (29/52) of isolates, and was present in the form of CI and chimeric elements. The success of the dissemination of the SCC*mec* IV element within MRSA has been attributed to a lack of fitness cost due its small size, where it has been shown that CA-MRSA strains harbouring SCC*mec* IV have a faster growth rate than HCA-MRSA strains harbouring other, larger, SCC*mec* types, and have similar growth rates to MSSA suggesting that there is no fitness cost to the *S. aureus* to carry SCC*mec* IV (Diep *et al.,* 2008). The emergence of these chimeric elements encoding both methicillin and fusidic acid resistance together with the most successful SCC*mec* element suggests that there is potential for further dissemination of these elements among other lineages of MRSA in the future.

While appropriate antimicrobial stewardship programmes ensure the correct use of topical agents for dermatological infections such as impetigo, studies have shown that often these agents are often used for non-indicated skin conditions (Lapolla *et al.,* 2011). In addition, the inappropriate and increased use of agents such as fusidic acid and mupirocin in the community have been related to increased resistance (Brown & Thomas, 2002; Livermore *et al.,* 2002). Despite a decrease in usage observed in hospitals in the UK, an increase in fusidic acid resistance among CA-MRSA has given rise to suggestions that inappropriate usage of the topical agent in the community may cause an increase in the ineffectiveness in the treatment of systemic infections in hospitals (Ellington *et al.,* 2015). Antimicrobial consumption in Ireland is monitored by the HPSC and systemic use of fusidic acid decreased during 2010-2014 (HPSC, 2015). No similar monitoring system is in place for the topical use of fusidic acid in Ireland and therefore it is not possible to determine the amount used in the community. However, based on the predominantly CA-MRSA genotypes identified in association with *fusC*-positive MRSA in the present study it is possible to speculate that the inappropriate topical usage of fusidic acid in the community in Ireland is selecting for the emergence and spread of *fusC*-positive CA-MRSA in Ireland.

Antimicrobial prescribing guidelines are based on phenotypic resistance patterns for a single agent with attempts to reduce antimicrobial resistance based on the independence of resistance mechanisms. In the current study, while resistance to erythromycin and aminoglycosides were detected, the majority of isolates (81.1%, 43/53) exhibited phenotypic resistance only to β-lactams and fusidic acid. Similar observations have been reported in the UK where MRSA recovered from BSIs and which exhibited resistance to only fusidic acid steadily increased between 2002-2013 (Ellington *et al.,* 2015). The SCC*mec* chimeric elements described here, carry not only the necessary *ccr* genes which

allow mobility of these elements, but also *mecA* and *fusC,* indicating that *S. aureus* has the potential to transfer multiple resistance genes in a single cassette which may lead to further increases in multidrug resistance in *S. aureus.* The presence of multiple resistance genes on a single element may be co-selected for by multiple drugs and attempts to reduce the selective pressure should take into consideration genetically linked resistance mechanisms.

Although licensed in Europe and Australia for a number of years, fusidic acid has only recently become available within the USA and therefore there are limited studies investigating resistance among MRSA recovered in the USA (Moellering *et al.,* 2011). Many of the predominant MRSA strains in the USA harbour *pvl* and, although this study did not investigate it, *pvl*-positive *S. aureus* strains in Ireland have previously been found to harbour *fusC* including CC1-MSSA and CC30-MRSA-IV (Shore *et al.,* 2014). The mobility of the SCC*mec* chimeric elements harbouring both *fusC* and *mecA,* along with adaptability of *S. aureus* in the presence of new selective pressures, suggests that in the near future, similar increasing fusidic acid resistance seen in Ireland, UK and Australia is possible within the USA and highlights the need for close monitoring of fusidic acid resistance within the USA. Furthermore, extensive studies are required to investigate the emergence of fusidic acid resistance in MSSA to determine if there is a predominant clone harbouring the SCC*fus* circulating in either the community or healthcare facilities or involvement of any other *ccr* genes assisting in the spread of *fusC*.

4.5 Conclusion

This part of the present study has highlighted the ability of *S. aureus* to readily adapt as the need arises in response to antimicrobial agents. The diversity of the chimeric SCC*mec-fusC* elements and the different lineages identified here suggest that *S. aureus* may favour smaller elements harbouring multiple resistance genes over larger composite elements. The use of genomic studies characterising resistance elements are necessary in order to comprehensively characterise and compare the elements and to attempt to prevent further spread.

Chapter 5

An investigation of the application of advance molecular typing techniques for enhanced tracking of MRSA

5.1 Introduction

The choice of typing technique used in an epidemiological investigation of any microorganism is dependent on the situation under investigation along with the context in which the method is to be used, the timescale and the geographical scale of its use (Stefani *et al.,* 2012; Sabat *et al.,* 2013a; Leopold *et al.,* 2014; Maiden *et al.,* 2014). An ideal typing technique should be rapid, inexpensive, highly reproducible, and easy to perform, should yield data that is easy to interpret and should utilise a standardised nomenclature to allow inter-laboratory comparison of strains. Furthermore, typing techniques should be able to link isolates together based on genealogical relationships while at the same time differentiate very closely related strains to identify transmission events (Maiden *et al.,* 2014). Finding an informative, accurate and efficient typing technique is especially difficult when attempting to track highly-clonal pathogens such as MRSA, which often exist in endemic situations in the nosocomial environment where differences between unrelated strains may not always be detected using conventional typing methods (Goering *et al.,* 2008; Stefani *et al.,* 2012; Leopold *et al.,* 2014; Maiden *et al.,* 2014).

The need for higher resolution characterisation of isolates has led to the development and use of a wide range of molecular methods for typing of MRSA. However, there are both advantages and disadvantages associated with many of the commonly used MRSA molecular typing methods and those associated with PFGE, MLST and *spa* typing are outlined in Table 5.1 (Sabat *et al.,* 2013; Maiden *et al.,* 2014).

The development of second generation sequencing techniques during the last decade, and the subsequent lowering of the associated costs and time involved, has resulted in the publication of a huge range of microbial whole-genome sequences. Based on these, various DNA microarray and RNA expression platforms have been developed for a number of

Table 5.1 Advantages and disadvantages of commonly used MRSA molecular typing techniques

Method	Principle	Advantages	Disadvantages
PFGE	• Genomic DNA is cleaved with a rare-cutting restriction endonuclease • The resulting restriction fragments, which are mostly large, are separated in an agarose gel by 'pulsed-field' electrophoresis in which the orientation of the electric field across the gel is changed periodically. • The separated DNA fragments are visualised in the gel as bands, and the resulting banding patterns are compared	• Excellent discriminatory power and high epidemiological concordance • Relatively inexpensive approach with excellent typeability and intra-laboratory reproducibility • Standardised approaches have allowed for inter-laboratory comparison of banding patterns • Insertions or deletions of mobile genetic elements as well as large recombination events within genomic DNA will result in changes in the PFGE patterns	• A technically demanding, labour-intensive and time-consuming method • Detects uncharacterised variations that occur in the chromosome • Lack of standardised nomenclature • May lack the resolution power to distinguish bands of nearly identical size (i.e. fragments differing from each other in size by less than 5%) • The analysis banding patterns is prone to subjectivity • Only useful for outbreak situations really as variation accumulates rapidly so can mask longer term relatedness • Continuous quality control and portability of data are limited compared to sequence-based methods
***spa* typing**	• Sequencing of the polymorphic X region of the protein A gene of *S. aureus* • Results in variable number of short repeats (24 bp) • Each identified repeat is associated to a code and a *spa*-type is deduced from the order of specific repeats	• Cost-effective method as only a single genes no larger than 800 bp requires sequencing • Relatively easy to perform • Excellent reproducibility • Online curator controlled database (http://www.spaserver.ridom.de/) allows easy sharing of data • Standardised international nomenclature • Useful in outbreak investigations and more long term studies • Strict criteria for internal and external quality assurance of data submitted to the database that is curated by SeqNet.org	• Lower discriminatory ability than PFGE • Based on single-locus typing is that it can misclassify particular types due to recombination and/or homoplasy

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Table 5.1 continued

Method	Principle	Advantages	Disadvantages
MLST	• Seven housekeeping genes are amplified by PCR and sequenced • For each locus, unique sequences (alleles) are assigned arbitrary numbers and, based on the combination of identified alleles (i.e. the 'allelic profile'), the sequence type (ST) is determined.	• Also, can infer MLST from *spa* type in most instances • The number of nucleotide differences between alleles is not considered • Examines only a small section of the genome • Data produced are unambiguous due to an internationally standardised nomenclature, and highly reproducible. MLST typing data is also used in the standard nomenclature of MRSA strains • Useful in long term studies of isolates from disparate geographic locations • Allele sequences and ST profiles are available in large central databases (http://pubmlst.org and www.mlst.net) that can be queried via the Internet • On-line software (eBURST) available for determination of the genetic relatedness between bacterial strains within a species as well as MLST-maps to track the isolates of each ST that have been recovered from each country plus the details of these isolates	• Requires sequencing of seven genes so is expensive in comparison to PFGE and *spa* typing • MLST is labour-intensive, time-consuming and insufficiently discriminatory for routine use in outbreak investigations and local surveillance

different organisms (Monecke & Ehricht 2005; Sabat *et al.,* 2013). One such platform developed for *S. aureus* is the *S. aureus* Genotyping Kit 2.0 DNA microarray (Alere Technologies GmbH) which allows the simultaneous array profiling of up to 96 isolates. The array covers 334 target sequences including 170 distinct genes and their allelic variants (Section 2.4.4). Arrays are scanned and, based on the hybridisation profile of the isolate, automatically assigned to a specific genetic lineage of MLST CC/ST and SCC*mec* type. In addition the presence or absence of antimicrobial resistance and virulence-associated genes are also determined (Shore *et al.,* 2012).

DNA microarray technology has been shown to be a faster, cheaper and a less labour intensive alternative to WGS. In outbreak situations involving *E. coli* O104:H4, a DNA microarray successfully differentiated clinically, temporally and geographically distinct isolates (Jackson *et al.,* 2012). However, no such study has been carried out evaluating the *S. aureus* Genotyping Kit 2.0 DNA microarray in outbreak situations. Instead, the array is more widely used for long-term epidemiological studies for characterising large collections of isolates recovered from specific regions (Monecke *et al.*, 2011; Monecke *et al.*, 2012a; Monecke *et al.,* 2012b; Uhlemann *et al.*, 2012; Rasmussen *et al.*, 2014; Shore *et al.*, 2014; Senok *et al.*, 2016).

Advances in WGS technology have also resulted in a plethora of studies that have utilised WGS for both small and large scale epidemiological investigations with highly discriminatory differentiation of isolates (Köser *et al*., 2012; Harris *et al.,* 2013; Leopold *et al*., 2014; Salipante *et al*., 2015). These have been primarily based on the determination of the relatedness of isolates based on SNV analysis, which requires complex bioinformatic analysis of the sequencing data using expensive computer software e.g. BioNumerics or Lasergene. Additionally, while WGS SNV analysis has been applied to outbreak situations, including those involving MSSA and MRSA, and to identify cases of cross transmission,

difficulties have arisen in determining an acceptable cut off for assigning isolate relatedness based on SNV differences in these settings (Köser *et al.*, 2012; Harris *et al.*, 2013; Price *et al.*, 2013; Kinnevey *et al.*, 2016). Lastly, SNV analysis considers each nucleotide polymorphism as a single event despite many common single recombination events resulting in several nucleotide changes (Leopold *et al.*, 2014).

In an attempt to overcome the limitations associated with WGS and to utilise the discriminatory power of WGS in clinical settings, gene-by-gene typing schemes, based on conventional typing methods have been developed. In particular, an allele-based approach for clustering of isolates, similar to that of MLST, due to the ease at which a centralised international database can be developed along with use of a standardised nomenclature has been suggested (Leopold *et al.*, 2014; Maiden *et al.*, 2014). Maiden *et al.* (2014) proposed a scheme which involved a comparison of the variable core genome (whole-genome MLST) of only isolates involved in a specific study group, region or institution.

In contrast, other gene-by-gene schemes utilise WGS data and compare all genes in the same set of species-specific core genes (core-genome MLST, cgMLST) allowing standardisation of the method and, more importantly, comparison of isolates outside of the immediate study group (Leopold *et al.*, 2014). In the case of S. *aureus*, the cgMLST target set is 1,861 genes which were selected using a gene-by gene comparison to identify all genes in the reference genome that were also present in the query 40 genomes with a sequence identity ≥90% and 100% overlap (Leopold *et al.*, 2014). Software, such as SeqSphere^{+} (Ridom GmbH) has been developed as an alternative to complex bioinformatic pipelines permitting advanced phylogenetic analysis automatically by assigning isolates to cgMLST types. SeqSphere^{+} excludes genes from the scheme which are <50 bp in length, those which lack transcription initiation codons (i.e. start codons) at the beginning of the gene or transcription termination codons (i.e. stop codons) at the end of the gene. A

homologous gene filter also discards all genes with fragments that occur in multiple copies within the genome (with identity 90% and more >100 bp overlap), while a gene overlap filter discards the shorter gene from the cgMLST scheme if the affected two genes overlap >4 bp (Leopold *et al.*, 2014). Furthermore, the development of centralised online databases (available at http://www.cgmlst.org/ncs) similar to those used for *spa* typing and conventional MLST allow for international sharing of data and for the development of a single publicly available central nomenclature service (Leopold *et al.*, 2014).

In Ireland ST22-MRSA-IV is the predominant nosocomial strain accounting for approximately 80% of MRSA recovered from BSIs each year (NMRSARL, 2014). Epidemiological tracking and differentiating of this clone in the Irish nosocomial setting is particularly challenging due to the fact that ST22-MRSA-IV is endemic and because of its highly-clonal nature (Goering *et al.*, 2008; Grundmann *et al.*, 2010; Shore *et al.*, 2010b). Previously it was reported that combining multiple conventional molecular typing techniques enhanced discrimination of ST22-MRSA-IV isolates (Shore *et al.*, 2010b; Creamer *et al.*, 2012; Sabat *et al.*, 2013). In particular, a combination of PFGE, *spa* and *dru* typing of ST22-MRSA-IVh isolates, in conjunction with key epidemiological data, successfully identified incidences of cross transmission between patients and their environments in four wards in a large hospital over a six-week period (Creamer *et al.*, 2012). However, combining several methods, such as *spa*, *dru* and PFGE typing is costly and time consuming, while the data analysis and interpretation is complex and therefore not ideal for routine use.

A subsequent study of a subset of the ST22-MRSA-IVh isolates investigated by Shore *et al*. (2010) utilised WGS SNV analysis to further investigate the transmission of isolates between patients and their environment within in a single ward. In that study, SNV analysis confirmed some previously identified incidences of transmission while refuting

others (Kinnevey *et al.,* 2016). Furthermore, it showed the involvement of all isolates (*n* = 41) in at least one potential transmission event with 228 pairwise comparisons of isolates differing by <40 SNVs, thus highlighting the transmissibility of ST22-MRSA-IVh within the healthcare environment and indicating that conventional methods may incorrectly identify or fail to identify cases of cross transmission (Kinnevey *et al.*, 2016). However, as discussed previously, SNV analysis is technically demanding and comparison of data with other laboratories or with historical isolates from the same environment is difficult and therefore is also not currently ideal for routine use in a clinical setting.

In addition to outbreaks caused by HCA-MRSA ST22-MRSA-IV, other strains are also routinely identified at the NMRSARL as part of outbreak investigations and conventional molecular typing methods often fail to differentiate between these isolates. In particular, as mentioned in Chapter 3, prolonged outbreaks associated with CA-MRSA ST5-MRSA-V and CC1-MRSA-IV have been recognised in two separate hospitals in Ireland but conventional *spa* typing could not differentiate the isolates.

The first aim of this part of the present study was to investigate the usefulness of DNA microarray profiling using the *S. aureus* Genotyping Kit 2.0 and array profiling system for differentiating MRSA isolates recovered during outbreak investigations, including HCA-MRSA ST22-MRSA-IV and CA-MRSA lineages CC5-MRSA-V and CC1-MRSA-IV. The second aim was to investigate the potential for implementation of WGS in a clinical environment for tracking the spread of MRSA, by evaluating cgMLST analysis of ST22-MRSA-IVh isolates that have previously been analysed in detail using traditional molecular typing methods and WGS SNV analysis (Kinnevey *et al.,* 2016).

5.2 Materials and methods

5.2.1 Isolate selection and study design

5.2.1.1 DNA microarray evaluation

Ninety-four MRSA isolates were investigated using DNA microarray profiling and were all recovered from patients or their environment in Irish hospitals during outbreak investigations in several different hospitals. This included:

- 23 MRSA isolates recovered between January and October 2014 during four separate outbreaks in three different hospitals, all of which exhibited phenotypic resistance profiles and *spa* types associated with ST22-MRSA-IV (Table 5.2);
- two MRSA isolates recovered in January 2010 from patients that had undergone surgery in the same operating theatre in a Dublin hospital and both of which exhibited indistinguishable resistance profiles and *spa* types associated with ST22-MRSA-IV (Table 5.2);
- 18 MRSA isolates recovered from screening specimens during an outbreak in a regional NICU between November 2011 and October 2012 and all of which exhibited an indistinguishable phenotypic resistance profile and *spa* type associated with CC5 (Table 5.3);
- 51 MRSA isolates recovered during an outbreak in a large Dublin teaching hospital between March 2013 and December 2015 and were assigned to two closely related *spa* types, t127 or t922, indicative of CC1-MRSA-IV (Table 5.4).

The culture media, growth and storage conditions for all isolates are described in Chapter 2, section 2.1.1. All isolates were previously confirmed as *S. aureus* and underwent AR typing against a panel of 25 antimicrobial agents and *spa* typing as detailed in Chapter 2, section 2.3.

Table 5.2 Molecular epidemiological typing data for 25 ST22-MRSA-IV isolates recovered during five outbreak investigations involving patients in Irish hospitals between 2010 and 2014

Incident & group[a]	No. of isolates	*spa* type	*spa* repeat succession[b]	DNA microarray resistance & virulence genes[c]	Phenotypic antimicrobial resistance[d]
Incident A September – October 2014					
A1	6	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(C), *egc*[e]	Ap, Cd, Cp, Er, Fd, Ln
A2	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, sec/l, egc,* IEC B[f]	Ap, Cp, Tp
A3	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(C), *sec/l, egc,* IEC B	Ap, Cp, Er, Ln, Tp
A4	2	t020	26-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, sec/l, egc,*	Ap, Cp
A5	1	t5893	26-23-23-13-23-31-29-17-31-29-17-25-24-25-16-28	*blaZ, erm*(C), *sec/l, egc,* IEC B	Ap, Cp, Er, Fd, Ln
Incident B September – October 2014					
B1	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ*, *erm*(C), *sec/l*, *egc,* IEC B	Ap, Cd, Cp, Er, Ln, St
B2	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ*, *erm*(C), *sec/l*, *egc,* IEC B	Ap, Cd, Cp, Er, Fd, Ln
B3	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ*, *erm*(C), *sec/l*, *egc*	Ap, Cd, Cp, Er, Fd, Ln
B4	1	t379	26-23-23-13-23-31-29-17- 25-17-25-16-28	*blaZ*, *erm*(C), *sec/l*, *egc*,	Ap, Cp, Er, Fd, Ln
Incident C January – March 2014					
C1	2	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, egc,* IEC B	Ap, Cd, Cp, Fd

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Table 5.2 continued

Incident & group[a]	No. of isolates	*spa* type	*spa* repeat succession[b]	DNA microarray resistance & virulence genes[c]	Phenotypic antimicrobial resistance[d]
C2	1	t515	26-23-23-13-23-31-29-17-31-29-17-25-16-16-28	*blaZ, egc,* IEC B	Ap, Cp
Incident D - July 2014					
D1	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(A)*, sec*, *sed, egc,* IEC B	Ap, Cd, Cp, Er, Fd, Ln
D2	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(A), *sed, egc,* IEC B	Ap, Cd, Cp, Er, Fd, Ln
D3	1	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(C), *egc,* IEC B	Ap, Cp, Er, Ln
D4	1	t2945	26-23-23-13-23-31-29-23-31-29-17-25-17-25-16-28	*blaZ, erm*(A), *egc,* IEC B	Ap, Cp, Er, Ln
D5	1	t14055	26-23-23-13-23-31-29-23-31-29-17-25-17-25	*blaZ, erm*(A), *sed, egc,* IEC B	Ap, Cp, Er, Ln
Incident E- January 2010					
E1	2	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	*blaZ, erm*(C), *egc*	Ap, Cp, Er, Ln

[a]Each incident was assigned a unique alphabetic designation and isolates within each incident were assigned to a group (numerical designation) if they exhibited indistinguishable *spa* types, DNA microarray profiles in terms of virulence and resistance gene content and phenotypic antimicrobial resistance profiles.

[b]Within each incident differences between *spa* repeat succession patterns are underlined.

[c]Antimicrobial resistance and virulence genes were detected using the *S. aureus* Genotypin Kit 2.0 (Alere, Germany).

[d]Phenotypic antimicrobial resistance was determined by antibiogram-resistogram typing using a panel of 25 antimicrobial agents including amikacin, ampicillin (Ap), cadmium acetate (Cd), chloramphenicol, ciprofloxacin (Cp), erythromycin (Er), ethidium bromide, fusidic acid (Fd) gentamicin,

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Table 5.2 continued

kanamycin, lincomycin (Ln), linezolid, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, propramide isoethionate, rifampicin, spectinomycin, streptomycin (St), sulphonamide, tetracycline, tobramycin, trimethoprim (Tp)and vancomycin.

[e]*egc*, the enterotoxin gene cluster including *seg, sei, sem, sen, seo* and *seu.*

[f]The immune evasion complex (IEC) type of each isolate was determined based on the combination of the IEC genes detected as described by van Wamel *et al.,* 2006. IEC type B: *sak, chp, scn.*

Table 5.3 Molecular epidemiological details of 18 CC5-MRSA-V isolates recovered during an outbreak in a neonatal intensive care unit over an 11-month period in one Irish hospital

Isolate no.	Patient date of birth	Specimen date	*spa* type[a]	DNA microarray analysis[b]			Phenotypic antimicrobial resistance[d]
				CC-SCC*mec* type	Resistance genes	Virulence genes[c]	
M11/0303	21/10/2011	04/11/2011	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M11/0304	19/11/2011	28/11/2011	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M11/0429	10/11/2011	12/12/2011	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M11/0428	10/11/2011	13/12/2011	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0013	02/12/2011	18/12/2011	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0096	22/02/2012	15/03/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0097	06/03/2012	20/03/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0215	28/06/2012	16/07/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb

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Table 5.3 continued

Isolate no.	Patient date of birth	Specimen date	*spa* type[a]	DNA microarray analysis[b]			Phenotypic antimicrobial resistance[d]
				CC-SCC*mec* type	Resistance genes	Virulence genes[c]	
M12/0337	26/08/2012	24/09/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0336	19/09/2012	26/09/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0350	03/10/2012	08/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0360	24/09/2012	14/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0376	26/08/2012	15/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0377	16/10/2012	18/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0383	Not known	19/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0384	Not known	19/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb
M12/0422	26/08/2012	26/11/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb

Continued overleaf

Table 5.3 continued

Isolate no.	Patient date of birth	Specimen date	*spa* type[a]	DNA microarray analysis[b]			Phenotypic antimicrobial resistance[d]
				CC-SCC*mec* type	Resistance genes	Virulence genes[c]	
M12/0388	08/02/1974	26/10/2012	t002	CC5-V	*blaZ, aacA-aphD, fosB*	*egc, sed, sej, ser, sea, sak, scn*	Ap, Gn, Kn, Tb

[a]*spa* repeat succession was determined from sequencing of the *spa* gene and interpreting the result data using StaphType software (Ridom, Münster, Germany).

[b]Antimicrobial resistance and virulence genes and clonal complex (CC) was determined using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies).

[c]The immune evasion complex (IEC) type of each isolate was determined based on the combination of the IEC genes detected as described by van Wamel *et al.,* 2006. IEC type D: *sea, sak, scn.*

[d]Antimicrobial resistance was determined by antibiogram-resistogram typing using a panel of 25 antimicrobial agents including amikacin (Ak), ampicillin (Ap) cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin (Gn), kanamycin (Kn), lincomycin, linezolid, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, propramide isoethionate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin (Tb), trimethoprim and vancomycin.

Table 5.4 Molecular epidemiological typing data of 51 CC1-MRSA-IV isolates associated with an outbreak in a surgical ward in a large Dublin teaching hospital between November 2013 and December 2015[a]

Group (*n*)[b]	*spa* type[c]	*spa* repeat succession	DNA Microarray		Antimicrobial resistance profile[e]
			Antimicrobial resistance genes[d]	Virulence genes[d]	
1 (27)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Er, Ln, Kn, Mp, Nm, St, Te
2 (12)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Cd(I), Er, Ln, Kn, Mp, Nm, St, Te
3 (1)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Er, Ln, Kn, Nm, St, Te
4 (1)	t922	07-23-21-16-33-13	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Cd(I), Er, Ln, Kn, Mp, Nm, St, Te
5 (6)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, tet*(K)	*seh*, IEC E (*sak, scn*)	Ap, Cd(I), Er, Ln, Kn, Nm, St, Te
6 (1)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, tet*(K)	*seh*, IEC E (*sak, scn*)	Ap, Er, Ln, Kn, Nm, St, Te
7 (1)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, fusB, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Er, Eb, Fd, Ln, Kn, Mp, Nm, St, Te
8 (1)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, aphA3, sat, lnu*(A)*, vgaA, aadD, ileS2, tet*(K)	*seh*, IEC E (*sak, scn*)	Ap, Er, Kn, Ln, Mp, Nm, St, Te, Tb

Continued overleaf

Table 5.4 continued

Group (*n*)[b]	*spa* type[c]	*spa* repeat succession	DNA Microarray		Antimicrobial resistance profile[e]
			Antimicrobial resistance genes[d]	Virulence genes[d]	
9 (1)	t127	07-23-21-16-34-33-13	*blaZ, erm*(C)*, fusB, aphA3, sat, ileS2, tet*(K)*, qacA*	*seh*, IEC E (*sak, scn*)	Ap, Eb, Er, Fd, Ln, Kn, Mp, Nm, St, Te

[a]One isolate in Group 1 was recovered from the ward environment while one isolate in Group 7 was recovered from a staff member. All other isolates were recovered from patients.

[b]Epidemiologically-related isolates that were indistinguishable by molecular typing methods i.e. *spa* typing, DNA microarray array detection of virulence and antimicrobial resistance genes and phenotypic antimicrobial resistance profiles were assigned to a group. *n*, the number of isolates in each group or subgroup.

[c]*spa* repeat succession was determined from sequencing of the *spa* gene and interpreting the result data using StaphType software (Ridom GmbH, Münster, Germany).

[d]Antimicrobial resistance and virulence genes were detected and clonal complex (CC) assigned using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies).

[e]Antimicrobial resistance was determined by antibiogram-resistogram typing using a panel of 25 antimicrobial agents including amikacin, ampicillin (Ap), cadmium acetate (Cd), chloramphenicol, ciprofloxacin, erythromycin (Er), ethidium bromide (Eb), fusidic acid (Fd), gentamicin, kanamycin (Kn), lincomycin (Ln), linezolid, mercuric chloride, mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate, propramide isoethionate, rifampicin, spectinomycin, streptomycin (St), sulphonamide, tetracycline (Te), tobramycin (Tb), trimethoprim and vancomycin. Intermediate resistance (I).

Isolates associated with ST22-MRSA-IV were investigated by DNA microarray profiling in this part of the present study. All other isolates recovered during outbreaks were investigated as part of a larger collection of 276 sporadically-occurring MRSA recovered from patients in Irish hospitals, the environment within healthcare facilities, GPs and a veterinary laboratory in Chapter 3. Within each outbreak incident, isolates with the same *spa* types, antimicrobial susceptibility patterns and DNA microarray profiles were assigned to the same group whilst any variation in *spa* type, antimicrobial susceptibility pattern or DNA microarray profile resulted in isolates being assigned to different groups.

5.2.1.2 Core-genome MLST evaluation

Previously generated whole-genome sequences for 41 ST22-MRSA-IVh isolates recovered during a six-week period in 2007 from patients (n = 19) and environmental sites (n = 22) in a single ward in a 700-bed hospital (Shore *et al.* 2010; Creamer *et al.* 2012, Kinnevey *et al*., 2016) were investigated using the SeqSphere^{+} software (Ridom GmbH) cgMLST scheme. This hospital ward consisted of 35 beds including five in single rooms, along with bays containing six, four or two beds (Creamer *et al.,* 2012). Cross-transmission events (CTEs) between patients, and between patients and their environment were previously designated based on conventional molecular epidemiological (CME) typing (Shore *et al*., 2010), and then subsequently by WGS SNV analysis (Kinnevey *et al.,* 2016). For CME typing, isolates were assigned to a CTE if they were recovered from ≥2 patients or from a patient and their environment in the same bay within a 3-week period (probable CTE) or from a patient and the environment of a different bay (possible CTE). These CTEs were then confirmed if the isolates differed by ≤1 typing method i.e. *spa, dru* or PFGE. This resulted in the identification of five CTEs (Shore, *et al.*, 2010; Creamer *et al.*, 2012) (Table 5.5). Additionally, five pairs of isolates, each consisting of a single patient

Table 5.5 Comparison of core-genome multilocus sequence type (cgMLST) Cluster Type assignment with conventional molecular epidemiological (CME) typing techniques for 41 ST22-MRSA-IVh isolates recovered from a single ward in a Dublin hospital during a six week period in 2007[a]

cgMLST Cluster Type[b]	Isolate no.	Date of isolation	Bay	Bed	Source	MRSA acquisition	Pairs[c]	CTE[d]	Combined CME typing (*spa-dru*-PFGE)
928	**M07/0353**	03/09/2007	B	15	Patient	OA	NA	CTE 4	t1214-dt10a-01039
	M07/0352	03/09/2007	B	15	Patient	OA	NA	CTE 4	t515-dt10a-01039
	M07/0319	30/07/2007	D	23	Patient	OA	Pair 2	NA	t515-dt10a-01042
	M07/0320	30/07/2007	D	23	Mattress	NA	Pair 2	NA	t515-dt10a-01042
1525	**M07/0334**	21/08/2007	D	23	Mattress	NA	NA	CTE 3	t032-dt10a-01018
	M07/0335	21/08/2007	D	23	Mattress	NA	NA	CTE 3	t032-dt10a-01018
	M07/0336	21/08/2007	D	26	Mattress	NA	NA	CTE 3	t032-dt10a-01018
	M07/0340	22/08/2007	B	10	Bedframe	NA	NA	CTE 2	t032-dt10a-01018
	M07/0346	22/08/2007	2	SO	Bedframe	NA	NA	NA	t032-dt10a-01018
	M07/0350	28/08/2007	D	27	Patient	HA	NA	CTE 3	t032-dt10a-01018
1526	**M07/0332**	17/08/2007	D	27	Patient	OA-K	NA	NA	t032-dt10j-01018
	M07/0415	04/10/2007	A	9	Patient	HA	NA	NA	t032-dt10j-01024
	M07/0338	22/08/2007	E	30	Pillow	NA	NA	NA	t032-dt10j-01146
1528	**M07/0337**	22/08/2007	E	29	Bedframe	NA	Pair 1[e]	NA	t022-dt10a-01039
	M07/0343	22/08/2007	B	14	Mattress	NA	NA	NA	t022-dt10a-01039
	M07/0357	04/09/2007	E	33	Bedframe	NA	NA	NA	t022-dt10a-01039
	M07/0330	15/08/2007	5	SO	Bedrail	NA	NA	NA	t032-dt10a-01039
	M07/0342	22/08/2007	B	15	Pillow	NA	NA	CTE 4	t032-dt10a-01039
	M07/0344	22/08/2007	B	15	Mattress	NA	Pair 5	CTE 4	t032-dt10a-01039
1529	**M07/0354**	03/09/2007	E	17	Patient	OA	NA	NA	t1214-dt10a-01039
	M07/0475	20/11/2007	B	SO	Air	NA	NA	NA	t1214-dt10a-01039
	M07/0356	03/09/2007	E	29	Patient	OA	NA	NA	t515-dt7i-01039

Continued overleaf

Table 5.5 continued

cgMLST Cluster Type[b]	Isolate no.	Date of isolation	Bay	Bed	Source	MRSA acquisition	Pairs[c]	CTE[d]	Combined CME typing (*spa-dru*-PFGE)
	M07/0327	09/08/2007	A	6	Patient	OA-K	NA	NA	t515-dt11j-01049
1530	**M07/0339**	21/08/2007	B	15	Patient	OA	Pair 5	CTE 2&3	t032-dt10n-01018
	M07/0341	22/08/2007	B	11	Bedframe	NA	NA	CTE 2	t032-dt10n-01018
	M07/0348	27/08/2007	B	14	Patient	HA	NA	CTE 2&3	t032-dt10n-01018
	M07/0323	31/07/2007	C	16	Patient	OA	NA	CTE 1	t032-dt10n-01024
	M07/0328	13/08/2007	B	15	Patient	OA	NA	CTE 1	t032-dt10n-01024
1531	**M07/0333**	20/08/2007	B	13	Patient	HA	Pair 1[e]	CTE 1	t032-dt10j-01024
1532	**M07/0355**	03/09/2007	E	30	Patient	OA	NA	CTE 5	t032-dt10a-01042
	M07/0358	10/09/2007	E	31	Patient	>72 h	NA	CTE 5	t032-dt10j-01042
1533	**M07/0322**	01/08/2007	E	18	Patient	OA	NA	NA	t032-dt10j-01030
1534	**M07/0351**	03/09/2007	A	7	Patient	OA	NA	NA	t032-dt10j-01024
1535	**M07/0359**	09/08/2007	C	16	Patient	OA	NA	NA	t2951-dt6e-01088
1536	**M07/0321**	30/07/2007	E	30	Mattress	NA	NA	NA	t515-dt10a-01039
1537	**M07/0326**	08/08/2007	C	21	Patient	OA	NA	NA	t032-dt7g-01018
1538	**M07/0329**	13/08/2007	E	31	Patient	HA	Pair 4	CTE 4	t1214-dt10a-01039
	M07/0331	15/08/2007	E	31	Pillow	NA	Pair 4	NA	t515-dt10a-01063
1539	**M07/0345**	22/08/2007	B	35	Mattress	NA	NA	NA	t032-dt10n-01018
1540	**M07/0324**	30/07/2007	A	6	Patient	OA	Pair 3	NA	t022-dt10p-01039
	M07/0325	30/07/2007	A	6	Mattress	NA	Pair 3	NA	t022-dt10p-01039

[a]All molecular epidemiological data for isolates, apart from cgMLST cluster type assignment was determined as part of previous studies (Shore *et al.* 2010; Creamer *et al.* 2012)

[b]cgMLST was determined using SeqSphere[+] software (Ridom GmbH, Münster, Germany) and involved a gene by gene comparison of 1861 genes to identify allelic variants between isolates on the core genome

Continued overleaf

Table 5.5 continued

[c]Each pair of isolates included in one isolate recovered from a patient and one recovered from their immediate ward environment. Isolates in two of these five pairs of isolates were previously implicated in the CTEs by CME typing.

[d]Isolates were previously assigned to a CTE if MRSA was recovered from ≥2 patients or from a patient and their environment in the same bay within a three-week period (probable CTE) or from a patient and the environment of a different bay (possible CTE). The CTEs were then confirmed using CME typing if the isolates differed by ≤1 typing method (i.e. *spa, dru,* PFGE). Among the 10 isolates assigned to the five pairs, four isolates were also implicated in three CTEs while the isolates associated with pairs 2 and 3 exhibited indistinguishable *spa, dru* and PFGE types but were not included in a CTE as the patient had been MRSA at the time of admission to the ward (Shore *et al.* 2010; Creamer *et al.* 2012).

[e]Isolates in pair 1 were recovered from a patient and an environmental sites in two separate bays. The patient was found to be positive for MRSA while in bay B but was subsequently moved to bay E where the subsequent environmental isolate was recovered allowing these two isolates to be classified as a pair.

Abbreviations: SO, single occupancy; NA, not applicable; PFGE, pulsed-field gel electrophoresis; >72 h, the patient's MRSA status was determined 72 h after admission to the ward; OA; the patient was positive for MRSA on admission to the ward; OA-K; the patients' MRSA positive status was known at the time of admission to the ward; HA, hospital-acquired MRSA.

isolate and one from their immediate ward environment were also investigated and two were designated as CTEs (Table 5.5).

The WGS SNV analysis involved comparison of the 41 ST22-MRSA-IVh isolates to a reference genome, which in this case, was the earliest recovered isolate (ERI; M07/0319). Isolates were assigned to a CTE if there was a ≤40 SNV difference between the isolates when both were compared to the ERI (Kinnevey *et al.,* 2016).

5.2.2 DNA microarray profiling

Whole-genomic DNA for DNA microarray profiling was extracted from isolates using the lysis buffer and solutions supplied with the *S. aureus* Genotyping Kit 2.0 and the Qiagen DNeasy kit as described in Chapter 2, section 2.4.1.3. DNA microarray profiling was carried out using the *S. aureus* Genotyping Kit 2.0 to investigate the presence of antimicrobial resistance genes, virulence genes and SCC*mec* associated genes as described in Chapter 2 section 2.4.4. Additionally, the DNA microarray was also used to assign isolates to MLST CC/ST and SCC*mec* types.

5.2.3 Analysis of whole-genome sequencing data using SeqSphere$^+$ cgMLST

The previously determined WGSs of the 41 ST22-MRSA-IV isolates were imported into the Ridom SeqSphere$^+$ software. This software allows for the automatic processing and analysing of WGS data and includes a cgMLST typing scheme which was established using all previously published *S. aureus* full genomes available in GenBank as of June 2013 (n = 40) with the *S. aureus* COL strain (GenBank NC_002951) used as a reference strain.

The ST22-MRSA-IVh isolate WGS data were assessed for quality using the SeqSphere^{+} software, i.e., the absence of premature stop codons and ambiguous nucleotides and a minimum sequence coverage of ≥10-fold over the whole genome. If a gene fulfilled all of these quality criteria, its complete sequence was analysed in comparison to that in the SeqSphere^{+} *S. aureus* COL reference strain and a numerical allele type was assigned by SeqSphere^{+}.

Each isolate was assigned to a Cluster Type and a minimum spanning tree (MST) was generated where isolates were clustered together based on the allelic profiles of isolates. Typing results generated from SeqSphere^{+} were compared to the inferences drawn from the data generated for SNV analysis and from CME typing.

5.3 Results

5.3.1 DNA microarray profiling for outbreak investigation

5.3.1.1 ST22-MRSA-IV outbreaks

A total of 25 ST22-MRSA-IV isolates which were associated with five different incidences (Incidents A-E, Table 5.2) in three Irish hospitals were investigated to determine if it was possible to further differentiate the isolates using the DNA microarray in conjunction with conventional phenotyping (AR typing) and *spa* typing. The molecular epidemiological typing details of isolates are shown in Table 5.2. All isolates were assigned to AR06 by AR typing and to ST22-MRSA-IV by DNA microarray profiling. All isolates exhibited resistance to ampicillin and ciprofloxacin with variation detected in relation to resistance to cadmium acetate (52%, 13/25), erythromycin and lincomycin (76%, 19/25), fusidic acid (56%, 14/25), trimethoprim (8%, 2/25) and streptogramin (4%, 1/25).

Using the DNA microarray, all isolates were found to harbour the β-lactamase gene *blaZ* and the enterotoxin gene cluster *egc* but variation was detected in relation to carriage of the MLS resistance genes *erm*(C) or *erm*(A) (76%, 19/25), enterotoxin genes *sec/l* (36%, 9/25), the immune evasion complex genes *sak, chp* and *scn* (IEC type B, 53%, 13/25) and the enterotoxin gene *sed* (12%, 3/25). The isolates were assigned to seven *spa* types with *spa* type t032 predominating (72%, 18/25). The *spa* type t020 accounted for just two isolates (8%) with each of the remaining *spa* types (t14055, t515, t2945, t379 and t5893), accounting for one isolate each.

Within each of the five ST22-MRSA-IV outbreak incidences, isolates that exhibited indistinguishable *spa* types, DNA microarray profiles in terms of virulence and resistance

gene content and antimicrobial resistance patterns were assigned to the same group and a unique alphanumeric group number (Table 5.2).

5.3.1.1.1 ST22-MRSA-IV INCIDENT A

During an eight-week period in 2014, eleven ST22-MRSA-IV isolates were recovered from patients attending a surgical ward in a large regional hospital. Phenotypically, all isolates exhibited resistance to ampicillin and ciprofloxacin while cadmium (9.1%, 1/11), erythromycin and lincomycin (72.7%, 8/11 each), fusidic acid (63.6%, 7/11) and trimethoprim (18.2%, 2/11) resistance varied (Table 5.2, Incident A). Three *spa* types were identified with 72.7% of isolates (8/11) being assigned to t032 with the remaining three isolates being assigned to t020 (18.2%, 2/11) and t5893 (9.1%, 1/11) (Table 5.2, Incident A). Comparison of the repeat succession of these *spa* types showed that t020 lacked three repeat units when compared to that of the t032 while t5893 differed from t032 by one unit (Table 5.2, Incident A). Using DNA microarray profiling, the isolates were found to harbour four different combinations of antimicrobial resistance and virulence genes with each group differing by the presence or absence of one to three genes, namely IEC B, *sec/l* and *erm*(C) (Table 5.2, Incident A).

By combining the *spa* types, DNA microarray profiles and antimicrobial resistance patterns, the 11 incident A isolates were assigned to five groups, two of which consisted of >1 isolate (Table 5.2, Incident A, Groups A1 and A4). The eight t032 isolates were assigned to three groups (Table 5.2, Incident A, Groups A1-A3) with different DNA microarray profiles with six of t032 isolates being assigned to a single group (Table 5.2, Incident A, Group A1). While the two t020 isolates yielded a different DNA microarray pattern to the t032 isolates (Table 5.2, Incident A, Group A4), the one t5893 isolate yielded an indistinguishable DNA microarray pattern to Group A3 t032 isolates (Table 5.2,

Incident A, Group A5). Isolates within each *spa*-DNA microarray group yielded a different antimicrobial resistance pattern, with differences detected due to resistance to cadmium, erythromycin, fusidic acid, trimethoprim and lincomycin (Table 5.2, Incident A).

5.3.1.1.2 ST22-MRSA-IV INCIDENT B

Over a three-week period in 2014, four MRSA isolates recovered from patients in a special care baby unit were investigated (Table 5.2, Incident B). All isolates exhibited phenotypic resistance to ampicillin, ciprofloxacin, erythromycin and lincomycin. Three isolates were also resistant to cadmium and fusidic acid while one was resistant to streptomycin. Three of the isolates were assigned to t032 while the remaining isolate was t379 which differed from t032 by three repeat units.

DNA microarray profiling revealed that all isolates harboured the same combination of antimicrobial resistance genes (*blaZ* and *erm*(C)) but two distinct combinations of virulence genes were identified which differed due to the absence of the IEC genes *sak*, *chp*, and *scn* (Table 5.2, Incident B).

By combining the *spa* types, DNA microarray profiles and antimicrobial resistance patterns, each of the four incident B isolates were assigned to a different group (Table 5.2, Incident B). Among the three t032 isolates, two harboured the same array genes but exhibited different antimicrobial resistance patterns (Table 5.2, Incident B, Groups B1 and B2) while one t032 isolate differed in the array genes (Table 5.2, Incident B, Group B3). The final Incident B Group (Group B4, Table 5.2) exhibited a different *spa* type and antimicrobial resistance pattern but harboured the same array genes as one of the t032 isolates (Table 5.2, Incident B, group B3).

5.3.1.1.3 ST22-MRSA-IV INCIDENT C

Three MRSA isolates recovered from three patients on a ward in a large tertiary referral adult teaching hospital in 2014 were investigated, two recovered in March (Group C1) and one in January (Group C2). The isolates were assigned to two *spa* types, t032 (n = 2) and t515 (n = 1), which differed from each other in three repeat succession units. Although DNA microarray profiling of the isolates yielded indistinguishable virulence and resistance gene profiles, differences were detected with regards to phenotypic resistance to cadmium and fusidic acid (Table 5.2, Incident C). Combining the results of the three typing methods grouped both isolates recovered in March together while the earlier isolate was distinct, exhibiting a different *spa* type and lacking phenotypic resistance to cadmium acetate and fusidic acid (Table 5.2).

5.3.1.1.4 ST22-MRSA-IV INCIDENT D

Five MRSA isolates, each recovered from patients attending an emergency department in a tertiary referral teaching hospital in July 2014 were investigated. Three of the isolates were assigned to *spa* type t032. The remaining isolates were assigned to t2945 and t14055 which differ from t032 by one and two repeat successions, respectively (Table 5.2, Incident D). Using DNA microarray profiling the three t032 isolates were differentiated into three groups based on the presence or absence of *erm*(A), *erm*(C), *sec* and *sed* (Table 5.2, Incident D, Groups D1-D3). The one t2945 isolate exhibited a different array profile to all other isolates while the t14055 isolate had the same pattern as one of the t032 isolate (Table 5.2, Incident D, Groups D4 and D5, respectively). All of the Incident D isolates exhibited resistance to ampicillin, ciprofloxacin, erythromycin and lincomycin. Two of the t032 isolates also exhibited resistance to cadmium acetate and fusidic acid (Table 5.2, Incident D, Groups D1 and D2).

5.3.1.1.5 ST22-MRSA-IV INCIDENT E

This incident included two isolates recovered from two patients who had undergone surgery in a Dublin hospital. Both isolates yielded indistinguishable *spa* types and DNA microarray profiles and antimicrobial resistance patterns and were therefore assigned to the same group (Table 5.2, Incident E).

5.3.1.2 CC5-MRSA-V outbreak

Eighteen MRSA isolates recovered as part of an outbreak in a NICU were investigated (Table 5.3). At the time of specimen collection, all patients except one were under three months of age and had been in the NICU since birth or shortly after. The remaining patient (M12/0388) was the mother of a baby (M12/0377) who had been found to carry the MRSA strain one week previously. All isolates were indistinguishable using all typing methods and were assigned to CC5, SCC*mec* V and *spa* type t002 (Table 5.3). The isolates exhibited phenotypic resistance to gentamicin, kanamycin and tobramycin encoded for by *aacA-aphD.* The isolates also carried *fosB* and *blaZ* encoding resistance to fosfomycin and ampicillin, respectively. With regards to virulence genes, the isolates carried *egc, sed, sej* and *ser* and IEC type D (*sea, sak* and *scn*).

5.3.1.3 CC1-MRSA-IV outbreak

Over a two-year period between 2013 and 2015, 51 MRSA isolates were recovered from 49 patients, one staff member and one environmental site in a surgical ward of a large Dublin teaching hospital. All isolates were assigned to CC1 and SCC*mec* IV by DNA microarray profiling. Isolates were assigned to different groups based on different combinations of *spa* types, DNA microarray resistance and virulence genes and phenotypic antimicrobial resistance pattern (Table 5.4, Groups 1-9). The majority of isolates (98%,

50/51) exhibited *spa* type t127 with one isolate being assigned to *spa* type t922 which differed from t127 by the lack of one repeat unit in t922. The 50 t127 isolates exhibited eight different combinations of DNA microarray genes and phenotypic resistance profiles. All isolates carried the same combination of virulence genes including *seh* and IEC type E (*sak* and *scn*) along with the *blaZ, erm*(C), *aphA3, sat* and *tet*(K) resistance genes but differed due to the presence or absence of additional antimicrobial resistance genes including *aadD, qacA*, *ileS2, lnu*(A) and *fusB* and resulted in four different gene combinations (Table 5.4).

Phenotypically, all t127 isolates exhibited resistance to ampicillin, erythromycin, lincomycin, kanamycin, neomycin, streptomycin and tetracycline. Differences in the phenotypic resistance patterns included resistance to cadmium acetate (28%, 14/50), mupirocin (84%, 42/50), fusidic acid (4%, 2/50) and ethidium bromide (2%, 1/50). While 42 of the isolates (84%, 42/50) carried *qacA*, only two (4%) exhibited phenotypic resistance to ethidium bromide.

The single isolate assigned to Group 6 was the only isolate recovered from a healthcare worker during the investigation and differed from the majority of isolates due to the lack of *ileS2* and *qacA* and a mupirocin susceptible phenotype (Table 5.4). Group 1, comprising of 27 isolates (52.9%), included a single isolate recovered from an environmental sample collected during the outbreak investigation.

5.3.2 Core-genome multilocus sequence typing for tracking ST22-MRSA-IV isolates

Core-genome MLST assigned the 41 ST22-MRSA-IVh isolates to ST22 and clustered the isolates into 16 distinct clusters (Cluster Types, Fig. 5.1). The number of cgMLST alleles that differed between each cluster ranged from 15-55 with an average of

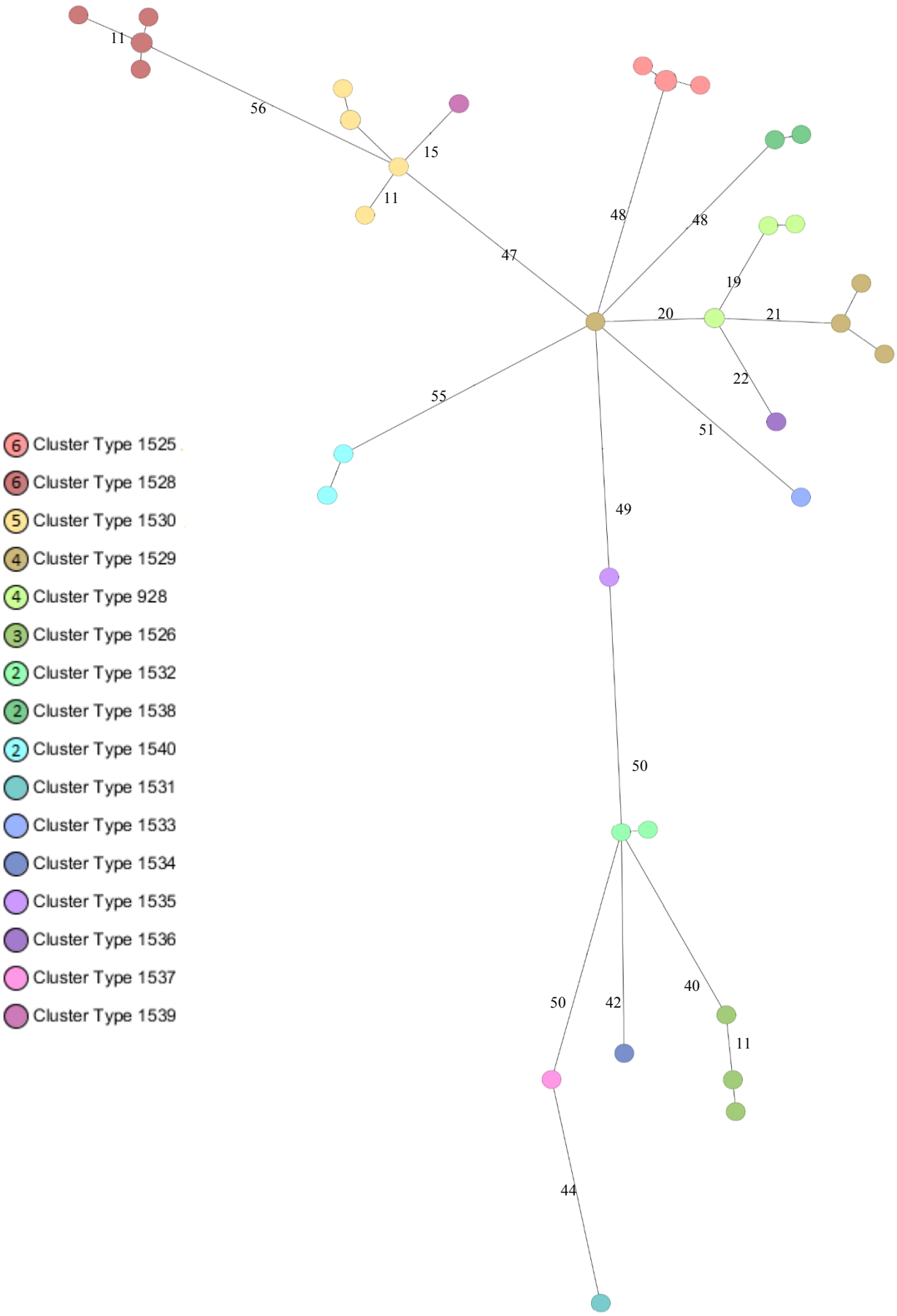
11
56
15
11
47
48
48
19
20
21
22
55
51
49
50
50
42
40
11
44
6 Cluster Type 1525
6 Cluster Type 1528
5 Cluster Type 1530
4 Cluster Type 1529
4 Cluster Type 928
3 Cluster Type 1526
2 Cluster Type 1532
2 Cluster Type 1538
2 Cluster Type 1540
Cluster Type 1531
Cluster Type 1533
Cluster Type 1534
Cluster Type 1535
Cluster Type 1536
Cluster Type 1537
Cluster Type 1539

Figure 5.1 Minimum spanning tree (MST) showing the Cluster Types assignments for 41 ST22-MRSA-IV isolates recovered from patients and their environments in a single ward in a Dublin hospital over a six-week period in 2007. The MST was generated based on the allele diversity as determined by analysis of 1,861 core-genome multilocus sequence typing (cgMLST) genes using the SeqSphere+ software (Ridom GmbH, Münster, Germany). Where more than one isolate was assigned to a Cluster Type the number of isolates is shown within the key. Numbers on the tree represent the number of allele differences between two nodes in the tree.

42 allelic differences between each cluster (Fig. 5.1). The range of allelic variants between isolates within Cluster Types ranged from 1 – 11 genes (Fig. 5.1). Nine clusters consisted of ≥2 isolates with the two largest clusters consisting of six isolates each (Cluster Types 1525 and 1528, Fig. 5.1). Seven clusters consisted of isolates from both patients and the hospital environment and five clusters (Cluster Types 928, 1526, 1529, 1530 and 1532) had multiple patient isolates (Table 5.5).

Only the two isolates assigned to CTE 5 by CME typing, and which had also been confirmed to be related using SNV, analysis were also assigned to the same Cluster Type (Cluster Type 1532) by cgMLST (Fig. 5.2). Three isolates were assigned to a CTE 1 based on CME typing but SNV analysis refuted this CTE with 86 (M07/0323 and M07/0328) and 43 (M07/0323 and M07/0333) SNV differences identified (Fig. 5.2). Using cgMLST 2/3 CTE 1 isolates, (M07/0323 and M07/0328) clustered together (Cluster Type 1530) while the remaining isolate (M07/0333) was separated into a different cluster (Cluster Type 1531) which differed from each other by 188 alleles (Fig 5.2).

CTE 2 included four isolates recovered from the environment (n = 2) and patients (n = 2) based on CME typing. The SNV analysis difference previously identified among these isolates ranged from 16-76 with only M07/0341 and M07/0348 confirmed by SNV analysis to represent a CTE (Fig. 5.2). The cgMLST confirmed that these two isolates, along with M07/0339 all clustered together in a single cluster (Cluster Type 1530) while M07/0340 was assigned to a different cluster (Cluster Type 1525) between which there were 95 allelic variations (Fig. 5.2).

Six isolates were assigned to CTE 3 based on CME typing. Among these, SNV analysis refuted the link between three of the isolates (M07/0335, M07/0336 and M07/0348) while confirming it for the remaining three isolates (M07/0334, M07/0339 and

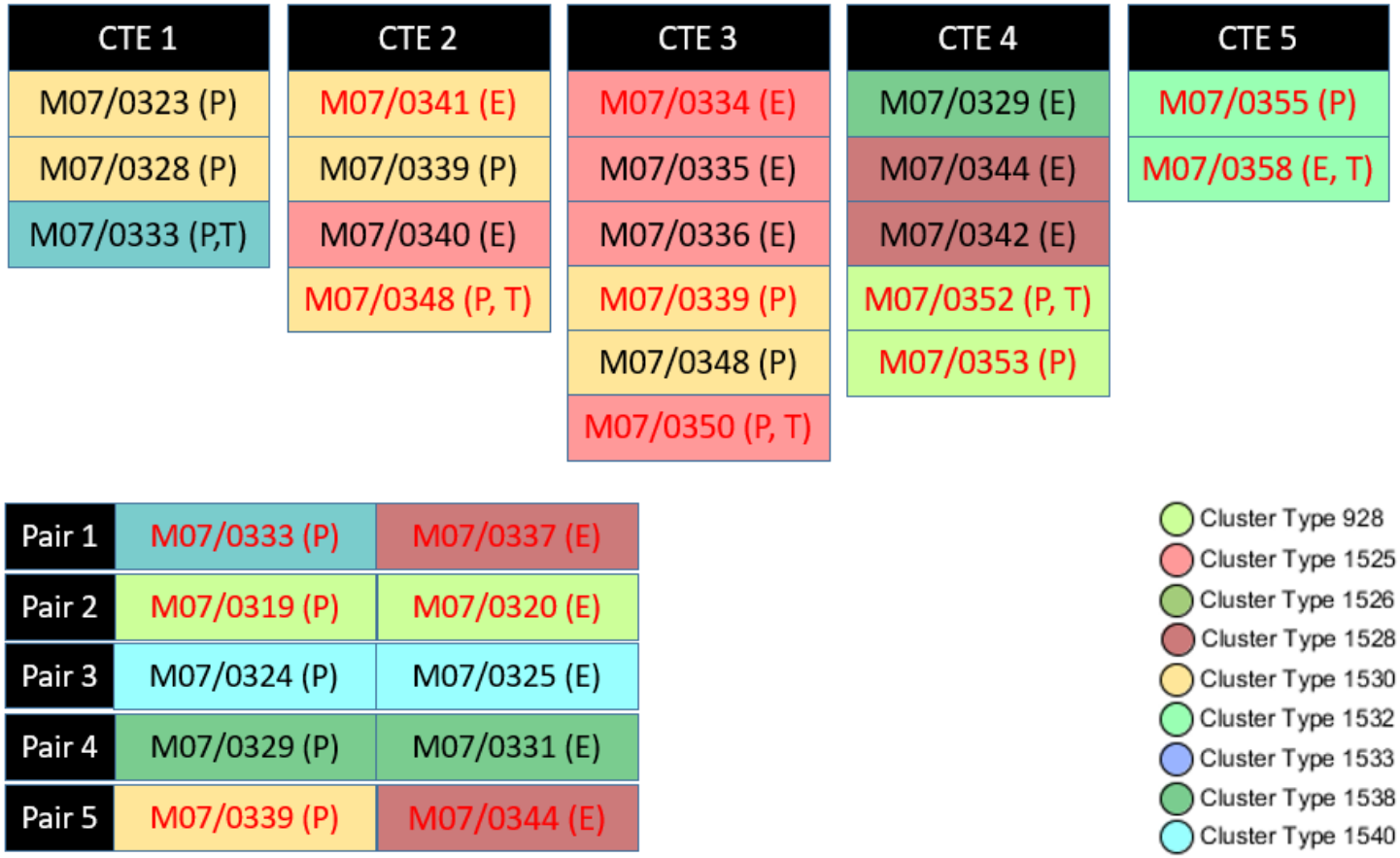

Figure 5.2 ST22-MRSA-IV isolates previously identified as being involved in cross-transmission events (CTEs) by conventional molecular epidemiological (CME) typing using a combination of epidemiological information and *spa, dru* and PFGE typing or identified as a pair of isolates

recovered from a patient and their immediate ward environment (Shore *et al.* 2010; Creamer *et al.* 2012). For each CTE, the putative source isolates recovered from patients (P) and their ward environment (E) as well as putative transmitted isolates (T) are shown. Whole-genome sequencing of the isolates followed by single-nucleotide variant (SNV) analysis subsequently confirmed (red text) or refuted (black text) the allocation of each isolate to the CTEs or pair, if isolates differed by ≤40 SNVs (Kinnevey *et al.* 2016). The colour for of each box represents the cluster to which the isolate was assigned following core-genome (cg) MLST using SeqSphere+ software (Ridom GmbH, Münster, Germany). The CME typing, SNV analysis and cgMLST analysis were only in agreement for a single CTE (CTE 5) while in each of the other CTEs the SNV analysis refuted the linking of the isolates by CME typing but cgMLST confirmed some of the initial CTEs while refuting others. Among the isolate pairs, SNV analysis refuted the original pairing of isolates in pair 3 and pair 4 while confirming those in pairs 1, 2 and 5. In contrast, cgMLST refuted the links identified within the pairs of isolates by SNV analysis but confirmed those refuted by SNV analysis.

M07/0350). In addition, two isolates in CTE 3 (M07/0339 and M07/0348) were also assigned to CTE 2 by CME typing. However, using cgMLST, these two isolates clustered together (Cluster Type 1530) with other isolates within CTEs 1 and 2 and not with any other CTE 3 isolates (Fig. 5.2). The remaining four isolates in CTE 3 (M07/0334, M07/0335, M07/0336 and M07/0350) clustered together using cgMLST along with the only isolate (M07/0340) from CTE 2 that did not cluster with other isolates within that CTE (Cluster Type 1525) (Fig. 5.2).

CTE 4 included five isolates, three of which were recovered from the environment with the remaining two were recovered from patients (Fig. 5.2). The SNV analysis refuted the association of the environmental isolates within this CTE (SNV range 84-355) while confirming the CTE between M07/0352 and M07/0353 (SNV difference of seven). Two of these environmental isolates (M07/0344 and M07/0342) were clustered together by cgMLST (Cluster Type 1528) as were the two isolates recovered from patients (M07/0352 and M07/0353) but in a different cluster (Cluster Type 928). The final CTE 4 isolate (M07/0329) clustered separately to the other isolates using cgMLST (Cluster Type 1538) (Fig. 5.2).

5.3.2.1 Comparison of the paired isolates previously identified by conventional molecular epidemiological typing and SNV analysis with cgMLST clustering

Previously, five pairs of isolates were identified based on the recovery of isolates from a patient and their immediate hospital environment (Fig. 5.2). Among these, CME typing found that isolates associated with Pairs 2 and 3 were indistinguishable to each other. Within the isolates in the remaining pairs (Pairs 1, 4 and 5) differed from each other by more than one CME typing method (Shore *et al.*, 2010b). SNV analysis identified $\leq$40

SNVs between 3/5 pairs of isolates (Pairs 1, 2 and 5) with SNV differences between patient and environmental isolates in each case identified as 20, 10 and 14, respectively. For the remaining two pairs of isolates (Pairs 3 and 4) the SNV differences were 100 and 99, respectively, and these isolates were deemed not to represent a CTE. A comparison of cgMLST clustering and SNV analysis showed only agreement for Pair 2 (M07/0319 and M07/0320) where both isolates exhibited a SNV difference of 10 and were assigned to the same cgMLST cluster (Cluster Type 928). In contrast, cgMLST clustered the isolates within Pairs 3 and 4 together and refuted the connection of the environment isolates with those from the patients in Pairs 1 and 5 (Fig. 5.2).

A pairwise comparison of the conventional CME typing data and SNV differences of ten arbitrarily selected isolates that differed by ≤40 SNV and by >40 SNV revealed that there was no correlation between the SNV differences and the differences recognised by CME typing (Kinnevey *et al.*, 2016). Based on cgMLST clustering, only two pairs of isolates were in agreement with SNV analysis of pairwise comparison of isolates for isolates differing by ≤40. While isolates included in pairwise comparison 3 and 6 (Table 5.6) were assigned to the same Cluster Type by cgMLST, none of the other pairs clustered together (Table 5.6). Pairs of isolates that differed by >40 SNV were also each assigned to different clusters (Table 5.6).

5.3.2.2 Additional isolate relatedness recognised using cgMLST clustering

Seventeen isolates which had not previously been assigned to a CTE or a Pair were assigned to Cluster Types with at least one other isolate (Table 5.5). Within the two largest clusters, Cluster Types 1525 and 1528, isolates which had not been previously linked as CTEs by CME typing, were recognised including one isolate in Cluster Type 1525 and four in Cluster Type 1528 (Table 5.5). Cluster Type 1528 was the only cluster to which

Table 5.6 Comparison of the conventional molecular epidemiological typing data, single-nucleotide variant (SNV) differences and cgMLST clusters identified among isolates within 10 arbitrarily selected pairwise comparisons (PC)[a] exhibiting ≤40 SNVs and >40 SNVs.

PC[a]	Compared isolates	*spa* type	*dru* type	PFGE	CME typing difference[a]	SNV difference[b]	cgMLST Cluster Type[c]
≤40 SNV differences							
1	M07/0325	t022	dt10p	01039	3	0	1540
	M07/0328	t032	dt10n	01024			1530
2	M07/0326	t032	dt7g	01018	2	4	1537
	M07/0328	t032	dt10n	01024			1530
3	M07/0319	t515	dt10a	01042	0	10	928
	M07/0320	t515	dt10a	01042			928
4	M07/0321	t515	dt10a	01039	1	1	1536
	M07/0329	t1214	dt10a	01039			1538
5	M07/0324	t022	dt10p	01039	3	15	1540
	M07/0336	t032	dt10a	01018			1525
6	M07/0355	t032	dt10a	01042	1	20	1532
	M07/0358	t032	dt10j	01042			1532
7	M07/0337	t022	dt10a	01039	0	27	1528
	M07/0359	t2951	dt6e	01088			1535
8	M07/0333	t032	dt10j	01024	2	33	1531
	M07/0342	t032	dt10a	01039			1528
9	M07/0341	t032	dt10n	01018	2	38	1530
	M07/0355	t032	dt10a	01042			1532
10	M07/0337	t022	dt10a	01039	3	39	1528
	M07/0415	t032	dt10j	01024			1526
>40 SNV differences							
1	M07/0335	t032	dt10a	01018	1	43	1525
	M07/0415	t032	dt10j	01024			1526

Continued overleaf

Table 5.6 continued

PC[a]	Compared isolates	*spa* type	*dru* type	PFGE	CME typing difference[a]	SNV difference[b]	cgMLST Cluster Type[c]
2	M07/0319	t515	dt10a	01042	2	54	928
	M07/0475	t1214	dt10a	01039			1529
3	M07/0328	t032	dt10n	01024	3	63	1530
	M07/0337	t032	dt10a	01039			1528
4	M07/0320	t515	dt10a	01042	2	78	928
	M07/0346	t032	dt10a	01018			1525
5	M07/0355	t032	dt10a	01042	1	75	1532
	M07/0351	t032	dt10j	01024			1534
	M07/0336	t032	dt10a	01018			1525
6	M07/0328	t032	dt10n	01024	2	96	1530
	M07/0338	t032	dt10j	01146			1526
7	M07/0339	t032	dt10n	01018	3	99	1530
	M07/0352	t515	dt10a	01039			928
8	M07/0321	t515	dt10a	01039	0	354	1536
	M07/0352	t515	dt10a	01039			928
	M07/0334	t032	dt10a	01018			1525
9	M07/0323	t032	dt10n	01024	2	209	1530
	M07/0332	t032	dt10j	01018			1526
10	M07/0324	t022	dt10p	01039	3	112	1540
	M07/0340	t032	dt10a	01018			1525

[a]The number of conventional molecular epidemiological (CME) typing methods that the isolates differed by including *spa* typing, *dru* typing and pulsed-field gel electrophoresis (PFGE).

[b]Pairwise comparisons were randomly selected by Kinnevey *et al.,* 2016 using the cut-off for isolates differing by ≤40 SNV and > 40 SNV for comparison to previously reported conventional molecular epidemiological typing data (Shore *et al.* 2010; Creamer *et al.* 2012).

[c]Core-genome multilocus sequence typing (cgMLST) Cluster Types were determined using SeqSphere software (Ridom GmbH, Münster, Germany) and involved a gene by gene comparison of 1861 genes to identify allelic variants between isolates on the core genome.

only environmental isolates were assigned while all other Cluster Types included isolates recovered from patient and the environment. Cluster Types 1526 and 1529 included isolates which had not previously been linked together based on CME. Cluster Type 928 included both isolates from Pair 2 along two isolates from CTE 4. The remaining three isolates from CTE 4 were assigned instead to cgMLST Cluster Types 1528 (M07/0342 and M07/0344) and 1538 (M07/0329) (Table 5.3). Cluster Type 1530 included isolates which had been assigned to CTE 1, 2 and 3 (Table 5.5).

5.4 Discussion

In an outbreak investigation the need to adequately differentiate between strains among isolates is essential in order to identify possible transmission events while also allowing the development of strategies to prevent further spread (Sabat *et al.,* 2013). Difficulty arises when attempts to differentiate isolates of a highly clonal pathogen in an endemic environment, are made using conventional methods which often lack adequate discriminatory power (Shore *et al.,* 2010; Kinnevey *et al.,* 2016). In the current study, recent advances in WGS were explored as a suitable alternative to *spa* typing and included the use of DNA microarray along with a WGS-based typing scheme involving genes located on the core genome.

5.4.1 Enhanced discrimination of isolates recovered during outbreak investigations using DNA microarray profiling

5.4.1.1 ST22-MRSA-IV

Five separate outbreak incidences of HCA ST22-MRSA-IV were investigated as part of the present study using DNA microarray profiling and, for 56% of isolates (14/25 isolates in 3/5 incidences), the DNA microarray was able to further differentiate isolates that otherwise would have been indistinguishable based on antimicrobial susceptibility testing and *spa* typing data alone. However, for 36% of ST22-MRSA-IV isolates (9/25 isolates in 3/5 incidents) the DNA microarray profiles of isolates were indistinguishable but differences were detectable by *spa* typing and antimicrobial susceptibility testing. The remaining two isolates were indistinguishable from each other using all three typing methods.

In Ireland, the most frequently recognised antimicrobial susceptibility differences among ST22-MRSA-IV isolates relate to fusidic acid and cadmium acetate (NMRSARL, 2014). Mutations in the *fusA* gene are the most commonly reported fusidic acid resistance mechanism in ST22-MRSA-IV (Castanheira *et al*., 2010; Reuter *et al.,* 2017). However, the DNA microarray does not detect resistance mechanisms arising from point mutations while cadmium resistance genes are not included on the array panel. Furthermore, in the current study two ST22-MRSA-IV isolates exhibited resistance to trimethoprim but were negative for *dfrS1* using the microarray. Additional trimethoprim resistance genes, such as *dfrG* and *dfrK* are not detected using the array. Therefore, despite the present study revealing the ability of the DNA microarray, in some instances, to enhance the discrimination of ST22-MRSA-IV in outbreak situations, its usefulness for typing of isolates of this strain is limited given the issues highlighted in terms on inability to detect mutational resistance mechanisms and additional resistance genes. This further highlights the need for additional investigation and application of WGS for ST22-MRSA-IV outbreak investigations, particularly in an endemic setting. However, as with all typing methods, it is of paramount importance that, where possible, epidemiological data is used to assist with the interpretation of typing results.

5.4.1.2 Non-ST22-MRSA-IV isolates recovered in outbreaks in Irish hospitals

Further differentiation of 18 CC5-MRSA-V isolates recovered during an outbreak in a NICU that were indistinguishable by *spa* typing and antimicrobial susceptibility testing, was not possible using the DNA microarray, indicating the spread of a single strain. However, the microarray did provide significant clinically-relevant information relating to the characterisation of this strain which is not frequently recognised within Ireland (NMRSARL, 2014). Currently, routine epidemiological typing techniques used in the NMRSARL are unable to determine SCC*mec* types or to assign isolates to MLST CCs. All

isolates in the outbreak were assigned to *spa* type t002 and an online database of *spa* types (available at http://www.spaserver.ridom.de/) assigned this *spa* type to CC5. However the microarray confirmed this CC assignment whilst also further characterising the SCC*mec* element (SCC*mec* V) and detecting a range of virulence and resistance genes (Table 5.3).

While CC5-MRSA-IV is frequently described throughout the world, CC5 harbouring SCC*mec* V is less frequently reported and is limited to strains recovered in Ireland, Germany and Abu Dhabi, many of which are *pvl*- positive (Monecke *et al.* 2011; Monecke *et al.* 2012b; Shore *et al.* 2014) and to strains also harbouring the SCC*fus* element in Saudi Arabia (Senok *et al.* 2016), neither characteristics which apply to the strain identified here. Furthermore, the presence of SCC*mec* type V suggests that these strains are indeed CA-MRSA strains and the outbreak has very likely arisen following the importation of this CA-MRSA lineage into the healthcare setting in Ireland.

A second prolonged outbreak spanning nearly two years and involving 51 CC1-MRSA-IV isolates was also investigated in the current study. The CC1-MRSA-IV isolates harboured multiple resistance genes including those encoding resistance to fusidic acid, mupirocin, aminoglycosides and tetracycline which resulted in patient management difficulties for clinical staff identifying suitable antimicrobial options for carriage eradication of this MRSA strain. Staff screening in this situation resulted in the detection of a CC1-MRSA-IV strain that was mupirocin susceptible (Group 7, Table 5.4). DNA microarray profiling and *spa* typing of this strain showed that it differed from the mupirocin resistant isolates only in relation to mupirocin resistance, and while the mupirocin resistant strain was limited to the outbreak ward in one hospital in Ireland, the susceptible strain has been recovered elsewhere in Ireland on a number of different occasions including in the community (NMRSARL, 2015).

CC1-MRSA-IV is a frequently reported CA-MRSA strain. Mainly associated with Western Australia, it is the predominant *pvl*-negative strain circulating in the area, however, it has also been reported sporadically in Germany and Egypt (Coombs *et al.,* 2006; Monecke *et al.,* 2011). In Ireland, *spa* type t127 is the most frequently recognised *spa* type among sporadically-occurring MRSA (NMRSARL, 2014). However, further characteristics of these strains were unknown prior to the present study due to the conventional typing methods used within the NMRSARL.

Similarly to that observed with the ST22-MRSA-IV isolates, use of the DNA microarray greatly improved the differentiation of isolates within the outbreak. Here, the improved discrimination among the CC1-t127-MRSA-IV, was mainly achieved due to the presence or absence of resistance genes, many of which are carried on MGEs such as plasmids. While isolates were assigned to nine different groups based on *spa,* array and AR group, it is not possible to determine if each group represents a single strain as many differ by the presence or absence of just one or two resistance genes. The relatively large number of resistance genes present in these strains suggests high antibiotic selective pressure, such as that present in a hospital, promoting resistance among the strain (Malachowa & Deleo, 2010).

In this outbreak investigation, recognition of the mupirocin susceptible CC1-MRSA-IV strains among staff caused difficulty in the case definition for the management of this outbreak, and further supports the rationale for periodic screening of staff members to identify asymptomatic carriers. In this case, while the mupirocin susceptible strain could represent the same strain as the outbreak strain having lost a plasmid encoding *ileS2,* it could also be a different strain and further studies using WGS are necessary in order to determine this. Transmission of MRSA between healthcare workers and patients has been widely reported but current Irish MRSA guidelines recommend staff screening only during

outbreak investigations (National Clinical Effectiveness Committee, 2013). Routine screening of staff has been suggested for settings where MRSA is endemic. Randomised periodic screening should also be considered in order to identify asymptomatic healthcare staff carrying MRSA (Otter & French, 2010).

Microarray assignment of isolates to a CC associated with a particular geographical region has previously proven useful in identifying a possible index case in Ireland. In an outbreak involving isolates assigned to ST772-MRSA-V, a clone which is frequently associated with the Bengal Bay geographic region, the index case was found to be a nurse of Indian origin who had recently been hospitalised in India (Brennan *et al.,* 2012). The role of healthcare workers in the transmission of MRSA is unclear (Ludlam *et al.,* 2010; Price *et al.,* 2016). Travel of staff between healthcare facilities is common in Ireland. While staff movement is more commonly associated with the Philippines and Australia, in recent years training programmes established by the Royal College of Physicians in the United Arab Emirates (UAE) has encouraged exchanges between medical professionals in the UAE and Ireland. While no known index case was identified in the outbreaks investigated in the present study, the assignment of strains to a clone which is not frequently recovered in Ireland suggests that screening of people beyond the patient cohort should be further considered as a source of importation, particularly in areas with employees and/or patients from diverse geographic locations.

While utilising DNA microarray profiling as an epidemiological typing method in outbreak situations has been shown to be somewhat useful in the current study, combining it with *spa* typing and phenotypic susceptibility testing yielded far greater discriminatory power. Instead however, the strength of the microarray profiling has been shown to be in the characterisation of isolates by the detection of virulence and resistance genes and

MLST/SCC*mec* typing, particularly for emerging strains, for which there is currently limited information available.

5.4.2 Comparison of cgMLST, WGS SNV analysis and conventional molecular epidemiological typing techniques to identify cross-transmission events involving ST22-MRSA-IVh recovered in an endemic setting

Due to recent advances in WGS technology, there are an increasing number of reports detailing the use of WGS for outbreak investigations (Köser *et al.,* 2012; Harris *et al.,* 2013; Leopold *et al.,* 2014; Salipante *et al.,* 2015). The availability of this technology, including the hardware and software required is, in many instances, limited to research facilities, but focus is moving towards making this technology more readily available in clinical facilities. The costs of WGS are reducing and software that permits easier interpretation of the data is now readily available. While microarray profiling is useful due to the amount of information it provides in a short time at a relatively low cost, limitations do exist, particularly regarding the need for constant updates to the array chip to ensure that newly described genes and alleles are included along with the lack of detection of resistance caused by mutations in specific genes. A comparison of WGS in conjunction with CME typing is necessary in order to determine the usefulness of WGS for use in a routine clinical laboratory.

While the utilisation of WGS SNV analysis has been applied in numerous studies to identify possible incidents of CTEs (Harris *et al.,* 2013; Price *et al.,* 2013a; Price *et al.,* 2013b, Price *et al.,* 2016; McGann *et al.,* 2016), other groups consider a gene by gene comparison approach to be more appropriate (Leopold *et al.,* 2014; Maiden *et al.,* 2014). However, while elsewhere cgMLST data has been shown to be largely in agreement with SNV analysis (Ugolotti *et al.,* 2016), this was not found to be the case in the current study.

Here isolates which had been previously assigned to CTEs based on either CME typing or SNV analysis (Shore *et al.,* 2010; Kinnevey *et al.,* 2014) were assigned to different cgMLST clusters with only three of the original pairs of isolates and one CTE being confirmed by cgMLST. SNV analysis requires comparison of all isolates in a collection against a reference while a pairwise comparison involves comparing two isolates directly (Azarian *et al.,* 2016). Kinnevey *et al.* (2016) identified possible paired comparison isolates with the comparison of isolates to a reference genome within the study which was the ERI, and, using a cut off of ≤40 SNV, found no concordance with the SNV difference between the isolates and previous CME for most isolates (Kinnevey *et al.,* 2016). The comparisons described in that study were determined by calculating the SNV difference between each pair of isolates where each pair were comprised of a test isolate and the ERI. Based on the currently available methods, ideally comparisons of isolates in WGS studies such as the study here would involve a multi stage approach initially with cgMLST followed by SNV comparison to further infer isolate relatedness. Further software developments are necessary in order to enable such comparisons.

This three-way comparison method of calculation has several shortcomings. The number of SNVs reported between the two isolates is not a true reflection on the differences between the two isolates but instead is the difference of the number of SNV between each of these isolates to the reference genome. In addition further analysis of the WGS data is necessary to determine if differences seen between any of the SNVs identified in the comparator isolates are the same as each other as opposed to differences to the reference genome. Finally a cut-off of ≤40 SNV has been applied to determine the link between comparator isolates since previously it has been shown that differences ranging from 23-40 SNV are indicative of epidemiological linkages between isolates. Elsewhere a cut-off of ≤30 SNV differences has been used to indicate putative transmission events.

However in previous studies this SNV difference is based on a direct pairwise comparison and does not include reference to the ERI.

Conventional molecular techniques such as PFGE examine genomic DNA using restriction endonucleases that cleave DNA infrequently, which results in a banding profile of the genome. *spa* typing and *dru* typing examine a single region of the genome but these methods have been shown to be highly discriminatory in epidemiological studies of MRSA (Goering *et al.* 2008; Shore *et al.* 2010b). In the current study, a comparison of cgMLST to a combination of *spa*, *dru* and PFGE typing showed good agreement between CME and cgMLST. The cgMLST Cluster Type 928 (Table 5.5) included four isolates, two isolates of *spa-dru*-PFGE type t515-dt10a-01042, one of type t515-dt10a-01039 and one t1214-dt10a-01039. Differences seen between the *spa* types (t515 and t1214) reflect a loss of a single repeat succession while the PFGE difference between 01042 and 01039 is a two band difference. Additionally all isolates in cgMLST cluster 1525 were indistinguishable by *spa*, *dru* and PFGE (Table 5.5).

Minor differences in the *spa* type repeat succession patterns, suggest genetic events including insertions, deletions or duplications (Hallin *et al.,* 2007). Similarly in PFGE profile, a single genetic event may result in one-three band differences (Tenover *et al.*, 1995). While in cases where isolates are not distinguished using CME typing, cgMLST has been shown to further differentiate the isolates and identify additional cases of acquisition (Moore *et al.,* 2015). Similarly in the current study cgMLST also permitted the identification of other possible CTEs which had previously been unrecognised. Core genome MLST identified two further probable CTEs (Cluster Type 1526 and 1529) which included seven isolates that had not been previously associated with a CTE (Table 5.5). Furthermore eight additional isolates, which had not been previously associated with a CTE, were also included in Cluster Types with isolates previously associated with a CTE.

A combination of CME typing and cgMLST in cases where CME typing is unable to adequately differentiate isolates may be an acceptable approach in clinical laboratories for the adoption of WGS for routine use. However, in these cases detailed epidemiological information is also necessary to assist in correctly identifying cases of transmission.

Although the increasing availability of WGS data due to advances in next generation sequencing technology has led to the increased availability of the methods, rapid data analysis and data transfer to non-expert users continue to be a challenge for clinical laboratories (Monecke *et al.,* 2016). Development of software such as SeqSphere^{+} allows rapid analysis of WGS data with minimal user intervention. In particular the software is developed to allow importation, assembly and analysis of short read FASTQ files generated from NGS sequencers such as the Illumina, PacBio or Ion Torrent.

The compatibility of strain nomenclature derived from WGS with CME typing is necessary in order to allow continued tracking of strains despite changes in the methodology. Core genome MLST correctly assigned each of the isolates included in the present study to ST22 however it did not allow the recognition of the SCC*mec* type. DNA microarray profiling assigns SCC*mec* types but it does not subtype SCC*mec* elements to the level achieved by conventional SCC*mec* typing by PCR (Shore *et al.,* 2010b). Additionally, while the microarray is able to recognise novel SCC*mec* elements and allelic variants based on unusual combination of the SCC*mec* associated genes, WGS is necessary to further investigate these combinations and determine the genetic organisation (Shore *et al.,* 2011a; Strauß *et al.,* 2016).

A large number of resistance and virulence genes expressed by *S. aureus* are carried on MGEs. Whole genome sequencing enables the detection of resistance genes and mutations and has been shown to correctly predict a phenotypic resistance profile against

clinically useful antimicrobial agents (Gordon *et al.,* 2014; Köser *et al.,* 2014). In order to determine the expression of resistance genes among *S. aureus*, it is necessary to identify all possible genes and the allelic variants but correlation of predicted resistance mechanism and phenotype is still needed along with software to identify such genes (Strauß *et al.,* 2016).

The SeqSphere^{+} software has been developed as an automated system for cgMLST to allow standardised interpretation of WGS data, a necessity for implementation in clinical diagnostics. However, recent research using SeqSphere^{+} for conventional bioinformatics of WGS data, has bridged the gap between DNA microarrays. The design of *ad hoc* cgMLST schemes within SeqSphere^{+} has utilised other fully annotated bacterial strains within GenBank thereby allowing SCC*mec* typing within a smaller collection of isolates. Using this method based on the combination of genes present, the SCC*mec* type was correctly identified in all incidences, and in some case was found to be a more sensitive method than the DNA microarray (Strauß *et al.,* 2016). In these cases however, skilled bioinformaticians are required to develop such *ad hoc* schemes and further upgrades of the software are required in order for these schemes to become more widely available.

Targets within the DNA microarray can only be detected in genes where the PCR-amplified region is complementary to the oligonucleotide probes. However, due to the hybridisation method utilised by the DNA microarray, there is no alternative to this approach. In contrast, web-based curator controlled databases such as those available for cgMLST (available at http://www.cgmlst.org/ncs) can be easily updated upon identification of novel allelic variants. Careful curation of allelic libraries is necessary in order to allow correct identification of alleles while also ensuring a standardised nomenclature is developed that can easily be exchanged between laboratories. An online,

curator-controlled database of all cgMLST types ensures standardisation of identification and nomenclature of these alleles.

5.5 Conclusion

While DNA microarray profiling has been shown to be useful for discriminating MRSA with regards to SCC*mec* type, MLST and resistance and virulence genes, its usefulness in an outbreak investigation of ST22-MRSA-IV is limited due to phenotypic resistance characteristics which arise due to mutations and alternative resistance genes among ST22-MRSA-IV isolates. DNA microarray profiling also fails to detect new allelic variants of genes due to the hybridisation principle of the technology. The use of only SNV analysis of WGS data in outbreak situations, although widely used to date, may not be the correct approach since, a single genetic event may lead to multiple SNVs within the genome which would be considered as multiple events. The development of user friendly rapid bioinformatics software such as SeqSphere^{+} for analysis of WGS has been shown in the present study to assist in the identification of possible CTEs and has shown promising compatibility with more conventional molecular epidemiological typing methods used in the tracking the spread of MRSA. Further studies are required to investigate the usefulness of the combined approach of cgMLST and pairwise SNV analysis of isolates for MRSA isolate discrimination.

Chapter 6

Evaluation of commercial chromogenic media for the detection of MRSA

6.1 Introduction

In the last two decades the epidemiology of MRSA has changed significantly, with an increasing prevalence of MRSA infections outside the healthcare environment in the community and more recently among livestock (Kinnevey *et al.,* 2014; Shore *et al.,* 2014). While the ST22-MRSA-IV clone predominates in Ireland and in many countries throughout Europe, (Grundmann *et al.,* 2014), an increased diversity of other strains including CA-*pvl* positive and -negative MRSA along with a small number of LA strains have also been reported (Kinnevey *et al.,* 2014; Shore *et al.,* 2014).

Just as the epidemiology of the organisms has changed, so too has the level of methicillin resistance among MRSA. Traditionally MRSA are defined as harbouring the *mecA* gene which can lead to homogenous or heterogeneous oxacillin resistance (Shore & Coleman, 2013). While homogenous resistance is easily detectable, few MRSA isolates express homogeneous oxacillin resistance and oxacillin disc diffusion often fails to detect heterogeneous MRSA populations. In addition oxacillin-susceptible *mecA*-positive *S. aureus* isolates have been reported worldwide (Kumar *et al.,* 2013) and similarly *mecA*-negative BORSA (Livermore, 1995) have further complicated the definition of MRSA.

Superimposed on this heterogeneous expression of methicillin resistance, recent reports have also identified a variety of MRSA strains of probable animal origin that encode a highly divergent methicillin-resistance gene termed *mecC*. Where once the detection of *mecA* was considered the gold standard for laboratory confirmation of MRSA, the emergence of *mecC* in infection in both humans and animals, now adds to the challenge of defining and detecting MRSA and in identifying MRSA-positive patients (Shore *et al.,* 2011a; Paterson *et al.,* 2012; Paterson *et al*., 2014b).

The rapid, accurate and cost-effective identification of MRSA is essential in any infection prevention and control strategy aimed at preventing MRSA transmission in a clinical setting, allowing appropriate patient management and, if required, implementation of appropriate antibiotic treatment. Patients colonised with MRSA at the time of admission are at a higher risk of infection while also acting as a reservoir for potential transmission to other patients (Matheson *et al.,* 2012). Screening for MRSA among those with increased risk has become important for the control of nosocomial infections and studies have shown that different laboratory procedures for the detection of MRSA from clinical samples impact on the isolation of MRSA (Brown *et al.,* 2005).

Culture-based methods are still the most widely used for MRSA detection and are based on the phenotypic detection of resistance to methicillin, oxacillin and cefoxitin (CLSI, 2013; EUCAST, 2013; Swenson *et al.,* 2005). While previously oxacillin resistance was the traditional method of detection, since 2001 disk diffusion using a 30-µg cefoxitin disc has proven to be more efficient in predicting *mecA* mediated methicillin resistance.

These phenotypic methods however can be labour intensive and time consuming (Brown *et al.,* 2005). Standard identification and susceptibility testing methods require 24 h of incubation, starting from a pure culture on agar medium for reliable automated identification and susceptibility testing systems such as the Phoenix System (Becton-Dickinson) and Vitek II system (bioMérieux). These systems have been shown to correctly identify MRSA with 98% sensitivity and >99% specificity after isolation in pure culture (Struelens, 2006). Alternatively molecular techniques for the direct detection of MRSA from clinical specimens have been developed but, although they offer improved TAT to detection, these tests are more expensive and not always feasible in diagnostic laboratories (Rossney *et al.,* 2008; Wassenberg *et al.,* 2010; Marlowe & Bankowski, 2011; Alipour *et al.,* 2014).

Since the 1990s a number of chromogenic media have become commercially available. These media utilise enzyme substrates, which, once hydrolysed, release coloured products and result in the target pathogens forming coloured colonies on the agar which are easily differentiated from normal flora. Often these chromogenic media are also selective further inhibiting the growth of normal flora (Perry & Freydière, 2007). These media have been developed for the detection of a range of different pathogens including *Candida*, *Listeria*, staphylococci, salmonellae, *E. coli* and also for the detection of various resistant organisms such as VRE, MRSA and extended spectrum β- lactamases (ESBLs).

Due to the improved TAT associated with the use of selective chromogenic culture media for one-step MRSA isolation and identification directly from clinical specimens, these media have now become common place in clinical diagnostics laboratories (Cunningham *et al.,* 2007; Morris *et al.,* 2012). However, the gold standard for such detection is broth enrichment followed by subculture to chromogenic media (National Clinical Effectiveness Committee, 2013) and the main disadvantage associated with this is the extended TAT for a positive result. With the wide-scale application of chromogenic media in diagnostic practice, the suitability of such media to ensure the correct detection of divergent MRSA strain types has come under review. However, whilst many studies have evaluated the use of chromogenic media for the direct recovery of MRSA from patient specimens (Morris *et al.,* 2012; Denys *et al.,* 2013; Veenemans *et al.,* 2013), few have undertaken a comparative evaluation of all available commercial media using a comprehensive and diverse collection of well characterised *S. aureus* strains (Malhotra-Kumar *et al.,* 2010) including those with the novel *mecC* gene and those expressing varying levels of methicillin resistance.

The purpose of this part of the present study was to evaluate the performance of commonly used chromogenic MRSA media using a diverse collection of well

characterised *S. aureus* isolates recovered in Ireland and Europe. The limit of detection (LoD) of four commercial chromogenic media for detecting MRSA was determined using MRSA strains representative of four SCC*mec* types i.e. II, IV, V and XI (Shore *et al.,* 2011; Kinnevey *et al.,*2014; Shore *et al.,* 2014). The performance of the media was also evaluated against a collection of genotypically diverse MRSA strains from hospitals, communities and livestock and representative of SCC*mec* types I-XIII, X and XI as well as MSSA and BORSA strains isolated from healthcare and community sources. An evaluation of the media was also undertaken using patient samples collected as part of the routine infection prevention and control procedures in a large teaching hospital.

6.2 Materials and methods

6.2.1 Limit of detection

Four MRSA isolates, representative of SCC*mec* types II, IV, V and XI (Table 6.1) were selected to investigate the LoD of the following four commercial MRSA chromogenic agars; MRSA Select II (BioRad), MRSA Brilliance 2 (Oxoid), Colorex MRSA (E & O Laboratories) and ChromID MRSA (bioMérieux). In each case, isolates were subcultured overnight on CBA at 37°C and then suspended in saline to a concentration equivalent to 0.5 McFarland standard. A ten-fold dilution series was prepared from $1.5x10^8$-10^0 CFU/ml and a 100 µl volume of each dilution was inoculated on to each of the MRSA chromogenic agars using a spiral plater (Don Whitley Scientific, Shipley, UK). This application was performed in triplicate for each isolate and plates were incubated as per the manufacturer's instructions. In each case, MRSA recovery was observed in accordance with the manufacturer's description of MRSA colony type i.e. pink colonies on MRSA Select II, Colorex and ChromID or blue colonies on MRSA Brilliance 2. The LoD for each media was recorded as the lowest bacterial concentration to give a positive culture result.

6.2.2 Evaluation of chromogenic media using a diverse collection of S. aureus isolates

The ability of the media to detect MRSA among a diverse collection of *S. aureus* isolates was also investigated. This included (i) MRSA isolates representing 10/11 SCC*mec* types (I-VIII, X & XI), (ii) *mecA* (*n* = 148) and *mecC* (*n* = 13) MRSA isolates representative of a range of genotypes and comprising of 149 MRSA isolates with oxacillin MICs ranging from 4 - >256 mg/L and 12 MRSA isolates with an oxacillin MIC ≤2.0 mg/L (MICs ranging from 0.125-2.0 mg/L), (iii) 34 MSSA isolates that lacked *mec*

Table 6.1 Limit of detection of MRSA isolates representative of four SCC*mec* types as determined using four chromogenic media

			Limit of detection CFU/ml				
Isolate no.	Genotype	*mec* gene	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2	Reference
AR07.4/0237	ST5-MRSA-II	*mecA*	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^4$	Shore et al., 2005
CA05	ST22-MRSA-IV	*mecA*	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^4$	Ma et al., 2002
WIS	ST8-MRSA-V	*mecA*	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^4$	Ito et al., 2004
M10/0061	ST130-MRSA-XI	*mecC*	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	Shore et al., 2011

Abbreviations: CFU/ml; colony forming units per millilitre, no; number

genes and were susceptible to oxacillin (oxacillin MIC range 0.5-2.0 mg/L) and (iv) 20 BORSA isolates which were *mec*-negative but which exhibited oxacillin MICs between 4-8 mg/L (Table 6.2). In each case isolates were suspended to a concentration equivalent to 0.5 McFarland standard, a 20 µl volume was inoculated on to each of the four commercial MRSA chromogenic agars, and plates were incubated and read as above. Quality control testing was performed on each medium using *S. aureus* control strains ATCC 43300 (MRSA) and ATCC 25923 (MSSA).

6.2.3 Evaluation of chromogenic media using patient samples

The ability of the chromogenic media to detect MRSA directly from patient samples was investigated using 228 swabs recovered from the nose, throat and groin of 76 in-patients at a 936-bed tertiary referral hospital in Dublin, Ireland. Specimens were collected as part of routine screening practices within the hospital. The 228 samples were initially inoculated on to MRSASelect (the earlier formulation of MRSASelectII) in accordance with the routine diagnostic procedures. The specimens were then inoculated on to the test chromogenic agars changing the order in which the media was inoculated for each sample. All suspect colonies recovered from the screening swabs that were consistent with the manufacturer's description of MRSA were tested for oxacillin susceptibility by disk diffusion (EUCAST, 2013) and investigated for the presence of *mec* and *nuc* genes using an in-house realtime PCR assay (see Chapter 2, section 2.4.3).

6.2.4 Statistical analysis

The ability of each chromogenic agar to correctly identify MRSA (sensitivity) and to exclude MSSA (specificity) was determined based on the number of correct results realised among the MRSA and MSSA isolates.

Table 6.2 Details of *Staphylococcus aureus* isolates used for the evaluation of the chromogenic media

Methicillin resistance phenotype	Genotype (*n*)[a]	*spa* types[a]	Reference[a]
MRSA	ST250-I (1)	ND	Shore *et al.*, 2005
	ST39-II (1)	t007	This study
	ST5-II (1)	ND	Shore *et al.*, 2005
	ST981-III (1)	ND	Shore *et al.,* 2008
	ST5-IV (15)	t1340, t002, t311, t688,	This study
	ST8-IV (7)	t008, t064	This study
	ST22-IV (90)	t513, t849, t032, t852, t022, t2235	This study
	ST30-IV (2)	t012	This study
	ST45-IV (6)	t620, t344, t727, t065, t230	This study
	ST53-IV (1)	t4545	This study
	ST88-IV (2)	t2622, t692	This study
	ST246-IV (1)	ND	Ma *et al.*, 2002
	ST8-V (1)	ND	Ito *et al.,* 2004
	ST59-V (1)	t316	This study
	ST398-V (3)	t011	This study Kinnevey *et al.*, 2014
	ST772-V (3)	t657	Brennan *et al.*, 2012
	ST5-VI (1)	ND	Oliveira *et al.*, 2006
	ST5-VII (1)	ND	Berglund *et al.*, 2008
	ST8-VIII (1)	ND	Zhang *et al.*, 2009
	ST398-X (1)	ND	Li *et al.*, 2011
	CC130-XI (13)	t1535, t978, t2345, t3218, t1736, t3570, t3391, t3256, t843, t8835, t267, t12399, t843, t373, t355	Petersen *et al.,* 2013
	ST1 (5)	t2246, t8698, t127	This study
	ST5 (1)	t045	This study
	ST59 (3)	t437	This study
	ST80 (3)	t044, t088	This study
	ST779 (1)	t878	This study
	ND (5)	t671, t6419, t2277, t579	This study
MSSA	ST8 (12)	t008, t190, t024	This study
	ST22 (2)	t005, t8281	This study
	ST1 (3)	t127	This study
	ST20 (1)	t164	This study
	ND (10)	t2365, t306, t1778, t334, t11018,	This study

Continued overleaf

Table 6.2 continued

Methicillin resistance phenotype	Genotype (*n*)[a]	*spa* types[a]	Reference[a]
		t2658	
	ST80 (1)	t088	This study
	ST2229 (1)	t701	This study
	ST7 (1)	t091	This study
	ST45/46 (1)	t065	This study
	ST15 (1)	t279	This study
	ND (1)	ND	This study
BORSA	ST8 (3)	t008	This study
	ST9 (1)	t100	This study
	ST7 (1)	t091	This study
	ST15 (1)	t084	This study
	ST398 (1)	t571	This study
	ND (2)	t164, t9222	This study
	ND (11)	ND	This study

[a]Isolates that were not part of previously published studies were identified as *S. aureus* and underwent susceptibility testing as part of routine work at the Irish National MRSA Reference Laboratory (NMRSARL). The genotypes i.e. the MLST clonal complex (CC) or sequence type (ST) were inferred from the *spa* type and/or the antibiogram-resistogram pattern based on previous experience at the NMRSARL. The presence or absence of *mecA* and *mecC* were determined using an in-house PCR assay. The CC130-MRSA isolates carried *mecC* and the remaining MRSA isolates carried *mecA*. Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; BORSA, borderline-oxacillin resistant *S. aureus*; ND, not determined.

6.3 Results

6.3.1 Limit of detection assay

The results of the LoD evaluation for the four MRSA strains on the four different chromogenic agars tested are shown in Table 6.1. MRSA Select II, Colorex MRSA and ChromID each had a LoD of $1.5x10^1$ CFU/ml for each SCC*mec* type investigated while MRSA Brilliance 2 had a higher MRSA LoD of $1.5x10^4$ CFU/ml for three of the four MRSA strains tested.

6.3.2 Performance of the chromogenic media using a diverse collection of S. aureus isolates

The MRSA strains representative of the different SCC*mec* types demonstrated good recovery with typical MRSA morphology on all four media tested. The performance data for the MRSA and MSSA isolates on the four chromogenic media is shown in Table 6.3. For the 161 MRSA isolates Colorex MRSA and ChromID MRSA were found to be 100% sensitive while MRSA Select II and MRSA Brilliance 2 demonstrated slightly lower sensitivity rates of 99% and 98%, respectively. Both media failed to detect one *mecA*-positive MRSA isolate exhibiting a oxacilin MIC of only 0.125 mg/L (*spa* type t2235), the Brilliance 2 media failed to detect an additional two MRSA isolates exhibiting susceptible MICs of 1.0 and 2.0 mg/L (*spa* types t002 and t3500, respectively). When compared with the predominant ST22-MRSA-IV *mecA* strain, *mecC* isolates included in this study yielded colonies equivalent in number and morphology on three of the four chromogenic agars tested and yielded a greater number of colonies on the Brilliance 2 agar. When tested with the 34 MSSA isolates, the specificity of the four chromogenic agars ranged between 73-85% (Table 6.3). The MRSA Select II exhibited the highest number of false positive results (9/34) while other chromogenic agars yielded 5-6 false positives (Table 6.4).

Table 6.3 Sensitivity and specificity of the four chromogenic media using MRSA and MSSA isolates[a] investigated in the present study

	Chromogenic medium			
	MRSA SelectII	**ChromID**	**Colorex**	**Brilliance 2**
Sensitivity[a]	99%	100%	100%	98%
Specificity[a]	73%	85%	85%	82%

[a]Sensitivity and specificity was determined for each of the four chromogenic agars with a collection of MRSA and MSSA isolates including (i) MRSA isolates representing 10/11 SCC*mec* types (I-VIII, X & XI), (ii) *mecA* (*n* = 148) and *mecC* (*n* = 13) MRSA isolates representative of a range of genotypes and comprising of 149 MRSA isolates with oxacillin MICs ranging from 4 - >256 mg/L and 12 MRSA isolates with an oxacillin MIC ≤2.0 mg/L (MICs ranging from 0.125-2.0 mg/L), (iii) 34 MSSA isolates that lacked *mec* genes and were susceptible to oxacillin (oxacillin MIC range 0.5-2.0 mg/L)

Table 6.4 Genotypes and oxacillin MICs of *mecA*- and *mecC*-negative MSSA and BORSA isolates yielding false positive results using the four chromogenic media

Isolate no.	Genotype[a]	Oxacillin MIC (mg/L)	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2
MSSA						
M11/0175	t306	2	POS	POS	POS	POS
M12/0147	ST80-t088	0.5	POS	POS	POS	NEG
M12/0272	ST7-t091	0.5	POS	NEG	NEG	NEG
M14/0220	ST45/46-t065	2	POS	NEG	NEG	NEG
M14/0248	t608	0.5	POS	POS	POS	POS
M14/0249	ST1-t127	0.5	POS	POS	POS	POS
M14/0250	t2828	1	POS	NEG	NEG	NEG
M14/0258	ST8-t008	0.5	POS	NEG	NEG	NEG
M14/0366	ND	2	POS	POS	POS	POS
M11/0281	t11018	0.5	NEG	NEG	NEG	POS
M14/0254	ST8-t008	1	NEG	NEG	NEG	POS
TOTAL			9	5	5	6
BORSA						
M05/0294	ND	4	POS	NEG	NEG	NEG
M07/0138	ND	4	POS	POS	POS	POS
M07/0376	ND	4	NEG	NEG	POS	NEG
M07/0377	ND	4	NEG	NEG	POS	NEG
M08/0079	ND	8	POS	POS	POS	POS
M12/0306	ST8-t008	8	POS	NEG	NEG	POS
M12/0355	ST8-t008	8	POS	NEG	POS	POS
M13/0626	t078	4	POS	NEG	NEG	NEG
M13/0629	ND	4	POS	POS	POS	POS
M14/0178	ST9-t100	4	POS	POS	POS	POS
M14/0179	ST9-t100	4	POS	NEG	NEG	POS
M14/0188	ST7-t091	8	POS	POS	POS	POS
M14/0260	ND	4	POS	POS	POS	POS
M14/0282	ST15/18-t084	4	POS	NEG	NEG	NEG
M14/0604	ST398-t571	8	POS	POS	POS	POS
M14/0784	ND	4	NEG	POS	POS	POS

Continued overleaf

Table 6.4 continued

Isolate no.	Genotype[a]	Oxacillin MIC (mg/L)	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2
TOTAL			13	8	8	11

[a]Where available genotypes are indicated by the MLST sequence type (prefix "ST") and/or the *spa* type (prefix "t"). The MLST STs were inferred from the *spa* type using the Ridom *spa* server (http://www.spaserver.ridom.de/) and/or based on previous experience at the Irish National MRSA Reference Laboratory.

Abbreviations: POS, positive; NEG, negative; MIC, minimum inhibitory concentration; mg/L, milligrams per litre; ND, not determined; MSSA, methicillin-resistant *S. aureus*; BORSA, borderline-oxacillin resistant *S. aureus.*

All MSSA isolates that generated false positives on the chromogenic agars, exhibited oxacillin susceptibilities representative of the range of oxacillin MIC values seen in the MSSA collection tested and belonged to a range of genotypes (Table 6.4).

As expected, due to their higher oxacillin MICs (4-8 mg/L), the BORSA isolates also proved challenging for the chromogenic agars. Once again MRSA Select II exhibited the highest number of false positive results, with 13 of the 20 BORSA isolates yielding suspect MRSA colonies. The other three chromogenic agars also produced a high number of false positives with Brilliance 2 demonstrating growth for 11 of the BORSA, while eight were recovered the on ChromID and Colorex media (Table 6.4).

6.3.3 Performance of the chromogenic media using patient samples

Of the 228 samples from 76 patients investigated, four patients were positive for MRSA. These findings were in agreement with the clinical microbiology laboratory results and were detected with all four chromogenic agars, with suspect colonies growing in sufficient numbers and with typical colony morphology that allowed ready detection despite the high number of plates inoculated. All isolates were subsequently confirmed as oxacillin resistant and carried the *mecA* gene.

6.4 Discussion

For the detection of MRSA all media performed well in the evaluation. With the exception of MRSA Brilliance 2 which demonstrated an LoD of 15,000 CFU/ml, the other three chromogenic agars, MRSA Select II, the Colorex MRSA and the ChromID, recovered all strains at cell densities as low as 15 CFU/ml. Previously reported studies of *mecC* isolates suggested that difficulties may arise in the laboratory detection of *mecC* MRSA isolates due to the low oxacillin MIC exhibited by some of these strains (Cartwright *et al.,* 2013; Paterson *et al.,* 2014; Skov *et al.,* 2014). However the *mecC* isolates included in this study grew well on each of the chromogenic media investigated, and showed even improved recovery on MRSA Brilliance 2 compared to HCA-MRSA strains such as ST22-MRSA-IV.

The chromogenic agars demonstrated excellent sensitivity ranging from 98-100%, with the Colorex media and the ChromID detecting the full collection of MRSA isolates investigated. The high sensitivities recorded here are in agreement with other MRSA chromogenic agar evaluation studies (Morris *et al.,* 2012; Denys *et al.,* 2013; Veenemans *et al.,* 2013). However, a challenge arose for the MRSA Select II and the MRSA Brilliance 2 media where, each failed to detect a small number of MRSA isolates (one and three isolates, respectively) exhibiting susceptible oxacillin MICs. A small increase in the prevalence of oxacillin-susceptible *mecA* positive isolates has been previously reported elsewhere (Kumar *et al.,* 2013) and similar isolates have been recovered in the NMRSARL in Ireland. As these strains can cause problems in the routine diagnostic laboratory, their successful recovery using the chromogenic media is essential.

When challenged with MSSA isolates, the chromogenic agars demonstrated reduced levels of specificity, ranging from 73-85%. This specificity rate is low in comparison to

other studies where specificity rates of 95-99% have been reported for chromogenic agars (Morris *et al.,* 2012; Denys *et al.,* 2013; Veenemans *et al.,* 2013). However, these other studies confined investigations to clinical specimens only, compared to the current study which included a diverse collection of MSSA strains. The recovery of these MSSA strains as presumptive MRSA isolates has implications for the diagnostic laboratory in terms of increasing the TAT and resource management (Morris *et al.,* 2012).

The reduced specificity of the chromogenic agars was once again demonstrated by the high recovery of BORSA with almost 50% of isolates being recovered on all four of the media. This high rate of false positivity can be expected with the BORSA isolates due to their high oxacillin MICs. Although a previous epidemiological investigation of BORSA isolates indicated that they are not implicated in patient-to-patient spread (Leahy *et al.,* 2011), their growth on this media further complicates the detection, prevention and control of true MRSA in the healthcare environment.

6.5 Conclusion

The early identification of MRSA and implementation of infection prevention and control procedures has been shown to reduce HCA infections. The high sensitivity of the chromogenic agars evaluated in this study, confirms the usefulness of this media in the one step detection and presumptive identification of MRSA. Whilst the recovery of a high number of MSSA and BORSA isolates is a concern, this is outweighed by the ability of the media to recover almost all MRSA including those which are oxacillin susceptible or harbour the novel *mecC* gene, isolates which may otherwise be missed in the diagnostic laboratory.

Chapter 7

General Discussion

7.1 Extensive diversity of sporadically-occurring MRSA in Ireland and the identification of community, health care and livestock associated lineages

Since it was first discovered over a half a century ago, MRSA has become a significant burden in healthcare facilities worldwide. The current predominant MRSA strain circulating in Irish healthcare facilities, ST22-MRSA-IV, accounts for approximately 80% of MRSA recovered from BSIs in Ireland each year (Fig. 1.7) and as such, extensive studies have been carried out characterising this clone (Rossney *et al.*, 2006; Mollaghan *et al.*, 2010; Shore, *et al.*, 2010; Jamrozy *et al.*, 2012). However, clonal displacement has occurred previously in Ireland and elsewhere, and is likely to occur again into the future (Shore *et al.*, 2008b; Jamrozy *et al.*, 2012; Monecke *et al.*, 2013; Shore *et al.,* 2014).

The purpose of the first part of the current study was to gain a greater understanding of sporadically-occurring MRSA strains in Ireland since these strains cause infections as well as having the potential to emerge as predominant strains. Detailed studies of sporadically-occurring MRSA, both worldwide and locally, are limited. In the present study, the use of the StaphyType microarray allowed the comprehensive characterisation of the genetic backgrounds, SCC*mec* types, virulence and antimicrobial resistance genes of sporadically-occurring MRSA strains from patients in Irish hospitals, their environments and the community. Among 276 isolates, the diversity detected was unprecedented with 86 *spa* types associated with 48 different STs or CCs and 18 different SCC*mec* types and subtypes detected.

Much of the diversity identified among these sporadic MRSA isolates spans many of the lineages previously recognised at a global level including CC1-MRSA-IV, CC5-MRSA-IV, CC30-MRSA-IV and CC45-MRSA-IV (Fig. 3.2), many of which are also

associated with HCA-MRSA in other regions or previously in Ireland (ST239-MRSA-III, ST8-MRSA-IIA,B,D and CC5-MRSA-IV) (Monecke *et al.*, 2011; Lim *et al.*, 2013). Additionally, strains that have been reported infrequently elsewhere were also identified in the current study (e.g. ST779-ΨSCC*mec-fus*-SCC$_{CRISPR}$, CC5-MRSA-V, CC45-MRSA-V, CC88-MRSA-I and CC97-MRSA-V), along with strains that had not been previously recognised in Ireland (ST772-MRSA-V) and the LA-MRSA lineages CC398-MRSA-IV, CC398-MRSA-V and CC130-MRSA-XI.

Among the 276 sporadically-occurring MRSA isolates investigated, 97.6% harboured multiple resistance genes indicating the extensive reservoir of resistance genes among MRSA in Ireland. This is in contrast to the currently endemic ST22-MRSA-IV which is commonly considered to be non-multidrug resistant, predominantly only exhibiting resistance to erythromycin and ciprofloxacin (McNicholas *et al.*, 2011). In addition, with the exception of CC130, CC97 and the majority of CC398, all other isolates harboured at least two virulence genes including enterotoxins, exfoliative toxins, ACME and IEC which are located on MGEs and can be transferred between strains. The success of *S. aureus* as a pathogen is largely attributed to its ability to acquire multiple antibiotic resistance genes and virulence genes enabling it to establish and maintain infections and to avoid the host immune system. Although sporadically-occurring within Ireland, many of the multiantibiotic resistant strains recognised here including ST772-MRSA-V and ST239-MRSA-III predominate in other regions (e.g. India and Taiwan, respectively) and as such have proven ability to emerge as predominant strains (Monecke *et al.*, 2011).

Different MRSA lineages have emerged as a result of genetic variation due to the acquisition of resistance and virulence genes, host adaptation or selective pressures (Deurenberg *et al.*, 2007; Bal *et al.*, 2016) . In particular the emergence and success of some MRSA strains has been attributed to the diversity of SCC*mec* (Bal *et al.*, 2016).

Interestingly, of the 18 different combinations of SCC*mec* genes identified among the 276 isolates, SCC*mec* IV predominated (62.7%, 173/276). Other smaller SCC*mec* elements including SCC*mec* V and variants (19.9%, 55/276) and SCC*mec* VI (1.1%, 3/276) were also detected. In contrast larger SCC*mec* elements were fewer in numbers with SCC*mec* I, II and III accounting for only 7.6% (21/276) of isolates and limited to only CC5 and CC8 (ST8 and ST239). The frequency and diversity of genetic backgrounds in which SCC*mec* IV and variants were associated within the present study provides further evidence that the small size of SCC*mec* IV (21 to 25 kb) may allow increased mobility of the element and therefore lead to greater diversity of *S. aureus* genetic backgrounds harbouring the element (Bal *et al.*, 2016). Compared to other SCC*mec* elements, SCC*mec* IV is smaller in size and as such has a much lower associated fitness cost compared to other larger SCC*mec* elements that are associated with traditional HCA-MRSA strains (Otto, 2012).

The epidemiology of MRSA has changed significantly over the last two decades with the emergence of CA- and LA-MRSA harbouring novel SCC*mec* elements. Coupled with the changing ways in which healthcare is delivered such as reduced lengths of hospital stays, day-only admittance and healthcare in the community, classical definitions of HCA, CA and LA- MRSA are no longer suitable. In the current study, lineages that would elsewhere be considered HCA-MRSA (ST239-MRSA-III) were recognised among patients in the community attending a general practitioner. Similarly, strains associated with CA-MRSA (ST772-MRSA-IV, CC1-MRSA-IV and CC5-MRSA-V) were found to cause outbreaks in Irish healthcare facilities and traditional LA-MRSA lineages were recovered from patients with no known animal risk factors (CC398-MRSA-IV and CC130-MRSA-XI).

While active surveillance, proper documentation and treatment of MRSA in healthcare facilities is effective in controlling MRSA within these settings, the extensive

diverse MRSA reservoirs beyond healthcare facilities and the community evident here confirms that within Ireland, healthcare facilities cannot become complacent in their infection prevention and control procedures despite a decreasing prevalence of MRSA in Irish hospitals. Furthermore it reaffirms the need to introduce and enhance these procedures outside of healthcare facilities adopting successful protocols seen in Scandinavia and the Netherlands involving a search-and-destroy approach. Additionally the improved monitoring of antibiotic usage in the community is essential in order to prevent future emergence of these multi-antibiotic resistant strains of MRSA in Irish hospitals.

In addition to the spread of CA-MRSA into healthcare facilities, incidences of the zoonotic spread of MRSA were also identified here highlighting the importance of animals as a potential reservoir for MRSA. The CC398 MRSA identified here appear to be predominantly of animal origin based on epidemiological evidence, the lack of IEC genes and the prevalence of *tet*(M). Among pigs and associated farm workers, epidemiological information, along with differences in resistance and virulence genes and the SCC*mec* elements indicated multiple incidences of importation of ST398 into Ireland. Additionally, along with zoonotic transmission from animals to humans, a case of human to animal transmission was also evident (Table 3.5). In the case of the CC130 isolate, it was recovered from an elderly patient in the community who had previously been hospitalised on several occasions and who lived adjacent to a farm. While the spread of CC398 MRSA from animals to humans via direct contact is of significant concern, CC398 MRSA have also been reported in retail meat products including pork (Hadjirin *et al.*, 2015) representing a further potential threat to public health and the reputation of the Irish agricultural sector.

In the current study, isolates investigated represent only a snapshot of the possible MRSA population circulating in Ireland. Isolates are representative of those submitted to the NMRSARL and since participation in the EARS-Net project require hospitals to submit only isolates recovered from BSIs, only a small proportion of those recovered from other sites are submitted to the NMRSARL for investigation. Here several incidences of importation of ST398 and ST772 were identified suggesting that not only is there poor infection prevention and control procedures within the community, but so too are there inadequate levels of biosecurity at country, farm and veterinary hospital level. Further efforts to understand the emergence and spread of high risk clones such as those identified here require extensive studies of a far greater collection of MRSA isolates while increased surveillance of MRSA among humans and animals is essential to prevent further spread of these strains into hospitals, community or the food chain in Ireland.

7.2 Identification of novel SCC*mec* chimeric elements and composite islands harbouring multiple resistance genes

In the present study WGS was used to determine the structure of SCC*mec* elements harbouring *fusC* (Chapter 4). Similar to other reports (Ellington *et al.*, 2015; Baines *et al.*, 2016), the current study found that SCC*mec*/SCC mediated the dissemination of *fusC* but the diversity of the *fusC*-positive MRSA strains identified was surprising when compared to other studies. While the increase in fusidic acid resistance in New Zealand has been associated with the emergence of ST5 (Baines *et al.*, 2016), no such similar trend is evident in Ireland based on the findings of this study where *fusC* was detected in association with strains belonging to six distinct CCs (Table 4.1). However, while like elsewhere, *fusC* was commonly associated with the SCC*mec* IV element (30.1%, 16/52) and a ΨSCC*mec-fus*-SCC_{CRISPR} element (36.5%, 19/52) it was also, for this first time, associated with SCC*mec*

I, V and VI. Additional differences were also observed among SCC*mec* IV chimeric elements harbouring *mecA* and *fusC* due to size differences. The diversity of the chimeric SCC*mec-fusC* elements and the different lineages identified here further supports earlier suggestions that *S. aureus* may favour smaller elements harbouring multiple resistance genes over larger composite islands. The recognition of elements recovered in Ireland with similarities to those described in New Zealand and the UK suggests intercontinental spread of strains and the rapid response that *S. aureus* has to selective pressures such as antimicrobial therapies.

Elsewhere it has been suggested that poor hygiene correlates with a higher prevalence of MRSA as well as improper use of antibiotics being associated with higher rates of MRSA carriage (Graveland *et al.*, 2010). In Ireland, hospital management have overall responsibility for controlling MRSA within their facility and the overall prevalence of MRSA recovered from BSIs has declined significantly in recent years (EARS-Net, 2015). However in the current study a significant number of isolates included were recovered from screening samples and were not associated with any infections. Although these patients were in-patients, many of the strains recovered from these screening samples were associated with CA-MRSA lineages (Monecke *et al.*, 2011). This suggesting possible inappropriate usage of antibiotics in the community is driving the emergence of CA-MRSA in Ireland with a lack of stringent infection control procedures in the community is leading to the importation of these strains into Irish healthcare facilities.

The characterisation of distinct SCC*mec-fus* chimeric elements in the current study, particularly among CA-MRSA strains, highlights the ongoing evolution of MRSA. It also provides further evidence that the overuse and misuse of fusidic acid in the community is driving the selection of *fusC*-encoding elements among MRSA. Lastly, this study has provided evidence that these elements have the potential to spread between different

MRSA strains and highlights the necessity to control MRSA both in hospital and community settings.

7.3 Nosocomial outbreaks caused by CA-MRSA lineages

In the current study several CA-MRSA lineages were found to cause outbreaks in nosocomial settings. The *pvl*-positive ST772-MRSA-IV strain (Chapter 3) and the *pvl*-negative CC5-MRSA-V (Chapter 5) were each associated with NICU outbreaks while the CC1-MRSA-IV strain (Chapter 5) was associated with an outbreak in a general medical ward. While again these isolates were mainly recovered from patient screening samples, of particular concern here is the range of resistance and/or virulence genes harboured by these strains as well as the lengthy duration of each outbreak. For example, the CC1-MRSA-IV outbreak isolates exhibited resistance to multiple clinically relevant antimicrobial agents including mupirocin, tetracycline and aminoglycosides and was recovered from 49 patients over a two-year period. The CC5-MRSA-V outbreak spanned 12 months affecting 17 patients. Finally, although the outbreaks involving the ST772-MRSA-IV strain were much shorter, the strain re-emerged following a short period of time when the staff member returned to work and the ST772 isolates exhibited multidrug resistance and were *pvl* positive.

Conventional genotyping techniques such as *spa* typing showed homogenous clones, and in many cases differences were only evident among strains when phenotypic resistance and the presence/absence of resistance or virulence genes detected using the DNA microarray were considered. The difficulty in differentiating these strains highlights the need for highly discriminatory epidemiological typing methods such as DNA microarray or WGS in the investigation of MRSA. For the CC1-MRSA-IV outbreak isolates, the majority exhibited high-level mupirocin resistance encoded for by *ileS2*

(Chapter 5). While this difference provided a useful putative marker to distinguish HCA and CA-CC1-MRSA-IV within the hospital environment, it highlights the adaptability of MRSA in environments where additional antibiotic selective pressures are present. Several other minor differences were also detected among CC1-MRSA-IV isolates using the microarray with regards to the presence or absence of resistance genes, namely *fusB, qacA* and *tet*(M), however these led to difficulties in determining the relationship between these isolates and the mupirocin resistant outbreak strain. With regards to the CC5-MRSA-V outbreak, although more discriminatory than conventional methods, DNA microarray still lacked the discriminatory power to differentiate isolates recovered over the 12 month outbreak. While epidemiological information would suggest that, in this case, there is transmission throughout the ward, prolonged periods (November 2011- November 2012) between the recovery of isolates may suggest multiple introductions of this strain into the unit and WGS is necessary to investigate this further.

Although initially developed as a research tool, recent developments in WGS technology has led to the suggestion that WGS will become the new gold standard for the genotyping of MRSA (Blomfeldt *et al.*, 2017). To date, WGS has provided significant insight into the origin and spread of antibiotic-resistance, the genetic basis of virulence, the emergence of lineages and the population structure of *S. aureus* (Lindsay, 2014; Lee *et al.*, 2015). Introduction of WGS as a diagnostic tool has the potential to provide rapid access to information surrounding resistance, virulence and host adaptation. Such knowledge, when applied to patient management and hospital procedures may help in limiting the spread of MRSA by timely adherence to strict infection control procedures.

Furthermore, the use of WGS could assist in the correct identification of problematic strains that may pose a threat to patient safety but are misreported in clinical laboratories. In particular, as seen in the evaluation of chromogenic media in the current

study (Chapter 6), the specificity of each media was reduced when isolates defined as BORSA were included. Among these isolates a significant number of false positives were detected (i.e. a test result which suggested that the isolate was an MRSA but molecular analysis of the isolate showed it to lack *mecA* and *mecC* and phenotypic analysis showed an elevated oxacillin MIC of 2.0-8.0 mg/L). While the raised MIC may be sufficient to guide patient management, the incorrect diagnosis has further implications on infection control procedures. Recent WGS of similar isolates in Scotland showed that indeed the isolates lacked *mecA* and *mecC* and instead resistance to β-lactamase-stable penicillins in this case was due to several single nucleotide substitutions in the transpeptidase domains of penicillin-binding proteins (PBPs) 1, 2 and 3 (Ba *et al.*, 2014).

This shift in the application of WGS to routine diagnostics is already evident throughout Europe. Numerous outbreaks caused by various organisms have been investigated by WGS (Köser *et al.* 2012; Miller *et al.* 2014; Gilchrist *et al.* 2015; Kong *et al.* 2016; McGann *et al.* 2016; Ugolotti *et al.* 2016). Furthermore, a number of studies have demonstrated the ability to predict antimicrobial susceptibility profiles from WGS data and bioinformatic software to automatically predict these resistances are in development (Köser *et al.*, 2012; Holden *et al.*, 2013; Gordon *et al.*, 2014; Bradley *et al.*, 2015; Lee *et al.*, 2015). The extensive research investigating resistance mechanisms in *S. aureus* has been a valuable resource and the adoption of WGS for clinical diagnostics of *S. aureus* may be far closer than for other microorganisms (Ellington *et al.*, 2016). However, further work is required. The instability of the MGEs harbouring *S. aureus* resistance genes have led to difficulties in predicting resistance to some antimicrobial agents such as erythromycin (encoded for by *erm* genes). Heterogeneous resistance exhibited in sub populations has also caused discrepancies particularly with regards to vancomycin, daptomycin and oxacillin.

Overall however, while studies have been positive with some as high 98.6% concordance, the methods in determining the resistance profile have varied using mainly either a MIC or zone size as a possible breakpoint. Recently, following a review of all published data surrounding WGS and predicting resistance profiles, EUCAST published recommendations that WGS prediction should be based on the epidemiological cut-off values (ECOFFs) wherever possible rather than just clinical breakpoints (Ellington *et al.*, 2016). Antimicrobial breakpoints are developed based on interactions between bacteria and agents including cellular permeability, target availability and enzymatic expression rather than just the presence or absence of the genes and while clinical breakpoints are determined to be clinically relevant, they are not considered to be epidemiologically relevant. In contrast, the ECOFF would include the upper limit of the wild type population possibly reducing the number of discrepancies between actual and predicted resistance profiles (Ellington *et al.*, 2016). Furthermore the adoption of a standardized method for predicting resistance profiles is the first step in the adoption of such technology in routine use.

7.4 Usefulness of cgMLST in tracking the transmission of ST22-MRSA-IV

In the current study, although the application of the StaphyType DNA microarray to outbreaks proved useful for some MRSA strains (CC1-MRSA-IV, ST772-MRSA-V and CC5-MRSA-V), it was not as useful for the endemic ST22-MRSA-IV strain. In the case of ST22-MRSA-IV, many of the differences detected between isolates recovered during outbreaks were based on differences seen in the phenotypic susceptibility testing (Table 5.2), primarily due to the resistance mechanisms associated with chromosomal mutations

including fusidic acid (*fusA*) and ciprofloxacin (*gyrA*) and which are not detected using the DNA microarray.

ST22-MRSA-IV accounts for a significant proportion of MRSA in Irish hospitals and so by default a significant number of outbreaks in healthcare facilities. The inability of the microarray to differentiate between ST22-MRSA-IV isolates is a serious limitation of this method. Similar to the need for standardisation for predicting antimicrobial resistance from WGS data, standardised methods and nomenclature in outbreak investigations are necessary before widespread adoption of WGS. Attempts to improve this, along with increased automation and the need for a standardised nomenclature system, has led to the development of a cgMLST scheme examining all the genes within the core genome of isolates. Furthermore, software to assist in the assignment of isolates to a cgMLST type has improved access to this technology. One such piece of software is SeqSphere^{+} which offers automated interpretation of WGS data and assignment of isolates to a cgMLST type. An evaluation of this software using WGS from isolates recovered from patients and their environment in a single hospital ward was undertaken in the current study. Although initial comparisons of results generated from this software to SNV analysis failed to group isolates in similar clusters, comparison of cgMLST with a combination of conventional molecular epidemiological typing methods (*spa*, *dru* & PFGE) gave similar results and also allowed the identification of other possible cases of transmission within the unit (Chapter 5).

Apart from ease of use and standarised interpretation, an additional advantage of the cgMLST typing scheme is its ability to type isolates on a similar basis to more widely used sequenced based methods such as MLST and *spa* typing. Conventional nomenclature of MRSA is based on the combination of the ST and the SCC*mec* type and there are a substantial number of reports describing MRSA utilising this nomenclature system. The

compatibility of newly generated information on MRSA derived from WGS with this previously generated information is necessary in order to allow continued tracking of strains despite changes in methodology. In particular, in the current study the cgMLST scheme successfully assigned all of the isolates to ST22 however since this method is based only on the genes within the core genome, the genes on the SCC*mec* element are excluded. While SCC*mec* typing can be determined using SeqSphere^{+} and other fully annotated bacterial strains within GenBank, this method requires greater user interaction and may reduce the standardisation of the cgMLST scheme.

While huge advances have been made in WGS much of this is based around research aspects and, from a diagnostic point of view further work is still required. The potential of WGS in outbreak investigation is only of importance if results are achievable in a time that is of relevance that it will influence patient management and of a guaranteed standard. While it has been suggested that a TAT of 48-96hr is achievable, these are based on predesigned studies rather than real time investigations (Price *et al.*, 2013; Kong *et al.*, 2016; Ugolotti *et al.*, 2016). Furthermore, many studies have been based in large sequencing centers with ample resources available. Additional evaluations of TATs are necessary to determine if these stated TATs are truly achievable in smaller resource-limited diagnostic laboratories. Independent quality assurance schemes are necessary to provide confidence in results achieved and issues surrounding data storage and management must be addressed in line with current legislative requirement.

7.5 Concluding comments

The adaptability of MRSA in response to environments in which it finds itself is worrying. Previously clear lines of separation between HCA-, CA- and LA-MRSA are becoming increasingly blurred with the importation of CA-MRSA strains into healthcare facilities and the zoonotic spread of LA-MRSA to humans. The current study is unique in that previously few studies have focused primarily on the detailed characterisation of sporadically-occurring MRSA strains. Limitations of traditional molecular epidemiological typing techniques limited the knowledge in Ireland of these circulating strains however advances in technology have led to the current enhanced study. While many of these strains are associated with CA-MRSA lineages, there is clear evidence of importation of these strains into healthcare facilities among a population of already vulnerable patients. It is evident that ongoing efforts to control MRSA with antibiotics are being hampered by the swift evolutionary responses of the bacteria to ensure survival of the fittest. The diversity seen with regards to resistance and virulence genes poses a significant threat to patient management options should these strains increase in frequency within healthcare facilities.

The ability of WGS to allow recognition of novel resistance mechanisms will undoubtedly assist in the future development of infection control and antibiotic stewardship programs. In addition, the ability of WGS to differentiate ST22-MRSA-IV has the potential to provide huge insight into tracking of this strain throughout hospitals and in particular during outbreaks and in the endemic situation that exists here. However significant validation and standardisation of these methods is essential to ensure that there can be widespread adoption of these techniques in diagnostic microbiology into the future.

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Appendix

Appendix Table 1 Phenotypic and genotypic characterisation of 276 sporadically-occurring MRSA isolates recovered from patients in Irish hospitals and in the community between 2001 and 2015

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
E3183	22/12/2006	39	Limerick	BSI	Ap, Fd	CC1-t127-IV & SCC*fusC*	*qacC, sdrM, fusC*	III	*seh, sek/q*, IEC D
E3202	06/01/2007	78	Offaly	BSI	Ap, Cd, Cp, Er, Fd, Ln, Sp	CC5-ST5-t045-II	*blaZ*, *erm*(A), *fosB*, *sdrM*	II	*tst, sed/j/r, egc*, IEC D
E3204	12/01/2007	52	Dublin	BSI	Ap, Er, Fd, Ln, Sp	CC1-t127-IV & SCC*fusC*	*blaZ, fusC, erm*(A), *sdrM*	III	*egc*, IEC D
E3209	06/01/2007	62	Dublin	BSI	Ak (I), Ap, Cd (I), Cp, Er, Fd, Kn, Ln, Nm, Tb	CC5-ST5-t463-II	*blaZ, erm*(A), *aadD, fosB, sdrM*	II	*tst, sed/j/r, egc*, IEC D
E3258	05/02/2007	82	Dublin	BSI	Ap, Cd(I), Er, Fd, Ln, Sp	CC1-t127-IV & SCC*fusC*	*blaZ, erm*(A), *fusC*, *sdrM*	III	*egc*, IEC D
E3280	18/02/2007	89	Dublin	BSI	Ap, Er, Ln, Sp	CC8-t008-IV	*blaZ, erm*(A), *fosB, sdrM*	II	*hlb*, ACME
E3409	12/05/2007	74	Waterford	BSI	Ap, Cd, Cp, Er, Fd, Ln	CC45-t727-IV	*blaZ, erm*(C), *merA/B, sdrM*	IV	*egc*
E3521	16/08/2007	63	Kildare	BSI	Ap, Er, Kn, Mc, Nm, PMA	CC5-t1781-IV	*blaZ,merA/B, msr*(A), *mph*(C*), aphA3, sat, fosB, qacA, sdrM*	IV	*egc*, IEC D
E3549	10/09/2007	56	Dublin	BSI	Ap, Cd (I), Cp(I), Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*egc*, IEC D
E3643	04/10/2007	29	Cork	BSI	Ap, Fd	CC45-t728- IV & *fus*C	*blaZ, fusC, sdrM*	I	*sec/l, sed/j/r, egc,* IEC B
E3827	11/04/2008	83	Cork	BSI	Ap, Cp, Er, Fd, Ln	CC22-t1084-IV	*blaZ, erm*(C)	I	*sec/l, egc*,

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
E3844	19/04/2008	37	Dublin	BSI	Ap, Cd, Cp, Er, Fd, Ln, Sp, Prl	CC1-t127-IV & SCC*fusC*	*blaZ, erm*(A), *fusC, sdrM*	III	IEC B *egc,* IEC D
E4063	05/03/2008	1	Dublin	BSI	Ap, Er, Ln, Rf, St, Su, Tp	CC8-t064- IV	*blaZ, erm*(C), *fosB, sdrM*	I	*seb, sek/q/r*, IEC E
E4217	26/04/2009	58	Limerick	BSI	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ*, *fusC*, *sdrM*	III	*etD/edinB*, IEC B, *seb*,
E4233	16/05/2009	44	Kildare	BSI	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B, *seb*, *sed/j/r*
E4298	03/08/2009	25	Limerick	BSI	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B
E4378	10/09/2009	73	Cork	BSI	Ap, Cd, Cp, Er, Fd, Ln, Te	CC45-t727- IV	*blaZ, merA/B, erm*(C), *ileS2, tet*(M), *sdrM*	IV	*egc*, IEC B
E4403	12/12/2009	67	Cork	BSI	Ap, Cp, Tp	CC8-ST8-t064-IV	*blaZ, fosB, sdrM*	I	*seb, sek/q/r*, IEC B
E4413	29/12/2009	7	Laois	BSI	Ap, Cd, Cp, Er, Fd, Ln	CC45-t727-IV	*blaZ, merA/B, erm*(C), *sdrM*	IV	*egc*, IEC B
E4433	01/10/2009	83	Cork	BSI	Ap, Fd	CC779-t878-	*blaZ, fusC, sdrM*	III	*etD/edinB*,

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
						ΨSCC*mec* *&fusC- SCC-* SCC_{CRISPR}			IEC B
E4434	28/10/2009	85	Cork	BSI	Ap, Cd, Cp, Er, Fd, Ln	CC22-t005-IV	*blaZ, erm*(C)	I	*sec, egc*, IEC B
E4449	01/02/2010	39	Limerick	BSI	Ap, Cd(I), Fd	CC779-t878-ΨSCC*mec* *&fusC- SCC-* SCC_{CRISPR}	*blaZ, fusC, sdrM*	III	*sed/j/r, etD/edinB*, IEC B
E4458	06/02/2010	50	Waterford	BSI	Ap, Er, Kn, Ln, Nm (I), St	CC1-t386-IV	*blaZ, erm*(A), *aphA3, sat, sdrM*	III	*seh,* IEC E
E4483	08/02/2010	80	Cork	BSI	Ap, Fd, Kn, Ln, Nm, Tb	CC5-ST5-t002-II	*blaZ, erm*(A), *aadD, fosB, sdrM*	II	*tst, sec/l, sed/j/r, egc,* IEC B
E4507	31/03/2010	77	Waterford	BSI	Ap, Cd, Cp, Fd, PMA, Mc	CC45-t727-IV	*blaZ, merA/B, sdrM*	IV	*egc*
E4524	21/04/2010	51	Offaly	BSI	Ap, Er, Fd, Ln	CC1-t16173-IV & SCC*fusC*	*blaZ, erm*(C), *fusC, sdrM*	III	*seh*, *sek/q*, *egc*, IEC D
E4550	08/05/2010	54	Waterford	BSI	Ap, Cd(I), Fd	ST779-t878-ΨSCC*mec* *&fusC- SCC-* SCC_{CRISPR}	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B, *seb*
E4620	01/04/2010	77	Waterford	BSI	Ap, Cd, Cp, Fd, Mc	CC45-t727-IV	*blaZ, merA/B, sdrM*	IV	*egc*
E4644	15/09/2010	4 m	Donegal	BSI	Ap, Cd, Cp, Er, Fd,Mp	CC59-ST59-t316-V	*blaZ, msr*(A), *ileS2, fusB, sdrM*	IV	*seb*, *sek/q*, IEC B
E4674	29/09/2010	75	Galway	BSI	Ap, Cd, Cp, Er, Ln,	CC5-t045-VI	*blaZ, merA/B,*	III	*egc*, IEC

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
					Mc, PMA, Prl		*erm*(C), *fosB, sdrM*		A
E4709	19/12/2010	51	Dublin	BSI	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B, *sed/j/r*
E4725	12/01/2011	1	Limerick	BSI	Ap, Cd(I), Gn, Kn, Tb	CC5-t002-V	*aac-aphD, fosB, sdrM*	II	*egc*, *sed/j/r*, IEC D
E4735	25/01/2011	87	Mullingar	BSI	Ap, Cd, Er, Eb, Ln, Mc, PMA	CC5-t002-IV	*blaZ, erm*(C),*fosB, merA/B, qacA, sdrM*	II	*egc*, IEC A
E4741	06/02/2011	42	Limerick	BSI	Ak (I), Ap, Cp, Er, Kn, Ln, Nm, Sp, Tb	CC5-ST5/ST225-t002-II	*blaZ, erm*(A), *aadD, fosB, sdrM*	II	*egc*, *sed/j/r*, IEC B
E4764	08/03/2011	67	Mayo	BSI	Ap, Cd, Cp (I), Er, Eb, Ln, Mc, PMA	CC5-t045- IV & *ccrB4*	*blaZ, merA/B, erm*(C), *fosB, qacA, sdrM*	II	*egc*, IEC A
E4767	04/03/2011	64	Kerry	BSI	Ak (I), Ap, Cd, Cp, Er, Eb, Fd, Gn, Kn, Ln, Nm, PMA, Sp, Tb, Te	CC8-ST8-t190-IIA/B/D	*blaZ, merA/B, erm*(A), *aacA-aphD, aadD, fusB, tet*(K), *sdrM, fosB*	I	IEC D
E4797	13/04/2011	68	Dublin	BSI	Ap, Er, Kn, Mc, Nm ,PMA	CC5-t002-IV	*blaZ, merA/B, msr*(A), *mph*(C), *aphA3, sat, fosB, sdrM*	II	*egc*, IEC D
E4810	27/04/2011	82	Mullingar	BSI	Ap, Cd, Er, Fd, Ln	CC1-t127-IV & SCC*fusC*	*blaZ, erm*(C), *fusC, sdrM*	III	*seh*, *sek/q*, *egc*, IEC D

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
E4825	02/05/2011	72	Cork	BSI	Ap, Cd(I)	CC1-t2279-IV	*blaZ, sdrM*	III	*seh*, *sek/q*, IEC D
E4841	13/04/2011	68	Dublin	BSI	Ap, Er, Kn, Ln, Mc(I), Nm, PMA	CC5-t002-IV	*blaz, merA/B, msr*(A)*, mphc, aphA3, sat, fosB, sdrM*	II	*egc*, *sed/j/r*, IEC D
E4846	28/06/2011	1	Cork	BSI	Ap, Cd, Cp, Er, Fd, Ln	CC22-t1802-IV	*blaZ, erm*(C)*, fosB*	I	*sed/j/r*, *egc*, IEC B
E4853	08/07/2011	58	Dublin	BSI	Ap, Cd, Cp, Fd	CC45-t727-IV	*blaZ, merA/B, sdrM*	IV	*egc*, IEC B
E4868	07/06/2011	87	Galway	BSI	Ap, Cd, Er, Eb, Gn, Kn, Ln, Mc, PMA, Prl, Tb, Tp,	CC5-t002-IV & *ccrB4*	*blaZ, merA/B, erm*(C)*, aacA-aphD, dfrS1, sdrM, fosB, qacA*	II	*egc*, IEC A
E4870	26/03/2011	74	Galway	BSI	Ap, Er, Cp(I), Cd, Eb, Ln, PMA, Prl	CC5-t002-IV & *ccrB4*	*blaZ, merA/B, erm*(C)*, sdrM, fosB, qacA*	II	*egc*, IEC A
E4871	25/06/2011	74	Galway	BSI	Ap, Cd, Cp (I), Er, Eb, Ln, Mc, PMA	CC5-t002-IV & *ccrB4*	*blaZ, merA/B, erm*(C)*, sdrM, fosB, qacA*	II	*egc*, IEC A
E4882	10/08/2011	59	Waterford	BSI	Ap, Er, Ln, Tp	CC5-t088-V	*erm*(C)*, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC A
E4905	07/09/2011	17	Cork	BSI	Ap, Cd (I), Fd	CC1-t2279-IV & SCC*fus*	*blaZ, sdrM, fusC*	III	*seh*, *sek/q*, *egc*, IEC D
E4916	25/09/2011	46	Donegal	BSI	Ap, Cd, Er, Eb, Mc, PMA, Prl	CC5-t002-IV & *ccrB4*	*blaZ, merA/B, erm*(C)*, sdrM, fosB,*	II	*egc*, IEC A

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
E4917	23/09/2011	85	Limerick	BSI	Ap, Cp, Er, Kn, Nm	CC8-ST8-t008-IV	*qacA blaZ, msr*(A)*, mphc, aphA3, sat, fosB, sdrM*	I	*sek/q*, *sed/j/r*, IEC B
E4963	11/01/2012	83	Dublin	BSI	Ap, Cd, Cp, Er, Fd, Ln	CC45-t727-IV	*blaZ, merA/B, erm*(C)*, fosB, sdrM*	I	*egc*
E4972	04/01/2012	64	Cork	BSI	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B, *sed/j/r*
E4984	08/02/2012	79	Waterford	BSI	Ap, Cd, Cp, Fd	CC45-t727-IV	*blaZ, fusB, sdrM*	IV	*egc*
E5096	28/07/2012	84	Waterford	BSI	Ap, Er, Tp	CC5-t088-V	*erm*(C)*, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC A
E5278	11/06/2013	55	Cork	BSI	Ap, Cd, Cp, Fd	CC45-t727-IV	*blaZ, sdrM*	III	*egc*
E5402	28/12/2013	55	Cork	BSI	Ap, Cp, Fd	CC22-t032-IV	*blaZ*	I	*egc*, IEC B
M06/0390	14/11/2006	34	Dublin	Persistent mastitis post-partum	Ap	CC5-t002-IV	*blaZ, fosB, sdrM*	II	*sec/l, sed/j/r*, *tst*, *egc*, IEC D
M07/0307	12/09/2007	1 d	Dublin	Routine surveillance	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B
M07/0383	05/10/2007	79	Waterford	Epidemiological typing	Ap	CC130-t12399-XI	*blaZ*$_{xi}$*, sdrM*	III	*hlb*

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M07/0393	16/10/2007	71	Waterford	Routine surveillance	Ap, Ak, Cd (I), Cp, Er, Gn, Kn, Ln, Mp, Nm, Prl, Tb	CC5-t067-IV & *ccrB4*	*blaZ, msr*(A)*, mphc, aphA3, sat, fosB, sdrM, aacA-aphD, aadD, ileS2, qacC*	II	*egc*, IEC A
M07/0425	19/02/2004	23	Dublin	Patient fell from horse, dislocated shoulder; no skin lesions; wound infection 2 weeks post-operative. MRSA nasal screen	Ap	CC5-t002-IV	*blaZ, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC A
M08/0218	01/08/2008	7 m	Cork	Baby boy from Vietnam; in ICU; severe CA pneumonia; pleural effusion.	Ap, Er, Ln	CC45-t026-IV	*blaZ, erm*(C)*, sdrM*	I	*sec/l*, *egc*, IEC B
M08/0390	01/08/2008	33	Dublin	Skin and soft tissue infection	Ap, Cd (I), Cp	CC8-ST8-t008-IV	*blaZ, fosB, sdrM*	I	*sek/q*, ser, IEC B, ACME
M08/0422	05/08/2008	24	Waterford	Routine surveillance	Ap, Cd, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B
M08/0515	15/12/2008	46	Waterford	Outbreak investigation	Ap, Fd, Cd (I)	CC8-t008-VI & *fusC*	*blaZ, fusC, fosB, sdrM*	I	IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M09/0091	24/02/2009	9	Waterford	Unknown	Ap, Cd, Fd	CC22-t005-IV	*blaZ*	I	*egc*
M09/0097	27/02/2009	65	Waterford	Routine surveillance	Ap, Cd, Cl, Cp, Er, Fd, Gn, Kn, Ln, Mp, Nm(I), Tb	CC45-t727-IV	*blaZ, merA/B, erm*(C)*, aacA-aphD, ileS2, cat, sdrM*	IV	*egc*, *sec/l*
M09/0169	08/06/2009	9 d	Dublin	Routine surveillance	Ap, Cd, Cl, Er, Kn, Ln, Mc, Nm, PMA, St, Su, Te, Tb	CC8-t037-III & SCC_{Hg}	*blaZ, merA/B, erm*(A)*, aadD, tet*(M)*, sdrM, cat, fosB, qacC*	I	*sek/q*, IEC D
M09/0189	08/05/2009	7 m	Dublin	Ear swab	Ak, Ap, Cp, Er, Gn, Kn, Ln, Nm, Sp, St, Te, Tb, Tp	CC8-ST239-t037-III	*blaZ, erm(A), aacA-aphD, aphA3, sat, tet*(M)*, fosB, sdrM*	I	*sek/q*, IEC D
M09/0190	17/06/2009	12	Dublin	Unknown	Ap, Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(A)*, aphA3, sat, sdrM*	III	*seh*
M09/0201	17/06/2009	65	Dublin	Routine surveillance	Ak (I), Ap, Cp, Er, Gn, Kn, Ln, Mp, Nm, Sp, Tb	CC5-ST5/ST225-t002-II	*blaZ, erm*(A)*, aacA-aphD, aadD, ileS2, fosB, qacC, sdrM*	II	*egc*, IEC A
M09/0295	23/11/2001	49	Sligo	Routine surveillance	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-*SCC_{CRISPR}	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B, *sed/j/r*
M09/0297	10/10/2005	25	Sligo	Routine surveillance	Ap, Kn, Nm, Tb	CC45-t015-IV	*blaZ, aadD*	I	*sec/l*, *egc*, IEC B
M09/0302	08/11/2007	58	Sligo	Routine surveillance	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-*SCC_{CRISPR}	*blaZ, fusC, erm*(C)*, fosB, sdrM*	III	*etD/edinB*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M09/0309	06/10/2008	49	Sligo	Routine surveillance	Ap, Er, Kn, Nm,	CC59-ST87-t216-IV	*blaZ, msr*(A), *mph*(C), *aphA3, sat, sdrM*	I	*seb*, *sek/q*, IEC B
M09/0341	16/12/2009	8 d	Drogheda	Outbreak investigation	Ap, Cd (I), Cp, Gn (I), Kn, Tb, Tp	CC5-t442-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*sed/j/r*, IEC E
M09/0344	07/12/2009	66	Galway	Routine surveillance	Ap, Cd, Cp, Er, Gn, Kn, Ln, Mc, Mp, PMA, Prl, Tb	CC5-t002-IV & *ccrB4*	*blaZ, merA/B, erm*(C), *aacA-aphD, aadD, ileS2, sdrM, fosB, qacA*	II	*egc*, IEC A
M10/0015	17/11/2009	43	Kildare	BSI	Ap, Cd(I)	CC5-t002-IV	*blaZ, sdrM, fosB*	II	*egc*, IEC F
M10/0022	15/01/2010	83	Kerry	Unknown	Ap, Cd, Er, Fd, Ln	CC1-t127-IV & SCC*fus*	*blaZ, erm*(C), *sdrM, fusC*	III	*seh*, *sek/q*, IEC D
M10/0029.1	07/01/2010	14 d	Cavan	Outbreak investigation	Ap, Cp, Er, Kn, Nm	CC8-ST8-t008-IV	*blaZ, msr*(A), *mph*(C), *aphA3, sat, sdrM, fosB*	I	IEC B
M10/0029.2	07/01/2010	14 d	Cavan	Outbreak investigation	Ap, Cp	CC8-t008-IV	*blaZ, sdrM, fosB*	I	IEC B
M10/0070	01/03/2010	72	Cork	Unknown	Ap, Gn, Kn, Tb	CC5-t002-IV	*blaZ, aacA-aphD, sdrM, fosB*	II	IEC E
M10/0217	08/06/2010	42	Dublin	Unknown	Ap, Cd	CC30-t018-IV	*blaZ, sdrM, fosB*	III	*tst*, *egc*, IEC E
M10/0220	25/06/2010	3 m	Donegal	Unknown	Ap, Cd, Fd,	CC5-t088-V	*blaZ, vga, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC A
M10/0244	15/07/2010	76	Dublin	Epidemiological typing	Ap, Gn, Kn, Mp, Tb	CC5-t002-IV	*aacA-aphD, ileS2, sdrM, fosB*	II	*tst*, *egc*, IEC F

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M10/0272	21/08/2010	10 d	Galway	Outbreak investigation	Ap, Er, Kn, Nm, Su, Te, Tb, Tp	CC8-ST8-t451-IV	*blaZ, msr*(A), *mph*(C), *aacA-aphD, aphA3, sat, dfrS1, tet*(M), *sdrM, fosB, qacA*	I	*seb*, *sek/q*, IEC D
M10/0273	21/08/2010	22 d	Galway	Outbreak investigation	Ap, Er, Kn, Nm, Su, Te, Tb, Tp	CC8-ST8-t451-IV	*blaZ, msr*(A), *mph*(C), *aacA-aphD, aphA3, sat, dfrS1, tet*(M), *sdrM, fosB, qacA*	I	*seb*, *sek/q*, IEC D
M10/0299	15/09/2010	15	Longford	Epidemiological typing	Ap, Cd(I), Er, Ln, Tp(I)	CC45-t065-IV	*blaZ, erm*(C), *dfrS1, sdrM*	I	*sec/l*, *egc*, IEC B
M10/0303	15/09/2010	4 m	Donegal	4 Month old baby, isolate from blood. 1 week post-operative correction of hip displacement.	Ap, Cd, Cp, Fd(I), Mp	CC59-ST59-t316-V	*blaZ, msr*(A), *fusB, ileS2, sdrM*	I	*seb*, *sek/q*, IEC B
M10/0343	12/10/2010	67	Sligo	Unknown	Ap, Cd, Fd	CC30-t012-IV & *fusC*	*blaZ, fusC, sdrM, fosB*	III	*tst*, *egc*, IEC B
M10/0352	19/10/2006	27	Dublin	Post-surgical infection	Ap, Cp, Er, Ln	CC22-t032-IV	*blaZ, erm*(C)	I	*egc*, *hlb*
M10/0353	24/04/2007	51	Dublin	Post-surgical infection	Ap, Cp, Er, Ln	CC22-t032-IV	*blaZ, erm*(C)	I	*egc*, *hlb*
M10/0372	17/11/2010	17	Dublin	Cesarean section in a maternity	Ap, Cd(I), Gn, Kn, Mc, PMA, Te, Tp, Tb	CC8-ST8-t1476-V	*blaZ, merA/B, aacA-aphD, sdrM, fosB,*	I	*sed/j/r*, *sek/q*, IEC

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
				hospital			*qacC*		B
M10/0375	31/08/2010	3 m	Dublin	Cesarean section in a maternity hospital	Ap, Cd(I), Cp, Er, Ln	CC22-NT-IV	*blaZ, erm(C)*	I	*egc*, IEC B
M11/0021	06/01/2011	85	Dublin	Unknown	Ap, Er, Ln, Sp, Tp	CC45-t065-IV	*blaZ, erm*(A)*, sdrM*	I	*egc*, IEC B
M11/0099	20/04/2011	29	Dublin	Routine surveillance	Ak(I), Ap, Cd, Cl, Er, Kn, Ln, Mc(I), Nm, PMA, St, Su, Te, Tb	CC8-ST239-t037-III & SCC_{Hg}	*blaZ, merA/B, erm*(A)*, aadD, tet*(K)*, tet*(M)*, sdrM, cat, fosB, qacC*	I	*sek/q*, IEC D
M11/0100	20/04/2011	52	Dublin	Routine surveillance	Ap, Cd, Cl, Er, Kn, Ln, Mc(I), Nm, PMA, Sm, St, Te, Tb	CC8-ST239-t037-III & SCC_{Hg}	*blaZ, merA/B, erm*(A)*, aadD, tet*(K)*, tet*(M)*, sdrM, cat, fosB, qacC*	I	*sek/q*, IEC D
M11/0111	29/04/2011	1 m	Dublin	Routine surveillance	Ap, Cd(I)	CC6-t701-IV	*blaZ, sdrM, fosB*	I	IEC E
M11/0114	11/05/2011	5 d	Dublin	Routine surveillance	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-*SCC_{CRISPR}	*blaZ, fusC, sdrM*	III	IEC B, etD, edinB
M11/0118	17/05/2011	30	Dublin	Routine surveillance	Ap, Fd	ST779-t878-ΨSCC*mec &fusC- SCC-*SCC_{CRISPR}	*blaZ, fusC, sdrM, fosB*	III	*etD/edinB*, IEC B, *seb*
M11/0127	23/05/2011	72	Kerry	Unknown	Ap, Cd (I), Fd	CC45-t4545-IV & *fusC*	*blaZ, fusC, sdrM*	I	*egc*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M11/0139	30/05/2011	69	Dublin	Routine surveillance; patient negative at admission	Ap	CC8-ST72-t126-IV	*blaZ, sdrM, fosB*	I	*egc*, IEC B
M11/0140	25/05/2011	66	Kerry	Unknown	Ap, Fd	CC5-t002-IV	*blaZ, sdrM, fosB*	II	*egc*, IEC F
M11/0141	26/05/2011	75	Kerry	Unknown	Ap, Fd, Kn, Nm, Tb	CC5-t002-I & *fusC*	*blaZ, aadD, fusC, fosB, qacC, sdrM*	II	*tst*, *sec/l*, *sed/j/r*, *egc*, IEC B
M11/0146	30/05/2011	63	Kerry	Unknown	Ap, Cd, Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*seh*, *sek/q*, IEC D
M11/0165	15/06/2011	16	Kerry	Unknown	Ap, Cd (I), Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*seh*, *sek/q*, IEC D
M11/0172	17/06/2011	62	Dublin	Patient has animal contacts	Ap, Er, Kn, Ln, Rf(I), Su, Te, Tb, Tp	CC8-t064-IV	*blaZ, erm*(C)*, aacA-aphD, dfrS1, tet*(M)*, sdrM, fosB*	I	*seb*, *sek/q*, *hlb*
M11/0183	11/07/2011	30	Dublin	Routine surveillance	Ap, Cp, Gn, Kn, Rf(I), Su, Tb, Tp	CC8-t11173-V	*blaZ, aacA-aphD, sdrM, fosB*	I	*seb*, *sek/q*, IEC D
M11/0197	15/07/2011	39	Sligo	Otitis externa	Ap, Cd, Fd, Prl (I)	CC30-t012 - IV & *fusC*	*blaZ, fosB, sdrM, fusC*	III	*tst*, *egc*, IEC B
M11/0202	25/07/2011	33	Dublin	Unknown	Ap, Cd, Fd	CC30-t382-IV & ccrC & *fus*	*blaZ, fusC, sdrM, fosB*	III	*tst*, *sec/l*, IEC B
M11/0203	06/07/2011	2 m	Dublin	Unknown	Ap, Cd, Fd	CC30-t021- IV & *fusC*	*blaZ, fusC sdrM, fosB,*	III	*tst*, *egc*, IEC B
M11/0204	25/07/2011	1 m	Dublin	Unknown	Ap, Fd	CC30-t021-IV & *fusC*	*blaZ, fusC, sdrM, fosB*	III	*tst*, *egc*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M11/0205	29/07/2011	35	Dublin	Routine surveillance	Ap, Cd, Fd	CC5-t067-IV	*blaZ, fosB, sdrM*	II	*tst*, IEC A
M11/0208	12/08/2011	17	Dublin	Unknown	Ap, Cd(I), Fd	ST779-t878-ΨSCC*mec &fusC- SCC-SCC*$_{CRISPR}$	*blaZ, fusC, sdrM*	III	*sed/j/r*, *etD/edinB*, IEC B
M11/0222	16/08/2011	1 m	Galway	Outbreak investigation	Ap, Cd(I), Er(I), Ln, Sp	CC5-t8892-I	*blaZ, erm*(C)*, sdrM, fosB*	II	*sed/j/r*, *egc*, IEC B
M11/0223	16/08/2011	34	Sligo	Unknown	Ap, Cd(I), Er, Ln	CC5-t8892-I	*blaZ, erm*(C)*, sdrM, fosB*	II	*sed/j/r*, *egc*, IEC A
M11/0224	15/08/2011	2 d	Sligo	Unknown	Ap, Cd(I), Er, Ln	CC5-ST5-t010-I	*blaZ, erm*(C)*, sdrM, fosB*	II	*sed/j/r*, *egc*, IEC A
M11/0229	23/08/2011	48	Galway	Patient from America with recurrent skin infection	Ap, Cp, Er, Ln, Sp	CC5-ST5/ST225-t002-II	*blaZ, sdrM, erm*(A)*, fosB*	II	*egc*, *sed/j/r*, IEC B
M11/0240	30/08/2011	56	Dublin	Epidemiological typing	Ap, Er, Ln, Sp, St, Su	CC8-ST250-t008-I	*erm*(A)*, sdrM, fosB*	I	*seb*, *sek/q*, IEC A
M11/0255	21/09/2011	67	Kerry	Unknown	Ap, Cd (I), Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*seh*, *sek/q*, IEC D
M11/0256	28/09/2011	64	Kerry	Unknown	Ap	CC6-t11019-IV	*blaZ, fosB*	I	IEC D
M11/0257	30/09/2011	33	Drogheda	Unknown	Ak, Ap, Cd, Cp, Er, Eb, Gn, Kn, Ln, Mc(I), Nm, PMA, Sp, St, Tb,	CC8-ST8-t190-IIA/B/D	*blaZ, merA/B, erm*(A)*, aacA-aphD, aadD, aphA3, sat,*	I	IEC D

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
					Tp		*sdrM, fosB, qacA*		
M11/0259	01/10/2011	72	Waterford	Unknown	Ak, Ap, Gn(I), Te, Tb	CC5-t1594-IV	*blaZ, aacA-aphD, tet*(M)*, sdrM, fosB*	II	*egc*, IEC F
M11/0260	06/01/2011	4 m	Dublin	Routine surveillance	Ap, Cp, Er, Fd, Ln	CC5-t311-V & *fusC*	*blaZ, fusC, erm*(C)*, sdrM, fosB*	II	IEC E, *seb*
M11/0263	10/10/2011	40	Dublin	Routine surveillance	Ap, Cd, Er, Kn, Ln ,Mc, Nm(I), PMA, St, Su, Te, Tb	CC8-ST239-t037-III & SCC_{Hg}	*blaZ, merA/B, erm*(A)*, aadD, tet*(K)*, tet*(M)*, sdrM, cat, fosB, qacC*	I	*sek/q*, IEC D
M11/0272	30/10/2011	38	Dublin	Epidemiological typing	Ap, Er, Gn, Kn, Ln, Rf, Te, Tb, Tp	CC8-t064-IV	*blaZ, erm*(C)*, aacA-aphD, dfrS1, tet*(M)*, sdrM, fosB*	I	*seb*, *sek/q*
M11/0280	01/04/2011	NK	Sligo	Nursing home resident nasal swab	Ap, Gn, Kn, Tb, Te, Tp	CC398-t011-IV	*blaZ, aacA-aphD, tet*(M)*, sdrM*	I	*hlb*
M11/0285	07/11/2011	77	Dublin	Epidemiological typing	Ap, Cd, Cp, Er, Gn, Kn, Mp, Tb	CC22-t022-IV	*blaZ, erm(C), aacA-aphD, ileS2*	I	*egc*, *sec/l*, IEC B
M11/0303	04/11/2011	14 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC G
M11/0304	28/11/2011	10 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC G
M11/0428	13/12/2011	1 m	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M11/0429	12/12/2011	1 m	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0011	03/01/2012	15	Dublin	15 Year old boy with pilonidal abscess. No healthcare contact or recent travel. Irish.	Ak(I), Ap, Cd, Er, Gn, Kn, Ln, Nm, Sp, Tb	CC30-ST36/39-t007-II	*blaZ, erm*(A)*, aac-aphd, aadD, sdrM, fosB, qacA*	III	*tst, sec/l, egc*, IEC E
M12/0013	18/12/2011	16 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0034	11/01/2012	24	Dublin	Routine surveillance	Ap, Cd (I)	CC80-t8154-IV	*blaZ, sdrM*	III	*seb*, *sek/q*, IEC B, etD, edinB
M12/0042	17/01/2012	48	Kerry	Unknown	Ap, Cd (I)	CC6-t304-IV	*blaZ, fosB, sdrM*	I	IEC E
M12/0051	27/01/2012	70	Kerry	Unknown	Ap, Cd (I), Cp, Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*seh*
M12/0094	09/03/2012	52	Kerry	Unknown	Ap, Cd (I), Fd	CC1-t2279-IV & SCC*fus*	*blaZ, sdrM, fusC*	III	sea, *seh*, *sek/q*, IEC D
M12/0096	15/03/2012	7 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC D
M12/0097	20/03/2012	14 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, fosB, sdrM*	II	*sed/j/r*, *egc*, IEC D

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M12/0155	08/05/2012	54	Dublin	Epidemiological typing	Ap, Cd(I), Cp, Er(I), Gn, Kn, Nm, Tb, Tp	CC1-ST772-t657-V	*blaZ, msr*(A), *mph(C), aacA-aphD, aphA3, sat, fosB, sdrM*	II	*egc*, sec, IEC H
M12/0167	28/05/2012	74	Sligo	Nasal swab; patient had no previous HCA risk factors	Ap, Fd	CC779-t11021Ψ SCCmec & *fusC*-SCC-SCC_{CRISPR}	*blaZ, fusC, sdrM, qacA*	III	*etD/edinB*, IEC B
M12/0170	31/05/2012	85	Dublin	Epidemiological typing	Ap, Cd, Cp, Fd	CC45-t727-IV	*blaZ, fusB, sdrM*	IV	*egc*
M12/0215	16/07/2012	12 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0271	03/08/2012	63	Kerry	Epidemiological typing	Ap	CC1-t2279-IV	*blaZ, sdrM*	III	*seh*, *sek/q*, IEC D
M12/0336	26/09/2012	7 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0337	24/09/2012	28 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0350	08/10/2012	5 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0360	14/10/2012	10 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*,

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
									IEC D
M12/0376	15/10/2012	11 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0377	18/10/2012	2 d	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0383	19/10/2012	Not known	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0384	19/10/2012	Not known	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0388	26/10/2012	37	Waterford	Outbreak investigation	Ak, Ap, Gn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0422	26/11/2012	3 m	Waterford	Outbreak investigation	Ap, Gn, Kn, Tb	CC5-t002-V	*blaZ, aacA-aphD, sdrM, fosB*	II	*egc*, *sed/j/r*, IEC D
M12/0443	01/09/2012	NK	Veterinary college	Belgian veterinarian	Ap, Er, Gn, Kn, Ln,Tb, Te, Tp	CC398-t011-IV	*blaZ, erm*(C), *aacA-aphD, tet*(M)	I	IEC B
M13/0106	07/02/2013	27	Kerry	Unknown	Ap, Cd, Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, sdrM*	III	*seh*, IEC E
M13/0113	05/02/2013	4	Limerick	Unknown	Ap	CC97-t12025-V	*blaZ, sdrM*	I	*hlb*
M13/0114	12/02/2013	89	Kerry	Unknown	Ap, Cd(I), Fd	CC45-t4545-IV & f*usC*	*blaZ, fusC, sdrM*	I	*egc*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M13/0127	27/02/2013	38	Kerry	Unknown	Ap, Cd(I), Fd	CC45-t4545 IV & f*usC*	*blaZ, fusC, sdrM,*	I	*egc*, IEC B
M13/0130	06/03/2013	81	Dublin	Epidemiological typing	Ap, Cd(I), Fd, Sp(I)	CC45-t2277 -IV & *fus*C	*blaZ, fusC, sdrM*	I	*sec/l*, *sed/j/r*, *egc*, IEC B
M13/0142	11/03/2013	19 d	Dublin	Unknown	Ap, Cd, Er, Kn, Mc, Nm, PMA, Su, Tp	CC5-t12117-IV	*blaZ, merA/B, msr*(A) *mph*(C), *aadD, aphA3, sat, dfrS1, ileS2, tet*(K), *fosB, sdrM*	III	*egc*, IEC A, *etA*
M13/0161	25/03/2013	30	Dublin	Australian	Ap	CC1-t127-IV	*blaZ, sdrM*	III	*seh*, *sek/q*, IEC D
M13/0166	21/03/2013	18 d	Waterford	Outbreak investigation	Ap, Cd(I), Tp	CC5-ST835-t1567-V & *ccrB4*	*fosB, sdrM*	II	sed/r/j, *egc*, IEC D
M13/0167	26/03/2013	2 m	Waterford	Outbreak investigation	Ap, Tp	CC5-ST835-t1567-V & *ccrB4*	*fosB, sdrM,*	II	sed/r/j, *egc*, IEC D
M13/0172	03/04/2013	81	Dublin	Epidemiological typing	Ap, Cp, Er, Fd, Ln, Rf	CC22-t032-IV	*blaZ, erm*(C)	I	sec, *egc*
M13/0404	13/06/2013	1	Dublin	Unknown	Ap, Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E
M13/0416	11/06/2013	42	Sligo	Wound swab post keyhole cholecystectomy No travel history.	Ap	CC45-t671-IV	*blaZ, sdrM,*	I	*egc*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M13/0417	12/06/2013	87	Sligo	Resident in long term care facility, wound swab from skin wound	Ap	CC5-t1340-IV	*fosB, qacC, sdrM,*	II	*sed/j/r*, *egc*, IEC B
M13/0423	04/07/2013	41	Kerry	Infected haematoma post hernia inguinal repair	Ap	CC1-t2279-IV	*blaZ, sdrM*	III	*seh*, *sek/q*, IEC D
M13/0466	23/07/2013	87	Donegal	Unknown	Ap, Er, Ln, Sp,	CC45-t563-V	*blaZ, erm*(A)*, qacC, sdrM*	I	*egc*, ACME
M13/0473	09/08/2013	40	Dublin	Unknown	Ap, Cd(I)	CC1-t127-IV	*blaZ, sdrM*	III	*seh*, *sek/q*, IEC D
M13/0476	08/08/2013	33	Dublin	Routine surveillance	Ap, Rf, Tp	CC88-t10785-IV	*blaZ, dfrS1, sdrM*	III	IEC B
M13/0478	15/08/2013	87	Galway	Epidemiological typing	Ap, Cp, Er, Fd, Mp	CC59-ST59-t10127-V	*blaZ, msr*(A)*, fusB, ileS2, qacC, sdrM*	I	*seb*, *sek/q*, IEC B
M13/0482	12/08/2013	4 d	Galway	Unknown	Ap, Fd	CC779-t878-ΨSCC*mec &fusC- SCC-SCC$_{CRISPR}$*	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B
M13/0483	09/08/2013	1 d	Galway	Unknown	Ap, Fd	CC779-t878-ΨSCC*mec &fusC- SCC-SCC$_{CRISPR}$*	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B
M13/0484	13/08/2013	1 d	Galway	Unknown	Ap, Fd	CC779-t878-ΨSCC*mec*	*blaZ, fusC, sdrM*	III	*etD/edinB*, IEC B

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
						&fusC- SCC-SCC_{CRISPR}			
M13/0493	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0494	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0495	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0496	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0497	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0498	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0499	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Er, Ln, Sp, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, sdrM*	I	*hlb*
M13/0500	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Cl, Te	CC398-t011-V	*blaZ, erm*(A)*, tet*(K)*, tet*(M)*, fexA, sdrM*	I	*hlb*
M13/0501	02/06/2013	NK	Veterinary College	Pig farmer	Ap, Cl, Er, Kn, Ln, Nm, Te, Tb, Tp	CC398-t011-V	*blaZ, erm*(B)*, aadD, tet*(K)*, tet*(M)*, fexA, sdrM*	I	*hlb*
M13/0509	01/05/2013	20 d	Dublin	Epidemiological typing	Ap, Cd(I), Er, Ln, Sp	CC88-t2622-IV	*blaZ, erm*(A)*, sdrM,*	III	*sec/l*, IEC C
M13/0543	16/09/2013	31	Dublin	Routine surveillance	Ap	CC5-t1567-IV	*blaZ*	II	*sed/j/r*, *seb*

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M13/0560	01/10/2013	71	Dublin	Epidemiological typing	Ap, Fd	CC45-t2277-IV & *fus*C	*blaZ, fusC, sdrM*	I	*sec/l*, *sed/j/r*, *egc*, IEC B
M13/0625	07/10/2013	82	Dublin	Epidemiological typing	Ap, Gn, Kn, Tb, Tp	CC1-ST772-t1839-V	*blaZ, aacA-aphD, fosB, sdrM*	III	*egc*, sec, IEC H
M13/0653	05/11/2013	75	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te, Tb	CC1-t127-IV	*blaZ, erm*(C), *lnu*(A), *vga, aadD, aphA3, sat, ileS2, tet*(K), *sdrM*	III	*seh*, IEC B
M13/0671	15/11/2013	77	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M13/0699	01/12/2013	10	Dublin	Child	ap, er, ln, ep, te, tp	CC398- t034- V	*blaz, erm*(A), *tet*(K), *tet*(M)	I	*hlb*
M14/0046	12/01/2014	93	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M14/0094	04/02/2014	48	Dublin	Unknown	Ap, Fd	CC5-t105-IV & *fusC*	*blaZ, fusC, fosB, sdrM*	II	*tst*, *sec/l*, *egc*, IEC D
M14/0103	16/02/2014	80	Dublin	Unknown	Ap, Er, Kn, Ln, Mp, Nm, Sp(I), St, Te	CC1-t127-IV	*blaZ, erm*(C), *vgaa, aphA3, sat, ileS2, tet*(K), *fosB, qacA, sdrM*	III	*seh*, IEC E
M14/0110	25/02/2014	18	Dublin	Epidemiological typing	Ap, Cd, Te, Tp	CC22-t5485-IV	*blaZ, dfrS1, tet*(K)	I	*tst*, *egc*, IEC D

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M14/0111	24/02/2014	4	Dublin	Epidemiological typing	Ap, Cd(I)	CC30-t5730-IV	*blaZ, fosB, sdrM*	III	*egc*, IEC B
M14/0125	26/02/2014	76	Dublin	Epidemiological typing	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0129	03/03/2014	29	Limerick	Epidemiological typing	Ap, Cp	CC22-t3387-IV	*blaZ,*	I	*egc*, IEC B
M14/0133	08/03/2014	10 d	Galway	Outbreak investigation	Ap, Er, Ln, Sp	CC88-t786-IV	*blaZ, erm*(A)*, sdrM*	III	*sec/l*, IEC E
M14/0134	11/03/2014	8 d	Galway	Outbreak investigation	Ap, Er, Ln, Sp	CC88-t786-IV	*blaZ, erm*(A)*, sdrM*	III	*sec/l*, IEC E
M14/0152	18/03/2014	78	Dublin	Leg ulcer	Ap, Cd	CC22-t4623-IV	*blaZ,*	I	*egc*, IEC B
M14/0153	15/03/2014	2 d	Galway	Outbreak investigation	Ap, Er, Ln,	CC22-t100-IV	*blaZ, erm*(C)	I	*sec/l*, *egc*, IEC B
M14/0182	25/03/2014	82	Dublin	Outbreak investigation	Ap, Cp, Er, Ln	CC22-t1653-IV	*blaZ, erm*(C)	I	*egc*, IEC B
M14/0186	07/04/2014	67	Dublin	Epidemiological typing	Ap, Er, Ln	CC22-t513-IV	*blaZ, erm*(C)	I	*egc*, *hlb*
M14/0242	06/05/2014	81	Dublin	Epidemiological typing	Ap, Cp, Er, Ln(I)	CC22-t032-IV	*blaZ, erm*(C)	I	*sec/l*, *egc*, *hlb*
M14/0273	13/05/2014	24	Galway	Epidemiological typing	Ap, Er, Fd	CC45-t4545-IV & *fusC*	*blaZ, erm*(C)*, fusC, sdrM*	I	*egc*, IEC B
M14/0274	13/05/2014	39	Galway	Epidemiological typing	Ap	CC45-t2642-IV	*blaZ, sdrM*	I	*egc*, IEC B
M14/0279	19/05/2014	75	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*,*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
							fosB, qacA, sdrM		
M14/0355	30/05/2014	70	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, vga, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0361	06/06/2014	83	Sligo	Unknown	Ap, Er, Ln	CC5-t1340-II trunc	*erm*(C)*, fosB, qacC, sdrM*	II	*sed/j/r*, *egc*, IEC B
M14/0373	11/05/2014	66	Dublin	Outbreak investigation	Ap, Er ,Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM,*	III	*seh*, IEC E
M14/0380	21/06/2014	33	Limerick	Unknown	Ap, Fd	CC1-t2279-IV & SCC*fus*	*blaZ, fusC, fosB, sdrM*	III	*seh*, *sek/q*, IEC D
M14/0422	25/06/2014	34	Dublin	Epidemiological typing	Ap, Er, Fd, Gn, Kn, Ln, Rf, Sp, St, Su, Tb	CC8-ST250-t1883-I	*erm*(A)*, aacA-aphD, fosB, sdrM*	I	IEC H
M14/0425	24/06/2014	77	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaz, erm*(C)*, vga, aphA3, sat, ileS2, tet*(K)*, fosB, qacA, sdrM*	III	*seh*, IEC E
M14/0465	01/08/2014	64	Dublin	Epidemiological typing	Ap, Cd	CC22-t891-IV	*blaZ*	I	*egc*, IEC B
M14/0466	02/07/2014	86	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0480	01/07/2014	71	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM,*	III	*seh*, IEC E
M14/0481	01/07/2014	72	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, vga, aphA3, sat, ileS2,*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M14/0586	08/07/2014	72	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*tet*(K), *fosB, qacA, sdrM, blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M14/0594	08/07/2014	80	Dublin	Outbreak investigation	Ap ,Cp, Er, Gn, Kn, Ln, Mp, Tb	CC22-t032-IV	*blaZ, erm*(C), *aacA-aphD, ileS2*	IV	*egc*, IEC B, *sec/l*
M14/0595	08/07/2014	59	Dublin	Outbreak investigation	Ap, Cd, Cp, Er, Fd, Ln, Sp	CC22-t032-IV	*blaZ, erm*(A)	I	*sec/l*, *egc*, IEC B
M14/0602	16/07/2014	80	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, PMA(I), St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M14/0603	16/07/2014	72	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA*	III	*seh*, IEC E
M14/0606	22/07/2014	49	Dublin	Unknown	Ap, Cd, Cp, Er, Ln	CC22-t515-IV	*blaZ, erm*(A)	I	*egc*, IEC B
M14/0656	28/07/2014	82	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM,*	III	*seh*, IEC E
M14/0660	28/07/2014	59	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M14/0664	05/08/2014	58	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M14/0681	14/08/2014	52	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, qacA,*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
							sdrM,		
M14/0695	21/08/2014	82	Dublin	Outbreak investigation	Ap, Er, Kn, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M14/0712	29/08/2014	90	Dublin	Outbreak investigation	Ap, Cp, Er, Gn, Kn, Mp, Tb	CC22-t3444-IV	*blaZ, erm*(A)	I	*sec/l*, *egc*, IEC B
M14/0713	01/11/2014	Not known	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0767	28/08/2014	34	Limerick	NK	Ap, Cd, Fd	CC45-t383-IV & *fusC*	*blaZ, fusC, sdrM*	I	sec, *sed/j/r*, *egc*, IEC B
M14/0834	01/10/2014	47	Dublin	Epidemiological typing	Ap, Cd, Cp	CC22-t4559-IV	*blaZ, vga, aphA3*	I	*egc*, IEC B
M14/0839	08/10/2014	10 d	Dublin	Routine surveillance	Ap, Te, Tp	CC22-t223-IV	*blaZ, dfrS1, tet*(K)	I	*tst*1, *egc*, IEC B
M14/0845	23/09/2014	26	Waterford	Epidemiological typing	Ap, Cd(I), Er, Kn, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, tet*(K)*, sdrM*	III	*seh*, IEC E
M14/0859	14/10/2014	91	Dublin	Epidemiological typing	Ap, Cp, Eb, Gn, Kn, Tb	CC22-t5121-IV	*blaZ, aacA-aphD, ileS2, qacA*	I	*sec/l*, *egc*, IEC B
M14/0861	13/10/2014	33	Donegal	NK	Ap, Cd(I), Fd	CC8-t14362-IV & V & *fusC*	*blaZ, fusC, fosB, sdrM,*	I	sed
M14/0865	17/10/2014	1 d	Waterford	Epidemiological typing.	Ap, Cd(I), Er, Kn, Nm ,Te	CC1-t2246-IV	*blaZ, erm*(C)*, aphA3, sat, tet*(K)*, sdrM*	III	*seh*, IEC E
M14/0868	16/10/2014	60	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Ln, Mp, Nm St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M14/0873	28/10/2014	31	Dublin	Routine surveillance	Ap, Cd	CC45-t728-IV	*blaZ, sdrM*	I	*tst1, sec/l, egc*, IEC B
M14/0876	22/10/2014	73	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M14/0877	27/10/2014	32	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Ln, Mp ,Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0878	23/10/2014	70	Dublin	Outbreak investigation	Ap, Cd(I), Er, Eb, Kn, Ln, Mp, Nm ,St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0881	06/10/2014	30	Cavan	Epidemiological typing	Ap, Cp	CC22-t2231-IV	*blaZ,*	I	*sec/l, egc*
M14/0885	30/10/2014	81	Dublin	Deep wound, swab	Ap, Cd, Cp, Te	CC88-t5562-V	*blaZ, sdrM*	III	*sec/l,* IEC E
M14/0892	22/10/2014	48	Dublin	Outbreak investigation	Ap, Cd(I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M14/0943	12/11/2014	17	Donegal	Vaginal swab	Ap, Cp, Tp	CC22-t14499-IV	*blaZ, dfrS1*	I	*sec/l, egc*, IEC B, ACME
M14/0958	22/11/2014	34	Cavan	Epidemiological typing	Ap, Cd, Cp ,Eb	CC22-t14500-IV	*blaZ,*	I	*egc*, IEC B
M14/0965	25/11/2014	62	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M14/0966	17/11/2014	75	Dublin	Outbreak	Ap, Er, Kn, Ln, Mp,	CC1-t127-IV	*blaZ, erm*(C)*, aphA3,*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
				investigation	Nm, St, Te		*sat, ileS2, tet*(K), *qacA, sdrM*		
M14/0967	28/10/2014	79	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M14/0968	25/11/2014	67	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M14/0992	19/12/2014	Not known	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E
M14/0993	02/12/2014	60	Dublin	Outbreak investigation	Ap, Er, Eb, Fd, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, fusB, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M15/0029	01/03/2013	33	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Tb	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E
M15/0030	25/02/2014	47	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Tb	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E
M15/0031	01/05/2014	81	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Tb	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E
M15/0068	20/01/2015	81	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M15/0138	11/02/2015	72	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, ileS2, tet*(K), *qacA, sdrM*	III	*seh*, IEC E
M15/0148	04/01/2015	49	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	III	*seh*, IEC E

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Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M15/0149	25/01/2015	51	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, tet*(K)*, sdrM*	III	*seh*, IEC E
M15/0154	16/02/2015	59	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t922-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM,*	III	*seh*, IEC E
M15/0206	10/03/2015	95	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M15/0221	17/03/2015	47	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M15/0222	24/03/2015	81	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M15/0223	24/03/2015	78	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M15/0245	01/04/2015	76	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, sdrM*	III	*seh*
M15/0283	26/04/2015	11 d	Waterford	Outbreak investigation	Ap, Cd, Er, Ln, Te	CC22-t790-IV	*blaZ, erm*(C)*, tet*(K)	I	*egc*, IEC B
M15/0337	27/05/2015	77	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M15/0429	06/07/2015	75	Dublin	Outbreak investigation	Ap, Cd (I), Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E

Continued overleaf

Appendix Table 1 continued

Isolate no.	Date collected	Age (y)[a]	County	Clinical information	Resistance profile[b]	CC-*spa*-SCC*mec*[c]	Resistance genes[d]	agr type[d]	Virulence genes[d,e]
M15/0540	31/07/2015	75	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA,*	III	*seh*, IEC E
M15/0541	23/08/2015	75	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, qacA, sdrM*	III	*seh*, IEC E
M15/0614	10/09/2015	89	Dublin	Outbreak investigation	Ap, Er, Eb, Fd, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, fusB, ileS2, qacC, sdrM*	III	*seh*, IEC E
M15/0637	10/09/2015	49	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	III	*seh*, IEC E
M15/0724	04/12/2015	88	Dublin	Outbreak investigation	Ap, Er, Kn, Ln, Mp, Nm, St, Te	CC1-t127-IV	*blaZ, erm*(C)*, aphA3, sat, ileS2, tet*(K)*, qacA, sdrM*	I	*seh*, IEC E

[a] y; years. The age of each patient in years with the exception of where the patient was less than one year of age and age is stated in months (m) or days (d).

[b] Resistance patterns determined for 276 MRSA isolates by antibiogram- resistogram typing where the number of isolates (n) exhibiting each profile are shown in parentheses. Abbreviations Ak, amikacin; Ap, ampicillin; Cd, cadmium acetate; Cl, chloramphenicol; Cp, ciprofloxacin;

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Appendix Table 1 continued

Er, erythromycin; Eb, ethidium bromide; Fd, fusidic acid; Gn, gentamicin; Kn, kanamycin; Ln, lincomycin; Mc, mercuric chloride; Mp, mupirocin; Nm, neomycin; Pma, phenyl mercuric acetate; Rf, rifampicin; Sp, spectinomycin; St, streptomycin; Su, sulphonamide; Te, tetracycline; Tb, tobramycin; Tp, trimethoprim. (I) indicates that the isolate exhibits intermediate resistance to the preceding antimicrobial agent.

[c]Multilocus sequence type (ST) and clonal complex (CC) and staphylococcal cassette chromosome (SCC) *mec* type as determined using the StaphyType DNA Microarray kit (Alere, Germany) and the *spa* type recognised for each isolate.

[d]Resistance and virulence genes detected using the *S. aureus* Genotyping Kit 2.0

[e]The immune evasion complex (IEC) type of each isolate was determined based on the combination of the IEC genes detected as described by van Wamel *et al.,* 2006. IEC type A: *sea, sak, chp, scn*; IEC type B: *sak, chp, scn;* IEC type C: *chp, scn*; IEC type D: *sea, sak, scn*; IEC type E: *sak, scn*; IEC type F: *sep, sak, chp, scn*; IEC type G: *sep, sak, scn*, IEC type H: *scn.* The enterotoxin gene cluster (*egc*) includes *seg, sei, sem, sen, seo* and *seu*.

Abbreviations: BSI; blood stream infection, ICU; intensive care unit, CA; community associated, HCA; healthcare associated.

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RESEARCH ARTICLE

The Emergence and Spread of Multiple Livestock-Associated Clonal Complex 398 Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Strains among Animals and Humans in the Republic of Ireland, 2010–2014

Gráinne I. Brennan[1,2], Yvonne Abbott[3], Aisling Burns[3], Finola Leonard[3], Brenda A. McManus[1], Brian O'Connell[1,4], David C. Coleman[2], Anna C. Shore[2,4]*

1 National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland, **2** Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin 2, Ireland, **3** Pathobiology, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, **4** Department of Clinical Microbiology, University of Dublin, Trinity College, St. James's Hospital, James's St., Dublin 8, Ireland

* anna.shore@dental.tcd.ie

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Abstract

Clonal complex (CC) 398 methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) are associated with carriage and infection among animals and humans but only a single case of CC398 MRSA has been reported in the Republic of Ireland (ROI). The present study investigated the molecular epidemiology of CC398 MRSA ($n = 22$) and MSSA ($n = 10$) from animals and humans in the ROI from 2010–2014. Isolates underwent antimicrobial susceptibility testing, *spa* typing, DNA microarray profiling and PCR for CC398-associated resistance genes. All MRSA underwent SCC*mec* IV or V subtyping. Four distinct CC398-MRSA incidents were identified from (i) a man in a nursing home (*spa* type t011-SCC*mec* IVa, immune evasion complex (IEC) negative), (ii) a horse and veterinarian who had recently travelled to Belgium (t011-IVa, IEC positive), (iii) pigs ($n = 9$) and farm workers ($n = 9$) on two farms, one which had been restocked with German gilts and the other which was a finisher farm (t034-V_T, IEC negative, 3/9 pigs; t011- V_T, IEC negative, 6/9 pigs & 9/9 farm workers), and (iv) a child who had worked on a pig farm in the UK (t034-V_T, IEC negative). Isolates also carried different combinations of multiple resistance genes including *erm*(A), *erm*(B), *tet*(K), *tet*(M) & *tet*(L), *fexA*, *spc*, *dfrG*, *dfrK aacA-aphD* and *aadD* further highlighting the presence of multiple CC398-MRSA strains. CC398 MSSA were recovered from pigs ($n = 8$) and humans ($n = 2$). CC398 MSSA transmission was identified among pigs but zoonotic transmission was not detected with animal and human isolates exhibiting clade-specific traits. This study highlights the importation and zoonotic spread of CC398 MRSA in the ROI and the spread of CC398 MSSA among pigs.

Competing Interests: The authors have declared that no competing interests exist.

Increased surveillance is warranted to prevent further CC398 MRSA importation and spread in a country that was considered CC398 MRSA free.

Introduction

Clonal complex 398 (CC398) livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) was first reported in 2005 from pigs, pig handlers and their close contacts in the Netherlands [1]. Subsequently it was identified from a range of livestock and livestock-derived food products and horses as well as in humans, predominantly those with contact with livestock, in several countries, particularly in regions with high-density pig farming in continental Europe, Canada, Asia and the USA [2]. While CC398 MRSA is predominantly associated with animal colonisation, serious human infections as well as spread to and within the healthcare system have been reported [2]. Methicillin-susceptible *S. aureus* (MSSA) belonging to CC398 have also been reported from animals and humans and have been associated with community- and healthcare-associated infections in humans, many without livestock contacts [3–6]. Phylogenetic studies have identified human and LA CC398 clades and have revealed that LA CC398 MRSA emerged from human CC398 MSSA via acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) element and tetracycline resistance genes *tet*(M) and loss of the phage-encoded immune evasion complex (IEC) genes [7, 8]. In human *S. aureus* strains (both MSSA and MRSA) the IEC genes are encoded in the genomes of a specific group of related lysogenic bacteriophages that integrate into and inactivate the *S. aureus* chromosomal beta-toxin gene *hlb* [9, 10]. Animal strains of *S. aureus* usually lack these bacteriophages and are IEC-negative.

Despite its prevalence in continental Europe and sporadic reports of CC398 MRSA in the UK (including Northern Ireland) among piglets, horses, turkeys, bovine bulk tank milk and retail pork [11–15], only a single case of CC398 MRSA has been reported in the Republic of Ireland (ROI), from an elderly man in a nursing home in 2012 and our pig population has remained CC398 free [16, 17]. Here we report molecular epidemiological evidence of the emergence and spread of CC398 MRSA and MSSA among animals and humans in the ROI, and evidence of the importation and zoonotic spread of CC398 MRSA.

Materials and Methods

Ethics statement

All isolates identified by the Irish National MRSA Reference Laboratory (NMRSARL) were collected as part of routine clinical care. The samples from the horse and the pig submitted for postmortem at the University College Dublin Veterinary Hospital (UVH) were collected as part of routine veterinary care. The UVH human samples were collected in compliance with UVH infection control policy and approved by University College Dublin Safety, Insurance, Operational and Compliance Office i.e the samples were collected by the person themselves and were processed as screening samples only. The extra samples collected from pigs on the farm were exempt from ethical review because they were part of a clinical investigation for the farmer. No medical records or identifying information about patients or owners were accessed as part of this study. The isolates and any relevant information about the cases was obtained and analysed in a fully anonymised and de-identified form.

Isolates

Thirty-two CC398 isolates, 22 MRSA and 10 MSSA, recovered in the ROI from animals (pigs, *n* = 17; horses, *n* = 1) and humans (*n* = 14) were investigated in the present study. The majority of isolates (*n* = 28) were identified at the UVH Microbiology Laboratory, which processes samples from UVH (a tertiary referral centre) and from private veterinary practitioners. The horse from which CC398 MRSA was isolated was one of 19 equine cases from which MRSA was isolated in the UVH Microbiology Laboratory between 2010 and 2014. CC398 MRSA was subsequently recovered from a nasal swab of the veterinarian attending the horse. The remaining UVH CC398 MRSA isolates were recovered from pigs on one farm (Farm A) or farm workers on two farms (Farms A and B) which were investigated due to the finding of CC398 MRSA in a pig from Farm A during a post mortem at UVH and an epidemiological link between Farms A and B. The porcine CC398 MSSA isolates were recovered from two farms which were investigated as part of a UVH research project where between 15% and 69% of pigs on Irish farms were MSSA positive (unpublished UVH data). CC398 MSSA represented 1.5% of MSSA isolated from one farm and 0.7% of MSSA isolated from another.

The remaining CC398 *S. aureus* isolates (*n* = 4) were identified at the NMRSARL between 2010 and 2014 as part of routine investigations. This represented 0.05% (2/3426) and 0.35% (2/574) of MRSA and MSSA isolates, respectively, investigated by NMRSARL, between 2010 and 2014. CC398 *S. aureus* represented 0.19% (4/2074) of *S. aureus* genotyped in the NMRSARL between 2010 and 2014. The NMRSARL investigates MRSA and MSSA isolates at the request of microbiology laboratories throughout Ireland and can include isolates recovered from various different patient and environmental sites from both hospital and community sources. It also analyses all MRSA bloodstream infection (BSI) isolates from patients in Irish hospitals that participate in The European Antimicrobial Resistance Surveillance Network (EARS-Net) project, which includes one isolate per patient per quarter from 26 participating hospitals.

Isolates were identified as *S. aureus* using the tube coagulase test as described previously [18] or a commercial latex agglutination assay (Pastorex Staph-Plus Bio-Rad, France). Isolates were initially assigned to CC398 by *spa* typing and this, and the species identification was confirmed by DNA microarray profiling (see below). Isolates were stored at -80°C on cryoprotective beads (Technical Service Consultants Ltd., UK).

Antimicrobial susceptibility testing

MRSA and MSSA were differentiated using Brilliance MRSA agar (Oxoid Ltd., Basingstoke, UK) or cefoxitin disks (30-μg) (Oxoid) using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and interpretive criteria [19, 20]. Isolates also underwent susceptibility testing against an additional 23 antimicrobial agents and heavy metals as described previously [21] according to EUCAST methodology [19] using previously described quality control strains, disk concentrations, and interpretive criteria [21]. In brief, where available, EUCAST disk concentrations and interpretive criteria were used [19, 21]. If not available, Clinical Laboratory Standards Institute (CLSI) disk concentrations and interpretive criteria were used, [21, 22] or for the remaining agents (including all heavy metals tested) the disk concentrations and interpretive criteria of Rossney *et al.* [21, 23] were used. The 23 agents tested were amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin, trimethoprim and vancomycin.

Molecular characterisation of isolates

Genomic DNA for all molecular tests was extracted from all isolates by enzymatic lysis using the buffers and solutions provided with the StaphyType DNA microarray kit (Alere Technologies GmbH, Jena, Germany) and the DNeasy Blood and Tissue kit (Qiagen, Crawley, West Sussex, UK). All isolates underwent DNA microarray profiling. The DNA microarray (version 2.0) consists of a DNA microarray chip adhered to each well of a microtitre strip; each chip consists of 334 *S. aureus* target sequences including species-specific, antimicrobial and heavy metal resistance, SCC*mec*, virulence-associated and typing genes [24, 25]. Data generated by the StaphyType arrays were analysed for the presence or absence of these genes using Arraymate software (Alere Technologies) which can assign *S. aureus* isolates to sequence types (STs) and/ CCs by comparing each isolate's DNA microarray results to those of a reference collection of previously characterised strains in the Arraymate database [25]. The DNA microarray primers, probes and protocols have been described previously in detail [24, 25].

All isolates were genotyped by *spa* typing and underwent PCRs for additional antimicrobial resistance genes commonly associated with CC398 but not included on the DNA microarray. Isolates found to harbour SCC*mec* by DNA microarray profiling underwent additional SCC*mec* typing PCRs. PCRs were performed using GoTaq Flexi DNA polymerase (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions and a G-storm GS1 (Applied Biosystems, Foster City, CA) or a Thermo Hybaid HBPX2 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) thermocycler. PCR products were visualised by conventional agarose gel electrophoresis. *spa* typing, which involves PCR and sequencing of the *S. aureus* protein A gene *spa* [26], was performed using the primers and thermal cycling conditions described by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet, www.seqnet.org.). *spa* typing PCR products were purified with the GenElute PCR clean-up kit (Sigma-Aldrich Ireland Ltd., Arklow, County Wicklow Ireland) and sequencing was performed commercially by Source Bioscience (Tramore, Waterford, Ireland) using an ABI 3730xl Sanger sequencing platform. The Ridom StaphType software version 1.3 (Ridom Gmbh, Wurzburg, Germany) was used for *spa* sequence analysis and assignment of *spa* types [27]. Antimicrobial resistance gene PCRs were performed using previously described primers and thermal cycling conditions and included detection of *spc*, *tet*(L), *erm*(T), *dfrK* and *dfrG*, [28–30]. Isolates with SCC*mec* IV were subtyped using a previously described multiplex PCR, which detects the SCC*mec* IV subtypes IVa-IVh [31]. Isolates with SCC*mec* V underwent *ccrC* allotype identification using a previously described multiplex PCR to differentiate between SCC*mec* type V (*ccrC2*) and V_T (*ccrC2* and *ccrC8*) [32]. Details of primers, thermal cycling conditions and control strains used are shown in S1 Table.

Results

Importation and zoonotic spread of multiple CC398 MRSA strains in the Republic of Ireland

The 22 CC398-MRSA isolates were from four epidemiologically distinct incidents, two of which included both human and animal isolates (Incidents 2 & 3, Table 1). All MRSA isolates were *spa* type t011 (18/22) or t034 (4/22), SCC*mec* types V_T (5C2 & 5 i.e. type 5 *ccr* genes (*ccrC1* allele 2), class C2 *mec* and class 5 *ccr* genes (*ccrC1* allele 8); 19/22) or IVa (2B i.e. type 2 *ccr* genes (*ccrAB2*) and class B *mec*; 3/22) and the majority lacked IEC genes (20/22) (Table 1). All isolates exhibited resistance to multiple classes of antimicrobial agents and carried multiple resistance genes including those encoding resistance to beta lactams (*blaZ* 22/22), tetracycline (*tet*(M) 22/22, *tet*(K) 18/22), *tet*(L) 6/22), macrolides, lincosamides and streptogramin B

Table 1. Epidemiological, phenotypic and genotypic characteristics of CC398 methicillin-resistant and methicillin-susceptible *S. aureus* (MRSA and MSSA) identified in the Republic of Ireland among animals and humans.

Methicillin resistance phenotype	Incident no.	Year	No. of isolates	Host	Sample site/ clinical presentation (*n*)	*spa* type [a]	IEC type	SCC*mec* type	Antimicrobial resistance pattern[b]	Antimicrobial resistance genes
MRSA	1	2011	1	Human	Nursing home resident nasal swab	t011	Negative	IVa	Ap, Gn, Kn, Tb, Te, Tp	*blaZ*, *aacA-aphD*, *tet*(M), *dfrK*
MRSA	2	2012	2	Horse & human	Horse umbilical abscess; veterinarian nasal swab	t011	B (*sak*, *chp* & *scn*)	IVa	Ap, Er, Gn, Ln, Kn, Tb, Te, Tp	*blaZ*, *erm*(C), *aacA-aphD*, *tet* (M), *dfrG*, *dfrK*
MRSA	3	2012 & 2013	18	Pig (*n* = 9) & human (*n* = 9)	Pig joint abscess-farm A (1); pig nasal swab- farm A (1)	t034	Negative	V_T	Ap, Er, Ln, Sp, Te, Tp	*blaZ*, *erm*(A), *tet* (K), *tet*(M), *dfrG*, *spc*
					Pig nasal swab-farm A (1)	t034	Negative	V_T	Ap, Er, Ln, Sp, Te, Tp	*blaZ*, *erm*(A), *tet* (M), *dfrG*, *spc*
					Pig nasal swab-farm A (1); pig farm worker nasal swab-farms A (2) & B (5)	t011	Negative	V_T	Ap, Er, Ln, Sp, Te	*blaZ*, *erm*(A), *tet* (K), *tet*(M), *spc*
					Pig farm worker nasal swab-farm A (1)	t011	Negative	V_T	Ap, Ch, Te	*blaZ*, *tet*(K), *tet*(M), *fexA*
					Pig farm worker nasal swab-farm A (1)	t011	Negative	V_T	Ap, Ch, Er, Kn, Ln, Nm, Tb, Te, Tp	*blaZ*, *erm*(B), *aadD*, *tet*(K), *tet* (M), *fexA*, *tet*(L), *dfrG*, *dfrK*
					Pig nasal swab-farm A (2)	t011	Negative	V_T	Ap, Ch, Er, Kn, Ln, Gn, Nm, Tb, Te, Tp	*blaZ*, *erm*(B), *aacA-aphD*, *aadD*, *tet*(K), *tet*(M), *fexA*, *tet*(L), *dfrG*, *dfrK*
					Pig nasal swab-farm A (1)	t011	Negative	V_T	Ap, Ch, Er, Ln, Nm, Tb, Te, Tp	*blaZ*, *erm*(A), *erm* (B), *aadD*, *tet*(M), *tet*(K) *fexA*, *tet*(L), *dfrG*, *dfrK*
					Pig nasal swab-farm A (1)	t011	Negative	V_T	Ap, Er, Gn, Kn, Ln, Nm, Sp, Tb, Te, Tp	*blaZ*, *erm*(A), *erm* (B), *aadD*, *tet*(M), *tet*(L), *dfrG*, *dfrK*, *spc*
					Pig nasal swab-farm A (1)	t011	Negative	V_T	Ap, Er, Kn, Ln, Nm, Sp, Te, Tb, Tp	*blaZ*, *erm*(A), *erm* (B), *aadD*, *tet*(K), *tet*(M), *dfrG*, *dfrK*, *tet*(L), *spc*
MRSA	4	2013	1	Human	Child skin abscess with family contact working with pigs	t034	Negative	V_T	Ap, Er, Ln, Sp, Te, Tp	*blaZ*, *erm*(A), *tet* (K), *tet*(M), *dfrG*, *spc*
MSSA	5	2010	3	Pig	Nasal swabs	t108 (1)	Negative	N/A	Ap, Cp, Sp, Te	*blaZ*, *tet*(M), *spc*
						t108 (1)	Negative	N/A	Ap, Cp, Er, Ln, Sp, Te	*blaZ*, *erm*(C), *tet* (M),
						t4854 (1)	Negative	N/A	Ap, Er, Ln, Sp, Te	*blaZ*, *erm*(C), *tet* (M), *spc*
MSSA	6	2010	5	Pig	Nasal swabs	t034	Negative	N/A	Ap, Cp, Er, Ln, Sp, Te	*blaZ*, *erm*(A), *tet* (M), *spc*

(*Continued*)

Table 1. (*Continued*)

Methicillin resistance phenotype	Incident no.	Year	No. of isolates	Host	Sample site/ clinical presentation (*n*)	*spa* type [a]	IEC type	SCC*mec* type	Antimicrobial resistance pattern[b]	Antimicrobial resistance genes
MSSA	7	2014	1	Human	BSI	t571	C (*chp* & *scn*)	N/A	Ap, Er	*blaZ*, *erm*(T)
MSSA	8	2014	1	Human	BSI	t011	D (*sea*, *sak* & *scn*)	N/A	Ap	*blaZ*

[a]*Spa* repeat successions: t011, 08-16-02-25-34-24-25; t034: 08-16-02-25-02-25-34-24-25; t108: 08-16-02-25-24-25; t571: 08-16-02-25-02-25-34-25; t4854: 08-16-02-25-24.

[b]The susceptibility of each isolate was determined against 23 antimicrobial agents including amikacin, ampicillin (Ap), cadmium acetate, chloramphenicol, ciprofloxacin (Cp), erythromycin (Er), ethidium bromide, fusidic acid, gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride, mupirocin, neomycin (Nm), phenyl mercuric acetate, rifampicin, spectinomycin (Sp), streptomycin, sulphonamide, tetracycline (Te), tobramycin (Tb), trimethoprim (Tp) and vancomycin.

Abbreviations: BSI, bloodstream infection; *n*, number of isolates; N/A, not applicable; IEC, immune evasion complex.

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(MLS_B) compounds (*erm*(A) 15/22, *erm*(B) 6/22, *erm*(C) 2/22), spectinomycin (*spc* 14/22), trimethoprim (*dfrG* 12/22, *dfrK* 9/22), aminoglycosides (*aacA-aphD* 5/22, *aadD* 6/22) and chloramphenicol (*fexA* 5/22) (Table 1). All isolates lacked Panton-Valentine leukocidin, enterotoxin, toxic shock toxin, exfoliative toxin and heavy metal resistance (*merA*, *merB*, *qacA* and *qacC*) genes and were susceptible to the heavy metals tested (cadmium acetate and ethidium bromide).

The first CC398 MRSA isolate has been reported previously [16] and was recovered in 2011 from a nursing home patient who had been a part-time cattle farmer and was *spa* type t011-SCC*mec* IVa (Incident 1, Table 1). Similar to the majority of other t011 isolates identified here, this isolate lacked IEC genes but harboured less resistance genes (Table 1). Incident 2 involved two t011-SCC*mec* IVa isolates recovered in 2012 from a horse and an attending Belgian veterinarian who had recently returned from Belgium. The veterinarian was tested for MRSA nasal carriage following identification of MRSA in the horse and was subsequently treated and successfully decolonised. Unlike Incident 1, these isolates harboured IEC genes and additional resistance determinants (*erm*(C) & *dfrG*) (Table 1).

Incident 3 yielded 18 isolates from two farms during 2012/2013. The initial farm A CC398 MRSA isolate was recovered from a pig joint abscess during a post mortem examination at UVH. Subsequently, the farm was visited and nasal swabs were collected from 100 pigs and five farm workers who had contact with the pigs. CC398 MRSA was recovered from 8/100 pigs and 4/5 farm workers sampled. This farm had been restocked prior to isolate recovery with Irish and German gilts. Farm B was a finisher unit for Farm A; all weaned pigs were transported from Farm A to Farm B at approximately 12 weeks of age. Isolates were recovered from nasal swabs of 5/10 Farm B workers. All isolates within Incident 3 harboured SCC*mec* V_T and lacked IEC genes. Three of the pig isolates (joint abscess and two nasal swabs) were *spa* type t034 and differed only in the absence of *tet*(K) in one nasal isolate (Table 1). Interestingly, the Incident 4 CC398-MRSA isolate, which was recovered from a child with a skin abscess, was indistinguishable in terms of *spa* and SCC*mec* type, lack of IEC and resistance gene content from 2/3 t034 pig isolates in Incident 3 (Table 1) but no epidemiological link between the isolates was identified. However, the child had worked with his father on a pig farm in the UK.

The remaining Incident 3 isolates were *spa* type t011 with seven different combinations of antimicrobial resistance genes identified (Table 1). However, one pig nasal t011-V_T isolate was

indistinguishable from 7/9 pig farmer nasal isolates and two pig nasal t011-V_T isolates were indistinguishable from each other due to carriage of the same combinations of antimicrobial resistance genes detected.

Distinct CC398 MSSA strains among animals and humans in the Republic of Ireland

Four distinct incidents involving CC398 MSSA, two from humans (BSIs, two isolates) and two from nasal carriage in pigs (eight isolates), were identified (Table 1). Isolates from animals and humans were distinguished from each other in *spa* types, IEC genes and antimicrobial resistance genes and phenotype (Table 1). The two human isolates were also distinct from each other; they were recovered from patients in two different hospitals, exhibited different *spa* types (t571 and t011) and harboured different combinations of IEC and resistance genes, with one isolate harbouring *erm*(T) (Incidents 7 and 8, Table 1). Incident 7 involved a 75-year old male and Incident 8 involved a 51-year old male but no additional information was available regarding these patients. Each pig CC398 MSSA incident consisted of multiple isolates recovered from two farms in 2010 and these isolates lacked IEC genes and harboured multiple resistance genes (Incidents 5 and 6, Table 1). Incident 5 and 6 isolates were phenotypically and genotypically distinct from each other (Table 1). Incident 5 isolates exhibited the same or closely related *spa* types and harboured similar resistance genes including *blaZ*, *tet*(M) and *spc* with 2/3 isolates also harbouring *erm*(C) and exhibiting ciprofloxacin resistance (Table 1). Incident 6 isolates exhibited a different *spa* type (t034) from Incident 5 isolates and although they harboured similar resistance genes and exhibited ciprofloxacin resistance, Incident 5 isolates harboured *erm*(A) and not *erm*(C). These t034 MSSA isolates were similar to the t034 MRSA isolates (Incident 3 & 4) but lacked *dfrG* and *tet*(K).

Discussion

This study revealed the emergence of multiple CC398-MRSA strains among animals and humans in the ROI as well as its importation and spread and highlights a combination of inadequate biosecurity at the level of country, farm and veterinary hospital. The CC398 MRSA identified here appear to be predominantly of animal origin based on epidemiological evidence, the lack of IEC genes and the prevalence of *tet*(M) [7]. The importation of gilts from Germany, where CC398 MRSA has been reported extensively among animals and humans [33–35], to restock one of the farms and subsequent spread to other pigs and farmers on this and an additional farm highlights the ability of CC398 MRSA to spread and the introduction of novel zoonotic organisms as a consequence of open border policies. These findings have implications for both human and animal health as well as the agricultural industry in the ROI. Firstly, the risk posed by contact with livestock needs to be considered when screening high-risk groups for MRSA on admission to Irish hospitals. Furthermore, animal MRSA infections are not notifiable in the ROI and there is no requirement to screen imported animals for MRSA. There is a need to reconsider this policy and to conduct further work to establish how widely CC398 MRSA has disseminated within the Irish pig industry. While the major threat identified here is the spread of CC398 MRSA from animals to humans via direct contact, CC398 MRSA have also been reported in retail meat products including pork [13, 36], representing a further potential threat to public health and the reputation of the Irish agricultural sector. The presence of IEC in the veterinarian and horse isolates and the recent travel of the veterinarian to Belgium where IEC-positive CC398-MRSA-IV have been reported [37] suggest human to animal transmission in this instance due to inadequate infection control measures within the veterinary hospital.

The extensive antimicrobial resistance of CC398 MRSA is also of concern. As is characteristic of CC398 MRSA, all isolates identified here harboured multiple antimicrobial resistance genes encoding resistance to a range of agents used in clinical and veterinary medicine (Table 1). This multidrug resistance compromises our ability to treat CC398 MRSA infections, and due to the previously reported plasmid location of many of these resistance genes [38–40], highlights the reservoir of resistance genes that exists among LA-MRSA and the potential of these genes to spread to other *S. aureus* strains in animals and humans. Interestingly many of these CC398-MRSA isolates also harboured multiple genes encoding resistance to a single agent including multiple tetracycline, trimethoprim, aminoglycosides and MLS_B resistance genes. While this may reflect the co-location of some of these genes on a single plasmid [38] it suggests significant pressure for the selection and maintenance of these resistance genes exists, particularly among isolates from pigs and farm workers, which carried the largest number of resistance genes (Table 1).

In the present study at least four distinct CC398 MRSA strains were identified based on *spa* and SCC*mec* typing and detection of IEC genes including (i) t011-IVa, IEC negative, (ii) t011-IVa, IEC positive, (iii) t034-V_T, IEC negative, (iv) t011-V_T, IEC negative, (Table 1). Within the t034- and t011-V_T isolates differences were detected in the combinations of antimicrobial resistance genes that they harboured. The three farm A and B t034-V_T isolates differed only in the absence of *tet*(K) in one isolate and two of these were indistinguishable from the child skin abscess t034-V_T isolate in terms of the antimicrobial resistance genes detected suggesting the possible spread of a single strain. However, this child had worked with his father on a pig farm in the UK, indicating that the CC398 MRSA infection may have been acquired through contact with pigs in the UK. Similar to the ROI, it is not known how widespread CC398 MRSA is among pigs in the UK with just three piglets reported to date with CC398 MRSA, two of which were assigned to the same *spa* type (t034) and a similar SCC*mec* type (V) to the child skin abscess CC398 MRSA isolate in the present study [11, 12]. The 15 farm A and B t011-V_T isolates harboured between four and 10 resistance genes each and were differentiated into seven groups based on the different combinations of these genes (Table 1). While the differences detected in resistance gene content may indicate the presence of multiple distinct t011-V_T CC398 MRSA strains, these differences may also represent the loss and gain of plasmids encoding resistance genes due to different selective pressures. Further studies using whole-genome sequencing are required to determine the precise relationship between these CC398 MRSA isolates with the same *spa* and SCC*mec* types but harbouring different combinations of antimicrobial resistance genes.

Based on molecular epidemiological typing, the animal and human CC398 MSSA isolates identified here were unrelated indicating their independent emergence and both harboured traits typical of animal and human CC398 MSSA clades, respectively [4, 5, 7] i.e. the human isolates were IEC positive and carried only one or two resistance genes with one harbouring *erm*(T) while the pig isolates were IEC negative and harboured multiple resistance genes. Just two human CC398 MSSA isolates, both from BSIs, were identified, in 2014. A low but increasing level of CC398 MSSA among human invasive infections have been reported elsewhere in Europe [5, 41]. While molecular epidemiological typing did not reveal the spread of CC398 MSSA from animals to humans or *vice versa*, it did reveal the spread of CC398 MSSA among pigs on two farms. Two of the *spa* types identified among the CC398 MSSA were also reported among the CC398 MRSA (t034 and t011) and these may be potential precursors for the emergence of CC398 MRSA. A recent study highlighted the presence of SCC*mec* remnants in CC398 MSSA and suggested that CC398 MRSA could emerge from these [6]. However no SCC*mec* genes were identified among the CC398 isolates identified here.

In conclusion, this study has, for the first time, revealed the importation and zoonotic spread of multiple multidrug resistant CC398 MRSA strains in the ROI and the spread of CC398 MSSA among pigs. It has also highlighted the reservoir of resistance genes that exists among CC398 MRSA that could potentially spread to other animal and human *S. aureus* strains. Increased surveillance of humans and animals in the ROI is warranted to prevent further CC398 MRSA importation and spread in a country that was, until recently, considered CC398 MRSA free.

Supporting Information

S1 Table. Details of primers, thermal cycling conditions and positive control strains used in the present study.
(PDF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: ACS GIB FL DCC BOC. Performed the experiments: GIB YA AB BAM. Analyzed the data: ACS GIB DCC FL AB YA. Contributed reagents/materials/analysis tools: DCC BOC GIB FL ACS. Wrote the paper: ACS. Edited and approved the manuscript: GIB FL DCC BOC BAM AB YA.

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Evaluation of commercial chromogenic media for the detection of meticillin-resistant *Staphylococcus aureus*

G.I. Brennan [a,b,*], C. Herra [c], D.C. Coleman [b], B. O'Connell [a,d], A.C. Shore [a,b]

[a] *National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland*
[b] *Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Ireland*
[c] *School of Biological Sciences, Dublin Institute of Technology, Dublin, Ireland*
[d] *Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin, Ireland*

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SUMMARY

Background: Selective chromogenic media allowing one-step meticillin-resistant *Staphylococcus aureus* (MRSA) isolation and identification are widely used. However, the changing epidemiology of MRSA means that the suitability of these chromogenic media requires investigation.

Aim: To evaluate the following chromogenic media — Colorex MRSA, MRSA Select II, ChromID MRSA, and MRSA Brilliance 2 — for the detection of divergent strain types.

Methods: We used a diverse collection of *S. aureus*, including strains harbouring the *mecC* gene, strains expressing varying levels of meticillin resistance, and isolates recovered from patient samples.

Findings: MRSA Select II, Colorex MRSA, and ChromID each grew at a density of 1.5×10^1 cfu/mL for each SCC*mec* type investigated. Brilliance 2 demonstrated growth at 1.5×10^1 cfu/mL for *mecC* MRSA but at a higher density (1.5×10^4 cfu/mL) for the three *mecA* MRSA strains. All four media demonstrated excellent sensitivity for MRSA detection (≥99%), but reduced levels of specificity (85–73%) when challenged with a range of meticillin-susceptible *S. aureus* (MSSA) isolates. High levels of false positives (~50%) were also obtained with all chromogenic media when tested with *mec*-negative borderline oxacillin-resistant *S. aureus* (BORSA) isolates.

Conclusion: Although false positives may be obtained with some strains of MSSA and BORSA, the high sensitivity of these media and their ability to recover almost all MRSA tested (including oxacillin-susceptible and *mec*C-positive strains) confirm the value of chromogenic agar in MRSA detection.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) are major healthcare-associated pathogens frequently associated with serious and sometimes life-threatening conditions. Meticillin resistance is mediated by an altered penicillin binding protein PBP2a encoded by *mec* and located on the staphylococcal

* Corresponding author. Address: National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland. Tel.: +353 1 4103662; fax: +353 1 4103666.
E-mail address: gbrennan@stjames.ie (G.I. Brennan).

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cassette chromosome *mec* (SCC*mec*) element. To date, 11 different SCC*mec* elements have been described in staphylococci corresponding to the emergence of a wide range of MRSA strains with different genetic backgrounds.[1]

In the last two decades the epidemiology of MRSA has changed significantly with an increasing prevalence of MRSA infections outside the healthcare environment in the community, and more recently among livestock.[2,3] In Ireland, MRSA is endemic in hospitals, and, as in many countries throughout Europe, the sequence type-SCC*mec* ST22-MRSA-IV clone predominates.[4] In addition, a diversity of other strains including community-associated *pvl* toxin-positive and -negative MRSA along with a small number of livestock-associated strains have also been reported in Ireland.[2,3]

Just as the epidemiology of MRSA has changed, so too has the level of meticillin resistance among MRSA. Traditionally MRSA are defined as having an oxacillin minimum inhibitory concentration (MIC) $\geq$4.0 mg/L or as harbouring the *mecA* gene encoding PBP2a.[1] However, few MRSA isolates express homogeneous oxacillin resistance. Oxacillin-susceptible *mecA*-positive *S. aureus* isolates have been reported worldwide.[5] Similarly, low-level oxacillin-resistant *mecA*-negative strains known as borderline oxacillin-resistant *S. aureus* (BORSA) isolates have further complicated the definition of MRSA.[6]

Superimposed on this heterogeneous expression of meticillin resistance, recent reports have also identified a variety of MRSA strains of probable animal origin that encode a highly divergent meticillin-resistance gene termed *mecC*. Where once the detection of *mecA* was considered the gold standard in laboratory confirmation of MRSA, the emergence of *mecC*-encoding strains in infection in both humans and animals adds to the challenge of defining and detecting an MRSA-positive patient.[7–9]

Regardless of the changing epidemiology of MRSA, rapid detection remains essential for the implementation of infection control procedures and effective patient management. The use of selective chromogenic culture media, which allow for one-step MRSA isolation and identification, has now become widespread practice.[10,11] With the frequent application of chromogenic media in diagnostic practice, the suitability of these media to ensure the correct detection of divergent MRSA strain types has come under review. However, whereas many studies have evaluated the use of chromogenic media for the direct recovery of MRSA from patient specimens, few have undertaken a comparative evaluation of all currently available commercial media using a comprehensive collection of diverse *S. aureus* strains, including those with the novel *mecC* gene and those expressing varying levels of meticillin resistance.[11–14]

The purpose of this study was to evaluate the performance of widely used chromogenic MRSA media using a diverse collection of *S. aureus* isolates recovered in Ireland and Europe. The limits of detection (LOD) of four commercial chromogenic media were determined using MRSA strains representative of four SCC*mec* types, i.e. II, IV, V, and XI.[2,3,7] The performance of the media was also evaluated against a collection of genotypically diverse MRSA strains from hospitals, communities, and livestock and representative of SCC*mec* types I–VIII, X, and XI as well as meticillin-susceptible *S. aureus* (MSSA) and BORSA strains isolated from healthcare and community sources. An evaluation of the media was also undertaken using patient samples collected as part of the routine infection prevention and control procedures in a large teaching hospital.

Methods

Limits of detection

Four MRSA isolates, representative of SCC*mec* types II, IV, and V (carrying *mecA*) and SCC*mec* XI (carrying *mecC*) (Table I) were selected to investigate the LOD of the following four commercial MRSA chromogenic agars: MRSA Select II (BioRad, Hercules, CA, USA), MRSA Brilliance 2 (Oxoid, Basingstoke, UK), Colorex MRSA (E & O Laboratories, Bonnybridge, UK), and ChromID MRSA (bioMérieux, Marcy l'Etoile, France). In each case, isolates were subcultured overnight on Columbia blood agar (Oxoid) at 37°C and then suspended in saline to a density equivalent to 0.5 McFarland standard. A ten-fold dilution series was prepared from 1.5×10^8–10^0 colony-forming units (cfu)/mL and a standard volume (100 μL) of each dilution was inoculated on to each of the MRSA chromogenic agars using a spiral plater (Don Whitley Scientific, Shipley, UK). This application was performed in triplicate for each isolate and plates were incubated as per the manufacturer's instructions. In each case, MRSA recovery was observed in accordance with the manufacturer's description of MRSA colony type, i.e. pink colonies on MRSA Select II, Colorex, and ChromID, or blue colonies on MRSA Brilliance 2. The LOD was recorded as the lowest bacterial density to give detectable growth on the chromogenic agar.

Evaluation of chromogenic media using a diverse collection of S. aureus isolates

The ability of the media to detect MRSA among a diverse collection of *S. aureus* isolates was also investigated. This included: (i) MRSA isolates representing 10/11 SCC*mec* types

Table I
Limits of detection of MRSA isolates representative of four *SCCmec* types as determined using four chromogenic media

Isolate no.	Genotype	*mec* gene	Lowest bacterial density (cfu/mL) at which growth was recorded[a]				Reference
			MRSA Select II	ChromID	Colorex	MRSA Brilliance 2	
AR07.4/0237	ST5-MRSA-II	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	16
CA05	ST22-MRSA-IV	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	17
WIS	ST8-MRSA-V	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	18
M10/0061	ST130-MRSA-XI	*mecC*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^1	7

MRSA, meticillin-resistant *Staphylococcus aureus*.
[a] The limit of detection was recorded as the lowest bacterial density to give detectable growth on chromogenic media.

(I–VIII, X and XI); (ii) *mecA*-positive ($n = 148$) and *mecC*-positive ($n = 13$) MRSA isolates representative of a range of genotypes and comprising 149 MRSA isolates with oxacillin MICs ranging from 4 to >256 mg/L and 12 MRSA isolates with an oxacillin MIC ≤2.0 mg/L (range: 0.125–2.0 mg/L); (iii) 34 MSSA isolates that lacked *mec* genes and were susceptible to oxacillin (MIC range: 0.5–2.0 mg/L); (iv) 20 BORSA isolates which were *mec* negative but which exhibited oxacillin MICs between 4 and 8 mg/L (Supplementary Table I). In each case, isolates were suspended to a density equivalent to 0.5 McFarland standard. A 20 μL volume was inoculated on to each of the four commercial MRSA chromogenic agars, and plates were incubated and read as above. Quality control testing was performed on each medium using *S. aureus* control strains ATCC43300 (MRSA) and ATCC25923 (MSSA).

Evaluation of chromogenic media using patient samples

The ability of the chromogenic media to detect MRSA directly from patient samples was investigated using 228 swabs recovered from the nose, throat, and groin of 76 inpatients at a 936-bed tertiary referral hospital in Dublin, Ireland. Specimens were collected as part of routine screening practices within the hospital. The 228 samples were initially inoculated on to MRSA Select (the earlier formulation of MRSA Select II) in accordance with the routine diagnostic procedures. The specimens were then inoculated on to the test chromogenic agars, changing the order in which the media were inoculated for each sample. All suspect colonies recovered from the screening swabs that were consistent with the manufacturer's description of MRSA were tested for oxacillin susceptibility by disc diffusion and investigated for the presence of *mec* and *nuc* genes using an in-house real-time polymerase chain reaction assay.[15]

Statistical analysis

The ability of each agar to correctly identify MRSA (sensitivity) and to exclude MSSA (specificity) was determined based on the number of correct results realized among the MRSA and MSSA isolates.

Results

Limits of detection assay

The results of the LOD evaluation for the four MRSA strains on the four different chromogenic agars tested are shown in Table I. The MRSA Select II, Colorex MRSA, and ChromID each yielded growth at a density of 1.5×10^1 cfu/mL for each SCC*mec*/*mec* gene type investigated. Brilliance 2 also demonstrated growth at 1.5×10^1 cfu/mL for the *mecC* MRSA but at a higher density (1.5×10^4 cfu/mL) for each of the three *mecA* MRSA strains tested. For each strain and each agar tested, the results from the three experiments were in agreement with each other.

Performance of the chromogenic media using a diverse collection of S. aureus isolates

The MRSA strains representative of the different SCC*mec* types demonstrated good recovery with typical MRSA morphology on all four media tested. The performance data for the MRSA and MSSA isolates on the four chromogenic media are shown in Table II. For the 161 MRSA isolates, Colorex MRSA and ChromID MRSA were found to be 100% sensitive whereas MRSA Select II and MRSA Brilliance 2 demonstrated slightly lower sensitivity rates of 99% and 98%, respectively. MRSA Select II and MRSA Brilliance 2 failed to detect one *mecA*-positive MRSA isolate, exhibiting an oxacillin MIC of only 0.125 mg/L (*spa* type t2235), and Brilliance 2 failed to detect an additional two MRSA isolates, exhibiting susceptible MICs of 1.0 and 2.0 mg/L (*spa* types t002 and t3500, respectively). When compared with the predominant ST22-MRSA-IV *mecA* strain, *mecC* isolates included in this study yielded colonies equivalent in number and morphology on three of the four chromogenic agars tested and yielded a greater number of colonies on the Brilliance 2 agar.

Table II
Sensitivity and specificity of the four chromogenic media using the MRSA and MSSA isolates

Variable	MRSA Select II	ChromID	Colorex	Brilliance 2
Sensitivity	99%	100%	100%	98%
Specificity	73%	85%	85%	82%

MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*.

When tested with the 34 MSSA isolates, the specificity of the four chromogenic agars ranged between 73% and 85% (Table II). The MRSA Select II exhibited the highest number of false-positive results (9 out of 25) whereas other chromogenic agars yielded five or six false positives (Table III). All isolates that generated false positives on the chromogenic agars exhibited oxacillin susceptibilities representative of the range of oxacillin MIC values in the MSSA collection tested and belonged to a range of genotypes (Table III).

As expected, due to their higher oxacillin MICs (4–8 mg/L), the BORSA isolates also proved challenging for the chromogenic agars. Once again MRSA Select II exhibited the highest number of false-positive results, with 13 out of 20 BORSA isolates yielding suspect MRSA colonies. The other three chromogenic agars also produced a high number of false positives with Brilliance 2 and Colorex demonstrating growth for 11 of the BORSA whereas eight were recovered on the ChromID (Table III).

Performance of the chromogenic media using patient samples

Of the 228 swabs investigated from 76 patients, six swabs from four patients were positive for MRSA (one patient nose and groin swab positive; two patients groin swab only positive; one patient throat swab only positive). These results were in agreement with the clinical microbiology laboratory results and were detected with all four chromogenic agars, with suspect colonies growing in sufficient numbers and with typical colony morphology that allowed ready detection despite the high number of plates inoculated. All isolates were confirmed as oxacillin resistant and carried the *mecA* gene.

Discussion

Although the decreasing level of invasive healthcare-associated MRSA infections in Europe and the USA is

Table III
Genotypes and oxacillin MIC values of *mecA*- and *mecC*-negative MSSA and BORSA isolates yielding false-positive results using the four chromogenic media

Isolate no.	Genotype[a]	Oxacillin MIC (mg/L)	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2
MSSA						
M11/0175	t306	2	pos	pos	pos	pos
M12/0147	ST80-t088	0.5	pos	pos	pos	neg
M12/0272	ST7-t091	0.5	pos	neg	neg	neg
M14/0220	ST45/46-t065	2	pos	neg	neg	neg
M14/0248	t608	0.5	pos	pos	pos	pos
M14/0249	ST1-t127	0.5	pos	pos	pos	pos
M14/0250	t2828	1	pos	neg	neg	neg
M14/0258	ST8-t008	0.5	pos	neg	neg	neg
M14/0366	ND	2	pos	pos	pos	pos
M11/0281	t11018	0.5	neg	neg	neg	pos
M14/0254	ST8-t008	1	neg	neg	neg	pos
Total			9	5	5	6
BORSA						
M05/0294	ND	4	pos	neg	neg	neg
M07/0138	ND	4	pos	pos	pos	pos
M07/0376	ND	4	neg	neg	pos	neg
M07/0377	ND	4	neg	neg	pos	neg
M08/0079	ND	8	pos	pos	pos	pos
M12/0306	ST8-t008	8	pos	neg	neg	pos
M12/0355	ST8-t008	8	pos	neg	pos	pos
M13/0626	t078	4	pos	neg	neg	neg
M13/0629	ND	4	pos	pos	pos	pos
M14/0178	ST9-t100	4	pos	pos	pos	pos
M14/0179	ST9-t100	4	pos	neg	neg	pos
M14/0188	ST7-t091	8	pos	pos	pos	pos
M14/0260	ND	4	pos	pos	pos	pos
M14/0282	ST15/18-t084	4	pos	neg	neg	neg
M14/0604	ST398-t571	8	pos	pos	pos	pos
M14/0784	ND	4	neg	pos	pos	pos
Total			13	8	8	11

MIC, minimum inhibitory concentration; MSSA, meticillin-susceptible *S. aureus*; BORSA, borderline-oxacillin resistant *S. aureus*; MRSA, meticillin-resistant *Staphylococcus aureus*; pos; positive, neg; negative; ND, not determined.
[a] Where available genotypes are indicated by the multi-locus sequence type (prefix 'ST') and/or the *spa* type (prefix 't'). The STs were inferred from the *spa* type using the Ridom *spa* server (http://www.spaserver.ridom.de/) and/or based on previous experience at the Irish National MRSA Reference Laboratory (NMRSARL).

encouraging, the changing epidemiology of this pathogen and the emergence of virulent strains in the community require rapid and sensitive laboratory detection.

For the detection of MRSA all media performed well in the evaluation. With the exception of MRSA Brilliance 2, which demonstrated growth at a higher density (15,000 cfu/mL) for the three *mecA* MRSA, the other three chromogenic agars – MRSA Select II, Colorex MRSA, and ChromID – recovered all strains (*mecA* and *mecC* MRSA) when challenged with lower densities at 15 cfu/mL. Previously reported studies of *mecC* isolates suggested that difficulties may arise in the laboratory detection of *mecC*-positive MRSA isolates due to the low oxacillin MIC exhibited by some of these strains.[9,19,20] However, the *mecC* isolates included in this study grew well on each of the chromogenic media investigated, and showed even improved recovery on MRSA Brilliance 2 compared to *mecA*- and healthcare-associated MRSA strains, e.g. ST22-MRSA-IV. The underlying reasons for this disparity between *mecA* and *mecC* MRSA strains on Brilliance 2 agar are unclear but may reflect differential interactions between the *mec* gene products and constituents of the medium.[21] This requires further study.

The chromogenic agars demonstrated excellent sensitivity ranging from 98% to 100%, with Colorex media and ChromID detecting the full collection of MRSA isolates investigated. The high sensitivities recorded here are in agreement with other MRSA chromogenic agar evaluation studies.[11–13] However, a challenge arose for the MRSA Select II and MRSA Brilliance 2 media: each failed to detect a small number of MRSA isolates (one and three isolates, respectively) exhibiting susceptible oxacillin MICs. A small increase in the prevalence of oxacillin-susceptible *mecA*-positive isolates has been previously reported elsewhere and similar isolates have been recovered in the National MRSA Reference Laboratory in Ireland.[5] As these strains can cause problems in the routine diagnostic laboratory, their successful recovery using the chromogenic media is essential.

When challenged with MSSA isolates the chromogenic agars demonstrated reduced levels of specificity, ranging from 73% to

85%. This specificity rate is low in comparison to other studies where specificity rates of 95–99% have been reported for chromogenic agars.[11–13] However, these other studies confined investigations to clinical specimens only, whereas the current study included a diverse collection of MSSA strains. The recovery of these MSSA strains as presumptive MRSA isolates has implications for the diagnostic laboratory in terms of increasing the turnaround time and resource management.[11]

The reduced specificity of the chromogenic agars was once again demonstrated by the high recovery of BORSA with almost 50% of isolates being recovered on all four of the media. This high rate of false positivity can be expected with the BORSA isolates due to their high oxacillin MICs. Although a previous epidemiological investigation of BORSA isolates indicated that they are not implicated in patient-to-patient spread, their growth on this medium further complicates the detection, prevention, and control of genuine MRSA in the healthcare environment.[22]

Despite the investigation of a relatively large number of patient samples (nose, throat, and groin from 76 patients, i.e. 228 samples) the rate of detection of MRSA was low (5.6%). However, this was similar to previous studies of patient samples from hospitalized patients in Ireland which showed MRSA prevalence rates of 8.5% and 7.6%.[23,24] Additionally, the results correlated with the clinical microbiology laboratory results for these samples, indicating that no false positives or false negatives were detected using any of the four chromogenic agars.

The early identification of MRSA and implementation of infection prevention and control procedures has been shown to reduce healthcare-associated infections. The high sensitivity of the chromogenic agars evaluated in this study confirms the usefulness of this medium in the one-step detection and presumptive identification of MRSA. Whereas the recovery of a high number of MSSA and BORSA isolates is a concern, the ability of the medium to recover almost all MRSA, including oxacillin-susceptible and *mecC*-positive strains, ensures appropriate management and treatment for MRSA-positive patients.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jhin.2015.10.019.

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Extensive Genetic Diversity Identified among Sporadic Methicillin-Resistant *Staphylococcus aureus* Isolates Recovered in Irish Hospitals between 2000 and 2012

Peter M. Kinnevey,[a] Anna C. Shore,[a,b] Grainne I. Brennan,[a,c] Derek J. Sullivan,[a] Ralf Ehricht,[d] Stefan Monecke,[d,e] David C. Coleman[a]

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

Clonal replacement of predominant nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) strains has occurred several times in Ireland during the last 4 decades. However, little is known about sporadically occurring MRSA in Irish hospitals or in other countries. Eighty-eight representative *pvl*-negative sporadic MRSA isolates recovered in Irish hospitals between 2000 and 2012 were investigated. These yielded unusual pulsed-field gel electrophoresis and antibiogram-resistogram typing patterns distinct from those of the predominant nosocomial MRSA clone, ST22-MRSA-IV, during the study period. Isolates were characterized by *spa* typing and DNA microarray profiling for multilocus sequence type (MLST) clonal complex (CC) and/or sequence type (ST) and SCC*mec* type assignment, as well as for detection of virulence and antimicrobial resistance genes. Conventional PCR-based SCC*mec* subtyping was undertaken when necessary. Extensive diversity was detected, including 38 *spa* types, 13 MLST-CCs (including 18 STs among 62 isolates assigned to STs), and 25 SCC*mec* types (including 2 possible novel SCC*mec* elements and 7 possible novel SCC*mec* subtypes). Fifty-four MLST-*spa*-SCC*mec* type combinations were identified. Overall, 68.5% of isolates were assigned to nosocomial lineages, with ST8-t190-MRSA-IID/IIE ± SCC_{M1} predominating (17.4%), followed by CC779/ST779-t878-MRSA-ψSCC*mec*-SCC-SCC_{CRISPR} (7.6%) and CC22/ST22-t032-MRSA-IVh (5.4%). Community-associated clones, including CC1-t127/t386/t2279-MRSA-IV, CC59-t216-MRSA-V, CC8-t008-MRSA-IVa, and CC5-t002/t242-MRSA-IV/V, and putative animal-associated clones, including CC130-t12399-MRSA-XI, ST8-t064-MRSA-IVa, ST398-t011-MRSA-IVa, and CC6-t701-MRSA-V, were also identified. In total, 53.3% and 47.8% of isolates harbored genes for resistance to two or more classes of antimicrobial agents and two or more mobile genetic element-encoded virulence-associated factors, respectively. Effective ongoing surveillance of sporadic nosocomial MRSA is warranted for early detection of emerging clones and reservoirs of virulence, resistance, and SCC*mec* genes.

Staphylococcus aureus colonizes the anterior nares of approximately 30% of the human population; it can give rise to a wide range of infections of skin and soft tissues, bones, joints, and prosthetic implants and can be responsible for a variety of toxinoses caused by specific toxins such as toxic shock toxin, enterotoxins, exfoliative toxins, and Panton-Valentine leukocidin (1). *Staphylococcus aureus* can evolve to methicillin-resistant *S. aureus* (MRSA) upon acquisition of a large staphylococcal chromosomal cassette (SCC) element harboring either the methicillin resistance gene *mecA* or *mecC* (SCC*mec*), both of which encode a modified penicillin-binding protein, PBP 2a (2–4).

Within SCC*mec*, *mecA* or *mecC* forms part of the *mec* gene complex, which may also harbor the *mec* regulatory genes *mecI* and *mecR1*, as well as insertion sequences and, in some instances, *blaZ* (1–3). Based on various combinations and truncations of the *mec* complex genes, five classes of the *mec* gene complex (A, B, C1, C2, and E) have been identified in MRSA (3, 5). In addition, each SCC*mec* element also harbors a chromosome cassette recombinase (*ccr*) gene complex, consisting of *ccrA* and *ccrB* together or *ccrC*; these genes encode polypeptides that catalyze site- and orientation-specific integration and excision of SCC*mec* into *orfX* within the *S. aureus* chromosome (1, 6). Seven types of *ccr* gene complex (types 1 to 5, 7, and 8) have been described to occur in MRSA, each with a different combination of *ccrA* and *ccrB* or *ccrC* alleles (5, 7). SCC elements that carry *ccr* genes but lack *mec* genes have also been described, as well as pseudo (ψ) SCC*mec* and SCC elements that lack *ccr* genes, individual SCC*mec* elements with multiple *ccr* genes, and composite islands (CIs) consisting of two or more elements (5).

Eleven SCC*mec* types (I to XI) have been described to date for MRSA, each with a different combination of *mec* class and *ccr* type (3). Numerous SCC*mec* subtypes have also been described for MRSA which differ from SCC*mec* types based on DNA sequence variation or the presence or absence of mobile genetic elements (MGEs) in the joining (J) regions, which are located outside the *ccr* and *mec* complexes (7). MRSA isolates often exhibit resistance to a range of antimicrobial agents that can be due to the carriage of multiple antimicrobial resistance genes located on MGEs, including transposons, plasmids, and SCC or SCC*mec* elements (8, 9).

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Address correspondence to David C. Coleman, david.coleman@dental.tcd.ie.
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The first report of MRSA appeared in the literature in 1961, shortly after the introduction of methicillin into clinical use; just 10 years later, in 1971, MRSA was first reported in Irish hospitals (10, 11). Following a major increase in the prevalence of MRSA in Irish hospitals in the late 1970s and during the 1980s and 1990s, it has now been endemic for more than 3 decades (12–17). Since 1999, the prevalence rate of MRSA among *S. aureus* strains causing bloodstream infections (BSIs) in Ireland has been monitored by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Annual rates of MRSA among *S. aureus* from BSIs in Ireland reached 42% (592 MRSA isolates among 1,412 *S. aureus* isolates) in 2006, the highest level reported to date, and declined in recent years, with a rate of 22.8% (242 MRSA isolates among 1,060 *S. aureus* isolates) reported for 2012 (18, 19).

Clonal replacement of predominant nosocomial MRSA strains has occurred several times in Ireland during the last 4 decades (20). The different MRSA lineages that predominated in Irish hospitals at different time periods have been well characterized, including ST250-MRSA-I/I-*pls* (where ST is multilocus sequence type) in the 1970s and early 1980s, ST239-MRSA-III/III-pI258/Tn*554* in the middle to late 1980s and early 1990s, and ST8-MRSA-IIA-IIE throughout the 1990s, together with ST36-MRSA-II and ST22-MRSA-IV in the late 1990s; since 2002, the ST22-MRSA-IV clone has dominated (17, 20). Prior to 1999, ST22-MRSA-IV was detected only sporadically among MRSA isolates in Ireland, but by 2003, it accounted for 80% of MRSA BSIs, and despite a decline in the proportion of *S. aureus* infections due to MRSA in recent years, it has continued to account for 70 to 80% of MRSA BSIs each year (19).

While a limited number of sporadically occurring MRSA clones from patients in Irish hospitals in the 1980s and 1990s have been characterized using multilocus sequence typing (MLST), SCC*mec* typing, and DNA microarray analysis, e.g., ST5-MRSA-II and ST247-MRSA-Ia, there have been no systematic detailed studies of the genetic diversity of sporadic MRSA strains in Ireland (20, 21). These account for approximately 20 to 30% of MRSA BSIs in Ireland each year, as well as being identified each year among non-BSI isolates submitted to the Irish National MRSA Reference Laboratory (NMRSARL) from patients in hospitals with a variety of infections and from patient and environmental screening samples (19). In fact, the number of sporadic MRSA isolates identified among BSIs in Ireland increased from 12.1% in 2005 to 23.1% in 2011 (19). Numerous studies have shown that many MRSA clones that occur sporadically or not at all in one geographic region are often prevalent in another region and vice versa (20, 22, 23). However, previous studies that have investigated sporadic MRSA populations are limited in terms of sample size and/or depth of analysis (24–27).

Due to the potential of sporadic MRSA strains to replace currently dominant MRSA clones and because they account for a significant proportion of MRSA infections in Ireland each year, it is essential that populations of new and emerging MRSA strains be monitored. In addition, sporadic MRSA strains may constitute a significant potential reservoir for virulence and resistance genes located on MGEs, in particular SCC*mec* elements. Therefore, the present study investigated the genotypes, SCC*mec* types, and virulence and resistance genes within 88 MRSA isolates representative of 1,663 *pvl*-negative sporadically occurring MRSA isolates from patients in Irish hospitals between 2000 and 2012. Isolates were investigated using *spa* typing, MLST, SCC*mec* typing, and DNA microarray profiling. The 88 sporadic MRSA isolates were selected at the NMRSARL based on unusual antibiogram-resistogram (AR) and/or pulsed-field gel electrophoresis (PFGE) typing patterns which were different from that of the endemic strain that predominated in Irish hospitals during the study period, i.e., ST22-MRSA-IV. All *pvl*-positive MRSA isolates from Irish hospitals and community sources submitted to the NMRSARL for examination during the same period have been investigated as part of a separate study (28).

MATERIALS AND METHODS

Bacterial isolates. MRSA isolates identified by the NMRSARL were deemed to be sporadic if they exhibited unusual AR and/or PFGE typing patterns which were different from those of the endemic strain in Irish hospitals during the study period, i.e., ST22-MRSA-IV. Unusual AR type patterns included those that were different from previously described ST22-MRSA-IV AR (AR06) type patterns. Unusual PFGE patterns were identified using the criteria of Tenover et al. (29) and differed from PFGE patterns previously identified among ST22-MRSA-IV isolates by ≥7 PFGE bands. Using these criteria, a total of 1,663 *pvl*-negative sporadic MRSA isolates were identified by the NMRSARL from patients in Irish hospitals between 2000 and 2012. Of the 1,663 isolates, 841 were investigated and determined to be *pvl* negative either by PCR, as described previously (30), or using an in-house real-time PCR assay. The remaining 822 isolates were not investigated for *pvl*, as they yielded AR and/or PFGE typing patterns indicative of strains not previously associated with *pvl*, e.g., AR13 and AR14, and therefore a *pvl*-negative status was inferred for these (30, 31). All *pvl*-positive isolates recovered in Ireland during the study period between 2000 and 2012 were investigated as part of a separate study (28). Eighty-seven of the 1,663 *pvl*-negative sporadic MRSA isolates, representative of approximately 5% of sporadic *pvl*-negative MRSA isolates identified each year from patients in Irish hospitals during the 12-year study period (see Table S1 in the supplemental material) and representing as diverse a range as possible of AR and/or PFGE typing patterns, were selected for detailed investigation. In addition, one *pvl*-negative MRSA isolate recovered from a patient in the community but who had previously been hospitalized on several occasions was also included for investigation. This isolate harbored *mecC* (SCC*mec* type XI) and was included because this clone was recovered sporadically in two patients in two separate hospitals in Ireland in 2010 (2) and has recently been reported in several other European countries (32). The majority of the MRSA isolates (73.8% [65/88]) selected for study were recovered from infections (89% [58/65] BSIs and 10.8% [7/65] skin and soft tissue infections [SSTIs]), 11% (10/88) were colonizing isolates from patient screening, and no information was available for the remainder (14.8% [13/88]). Isolates were identified as *S. aureus*, and methicillin resistance was confirmed as described previously (30). Isolates were stored on Protect beads (Technical Service Consultants Limited, Heywood, United Kingdom) at −70°C prior to subsequent detailed analysis.

DNA microarray analysis. The 88 sporadic MRSA isolates were investigated by DNA microarray profiling using the StaphyType kit (Alere Technologies GmbH, Jena, Germany). The StaphyType kit consists of individual DNA microarrays mounted in 8-well microtiter strips which detect 333 *S. aureus* gene sequences and alleles, including species-specific, antimicrobial resistance and virulence-associated genes, SCC*mec* genes, typing markers, and a staining control (33, 34). ArrayMate software (version 2012-01-18) (Alere Technologies) was used to analyze data generated by the microarray system and to assign isolates to inferred sequence types (STs) and/or clonal complexes (CCs) by comparing the DNA microarray profile results of test isolates to microarray profiles of an extensive range of reference strains held in the ArrayMate database that have been previously typed by MLST (33, 34). The DNA array can assign all isolates investigated to the correct MLST clonal complex (CC) with a 98% correlation with STs assigned by MLST (21). Genomic DNA for use with the DNA microarray was extracted from all isolates by enzymatic lysis using the buffers and

solutions provided with the StaphyType kit and the Qiagen DNeasy blood and tissue kit (Qiagen, Crawley, West Sussex, United Kingdom). The primers, probes, and protocols for this DNA microarray system have been described in detail previously (34).

In order to visualize the similarities between the 88 sporadic isolates investigated (although not necessarily true phylogenetic relationships), a network tree was constructed using the complete DNA microarray hybridization profile data of the isolates using the software program SplitsTree, version 4.11.3 (35), as described previously (1). Array hybridization profiles of the isolates were converted into a series of strings of letters that can be handled by the software as sequences. For comparison, array profiles of 3,139 MRSA isolates representative of MRSA isolates globally that were characterized in a previous study were included for comparison (1).

Molecular typing. Genomic DNA for *spa* typing, MLST, and SCC*mec* typing was extracted from each isolate using enzymatic lysis and the DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions. Unless otherwise stated, PCRs were performed using GoTaq Flexi DNA polymerase (Promega Corporation, Madison, WI) according to the manufacturer's instructions and using the published methods for each PCR protocol as described below. PCR amplifications were performed in a G-storm GS1 thermocycler (Applied Biosystems, Foster City, CA). PCR products were visualized by conventional agarose gel electrophoresis and purified with the GenElute PCR cleanup kit (Sigma-Aldrich Ireland Ltd., Arklow, County Wicklow, Ireland).

All isolates underwent *spa* typing using the primers and thermal cycling conditions described by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet [http://www.seqnet.org]). Sequencing was performed commercially by Geneservice Limited (Source Bioscience, Dublin, Ireland) using an ABI 3730xl Sanger sequencing platform. Sequences were analyzed and were assigned to *spa* types using the Ridom StaphType software program, version 1.3 (Ridom GmbH, Wuerzburg, Germany) (36).

Although all isolates were assigned to STs and/or CCs using the DNA microarray, this system became available only during the latter half of the study. Prior to 2006, MLST had been performed on a subset of sporadic MRSA isolates ($n = 27$) representative of different *spa* types (Table 1). MLST was performed as described previously (37), sequences were analyzed using BioNumerics software, version 7.1 (Applied Maths, Ghent, Belgium), and alleles and STs were assigned using the MLST database (http://www.mlst.net/).

Fifty-two sporadic MRSA isolates underwent additional PCR-based SCC*mec* typing to distinguish between SCC*mec* types and subtypes when the DNA microarray was unable to further differentiate them. This included (i) SCC*mec* IV subtyping using the method previously described by Milheirico et al. (38), (ii) SCC*mec* IIA and IIB differentiation using a novel primer, Tn*554*r (5′-GATAGCAGTATGCCTTAATG-3′), targeting Tn*554*, which is present only in SCC*mec* IIA, and a previously described *ccrAB2* forward primer (α2) (39), (iii) SCC*mec* IIIA and IIIB differentiation using a multiplex PCR previously described by Oliveira and de Lencastre (40), and (iv) *ccrC* allotype identification using a previously described multiplex PCR to differentiate between SCC*mec* types V (*ccrC2*) and V_T (*ccrC2* and *ccrC8*) (41). In addition, two isolates harboring potentially novel SCC*mec* types were also further investigated using two previously described multiplex PCR schemes targeting the *mec* class and the *ccr* gene complexes (39). Finally, one isolate underwent PCR to confirm the presence of *mecC* and long-range PCR to confirm the presence of SCC*mec* XI using previously described primers (2). Long-range PCRs were performed using the Expand Long Template PCR system (Roche Diagnostics GmdH, Lewes, East Sussex, United Kingdom). The following MRSA control reference strains and clinical isolates were used as positive controls for SCC*mec* typing: AR07.4/0237 (SCC*mec* IIA/B) (20), E0898 (SCC*mec* III) (20), CA05 (SCC*mec* IVa) (42), 8/63-P (SCC*mec* IVb) (42), JCSC/4788 (SCC*mec* IVc) (43), JCSC/4469 (SCC*mec* IVd) (44), M04/0177 (SCC*mec* IVg) (17), E1749 (SCC*mec* IVh) (17), WIS (SCC*mec* V) (45), PM1 (SCC*mec* V_T) (41), and M10/0061 (SCC*mec* XI) (2).

RESULTS

Genotyping. Fifty-four different combinations of MLST CC/ST, *spa* types, and SCC*mec* types were identified among the 88 isolates, 41 of which were each represented by just one isolate (Table 1). The most prevalent type combination was CC8/ST8-t190-MRSA-IID and SCC_{M1} (11.4% [10/88]), followed by CC779/ST779-t878-MRSA-ψSCC*mec*-SCC-SCC_{CRISPR} (6.8% [6/88]) and CC22/ST22-t032-MRSA-IVh (5.7% [5/88]). Three type combinations occurred in 4.5% (4/88) of isolates, including CC8/ST8-t190-MRSA-IIE and SCC_{M1}, CC30/ST36-t018-MRSA-II, and CC45/ST45-t727-MRSA-IVa. Seven type combinations occurred in 2.3% (2/88) of isolates each, including the combination CC1-t127-MRSA-IVa and SCC*fus* and the combinations ST1-t2279-MRSA-IVa and SCC*fus*, CC1-t2279-MRSA-IVa, ST5-t045-MRSA-II, ST5-t242-MRSA-V_T, CC8/ST8-t008-MRSA-IVa, and ST59-t316-MRSA-V (harboring *ccrC8*) (Table 1). A total of 37 *spa* types and 13 MLST-CCs were identified (Table 1). The identification of STs using MLST or the DNA microarray or both was possible for 63/88 isolates, resulting in 17 STs, 4 of which were novel (Table 1). Overall, isolates belonging to CC8 predominated (24/88 [27.3%]), followed by isolates belonging to CC5 (17/88 [19.3%]), CC1 (10/88 [11.4%]), CC22 (9/88 [10.2%]), CC45 (9/88 [10.2%]), CC779 (6/88 [6.8%]), CC30 (6/88 [6.8%]), and CC59 (2/88 [2.3%]). The remaining CCs (6, 78, 398, 361, and 130) were each represented by one isolate (Table 1).

A total of 25 SCC*mec* types and subtypes were identified, including SCC*mec* IVa (20.5% [18/88]), which was the most prevalent, followed by SCC*mec* IID and SCC_{M1} (11.4% [10/88]), SCC*mec* II (10.2% [9/88]), SCC*mec* IVh (10.2% [9/88]), ψSCC*mec*-SCC-SCC_{crispr} (6.8% [6/88]), SCC*mec* IIE and SCC_{M1}, SCC*mec* V_T, and SCC*mec* IVa and SCC*fus* (4.5% [4/88]), SCC*mec* IVc (3.4% [3/88]), and SCC*mec* IIIB and SCC*mec* IVg (2.3% [2/88]); six SCC*mec* types were detected in just one isolate, including SCC*mec* types IID and III, SCC*hg* IIIA, and SCC*mec* IVd, VI, and XI (Table 1).

Two isolates (CC5/ST100-t002 and CC45/ST45-t747) carried possible novel SCC*mec* elements, because *mecA* was identified, but no *ccr* gene could be detected by the DNA microarray or by PCR-based SCC*mec* typing (Table 1). The remaining nine isolates harbored six possible novel SCC*mec* subtypes (10.2% [9/88]). Of these, three isolates harbored SCC*mec* elements assigned to previously described SCC*mec* types, but additional *ccr* genes were also identified, i.e., SCC*mec* I and *ccrC* (ST5-t109), SCC*mec* II and *ccrC* (ST36-t018), and SCC*mec* IV (nonsubtypeable) and *ccrB4* (CC5-t067) (Table 1); two isolates (CC5-t002 and ST930-t002) harbored SCC*mec* IV elements that could not be subtyped (Table 1).

Two novel SCC*mec* V or V_T variants were identified in four isolates due to the carriage of a class C *mec* complex but unusual combinations of *ccr* genes. The SCC*mec* V or V_T elements described in the literature to date have been described as harboring class C *mec* and (i) *ccrC* allele *ccrC1* in SCC*mec* V (5C) in MRSA strain WIS (45), (ii) *ccrC8* and *ccrC10* in SCC*mec* V (5C2&5) in MRSA strain UMCG-M4 (46), or (iii) *ccrC2* and *ccrC8* in SCC*mec* V_T (5C2&5) in MRSA strain PM1 (41, 47). However, four isolates in the present study carried class C *mec*, but one harbored *ccrC2* only (CC5-t002) and three isolates carried *ccrC8* only (CC5-t442 and two ST59-t316) (Table 1).

Overall, SCC*mec* IV types and subtypes predominated and accounted for 45.5% (40/88) of isolates, followed by SCC*mec* II

TABLE 1 Molecular characteristics of 88 sporadic MRSA isolates recovered from patients in Irish hospitals between 2000 and 2012

Isolate reference number(s)[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	Capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Locations where similar isolates have been reported (reference)
52, 88	CC/ST1-t2279	IVa (2)	III	8	D (1), E (1)	*blaZ* (2), *fusC* (1), *sdrM* (2)	*sea* (1), *sek* (2), *seq* (2), *seh* (2)	Western Australia (1)
62, 86	CC1-t2279[c]	IVa and SCC*fus* (2)	III	8	D	*blaZ* (2), *fusC* (2), *sdrM* (2)	*sea* (2), *sek* (2), *seq* (2), *seh* (2)	Malta (68)
48	CC1-t386	IVa (1)	III	8	E	*blaZ*, *erm*(C), *aphA3*, *sat*, *sdrM*	*seh*	Germany (1)
7	CC1-t127	IVa (1)	III	8	D	*blaZ*, *sdrM*	*sea* (1), *sek*, *seq*, *seh*	Western Australia (1)
38, 41	CC1-t127	IVa and SCC*fus* (2)	III	8	D	*blaZ* (2), *erm*(A) (2), *fusC* (2), *sdrM* (2)	*sea* (2), *sek* (2), *seq* (2), *seh* (2)	Malta (68)
29	CC1/ST1336-t127[c]	IVc (1)	III	8	D	*blaZ*, *tet*(K), *sdrM*	*sea*, *seb*, *sek*, *seq*, *seh*	None
66	CC1/ST1115-t127[c]	IVa (1)	III	8	E	*blaZ*, *erm*(C), *aphA3*, *sat*, *tet*(K), *sdrM*	*seh*	None
8, 19	CC/ST5-t045	II (2)	II	5	D (1), neg[e] (1)	*blaZ* (2), *erm*(A) (2), *aadD* (2), *sdrM* (2), *fosB* (2), *qac*(A) (1)	*tst* (2), *sea* (1), *egc* (2), *sed* (1), *sej* (1), *ser* (1)	None
32, 68	CC/ST5-t242[c]	V_T (2)	II	5	B	*blaZ* (2), *aacA-aphD* (2), *sdrM* (2), *fosB* (2)	*egc* (2)	USA (69)
85	CC5-t002	V_T (harboring *ccrC2*) (1)	II	5	G	*blaZ*, *aacA-aphD*, *sdrM*, *fosB*	*egc*, *sep*, *sed*, *sej*, *ser*	None
75	CC5-t002	II (1)	II	5	F	*erm*(A), *aacA-aphD*, *aadD*, *mupA*, *sdrM*, *fosB*, *qac*(C)	*egc*, *sep*	Pandemic (1)
72	CC5-t002	IVc (1)	II	5	F	*blaZ*, *sdrM*, *fosB*	*egc*, *sep*, *sed*, *sej*, *ser*	Denmark (38)
79	CC5-t002	IV (nonsubtypeable) (1)	II	5	F	*blaZ*, *sdrM*, *fosB*	*egc*, *sep*	Pandemic (1)
64	CC5/ST930[c,d]-t002[b]	IV (nonsubtypeable) (1)	II	5	B	*blaZ*, *erm*(C), *aacA-aphD*, *sdrM*, *fosB*	*egc*	None
21	CC5/ST100-t002[c,d]	Novel 1 (*mecA* only detected) (1)	II	5	E	*blaZ*, *aacA-aphD*, *sdrM*, *fosB*	*egc*	None
71	CC5-t067	IV (nonsubtypeable) & *ccrB4* (1)	II	5	F	*blaZ*, *msrA*, *mph*(C), *aacA-aphD*, *aadD*, *aphA3*, *sat*, *mupA*, *sdrM*, *fosB*, *qac*(C)	*egc*, *sep*	Spain (70)
55	CC5-t088	V_T (1)	II	5	D	*erm*(C), *sdrM*, *fosB*	*sea*, *egc*, *sed*, *sej*, *ser*	None
14	CC/ST5-t109[c,d]	I and *ccrC* (1)	II	5	B	*blaZ*, *erm*(A), *aacA-aphD*, *aphA3*, *sat*, *tet*(K), *sdr*(M), *fosB*, *qac*(A)	*egc*	None
67	CC5/ST1435[d]-t242[c]	V_T (1)	II	5	B	*blaZ*, *aacA-aphD*, *sdrM*,*fosB*	*egc*	None
78	CC5-t442	V (harboring *ccrC8*) (1)	II	5	E	*blaZ*, *aacA-aphD*, *sdrM*, *fosB*	*egc*, *sed*, *sej*, *ser*	Australia (71)
37	CC/ST5-t463	II (1)	II	5	A	*blaZ*, *erm*(A), *aadD*, *sdrM*, *fosB*	*tst*, *sea*, *egc*, *sed*, *sej*, *ser*	None
40	CC5-t1781	IVa (1)	II	5	G	*blaZ*, *msrA*, *mph*(C), *aphA3*, *sat*, *sdrM*, *fosB*, *qac*(C)	*egc*, *sep*, *sej*, *ser*	Germany, Canada (spa.ridom.de)
82	CC6-t701	IVh (1)	I	8	E	*blaZ*, *sdrM*, *fosB*	neg	Australia, Abu Dhabi, Hong Kong (72)
2, 6, 9, 13, 17, 24, 27, 50, 15, 16	CC/ST8-t190	IID and SCC_{M1} (10)	I	5	D (8), neg (2)	*blaZ* (10), *erm*(A) (10), *aacA-aphD* (10), *aadD* (1), *aphA3* (4), *sat* (4), *fusB* (1), *tet*(K) (1), *sdrM* (10), *cat* (1), *fosB* (10), *qacA* (9)	*sea* (8), neg (2)	None
5, 12, 60, 43	CC/ST8-t190	IIE and SCC_{M1} (4)	I	5	D (3), neg (1)	*blaZ* (4), *erm*(A) (4), *aacA-aphD* (4), *aadD* (1), *aphA3* (3), *sat* (3), *mupA* (1), *sdrM* (4), *fosB* (4), *qacA* (4)	*sea* (3), neg (1)	None
26	CC/ST8-t190[c]	IID (1)	I	5	A	*erm*(A), *aacA-aphD*, *aphA3*, *sat*, *sdrM*, *fosB*	*tst*, *sea*	None
56	CC/ST8-t190[c]	VI (1)	I	5	E	*blaZ*, *erm*(A), *aphA3*, *sat*, *sdrM*, *fosB*	neg (1)	None
39, 53	CC8-t008	IVa (2)	I	5	B (1), neg (1)	*blaZ* (2), *erm*(A) (1), *msrA* (1), *mph*(C) (1), *aphA3* (1), *sat* (1), *sdrM* (2), *fosB* (2)	ACME-*arc* (2)	USA (1)
42	CC/ST8-t064[c]	IVa (1)	Ì	5	E	*blaZ*, *erm*(C), *sdrM*, *fosB*	*seb*, *sek*, *seq*	USA, Switzerland (73, 74)
35	CC/ST8-t4268	IVd (1)	I	5	D	*blaZ*, *erm*(C), *aacA-aphD*, *dfrS1*, *tet*(M), *sdrM*, *fosB*, *qac*(A), *qac*(C)	*sea*, neg	None
61	CC/ST8-t1209[c]	IIIB (1)	I	8	neg	*blaZ*, *erm*(A), *aacA-aphD*, *aadD*, *tet*(M), *sdrM*, *fosB*, *qac*(A)	*sek*, *seq*	None
31	CC8/ST239-t030[c]	IIIB (1)	I	8	neg	*blaZ*, *erm*(A), *tet*(M), *sdrM*, *fosB*, *qac*(A)	*sek*, *seq*	Pandemic (75)
22	CC8/ST239-t037	IIIA (1)	I	8	D	*blaZ*, *erm*(A), *aacA-aphD*, *aphA3*, *sat*, *tet*(M), *sdrM*, *fosB*, *qac*(A)	*sea*, *sek*, *seq*	Pandemic (1)
18	CC8/ST239-t037[c]	III and SCC*hg* (1)	I	8	D	*blaZ*, *erm*(A), *aacA-aphD*, *tet*(K), *tet*(M), *sdrM*, *aphA3*, *sat*, *fosB*, *qac*(A)	*sea*, *sek*, *seq*	Pandemic (1)
11, 20, 25, 10, 28	CC/ST22-t032[c]	IVh (5)	I	5	B (3), neg (2)	*blaZ* (5), *erm*(C) (4), *aphA3* (1), *sat* (1), *qac*(A) (1)	*egc* (4), *sec* (2), *sel* (2)	Pandemic (1)

33	CC/ST22-t032[c]	IVg (1)	I	5	B	*blaZ*, *erm*(C)	*egc*, *sec*, *sel*	Pandemic (1)
30	CC/ST22-t022[c]	IVh (1)	I	5	B	*blaZ*, *erm*(C)	*egc*, *sec*, *sel*	Pandemic (1)
36	CC/ST22-t2951	IVh (1)	I	5	B	*blaZ*, *erm*(C), *lnu* (A), *aacA-aphD*, *aadD*, *mupA*, *cat*, *fosB*, *qac*(C)	*egc*	None
51	CC22-t1802	IVh (1)	I	5	B	*blaZ*, *erm*(C), *fosB*	*egc*, *sed*	None
57, 1, 3, 4	CC/ST36-t018[c]	II (4)	III	8	A (4)	*blaZ* (4), *erm*(A) (4), *aadD* (2), *sdrM* (4), *fosB* (4)	*tst* (3), *sea* (4), *sed* (1), *egc* (4)	UK (76)
63	CC/ST36-t018[c]	II and *ccrC* (1)	III	5	A	*blaZ*, *erm*(A), *aacA-aphD*, *aadD*, *sdrM*, *fosB*	*tst*, *sea*, *egc*	None
34	CC30/ST36-t012	II (1)	III	5	B	*blaZ*, *erm*(A), *aacA-aphD*, *aadD*, *mupA*, *sdrM*, *fosB*	*tst*, *egc*	UK (76)
23, 45, 54, 87	CC/ST45-t727	IVa (4)	IV	8	B (2), neg (2)	*blaZ* (4), *erm*(C) (2), *fusB* (4), *mupA* (1), *tet*(M) (2), *sdrM* (4), *fosB* (1)	*egc* (4)	Hong Kong, Australia (1)
58	CC/ST45-t727[c]	Novel 2 (*mecA* only detected) (1)	IV	8	neg	*blaZ*, *sdrM*	*egc*	None
77	CC/ST45-t132	IVa (1)	I	8	B	*blaZ*, *sdrM*	*egc*	Germany, Belgium (1)
73	CC/ST45-t026	IVa (1)	I	8	B	*blaZ*, *erm*(C), *sdrM*	*egc*, *sec*, *sel*	Germany, Belgium (1)
81	CC/ST45-t065	IVa (1)	I	8	B	*blaZ*, *erm*(A), *sdrM*	*egc*	Germany, Belgium (1)
76	CC/ST45-t015	IVc (1)	I	8	B	*blaZ*, *aadD*, *sdrM*	*egc*, *sec*, *sel*	Germany, Belgium (1)
43, 80	CC/ST59-t316[c]	V (harboring *ccrC8*) (2)	I	8	B	*blaZ* (2), *msrA* (2), *fusB* (2), *mupA* (2), *sdrM* (2)	*seb* (2), *sek* (2), *seq* (2)	Australia, Taiwan (1)
83	CC88/ST88-t186	IVa (1)	III	8	E	*blaZ*, *erm*(A), *sdrM*	*sec*, *sel*	Australia, Japan (1, 57)
70	CC130-t12399	XI (1)	III	8	neg	*sdrM*	neg	Europe, UK (32)
59	CC/ST361-t315[c]	IVg (1)	I	8	B	*blaZ*, *aacA-aphD*, *aphA3*, *sat*, *tet*(K), *sdrM*, *fosB*	*egc*	Western Australia (32)
84	CC/ST398-t011	IVa (1)	I	5	neg	*blaZ*, *aacA-aphD*, *tet*(M), *sdrM*	neg	Hong Kong, Belgium, Germany (1)
65, 69, 74, 44, 46, 47	CC/ST779-t878	ψSCC*mec*-SCC-SCC_{CRISPR} (6)	III	5	B	*blaZ* (6), *aadD* (1), *fusC* (6), *mupA* (1), *sdrM* (6)	*etD* (6), *edinB* (6), *seb* (1), *sed* (1), *sej* (1), *ser* (1)	Uk, Ireland, France, Australia (1, 6)

[a] Isolate reference numbers were assigned to each individual sporadic MRSA isolate for inclusion in network trees constructed using complete DNA microarray profile data for all 88 sporadic MRSA isolates using the SplitsTree software package (1, 35) (Fig. 1).

[b] IEC types were assigned as described previously. IEC type A includes *sea*, *sak*, *chp*, and *scn*; IEC type B includes *sak*, *chp*, and *scn*; IEC type C includes *chp* and *scn*; IEC type D includes *sea*, *sak*, and *scn*; IEC type E includes *sak* and *scn*; IEC type F includes *sep*, *sak*, *chp*, and *scn*; and IEC type G includes *sep*, *sak*, and *scn* (48). The number of isolates with each IEC type are indicated only when more than one IEC type was identified within a given type combination.

[c] MLST was performed on the isolates indicated before the DNA microarray became available. Isolates were selected for MLST based upon *spa* typing results.

[d] Novel MLST sequence types detected.

[e] neg, negative.

(29.5% [26/88]), SCC*mec* V (9% [8/88]), pseudo element ψSCC*mec*-SCC-SCC$_{crispr}$ (6.8% [6/88]), and SCC*mec* III (3.4% [3/88]) (Table 1). The majority of isolates carried *mecA*, with just one isolate (CC130-t12399) carrying *mecC*.

Virulence-associated genes. Immune evasion cluster (IEC) genes were detected among 84.1% (74/88) of sporadic MRSA isolates and included *scn* (84% [74/88]), *sak* (84% [74/88]), *chp* (44.3% [39/88]), *sea* (34.1% [30/88]), and *sep* (6.8% [6/88]) (Table 1). The most common IEC type as defined by Van Wamel et al. was IEC type B (34.1% [30/88]), followed by D (28.4% [25/88]), E (9.1% [8/88]), A (6.8% [6/88]), F (4.5% [4/88]), and G (1.1% [1/88]) (Table 1) (48). Clonal complex 5 exhibited the most IEC types, including IEC types A, B, and D to G, while CC22, CC45, CC59, and CC779 harbored IEC type B only. CC1, CC8, and CC30 harbored multiple IEC types, and all CC8 MRSA isolates harboring SCC*mec* IID plus SCC$_{M1}$ and SCC*mec* IIE plus SCC$_{M1}$ elements that harbored IEC genes exhibited IEC type D, and this association has been reported previously (Table 1) (21). The accessory gene regulator (*agr*) allele I was the most dominant *agr* type (47.7% [42/88]), followed by *agr* III (27.3% [24/88]), *agr* II (19.3% [17/88]), and *agr* IV (6.8% [6/88]) (Table 1). The capsule gene type 5 predominated and was detected in 60.2% (53/88) of isolates (Table 1).

The virulence-associated genes detected among the isolates belonging to the different CCs are shown in Table 1. The most common toxin genes detected were the enterotoxin gene cluster (*egc*), which was detected in 48.9% (43/88) of sporadic isolates belonging to six CCs, and the enterotoxin A gene *sea*, which was detected in 34.1% (30/88) belonging to four CCs (1, 5, 8, and 30). The enterotoxin genes *sek* and *seq* were harbored by 16% (14/88) of isolates (CC1, -8, and -59), and 11.4% (10/88) of isolates (all CC1) harbored the enterotoxin gene *seh*. The toxic shock toxin gene *tst* was detected in 9% (8/88) of isolates, all of which belonged to CC30 (83.3% [/6]) or CC5 (15.8% [3/19]). The enterotoxin genes *sec* and *sel* were harbored by 7.9% (7/88) of isolates from three CCs (22, 45, and 78), and *seb* was detected in 4.5% (4/88) of isolates from three CCs (1, 8, and 59). Various combinations of the enterotoxin genes *sed*, *sej*, and *ser* were detected in 11.4% (10/88) of isolates (CC5, ST30, CC22, and CC779). The ACME-*arc* genes were detected in 2.3% (2/88) of isolates (both ST8-MRSA-IVa). The exfoliative toxin gene *etD* and the epidermal cell differentiation inhibitor gene, *edinB*, were detected among all ST779-MRSA isolates (6.8% [6/88]), and the *sep* enterotoxin gene was detected in 6.8% (6/88) of isolates in one CC (CC5).

Antimicrobial resistance genes. The antimicrobial resistance genes detected among the isolates belonging to the different CCs are shown in Table 1. The most prevalent antimicrobial resistance genes detected among the 88 sporadic MRSA isolates other than *mec* were the beta-lactamase resistance gene *blaZ* (96.6% [84/88 isolates]) and *sdrM*, encoding an unspecific efflux pump (89.8% [79/88]). The *erm*(A) gene (encoding resistance to macrolides, lincosamides, and streptogramin B compounds) was detected in 40.9% of isolates (36/88) belonging to 6/13 CCs (CC1, -5, -8, -30, and -45 and ST88). The aminoglycoside resistance gene *aacA-aphD* (encoding resistance to amikacin, gentamicin, kanamycin, and tobramycin) was detected in 39.8% of isolates (35/88) in 6/13 CCs (CC5, -8, -22, and -30, ST361, and ST398). Other significant antimicrobial resistance genes detected included the fusidic acid resistance genes *fusB* and *fusC*, which were detected in 7.9% (7/88) and 11.4% (11/88) of isolates, respectively. The *fusB* gene was detected in three CCs (8, 45, and 59), and the *fusC* gene was detected in two CCs (1 and 779). The mupirocin resistance gene, *mupA*, was present in 10.2% of isolates (9/88) belonging to seven different CCs (5, 8, 22, 30, 45, 59, and 779).

Two or more resistance genes that encoded resistance to commonly used antimicrobial agents, including aminoglycosides, macrolides-lincosamides, tetracycline, fusidic acid, and mupirocin, were detected among 55.7% (49/88) of isolates and included isolates from all CCs except for the CCs represented by one isolate only (6, 78, 398, 361, and 130) (Table 1).

Similarities between sporadic and global isolates based on microarray data. Figure 1a shows a graphic representation of the diversity detected among the 88 sporadic MRSA isolates based on DNA microarray profiles. SplitsTree analysis using the transformed microarray profile data separated all 88 isolates into their MLST CCs, and within each CC, similar isolates were grouped closely together. For example, two closely related ACME-*arc*-positive ST8-MRSA-IVa isolates (isolates 39 and 53) (Table 1) clustered together and were separate from a more distantly related ACME-*arc*-negative ST8-MRSA-IVd isolate (isolate 35) (Table 1). Figure 1b shows a graphic representation of the relationships between the 88 sporadic MRSA isolates based on array profile data relative to a very large population of global MRSA isolates (n = 3,139). Within the 88 sporadic MRSA isolate population, isolates with specific CCs distributed among global isolates with the same CC in each case (Fig. 1b).

DISCUSSION

Many detailed investigations of the predominant nosocomial MRSA clones prevalent in different regions of the world have been reported, whereas in-depth systematic investigations of sporadically occurring MRSA clones are scarce. The highly clonal ST22-MRSA-IV strain continues to predominate in Irish hospitals, but the prevalence of sporadically occurring MRSA from BSIs increased 2-fold between 2005 and 2011 (19). This study is the first to investigate in detail the molecular epidemiology of sporadic MRSA isolates in Irish hospitals, and it has revealed extensive diversity in genetic backgrounds, SCC*mec* elements, and virulence and resistance genes. Comparative analysis of DNA microarray data from the 88 sporadic isolates investigated and the corresponding data from 3,139 global MRSA isolates revealed that the relationships between the sporadic MRSA isolates from patients in Irish hospitals reflects the relationships between global MRSA isolates (Fig. 1). An apparently reduced diversity of SCC*mec* elements in the 88 sporadic MRSA isolates compared to the global MRSA population likely reflects the reduced biodiversity associated with a restricted/insular geographic location (Fig. 1).

A total of 54 MLST, *spa*, and SCC*mec* type combinations were identified among the 88 sporadic MRSA isolates investigated, with 49 isolates (55.7%) carrying genes encoding resistance to two or more commonly used antimicrobial agents and 40 (38.6%) harboring two or more virulence-associated genes previously reported to be located on MGEs. Isolates belonging to CC8/ST8-t190-IID/IIE ± SCC$_{M1}$ predominated. Previous studies have demonstrated a reduced fitness associated with larger SCC*mec* elements (49, 50), and we previously speculated that the potential fitness cost associated with carrying a large SCC*mec*-SCC composite island (CI) may have contributed to the decline of ST8-MRSA-IIA-IIE and SCC$_{M1}$ (21) in Irish hospitals. However, the many resistance genes detected among isolates of this clone may also

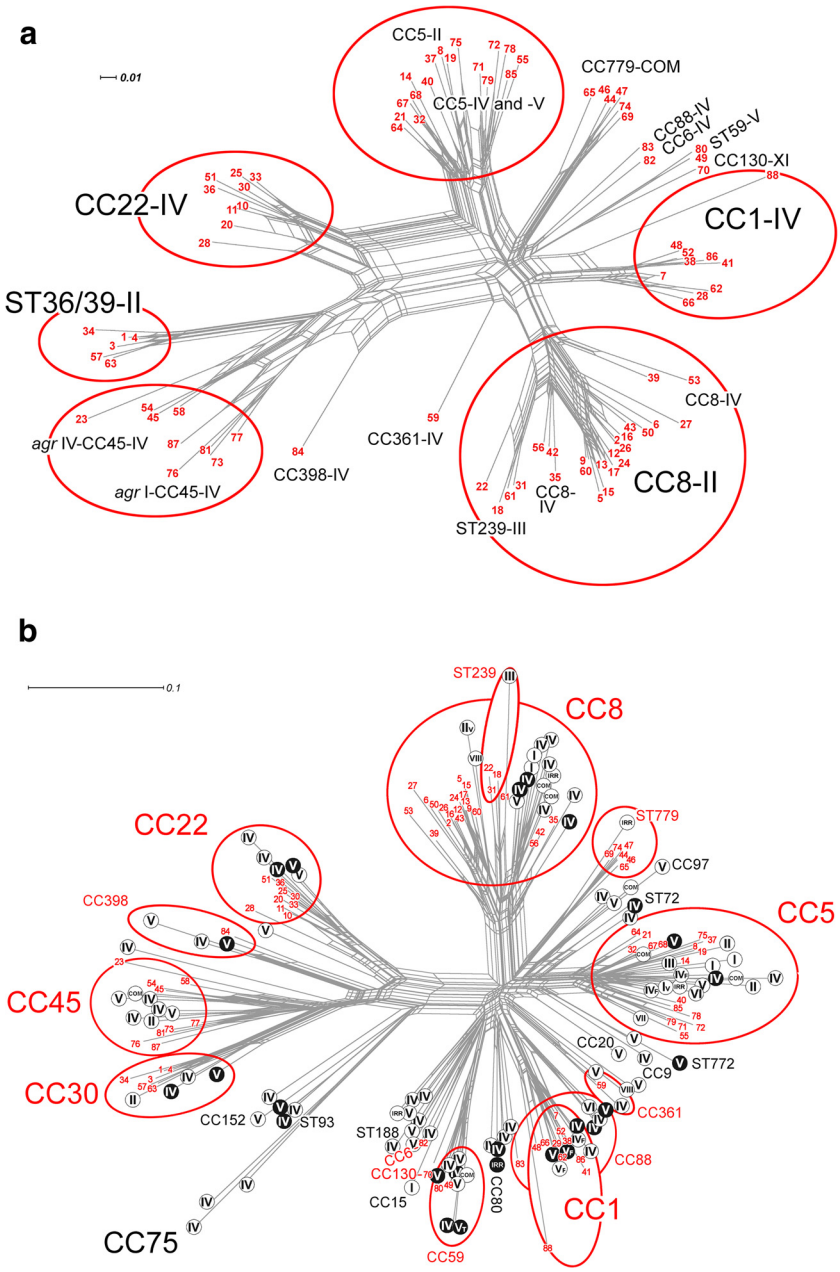

FIG 1 Network trees generated using the SplitsTree, version 4.11.3, software program (1, 35) and the StaphyType DNA microarray profiles as described previously (1) to visualize the similarities and relationships between CCs and mobile genetic elements, including SCC*mec*, for the 88 sporadic MRSA isolates investigated and a global population of MRSA isolates. (a) Network tree showing the relationships between the 88 sporadic MRSA isolates investigated. (b) Network tree showing the relationships between the 88 sporadic MRSA isolates investigated in the present study and a previously described global collection of MRSA isolates (n = 3,139) (1). Each sporadic MRSA isolate investigated in the present study is indicated with a number in red (numbers 1 to 88); the details of isolates represented by each number are listed in Table 1. The major CCs identified among isolates in the current study and the previous global study are circled in red, and Roman numerals indicate SCC*mec* types. In panel b, CCs and STs that were exhibited by MRSA strains from this study are shown in red, and if a CC or ST was not exhibited by any of the 88 sporadic MRSA strains, it is shown in black. MRSA strains from the previously described global population (1) that lacked the Panton-Valentine leukocidin toxin genes *lukF/S-PV* (*pvl*) are indicated using black letters on a white background, and *pvl*-positive MRSA strains are indicated using white letters on a black background. The scale bars show how the length of a branch translates in sequence divergence. The unit is divergent nucleotides divided by the length of the sequence analyzed. Abbreviations: IRR, irregular SCC*mec* elements; COM, composite or multiple SCC*mec* elements.

have contributed to its decline due to reduced fitness, but other lineage-specific factors may also have contributed (51).

The second most prevalent clone, ST779-t878-MRSA-ψSCC*mec*-SCC-SCC$_{crispr}$, also carried multiple resistance genes and a large SCC-CI element that may have originated in coagulase-negative staphylococci (CoNS). We recently reported the emergence of this clone in Irish hospitals (6), and ST779-MRSA has also been reported sporadically in Australia, Canada, Germany, Malaysia, Thailand, the United Arab Emirates, and the United Kingdom (http://saureus.mlst.net/) (2, 6, 24, 52). Fitness

costs associated with the carriage of a large SCC-CI and multiple MGE-located resistance genes may curtail the widespread emergence of this clone in the absence of selective pressure.

Extensive diversity was detected among the SCC*mec* elements harbored by the sporadic MRSA isolates and included 25 different SCC*mec* types and subtypes encompassing types and/or subtypes of SCC*mec* types I to VI, SCC*mec* type XI, two possible novel SCC*mec* elements, and six possible novel SCC*mec* subtypes. SCC*mec* type IV predominated, accounting for almost half of the isolates. Since SCC*mec* IV is also the SCC*mec* type of the ST22-MRSA-IV clone endemic in Irish hospitals for the last decade (17), it is clear that SCC*mec* IV is the dominant SCC*mec* element among all nosocomial MRSA isolates in Ireland. However, eight different subtypes were identified among the sporadic isolates, with SCC*mec* IVa being the most common (20.5% [18/40]). In contrast, SCC*mec* IVh predominates among isolates of the endemic ST22-MRSA-IV clone (17). It is not possible to discriminate between most SCC*mec* IV subtypes using the DNA microarray, and considering that these are associated with particular pandemic MRSA clones, e.g., SCC*mec* IVh in ST22 and SCC*mec* IVa in ST8/USA300, it is essential that detailed SCC*mec* IV subtyping be performed to ensure effective tracking and typing of these clones.

SCC*mec* V and V_T subtyping identified novel SCC*mec* V subtypes and provided further evidence of the diversity present in SCC*mec* V elements (46), including *ccrC* alleles. The CC59-MRSA-V clone usually harbors two *ccrC1* complexes (*ccrC1* allele 2 and *ccrC* allele 8) (47). However, the two CC59-MRSA-V isolates and a CC5-t442-MRSA isolate identified in the study harbored only *ccrC1* allele 8. Additionally, a CC5-t002-MRSA isolate harbored an SCC*mec* V element with just one *ccrC* allotype, *ccrC2*. These may represent possible SCC*mec* V variants or precursors in two separate CCs, CC5 and CC59.

The majority of isolates investigated had genotypes generally considered to be health care associated, including ST8-MRSA-IID/IIE ± SCC_{M1}, ST239-MRSA-III, ST36-MRSA-II, ST22-MRSA-IV, ST45-MRSA-IV, ST5-MRSA-II, and ST361-t315-MRSA-IVg (1, 53), each of which, apart from the ST361 MRSA-IVg isolate, was previously identified in Ireland as either predominant or sporadic strains (2, 20). Many of these clones predominate or have predominated in hospitals in other countries, and no major differences were noted between these isolates and those reported previously (1). A number of isolates with CC/ST and SCC*mec* type combinations commonly associated with *pvl*-positive community-associated MRSA (CA-MRSA) clones were also detected, including CC1-MRSA-IV (1, 54), CC59-MRSA-V (47, 55), ST8-t008-MRSA-IVa (1), CC5-MRSA-IV (1), CC5-MRSA-V (56), and CC88/ST88-t186-MRSA-IVa (1, 57). It should be noted that potential CA-MRSA-associated clones may be underrepresented in the present study due to the exclusion of *pvl*-positive sporadic MRSA isolates. The prevalence of CA-MRSA (both *pvl* positive and negative) among patients in Irish hospitals remains to be determined.

This study also found further evidence of the possible zoonotic spread of MRSA in Ireland. First, a CC130-MRSA-XI isolate recovered in 2007 was identified. We previously reported the recovery in 2010 of two sporadic CC130-MRSA-XI isolates from separate hospitals (2). The newly identified isolate exhibited a previously unreported *spa* type (t12399) harboring two additional *spa* repeats compared to *spa* type t843 exhibited by the CC130 MRSA isolates recovered in 2010 (2). The isolate was recovered from an elderly patient in the community who had previously been hospitalized on several occasions and who lived adjacent to a farm. Since its first detection, SCC*mec* XI has been reported sporadically among MRSA isolates belonging to a number of animal-associated MRSA lineages (predominantly CC130) in many different European countries from human and animal sources (32), and several studies have provided evidence for the zoonotic spread of these strains (58, 59). Other clones of possible animal origin were also identified, all recovered between 2007 and 2011, including the equine-associated ST8-t064-MRSA-IVa clone (60, 61), as well as the livestock-associated clone ST398-t011-MRSA-IVa and CC6-MRSA-IVh, which has been linked with camels (1, 62, 63). These findings highlight the importance of animals as a reservoir for MRSA and for effective surveillance to minimize the spread of these clones in hospitals.

The prevalence and diversity of resistance and virulence genes identified among the sporadic MRSA isolates also highlight the extensive reservoir of these genes that exist within the population of Irish MRSA. This, coupled with the range of genetic backgrounds of the isolates, highlights the potential for spread of these resistance genes and thus our ability to treat MRSA colonization and infection. For example, a high rate of the carriage of macrolide (57.9%) and aminoglycoside (43.4%) resistance genes was observed among isolates belonging to an extensive range of genetic backgrounds. Additionally, the high-level mupirocin resistance gene *mupA*, known to be carried on conjugative plasmids (64), was identified in 9/88 (10.2%) isolates belonging to seven different genetic backgrounds. Mupirocin is commonly used for MRSA nasal decolonization, and previous reports from Ireland have reported high-level mupirocin resistance rates among MRSA isolates from BSIs, ranging from 1.4% between 1999 and 2005 to 3.1% in 2011, predominantly among ST22-MRSA-IV and ST8-MRSA-IIA-IIE isolates (19). Lastly, in Ireland the rate of phenotypic fusidic acid resistance among MRSA isolates from BSIs increased from <10% to 34% between 1999 and 2011 (19). In the present study, 18/88 (20.5%) sporadic isolates harbored either the plasmid-located *fusB* gene or the SCC-associated *fusC* gene. More stringent use of these antimicrobial agents is warranted so that resistance does not become more widespread.

Few studies focused primarily on the detailed characterization of sporadic MRSA isolates. The main emphasis of most studies that reported such isolates concentrated on identifying the main MRSA lineages present in large populations of MRSA isolates from particular countries or from several hospitals (20, 65, 66). For example, while reporting the clonal replacement of CC5/ST228-MRSA-I and CC5-MRSA-II by the emerging CC22-MRSA-IV and CC45-MRSA-IV clones as the predominant nosocomial strains over an 11-year period in a German tertiary care hospital, Albrecht et al. also identified 17 *pvl*-negative sporadic MRSA isolates among 778 isolates investigated, including CC7-MRSA-IV, CC97-MRSA-IV, CC88-MRSA-IV, and CC30/ST36-MRSA-II (67), the former two of which were also identified in the present study. The diversity identified among the Irish sporadic MRSA isolates investigated in this study spans most of the lineages seen at the global level (Fig. 1). This may be because the strains, or at least some of them, have at some stage been endemic in Ireland since their evolutionary origin. However, it is important to emphasize that the origin of some MRSA strains can be polyphyletic resulting from multiple transmissions of identical or similar SCC*mec* elements from MRSA or CoNS into methicillin-susceptible *S. aureus* (MSSA) of one clonal lineage (1). Recur-

rent importation of MRSA strains from other countries is also likely to have been another significant factor contributing to the diversity found among the sporadic MRSA isolates. The latter suggestion is reflected by the findings of a recent study from this laboratory on *pvl*-positive MRSA recovered in Ireland over the last decade that revealed frequent importation of MRSA strains, particularly in recent years (28). While the increasing prevalence of sporadic MRSA strains in Ireland may be due to an increase in their importation or to the local emergence of strains, the decreasing prevalence of ST22-MRSA-IV in Irish hospitals may also have contributed, allowing for the emergence of these sporadic MRSA with enhanced virulence and resistance potential. However, further studies of both sporadic and endemic MRSA as well as MSSA are required in order to determine this.

In conclusion, the diversity detected among the 88 representative sporadic MRSA isolates, including SCC*mec* and SCC-associated elements and virulence-associated and antimicrobial resistance genes, and the number of different genetic lineages identified by MLST, *spa* typing, and DNA microarray analysis provide further evidence of the need for effective surveillance of this genetically diverse reservoir. Exchange of genetic material between these and other more prevalent MRSA strains may contribute to the emergence of successful MRSA strains in the future. Shore et al. previously demonstrated that there is a history of strain replacement approximately once per decade in Ireland, and therefore, it is important that emerging MRSA strains be detected early (20). The ST22-MRSA-IV clone has predominated for more than a decade in Irish hospitals, and its recent decline in prevalence suggests that a novel strain(s) may emerge in the near future.

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Panton-Valentine Leukocidin-Positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 Clones, Frequent Importation of Clones, Temporal Shifts of Predominant Methicillin-Resistant *S. aureus* Clones, and Increasing Multiresistance

Anna C. Shore,[a,b] Sarah C. Tecklenborg,[a] Gráinne I. Brennan,[c] Ralf Ehricht,[d] Stefan Monecke,[d,e] David C. Coleman[a]

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

There has been a worldwide increase in community-associated (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) infections. CA-MRSA isolates commonly produce the Panton-Valentine leukocidin toxin encoded by the *pvl* genes *lukF-PV* and *lukS-PV*. This study investigated the clinical and molecular epidemiologies of *pvl*-positive MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates identified by the Irish National MRSA Reference Laboratory (NMRSARL) between 2002 and 2011. All *pvl*-positive MRSA ($n = 190$) and MSSA ($n = 39$) isolates underwent antibiogram-resistogram typing, *spa* typing, and DNA microarray profiling for multilocus sequence type, clonal complex (CC) and/or sequence type (ST), staphylococcal cassette chromosome *mec* type assignment, and virulence and resistance gene detection. Where available, patient demographics and clinical data were analyzed. The prevalence of *pvl*-positive MRSA increased from 0.2% to 8.8%, and that of *pvl*-positive MSSA decreased from 20% to 2.5% during the study period. The *pvl*-positive MRSA and MSSA isolates belonged to 16 and 5 genotypes, respectively, with CC/ST8-MRSA-IV, CC/ST30-MRSA-IV, CC/ST80-MRSA-IV, CC1/ST772-MRSA-V, CC30-MSSA, CC22-MSSA, and CC121-MSSA predominating. Temporal shifts in the predominant *pvl*-positive MRSA genotypes and a 6-fold increase in multiresistant *pvl*-positive MRSA genotypes occurred during the study period. An analysis of patient data indicated that *pvl*-positive *S. aureus* strains, especially MRSA strains, had been imported into Ireland several times. Two hospital and six family clusters of *pvl*-positive MRSA were identified, and 70% of the patient isolates for which information was available were from patients in the community. This study highlights the increased burden and changing molecular epidemiology of *pvl*-positive *S. aureus* in Ireland over the last decade and the contribution of international travel to the influx of genetically diverse *pvl*-positive *S. aureus* isolates into Ireland.

Usually, methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be a health care-associated (HCA) pathogen, and it is frequently responsible for serious and often life-threatening infections in individuals with established risk factors, such as prolonged hospital stay and antibiotic usage, older age, recent surgery, or an immunocompromised state. Health care-associated MRSA isolates have been found to belong to five distinct clonal lineages, typically harbor the staphylococcal cassette chromosome *mec* (SCC*mec*) type I, II, or III, (or less frequently, SCC*mec* type IV, VI, or VIII), and often exhibit resistance to multiple classes of antimicrobial agents (1).

However, during the last decade, there has been a concurrent worldwide increase in the prevalence of community-associated (CA) MRSA infections among otherwise healthy individuals, often children and young adults, who exhibit none of the HCA risk factors (2, 3). These consist predominantly of skin and soft tissue infections (SSTIs) but also include necrotizing pneumonia, necrotizing fasciitis, and sepsis (2, 4–6). The pathogenesis of CA-MRSA has in some studies been attributed to the ability of these organisms to express the Panton-Valentine leukocidin (PVL) toxin (3, 7). Panton-Valentine leukocidin-positive MRSA infections have been reported in many different populations, particularly those in close contact or in poor socioeconomic situations (2).

Panton-Valentine leukocidin is a bicomponent beta-barrel toxin that causes leukocyte lysis or apoptosis via pore formation (8). PVL is encoded by two genes, *lukF-PV* and *lukS-PV*, which are carried on a variety of lysogenic bacteriophages (9). While outbreaks of PVL-producing methicillin-susceptible *S. aureus* (MSSA) isolates were reported in the 1950s and 1960s (10), PVL was first reported in newly emerging CA-MRSA strains in the 1990s (4, 11). While not all CA-MRSA isolates produce PVL, and there are conflicting data regarding the role of PVL in the pathogenesis of CA-MRSA infection, it is clear that the success of some CA-MRSA clones is associated with PVL, albeit not exclusively (12).

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Address correspondence to David C. Coleman, david.coleman@dental.tcd.ie.
A.C.S. and S.C.T. contributed equally to this article.
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TABLE 1 Numbers of *pvl*-positive MRSA and MSSA isolates identified each year between 2002 and 2011 by the Irish National MRSA Reference Laboratory

yr	MRSA isolates			MSSA isolates		
	No. identified by NMRSARL	No. investigated for *pvl*[a]	No. (%) confirmed *pvl* positive[b]	No. identified by NMRSARL	No. investigated for *pvl*[a]	No. (%) confirmed *pvl* positive[b]
2002	497	8	1 (0.2)[c]	1	1	1 (100)
2003	599	17	4 (0.7)[c]	0	0	0 (0)
2004	724	134	10 (1.4)[c]	15	14	3 (20)
2005	827	112	9 (1.1)[c]	43	30	5 (11.6)
2006	869	110	12 (1.4)	41	31	5 (12.2)
2007	782	120	17 (2.2)	42	29	6 (14.3)
2008	747	179	37 (5.0)	58	53	7 (12.1)
2009	605	187	32 (5.3)[d]	44	35	5 (11.4)
2010	596	160	28 (4.7)[d]	77	61	5 (6.5)
2011	456	190	40 (8.8)[d]	81	61	2 (2.5)
Total	6,702	1,217	190 (2.8)	401	315	39 (9.7)

[a] An isolate was selected for *pvl* investigation if it was from a suspected *pvl*-associated infection or, for MRSA only, if the isolate exhibited an antibiogram-resistogram (AR) and pulsed-field group (PFG) pattern distinct from that of previously or currently predominant *pvl*-negative health care-associated MRSA clones, e.g., AR-PFG 06-01, indicative of ST22-MRSA-IV, or AR-PFG 13/14-00, indicative of ST8-MRSA-IIA-E ± SCC_{M1}.

[b] The values shown in parentheses indicate the percentages of *pvl*-positive MRSA or MSSA isolates identified among the total number of MRSA or MSSA isolates investigated by the NMRSARL each year during the study period.

[c] The MRSA isolates recovered between 2002 and 2005 were described previously (30). One MRSA isolate (E1760) from that study was excluded because *pvl* was not detected, despite several attempts using PCR and DNA microarray profiling.

[d] One, eight, and nine *pvl*-positive isolates recovered in 2009, 2010, and 2011, respectively, were described previously (31).

MRSA isolates carrying the PVL toxin genes (*pvl*) are predominantly genetically distinct from HCA-MRSA, as they belong to more diverse clonal lineages and harbor the smaller SCC*mec* elements type IV, V, or V_T, and they are frequently not multiresistant (1, 3, 13). Different *pvl*-positive MRSA clones predominate in different regions, e.g., sequence type 8 (ST8)-MRSA-IV (USA300) in the United States (14), ST59-MRSA-V_T in Asia (13, 15), ST30-MRSA-IV in New Zealand (16), ST93-MRSA-IV in Australia (17), ST80-MRSA-IV in Europe (18) and the Middle East (1), ST88-MRSA-IV in Africa (19), and ST22-MRSA-IV and ST772-MRSA-V in India (20). However, recent studies highlighted the complex and changing epidemiology of *pvl*-positive MRSA, including (i) considerable variation in the prevalence rates of *pvl*-positive MRSA in different regions of the world (2, 17), (ii) the increasing prevalence and polyclonal population structure of *pvl*-positive MRSA isolates in Europe (1, 21, 22), (iii) the increasing prevalence of ST8-MRSA-IV in Europe and the decreasing prevalence of ST80-MRSA-IV (21), (iv) the increasing prevalence of multiresistant *pvl*-positive MRSA (22), and (v) the spread of *pvl*-positive MRSA into hospitals (14, 23–25). Furthermore, there has been an increasing frequency of reports of infections associated with *pvl*-positive MSSA (26, 27) that produce similar clinical presentations as *pvl*-positive MRSA, and the former are a potential reservoir for the emergence of *pvl*-positive MRSA.

In Ireland, MRSA is endemic in hospitals, and since 2002, the *pvl*-negative ST22-MRSA-IV clone has accounted for 70 to 80% of MRSA from bloodstream infections (BSIs) each year (28, 29). Between 1999 and 2005, a prevalence rate of 1.8% was reported for *pvl*-positive MRSA in Ireland, and six distinct *pvl*-positive MRSA clones (ST30, ST8, ST22, ST80, ST5, and ST154, all harboring SCC*mec* IV) were identified, some of which were probably imported (30). In 2011, we reported multiple importations of the multiresistant *pvl*-positive ST772-MRSA-V clone into Ireland and a cluster of this clone in a neonatal intensive care unit (NICU) in an Irish hospital (31). However, there have been no published data on the overall prevalence and molecular epidemiological characteristics of the *pvl*-positive MRSA population in Ireland since 2005 and only a single report of a familial outbreak of *pvl*-positive MSSA in Ireland; no molecular epidemiological typing of the isolates was undertaken (32). The purpose of the present study was to investigate the clinical and molecular epidemiologies of *pvl*-positive MRSA and MSSA identified by the Irish National MRSA Reference Laboratory (NMRSARL) between 2002 and 2011.

MATERIALS AND METHODS

Bacterial isolates. The NMRSARL investigated 7,103 *S. aureus* isolates (6,702 MRSA and 401 MSSA) between 2002 and 2011, of which 1,531 were examined for the presence of the *lukF-PV* and *lukS-PV* genes (*pvl*) (Table 1). An isolate was selected for *pvl* investigation if it was recovered from a suspected *pvl*-associated infection; for MRSA only, an isolate was selected if it exhibited an antibiogram-resistogram (AR) pattern and/or pulsed-field group (PFG) distinct from that of previously or currently predominant *pvl*-negative health care-associated MRSA clones, e.g., AR-PFG 06-01, indicative of ST22-MRSA-IV or AR-PFG 13/14-00, indicative of ST8-MRSA-IIA-E ± SCC_{M1} (28, 33). Of the 1,532 isolates investigated for *pvl* (1,217 MRSA and 315 MSSA), 229 (190 MRSA and 39 MSSA) were *pvl* positive and were investigated further (Table 1). This included 24/25 previously described *pvl*-positive MRSA isolates recovered between 2002 and 2005 (30) and 18 previously described *pvl*-positive ST772-MRSA-V isolates recovered between 2009 and 2011 (31). One MRSA isolate (E1760) previously reported as *pvl* positive (30) was excluded from the present study because *pvl* was not detected despite several attempts using PCR and DNA microarray profiling. Only one isolate per patient was investigated unless AR and pulsed-field gel electrophoresis typing indicated the presence of a second strain from a particular patient. When possible, patient demographics and clinical data were collected from isolate submission forms, telephone follow-ups, and follow-up questionnaires. The isolates were defined as clusters if they were recovered from members of one family/household, within a hospital, or both. Within each cluster, the isolates were recovered between 3 months and 2 years

apart. Each isolate within a cluster was recovered from a different person or environmental source. This paper does not include any identifying or potentially identifying patient information.

Confirmation of isolates as *S. aureus*, methicillin susceptibility testing, and detection of the *lukF-PV* and *lukS-PV* genes. On receipt by the NMRSARL, all *S. aureus* isolates were inoculated onto Protect beads (Technical Service Consultants Ltd., Heywood, United Kingdom) and stored at −70°C prior to subsequent investigation. The isolates were confirmed to be *S. aureus* using the tube coagulase test, and methicillin resistance was investigated with 10-μg and 30-μg cefoxitin discs (Oxoid Ltd., Basingstoke, United Kingdom), as described previously (30). The detection of the *lukF-PV* and *lukS-PV* genes was performed by PCR, as described previously (4); isolates recovered in the final quarter of 2011 were tested using an in-house real-time PCR assay designed to detect the *mecA*, *nuc*, and *pvl* genes. The identification of isolates as *S. aureus*, the presence or absence of *mecA*, and the presence of the *lukF-PV* and *lukS-PV* genes were also confirmed in all isolates using DNA microarray profiling, as described below.

Phenotypic and genotypic characterization of *pvl*-positive *S. aureus* isolates. All 229 *pvl*-positive *S. aureus* isolates underwent antimicrobial susceptibility testing, *spa* typing, and DNA microarray profiling. For the 18 *pvl*-positive ST772-MRSA-V isolates included in the study, this analysis was performed previously, and three of these isolates also underwent multilocus sequence typing (MLST) (31). The 24 previously described *pvl*-positive MRSA isolates recovered between 2002 and 2005 included in the study underwent previous antimicrobial susceptibility, MLST, SCC*mec* typing, and toxin gene profiling for a limited number of toxin genes (30).

Antimicrobial susceptibility testing. The susceptibility of each isolate to a panel of 23 antimicrobial agents was determined by disk diffusion, as described previously (30). The antimicrobial agents tested were amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampin, spectinomycin, streptomycin, sulfonamide, tetracycline, tobramycin, trimethoprim, and vancomycin.

DNA microarray analysis. DNA microarray analysis was performed on all isolates using the StaphyType kit (Alere Technologies GmbH, Jena, Germany), which simultaneously detects 333 *S. aureus* gene targets, including species markers, antimicrobial resistance and virulence-associated genes (including *lukF-PV*, *lukS-PV* and *mecA*), and SCC*mec*-associated genes and typing markers allowing isolates to be assigned to MLST sequence types (STs) and/or clonal complexes (CCs), and SCC*mec* types (34, 35). The DNA microarray procedure was performed according to the manufacturer's instructions.

PCR detection of antimicrobial resistance genes. Isolates that exhibited phenotypic resistance to particular antimicrobial agents for which associated resistance genes were not detected by the DNA microarray, or for which resistance genes were detected but partial or none of the associated resistance phenotypes were detected, were further investigated by PCR to confirm the presence or absence of these resistance genes. These investigations included PCRs using previously described primers to detect *mupA* (36), *aphA3* (37), *aacA-aphD* (37), *fusB* (38), *tet*(K) (36), *tet*(M) (36), *aadD* (39), and *qacA* (40), and also novel primers to detect *qacC*, *msr*(A), *dfrS1*, *lnu*(A), *mph*(C), and *blaZ* (see Table S1 in the supplemental material).

Statistical analysis. A two-sample *z* test was used to assess the significance of the difference between the two population proportions. A *P* value of ≤0.05 was considered significant.

RESULTS

A total of 229 *pvl*-positive *S. aureus* isolates were identified by the NMRSARL between 2002 and 2011, including 190 MRSA and 39 MSSA isolates representing 2.8% and 9.7% of all MRSA and MSSA isolates, respectively, submitted to the NMRSARL during this time (Table 1). Overall, the prevalence of *pvl*-positive MRSA among all MRSA isolates submitted to the NMRSARL increased significantly during the study period ($P < 0.0005$) from 0.2% in 2002 (1/497) to 8.8% (40/456) in 2011, with those two specific years recording the lowest and highest prevalence rates, respectively (Table 1). In contrast, for *pvl*-positive MSSA, the prevalence rate among all MSSA isolates submitted to the NMRSARL decreased significantly ($P < 0.0005$) from 20% in 2004 (3/15) to 2.5% (2/81) in 2011 (Table 1).

Genotyping. The *pvl*-positive MRSA ($n = 190$) and MSSA ($n = 39$) isolates were assigned to 11 and five MLST clonal complexes (CCs), respectively (Table 2). For MRSA, the isolates were assigned to either SCC*mec* type IV (79.5% [151/190]) or V (20.5% [39/190]), and to 16 genotypes (CC/ST-SCC*mec* types) (Table 2), with CC/ST8-MRSA-IV predominating (33.7% [64/190]), followed by CC/ST30-MRSA-IV (21.1% [40/190]), CC/ST80-MRSA-IV (14.2% [27/190]), CC1/ST772-MRSA-V (13.2% [25/190]), CC/ST22-MRSA-IV (6.3% [12/190]), ST59/952-MRSA-V (4.7% [9/190]), ST93-MRSA-IV (3/190 [1.6%]), and CC1-MRSA-IV (1.1% [2/190]) (Table 2). The remaining eight MRSA genotypes were each represented by one isolate only (Table 2).

Among the *pvl*-positive MSSA isolates, CC30 was the dominant clone, with 38.5% (15/39) of the isolates being assigned to this genotype (Table 2). CC22-MSSA accounted for 25.6% (10/39) of MSSA isolates, while CC121-MSSA, CC1-MSSA, and CC88-MSSA accounted for 18% (7/39), 10.3% (4/39), and 7.7% (3/39) of the isolates, respectively (Table 2).

Temporal changes in the predominant clonal types of *pvl*-positive MRSA. Fig. 1 shows the percentage of *pvl*-positive MRSA isolates assigned to each genotype for each year between 2002 and 2011. Ten of the genotypes identified between 2006 and 2011 were not identified between 2002 and 2005, including ST93-MRSA-IV, CC/ST59-MRSA-IV/V, and ST772-MRSA-V. The latter was identified for the first time in 2009, when it accounted for just 3.1% (1/32) of the isolates, but this increased to 28.6% (8/28) in 2010, and it was the predominant genotype in 2011, accounting for 40% (16/40) of *pvl*-positive MRSA isolates (Fig. 1).

The CC/ST30-MRSA-IV clone predominated and was at its most prevalent in 2004, when it accounted for 70% of *pvl*-positive MRSA isolates (7/10). Subsequently, the prevalence of CC/ST30-MRSA-IV varied significantly each year between 2005 and 2011, accounting for 33.3% (3/9) of the isolates in 2005 but just 5% (2/40) of the isolates in 2011 (Fig. 1). The CC/ST8-MRSA-IV clone predominated and was at its most prevalent in 2005, when it accounted for 66.7% of the isolates (6/9); afterwards, however, the prevalence of this clone varied dramatically each year between 2006 and 2011, e.g., despite a decrease to 33.3% (4/12) in 2006, a rise in the prevalence of this clone was noted between 2006 and 2009 to 46.9% (15/32), followed by an overall decrease to 27.5% (11/40) in 2011 (Fig. 1).

Apart from 2002, when just one *pvl*-positive MRSA isolate was identified and was assigned to CC80/ST80-MRSA-IV, the highest prevalence of this clone was in 2007, when it accounted for 47.1% (8/17) of the isolates. Subsequently, the prevalence of this clone declined, and by 2011, it accounted for just 2.5% of the isolates (1/40) (Fig. 1).

Prior to 2008, only one *pvl*-positive ST22-MRSA-IV isolate had been detected (in 2003). Despite the low numbers of the isolates, an increase in the prevalence of *pvl*-positive ST22-MRSA-IV was

TABLE 2 Phenotypic and genotypic typing data for *pvl*-positive MRSA ($n = 190$) and MSSA ($n = 39$) isolates identified by the Irish National MRSA Reference Laboratory between 2002 and 2011

Typing category results (n):									
	Genotype[a]						Antibiotic resistance profiles		Virulence genes (% indicated when not 100%)[e]
CC[a]	MRSA	MSSA	*spa*[b]	*agr*[a]	Capsule[a]	IEC[a,b,c]	Phenotype (% indicated when not 100%)[d]	Genotype (% indicated when not 100%)	
1	CC1-MRSA-IV (2)		t128 (1), t8968 (1)	III	8	D (1), B (1)	AMP, CAD, TET (50)	*blaZ*, *sdrM*, *tet*(K) (50)	*sea* (50), *sec&sel* (50), *sek&seq* (50), *seh*
	ST772-MRSA-V (25)		t657 (24), t345 (1)	II	5	Novel: *scn&sea*	AMP, AMI, CAD (88), CIP, ERY, GEN, KAN, NEO, TOB, TMP	*blaZ*, *sdrM*, *msr*(A), *mph*(C), *aacA-aphD*, *aphA3&sat*, *fosB*	*sea*, *sec&sel*, *egc*
	CC1-MRSA-V (1)		t127	III	8	D	AMP, FUS, GEN, KAN, NEO, TOB, TET	*blaZ*, *sdrM*, *aacA-aphD*, *aphA3&sat*, *tet*(K), *tet*(M), *fusC*	*sea*, *sek&seq*, *seh*
		CC1-MSSA (4)	t127 (2), t177 (1), t12303 (1)	III	8	D	AMP, CAD (75), FUS, KAN (25), NEO (25)	*blaZ*, *sdrM*, *aphA3&sat* (25), *ileS2* (50), *fusC*, *qacA* (50)	*sea*, *sek&seq*, *seh*
5	CC5-MRSA-V (1)		t311	II	5	A	AMP, CAD, TET, TMP	*blaZ*, *sdrM*, *tet*(K), *dfrS1*, *fosB*	*sea*, *edinA*, *egc*
	CC5-MRSA-IV (1)		t311	II	5	A	AMP, CAD	*blaZ*, *sdrM*, *fosB*	*sea*, *edinA*, *egc*
8	CC/ST8-MRSA-IV (64)		t008 (47), t051 (4), 121 (3), t068 (2), t4229 (1), t304 (1), t024 (1), t681 (1), t4306 (1), t11157 (1), t596 (1), t1635 (1)	I	5	B (63), neg (1)	AMI (21.8), AMP, CAD (20.3) (I), CHL (1.5), CIP (46.9), ERY (84.3), GEN (1.5), KAN (81.3), LIN (3.1), MC (6.3), MUP (1.5), NEO (81.3), PMA (1.5), TOB (1.5), TET, (9.4) TMP (3.1), LNZ (1.6)	*blaZ* (90.6), *sdrM*, *tet*(K) (9.4), *lnu*(A) (3.1), *msr*(A) (84.3), *mph*(C) (84.3), *aacA-aphD* (1.5), *aphA3&sat* (81.3), *fosB* (100), *merA&merB* (6.3), *qacC* (4.7), *ileS2* (1.5), *cfr&fexA* (1.5), *dfrS1* (3.1)	*sek&seq* (96.9), *sed*, *sej&ser* (4.7), ACME (89.1)
	CC8-MRSA-V (1)		t008	I	5	B	AMP	*sdrM*, *fosB*	*sek&seq*, ACME
22	CC/ST22-MRSA-IV (12)		t852 (7), t2480 (1), t3107 (1), t4463 (1), t5422 (1), t005 (1)	I	5	B (11), neg (1)	AMI (25), AMP, CAD (50), CIP (75), ERY (66.7), GEN (25), KAN (91.7), NEO (33.3), TOB (91.7), TMP (66.7)	*blaZ*, *aacA-aphD* (91.7), *dfrS1* (66.7), *erm*(C) (66.7), *aadD* (75)	*egc*
		CC22-MSSA (10)	t005 (7), t891 (2), t1869 (1)	I	5	B	AMI (10), AMP, CAD (60), FUS (10), GEN (80), KAN, TOB (90), TMP (90)	*blaZ*, *aacA-aphD*, *dfrS1* (90)	*egc*
30	CC/ST30-MRSA-IV (40)		t019 (22), t012 (12), 3800 (2), t122 (1), t275 (1), t318 (1), t9904 (1)	III	8	B (37), A (2), neg (1)	AMP, CAD (82.5), CIP (5), FUS (35), TET (2.5), TMP (2.5)	*blaZ*, *sdrM*, *tet*(K) (2.5), *fosB*, *dfrS1* (2.5), *fusC* (35)	*sea* (5), *tst* (35), *egc*
		CC30-MSSA (15)	t021 (6), t318 (4), t990 (1), t3502 (1), t1055 (1), t11156 (1), t433 (1)	III	8	A (2), B (11), neg (3)	AMP (73.3), CAD, CIP, ERY (6.7), TET (13.3), TMP (13.3)	*blaZ* (73.3), *mph*(C) (6.7), *sdrM*, *tet*(K) (13.3), *fosB*, *dfrS1* (13.3)	*sea* (13.3), *sec&sel* (6.7), *sek&seq* (6.7), *tst* (13.3), *egc* (86.7)
45	CC45-MRSA-V (1)		t620	I	8	B	AMP, TET	*blaZ*, *sdrM*, *tet*(K)	*sec&sel*, ACME, *egc*
59	ST59/952-MRSA-V (9)		t437	I	8	C	AMP, CAD (20), CHL (88.9), ERY (88.9), KAN (88.9), LIN (88.9), NEO (88.9), STR (80), TET (60)	*blaZ* (11.1), *sdrM*, *tet*(K) (66.7), *aphA3&sat* (88.9), *erm*(B) (88.9), *cat*-pC223 (88.9)	*seb*, *sek&seq*
	ST59-MRSA-IV (1)		t437	I	8	A	AMP, ERY, KAN, LIN, NEO, STR	*blaZ*, *sdrM*, *aphA3&sat*, *erm*(B)	*sea*, *seb*, *sek&seq*
80	CC80/ST80-MRSA-IV (27)		t044 (21) t376 (5) t131 (1)	III	8	E	AMI (3.7), AMP, CAD (85.2), CHL (3.7), CIP (3.7), ERY (40.7), FUS (74.1), KAN, NEO, TET (77.8), TMP (3.7)	*blaZ* (77.8), *sdrM*, *dfrS1* (3.7), *tet*(K) (70.4), *aphA3&sat*, *fusB* (77.8), *erm*(C) (40.7), *tet*(K) (70.4), *cat*-pC221 (3.7), *dfrS1* (3.7)	*etD*, *edinB*

88		CC88-MSSA (3)	t186 (2) t448 (1)	III	8	F	AMP, CAD (66.7), ERY (33.3), TET (66.7), TMP (33.3)	*blaZ*, *sdrM*, *tet*(K) (66.7), *erm*(C) (33.3), *dfrS1* (33.3)	*sek*&*seq* (33.3), *sep*
93	ST93-MRSA-IV (3)		t3949 (1) t1819 (1) t202 (1)	III	8	B	AMP, CAD (66.7), ERY (33.3)	*blaZ*, *sdrM*, *msr*(A) (33.3), *mph*(C) (33.3), *qacC* (33.3)	*CM14*
121		CC121-MSSA (7)	t159 (5), t435 (2)	IV	8	E	AMP, CAD (28.6) (1), CHL (14.3), ERY (57.1), LIN (14.3), STR (14.3), TET (28.6), TMP (28.6)	*blaZ*, *sdrM*, *tet*(K) (28.6), *fosB*, *erm*(C) (57.1), *cat*-pC221 (14.3), *dfrS1* (28.6)	*seb* (57.1), *egc*, *CM14*
152	ST152-MRSA-V (1)		t355	I	5	E	AMP, CAD, GEN, KAN, TOB	*blaZ*, *sdrM*, *aacA-aphD*	*etD*&*edinB*
154	ST154-MRSA-IV (1)		t667	III	8	Neg	AMP, CAD, CIP, SPC, TET	*blaZ*, *sdrM*, *tet*(M), *cat*-pMC524	None detected

[a] The StaphyType DNA microarray kit (Alere Technologies) was used for assigning the isolates to a multilocus sequence type (MLST) sequence type (ST) and/or a clonal complex (CC), a staphylococcal cassette chromosome *mec* (SCC*mec*) type (for MRSA only), accessory gene regulator (*agr*), capsule, and immune evasion complex (IEC) types. Forty-three MRSA isolates previously underwent MLST and SCC*mec* typing, namely, 18 ST772-MRSA-V, two ST22-MRSA-IV, 11 ST30-MRSA-IV, eight ST8-MRSA-IV, two ST80-MRSA-IV, one ST154-MRSA-IV, and one ST5-MRSA-IV isolates (30, 31).

[b] The number of isolates (*n*) represented by each *spa* type or IEC type are indicated in parentheses only when more than one *spa* or IEC type was identified within a genotype.

[c] Immune evasion complex (IEC) types were defined as described previously (59): 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*; novel, novel IEC type consisting of *sak* and *sea* (41); neg (negative), no IEC genes detected.

[d] The susceptibility of each isolate to a panel of 23 antimicrobial agents was determined by disk diffusion, as described previously (30). The antimicrobial agents tested were amikacin (AMI), ampicillin (AMP), cadmium acetate (CAD), chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), ethidium bromide (ETBR), fusidic acid (FUS), gentamicin (GEN), kanamycin (KAN), lincomycin (LIN), mercuric chloride (MC), mupirocin (MUP), neomycin (NEO), phenyl mercuric acetate (PMA), rifampin, spectinomycin (SPC), streptomycin (STR), sulfonamide, tetracycline (TET), tobramycin (TOB), trimethoprim (TMP), and vancomycin. The ST8-MRSA-IV *cfr*-positive isolate M05/0060 was tested for linezolid resistance (LNZ), as described previously (42).

[e] Excluding *lukF-PV* and *lukS-PV*, which were detected in all isolates.

noted between 2009 (3.1% [1/32]) and 2011 (12.5% [5/40]) (Fig. 1). The first ST59/952-MRSA-V isolates were detected in 2006, and a small number of the isolates of this clone were subsequently detected each year apart from 2010 (Fig. 1). The highest prevalence of this clone occurred in 2009 (12.5% [4/32]). Only three ST93-MRSA-IV isolates were identified, one in 2009 and two in 2011. All other clones were represented by one or two isolates only (Fig. 1).

Characteristics of *pvl*-positive *S. aureus* isolates. The virulence and resistance gene profiles of the isolates identified within each genotype of *pvl*-positive MRSA and MSSA are shown in Table 2, and the main characteristics of the isolates within lineages, i.e., CCs, represented by more than one isolate, are described below.

CC1. The majority of CC1/ST772-MRSA-V isolates exhibited *spa* type t657 (96% [24/25]), and all exhibited resistance to multiple antimicrobial agents, including ciprofloxacin, trimethoprim, erythromycin, and aminoglycosides, the latter two encoded by *msr*(A) and *mph*(C) and by *aacA-aphD* and *aphA3*, respectively. The enterotoxin genes *sec*&*sel* ("&" denotes linked genes) and *egc*, as well as the novel immune evasion complex (IEC) type consisting of *scn* and *sea*, were identified in all ST772-MRSA-V isolates (41).

The other CC1 genotypes identified (CC1-MRSA-IV, CC1-MRSA-V, and CC1-MSSA) exhibited different *spa*, *agr*, capsule, and IEC types from those of CC1/ST772-MRSA-V. The CC1-MRSA-V isolate also exhibited resistance to multiple antimicrobial agents and carried multiple resistance genes, but apart from aminoglycoside resistance encoded by *aphA3* and *aacA-aphD*, these were different from those detected in CC1/ST772-MRSA-V and included tetracycline resistance encoded by *tet*(K) and *tet*(M) and fusidic acid resistance encoded by *fusC*. The two CC1-MRSA-IV isolates carried fewer resistance genes, with just one isolate carrying *tet*(K). The CC1-MRSA-IV/V isolates lacked *egc*, but various other enterotoxin genes were detected, including *sea*, *sec*&*sel*, *sek*&*seq*, and *seh*.

Of the four CC1-MSSA isolates identified, two exhibited the same *spa* type, t127, as the CC1-MRSA-V isolate. Multiple resistance genes were detected among these isolates, including *aphA3*, *fusC*, *ileS2*, and *qacA*, but for the latter two, phenotypic resistances to mupirocin and quaternary ammonium compounds were not detected. Toxin genes similar to those detected in CC1-MRSA were detected among the CC1-MSSA isolates, namely, *sea*, *sek*&*seq*, and *seh*. In fact, *seh* was unique to CC1 and was detected in all isolates except those belonging to ST772.

CC5. The two CC5 MRSA isolates identified, one with SCC*mec* IV and the other with SCC*mec* V, exhibited the same *spa*, *agr*, capsule, and IEC types. Only the CC5-MRSA-V isolate carried *dfrS1* and *tet*(K) and exhibited resistances to trimethoprim and tetracycline, respectively, and both isolates carried *sea*, *egc*, and the epidermolytic toxin gene *edinA*.

CC8. Although 12 *spa* types were identified among the CC/ST8-MRSA-IV isolates, t008 predominated (73.4% [47/64]). The majority of CC/ST8-MRSA-IV isolates exhibited resistances to kanamycin and neomycin encoded by *aphA3* and to erythromycin encoded by *msr*(A), and almost half of the isolates were resistant to ciprofloxacin. Slightly <10% of the CC/ST8-MRSA-IV isolates were tetracycline resistant and carried *tet*(K). One isolate carried *cfr* and *fexA* and exhibited chloramphenicol and linezolid resistances (42). The majority of the isolates carried the enterotoxin

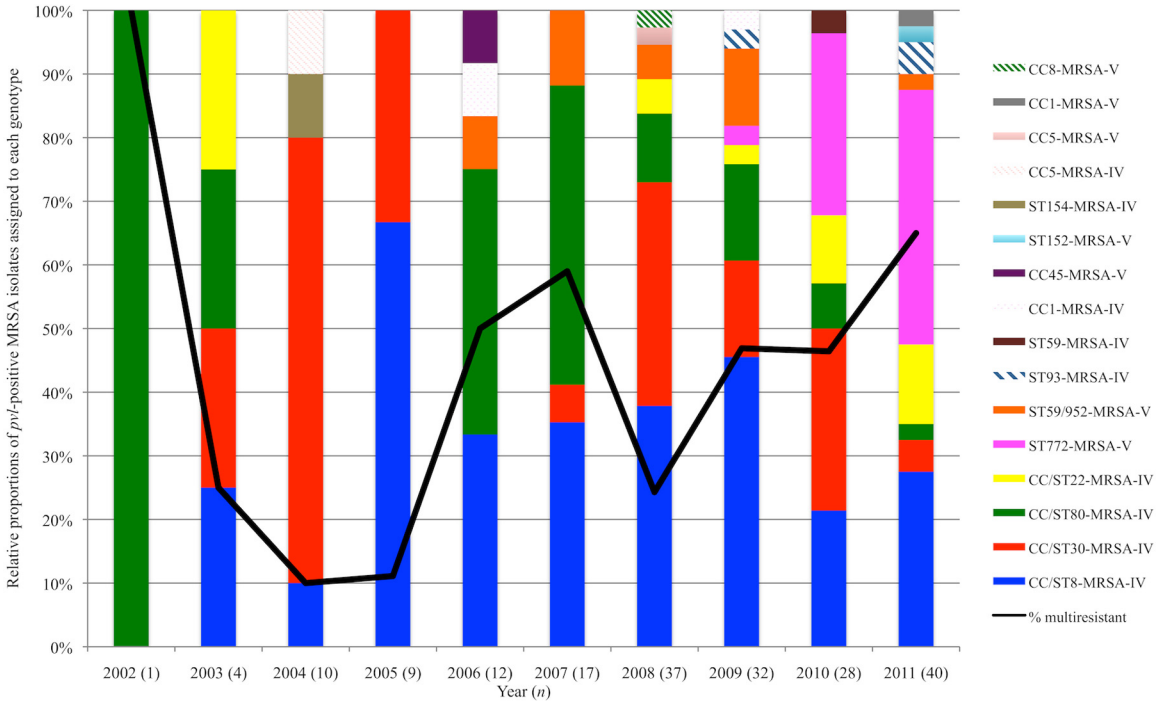

FIG 1 The relative proportions of the 190 *pvl*-positive MRSA isolates identified by the Irish National MRSA Reference Laboratory between 2002 and 2011 assigned to each genotype each year during the study period and the annual percentage of these MRSA isolates that exhibited multiresistance during this time period (black line). Multiresistant MRSA isolates were defined as those exhibiting resistance to three of more classes of commonly used antimicrobial agents, including fluoroquinolones, aminoglycosides, macrolides/lincosamides, tetracyclines, fusidic acid, and mupirocin (22). Numbers in parentheses (*n*) indicate the numbers of *pvl*-positive MRSA isolates identified each year.

genes *sek* and *seq* and the arginine catabolic mobile element (ACME), and although they were less common, *sed*, *sej*, and *ser* were also identified.

The one remaining CC8-MRSA t008 isolate harbored SCC*mec* V and did not exhibit resistance to multiple antimicrobial agents or harbor multiple resistance genes, but *sek*&*seq* and ACME were detected. ACME was only identified in one non-CC/ST8-MRSA isolate (CC45-MRSA-V).

CC22. The *spa* types t852 and t005 predominated among the CC22 MRSA (58.3% [7/12]) and MSSA (70% [7/10]) isolates, respectively. Only one *spa* type, t005, was common to CC22 MRSA and MSSA, but only one t005 MRSA isolate was identified. Among the CC22-MRSA-IV isolates, resistances to aminoglycosides encoded by *aacA-aphD* and *aadD*, trimethoprim encoded by *dfrS1*, erythromycin encoded by *erm*(C), and ciprofloxacin were common. No ciprofloxacin-resistant CC22 MSSA isolates were identified, but they all exhibited aminoglycoside resistance encoded by *aacA-aphD*; the majority were resistant to trimethoprim and carried *dfrS1*, and one isolate exhibited resistance to fusidic acid, which was probably due to mutations in *fusA*, as neither *fusB* or *fusC* were detected. However, not all CC22 isolates harboring *aacA-aphD* and *aadD* exhibited resistance to all of the appropriate aminoglycoside antimicrobial agents. All CC22 isolates carried *egc*, but no other toxin genes were detected.

CC30. The majority of CC/ST30-MRSA-IV isolates were assigned to *spa* type t019 (55% [22/40]) or t012 (30% [12/40]). Fewer than half of the isolates were resistant to fusidic acid encoded by *fusC*, and resistances to tetracycline and trimethoprim encoded by *tet*(K) and *dfrS1*, respectively, were detected in one isolate each. All CC/ST30-MRSA-IV isolates carried *egc*, and 35% (14/40) carried the toxic shock toxin gene *tst*, with only two isolates harboring *sea*.

Among the CC30 MSSA *spa* types, t021 (40% [6/15]) and t318 (26.7% [4/15]) predominated. The latter *spa* type (t318) was the only common *spa* type detected among CC30 MRSA and MSSA but was only detected in one CC30-MRSA isolate. While no fusidic acid resistance phenotype or genes were detected among the CC30-MSSA, resistances to tetracycline, trimethoprim, and erythromycin encoded by *tet*(K), *dfrS1*, and *mph*(C), respectively, were identified in one or two isolates each. CC30-MSSA isolates carried the greatest range of toxin genes, i.e., the enterotoxin genes *sek*&*seq*, *egc*, *sea*, *sec*&*sel*, and *tst*, but apart from *egc*, which was detected in the majority of CC30 MSSA, each of these were found in one or two CC30-MSSA isolates only. Overall, *tst* was unique to CC30 isolates.

CC59. All ST59/952-MRSA-V isolates exhibited a single *spa* type, t437, and the majority of the isolates exhibited resistances to multiple antimicrobial agents and carried multiple resistance genes, with erythromycin and lincomycin resistances encoded by *erm*(B), kanamycin and neomycin resistances encoded by *aphA3*, and chloramphenicol resistance encoded by *cat*-pC223. Tetracycline resistance encoded by *tet*(K) was also common among these isolates. All ST59/952-MRSA-V isolates carried the enterotoxin genes *seb* and *sek*&*seq*.

The one ST59-MRSA-IV isolate identified carried fewer resistance genes, but *aphA3* and *erm*(B) encoding resistances to aminoglycosides and erythromycin, respectively, were detected. Simi-

lar to the ST59/952-MRSA-V isolates, the ST59-MRSA-IV isolate carried *seb* and *sek*&*seq*, but *sea* was also detected.

CC80. The majority of the CC/ST80-MRSA-IV isolates exhibited *spa* type t044 (77.8% [21/27]). All isolates exhibited resistances to kanamycin and neomycin, encoded by *aphA3*. Resistances to tetracycline, fusidic acid, and erythromycin encoded by *tet*(K), *fusB*, and *erm*(C), respectively, were also common. However, for a small number of the isolates, *tet*(K) and *fusB* were identified but the appropriate resistance phenotype was not detected. Chloramphenicol and trimethoprim resistances encoded by *cat*-pC221 and *dfrS1*, respectively, as well as ciprofloxacin resistance, were detected in only one isolate each. All CC/ST80-MRSA-IV isolates harbored the exfoliative toxin gene *etD* and the epidermolytic toxin gene *edinB*, which were identified in only one other isolate (ST152-MRSA-V).

CC88. Only three CC88 isolates, all MSSA, were identified and were assigned to two *spa* types. These isolates were the only isolates found to harbor IEC type F (*sep*, *sak*, *chp*, and *scn*). Two isolates exhibited tetracycline resistance and carried *tet*(K), with only one isolate each exhibiting resistances to erythromycin and trimethoprim, encoded by *erm*(C) and *dfrS1*, respectively. The enterotoxin genes *sek*&*seq* were detected in one CC88-MSSA isolate.

ST93. The three ST93-MRSA-IV isolates each exhibited a different *spa* type. Erythromycin resistance encoded by *msr*(A) and *mph*(C) was detected in one isolate only. The *qacC* gene was also detected in one isolate but the isolate did not exhibit resistance to ethidium bromide. The enterotoxin gene homolog *CM14* was the only toxin gene detected among ST93-MRSA-IV isolates.

CC121. All CC121 isolates identified were MSSA, and the majority exhibited *spa* type t159 (71.4% [5/7]). Only CC121-MSSA isolates exhibited *agr* type IV. Just over half of the isolates exhibited erythromycin resistance encoded by *erm*(C), and tetracycline, trimethoprim, and chloramphenicol resistances encoded by *tet*(K), *dfrS1*, and *cat*-pC221, respectively, were also detected among CC121-MSSA. All CC121-MSSA isolates harbored *egc* and *CM14*, and just over half also carried *seb*.

Multiresistant *pvl*-positive MRSA. Multiresistance was identified among MRSA isolates only and was defined as phenotypic resistance to three or more classes of commonly used antimicrobial agents tested, including fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin/kanamycin/neomycin/tobramycin), macrolides/lincosamides (erythromycin/lincomycin), tetracyclines, fusidic acid, and mupirocin (22). Using this criterion, 43.7% (83/190) of *pvl*-positive MRSA isolates were multiresistant. These multiresistant *pvl*-positive MRSA isolates were assigned to six genotypes, with the majority belonging to CC/ST8-MRSA-IV (30.1% [25/83]), ST772-MRSA-V (30.1% [25/83]), and CC/ST80-MRSA-IV (25.3% [21/83]), with a small number of multiresistant isolates also belonging to CC/ST22-MRSA-IV (7.2% [6/83]), ST59/952-MRSA-V (6% [5/83]), and CC1-MRSA-V (1.2% [1/83]) (see Fig. S1 in the supplemental material). An increase in the prevalence of multiresistant *pvl*-positive MRSA was observed between 2004 (10% [1/10]) and 2007 (59% [10/17]) ($P < 0.02$), and despite a decline in 2008 (24.3% [9/37]), this prevalence increased again between 2008 and 2011 to 65% (26/40) ($P < 0.001$) (Fig. 1). In fact, the highest prevalence of multiresistance among *pvl*-positive MRSA isolates was observed in 2011, and this was predominantly associated with isolates within ST772-MRSA-V (61.5% [16/26]) and to a lesser extent, CC/ST8-MRSA-IV (19.2% [5/26]), CC/ST22-MRSA-IV (11.5% [3/26]), ST59-MRSA-V (3.8% [1/26]), and CC1-MRSA-V (3.8% [1/26]).

Patient demographics. Of the 229 isolates investigated, 216 (94.3%) were from patients, nine (3.9%) from health care staff, and four (1.8%) from environmental sources. Information pertaining to whether the *S. aureus* isolates were from patients based in the community or in hospitals were available for 175/216 isolates, 69.7% (122/175) of whom were based in the community.

Sex and age. Gender data were available for patients, from whom 189 isolates were recovered, of which 52.4% (99/189) were from females (Table 3). There was no significant difference between the genders of patients associated with *pvl*-positive MRSA and MSSA isolates, with 52.2% (83/159) and 47.8% (76/159) of MRSA isolates being associated with females and males, respectively, and 53.3% (16/30) and 46.7% (14/30) of MSSA isolates being associated with females and males, respectively. The ages of patients from whom *pvl*-positive *S. aureus* isolates were recovered ranged from <1 month to 98 years, and the median age was 30 years (age data were available for 193 patients). Seventy percent (136/193) of the isolates were from patients who were <40 years of age (Table 3).

Isolate clusters. No clusters were identified among *pvl*-positive MSSA isolates, but seven isolate clusters were identified among *pvl*-positive MRSA isolates, either from two or more members of one family/household, within a hospital, or both (Table 3). Within each cluster, the isolates were recovered between 3 months and 2 years apart, and the isolates were represented by a single genotype, with indistinguishable *spa* and DNA microarray profiles in each case. ST772-MRSA-V accounted for almost half of all cluster-associated isolates identified (48.6% [17/35]) (Table 3).

International travel or country of origin outside of Ireland. Thirty-five individuals from whom *pvl*-positive isolates were recovered were known to have recently traveled internationally or had a country of origin other than Ireland (Table 3). Recent travel ranged from 2 weeks to 1 year prior to the recovery of the *pvl*-positive *S. aureus* isolates, but for the majority of patients, the time period since travel was not defined. The genotypes most commonly associated with international travel or country of origin other than Ireland were ST8-MRSA-IV (seven isolates), ST772-MRSA-V (six isolates), and ST30-MRSA-IV (five isolates) (Table 3). The ST8-MRSA-IV and ST30-MRSA-IV isolates were identified from patients with links to multiple regions worldwide, while the ST772-MRSA-V isolates were associated exclusively with India (Table 3).

Overall, the most common travel destination or region of origin for patients with *pvl*-positive *S. aureus* was Asia (15 isolates), followed by Africa (six isolates) and the United States (4 isolates) (Table 3).

Clinical presentations. Clinical data were available for 159 isolates (135 MRSA and 24 MSSA) (see Fig. S2 in the supplemental material). The most common infections were SSTIs (60.4% [96/159]), including unspecified SSTIs, abscesses, boils, furuncles, bursitis, folliculitis, sinusitis, eye and ear infections, inguinal lymphadenitis, and wound infections. SSTIs were associated with isolates from all except three genotypes: CC1-MSSA ($n = 4$), ST152-MRSA-V ($n = 1$), and ST59-MRSA-IV ($n = 1$). More serious manifestations were also identified, including BSIs (10.7% [17/159] of the isolates including ST59-MRSA-IV, ST59/952-MRSA-V, ST30-MRSA-IV, ST22-MRSA-IV, ST8-MRSA-IV, CC1-MRSA-IV, ST772-MRSA-V, and CC30-MSSA), pneumonia

TABLE 3 Patient demographics associated with *pvl*-positive MRSA and MSSA identified by the Irish National MRSA Reference Laboratory between 2002 and 2011

Patient characteristics	No. of isolates	Genotype[a]
Gender		
Male	90	NA
Female	99	NA
Data not available	36	NA
Age group (yr)		
0–9	35	NA
10–19	19	NA
20–29	40	NA
30–39	42	NA
40–49	23	NA
50–59	11	NA
60–69	9	NA
70–79	7	NA
80–89	4	NA
90–99	3	NA
Data not available	32	NA
No. of clusters[b]		
1 (household)	4	CC/ST30-MRSA-IV
2 (household)	3	CC/ST30-MRSA-IV
3 (household)	4	CC/ST8-MRSA-IV
4 (household)	3	CC/ST8-MRSA-IV
5 (household)	4	CC80-MRSA-IV
6 (hospital)	6	ST772-MRSA-V
7 (hospital and household)[c]	11	ST772-MRSA-V
International travel to or country/region of origin		
United States	1	CC1-MRSA-IV
	3	ST8-MRSA-IV
Africa	1	ST8-MRSA-IV
	1	CC5-MRSA-IV
	2	ST30-MRSA-IV
	1	CC121-MSSA
	1	CC5-MRSA-V
The Middle East	1	ST80-MRSA-IV
India	1	ST772-MRSA-V
	5	ST772-MRSA-V
Far East Asia	1	ST80-MRSA-IV
	1	ST154-MRSA-IV
	3	ST30-MRSA-IV
	1	CC121-MSSA
	1	ST59-MRSA-V
	1	ST8-MRSA-IV
Australia	1	CC22-MRSA-IV
	1	ST93-MRSA-IV
New Zealand	1	ST8-MRSA-IV
Brazil	1	ST8-MRSA-IV
Czech Republic	1	CC1-MRSA-IV
Undefined (outside of Ireland)	2	ST59/952-MRSA-V
	1	ST22-MRSA-IV
	1	CC22-MSSA
	1	CC30-MSSA

[a] NA, not applicable; CC, clonal complex; ST, sequence type.
[b] Isolates were defined as clusters if they were recovered from members of one family/household, within a hospital, or both. Within each cluster, isolates were recovered between 3 months and 2 years apart. Each isolate within a cluster was recovered from a different person or environmental source.
[c] The 11 *pvl*-positive ST772-MRSA-V isolates in cluster 7 were described previously (31).

(3.1% [5/159] of the isolates including CC30-MSSA, ST772-MRSA-V, CC/ST8-MRSA-IV, and CC/ST80-MRSA-IV), osteomyelitis (1.3% [2/159] of the isolates including CC121-MSSA and CC1-MSSA), necrotizing pneumonia (1.3% [2/159] of the isolates belonging to CC/ST8-MRSA-IV and CC1 MSSA), necrotizing fasciitis (0.6% [1/159] of the isolates belonging to CC30-MSSA), and endocarditis (0.6% [1/159] isolates belonging to CC22/ST22-MRSA-IV). Thirty-one isolates were recovered during patient screenings (nose, throat, and/or perineum sites) during hospital outbreaks or from persons with close contact with patients with *pvl*-positive *S. aureus*.

DISCUSSION

This study reports several novel findings in relation to *pvl*-positive MRSA, including an increase in the prevalence and diversity of *pvl*-positive MRSA isolates submitted to the NMRSARL between 2002 and 2011 and several temporal shifts in the predominant clonal types. A 44-fold increase in the prevalence of *pvl*-positive MRSA, from 0.2% to 8.8%, was observed between 2002 and 2011 (Fig. 1). While these findings may reflect a true increase in the prevalence of *pvl*-positive MRSA in Ireland over the last decade, enhanced clinical and laboratory awareness of *pvl* probably also contributed to the higher rate. A relatively low but increasing prevalence of *pvl*-positive MRSA has also been reported from Austria and Germany during the last decade (43, 44).

The polyclonal *pvl*-positive MRSA population structure identified in Ireland and in other European countries (21, 22, 43, 45) contrasts starkly with that in the United States and Australia, where single epidemic *pvl*-positive clones predominate, specifically ST8-MRSA-IV/USA300 and ST93-MRSA-IV, respectively (17, 46). Many reasons have been proposed for this difference between the United States and Europe, including environmental, host, social, economic, and cultural factors (2, 21). However, direct evidence for these is somewhat lacking, and many of the risk factors identified for *pvl*-positive MRSA/CA-MRSA in the United States may also apply to various communities in Europe (2). In the present study, while such specific parameters were not investigated, six familial/household outbreaks of *pvl*-positive MRSA were identified. Furthermore, links between several *pvl*-positive *S. aureus* isolates and patients with recent foreign travel to or ethnic origin from outside of Ireland were also identified, highlighting the continuing role of strain importation on the variety of *pvl*-positive MRSA strains found in Ireland.

While the prevalence of different *pvl*-positive MRSA clones identified in the present study, together with precise temporal shifts of predominant clones that are unique to Ireland, similarities and differences were noted in comparison with polyclonal *pvl*-positive MRSA populations observed in other European countries. For example, a decline in the incidence of the *pvl*-positive European clone ST80-MRSA-IV has been noted recently across Europe in association with an increase in ST8-MRSA-IV/USA300 (21). In the present study, an increase in the prevalence of ST8-MRSA-IV/USA300 was observed between 2006 and 2009, and it predominated in 2008 and 2009, decreased in 2010, and was the second most common clone in 2011, surpassed only by ST772-MRSA-V. The emergence of ST772-MRSA-V as the dominant *pvl*-positive MRSA clone in 2011 in Ireland reflects a similar situation in the United Kingdom, where ST772-MRSA-V was the predominant multiresistant *pvl*-positive clone between 2005 and 2008 (22). The predominance of ST772-MRSA-V and ST8-MRSA-IV/USA300 in the *pvl*-positive MRSA isolates in Ireland is of concern, as both clones appear to be highly transmissible, with a propensity to spread worldwide and displace hospital strains (14, 20). In the present study, ST772-MRSA-V was found in association with two

separate hospital clusters and one familial cluster, and ST8-MRSA-IV/USA300 was found in association with three family clusters; both of these strains were found to have been imported frequently into Ireland. In addition, genetic characteristics that may enhance the virulence or ability of these clones to spread have been identified in this and other studies, including ACME and the enterotoxin genes *sek*&*seq* in ST8-MRSA-IV/USA300 and an *sea*- and *pvl*-encoding bacteriophage (41), and also multiple other enterotoxin genes in ST772-MRSA-V (Table 2). Lastly, isolates belonging to both clones can exhibit multiresistance (22), and all ST772-MRSA-V and 38.5% of ST8-MRSA-IV/USA300 isolates investigated in this study were multiresistant.

While the overall numbers of *pvl*-positive ST22-MRSA-IV isolates in this study were low, a 4-fold increase was noted between 2009 and 2011 (Fig. 1). Worryingly, *pvl*-positive ST22-MRSA-IV has been associated with hospital and community outbreaks elsewhere (47–49), and it now predominates together with ST772-MRSA-V in hospitals in India (20). Although *pvl*-negative ST22-MRSA-IV is currently predominant in Irish hospitals (mainly *spa* type t032 [28]), *pvl*-positive ST22-MRSA-IV was genetically distinct (*spa* type t852) in the present study, indicating the independent evolution of these strains.

CC/ST30-MRSA-IV was the second most common *pvl*-positive MRSA clone identified, accounting for 21.1% of all isolates during the study period and predominating several times across the duration of the study (Fig. 1). Isolates of this pandemic clone are also common in the United Kingdom and have been associated with a hospital outbreak in which the probable index case was a staff member who had recently traveled to the Philippines (24, 50, 51). In the present study, a link between travel to or ethnic origin in Asia or Africa was identified for several CC/ST30-MRSA-IV isolates, emphasizing the role of travel in its spread. CC/ST30-MRSA-IV isolates were also associated with two familial outbreaks, indicating further its propensity to spread. In the present study, the prevalence of CC/ST30-MRSA-IV declined from 70% to 0% between 2004 and 2006 and from 28.6% to 5% between 2010 and 2011 (Fig. 1). A decline in the prevalence of this once-predominant clone among CA-MRSA was also recently reported in New Zealand, where it was replaced by *pvl*-negative ST5-MRSA-IV (52). It is now well established that not all CA-MRSA isolates carry *pvl*. In the present study, 70% of *pvl*-positive *S. aureus* isolates for which information was available were from patients in the community, indicating that CA *S. aureus* had emerged as a significant problem in Ireland. However, the true burden of CA *S. aureus* infections in Ireland will only be fully understood when *pvl*-negative and *pvl*-positive CA *S. aureus* isolates are investigated systematically together with detailed epidemiological information.

The diversity of *pvl*-positive MRSA clones increased in the second half of the study period, with 10/16 MRSA genotypes identified for the first time between 2006 and 2011, including CC/ST59-MRSA-V, ST93-MRSA-IV, and ST772-MRSA-V. Links between several isolates of these clones and the regions where they predominated were also noted. Although an increase in the Taiwanese clone (CC/ST59-MRSA-V), from 8.3% in 2006 to 12.5% in 2009, was observed, the number of isolates recovered each year remained low throughout (between one and four isolates each year). CC/ST59-MRSA-V is among the predominant CA-MRSA clones in some northern European countries (21). In contrast, similar to in the present study, ST93-MRSA-IV has only been reported sporadically in Europe (21, 53) but is the dominant *pvl*-positive MRSA strain in Australia, where it has spread into health care facilities (54). Increasing reports of outbreaks of *pvl*-positive MRSA, particularly in NICUs, highlights the ability of these strains to spread among vulnerable patient groups in hospitals (24, 25, 47, 48). In the present study, two NICU clusters in separate hospitals were due to the recently emerged *pvl*-positive multiresistant ST772-MRSA-V clone. In fact, 30% of *pvl*-positive isolates for which information was available were from patients in hospitals, a situation that requires close monitoring so that *pvl*-positive MRSA does not become widespread in Irish hospitals.

Despite a decrease in 2008, an overall 6-fold increase in the prevalence of multiresistant *pvl*-positive MRSA was identified between 2004 and 2011 (Fig. 1). Similarly, a 12.3-fold increase in the prevalence of multiresistant *pvl*-positive MRSA was noted in the United Kingdom between 2006 and 2008 (22). Both in Ireland and the United Kingdom, this was largely due to the emergence and predominance of ST772-MRSA-V, and in Ireland only, to the continued prevalence of ST8-MRSA-IV/USA300. Also of concern is the high prevalence of ciprofloxacin resistance identified among multiresistant *pvl*-positive MRSA isolates (67.1%). All of these findings highlight how non-multiantibiotic resistance and susceptibility to ciprofloxacin can no longer be considered to be reliable markers for *pvl*-positive MRSA.

This study has for the first time provided important insights into the molecular epidemiology of *pvl*-positive MSSA in Ireland. The prevalence of *pvl*-positive MSSA decreased 8-fold, from 20% in 2004 to 2.5% in 2011, and it accounted for only 17% of all *pvl*-positive isolates identified during the study period. In contrast, in the United Kingdom, the prevalence of *pvl*-positive MSSA increased 9-fold between 2005 and 2010, accounting for 61.5% of all *pvl*-positive *S. aureus* in 2009 (26); in Africa, *pvl*-positive MSSA is also common, with 57% of MSSA isolates in one study being identified as *pvl* positive (27). However, MSSA isolates are not routinely referred to the Irish NMRSARL, and the number of MSSA isolates referred each year during our study was low (Table 1). Additional studies are required in order to determine the true burden of *pvl*-positive MSSA in Ireland.

The results of this study also suggest that the importation of *pvl*-positive MRSA strains is more significant than the local emergence of *pvl*-positive MRSA from *pvl*-positive MSSA, with only 1.6% (3/189) of the MRSA isolates exhibiting the same *spa* type as the MSSA isolates. Due to the greater abundance of these *spa* types among *pvl*-positive MSSA, it is reasonable to speculate that this small number of *pvl*-positive MRSA isolates may have evolved from the *pvl*-positive MSSA isolates by the acquisition of SCC*mec* rather than the loss of SCC*mec* by MRSA, although both alternatives are possible.

Similar to a recent study in the United Kingdom, CC22 and CC30 were the most common *pvl*-positive MSSA clones identified in our study, accounting for 64.1% of the isolates (26). While not reported previously in the United Kingdom (26), the CC121-MSSA clone that accounted for 17.9% of *pvl*-positive MSSA isolates in the present study is a pandemic clone (55, 56). Interestingly, a link to Africa and the Far East was noted for 2/7 CC121 MSSA isolates, where that clone has been shown to dominate (56, 57). CC88-MSSA accounted for just 7.6% of the *pvl*-positive MSSA isolates and was reported previously in India (58), but isolates of this lineage are more commonly reported as MRSA with SCC*mec* IV, particularly in Africa (19).

In conclusion, while this study highlights the changing molecular epidemiology of *pvl*-positive MRSA and MSSA in Ireland over the last decade, it is clear that the actual burden of *pvl*-positive and CA *S. aureus* infections in Ireland may be even higher, since this study investigated only *pvl*-positive isolates and only those submitted to the NMRSARL. There is a need for ongoing and systematic surveillance of *pvl*-positive and CA *S. aureus* infections in communities and hospitals in Ireland, together with obtaining detailed epidemiological information, in order to fully understand the burden of *S. aureus* infections that exists.

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

Peter M. Kinnevey,[a] Anna C. Shore,[a,b] Grainne I. Brennan,[a,c] Derek J. Sullivan,[a] Ralf Ehricht,[d] Stefan Monecke,[d,e] Peter Slickers,[d] David C. Coleman[a]

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[h] N/A, information not available.

[i] BSI, bloodstream infection.

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

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

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

RESULTS

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

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

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

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

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

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

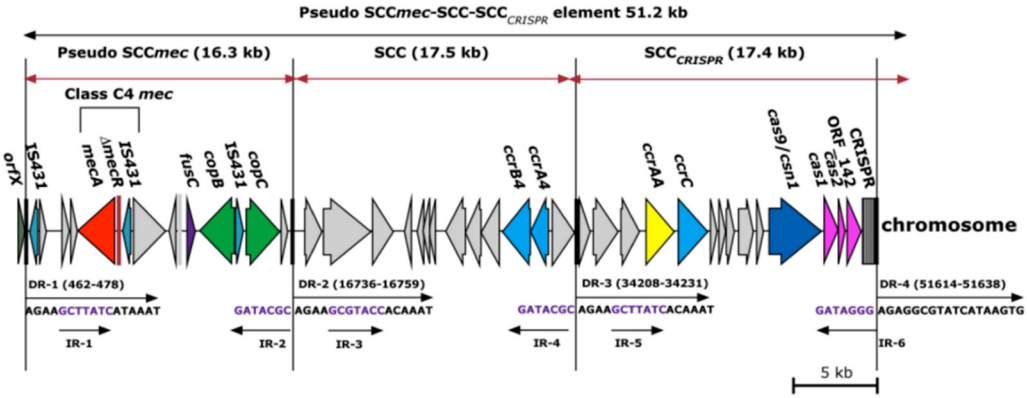

FIG 1 Schematic diagram showing the genetic organization of the novel composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element harbored by the ST779/t878 MRSA isolate M06/0171 (GenBank accession number HE980450). The 51-kb composite pseudo SCC*mec*-SCC-SCC$_{CRISPR}$ element, as well as each of the individual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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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Emergence of Hospital- and Community-Associated Panton-Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* Genotype ST772-MRSA-V in Ireland and Detailed Investigation of an ST772-MRSA-V Cluster in a Neonatal Intensive Care Unit

Gráinne I. Brennan,[a] Anna C. Shore,[b,c] Suzanne Corcoran,[d] Sarah Tecklenborg,[b] David C. Coleman,[b] and Brian O'Connell[a,c]

National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland[a]; Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College, Dublin, Ireland[b]; Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin, Ireland[c]; and Microbiology Department, Rotunda Hospital, Dublin, Ireland[d]

Sequence type 22 (ST22) methicillin-resistant *Staphylococcus aureus* (MRSA) harboring staphylococcal cassette chromosome *mec* (SCC*mec*) IV (ST22-MRSA-IV) has predominated in Irish hospitals since the late 1990s. Six distinct clones of community-associated MRSA (CA-MRSA) have also been identified in Ireland. A new strain of CA-MRSA, ST772-MRSA-V, has recently emerged and become widespread in India and has spread into hospitals. In the present study, highly similar MRSA isolates were recovered from seven colonized neonates in a neonatal intensive care unit (NICU) in a maternity hospital in Ireland during 2010 and 2011, two colonized NICU staff, one of their colonized children, and a NICU environmental site. The isolates exhibited multiantibiotic resistance, *spa* type t657, and were assigned to ST772-MRSA-V by DNA microarray profiling. All isolates encoded resistance to macrolides [*msr*(A) and *mpb*(BM)] and aminoglycosides (*aacA-aphD* and *aphA3*) and harbored the Panton-Valentine leukocidin toxin genes (*lukF-PV* and *lukS-PV*), enterotoxin genes (*sea*, *sec*, *sel*, and *egc*), and one of the immune evasion complex genes (*scn*). One of the NICU staff colonized by ST772-MRSA-V was identified as the probable index case, based on recent travel to India. Seven additional hospital and CA-ST772-MRSA-V isolates recovered from skin and soft tissue infections in Ireland between 2009 and 2011 exhibiting highly similar phenotypic and genotypic characteristics to the NICU isolates were also identified. The clinical details of four of these patients revealed connections with India through ethnic background or travel. Our study indicates that hospital-acquired and CA-ST772-MRSA-V is currently emerging in Ireland and may have been imported from India on several occasions.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most common causes of hospital-acquired infection worldwide, with elderly patients at increased risk of infection. Neonates in intensive care units are also at increased risk of MRSA infection or colonization. Risk factors for this group are premature birth, low birth weight, chronic underlying disease, prolonged exposure to antibiotics, and invasive or surgical procedures (35). Since the 1990s the emergence and increasing prevalence of community-associated MRSA (CA-MRSA) outside the health care environment have highlighted the changing epidemiology of MRSA (24). CA-MRSA has been associated with colonization of healthy individuals but can also cause skin and soft tissue infections and life-threatening necrotizing pneumonia in children and adults with no predisposing risk factors (12, 24). Outbreaks of CA-MRSA have been reported in specific community settings and groups, including among those in prisons, crèches, gymnasia, and military bases and among Australian Aborigines and Native Americans (7, 12).

Methicillin-resistant *S. aureus* has acquired a mobile genetic element carrying the methicillin resistance genes *mecA* that has been termed the staphylococcal cassette chromosome *mec* (SCC*mec*). Eleven different SCC*mec* elements have been described to date (19, 21, 39). Recently, a new type of MRSA of animal origin harboring a novel and highly divergent *mecA* gene was identified in Ireland, the United Kingdom, and Germany (10, 18, 39).

Community-associated MRSA is generally genetically distinct from hospital-acquired MRSA (HA-MRSA) and is characterized by the presence of small SCC*mec* elements, usually SCC*mec* type IV, and to a lesser extent SCC*mec* type V. Community-associated-MRSA is often less resistant to antibiotics than HA-MRSA and often expresses specific toxins and virulence factors, such as Panton-Valentine leukocidin (PVL) and phenol-soluble modulins (41). PVL is a bicomponent pore-forming cytolytic toxin encoded by the *lukF-PV* and *lukS-PV* genes, which are carried by a group of specific bacteriophages (46).

The emergence of CA-MRSA clones was originally thought to be continent specific, but intercontinental spread of several CA-MRSA clones has been observed and new clones have also emerged (23). Recently, a PVL-positive CA-MRSA clone, ST772-MRSA-V, that is relatively multiantibiotic resistant compared to other CA-MRSA clones was identified in several countries. Methicillin-susceptible ST772 *S. aureus* was originally reported in Bangladesh, but this was quickly followed by reports of ST772-MRSA-V in India and Malaysia and subsequently in England, Italy, Australia, Germany, Hong Kong, and Abu Dhabi (1, 13, 15,

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Address correspondence to Gráinne I. Brennan, gbrennan@stjames.ie.

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24, 26, 34). Many patients identified with ST772-MRSA-V outside India had familial links in, or travel history to or from, India, resulting in the ST772-MRSA-V clone being dubbed the Bengal Bay clone (15, 24, 26). In addition, ST772-MRSA-V is increasingly prevalent in India, where it has spread into hospitals and, along with ST22-MRSA-IV, has displaced the previously predominant nosocomial ST239-MRSA-III clone (13). There have also been a number of other reports where CA-MRSA was associated with hospital-acquired infections and outbreaks among adults and neonates (11, 27, 28, 34, 36, 41, 45). It has been speculated that CA-MRSA infection among neonates is acquired through contact with colonized adults (36).

The spread of these CA-MRSA isolates in both community and nosocomial settings has led to revised infection prevention and control guidelines to include environments such as gymnasia, child care facilities, and prisons (29). The constant influx of patients, visitors, and health care workers, who constitute a reservoir for CA-MRSA, into hospitals places additional burdens on staff attempting to control the spread of HA-MRSA (28). Infection control interventions that are successful in the control of HA-MRSA may be useful in the control of CA-MRSA outbreaks in hospitals; however, these may need to be modified to include more emphasis on the involvement of health care workers in outbreaks (11, 22, 27).

MRSA has been endemic in Irish hospitals for almost 4 decades, with a major shift in the predominant clonal type occurring in each decade (37). Since 2002, isolates belonging to ST22-MRSA-IV have predominated as the cause of nosocomial infections (33, 37, 38). Several genotypes of PVL-positive CA-MRSA have also been reported in Ireland and have been linked to the importation of different strains, with ST30-MRSA-IV and ST8-MRSA-IV predominating (33).

Here we report the recent emergence of ST772-MRSA-V in Irish hospitals and in the community. The study describes the detailed characterization by DNA microarray profiling and *spa* typing of closely related isolates of PVL-positive MRSA ST772-MRSA-V recovered in 2010 and 2011 from colonized neonates and staff in a neonatal intensive care unit in an Irish maternity hospital and additional epidemiologically unrelated isolates of this strain from cases of HA and CA infections in Ireland between 2009 and 2011.

MATERIALS AND METHODS

Hospital setting. The neonatal intensive care unit (NICU) described in this report is located in a 194-bed maternity hospital in Dublin, Ireland. The neonatal unit is a 36-bed unit consisting of three air-conditioned wards and a separate isolation wing. Babies admitted to the unit include those born prematurely (before 37 weeks), those with congenital abnormalities, and any baby who has problems identified immediately after birth or who subsequently becomes ill.

MRSA surveillance and description of the cluster. In October 2010, a skin swab from the chin of a baby in the NICU yielded MRSA. Subsequent screening swabs from the nose and umbilicus yielded MRSA organisms that were characterized as *spa* type t657 and ST772-MRSA-V. Within 2 weeks, two additional babies also yielded ST772-MRSA-V. Isolates were recovered from a nasal screening swab of the first additional baby and from nasal and umbilicus screening swabs from the second. In an attempt to identify the source of the MRSA, nasal screening swabs were collected from NICU staff who were closely involved in the care of the neonates, along with the parents of each baby positive for MRSA. At that time a source was not identified, and there were no further ST772-MRSA-V isolates recovered from patients in the NICU until March 2011, when nose and umbilicus screening swabs from two babies yielded additional ST772-MRSA-V isolates. In May, a further two babies yielded ST772-MRSA-V, one from an umbilicus swab and one from nasal and umbilicus swabs.

Following the isolation of the ST772-MRSA-V strain in March 2011, extensive supervised staff screening was undertaken. Nose, throat, groin, and/or axilla screening specimens were collected from 148 hospital staff, including medical, nursing, and midwifery staff, maternity care assistants, household cleaning staff, administrative staff, radiology staff, social workers, and biomedical engineers. In addition, parents of MRSA-positive babies were screened, and environmental specimens were also collected from horizontal surfaces within the unit around the cots of MRSA-positive babies and from staff areas.

Confirmation of isolates as MRSA. All MRSA isolates recovered from patients in the NICU during the 7-month period were submitted to the Irish National MRSA Reference Laboratory (NMRSARL) for epidemiological typing. On receipt of isolates at NMRSARL, all MRSA isolates were inoculated onto Protect beads (Technical Service Consultants Ltd., Heywood, United Kingdom) and stored at −70°C prior to subsequent investigation. Isolates were confirmed as *S. aureus* by using the tube coagulase test, and methicillin resistance was detected using 10-μg and 30-μg cefoxitin disks (Oxoid Ltd., Basingstoke, United Kingdom).

AR typing. All isolates underwent antibiogram-resistogram (AR) typing using the Clinical and Laboratory Standards Institute (CLSI) standardized disk diffusion methodology as described previously (33). Antibiogram-resistogram typing involved determining the resistance of isolates to a panel of 23 antimicrobial agents, including amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampin, spectinomycin, streptomycin, sulfonamide, tetracycline, tobramycin, trimethoprim, and vancomycin (30).

Biotyping. All MRSA isolates were characterized by a biotyping method that investigated hydrolysis of urea, hydrolysis of Tween 80, and pigment production, all as described previously (32).

DNA macrorestriction digestion analysis. DNA macrorestriction digestion analysis followed by pulsed-field gel electrophoresis (PFGE) was performed on all MRSA isolates as described previously (33). Banding patterns were analyzed using the GelCompar software package (version 4.1; Applied Maths, Belgium) (32), and the final interpretation of the differences between PFGE patterns was performed as recommended by Tenover et al. (44).

Staphylococcal protein A (*spa*) typing. *spa* typing, which involves PCR amplification and sequencing of the variable X region in the *S. aureus* protein A gene, *spa*, was performed as previously described (17) on MRSA isolates associated with the cluster and that exhibited an indistinguishable banding pattern when investigated by PFGE. The Ridom StaphType software (Ridom GmbH, Würzburg, Germany) was used for *spa* sequence analysis to identify the repeat successions of the *spa* gene and to assign it a *spa* type.

DNA microarray analysis. All MRSA isolates associated with the cluster were investigated by DNA microarray analysis using the StaphyType kit (Alere Technologies GmbH, Jena, Germany) to detect 334 *S. aureus* gene sequences and alleles, including antimicrobial resistance genes, virulence-associated genes, and typing markers (23). The array also assigned isolates to a multilocus sequence type (ST) and/or clonal complex (CC) and SCC*mec* type (25).

MLST. Three isolates underwent multilocus sequence typing (MLST) to confirm the ST of the isolate, and MLST was performed as described previously (16, 40). This included two isolates representative of the predominant *spa* type identified among the isolates (M11/0092 and M11/0085) as well as the one isolate exhibiting a different but closely related *spa* type (M10/0333).

Prevalence of ST772-MRSA-V in Irish hospitals. The database of isolates submitted to the NMRSARL was examined for other isolates that

exhibited an AR pattern or *spa* type indistinguishable from the ST772-MRSA-V isolate from the NICU cluster. Seven additional isolates were identified and investigated by *spa* typing and DNA microarray analysis and, where possible, clinical information regarding diagnosis, travel history, and ethnicity was collected retrospectively.

RESULTS

Cluster investigation. Over the 7-month period of the investigation (October 2010 to May 2011), seven babies in the NICU were found to be colonized by ST772-MRSA-V, i.e., none had clinical or other evidence to suggest MRSA infection. All parents and staff members screened for MRSA after the first isolates were recovered in October 2010 were found to be negative for MRSA. During the second episode of staff screening in March 2011, 148 staff were screened, and 5 were identified as MRSA carriers, 2 of whom were found to carry a strain indistinguishable from ST772-MRSA-V recovered from the neonates. Of the two staff members, one was from India (and yielded isolate M11/0092) and one was from Ireland (and yielded isolate M11/0097); both had worked in the NICU at the time when the babies yielded positive MRSA cultures.

Epidemiological investigation indicated that the staff member of Indian background (who yielded isolate M11/0092) was the probable index case due to her intimate involvement in the care for all babies who were colonized with ST772-MRSA-V. The staff member had worked in the NICU for a number of years. However, she had been hospitalized during a visit to India, where she had returned to give birth shortly prior to the recovery of the first ST772-MRSA-V isolate. Prior to decolonization treatment, ST772-MRSA-V was recovered from her nose, groin, and axilla, but she had no symptoms of infection. Following decolonization treatment, undertaken by the occupational health department, screening swabs were negative for MRSA on two occasions. However, the ST772-MRSA-V strain was recovered from a groin swab from a third set of screening swabs. Family contacts of the probable index case were screened, and a nasal swab from her India-born child yielded M11/0167. Table 1 shows the epidemiological characteristics of all ST772-MRSA-V isolates recovered during the NICU investigation.

The decolonization protocol applied to staff members consisted of a 5-day course of 2% mupirocin nasal ointment applied to the inner surface of each nostril three times daily and use of an octenidine-based shower gel/shampoo. Babies over 36 weeks' gestation were also bathed in octenidine and treated with mupirocin. One of the staff members (who yielded isolate M11/0097) colonized with the ST772-MRSA-V strain was successfully decolonized. The probable index case proved difficult to decolonize and as such underwent the decolonization protocol twice. Following decolonization, a screening throat swab collected from her child yielded the same ST772-MRSA-V strain. Treatment for this throat carriage was not possible by use of a mouth wash due to the age of the child.

Environmental screening. Following environmental screening of approximately 30 areas, including the main reception, staff kitchen, and clinical areas, MRSA was recovered from 1 area of the NICU, a horizontal surface in the area of one baby associated with the ST772-MRSA-V cluster. The environmental cleaning regimen, which included the cleaning of all equipment in the patient zone area (e.g., monitors, suction machines, intravenous pumps, canopy of the incubator, etc.) with detergent wipes and which was carried out daily within the unit, was increased to twice daily.

Characterization of MRSA isolates. The 11 MRSA isolates from patients, staff members, the child of the probable index case, and the environmental isolate from the NICU exhibited a similar multiantibiotic resistance phenotype (Table 1) and were positive for Tween 80 and urease hydrolysis. The PFGE banding patterns exhibited by these 11 isolates were indistinguishable. All MRSA isolates exhibited *spa* type t657, and DNA microarray analysis assigned the isolates to CC1 and ST573/772 with SCC*mec* type V. MLST of three isolates investigated confirmed the genotype as ST772. All isolates harbored multiple antibiotic resistance genes, including those encoding resistance to macrolides [*msr*(A) and *mpb*(BM)] and aminoglycosides (*aacA-aphD* and *aphA3*) (Table 1). All isolates were positive for the PVL genes *lukF-PV* and *lukS-PV*, the enterotoxin genes *sea*, *sec*, and *sel*, and the enterotoxin gene cluster (*egc*) along with the immune evasion complex (IEC) gene *scn*, but the beta-hemolysin toxin gene *hlb* was not detected either in its intact or disrupted form (Table 1). Genes encoding adhesion and biofilm factors were also detected (Table 1).

Prevalence of ST772-MRSA-V in Irish hospitals. Comparison of the AR pattern of the ST772-MRSA-V strain recovered in the NICU to all MRSA isolates submitted to the NMRSARL since 2000 showed that a similar AR pattern had previously been identified among seven additional sporadic PVL-positive isolates, two recovered from patients in two separate Irish hospitals and five from community sources (Table 1). These isolates were confirmed as ST772-MRSA-V by DNA microarray analysis. Six isolates were assigned *spa* type t657, while one was assigned *spa* type t345 (Table 1). MLST of the latter isolate confirmed it as ST772. The DNA microarray data for these isolates were indistinguishable from those of the ST772-MRSA-V isolates recovered in the NICU (Table 1).

Three of the seven patients from whom the isolates were recovered had Indian parents, while one other patient had traveled to India as part of his work (Table 1). The latter patient had a history of recurrent boils, and at the time of sampling had boils on his elbows, buttocks, thighs, and face. Five of the seven ST772-MRSA-V isolates were recovered from samples submitted by general medical practitioners in the community; however, two patients acquired the strain while in hospital. There was no known epidemiological association between any of the seven patients from whom the additional ST772-MRSA-V isolates were recovered.

DISCUSSION

The present study describes the detection and detailed molecular characterization of a cluster of the PVL-positive and multiantibiotic-resistant CA-MRSA strain ST772-MRSA-V among staff and patients in an Irish neonatal intensive care unit and the subsequent retrospective identification of isolates of this strain from patients in other hospitals and in the community in Ireland. Epidemiological data identified the likely source of the ST772-MRSA-V cluster in the NICU as a staff member from India. The use of *spa* typing in conjunction with the DNA microarray was essential in directing the epidemiological investigation, identifying the likely source and, hence, the implementation of control measures that led to prevention of further spread of the ST772-MRSA-V strain. Antibiogram-resistogram typing indicated that there was a possible outbreak or a cluster of MRSA within the NICU, and this was confirmed by PFGE and DNA microarray analysis. However, the ST772-MRSA-V isolates recov-

TABLE 1 Epidemiological, clinical, phenotypic, and genotypic characteristics of hospital-associated and community-associated ST772-MRSA-V isolates recovered in Ireland between 2009 and 2011

Hospital	Isolate no.	MRSA-positive site	Age	Ethnicity	Relevant clinical information	Location acquired/source	*spa* type	Antimicrobial resistance pattern[a]	Antimicrobial resistance genes[b]	Virulence-associated genes[b]	*agr*/capsule type[b]
H1	M10/0338	Nasal, umbilicus, perineum	7 days	Irish	Carriage cluster in NICU	Inpatient	t657	AMI,[c] AMP, CAD,[d] CIP,[e] ERY[f] GEN, KAN, NEO, TOB, TMP	*blaZ*, *msr*(A), *mph*(C), *aacA-aphD*, *aphA3* and *sat*, *fosB*, *sdrM*	*lukF-PV* and *lukS-PV*, *sea*, *sec*, and *sel*, *egc*[h] *scn*, *bbp*, *cflA*, *clfB*, *cna*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *icaA*, *icaC*, *icaD*, *sdrC*, *sdrD*, *vwb*, *sasG*	*II*/5
H1	M10/0342	Nasal	15 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M10/0349	Nasal, umbilicus	20 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M11/0082	Nasal, umbilicus	3 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M11/0085	Nasal, umbilicus	24 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M11/0107	Umbilicus	9 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M11/0120	Nasal, umbilicus	9 days	Irish	Carriage cluster in NICU	Inpatient	t657				
H1	M11/0092	Nasal, axilla, groin	29 yrs	Indian	Staff member, probable index case	Staff working in NICU	t657				
H1	M11/0097	Unknown	35 yrs	Irish	Staff member	Staff working in NICU	t657				
H1	M11/0167	Nasal	16 mos	Indian	Child of probable index case	Family contact of colonized NICU staff member	t657				
H1	M11/0093	Environment	NA[g]	NA[g]	Horizontal surface in NICU	NA[g]	t657				
NA[g]	M11/0035	Unknown	9 yrs	Indian	Pustule on back	Community associated					
H2	M09/0243	Unknown	82 yrs	Unknown	In patient with no previously positive MRSA screen	Patient	t657				
NA[g]	M10/0045	Unknown	29 yrs	Unknown	Unknown	Community associated	t657				
H3	M10/0203	Unknown	96 yrs	Unknown	Eye swab	Inpatient	t657				
NA[g]	M10/0131	Unknown	28 yrs	Indian	Unknown	Community associated	t657				
NA[g]	M10/0361	Unknown	18 mos	Indian	Ear swab	Community associated	t657				
NA[g]	M10/0033	Unknown	22 yrs	Irish with history of travel to India	History of recurrent boils and abscesses	Community associated	t345				

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

[b] Antimicrobial resistance genes, virulence-associated genes, *agr* type, and capsule type were determined using the StaphyType DNA microarray kit (Alere, Germany).

[c] Fourteen of 18 isolates exhibited moderate resistance to amikacin, while 4 isolates (M11/0035, M10/0045, M10/0131, and M10/0203) were susceptible.

[d] Four of 18 isolates exhibited resistance to cadmium acetate (M10/0342, M10/0349, M10/0131, and M11/0107), 2 (M11/0035 and M10/0033) were susceptible, and the remaining 12 isolates exhibited moderate resistance.

[e] Seventeen of 18 isolates exhibited resistance to ciprofloxacin, and 1 isolate (M11/0035) was susceptible.

[f] Eleven isolates exhibited moderate resistance to erythromycin.

[g] NA, not applicable.

[h] *egc*, evasion gene cluster consisting of *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*.

ered from the NICU were not associated with infections. All ST772-MRSA-V isolates identified in this study exhibited the same multiantibiotic-resistant AR pattern, which was distinctly different from that exhibited by the most frequently occurring MRSA clone in Irish hospitals (ST22-MRSA-IV) (3). Based on *spa* typing, all of the isolates apart from one were assigned to *spa* type t657. The remaining isolate was assigned to *spa* type t345, which differs in one repeat unit only from *spa* type t657. DNA microarray analysis and MLST assigned the isolates as ST772-MRSA-V and showed that they harbored similar virulence and antimicrobial resistance genes to each other and to previously reported ST772-MRSA-V isolates. However, unlike some of the recently reported ST772-MRSA-V isolates, the isolates in the present study lacked the antimicrobial resistance genes *erm*(C) and *tet*(K) (24) and the enterotoxin genes *sek* and *seq* (34). The absence of DNA microarray signals corresponding to the presence of either the disrupted or complete beta-hemolysin gene *hlb* in the ST772-MRSA-V isolates in this and previous studies (24, 34) is interesting. Most human *S. aureus* isolates, including MRSA, harbor a disrupted *hlb* gene due to insertional inactivation during lysogenization by *hlb*-converting bacteriophages (8, 9). These findings indicate the possible presence of mutations in the primer or probe binding sites within *hlb* used with the DNA microarray system in isolates of this strain. The presence of the IEC gene *scn*, which is carried on bacteriophages that integrate within *hlb*, suggests that *hlb* is present in a truncated form in these isolates. Additional studies are under way to investigate this further.

Unlike other ST772-MRSA-V isolates reported in the literature (1, 11, 13, 15, 24, 26, 34), those recovered within the Irish maternity hospital NICU were not associated with any known clinical diagnosis of infection and were most frequently recovered from screening specimens of patients within the NICU. In contrast, the seven ST772-MRSA-V isolates recovered from patients in other Irish hospitals ($n = 2$) and from patients in the community ($n = 5$) and for which there was clinical information available were found to be associated with skin and soft tissue infections.

The increased spread of CA-MRSA in India has been associated with severe soft tissue infections; however, there has been an increase in the number of cases associated with bacteremias affecting neonates (13). The decreasing prevalence of the HA-MRSA strain ST239-MRSA-III in hospitals in India since 2006, coupled with an increase in prevalence of ST22-MRSA-IV and ST772-MRSA-V, has led to the suggestion that these strains may be replacing the ST239-MRSA strain in Indian hospitals (13). Within Ireland it has been shown that previously predominant strains of MRSA in Irish hospitals have also been replaced at different time periods by different strains. In 1989 the most frequently occurring strain was ST239-MRSA-III, while in 1993 this had changed to ST8-MRSA-II (31, 37). The most recent change in strains occurred in 1998, when the frequency of ST22-MRSA-IV had increased, and this strain now accounts for 80% of MRSA bloodstream infections investigated in the NMRSARL under the European Antimicrobial Resistance Surveillance Network (EARS-Net; previously EARSS) in 2003 (32). Although the predominant MRSA strain causing bloodstream infections has not changed since 1998, the proportion of *S. aureus* isolates that are resistant to methicillin fell from 41.9% in 2006 to 24.3% in 2010 (4). The ST22-MRSA-IV clone could be displaced at some stage in the future, possibly by ST772-MRSA-V, which, as the situation in India has shown, is capable of displacing previously successful nosocomial MRSA strains.

Transmission of MRSA between health care workers and patients has been reported on many occasions (2, 5, 6, 20, 42, 48). Current Irish guidelines recommend that staff be screened for MRSA carriage only as part of an outbreak investigation (43). However, it has been suggested that there are higher colonization rates in settings where MRSA is endemic, and randomized periodic screening may be required to identify asymptomatic persons carrying MRSA (7, 14). Similar to the practice in the Netherlands of screening all patients who have previously been hospitalized in foreign countries, identifying and screening high-risk staff with previous hospitalization or a MRSA-positive family contact should be considered (47).

The toddler associated with the index case in the present study yielded MRSA from a throat swab, and as it is not possible to treat a toddler for carriage of MRSA in the throat, this very likely led to recolonization of the health care worker, thus posing many challenges regarding further management.

The emergence of a PVL-positive and multiantibiotic-resistant MRSA strain in the community and hospitals in Ireland is a worrying development, and enhanced surveillance is vital to ensure that these strains do not spread. The familial links or travel histories of a number of patients from which the ST772-MRSA-V strain was recovered in the present study suggest that the isolates recovered in Ireland may have been imported from India and that this may have occurred on a number of different occasions. Foreign travel as well as the employment of health care staff from foreign countries may lead to further importation incidents of this or other MRSA strains into Ireland. Rapid and informative molecular typing, such as that provided by DNA microarray profiling, is essential for the early identification of MRSA strains and for prevention of these strains spreading in hospitals.

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