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Molecular Typing of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* and Coagulase-Negative Staphylococci Recovered in Irish Hospitals and Characterisation of SCC*mec* and SCC*mec*-Containing Composite Islands

A thesis submitted to the University of Dublin in fulfilment of the requirement for the degree Doctor of Philosophy by

Emily Deasy

April 2014

Microbiology Research Unit,
Division of Oral Biosciences,
Dublin Dental University Hospital,
University of Dublin,
Trinity College Dublin

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Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of infections in animals and humans. The methicillin resistance gene *mecA* is located on a mobile genetic element termed the staphylococcal cassette chromosome *mec* (SCC*mec*). Composite islands (CIs) consisting of two or more SCC*mec*, SCC or SCC-like elements also occur in MRSA and characterisation of these is essential for accurate typing and tracking of MRSA. Coagulase-negative staphylococci (CoNS) are thought to be a reservoir for SCC*mec* and other genes in *S. aureus*. CoNS and methicillin-susceptible *S. aureus* (MSSA) are leading causes of healthcare-associated infections (HCAIs), but detailed molecular characterisation of both is lacking from Ireland. CoNS often harbour the arginine catabolic mobile element (ACME) that enhances survival on skin. Over the last decade one highly-clonal MRSA lineage, ST22-MRSA-IV, has predominated making tracking of MRSA in Irish hospitals very difficult. The high-throughput StaphyType DNA microarray offers an alternative typing method for *S. aureus* by assigning isolates to a multilocus sequence type (MLST) and/or clonal complex (CC) and also detects a wide range of virulence, resistance and SCC*mec*/SCC genes. Combining microarray profiling and *spa* typing has shown potential for enhancing discrimination of ST22-MRSA-IV isolates and for detecting SCC*mec*, resistance genes and ACME in CoNS.

The five aims of the present study were as follows:

- To investigate two methicillin resistant clinical *S. aureus* isolates that lacked *mecA* by conventional PCR for the possible presence of a novel *mec* gene.
- To investigate the genetic organisation of a possible novel CI among MRSA from Irish hospitals.
- To investigate the SCC*mec*, SCC, resistance genes and ACME in CoNS from patients in Irish hospitals by microarray profiling and to perform molecular typing of the two most clinically-relevant species identified including *S. epidermidis* and *S. haemolyticus*.
- To perform a comparative molecular analysis of MSSA and MRSA isolates from bloodstream infections (BSIs) and healthy carriers (HCs).
- To investigate the combination of microarray profiling and *spa* typing to improve discrimination of ST22-MRSA-IV isolates from patients, environmental sites and healthcare workers in one Dublin hospital.

The two *mecA*-negative MRSA isolates were identified as CC130-MRSA. Whole-genome sequencing of one isolate (M10/0061) identified a novel *mec* gene, *mecC*, with 62% amino acid identity to *mecA* and a novel 30 kb SCC*mec* element encoding highly divergent *mec* and *ccr* genes and an arsenic resistance operon. This element was designated SCC*mec* XI and consisted of a novel *mec* (class E *mec*: *blaZ-mecC-mecR1-mecI*) and *ccr* (type 8: *ccrA1B3*) complex. SCC*mec* XI was also identified in the second CC130 MRSA isolate (M10/0148) by PCR and sequencing. Previously reported CC130 have mainly been reported in animals, suggesting an animal origin of these isolates. The highly divergent nature of SCC*mec* XI relative to other SCC*mec* elements indicates that it may have originated in another taxon. Other studies from Europe have also revealed the emergence SCC*mec* XI MRSA in several animal lineages in both animals and humans.

A previous study identified the *S. epidermidis ccrAB4* gene in ST8-MRSA-IIA-IIE and IVE/IVF (*spa* type t190) from Irish hospitals. In the present study whole-genome sequencing of an ST8-MRSA-IIE isolate (AR13.1/3330.2) identified a 14 kb *ccrAB4*-SCC element downstream and adjacent to SCC*mec* IIE. The SCC exhibited 99% DNA identity to SCC_{M1} previously identified as part of a CI in Danish ST8 MRSA (*spa* type t008) isolates and was similar to the region between SCC_{pbp} and IS*431* in SCC-CI in *S. epidermidis* ATCC12228. Eighty-five isolates were tested for the *S. epidermidis ccrAB4* gene by PCR including 63 ST8-

MRSA-IIA-IIE, 20 ST8-MRSA-IVE-IVF isolates and one isolate each of ST94-MRSA-IV and ST22-MRSA-IV that yielded signals for *ccrAB4* by microarray profiling and 94% (80/85) of isolates were positive. Long-range PCRs and primers based on SCC_{M1} were used to investigate one isolate representative of each of these *ccrAB4*-positive genotypes for SCC_{M1} and its location downstream of SCC*mec* was confirmed for all except the ST8-MRSA-IVE/IVF isolates. The ST22-MRSA-IV isolate harboured ACME in addition to SCC_{M1}. In total nine novel SCC*mec*-SCC CIs were identified, which provided further evidence of the horizontal transfer and recombination of SCC elements among staphylococci.

Identification of 53 CoNS isolates from patients in Irish hospitals by 16S rDNA sequencing yielded *S. epidermidis* (56.6%), *S. hominis* (18.9%), *S. haemolyticus* (13.2%), *S. capitis* (7.5%) and *S. warneri* (3.8%). Microarray profiling identified SCC and SCC*mec*-associated genes in 88.7% (47/53) of isolates and 71.7% (38/53) harboured *mecA*. Previously identified SCC*mec* types were identified in 34.1% (16/47) of SCC*mec*/SCC-positive isolates, while 65.9% (31/47) harboured possible novel SCC and SCC*mec* types and subtypes. SCC*mec* IV predominated among *S. epidermidis*, class A *mec* and *ccrAB1* among *S. hominis* and *ccrAB4* among *S. haemolyticus*. Each isolate harboured at least one antimicrobial resistance gene with *blaZ* (92.5%) and *qacA* (77.4%) predominating. ACME was detected in 11.3% of isolates. MLST analysis assigned the majority of *S. epidermidis* to the predominant nosocomial lineage, CC2, and all *S. haemolyticus* isolates were assigned to CC1. These results provide further evidence that specific CoNS species are a reservoir for SCC*mec* genes as well as antimicrobial resistance genes and ACME in *S. aureus*.

A total of 83 MRSA and 85 MSSA from BSIs and 53 MRSA and 98 MSSA from HCs were investigated by *spa* typing and microarray profiling. The MRSA were assigned to six CCs and 34 *spa* types and the MSSA to 33 CCs and 100 *spa* types. ST22-MRSA-IV predominated among both groups of MRSA isolates (83.8%, 114/136) and CC30-MSSA (33.7%, 61/181) and CC45-MSSA (18.2%, 33/181) among the MSSA. Similar genotypes were detected among MRSA from BSIs and HCs and among MSSA from BSIs and HCs. Unsurprisingly MRSA harboured more antimicrobial resistance genes than the MSSA isolates, but MSSA isolates harboured more virulence genes.

Analysis of 178 MRSA from the hands of healthcare workers (HHCWs) (n = 38), patients (n = 82) and environmental sites (n = 61) in one Irish hospital was undertaken by *spa* typing and microarray profiling. ST22-MRSA-IV predominated (178/181 isolates). The *spa* type t032 predominated among isolates from HHCWs (63.2%, 24/38) and patients (47.6%, 39/82), but t557 predominated among environmental isolates (40.9%, 25/61). Microarray profiling identified 12 variable virulence and resistance genes among ST22-MRSA-IV isolates and each combination of variable genes was assigned a microarray group (MG) number, yielding 19 MGs. Isolates with the *spa* type, t032, were assigned to 14 MGs. Among the 66 possible pairwise combinations of these 14 MGs, isolates from 12 were deemed to be closely related (differed by one gene/gene cluster), three were deemed to be related (differed by two genes/gene clusters) and 51 were deemed to be different (differed by ≥ 3 or more genes). Analysis of *spa* type/MG combinations exhibited by isolates from three wards identified similar ST22-MRSA-IV strains from patients, environmental sites and HHCWs. The combination of *spa* typing and microarray profiling enhanced ST22-MRSA-IV discrimination.

This study has revealed unprecedented diversity among SCC*mec*, SCC and CIs among staphylococci with the identification of a novel *mec* gene and SCC*mec* element and several novel CIs in MRSA as well as a large range of SCC*mec* and SCC genes among CoNS. Additionally, the higher virulence potential of MSSA compared to MRSA was revealed as well as the usefullness of array profiling for characterisation of MRSA, MSSA and CoNS.

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Abbreviations

aa	amino acid
ACME	arginine catabolic mobile element
agr	accessory gene regulator
AR	antibiogram-resistogram
BLAST	basic local alignment search tool
bp	base pair
BSI	bloodstream infection
BURP	based upon repeat pattern
ca.	approximately
cap	capsule
CBA	Columbia blood agar
CA-MRSA	community-associated MRSA
CC	clonal complex
ccr	cassette chromosome recombinase gene complex
CDC	Centers for Disease Control and Prevention
cDNA	complementary DNA
CHEF	clamped homogenous electrical field
CHIP	chemotaxis inhibitory protein
CI	composite island
CLSI	Clinical and Laboratory Standards Institute
cm^2	centimetre squared
CoNS	coagulase-negative staphylococci
Ct	threshold cycle
dlv	double locus variant
DNA	deoxyribonucleic acid
DR	direct repeat

EARS-Net	European Antimicrobial Resistance Surveillance Network (Formerly EARSS)
EDTA	ethylenediaminetetraacetic acid
e.g.	for example
egc	enterotoxin gene cluster
et al.	and others
etc.	*et cetera*
EVD	external ventricular device
FBS	foetal bovine serum
g	gram
GrMSSA	gentamicin-resistant methicillin-susceptible *Staphylococcus aureus*
h	hours
HA-MRSA	healthcare-associated MRSA
HC	healthy carriers
HHCWs	hands of healthcare workers
HRP	horseradish peroxidase
hVISA	heterogenous vancomycin-intermediate *Staphylococcus aureus*
H_2O_2	hydrogen peroxide
I	intermediate
i.e.	that is
IEC	immune evasion cluster
IR	inverted repeat
IS	insertion sequence
ISS	integration site sequences
IWG-SCC	International Working Group-Staphylococcal Cassette Chromosome
J region	junkyard region

kb	kilobase pair
L	litre
L agar	Luria-Bertani agar
LA-MRSA	livestock-associated MRSA
M	molar
mec	methicillin resistance gene
mg	milligram
MG	microarray group
MGE	mobile genetic element
MH	BBL Mueller-Hinton II agar
MIC	minimum inhibitory concentration
min	minute
ml	millilitre
MLST	multilocus-sequence typing
mM	millimolar
MRSA	methicillin-resistant *Staphylococcus aureus*
MRSE	methicillin-resistant *Staphylococcus epidermidis*
MRSH	methicillin-resistant *Staphylococcus haemolyticus*
MRSHo	methicillin-resistant *Staphylococcus hominis*
MSCRAMMs	microbial surface components recognizing adhesive matrix molecules
MSSA	methicillin-susceptible *Staphylococcus aureus*
MSSE	methicillin-susceptible *Staphylococcus epidermidis*
ng	nanogram
NHCWs	nares of healthcare workers
NMRSARL	National MRSA Reference Laboratory
NT	non-typeable

NT	non-typeable
ORF	open reading frame
PBS	phosphate buffer saline
PBPs/PBP2′	penicillin binding proteins/ penicillin binding protein 2′
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PFT	pulsed-field type
PVL	Panton-Valentine leukocidin
qRT-PCR	quantitative real-time PCR
R	resistant
RNA	ribonucleic acid
RPLA	reverse passive latex agglutination
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid
s	seconds
S	susceptible
sea	enterotoxin A
sak	staphylokinase
SCIN	staphylococcal complement C3 convertase inhibitor
SCC	staphylococcal cassette chromosome
SCC-CI	staphylococcal cassette chromosome-composite island
SCC*mec*	staphylococcal cassette chromosome *mec*
SeqNet	European Network of Laboratories for Sequenced Based Typing of Microbial Pathogens
SID	Simpson's index of diversity
slv	single locus variant

SNP	single nucleotide polymorphosim
ST	sequence type
SSTI	skin and soft tissue infection
SSSS	staphylococcal scalded skin syndrome
TBE buffer	tris-borate/EDTA electrophoresis buffer
TE buffer	tris-EDTA buffer
TMB	tetramethyl benzidine
TSA	trypticase soy agar
TSST-1	toxic shock syndrome toxin
µg	microgram
µM	micromolar
UK	United Kingdom
µl	microlitre
Unf	unfamiliar
USA	United States of America
UV	ultraviolet
v/v	% volume in volume: express the number of millilitres of an active constituent in 100 milliliters of solution
w/v	% volume in volume: express the number of grams of an active constituent in 100 milliliters of solution
%	percentage
≥	greater than or equal to
≤	less than or equal to
>	greater than
<	less than
°C	degrees centigrade
n	number

α	alpha
β	beta
γ	gamma
′	prime
ψSCC	pseudo SCC
ψSCC*mec*	pseudo SCC*mec*

Publications

Some of the original work presented in this thesis has been published in international research journals subject to peer review as listed below. Copies of these publications are included at the end of this thesis.

Shore AC, Brennan, OM, Rossney AS, **Deasy EC**, Kinnevey P, Ehricht R, Slickers P, Monecke S, Coleman DC. 2012. An investigation of the usefulness of a DNA microarray for typing MRSA isolates recovered over a 30-year period in Irish hospitals. *Antimicrobial Agents and Chemotherapy*. **56** (8): 5340-5355.

Shore AC, **Deasy EC**, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC. Detection of Staphylococcal Cassette Chromosome *mec* Type XI Encoding Highly Divergent *mecA*, *mecI, mecR1, blaZ* and *ccr* Genes in Human Clinical Clonal Complex 130 Methicillin-Resistant *Staphylococcus aureus*. 2011. *Antimicrobial Agents and Chemotherapy*. **55** (8): 3765-3773.

Creamer E, Shore AC, **Deasy EC**, Galvin S, Dolan A, Walley N, McHugh S, Fitzgerald-Hughes D, Sullivan DJ, Cunney R, Coleman DC, Humphreys H. Air and surface contamination patterns of methicillin-resistant *Staphylococcus aureus* on eight acute hospital wards. 2014. *Journal of Hospital Infections.* **86** (3): 201-208

Chapter 1

General Introduction

1.1 Staphylococci

Staphylococci are Gram-positive bacteria that belong to the *Micrococcaceae* family. They are catalase positive, facultative anaerobes and appear as cocci when viewed under the microscope and grow in clusters. More than 40 species of staphylococci have been described to date (Widersrom *et al.*, 2010) and they can be differentiated into two broad groups, coagulase-positive and coagulase-negative, based on their ability or inability, respectively, to produce coagulase, an enzyme that catalyses the conversion of fibrinogen to fibrin.

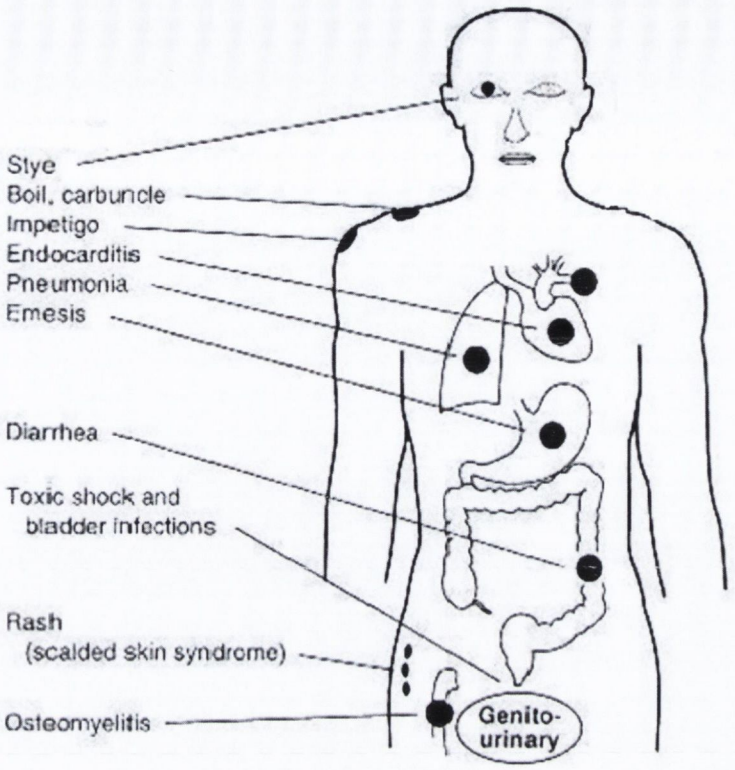
1.1.1 Coagulase-positive staphylococci: *Staphylococcus aureus*

Staphylococcus aureus is the best known coagulase-positive staphylococcal species and it is the most pathogenic of all the staphylococci. It forms part of the normal microbial flora of humans and animals but it is also an important pathogen. Approximately 30% of healthy individuals carry *S. aureus* in the anterior nares, although multiple body sites can be colonised including the throat, axilla, perineum, the gastrointestinal tract and the groin (Van Belkum *et al.*, 2009). However, once host defenses become compromised such as damage to the skin or mucous membrane barriers, *S. aureus* can cause a wide range of infections from skin and soft tissue infections (SSTIs), to food poisoning, toxic shock, bloodstream infections, endocarditis, osteomyeltis, septic athritis, pneumonia and wound infections (Lowy, 1998) (Figure 1.1). It is one of the most common causes of healthcare-associated infections (HCAIs), and it has also emerged as a significant cause of infections among individuals in the community (Said-Salim *et al.*, 2003) and among animals (Wesse *et al.*, 2010).

1.1.2 Coagulase-negative staphylococci (CoNS)

Coagulase-negative staphylococci (CoNS) form part of the normal microbial flora of human and animal skin and mucous membranes (Piette *et al.*, 2009) and are often

(a)



(b)

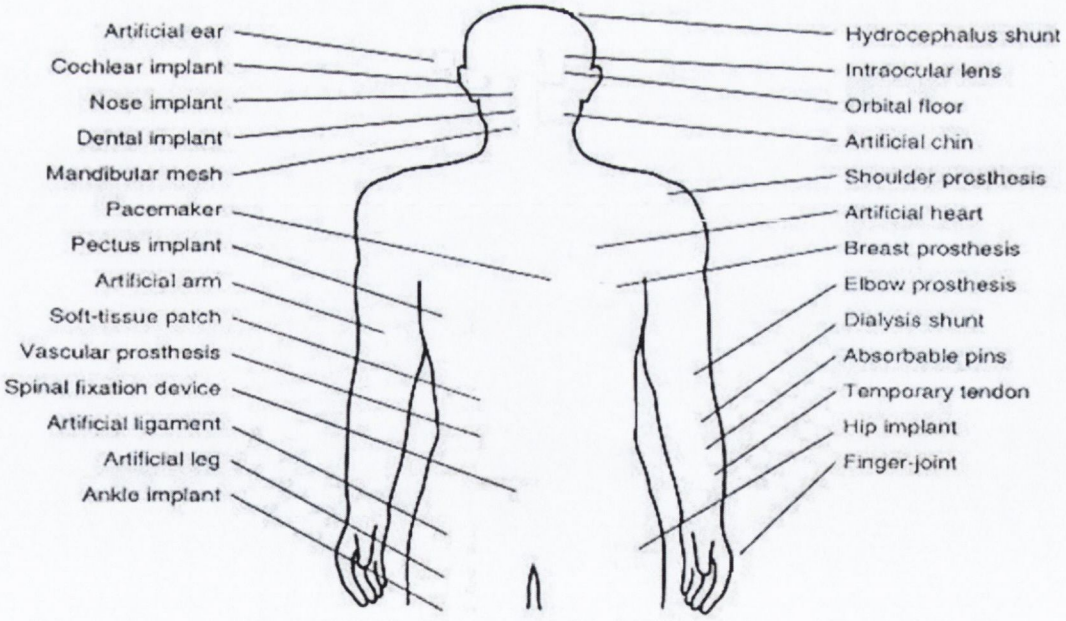


Figure 1.1. Examples of the range of human infections caused by S*taphylococcus aureus* (panel a) and the sites of implanted and indwelling devices associated with coagulase-negative staphylococci (CoNS) infections (panel b). Adapted from Foster, 1996.

considered to be contaminants of samples sent to diagnostic laboratories rather than the causative agents of infections (von Eiff *et al.*, 2002). However, CoNS are frequently associated with opportunistic human and veterinary infections particularly in neonates, immunocompromised patients and patients with indwelling and implanted devices (Piette *et al.*, 2009) (Figure 1.1). Over 40 species of CoNS have been described to date (Widersrom *et al.*, 2010), but clinically, *S. epidermidis* is the most relevant and the most common cause of infections of prosthetic and implanted devices in humans (Vuong *et al.*, 2002; Otto, 2009). *Staphylococcus haemolyticus* is commonly reported as the second most clinically-relevant CoNS species and has been associated with endocarditis, septicaemia, peritonitis, and wound, bone and joint infections particularly in neonates and patients with haematological disorders (Froggat *et al.,* 1989, Takeuchi *et al.*, 2005). Other CoNS species can also cause a variety of infections e.g. *Staphylococcus saprophyticus* has been associated with urinary tract infections (Raz *et al.*, 2005), *Staphylococcus lugdunensis* with arthritis, catheter-related infections, endocarditis and prosthetic joint infections (Frank *et al.*, 2008; Chung *et al.,* 2012) and *Staphylococcus capitis* with endocarditis (Kamalakannan *et al.*, 2004; Cone *et al.,* 2005).

1.2 Antimicrobial resistance in staphylococci

1.2.1 Antimicrobial resistance in *S. aureus*

Antimicrobial resistance in *S. aureus* was first described in the 1940's shortly after the introduction of penicillin into clinical practice (North & Christie, 1946). Penicillin is a β-lactam antibiotic and penicillin resistance is due to the production of a penicillinase enzyme (β-lactamase) that degrades the β-lactam ring of this antmicrobial agent (North & Christie, 1946). This led to the development of the penicillinase-resistant semi-synthetic β-lactam antibiotics such as methicillin, but within two years of the introduction of methicillin into clinical practise methicillin-resistant *S. aureus* (MRSA) was identified

(Jevons, 1961). Methicillin-resistant *S. aureus* exhibit phenotypic resistance to methicillin and related β-lactam antibiotics including cefoxitin and oxacillin but they can also be resistant to multiple clinically used antimicrobial agents including aminglycosides, fluroquinolones, macrolides, lincosamides, trimethoprim, linezolid, tetracycline, fusidic acid, mupirocin and glycopeptides (Kali *et al.*, 2013). Multi-resistant MRSA strains became a major nosocomial problem worldwide in the 1970s and 1980s (Shanson *et al.*, 1981) and today MRSA is one of the leading causes of HCAIs (Kali *et al.*, 2013).

1.2.2 Antimicrobial resistance in CoNS

Methicillin resistance is more common among CoNS than MRSA (Monkolrattanotahi *et al.*, 2004). Resistance to other antimicrobial agents including macrolides, glycopeptides, lincosamides, trimethoprim, tetracycline, linezolid, fusidic acid and quaternary ammonium compounds has also been reported to be more common among CoNS, especially *S. epidermidis,* than *S. aureus* (Koksal *et al.*, 2009; Bouchami *et al.,* 2011; LeBouter *et al.,* 2011; Zhang *et al.*, 2011; Mendes *et al.,* 2012; Cabrara-Contreras *et al.*, 2013).

1.2.3 Mechanisms of resistance in staphylococci

Resistance to antimicrobial agents can arise either through spontaneous mutations in the genes targeted by the antimicrobial agent or acquisition of antimicrobial resistance genes. These antimicrobial resistance genes are often carried on mobile genetic elements (MGEs) that can be horizontally transferred and include plasmids, transposons and staphylococcal cassette chromosome (SCC) elements (Malachowa & DeLeo, 2010). These antimicrobial resistance genes can encode (i) enzymes that modify the drug rendering it inactive, (ii) enzymes that modify the drug target site, (iii) proteins that act as efflux pumps that transport the antimicrobial agent out of the cell or (iv) alternative proteins that the antimicrobial agent will bind to instead of the target site. Examples of the target sites of

clinically relevant antimicrobial agents and resistance mechanisms in staphylococci are shown in Table 1.1. The location of many of these antimicrobial resistance genes on MGEs, the presence of similar resistance genes in *S. aureus* and CoNS and the fact that many of these resistance genes are more common among CoNS than *S. aureus*, has led many researchers to speculate that CoNS are a reservoir for antimicrobial resistance genes in *S. aureus* (Zong *et al.*, 2011). Despite this, detailed studies of the extensive range of antimicrobial resistance genes and phentoypes that exist in CoNS are lacking with most studies focusing on particular genes or phenotypes (LeBouter *et al.*, 2011; Bouchami *et al.*, 2011; Zhang *et al.*, 2011).

1.3 Staphylococcal chromosome cassette (SCC)

1.3.1 SCC*mec*

Methicillin inhibits bacterial growth by binding to the enzymatic site of penicillin binding proteins (PBPs) that catalyse cross-linking or transpeptidation of the bacterial cell wall (Labischinski, 1992). This results in weakening of the structure of the cell wall, release of the cytoplasmic contents of the cell and ultimately cell death. MRSA and methicilln-resistant CoNS (MR-CoNS) arise from methicillin-susceptible *S. aureus* (MSSA) and methicillin-susceptible CoNS (MS-CoNS), respectively, following acquisition of the methicillin resistance gene *mecA*. The *mecA* gene encodes an alternative PBP termed PBP2a that has decreased affinity for β-lactam antibiotics and is only bound at levels in excess to those used in clinical practice (Hartman *et al.*, 1984). This alternative PBP allows cell wall biosynthesis to continue despite the inactivation of the four native PBPs by methicillin (Hartman *et al.*, 1984; Reynolds *et al.*, 1985).

The *mecA* gene is carried on a large MGE designated SCC*mec* (Ito *et al.*, 1999) (Figure 1.2). SCC*mec* elements are characterised by (i) a *mec* complex that consists of *mecA* and, when present, its regulatory genes *mecI* and *mecR1*, as well as insertion

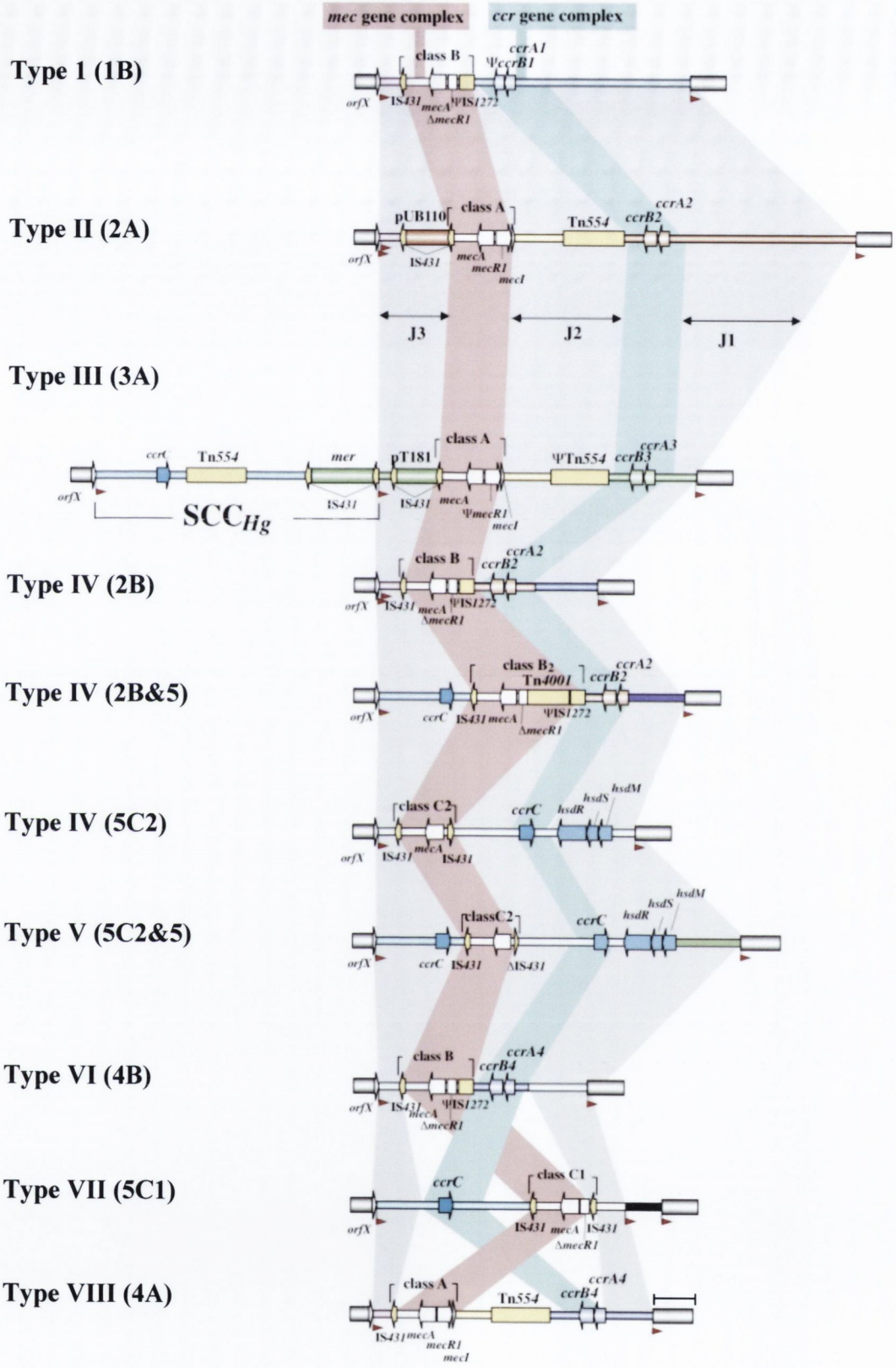
sequences, (ii) cassette chromosome recombinase (*ccr*) genes that are necessary for accurate and site-specific integration and excision of SCC*mec* into and out of the staphylococcal genome, (iii) integration into the integration site sequence (ISS) located at the 3' end of an open reading frame (ORF) designated *orfX* encoding a ribosomal methyltransferase (Boundy *et al.*, 2013), (iv) inverted and direct repeat (IR and DR, respectively) flanking sequences that are involved in the integration and excision of SCC*mec* (Wang *et al.*, 2012) and (v) joining regions (J1, J2 and J3) outside of the *mec* and *ccr* gene complexes that can harbour integrated plasmids and transposons carrying additional resistance determinants (IWG-SCC, 2009).

1.3.1.1. SCC*mec* in MRSA

Three classes of the *mec* gene complex (A-C) that differ in their genetic organisation, have been described in MRSA (IWG-SCC, 2009). Each has an intact copy of *mecA*, a copy of the insertion sequence IS*431* and, when present, complete or truncated *mec* regulatory genes, *mecI* (a repressor), *mecR1* (a sensor inducer) and the recently identified repressor gene, *mecR2* (previously designated *xylR*) (Arede *et al.*, 2012). Five *ccr* gene complexes, designated *ccrA1B1*, *ccrA2B2*, *ccrA3B3*, *ccrA4B4* and *ccrC* have been identified among MRSA. Based on the different combinations of *ccr* and *mec* complex genes, ten SCC*mec* types (I-X) have been described in MRSA in the published literature, at the outset of this study (Figure 1.2). Numerous SCC*mec* subtypes have also been reported and are defined based on variation in the J region, due to mutations or the integration of plasmids or transposons, and are designated either using a lower or uppercase alphabetic designation or a numerical designation after the SCC*mec* type name (Ito *et al.*, 2001; Ma *et al.*, 2002; Ito *et al.*, 2004; Boyle-Vavra *et al.*, 2005; Shore *et al.*, 2005; Oliveira *et al.*, 2006; Berglund *et al.*, 2008; IWG-SCC, 2009; Zhang *et al.*, 2009).

Table 1.1. Examples of antimicrobial agents used to treat staphyloccal infections, their target sites and mechanisms of resistance in staphylococci

Antimicrobial agent(s)	Target	Resistance mechanism	Reference
Methicillin, oxacillin and cefoxitin	Peptidoglycan biosynthesis i.e. PBPs	Alternative binding site, PBP2a; antibiotics only bound at levels in excess of the therapeutic range	Hartman *et al.*, 1984
Aminoglycosides	Protein synthesis i.e. 30S ribosomal subunit	Alteration of binding site in 30S ribosomal subunit. Enzymatic inactivation of antimicrobial agent (*aadA-aphD*, *aadD* and *aphA3*)	Rouch *et al.*, 1987; Derbise *et al.*, 1996; Schwarz *et al.*, 2011
Fusidic acid	Protein synthesis i.e. elongation factor G (EF-G)	Reduced affinity for EF-G due to mutations in gene encoding EF-G (*fusA* and *fusE*). Protection of EF-G due to *fusB*, *fusC* and *fusD*	O'Brien *et al.*, 2002; O'Neill et al., 2006; O'Neill *et al.*, 2007
Linezolid	Protein synthesis i.e. 23S ribosomal subunit	Mutations in 23S. Methylation of 23S rRNA due to *cfr*	Schwarz *et al.*, 2000; Tsiodras *et al.*, 2001; Kehrenberg *et al.*, 2006; Long *et al.*, 2006
Vancomycin	Peptidoglycan biosynthesis i.e. D-ala-D-ala	Alternative subunit for peptidoglycan biosynthesis encoded by *vanA* and *vanB*	Arther *et al.*, 1996; Courvalin *et al.*, 2006
Macrolides and lincosamides	Protein synthesis i.e. 50S ribosomal subunit	Methylation of target site (*erm*(A), *erm*(B) and *erm*(C)). Enzymatic inactivation of the antimicrobial agent (*msr*(A), *mph*(C), and *lun*(A))	Horiniuchi *et al.*, 1982; Murphy *et al.*, 1985; Luna *et al.*, 2002; Kadlec *et al.*, 2010; Schwarz *et al.*, 2011; Loranzo *et al.*, 2012
Tetracycline	Protein synthesis i.e. 30S ribosomal subunit	Efflux pump encoded by *tet*(K) and *tet*(L). Enzymatic modification of 30S ribosomal target site encoded by *tet*(M) and t*et*(O)	Nesin *et al.*, 1990; Robert *et al.*, 1996; Kadlec *et al.*, 2010
Mupirocin	Protein synthesis i.e. isoleucyl tRNA synthetase	Low level resistance due to mutation in target binding site. High level resistance due to acquisition of *mupA* or *mupB* that encodes an alternative binding site.	Hodgson *et al.*, 1994; Perez-Roth *et al.*, 2010; Seah *et al.*, 2012


mec gene complex
ccr gene complex
Type 1 (1B)
class B
ccrA1
ΨccrB1
orfX
IS431
mecA
ΨIS1272
ΔmecR1
Type II (2A)
pUB110
class A
Tn554
ccrB2
ccrA2
IS431
mecA
mecR1
mecI
J3
J2
J1
Type III (3A)
ccrC
Tn554
mer
pT181
ΨTn554
ccrB3
ccrA3
SCC$_{Hg}$
ΨmecR1
Type IV (2B)
Type IV (2B&5)
class B$_2$
Tn4001
Type IV (5C2)
class C2
hsdS
hsdM
hsdR
Type V (5C2&5)
classC2
Type VI (4B)
ccrB4
ccrA4
Type VII (5C1)
class C1
Type VIII (4A)

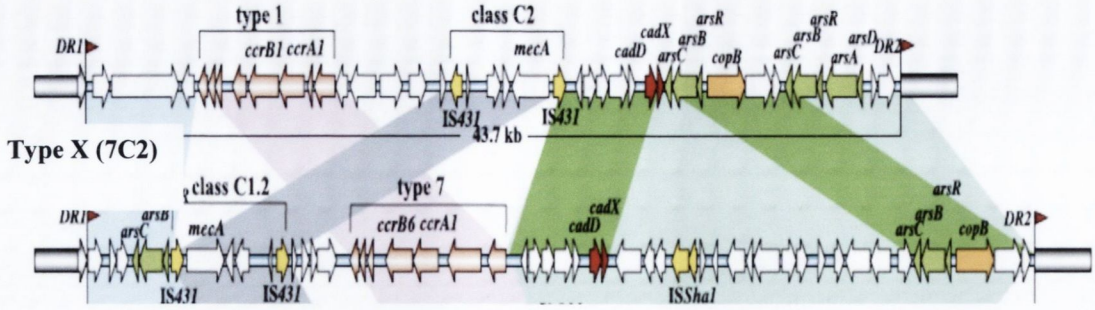


Figure 1.2. Schematic diagram showing the genetic organisation of SCC*mec* I, NCTC10442 (Genbank accession number AB033763); SCC*mec* II, N315 (D86934); SCC*mec* type III*mer*, 85/2082 (AB037681); SCC*mec* type IV, CA05 (AB0633172) and ZH47 (AM292304); SCC*mec* type V, CA05 (AB063172), SCC*mec* type VI, HDE288 (AF411935); SCC*mec* type VII, JCSC6082 (AB373032) and SCC*mec* type VIII, C10682 (C10682), SCC*mec* IX, JCSC6943 (AB505630) and SCC*mec* X, JCSC6945 (AB478780). The number and alphabetic designations in parenthesis after the SCC*mec* type name indicate the *ccr* and *mec* complex types characteristic of each SCC*mec* type i.e 1B after SCC*mec* I refers to *ccrAB1* and class B *mec*. Adapted from IWG-SCC, 2009 (SCC*mec* I-IX) and Li *et al.*, 2011 (SCC*mec* X & XI).

1.3.1.2 SCC*mec* in CoNS

SCC*mec* elements in CoNS have not been studied in as much detail as in MRSA. However, SCC*mec* elements are more abundant and more diverse among CoNS, and while similar SCC*mec* elements have been identified in CoNS and MRSA (Bloemendal *et al.*, 2010, Barbier *et al.*, 2010, Smyth *et al.*, 2010), many CoNS harbour SCC*mec* elements not identified to date in MRSA. Many studies have reported non-typeable SCC*mec* elements in CoNS in which the *ccr* and/or *mec* gene complexes are not detected (Wisplinghoff *et al.*, 2003; Hanssen *et al.,* 2006; Barbier *et al.*, 2010; Bloemendal *et al.*, 2010; Garza-Gonzalez *et al.,* 2010; Smyth *et al.*, 2010). Additionally, putative ancestral SCC*mec* elements and *mec* genes have been identified in CoNS and related species (see section 1.3.3). This has led many researchers to speculate that CoNS are a reservoir for SCC*mec* in *S. aureus* (Wu *et al.,* 1998; Schnellmann *et al.*, 2006) but further studies are required to determine the diversity and abundance of SCC*mec* among CoNS in order to fully understand the reservoir of SCC*mec* elements that exist.

1.3.2 SCC, SCC-like elements and composite islands

Various SCC and SCC-like elements have also been described in CoNS and *S. aureus* and include (i) SCC elements that lack *mecA* but carry *ccr* genes, (ii) pseudo SCC*mec* (ψSCC*mec*) elements that carry a *mec* gene complex but lack *ccr*, and (iii) pseudo SCC (ψSCC) elements (e.g. the arginine catabolic mobile elements (ACME)) that lack *ccr* and *mec* genes and are normally found as part of a composite island (Shore & Coleman, 2013) (Table 1.2). Composite islands (CIs) have also been described in staphylococci and consist of two or more SCC*mec* and/or atypical SCC*mec* elements (Ito *et al.*, 2001; Monkolrattanothai *et al.*, 2004; Takeuchi *et al.*, 2005; Bartels *et al.*, 2011; Shore *et al.*, 2011b; Kinnevery *et al.*, 2013). Similar to SCC*mec* elements, SCC, SCC-like elements and

CIs insert into *orfX* using *ccr* genes for excision and integration and are flanked by IR and DR sequences (IWG, 2009).

A number of CIs have been described to date in staphylococci, ranging in size from 45 to 91 kb (Ito *et al.*, 2001; Mongkolrattanothai *et al.*, 2004; Takeuchi *et al.*, 2005; Bartels *et al.*, 2011; Shore *et al.,* 2011b; Kinnevey *et al.,* 2013). In CoNS, SCC-CIs have been described and fully sequenced in *S. epidermidis* strain ATCC12288 and *S. haemolyticus* strain JCSC1435. The *S. epidermidis* SCC-CI is the best characterised CI in CoNS. It is 91 kb in size and consists of two SCC elements, one with *ccrAB4* and the other *ccrAB2*, the latter element also carrying *tagF* and *pbp* encoding a teichoic acid synthesis protein and a penicillin binding protein, respectively, as well as ACME (Mongkolrattanothai *et al.*, 2004). A previous study revealed the possible presence of a SCC-CI in Irish ST8-MRSA-IIA-E and IVE/F isolates due to the detection and localisation of *ccrAB4* adjacent to the SCC*mec* element in isolates of this clone (Shore *et al.,* 2008). Interestingly, the *ccrAB4* gene exhibited 100% DNA sequence identity to that of the *S. epidermidis* SCC-CI suggesting the presence of a SCC-CI in these isolates that may have originated in CoNS (Shore *et al.*, 2008). However, detailed sequence analysis of this SCC-CI ST8-MRSA-IIA-E and IVE/F isolates is required in order to confirm this.

The diversity of CIs in staphylococci highlight the complex evolution of SCC*mec*, SCC and SCC-like elements and how multiple antimicrobial resistance, and in some case virulence genes, can be carried on these elements. Ideally, the complete characterisation and nucleotide sequencing of these elements is necessary to provide insights into the evolution of SCC*mec* in MRSA. Additionally, CIs carrying multiple *ccr* genes can present a challenge to SCC*mec* typing and require detailed analysis so that accurate typing can be performed.

Table 1.2. Examples of fully sequenced SCC elements, pseudo SCC and pseudo SCC*mec* elements identified in staphylococci

Element type (definition)[a]	Example (size)	Associated staphylococcal species	Characteristic genes			Reference
			ccr type	*mec* complex genes	Additional antibiotic resistance or virulence genes	
SCC (*ccr* but no *mec* complex)	SCC_{M1} (14 kb)	*S. aureus*	*ccrAB4*	None	None	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
	$SCC_{M10/1071}$ (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*	Ψ*ccr*	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* & *tag4*	Monkolrattanothai *et al.*, 2004
	$SCC_{ATCC12228}$ (38 kb)	*S. epidermidis*	*ccrAB4*	None	*copA*, mercury & cadmium resistance gene cluster	Katayama *et al.*, 2003
Pseudo (ψ) SCC*mec* (*mec* gene complex but lack of *ccr* genes)	ψSCC*mec* II.5 (ca. 21 kb)	*S. aureus*	None	*mecA* & Δ*mecR1*	*ant*(4')	Han *et al.*, 2009
	$\psi SCCmec_{16691}$ (11 kb)	*S. aureus*	None	IS*431*, *mecA*, *mecR1* & *mecI*	None	Chen *et al.*, 2010
	$\psi SCCmec_{M06/0171}$ (16 kb)	*S. aureus*	None	IS*431*, *mecA*, Δ*mecR1*& IS*431*	*copB*, *copC* & *fusC*	Kinnevey *et al.*, 2013
	ψSCC*mec*(*h1435*)[b]	*S. haemolyticus*	None	*mecA* & Δ*mecR1*	*cadD*, *cadX*, *arsC*, *arsB*, & *arsR*	Takeuchi *et al.*, 2005

Continued overleaf

Table 1.2 continued. Examples of fully sequenced SCC elements, pseudo SCC and pseudo SCC*mec* elements identified in staphylococci

Element Type (definition)[a]	Example (size)	Associated staphylococcal species	Characteristic genes			Reference
			ccr type	*mec* complex genes	Additional antibiotic resistance or virulence genes	
Pseudo (ψ) SCC (Lack of *ccr* and *mec* genes)	SCC-like or IE25923 (5.9 kb)	*S. aureus*	None	None	None	Ito *et al.*, 2001; Ito *et al.*, 2003; Jansen *et al.*, 2006
	CC_{V14} (ca: 5 kb)	*S. aurues*	None	None	None	Arakere *et al.*, 2009
	CC6082 (5.5 kb)	*S. aurues*	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
	ψSCC*h1*, ψSCC*h2*, ψSCC*h3* & ψSCC*h4*[b]	*S. haemolyticus*	None	None	*kdp* (SCC*h1*)	Takeuchi *et al.*, 2005
	ACME, ΔACME or ψSCC*arc* (12-34 kb)	Various staphylococcal species	None	None	*arc* & *opp* operons (*opp* not presence in ΔACME/ψSCC*arc*)	Diep *et al.*, 2006; Shore *et al.*, 2011b; Bartels *et al.*, 2011

Adapted from Shore & Coleman, 2013.

[a] Each of these elements share the common feature of integration into the ISS with *orfX* and are flanked by direct and inverted repeats but each also has unique characteristics which are indicated in parenthesis after the element type.

1.3.3 Evolution of SCC*mec* and SCC elements

Several studies have identified possible ancestral SCC*mec* and SCC elements as well as *mec* gene homolgues among CoNS and related species including *Staphylococcus sciuri, Staphylococcus fleuretii*, *Staphylococccus vitulinus*, and *Macrococcus caseolyticus* (Wu *et al.,* 1998; Schnellmann *et al.*, 2006; Tsubakishita *et al.*, 2010). Numerous SCC elements have been identified among CoNS, in particulary *S. hominis*, suggesting that the assembly of SCC*mec* may have occurred here (Bouchami *et al.*, 2011a). A *mec* gene was identified in *M. caseolyticus* that exhibited 72% animo acid identity to *mecA* in MRSA. This *M. caseolyticus mec* gene was localised to a transposon and formed part of a *mec* gene complex that consisted of the regulatory genes, *mecR1* and *mecI,* and for the first time, *blaZ* (Tsubakishita *et al.*, 2010). The *mec* gene complex in *M. caseolyticus* was identified adjacent to a non-*mecA* SCC transposon that was separated by a DR form the *mec* gene complex (Tsubakishita *et al.,* 2010a). The identification of *mecA* genes in the CoNS species *S. sciuri, S. vitulinus* and *S. fleuretti* also suggests that SCC*mec* may have originated in CoNS (Tsubakishita *et al.,* 2010b). The *mecA* gene is ubiquitous among *S. sciuri* but isolates of this species do not express methicillin resistance, leading researchers to speculate that *mecA* may have had another function such as cell wall biosynthesis in this species (Couto *et al.*, 1996). The *mecA* gene identified among *S. fleuretti* isolates was found as part of a putative precursor of the class A *mec* complex (Tsubakishita *et al.,* 2010b). Although all of this is evidence of potential precursors to SCC*mec* among CoNS and related species, the precise steps in the evolution of SCC*mec* have not yet been identified in CoNS and MRSA and no potential *mecA* precursors have yet been identified in MRSA.

1.4 Virulence factors

1.4.1 Virulence factors in *S. aureus*

The ability of *S. aureus* to cause a wide variety of infections is due to a large extent to the extensive range of virulence factors that it can express (Table 1.3). These virulence factors are involved in colonisation and infection of host tissues, evasion of the host immune system and can cause damage to host cells and tissues (Foster *et al.,* 1998). For the initiation of *S. aureus* colonisation, surface proteins, collectively termed microbial surface components recognising adhesive matrix molecules (MCSRAMMs), play an important role by promoting attachment to prosthetic materials and the extracellular matrix of the host such as laminin, collagen, fibronectin and fibrinogen (Foster *et al.,* 1998; Foster, 2009) (Table 1.3).

Once adherence of the *S. aureus* cells to host tissues or prosthetic materials has occurred, the organism can grow and persist and successfully evade the immune system. It can evade the immune system by expressing several structural and soluble proteins that reduce the effectiveness of neutrophils and macrophages. These include capsular polysaccharide, protein A, the lysogenic bacteriophage-encoded immune evasion complex (IEC) proteins, enterotoxin A (SEA), staphylokinase (SAK), chemotaxis inhibitory protein (CHIP), staphylococcal complement inhibitor (SCN) and staphyloccoal enterotoxin-like protein (SEP), as well as proteins involved in biofilm formation (Foster *et al.*, 1998; van Wammel *et al.*, 2006) (Table 1.3).

Staphylococcus aureus can also express many different enzymes and toxins that can damage and destroy host tissues and cells. These include extracellular enzymes such as lipase, protease and hyaluronidase and the membrane damaging toxins α-haemolysin and β-haemolysin which damage the membranes of platelets and monocytes and erythrocytes, respectively (Abdelnour *et al.*, 1993; Dinges *et al.*, 2000) (Table 1.3). Other toxins

Table 1.3. Examples of major virulence factors that can be expressed by *Staphlococcus aureus*

Virulence factor	Biological effect	Genes
Adhesion		
Fibronection binding protein[a]	Attachment to fibronection in host cell	*fnbpA, fnbpB*
Collagen binding protein[a]	Attachment to collagen in host cells	*cna*
Fibrinogen binding protein[a]	Attachment to fibrinogen and protects against macrophage phagocytosis	*clfA, clfB*
Elastin-binding protein	Attachment to tissues that are abundant in elastin	*ebpS*
Persistence		
Polysaccharide intercellular adhesion	Biofilm formation	*icaA, icaB, icaC, icaD*
Arginine catabolic mobile element (ACME)	Enhanced survival on the skin	*arcA, arcB, arcC, arcD*
Evasion of the immune system		
Capsular polysaccharide	Inhibits chemotaxis and phagocytic engulfment	*capH, capI, capJ, capK*
Protein A[a]	Inhibits antibody mediated clearance and interacts with platelets	*spa*
Immune evasion complex (IEC)	Inhibits immune response	*sea, sak, chp, scn, sep*
Damage to tissue and cells		
Panton-Valentine leukocidin (PVL)	Lysis of human leukocytes	*lukF-pvl, lukS-pvl*
Lipase	Hydrolyses lipids	*lip*
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 cytokine and cause damage to host tissue	*sea- seu*
Toxic shock toxin	Stimulate T cells to release cytokine and cause damage to host tissue	*tst*
Exfoliative toxins	Bind to desmosomes and break up the epidermal layers	*etA, etB, etD*

[a] These virulence factors belong to the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)

produced by some *S. aureus* isolates include the leukocidin toxins, the superantigens and the exfoliative toxins. The leukocidin toxins, include the bi-component pore-forming cytolytic toxin Panton-Valentine leukocidin (PVL), which is strongly associated with community-acquired (CA) MRSA (Boyle-Vavra *et al.,* 2007), result in the destruction of mononuclear and polymorphonuclear cells (Rossney *et al.,* 2007). The superantigens include the toxic shock syndrome toxin (TSST-1) and the staphylocoal enterotoxins (Fraser *et al.*, 2008) and cause non-specific T-cell activation resulting in massive release of cytokines. Lastly, the exfoliative toxins (ETA, ETB and ETD) are serine proteases that cause widespread blistering and loss of epidermis and can cause a severe skin condition termed staphylococcal scalded skin syndrome (Ladhani, 2003), and are associated with the epidermal cell differentiation inhibitior toxins (EDINA, EDINB, EDINC) that damage endothelial cells (Munro *et al.*, 2010) (Table 1.3). Some recently described virulence-factors in *S. aureus* include the SCC*mec*-associated cytolytic toxin phenol soluble modulin (PSM) (Queck *et al.*, 2009) and the arginine catabolic proteins encoded by ACME (Diep *et al.*, 2006). While the precise role of ACME has not yet been determined results from animal model studies indicate that it improves bacterial fitness and the ability to colonise skin and mucous membranes (Diep *et al.*, 2008).

Similar to antimicrobial resistance genes, many *S. aureus* virulence factors are located on MGEs including plasmids, bacteriophages, pathogenicity islands, genomic islands, SCC*mec* and SCC elements (Malachowa *et al.*, 2010). Some examples of MGE that carry virulence factors include the following: beta-toxin gene (*hlb*)-inactivating bacteriophages that encode IEC genes (van Wammel *et al.*, 2006); the pathogenicity islands that carry *tst* (SaPI1, SaPI2, SaPIbov1, SaPI3, SaPIn1/m1) (Kreiswirth *et al.,* 1989; Lindsay *et al.*, 1998; Ubeda *et al.,* 2003); the three genomic islands that have been reported to date in *S. aureus* including νSAα (*lpl,* and *ssl*), νSAβ (*seg*, *sei, sem, sen, seo, seu, lukD, lukE,* and *spl*) and νSAγ (*edinB, etD* and *hla*) (Baba *et al.,* 2002; Gill *et al.*, 2005).

1.4.2 Virulence factors in CoNS

Less is known about the mechanism of pathogenesis of CoNS but, in general, they are considered to be less virulent and harbour fewer virulence genes than *S. aureus* (von Eiff *et al.*, 2002). Survival of CoNS in the host is primarily due to biofilm formation, which protects the organism from antibiotics and host immune responses. For example, the production of polysaccharide intercellular adhesin (PIA), encoded by the *ica* gene cluster in *S. epidermidis*, can aid in adherence and initiation of foreign device infections (Vuong *et al.*, 2004).

Although a small number of studies have reported enterotoxins in CoNS, they appear to be rare, and to be mainly associated with CoNS from food sources, such as milk or meats (Pepe *et al.*, 2006; Veras *et al.*, 2008; Zell *et al.*, 2008). A recent report identified the first enterotoxin-bearing pathogenicity island in *S. epidermidis* that was shown to harbour the enterotoxin C and L genes (Madhusoodanan *et al.*, 2011). The SCC*mec* associated cytolytic toxin PSM-*mec* has also been reported among CoNS. Lastly, there have been numerous reports of the prevalence of ACME among different CoNS, in particular *S. epidermidis*, *S. haemolyticus* and *S. capitis* and many researchers have speculated that ACME originated in CoNS (Barbier *et al.*, 2010; Diep *et al.*, 2006; Miragaia *et al.*, 2009; Pi *et al.*, 2009). However, a small number of studies have revealed that ACME is more common among colonising rather than invasive CoNS isolates but further studies of CoNS from different types of infections and from colonisation are required in order to investigate this further (Montgomery *et al.*, 2009).

1.5 Typing of staphylococci

Effective and informative typing of both *S. aureus* and CoNS isolates is essential for detecting and tracking outbreaks and for determining the relatedness of isolates in both local and global epidemiological investigations. During the 1980s and early 1990s, typing

of bacteria primarily involved the use of phenotypic typing methods. During this time bacteriophage typing (Parker, 1972) and antimicrobial susceptibility testing (Rossney *et al.*, 1994) were predominantly used for typing *S. aureus*. While antimicrobial susceptibility testing can play a role in the initial typing of *S. aureus* and in determining an isolate's susceptibility to different antimicrobial agents, phenotypic typing methods are rarely used today and have been replaced by genotypic typing methods such as pulsed-field gel electrophoresis, DNA sequencing-based typing methods and more recently whole-genome based typing methods, that provide more informative and accurate typing of isolates. Methods used in the present study for typing of staphylococcal isolates are discussed in the following sections and are summarised in Table 1.4.

1.5.1 Antibiogram-resistogram (AR) typing of MRSA

Antibiogram-resistogram (AR) typing is a method used by the Irish National MRSA Reference Laboratory (NMRSARL) for the initial typing of MRSA isolates. It involves determining the susceptibility of each isolate to a panel of 23 antimicrobial agents using disk diffusion and recommendation of the Clinical and Laboratory Standards Institute (CLSI) (Rossney *et al.*, 1994a; Rossney *et al.*, 1994b; Rossney *et al.,* 2007) or, more recently, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology (personal communication Grainne Brennan, NMRSARL). The panel of antimicrobial agents includes both clinically used and non-clinical antimicrobial agents and chemicals so as to reduce the effect of selective pressure that the different agents and chemicals used in clinical practice may have on each isolate's AR pattern (Rossney *et al.,* 1994). Distinct AR patterns are assigned an AR type number eg: AR06, AR13 and AR14. Variations within the patterns are assigned to subtypes e.g. AR06.1, AR13.2 and AR14.1 (Rossney *et al.*, 1994a). An AR pattern that shares no similarity to known AR patterns is classified as Unfamiliar (Unf). Isolates that display susceptibility patterns which are similar to a particular AR type, but which are urease-positive are

described at Non Typeable (NT); e.g. the susceptibility pattern of the most prevalence type is Ireland is AR06, which is urease negative, but if an isolate yields an AR susceptibility pattern that is normally assigned to AR06 but is urease-positive, it is assigned as NT. AR patterns are subject to change due to the loss or acquisition of, or spontaneous mutations in, a resistance gene(s) and genotypic analysis of isolates exhibiting Unf and NT AR patterns need to be performed before new AR types are assigned. AR typing is easy to preform, relatively inexpensive and provides important information on the antimicrobial susceptibility of an isolate. However, AR patterns are subject to change due to selective pressure and loss and gain of resistance determinants, it offers a low discriminatory power and AR typing is not widely used outside of Ireland, so it is not possible to compare AR types internationally (Table 1.4). In addition, its use is currently restricted to MRSA as it not used for typing MSSA or CoNS isolates.

1.5.2 Pulsed-field gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory typing method and was, until recently, considered to be the gold standard method for typing *S. aureus* and CoNS isolates, in particular *S. epidermidis*, *S. haemolyticus* and *S. hominis*, in outbreak situations as it detects uncharacterised variation that accumulates rapidly (Fessler *et al.*, 2010; Kristof *et al.*, 2011; Botelho *et al.*, 2012; Mendoza-Olazaron *et al.*, 2013; Pereira *et al.*, 2013). During PFGE high molecular weight staphylococcal total cellular DNA is digested using a rare-cutting restriction endonuclease such as *SmaI*, which is frequently used for PFGE of *S. aureus*. The digested DNA is embedded in agarose plugs and subjected to agarose gel electrophoresis using a clamped homogenous electrical field (CHEF) electrophoresis system. The resulting banding patterns can be inspected visually and interpreted using the criteria developed by Tenover *et al.* (1995). Computer-assisted analysis of banding patterns can also be performed. Tenover *et al.* (1995) proposed the following criteria for interpreting PFGE banding patterns in outbreak situations over a

Table 1.4. Summary features of methods commonly used for typing of *S. aureus* and relevant to the present study

Typing method	Principle	Strengths	Weaknesses
Antibiogram-resistogram (AR) typing	Antimicrobial susceptibility testing	Standard nomenclature, interlaboratory portability, inexpensive	Low discriminatory power, not used outside of the Irish NMRSARL, used for MRSA only
Pulsed-field gel electrophoresis (PFGE)	Genomic DNA digestion using rare-cutting endonucleases and electrophoresis with periodic changing of the electric field direction	High discriminatory power, widely used	Time consuming, technically difficult, multiple nomenclatures, limited interlaboratory portability, useful only in outbreak/short-term studies
Multilocus sequence typing (MLST)	Sequencing of internal fragments of seven housekeeping genes	Standard nomenclature, interlaboratory portability, used for *S. aureus* clone nomenclature, useful for inferring evolutionary relatedness of isolates	Low throughput, high cost, only useful in long-term studies
spa typing	Sequencing of the variable X region in the protein A gene *spa*	Rapid, high throughput, standard nomenclature, interlaboratory portability, higher discriminatory power than MLST but lower than PFGE, useful in both long and short term studies, can infer MLST	Lower discriminatory power than PFGE
SCC*mec* typing	PCR analysis of the *ccr* and *mec* complex	Standard nomenclature, interlaboratory portability, used for MRSA clone nomenclature	Time consuming, high cost, low discriminatory power (but improves when combined with MLST and/or *spa* typing)
DNA microarray profiling	Simultaneous detection of 334 gene targets	Rapid, high throughput, genotype assignment and detection of an extensive range of SCC/SCC*mec*, virulence & resistance genes.	High cost
Whole-genome sequencing	Sequencing of entire genome	High discriminatory power, examines the entire genome	Relatively high cost, complex data analysis and interpretation

Abbreviations: NMRSARL, National MRSA Reference Laboratory.
Adapted from Stefani *et al.*, 2012.

short period of time: isolates that yield (i) indistinguishable PFGE banding patterns belong to the same strain; (ii) PFGE banding patterns that differ by between one and three bands are considered to be closely related, as it is likely that a single genetic event such as the acquisition or loss of a MGE or a spontaneous mutation resulted in these changes to the banding pattern; (iii) PFGE banding patterns that differ by between four to six bands are considered to be possibly related, as this reflects two genetic events; (iv) PFGE banding patterns with six or more band differences are considered to be unrelated, as this reflects three or more genetic events (Tenover *et al.*, 1995).

In the Irish NMRSARL each PFGE banding pattern is assigned a five-digit pulsed-field type (PFT) number and the first two digits of the PFT are then abbreviated to a pulsed-field group (PFG) number e.g. PFG-00, PFG-01, PFG-02 (Rossney *et al.*, 2006). Isolates that exhibit PFGE banding patterns that differ by less than or equal to six PFGE bands are assigned to the same PFG (Rossney *et al.*, 2006). The AR and PFG of each isolate are then combined to give each isolate an AR-PFG designation. For example, in Ireland for the past several years the most common nosocomial MRSA clone exhibits AR type 06 and PFG 01 and is therefore designated AR-PFG 06-01 (Rossney *et al.*, 2006).

PFGE has a high discriminatory power, but it is time-consuming process and interpretation of patterns is subjective, which can lead to difficulty in comparing results between different laboratories. Furthermore, currently there is no international consensus nomenclature for PFGE, despite several attempts to develop one. As in other countries until recently, PFGE was routinely used in the Irish NMRSARL and all MRSA isolates submitted to the laboratory underwent PFGE. However, the development of DNA sequencing and whole-genome based typing methods offer fast, accurate, unambiguous typing of isolates and the data can be easily compared between laboratories (Table 1.4). In many laboratories these methods have now replaced PFGE for typing of staphylococci, in particular *S. aureus*.

1.5.3 Mulitlocus sequence typing (MLST)

Multilocus sequence typing involves PCR amplification and sequencing of the internal fragments of seven unlinked housekeeping genes and is widely used for typing of many different microbial pathogens, including staphylococci (Enright *et al.*, 2000; Thomas *et al.*, 2007). It is particularly useful for determining the clonal relatedness of isolates in long-term and global epidemiological investigations due to the stability of housekeeping genes over time (Saunders & Holmes, 2007; Rijnders *et al.*, 2009; Wagennaar *et al.*, 2009; Argudin *et al.*, 2011) (Table 1.4). In addition, an international nomenclature has been developed for *S. aureus*, where a clone is defined based on its sequence type (ST) and/or clonal complex (CC) and, for MRSA, the SCC*mec* type (see section 1.5.5). MLST schemes have been developed for *S. aureus*, *S. epidermidis* and *S. haemolyticus* and the housekeeping genes used for these schemes are listed in Table 1.5. (Enright *et al.*, 2000; Thomas *et al.*, 2007; Cavanagh *et al.*, 2012). For *S. aureus* and *S. epidermidis* the sequenced fragments of the seven genes are compared to those in the web-based databases http://saureus.mlst.net/ and http://sepidermidis.mlst.net/, respectively. Each distinct sequence of a gene is assigned an allelic number. Using the same databases, each combination of the seven housekeeping loci is assigned a ST number (Enright *et al.*, 2000). The STs can be assigned to a MLST lineage or CC based on their evolutionary relatedness as determined using eBURST (Based Upon Repeat Sequence Types) (Feil *et al.*, 2004). The isolates are assigned to the same CC if a minimum of 5/7 MLST alleles are identical to at least one other ST within the CC (Enright *et al.*, 2000). While a MLST scheme has been developed for *S. haemolyticus* only a limited number of *S. haemolyticus* isolates have been investigated using this method and there is currently no web-based database available, making inter-laboratory comparison of results difficult. The development of MLST schemes for other clinically relevant CoNS species, such as *S. hominis*, should also be considered in the future.

Table 1.5. Housekeeping genes and their protein products used for MLST schemes for *S. aureus, S. epidermidis*, and *S. haemolyticus*

Species[a]	Gene(s)	Products
Staphylococcus aureus	*arcC*	Carbamate kinase
	aroE	Skikimate dehydrogenase
	glp	Glycerol kinase
	gmk	Guanylate kinase
	pta	Phosphate acetyltransferase
	tpi	Triosephosphate isomerase
	yqiL	Acetyl coenzyme A acetyltransferase
Staphylococcus epidermidis	*arcC*	Carbamate kinase
	aroE	Skikimate dehydrogenase
	gtr	ABC transporter
	mutS	DNA mismatch repair protein
	pyrR	Pyrimidine operon regulatory protein
	tpiA	Triosephosphate isomerase
	yqiL	Acetyl coenzyme A acetyltransferase
Staphylococcus haemolyticus	*arc*	Carbamate kinase
	SH 1200	Ser A: D-3-phosphoglycerate dehydrogenase
	hemH	Ferrochelatase
	leuB	3-isopropymalate dehydrogenase
	SH 1431	Cell surface elastin binding protein
	cfxE	Ribulose 5-phosphate epimerase
	Ribose ABC	ABC transporter

[a] Web-based databases are available for MLST schemes for *S. aureus* and *S. epidermidis* only (www.mlst.net)

1.5.4 *spa* typing

Sequencing of the variable region of the *S. aureus* protein A gene or *spa* typing is widely used for typing of *S. aureus* isolates including both MRSA and MSSA (Shopsin *et al.*, 1999). It involves PCR amplification and sequencing of the polymorphic X region of the *spa* gene which consists of variable number of tandem repeat units, each of between 21-27 bp in size. Variation in this region is due to duplication and deletion of repetitive units and also point mutations within the repeat units (Shopsin *et al.*, 1999; Mellmann *et al.*, 2007). Following sequencing of the *spa* gene the sequences are compared to those in a web-based database (http://spa.ridom.de/) and each isolate is assigned a *spa* type number based on the sequences and order of the tandem repeat units. As of the 30th of November 2013 this database consisted of 13,110 *spa* types and 625 distinct *spa* repeat units.

spa typing is useful for typing of *S. aureus* in both short- and long-term epidemiological investigations, offers greater discrimination than MLST and, in most instances, an isolate's MLST ST and/or CC can be inferred from the *spa* type (Table 1.4). The clonal relatedness of isolates can also be inferred by *spa* typing using the Based Upon Repeat Pattern (BURP) algorithm (Mellmann *et al.*, 2007). While *spa* typing does not offer as much discrimination of isolates as PFGE, it is rapid, cost effective and offers excellent interlaboratory comparability and in many *S. aureus* reference laboratories *spa* typing has now replaced MLST and PFGE as a routine typing tool for *S. aureus* (Grundmann *et al.*, 2010) (Table 1.4).

1.5.5 Staphylococcal Chromosome Cassette *mec* (SCC*mec*) typing

Staphylococcal chromosome cassette *mec* (SCC*mec*) typing involves identifying the *mec* and *ccr* type of a staphylococcal isolate and thus the SCC*mec* type and, in some instances, characterising the genetic organisation of the J region in order to determine the SCC*mec* subtype. SCC*mec* typing is predominanly performed using PCR and several

different schemes have been developed for MRSA and can be time consuming and expensive (Table 1.4) (Oliveria *et al.*, 2002; Kondo *et al.*, 2007; Milheirico *et al.*, 2007; Chen *et al.*, 2009). However, none of these schemes detect all SCC*mec* types and subtypes reported to date in MRSA.

No SCC*mec* typing schemes have been developed specifically for use with CoNS, and SCC*mec* typing of CoNS is predominantly performed using MRSA SCC*mec* typing schemes (Hanssen *et al.*, 2004; Fesser *et al.*, 2010; Bouchami *et al.,* 2011). However, due to the additional diversity detected among SCC*mec* elements in CoNS this may not be appropriate as using these schemes many CoNS have been found to carry non-typeable SCC*mec* elements e.g. the *ccr* and/or *mec* complex genes are not detected or novel combinations of these genes are identified (Wisplinghoff *et al.*, 2003; Hanssen *et al.,* 2006; Barbier *et al.*, 2010; Bloemendal *et al.*, 2010; Garza-Gonzalez *et al.,* 2010; Smyth *et al.*, 2011). There is a need for SCC*mec* typing schemes to be developed that will provide more accurate typing of SCC*mec* in CoNS and in order to do this more studies of the variety of *ccr* and *mec* genes and SCC*mec* types in CoNS need to be performed.

Combining SCC*mec* typing with MLST has been shown to be highly informative for typing of MRSA as it allows even greater differentiation between isolates belonging to the same clone (Robinson & Enright, 2002). The combination of the MLST, the methicillin resistance phenotype and the SCC*mec* type of a clone is use internationally for MRSA clone nomenclature, e.g MRSA isolates that belong to ST22 and harbour SCC*mec* type IV are designated ST22-MRSA-IV (IWG-SCC, 2009). In many recent studies, the SCC*mec* type and ST of MRSA isolates were combined with the *spa* type for strain nomenclature (Enright *et a*l., 2002; Robinson & Enright, 2004; Saunder & Holmes, 2007).

1.5.6 DNA microarray analysis of *S. aureus* isolates

Major advances in recent years in high-throughput DNA sequencing technologies have resulted in the publication of whole-genome sequence of numerous *S. aureus* strains. Using these sequences a high-throughput DNA microarray platform (The StaphyType Kit, Alere, Jena, Germany) has recently been developed that can be used for screening a large number of *S. aureus* isolates within a short period of time while still providing detailed information on the genetic composition of *S. aureus* isolates. This DNA microarray involves the simultaneous detection of 334 *S. aureus* genes and alleles, including species-specific markers, antimicrobial resistance and virulence genes, toxins, capsule and *agr* types and microbial surface components recognising adhesive matrix molecules (MSCRAMM's) (Monecke *et al.*, 2008) (Table 1.6) and assigns isolates to CCs/STs and SCC*mec* types (Monecke *et al.*, 2011). A schematic diagram of the DNA microarray process is shown in Figure 2.1, Chapter 2.

The DNA microarray is a high-throughput *S. aureus* typing system that has a high discriminatory power and excellent interlaboratory comparability (Shore *et al.*, 2012). The DNA microarray is less time consuming than MLST and SCC*mec* typing but provides the same information as these typing methods in addition to the detection of clinically relevant virulence and resistance genes. To date, there have been no published studies that have used this DNA microarray platform for investigating CoNS isolates. Due to the reservoir of SCC*mec* and resistance genes that studies have identified among CoNS (Monkolrattanotahi *et al.*, 2004; Zong *et al.*, 2011), the DNA microarray offers the potential of the simultaneous detection of an extensive range of SCC*mec* and antimicrobial resistance genes among CoNS. However, it is important to note that the array was developed for screening *S. aureus* isolates based on whole genome sequences of reference *S. aureus* strains and no studies to date have used the array to detect SCC*mec* and antimicrobial resistance genes in CoNS.

1.5.7 Whole-genome sequencing

Recent advances in whole-genome sequencing technology have also resulted in a major reduction in the cost of high-throughput whole-genome sequencing (WGS) and as a result a small number of studies have highlighted the usefulness of WGS for typing of *S. aureus*. Harris *et al.* (2012) used whole-genome sequence single-nucleotide polymorphism (SNP) analysis to investigate MRSA isolates from an outbreak in a UK hospital. The study identified 26 cases of MRSA carriage and showed transmission between patients, wards and the community. Harrison *et al.* (2013) also used WGS SNP analysis to confirm the zoonotic transmission of MRSA isolates. While WGS as a typing method provides a high discriminatory power, the main barrier to its widespread use relates to the detailed bioinformatic analysis of data that is required. Until easy-to-use automated programs are developed that can analyse such data and provide information on the presence of antimicrobial resistance and virulence genes, SCC*mec* and genotype, WGS will not be used for routine typing.

1.6 Evolution and epidemiology of staphylococci

MRSA and MR-CoNS have emerged from MSSA and MS-CoNS, respectively, on multiple independent occasions following acquisition of SCC*mec* (Enright *et al.*, 2002; Hallin *et al.*, 2007; Argudin *et al.*, 2009; Zong *et al.*, 2011). CoNS are a predominantly a nosocomial problem particularly among the immunocompromised and those with implanted prosthetic and medical devices. Similarly, MRSA has also traditionally been considered to chiefly be a HCA pathogen causing serious and life-threatening infections among already sick and vulnerable individuals. However, since the late 1990s MRSA have also emerged as a significant problem among otherwise healthy individuals, often children and young adults in the community and among animals.

Table 1.6 *Staphylococcus aureus* genes detected using the StaphyType DNA microarray[a]

Group	Gene Target	Description	Location
S. aureus species markers	*coa*	Staphylocoagulase	Core variable
	eno	Enolase phosphopyruvate hydratase laminin binding protein	Core genome
	fnbA	Fibronectin-binding protein A	Core variable
	gapA	Glyceraldehyde 3-phosphate dehydrogenase	Core genome
	katA	Catalase A	Core genome
	nuc	Thermostable nuclease	Core genome
	spa	IgG binding protein A	Core genome
	saeS	Histidine protein kinase	Core genome
	sarA	Staphylococcal accessory regulator A	Core genome
	sbi	IgG-binding protein	Prophage
	vraS	Sensor protein	Core genome
agr typing	*agr* I-IV	Accessory gene regulators genes; alleles I-IV	Core genome
SCC/SCC*mec*	*ccrA*1-4	Cassette chromosome recombinase A alleles	SCC/SCC*mec*
	ccrAA-MRSAZH47	Cassette chromosome recombinase A alleles, specific for MRSAH47	SCC/SCC*mec*
	ccrAA-85-2082	Cassette chromosome recombinase AA, marker for *ccrC*	SCC/SCC*mec*
	*ccrB*1-4	Cassette chromosome recombinase B alleles	SCC/SCC*mec*
	ccrC-85-2082	Cassette chromosome recombinase C	SCC/SCC*mec*
	*kdp*A-E	Potassium-translocating ATPase A-E	SCC*mec*
	mecA	Methicillin resistance	SCC*mec*
	mecI	Methicillin-resistance regulatory protein	SCC*mec*
	mecR1	Signal transducer protein MecR1	SCC*mec*
	merA	Mercuric reductase Hg (II) reductase	SCC*mec*/pI*258*
	merB	Mercuric reductase Hg (II) reductase	SCC*mec*/pI*258*
	Q9XB68-*dcs*	Hypothetical protein	SCC*mec*
	ugpQ	Glycerophosphoryl diester phosphodiesterase	SCC*mec*
	xylR	Xylose repressor	SCC*mec*
Resistance genes	*aacA-aphD*	Gentamicin, kanamycin and tobramycin	Transposon e.g. Tn*4001*
	aadD	Amikacin, kanamycin, neomycin and tobramycin resistance	Plasmid e.g. p*UB110*
	aphA3 & sat	Kanamycin, neomycin and streptothricin resistance	Transposon e.g. Tn*5405*
	blaI	Beta-lactamase repressor	Plasmid e.g. p*I258*
	blaR1	Beta-lactamase regulatory protein	Plasmid e.g. p*I258*
	blaZ	Beta-lactamase penicillinase	Plasmid e.g. p*I258*
	cfr	Chloramphenicol, florfenicol and clindamycin resistance	Plasmid e.g. p*SCFS*3
	dfrS1	Mediates trimethoprim resistance	Transposon e.g. Tn*4003*
	erm(A)	Macrolide-lincosamide-streptogramin B resistance	Transposon e.g Tn*554*
	erm(B)	Macrolide-lincosamide-streptogramin B resistance	Plasmid e.g p*I258*
	erm(C)	Macrolide-lincosamide-streptogramin B resistance	Plasmid e.g. p*E194*
	fexA	Chloramphenicol and florfenicol resistance	Transposon e.g. Tn*558*
	fosB	Fosfomycin resistance	Core variable
	fosB-plasmid	Fosfomycin resistance	*fosB* Plasmid
	fusB	Fusidic acid resistance	Plasmid
	fusC	Fusidic acid resistance	SCC/SCC*mec* e.g.SCC*fus*
	lnu(A)	Lincosamide resistance protein	Plasmid e.g. p*UR5425*
	mef(A)	Macrolide efflux pump A	Transposon
	mpb(C)	Macrolide 2'-phosphotransferase II	Core genome
	msr(A)	Erythromycin resistance	Core genome
	mupA/ileS2	Mupirocin resistance	Plasmid eg. *ileS2*-plasmid
	qacA/C	Quaternary ammonium compounds. Ethidium Bromide, Antiseptic resistance protein	Plasmid e.g. p*SK57*
	sdrM	Efflux pump	Core genome
	tet(K)	Tetracycline efflux protein	Plasmid e.g. p*T181*
	tet(M)	Tetracycline resistance	Transposon e.g. Tn*916*
	vanA/B/Z	Vancomicin/teicoplain-A type resistance protein	Plasmid e.g: p*LW*043
	vat(A)	Virginiamycin A acetyltranferase	Plasmid e.g. p*IP*630
	vat(B)	Acetyltransferase inactivating streptogramin A	Plasmid e.g. p*UB110*
	vgaA	Streptogramin A, lincosamide, pleuromutilin resistance	Plasmid e.g. p*I680*
	vgaB	Virginiamycin B hydrolase streptogramin lyase	Plasmid e.g. p*I680*
Virulence factors	*arc*A/B/C/D	Arginine/ornithine genes	ACME
	aur	Aureolysin secreted zinc-metalloproteinase	Core genome
	chp	Chemotaxis-inhibiting protein	*hlb* converting phage
	edinA	Epidermal cell differentiation inhibitor precursor	Pathogenicity island
	edinB	Epidermal cell differentiation inhibitor B	Pathogenicity island
	edinC	Epidermal cell differentiation inhibitor C	Pathogenicity island

Continued overleaf

Table 1.6 continued. *Staphylococcus aureus* genes detected using the StaphyType DNA microarray[a]

Group	Gene Target	Description	Location
Virulence factors	*etA/B/D*	Exfoliative toxin A/B/D	Pathogenicty island
	hl	Putative membrane protein	Core genome
	hla	Haemolysin alpha toxin	Core genome
	hlb	Haemolysin bata	Core genome
	hld	Haemolysin delta	Core genome
	hlgA	Haemolysin gamma	Core genome
	lukD, E, F, S, X, Y	Leukocidins	Genomic island
	lukF-PV, *lukS*-PV	Panton-Valentine leucocidin	Prophage
	sak	Staphylokinase	*hlb* converting phage
	scn	Staphylococcal complement inhibitor	*hlb* converting phage
	sea	Enterotoxin A	*hlb* converting phage
	seb, sek and seq	Enterotoxin B, K and Q	Pathogenicity island
	sec and sel	Enterotoxin C and L	MBE gene cluster
	sed, sej and ser	Enterotoxin D, J and R	Plasmid e.g. p*IB485*
	see	Enterotoxin E	Pathogenicity island
	seg, sei, sem, sen, seo, seu	Enterotoxin G, I, M, N, O, U	Pathogenicity island
	seh	Enterotoxin H	Transposon
	splA	Serine protease A	Genomic island
	splB	Serine protease B	Genomic island
	splE	Serine protease-like exoprotein E	Genomic island
	sspA	Extracellular serine protease	Core genome
	sspB	Papain-like cysteine protease	Core genome
	sspP	Papain-like cysteine protease	Core genome
	tst	Toxic shock syndrome toxin-1	Pathogenicity island
Capsular/ biofilm	*bap*	Surface protein involved in biofilm formation	Transposons
	cap5	Capsular polysaccharide allele, serotype 5	Genomic island
	cap8	Capsular polysaccharide allele, serotype 8	Genomic island
	icaA	Intercellular adhesion protein A	Gene cluster
	icaC	Intercellular adhesion protein B	Gene cluster
	icaD	Intercellular adhesion protein D	Gene cluster
MSCRAMM/ adhesion	*bbp*	Bone sialoprotein-binding protein	Core genome
	clfA	Clumping factor A	Core genome
	clfB	Clumping factor B	Core genome
	cna	Collagen-binding adhesion	Genomic island
	ebh	Cell wall associated fibronectin-binding protein	Core genome
	ebpS	Cell surface elastin binding protein	Core genome
	fib	Fibrinogen binding protein	Core genome
	fnbB	Fibronectin binding protein B	Genomic island
	sasG	*S. aureus* surface protein G	Genomic island
	sdrC	Serine aspartate repeat protein C	Core genome
	sdrD	Serine aspartate repeat protein D	Core genome
SSL genes	*setB*	Signal peptide	Core variable
	setC	Putative staphylococcal enterotoxin	Transposons
	*ssl*01-11 alleles	Staphylococcal superantigen-like proteins	Genomic island
Immunevasion and Misc.	*hsdS*	Type I site-specific deoxyribonuclease subunit	Core genome
	hysA	Hyaluronate lyase	Genomic island
	isaB	Immunodominant antigen B	Core variable
	isdA	Haeme-binding protein	Gene cluster
	lmrP	Putative transporter protein	Core genome
	mprF	Virulence factor which mediates insensitive to defensins	Core variable
	Q2YUB3	Multidrug resistance protein	Core genome
	Q7A4X2	Hypothetical protein	Core genome

[a] DNA microarray is developed by Alere Technologies, GmbH, Jena, Germany
Table adapted from Monecke *et al.*, 2008.
Abbreviations; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; SLL, staphylococcal superantigen-like.

1.6.1 Healthcare-associated MRSA

Since MRSA was first reported in the UK in 1961, it has become a pandemic problem causing infections among patients in hospitals worldwide (Jevons, 1961; Kali *et al.*, 2013). Prevalence rates of MRSA among *S. aureus* isolates from hospitalised patients vary widely between countries, ranging from very low to very high e.g. for some Scandinavian countries the rate of MRSA among *S. aureus* BSIs is <1% (EARS-net, 2013) (Figure 1.3), while a study undertaken by the National Nosocomial Infections Surveillance system in the USA revealed that >60% of *S. aureus* isolates from patients in intensive care units were MRSA (NNIS, 2004; Boucher *et al.*, 2008). Overall in Europe, the highest prevalence rates of MRSA among *S. aureus* BSIs are found in Southern European countries such as Italy, Greece, Romania and Portugal and the lowest rates are found in Northern European countries such as The Netherlands, Denmark, Norway and Sweden (Figure 1.3).

The majority of nosocomial MRSA infections worldwide are caused by clones belonging to five major MRSA lineages or CCs as defined by MLST (see section 1.5.3) i.e. CC5, CC8, CC22, CC30 and CC45 (Chambers *et al.*, 2009; Enright *et al.*, 2000). MRSA isolates exhibiting different STs belonging to these lineages predominate in different regions but the most predominant and widespread HCA-MRSA clones include ST5-MRSA-II (CC5), ST8-MRSA-II (CC8), ST239-MRSA-III (CC8), ST22-MRSA-IV (CC22), ST36-MRSA-II (CC30) and ST45-MRSA-II (CC45) (Enright *et al.*, 2002; Monecke *et al.*, 2011).

1.6.2 Community-associated MRSA (CA-MRSA)

In 1993 the first case of MRSA acquired in the community was reported in Western Australia from a patient with an SSTI (Udo *et al.*, 1993). This was followed in the late 1990s by the deaths of four previously healthy children in the USA and marked the

beginning of the worldwide emergence of CA-MRSA (Herold *et al.*, 1998; CDC, 1999; Vandenesch *et al.*, 2003; O'Brien *et al.*, 2004; Tristan *et al.*, 2006; Maree *et al.*, 2007; David & Daum, 2010; Khokhlova *et al.*, 2012; Sanchini *et al.*, 2012).

Epidemiologically MRSA isolates are considered to be CA-MRSA if they are recovered in an outpatient setting or from patients within 48 h of admission to a hospital (Deurenberg *et al.*, 2007). In addition, patients must lack the HCA risk factors, i.e. the MRSA must not have been acquired by the patient as a result of exposure to the healthcare system, such as previous hospitalisation. In addition, CA-MRSA tend to be genetically distinct from HCA-MRSA and have not emerged from HCA-MRSA but from MSSA in the community following acquisition of SCC*mec* (Okuma *et al.*, 2002; Fey *et al.*, 2003). CA-MRSA predominantly carry the smaller SCC*mec* types IV and V, which is thought to enhance the fitness CA-MRSA (David *et al.*, 2010). Until recently, the majority of CA-MRSA were considered to be non-multiresistant but the prevalence of multiresistant CA-MRSA strains has increased in some regions (Ellington *et al.*, 2010). The spread of CA-MRSA into hospitals has also been reported and in the USA one CA-MRSA clone, ST8-MRSA-IV (USA300), has replaced ST5-MRSA-II as the predominant strain in some hospitals (Tenover & Goering, 2009).

Community-associated MRSA are predominantly associated with SSTIs but can also be associated with serious and life threatening infections such as nectrotising pneumonia and fasciitis among otherwise healthy children and adults (Herold *et al.*, 1998; Vandenesch *et al.*, 2003; Boyle-Vavra & Daum, 2007; DeLeo *et al.*, 2010; Tattevin *et al.*, 2012). The success and virulence of some CA-MRSA clones has been linked to the expression of the cytolytic toxin PVL encoded by *pvl* (Vandenesch *et al.*, 2003; Boyle-Vavra and Daum, 2007). However, not all CA-MRSA carry *pvl* and there is conflicting data regarding the role of *pvl* (Vandenesch *et al.*, 2003; Brien *et al.*, 2004; Said-Salim *et al.*, 2005; Diep *et al.*, 2006; Boyle-Vavra and Daum, 2007; Rossney *et al.*, 2007).

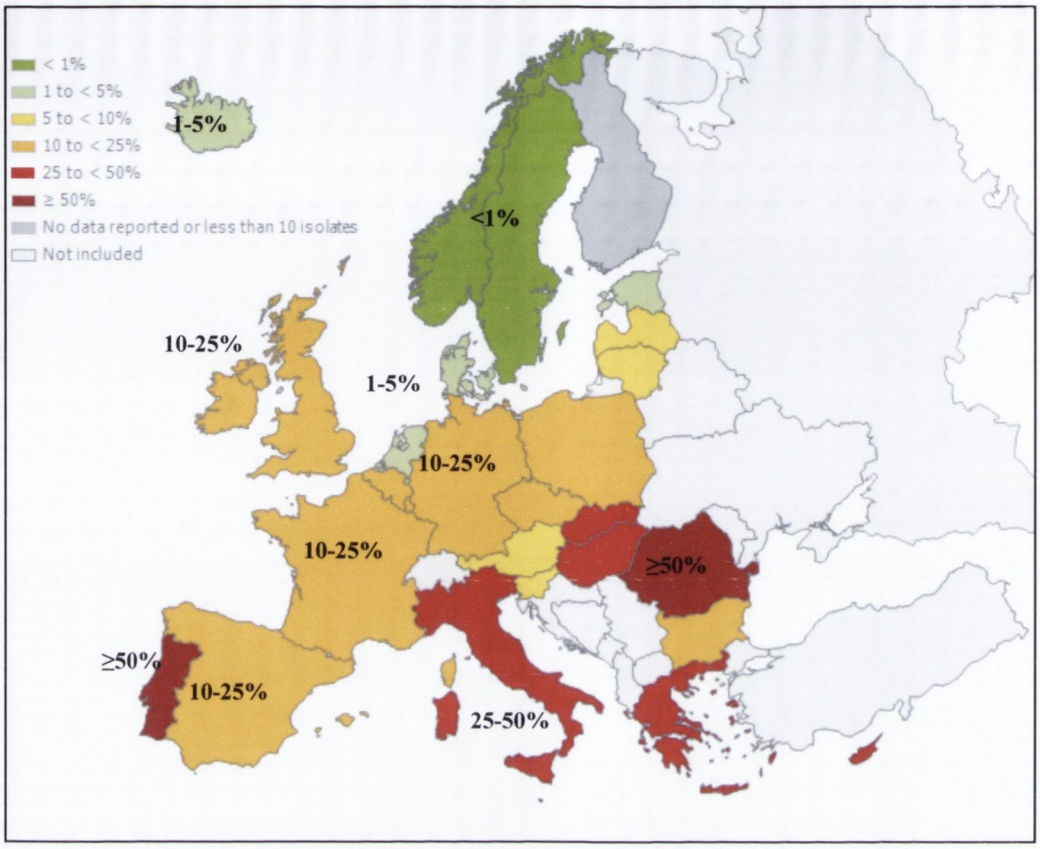


Figure 1.3. Percent prevalence rate of MRSA among *S. aureus* isolates from patients with bloodstream infections in hospitals in Europe in 2011. Adapted from EARS-Net, 2012.

1.6.3 Animal-associated MRSA

MRSA was first isolated from an animal source in 1971, when MRSA was recovered from a milk sample from a dairy herd in Belgium (Devriese *et al.,* 1972). Since then MRSA has been isolated from a number of animal sources including pigs, cows, horses, and companion animals such as cats and dogs (Smith *et al.*, 1989; Cefai *et al.*, 1994; Scott *et al.*, 1998; O' Mahony *et al.*, 2005; Moodley *et al.*, 2006; Abbott *et al.*, 2010; Catry *et al.,* 2010). Several studies have identified the zoonotic spread of MRSA from animals and humans, including those belonging to CC5, CC9, CC30, CC97 and CC151 (Sung *et al.*, 2008; Guardabassi *et al.,* 2009; Hasman *et al.*, 2010). While companion animals such as cats and dogs have been shown to carry MRSA strains similar to strains usually isolated from humans e.g. ST22-MRSA-IV, those from larger animals and livestock appear to have originated in animals.

The term livestock-associated (LA)-MRSA was coined in 2005 when the CC398 MRSA clone emerged among pigs and pig handlers in the Netherlands (Voss *et al.,* 2005). Subsequently, CC398 MRSA emerged in other European countries and then worldwide among pigs and other livestock including horses and poultry and among humans with close contact with animals (Witte *et al.*, 2007; Graveland *et al.*, 2011; Asai *et al.*, 2012; Lim *et al.,* 2012). In the USA and Europe CC398 and ST9 predominate among LA-MRSA, respectively (Graveland *et al.*, 2011). Several studies have also reported MRSA contamination of food intended for human consumption, including pork and beef (Loo *et al.,* 2007; Pu *et al.*, 2009; Weese *et al.*, 2010). While in some instances this has been shown to be due to the contamination of meat products by MRSA from humans (Pu *et al.,* 2009; Weese *et al.*, 2010), other studies have found food products to be contaminated with MRSA clones that have emerged from animals (Loo *et al.*, 2007; Kluytmans *et al.*, 2010).

The spread of MRSA from animals to humans is a cause for concern, not least because of the prevalence of multiresistant MRSA strains among animals (Fessler *et al.*, 2011; Kadlec *et al.,* 2012). This highlights another potential source of MRSA infections in humans as well a reservoir for resistance and SCC*mec* genes in MRSA in humans and warrants close surveillance.

1.6.4 MSSA

Similar to MRSA, MSSA are a major cause of infections among patients in healthcare facilities and in the community as well as among animals (Jorgense *et al.*, 2005; Sung *et al.*, 2008; Low *et al.,* 2009; Zhoa *et al.*, 2012). MSSA belong to more diverse genetic backgrounds than MRSA (Argudin *et al.*, 2009; Lim *et al.*, 2012; Jimenez *et al.,* 2013). A number of studies have revealed that MSSA often carry more virulence genes than MRSA (Hallin *et al.*, 2007; Sila *et al.*, 2009). In addition, in many regions, including Ireland (see section 1.7.2), MSSA are a more common cause of BSIs than MRSA. Despite this, the molecular characteristics and population structure of MSSA is not as widely studied as MRSA and there is a need for further detailed studies.

1.6.5 CoNS

Although, data from the Centers for Disease Control and Prevention (CDC) during 2006 and 2007 revealed that CoNS together with *S. aureus* were the most common cause of HCAIs in the USA (Hidron *et al.,* 2008), there is only limited information available on the population structure and molecular characteristics of CoNS (Thomas *et al.*, 2007; Cavanagh *et al.*, 2012; Piessens *et al.*, 2012). Using MLST, one major lineage of *S. epidermidis*, CC2, has been found to predominate worldwide in both hospitals and communities (Winderstom, 2010; Miragaia *et al.*, 2007; Mendes *et al.*, 2012; Du *et al.*, 2013). A large number of clones have been identified within CC2, and the high level of genetic diversity within this lineage has been attributed to frequent recombination and

transfer of MGEs (Miragaia *et al.*, 2007). Cavanagh *et al.* (2012) revealed that most *S. haemolyticus* isolates also belong to a single lineage, designated CC1, but that there is a high level of genetic conservation in core *S. haemolyticus* genome genes. CoNS have been shown to harbour an extensive and diverse range of SCC*mec* elements and genes but recent studies have also provided evidence that specific SCC*mec* types and genes predominate within different CoNS species e.g. SCC*mec* IV in *S. epidermidis* and SCC*mec* V/*ccrC* and/or class C *mec* in *S. haemolyticus* (Wisplighoff *et al*, 2003; Zhang *et al.*, 2008; Fessler *et al.*, 2010; Garza-Gonzalez *et al.*, 2010a; Garza-Gonzalez, *et al.*, 2010b, Barbier *et al.*, 2010; Bloemendaal *et al.*, 2010; Barros *et al.*, 2011; Bouchami *et al.*, 2012). Further studies of individual CoNS species are warranted in order to provide further insights into both the population structure of clinically relevant CoNS species and the evolution of SCC*mec*.

1.7 Staphylococci in Ireland

1.7.1 MRSA in Ireland

Unsurprisingly, in Ireland, the molecular epidemiology of *S. aureus*, in particular MRSA, has also been studied in far more detail than MSSA and CoNS. MRSA was first reported in Ireland in 1971, 10 years after it was first reported in the UK (Hone & Keane, 1974) and has been endemic in Irish hospitals since the 1980s (Coleman *et al.*, 1985; Carroll *et al.*, 1989; Rossney *et al.*, 2002; Rossney *et al.*, 2003; Rossney *et al.*, 2006). The prevalence rate of MRSA among *S. aureus* isolates from BSIs peaked in 2006 at 42%, but decreased to 23.7% in 2011 (EARS-Net, 2011) (Figures 1.3 and 1.4).

Molecular typing of MRSA from Ireland has shown that different MRSA clones have predominated in Irish hospitals at different time periods (Figure 1.4). In the 1970's and early 1980s the ST250-MRSA-I/I-*pls* predominated, followed by ST239-MRSA-III*Hg*/III*Hg*-p*1258*/Tn*554* in the mid 1980's and early 1990s and ST8-MRSA-II

(harbouring SCC*mec* IIA to IIE) in the mid to late 1990's, during which time the ST36-MRSA and ST22-MRSA-IV clones also emerged (Coleman *et al.*, 1985; Carroll *et al.*, 1989; Humphreys *et al.*, 1989; Shore *et al.*, 2005). Since 2002, ST22-MRSA-IV has predominated, accounting annually for 70-80% of all MRSA isolates from BSIs (NMRSARL, 2011). ST22-MRSA-IV is endemic in Irish hospitals and differentiating between strains of this clone is difficult as they exhibit a limited number of PFGE patterns, *spa* and *dru* types and the majority carry SCC*mec* IVh (Shore *et al.*, 2010). The combination of PFGE patterns, *spa* and *dru* types and assignment of isolates to dendrogram groups (DG) in order to avoid over interpretation of the results, has been shown to enhance discrimination of ST22-MRSA-IV isolates (Shore *et al.*, 2010). Interestingly, this combination of typing methods has been used to identify indistinguishable and closely related ST22-MRSA-IV strains in the environment and among patients in an Irish hospital, highlighting the role that environmental contamination plays in the transmission of MRSA between patients. Although this method offers a high discriminatory power for ST22-MRSA-IV isolates, the use of three typing methods is time consuming, labour intensive and costly. A previous study from this laboratory has shown that the combination of *spa* typing and DNA microarray profiling also offers a high discriminatory power for ST22-MRSA-IV isolates (Brennan, 2013), but further studies are required in order to determine the usefulness of this method for tracking ST22-MRSA-IV.

Other key features regarding the evolution and epidemiology of MRSA in Ireland are shown in Figure 1.4. This includes the emergence of CA-MRSA in Irish CC22, C30 and CC8 strains (Rossney *et al.*, 2007), the identification of CC5, CC8 and CC22 MRSA in cats, dogs and horses (O'Mahony *et al.*, 2005; Moodley *et al.*, 2006; Abbott *et al.*, 2010), the finding of the linezolid and multidrug resistance gene *cfr* in an isolate of the CA-MRSA clone USA300/ST8-MRSA-IVa (Shore *et al.*, 2010), the identification of MRSA isolates with reduced susceptibility to vancomycin (hVISA) (Rossney *et al.*, 2003)

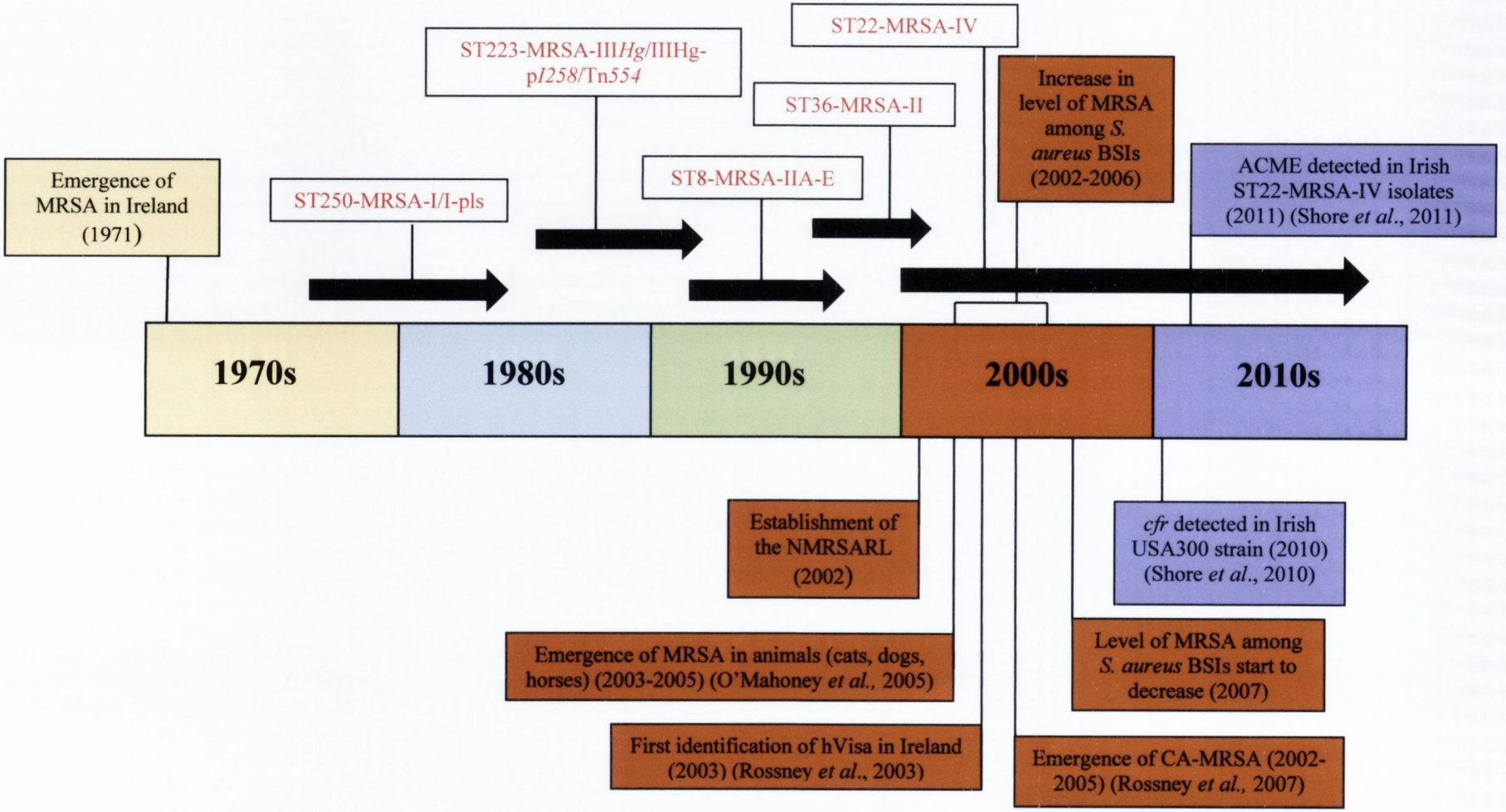

Emergence of MRSA in Ireland (1971)
ST250-MRSA-I/I-pls
ST223-MRSA-III*Hg*/IIIHg-p*I258*/Tn*554*
ST8-MRSA-IIA-E
ST36-MRSA-II
ST22-MRSA-IV
Increase in level of MRSA among *S. aureus* BSIs (2002-2006)
ACME detected in Irish ST22-MRSA-IV isolates (2011) (Shore *et al.*, 2011)
1970s
1980s
1990s
2000s
2010s
Establishment of the NMRSARL (2002)
Emergence of MRSA in animals (cats, dogs, horses) (2003-2005) (O'Mahoney *et al.*, 2005)
First identification of hVisa in Ireland (2003) (Rossney *et al.*, 2003)
Emergence of CA-MRSA (2002-2005) (Rossney *et al.*, 2007)
Level of MRSA among *S. aureus* BSIs start to decrease (2007)
cfr detected in Irish USA300 strain (2010) (Shore *et al.*, 2010)

Figure 1.4. Time line of the emergence of MRSA clones in Ireland. The MRSA clones that predominated in Irish hospitals at different time periods are shown in red font.

Abbreviations: MRSA, methicillin-resistance *Staphylococcus aureus*; ST, sequence type; BSIs, bloodstream infections; CA-MRSA, community-associated MRSA; NMRSARL, national MRSA reference laboratory; hVISA, hetero-vancomycin-intermediate *Staphylococcus aureus*; ACME, arginine catabolic mobile element.

and the identification of ACME with a genetic organisation similar to that found in *S. epididermidis* in ST22-MRSA-IV isolates (Shore *et al.*, 2011).

1.7.2 MSSA in Ireland

Since 2006, the prevalence of MRSA among *S. aureus* BSIs has decreased while the prevalence of MSSA has increased from 58% in 2006 to 72.2% in 2012 (EARS-net, 2006; EARS-Net, 2013). Despite this, very few studies have investigated the molecular characteristics of MSSA in Ireland. Two studies have been performed that have investigated MSSA from humans in Ireland, one from invasive infections and the other from the nares of healthy carriers and both revealed extensive diversity among MSSA in (Collery *et al.,* 2008; Grundmann *et al.*, 2009). However, different typing methods were used in the two studies i.e. *spa* typing of the invasive isolates and multiple locus variable number tandem repeat analysis (MLVA) and MLST of the nasal isolates, so comparisons between the two studies are difficult (Collery *et al.,* 2008; Grundmann *et al.*, 2009). Also neither study investigated antimicrobial resistance genes among MSSA in Ireland and only one of the studies investigated of virulence genes (Collery *et al.,* 2008), albeit a relatively small number. Lastly, Smyth *et al.* (2010) investigated MSSA from a range of animals from around the world including MSSA isolates recovered from cows in Ireland. These MSSA isolates from cows were found to belong to three MLST CCs predominaly considered to be animal-associated lineages, namely CC97, CC133 and CC151.

Due to the threat posed by the increasing prevalence of MSSA BSIs in Ireland, that MSSA carriage is a risk factor for subsequent MSSA infection and that MRSA arise from MSSA further studies of MSSA in Ireland are warranted.

1.7.3 CoNS in Ireland

A point prevalence study of HCAIs in Ireland in 2012 revealed that CoNS were the third most common cause of BSIs (13%), after *S. aureus* (19%) and *Escherichia coli* (16%)

(Burne *et al.*, 2012). The individual CoNS species recovered from these BSIs were not identified. Further studies investigating CoNS in Ireland are needed as there are no published studies that provide insights into the molecular characteristics of CoNS in Ireland. In addition, detailed studies of the SCC*mec* and antimicrobial resistance genes carried by CoNS are needed to investigate this possible reservoir for these genes in MRSA.

1.8 Aims of the present study

1. Prior to the present study, all MRSA isolates investigated that were resistant to methicillin and all beta-lactam antimicrobial agents were found to carry *mecA*. While an alternative *mec* gene and allelic variants have been reported in CoNS and related species no alternative *mec* genes have been reported in MRSA. During the course of the present study two human clinical *S. aureus* isolates were identified by the NMRSARL that were phenotypically methicillin resistant and produced PBP2a but lacked *mecA* by conventional *mecA* PCR. **The first aim of the present study was to investigate these two isolates for the possible presence of a novel *mec* gene.**

2. A previous study revealed the presence of *ccrAB4* outside of SCC*mec* in Irish ST8-MRSA-IIA-E and IVE/IVF isolates that was similar to *ccrAB4* identified in a SCC-CI in *S. epidermidis*. Detailed analysis of potential CIs are needed to permit accurate typing methods to be developed and to provide insights into the evolution of SCC*mec* and MRSA. **The second aim of the present study was to comprehensively characterise the region surrounding *ccrAB4* in Irish ST8-MRSA-IIA-E and IVE/IVF isolates and to determine the similarity between any CIs identified and the *S. epidermidis* SCC-CI.**

3. Detailed molecular analysis of CoNS from infections is warranted as they are a major cause of HCAIs and a potential reservoir for virulence, resistance, SCC and SCC*mec* genes in *S. aureus*. **The third aim of the present study was to characterise the SCC*mec* and SCC elements, antimicrobial resistance genes and ACME among clinical CoNS**

isolates recovered from patients in Irish hospitals and to undertake molecular typing of the most clinically relevant species identified.

4. MSSA are more a common cause of BSIs than MRSA but there is a lack of molecular analysis of MSSA, both in terms of their population structure and the range of virulence, resistance and SCC*mec* genes that they harbour. **The fourth aim of the present study was was to perform a comparative molecular analysis of MSSA isolates recovered from both healthy carriers and from BSIs using *spa* typing and DNA microarray profiling and to compare the results to those from MRSA isolates recovered from similar group's i.e. healthy carriers and BSIs.**

5. Lastly, the ST22-MRSA-IV clone predominates in Irish hospitals accounting for 70-80% of MRSA from *S. aureus* BSIs each year. ST22-MRSA-IV isolates are highly clonal and this, coupled with the endemic presence of this clone in Irish hospitals, hinders accurate molecular epidemiological typing and tracking of isolates of this clone. The StaphyType DNA microarray offers an alternative typing method but its usefulness for typing ST22-MRSA-IV, particularly when combined with *spa* typing, requires further investigation. In addition, transmission of MRSA is a complex process, involving healthcare workers, patients and the environment, and the development of accurate and informative typing methods would allow us to better understand the role each of these play in the transmission process. **Therefore, the final aim of the present study was to investigate the genetic relatedness of ST22-MRSA-IV isolates recovered from healthcare workers, patients and environmental sites in one Dublin hospital using *spa* typing and DNA microarray profiling and to assess the role that each of these sources play in the transmission of *S. aureus* in hospitals.**

Chapter 2

Materials and Methods

2.1 General microbiological methods

2.1.1 Bacterial culture and storage

All bacterial isolates, unless otherwise stated, were cultured on Trypticase Soy Agar (TSA) plates (Oxoid Ltd., Hampshire, UK), or Columbia Blood Agar (CBA) plates (Fannin Ltd., Dublin, Ireland), with overnight incubation (15 h) in a static incubator (Gallenkamp, Leicester, UK) at 37°C. For long-term storage, isolates were stored at -70°C in individual preserver vials (Protect Bacterial Preservation Systems, Technical Services Ltd., UK). Isolates were cultured by removing a single bead from the vial using a sterile inoculating wire and inoculating a TSA plate followed by overnight incubation in a static incubator at 37°C.

2.1.2 Chemicals, oligonucleotides and enzymes

All chemicals used, unless otherwise specified, were of analytical grade or molecular biology grade and were purchased from the Sigma-Aldrich Chemical Co. (Tallaght, Dublin, Ireland). Custom synthesised oligonucleotides were also purchased from Sigma-Aldrich at a concentration of 100 μM and diluted to a concentration of 10 μM using molecular biology reagent water (Sigma-Aldrich) and stored at -20°C. DNA molecular weight markers (100 bp and 1 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 stock of 1 x and was used to track the migration of DNA samples on agarose gels during electrophoresis. All enzymes were purchased from Promega and stored at -20°C. Proteinase K, lysozyme and lysosptaphin solutions were prepared using molecular biology reagent water (Sigma-Aldrich) at concentrations of 20 mg/ml, 25 mg/ml and 1 mg/ml, respectively.

2.1.3 Water, buffers and solutions

The Milli-Q water Biocel system (Millipore Ireland, Carrigtwohill, Co. Cork, Ireland) was used to provide ultra-pure water for the preparation of buffers and agarose gels. Molecular biology reagent water was purchased from Sigma-Aldrich and was used in all PCR reactions, DNA elutions and dilutions. Tris-borate/EDTA (TBE) buffer was used at 0.5 x concentration as a running buffer for agarose gel electrophoresis and for preparing agarose gels. This was prepared at 10 x concentration and consisted of 0.45 M Trizma base, 0.45 M boric acid and 0.01 M EDTA, pH8. This was diluted in ultrapure water to 0.5 x concentration. Tris-EDTA (TE) buffer was used routinely as a DNA buffer and consisted of 10 mM Tris-HCL and 1 mM EDTA, pH 8.

2.1.4 Disposable laboratory plastic ware

Sterile disposable plastic triple-vented Petri dishes (90 mm diameter) (Cruinn Diagnostics Ltd., Dublin, Ireland) were used for preparing agar plates for culturing bacterial isolates. Individually wrapped sterile disposable plastic pipettes (Cruinn Diagnostics Ltd.) were used to measure and transfer liquid volumes of between 1 and 25 ml. For volumes of between 1 µl and 1 ml, sterile StarLab plastic pipette tips (Starlab UK Ltd., Milton Keynes, UK) were used with variable volume Gilson pipettes (Gilson Inc., Middleton, WI, USA). Sterile microfuge tubes (Eppendorf UK Ltd., Stevenage, UK) were used to hold any volumes below 1.5 ml., Filter pipette tips (Starlab UK Ltd.) and sterile 0.2 ml PCR tubes (Molecular BioProducts, CA, USA) were used for PCR experiments.

2.2. Bacterial isolates

Six groups of staphylococcal isolates, consisting of a total of 405 MRSA, 187 MSSA and 53 CoNS, were investigated as part of this study. An outline of each group of isolates is provided in Table 2.1 and further details are provided in individual chapters describing the use of the isolates.

Table 2.1. Staphylococcal isolates investigated as part of the present study

No. & species of isolates	Source	Year of isolation	Purpose of study	Chapter
2 MRSA	Patients in 2 Irish hospitals	2010	Investigation of isolates that exhibited resistance to oxacillin and cefoxitin and produced PBP2a but failed to yield any *mecA* amplimer by conventional *mecA* PCR	3
86 MRSA & 6 MSSA	Patients in Irish hospitals	1989-2008	Investigation of *S. aureus* isolates for the presence of the *S. epidermidis* SCC-CI *ccrAB4* gene and additional associated genes	4
53 CoNS	Patients in 2 Irish hospitals	2004-2006 2010-2011	Characterisation of SCC*mec* elements & antimicrobial resistance genes in CoNS	5
53 MRSA	Anterior nares of healthcare workers in a Dublin hospital	2002-2009	Comparative molecular analysis of MRSA and MSSA from healthy carriers and bloodstream infections	6
98 MSSA	Anterior nares of undergraduate students attending a Dublin University. Samples were collected during a laboratory practical	1999-2005 2009-2013	Comparative molecular analysis of MRSA and MSSA from healthy carriers and bloodstream infections	6
83 MRSA & 83 MSSA	Patients in Irish hospitals with BSIs	2006-2007	Comparative molecular analysis of MRSA and MSSA from healthy carriers and bloodstream infections	6
82 MRSA	Patients in Irish hospitals	2009	Comparative molecular analysis of MRSA from patients, environmental sites and healthcare workers in a Dublin hospital	6
61 MRSA	Environmental sites in an Irish hospital	2009	Comparative molecular analysis of MRSA from patients, environmental site and healthcare workers in a Dublin hospital	6
38 MRSA	Hands of healthcare workers in a Dublin hospital	2009	Comparative molecular analysis of MRSA from patients, environmental site and healthcare workers in a Dublin hospital	6

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; CoNS, coagulase negative staphylococci; SCC-CI, staphylococcal chromosome cassette composite island; BSIs, bloodstream infections.

2.3 Identification of isolates

2.3.1 *Staphylococcus aureus*

All isolates were identified as *S. aureus* at the Irish National MRSA Reference Laboratory, (NMRSARL, St. James's Hospital, James's Street, Dublin 8, Ireland) by detecting (i) staphylocoagulase production using the tube coagulase method which detects extracellular coagulase (Rossney *et al.*, 1990) and (ii) clumping factor, protein A and *S. aureus* specific capsular polysaccharides using the Pastorex™ Staph-Plus kit (Bio-Rad, Hercules, California, USA). If an isolate produced neither staphylocoagulase nor clumping factor, identification was based on the detection of thermostable deoxyribonucleases and if necessary, biochemical identification using the API Staph identification system (bioMérieux, sa, Marcy-l'Etoile, France) was also performed (Rossney *et al.*, 1990).

2.3.2 Coagulase-negative staphylococci (CoNS)

The identification of CoNS isolates was performed by the clinical diagnostic laboratories in the hospitals in which they were recovered. Coagulase-negative isolates were provisionally identified by failure to agglutinate using the Pastorex™ Staph-plus *S. aureus* identification kit. The identification of isolates as CoNS was confirmed by the tube coagulase test by the absence of a clot. Isolates were tested for the production of catalase to confirm that they were staphylococcal species. This was done by adding a loopful of overnight culture into 5% (v/v) hydrogen peroxide (H_2O_2) and looking for the generation of bubbles, as catalase breaks down H_2O_2 into oxygen and water.

2.4 Antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE)

All antimicrobial susceptibility testing (unless otherwise stated), as well as PFGE, was performed at the NMRSARL.

2.4.1 Detection of methicillin resistance

The detection of methicillin resistance in all staphylococcal isolates (both *S. aureus* and CoNS) was performed as described previously (Rossney *et al.*, 2007). In brief, this involved (i) using 10 μg and 30 μg cefoxitin disks (Oxoid) and Clinical and Laboratory Standards Institute (CLSI) methodology for zone breakpoints (CLSI, 2006), on BBL Mueller-Hinton II agar (MH) (Becton Dickinson and Company, Sparks, MD, USA) and incubation at 35°C for 18-24 h, (ii) testing isolates that appeared susceptible to either concentration of cefoxitin against 1 μg and 5 μg oxacillin disks on Columbia Blood Agar (CBA) at 30°C for 18-24 h, (iii) using the oxacillin MIC E-test™ system consisting of antibiotic gradient strips (AB Biodisk, Solna, Sweden), (iv) testing all isolates for PBP2a production using the Mastalex™-MRSA agglutination kit (Mast Diagnostics, Bootle, UK), and (v) testing any isolates exhibiting an oxacillin MIC of ≤ 4 mg/l for the presence of *mecA* by PCR using PCR primers and conditions described previously (Oliveira and de Lencaster *et al.*, 2002).

2.4.2 Antibiogram-resistigram (AR) typing

Antibiogram-resistogram typing was performed on MRSA isolates by determining their susceptibility to a panel of 23 antimicrobial agents using disk diffusion (Rossney *et al.*, 1994), CLSI methodology and the breakpoints described by Rossney *et al.* (2007) for each antimicrobial agent (Table 2.2).

Each isolate was assigned an AR type or subtype number based on the AR pattern obtained (Table 2.3). These were assigned chronologically by the NMRSARL and 46 distinct AR types have been assigned to date i.e. AR01-AR46. AR subtypes were assigned to isolates that exhibited a slight variation to an existing AR type and were designated using a third numeral in the AR type e.g. AR06.1. An isolate was classified as "unfamiliar (Unf)" if it yielded an AR pattern with no similarity to any known AR type. An isolate was

Table 2.2 Antimicrobial agents used for antibiogram-resistogram (AR) typing

Antimicrobial agent	Antimicrobial agent abbreviation	Concentration (µg/disk)	Zone breakpoints[a]		
			R (mm)	I (mm)	S (mm)
Amikacin	Ak	30	14	15-19	20
Ampicillin	Ap	10	28	*	29
Cadmium acetate	Cd	130	10	11-15	16
Chloramphenicol	Cl	30	12	13-17	18
Ciprofloxacin	Cp	5	15	16-20	21
Erythromycin	Er	15	13	14-22	23
Ethidium bromide	EB	60	13	14	15
Fusidic acid	Fd	10	23	24-26	27
Gentamicin	Gn	10	12	13-14	15
Kanamycin	Kn	30	13	14-17	18
Lincomycin	Ln	2	14	15-16	17
Mercuric chloride	Mc	10	13	14	15
Mupirocin	Mp	5	12	13-19	20
Mupirocin	Mp	200	15	16-29	30
Neomycin	Nm	30	15	16-17	18
Phenyl mercuric acetate	Pma	10	24	25-28	29
Rifampicin	Rf	5	16	17-19	20
Spectinomycin	Sp	500	13	14-19	20
Streptomycin	Sm	25	13	14-15	16
Sulfonamide	Su	300	12	13-16	17
Tetracycline	Te	30	14	15-18	19
Tobramycin	Tb	10	17	18	19
Trimethoprim	Tp	5	10	11-15	16
Vancomycin	Va	30	14	*	15

[a] Zones of growth inhibition (diamater) around each disc impregnated with antimicrobial agent were recorded in mm and interpreted as R, I and S according to CLSI guidelines for zone diameters.

Abbreviations: R, resistant; I, intermediate resistance; S, susceptible.

* No intermediate breakpoint (CLSI, 2009; Rossney *et al.*, 2007).

Table 2.3. Antibiogram-resistogram (AR) types and patterns of MRSA isolates investigated in the present study

AR type or subtype[a]	AR pattern
AR05.5	ApCdErFdKnMcNmPmaSmSpTbTe
AR06.1	Ap
AR06.1 + Cp	ApCp
AR06.1 + Cp & Cd	ApCpFd
AR06.3	ApCd
AR06.3 + Cp	ApCdCp
AR06.3 + Cp & Er	ApCdCpEr
AR06.3 + Cp & Fd	ApCdCpFd
AR06.5	ApCdEr
AR06.5 + Cp	ApCdCpEr
AR06.5 + Cp & Mp	ApCdCpErMp
AR06.5 + Cp & Cl	ApCdCpClEr
AR06.5 + Cp	ApCdCpEr
AR06.5 + Cp & Rf	ApCdCpErRf
AR06.5 + Fd	ApCdErFd
AR06.5 + Cp, Fd & Te	ApCdCpErFdTe
AR06.6	ApEr
AR06.6 + Fd	ApErFd
AR06.6 + Cp & Te	ApCpErTe
AR06.6 + Cp & Fd	ApCpErFd
AR07	ApCdCpErKnLnMnSpTb
AR07.3	ApCpErFdKnNmSpTb
AR07.4	ApCpErKnLnNmSpTb
AR13	Ak^{I}ApCdCpEbErGnKnLnMcNmPmaSmSpTb
AR13.1	Ak^{I}ApCdCpEbErGnKnLnMcMp^{I}NmPmaSmSpTb
AR14	Ak^{I}ApCdCpEbErGnKnMcNmPmaSmSpTb
AR14.1	Ak^{I}ApCdCpEbErGnKnMcNmPmaSmSpTbTp
AR14.2	Ak^{I}ApCdCpEbErGnKnMcMp^{m}NmPmaSmSpTb
AR14.4	Ak^{I}ApCdCpEbErGnKnMcNmPmaSmSpTbTp
AR14.4 + Fd	Ak^{I}ApCdCpEbErFd^{I}GnKnMcNmPmaSmSpTbTp
AR14.4 + Mp	Ak^{I}ApCdCpEbErGnKnMcMpNmPmaSmSpTbTp
AR43	ApCd^{I}CpErKnNmSmSpTp
AR43 + Eb	ApCd^{I}CpEbErKnNmSmSpTp
AR43 + Te	ApCd^{I}CpErKnNmSmSpTeTp
AR44	AkApCdCpEbErGnKnLnMcNmPmaSmSpSuTbTeTp
New03	ApCdCpEbErGnKnLnMcMp^{I}PmaSpTb
New03	ApCdCpEbErGnKnLnMcPmaSpTb
Unfamiliar	AkApCdClCpErGnKnRfSmSpTbTe
Unfamiliar	Ak^{I}ApCdGnKnMcNmSmTbTe
Unfamiliar	ApCdCpErGnKnMpTb
Unfamiliar	ApCdTeTp
Unfamiliar	ApCpErGnKnLnNmSuTbTeTp

[a]AR type followed by +; additional resistance phenotype associated with AR type. An isolate was classified as "unfamiliar (Unf)" if it had an AR pattern with no similarity to any known AR type.

Abbreviations: I, intermediate resistance level; Ak, amikacin; Ap, ampicillin; Cd, cadmium acetate/cadmium nitrate; Cl, chloramphenicol; Cp, ciprofloxacin; Eb, ethidium bromide; Er, erythromycin; Fd, fusidic acid; Gn, gentamycin; Kn, kanamycin; Ln, lincomycin; Mc, mercuric chloride; Mp, mupirocin; Nm, neomycin; Pma, phenyl mercuric acetate; Rf, rifampicin; Sm, streptomycin; Su, sulphonamide; Tb, tobramycin; Te, tetracycline and Tp, trimethoprim. Resistance to vancomycin was not observed in any AR type.

designated "No Type (NT)" if it exhibited an AR pattern indicative of a certain AR type but was positive for the production of urease. Urease production was investigated in all MRSA isolates by testing for the hydrolysis of urea on Christensen's urea agar slopes containing urea base agar (Oxoid) with 2% (w/v) urea (Merck, Darmstadt, Germany) followed by incubation at 35°C for 48 h. Isolates belonging to the most prevalent AR type among nosocomial MRSA in Irish hospitals, AR06, are urease negative. However an MRSA isolate that exhibits a susceptibility pattern related to AR06 but which is urease positive is reported as NT as molecular typing has shown these to be unrelated to AR06 isolates (Rossney *et al.*, 2006).

Antimicrobial-susceptibility testing of CoNS and MSSA isolates was also performed using the AR typing method but isolates were not assigned an AR type.

2.4.3 Pulsed-Field Gel Electrophoresis (PFGE) of MRSA

Pulsed-field gel electrophoresis was performed as described previously (Rossney *et al.*, 2007) and involved the extraction and digestion of high molecular weight chromosomal DNA embedded in agarose plugs with the rare-cutting restriction endonuclease *Sma*I (Promega). Digested DNA was subjected to agarose gel electrophoresis using the clamped homogenous electrical field (CHEF) electrophoresis system and the CHEF DRIII PFGE system (Bio-Rad) with 0.5x TBE as the electrophoresis buffer (see section 2.1.3), which was maintained at 4°C during electrophoresis by recirculation through a Bio-Rad chiller (CHEF DRIII) (Bio-Rad). Following this, the agarose gels were stained for 30 min with 0.5 mg/l ethidium bromide, destained for 15 min in distilled water and then examined using a model 97-0108-08 UV transilluminator (UVP, Upland, CA, USA). Each different PFGE pattern was assigned a 5-digit pulsed-field type (PFT) number following visual inspection and computer-assisted analysis of a gel images using the Gel Compar software (Bionumerics, Version 5.10 and Gel Compar II, Applied Maths NV,

Belgium). Patterns were visually inspected to ensure accurate assignment of banding patterns and pattern differences were interpreted according to the criteria described by Tenover *et al.* (1995). Related PFTs were assigned blocks of 1000 numbers starting at 00001 i.e. a particular series of related PFTs are in the 00001-00999 group and the next group of related PFTs were in the group 01000-01999 etc. Groups of related PFTs that differed by ≤ six bands were assigned to a pulsed field group (PFG) and were referred to by the first two numbers of their PFT e.g. PFG-00, PFG-01, PFG-02, etc. Sporadic patterns were assigned numbers within the series 99,000-99,999 in blocks of 100 numbers so that numbers are reserved for each sporadically occurring pattern to cater for future variants of such a pattern (Rossney *et al.,* 2006).

2.5 Molecular methods

2.5.1 Extraction of whole-genomic DNA

Extraction of total-cellular DNA for all PCR applications apart for DNA microarray analysis (section 2.5.4.1) was performed as follows. Each isolate was cultured as described above (section 2.1.1). A single isolated colony was selected from a TSA plate and lawned onto a fresh TSA plate using a sterile wire inoculating loop and incubated overnight in a static incubator (Gallenkamp) at 37°C. Following 18 h incubation, the cells were lysed by adding a 2.5 cm^2 area of culture growth of each isolate from lawned TSA plates using a sterile bacteriological loop (Greiner Bio-One GmbH, Germany) into 200 µl of lysis buffer containing 1 x TE buffer (section 2.1.3), 0.2 mg/ml lysostaphin and 0.02 mg/ml lysozyme. This was incubated with shaking (250 rpm) for 2-3 h at 37°C. Following lysis, the DNeasy Blood and Tissue kit (Qiagen, Crawley, UK) was used according to the manufacturer's instructions. Proteins and nucleases were removed by adding 25 µl of proteinase K and 200 µl of buffer AL (both supplied with the Qiagen DNeasy Blood and Tissue kit) and incubation at 70°C for 30 min. The released DNA was then bound to a mini-column inside a collection tube. The column contains a silica gel membrane that

binds DNA and allows cellular debris to wash through. The membrane was washed twice with buffers (supplied with kit) containing ethanol and salt to wash cellular debris from the column while the DNA remained membrane bound. The DNA was finally eluted into 200 µl of elution buffer with centrifugation at 15,970 x *g* for 1 min using an Eppendorf model 5417C centrifuge fitted with an F-45-30-11 rotor (Eppendorf, Hamburg, Germany). The DNA was stored at 4°C (for storage of four to six weeks) or at -20°C (for long-term storage, > 6 weeks). The quality of the total genomic DNA was assessed following electrophoresis in 0.8% (w/v) agarose gels. The concentration of genomic DNA (ng/µl) was measured using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc, Massachusetts, USA).

2.5.2 Polymerase chain reaction (PCR) and sequencing of PCR products

Whole-genomic DNA extracted as described in section 2.5.1 was used as a template for PCRs. All PCRs with expected ampilnmers less than 5 kb were performed using Go Taq DNA polymerase (Promega) according to the manufacturer's instructions. PCR's with expected amplimers greater than 5 kb were performed using the Expand long template PCR system (Roche Diagnostics Ltd., Lewes, East Sussex, United Kingdom) according to the manufacturer's instructions. DNA was amplified in a G-storm GSI thermocycler (Life Technologies, Corp. California, USA). All PCR products were visualised by agarose gel electrophoresis and were purified using the GenElute PCR Clean-up kit (Sigma-Aldrich). Sequencing of target genes was performed commercially by Geneservice (Source Bioscience, Dublin, Ireland) using the dideoxy chain termination method of Sanger *et al.* (1977) and an ABI 3730xl Sanger DNA analyser with the and a ABI BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA). DNA sequences were analysed using the BioNumerics and DNA strider version 1.3f11 (CEA Saclay, Gif-sur-Yvette, France) software packages or for *spa* typing, using the Ridom StaphType software program, version 1.3 (Ridom GmbH, Wutzburg,

Germany) (section 2.5.2.1). Homology searches were performed using BLAST software (http://ncbi.nih.gov/BLAST). Alignment of DNA and amino acid sequences were performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

2.5.3 *spa* typing

Amplification of the *spa* gene was performed as described previously (Shore *et al.*, 2008) using the primers [113f (5'-TAAAGACGATCCTTCGGTGAGC-3') and 1514r (5'-CAGCAGTAGTGCCGTTTGCTT-3')] and thermal cycling conditions (80°C for 5 min, 35 cycles of 94°C for 45 s, 60°C for 45 s and 72°C for 75 s, followed by an elongation step at 72°C for 10 min) described previously by the European Network of Laboratories for Sequence Based Typing (SegNet) (http://www.segnet.org/). Amplimers were sequenced commercially by Geneservice (section 2.5.2) and the Ridom Staphtype software program, version 1.3 (Ridom Gmbh, Wutzberg, Germany) was used for chromatogram analysis, the detection of repeat successions and *spa* repeat and type assignment. Clustering of *spa* types was carried out using Based Upon Repeat Patterns (BURP) analysis in the Ridom StaphType software to assess the clonal relatedness of isolates (Mellmann *et al.*, 2007). The *spa* types were clustered together using default parameters which included (i) excluding any *spa* types that contained four or less repeats, as this was deemed to be too few repeats to infer evolutionary relatedness from and (ii) clustering *spa* types if they had a cost value less than four where the cost value accounts for the number of evolutionary steps between *spa* types. The founder of each BURP cluster that consisted of at least three different *spa* types was determined based on the *spa* type that shared the highest DNA sequence identity with the greatest number of *spa* types within the cluster as determined by the cost value.

2.5.4. DNA microarray analysis

DNA microarray analysis was performed using the Staphy-Type genotyping kit (Alere Technologies GmbH, Jena, Germany) according to the manufacturer's instructions and as outlined in Figure 2.1. The Staphy-Type kit consists of an eight-well microtitre strips each containing an individual DNA microarray chip which detects 185 *S. aureus* targets (334 genes and alleles) including SCC*mec*-assoicated genes, species markers, virulence and antimicrobial resistance genes and assigns isolates to multilocus sequence types (STs) and/or clonal complexes (CCs).

2.5.4.1 Extraction of whole-genomic DNA for DNA microarray analysis

Genomic DNA for use with the DNA microarray was extracted from isolates using the Qiagen DNeasy kit and according to the Staphy-Type kit manufacturer's instructions Each isolate was cultured on a CBA plate for 18 h at 37°C in a static incubator and following incubation an individual colony of each isolate was selected with a sterile inoculating loop, lawned onto a fresh CBA plate and incubated for 18 h at 37°C in a static incubator. The cells were then lysed by adding a 2.5 cm^2 area of culture growth to 200 µl lysis buffer A1 and 1.7 µl lysis enhancer (buffer A2) both of which were supplied with the DNA microarray StaphyType kit. The cell suspension was vortexed and incubated with shaking (250 rpm) at 37°C for 30-60 min. Following lysis, protein was removed by adding 25 µl of proteinase K and 200 µl of AL buffer (both supplied with the Qiagen DNeasy Blood and Tissue kit) and incubation at 70°C for 30 min. Genomic DNA was extracted using the DNeasy Blood and Tissue kit as described in section 2.5.1. The DNA was eluted with 50 µl Sigma-Aldrich molecular biology-grade water and 10 min incubation at room temperature followed by centrifugation at 15,970 x *g* for 1 min. The DNA samples were concentrated to remove any residual ethanol by an evaporation step in a QB dry heating block (Thomas Scientific, New Jersey, USA) at 70°C for 30 min with the caps of sample

microfuge tubes removed. The concentration of the DNA was measured using a Nanodrop 2000c spectrophotometer. The quality of the DNA samples and the absence of RNA were assessed by electrophoresing a 1:50 dilution of each sample through a 0.8% (w/v) agarose gel. The extracted DNA was then stored as described in section 2.5.1.1. The concentration of each DNA sample was measured using a Nanodrop 2000c spectrophotometer and diluted with ultrapure water to a concentration of between 0.5-1.5 µg in 5 µl for linear PCR amplification and biotin labelling.

2.5.4.2 Linear PCR amplification

A linear PCR amplification and labelling reaction was performed on template DNA, prepared as described in section 2.5.4.1, by using thermally synchronised primer elongation reactions involving a labelling buffer and enzyme supplied with the Staphy-Type kit (B1 and B2, respectively) and a G-storm GSI thermocycler. This linear PCR reaction allowed all target genes to be amplified and labelled simultaneously using a single primer for all 334 gene targets simultaneously and biotin-16-dUTP, respectively. A PCR master mix was prepared by combining 4.9 µl of B1 and 0.1 µl of B2 per sample. A 5 µl aliquot of this was added to 5 µl of DNA and the following thermocycling conditions were used: 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.

2.5.4.3 Array prewashing and hybridisation

A hybridisation mixture was prepared by adding 90 µl of the hybridisation buffer (buffer C1, supplied with the StaphyType kit) to each biotin-16-dUTP labelled PCR product (10 µl). The wells of the microarray strip was prepared for use by washing with 200 µl ultra-pure water, followed by the addition of 100 µl hybridisation buffer (C1) and incubation at 55°C for 2 min with shaking at 550 rpm in a BioShake iQ thermoshaker (Q

Culture *S. aureus* isolates on Columbia blood agar for 18 h at 37°C

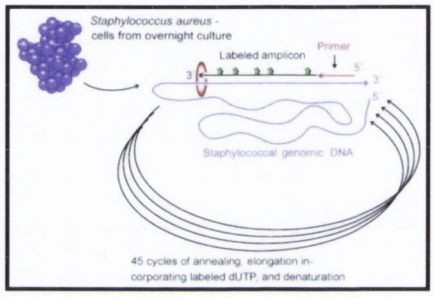
↓

Whole-genomic DNA extraction

Buffers and solutions supplied with the DNA microarray kit (Alere) and Qiagen DNeasy kit

↓

Linear PCR amplification

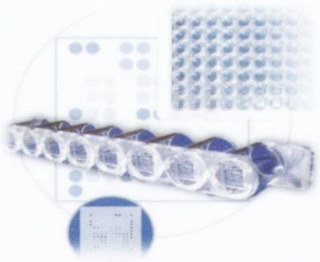


4.9 µl labelling buffer (B1*)
0.1 µl labelling enzyme (B2*)
5 µl DNA (0.5-1.5 µg)

Thermocycling conditions:
96°C- 5 min
45 Cycles:
96°C - 20 s
50°C – 20 s
72°C – 30 s

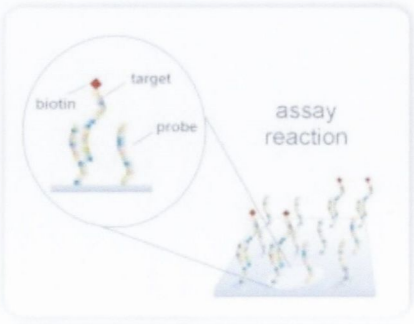
↓

Array pre-washing and hybridisation



Add 90 µl of hybridisation buffer (C1*) to each PCR reaction

Add 100 µl of hybridisation buffer to each array chip & incubate at 55°C at 550 rpm for 2 min.



Discard the hybridisation buffer & transfer each hybridisation mixture to a prewashed well of an arraystrip; cap the wells & incubate at 55°C at 550 rpm for 1 h.

↓

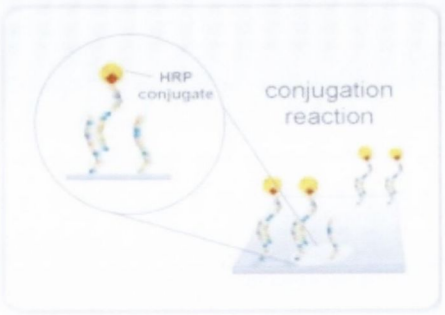
Conjugation and precipitation staining

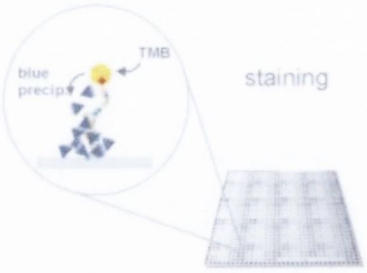
Discard the hybridisation mixture & add wash array strip with 200 µl of washing buffer 1 (C2*) three times

Add 100 µl of 1:100 of HRP-streptavidin (C3*) and conjugate buffer (C4*) to each well; incubate at 30°C at 550 rpm for 10 min.

Remove the HRP-conjugate solution & rinse array with 200 µl of washing buffer 2 (C5*)

Add 100 µl of tetramethylbenzidine (D1*) to each well; incubate at room temperature without agitation for 5 min.





Chip scanning and Automated data analysis

Discard the HRP substrate completely from each well.

Place the arraystrip in the ArrayMate reader

The Iconoclust software interprets the raw data as a positive, negative or ambiguous, based on a previously described algorithm.

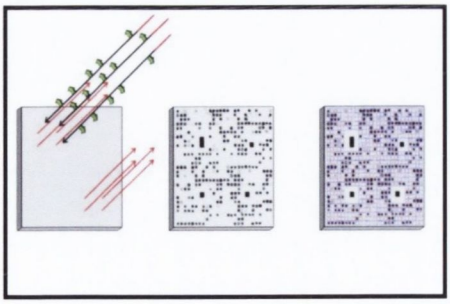


Figure 2.1. Schematic diagram illustrating the DNA microarray process. Regents marked with an astericks (*) were-supplied with the Staphy-Type kit. Once the whole genomic DNA is extracted, a linear PCR amplification using a single primer for all 334 gene targets and

labelled with biotin-16-dUTP is performed. The biotin-16-dUTP labeled amplicons are hybridised to the arrays chips followed by a conjugation reaction with horseradish peroxidase-conjugate (HRP-conjugate) with the final step of precipitation with the substrate tetramethylbenzidine (TMB). The array chips 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.

Instruments, Jena, Germany). The prepared hybridisation mixture (C1 and PCR reaction, total volume 100 µl) was then transferred to a well of a prewashed array strip, the wells were capped and the strip was incubated for 1 h at 55°C with shaking at 550 rpm in the thermoshaker to allow hybridisation of the PCR products to the array probes on the array chip.

2.5.4.4 Conjugation, precipitation staining, chip scanning and automated data analysis

Following the hybridisation step the hybridisation mixture was removed completely from the wells and three successive wash steps was performed by the addition and removal of 200 µl of washing buffer 1 (C2, Staphy-Type kit). The biotin-16-dUTP bound target molecules were then labelled by the addition of a horseradish-peroxidase (HRP)-conjugate which consisted of reagent C3 (supplied with the StaphyType kit) containing HRP-streptavidin and a conjugate buffer (C4, supplied with the StaphyType kit) in a 1:100 ratio. The HRP-conjugate mixture was added to each well of the mircoarray strip, incubated at 30°C at 550 rpm for 10 min and removed before a final wash step which was performed using 200 µl washing buffer 2 (C5, supplied with the StaphyType kit). Once washing buffer 2 was fully removed, staining of the bound biotin-HRP conjugate was performed using 100 µl of the HRP substrate, tetramethylbenzidine (D1, supplied with the StaphyType kit), and incubation at room temperature for 5 min without agitation, resulting in a blue/green reaction product due to the reaction between the substrate and the bound HRP-conjugate. The D1 reagent was then discarded completely and each strip was analysed using the ArrayMate reader and Iconoclust software (Alere). The ArrayMate Reader recorded an image of the chip and the software interpreted the raw data as a positive, negative or ambiguous, based on a previously described algorithm (Monecke *et al.,* 2008). The breakpoints for the positive, negative and ambiguous signals are defined as an average of each individual signal for the control spots (biotin-labelled DNA

immobilised on the array) and species markers (ribosomal probes, *femA, gapA, katA, coa, spa, sbi, eno, nuc, fnbA, sarA, and vraS*). Values <25% are assigned as negative, values between 25-33% are assigned as ambiguous and values >33% are assigned as positive (Monecke *et al.*, 2008). The Iconoclust software (Alere) also compared the DNA microarray profiles of each isolate to *S. aureus* reference isolates in the ArrayMate database previously characterised and MLST typed in order to assign each isolate to a MLST CC and ST and a SCC*mec* type (Monecke *et al.*, 2008).

2.5.5 Whole-genome sequencing

DNA extraction for whole-genome sequencing was performed as described in section 2.5.1.2. High-throughput *de novo* sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, UK) using the Illumina Genome Analyzer system (Illumina HiSeq 2000 platform, Illumina, Essex, UK). The average coverage across the genomes investigated was 40X. The individual reads for each isolate were assembled into contigs using a Velvet de novo genome assembler (version 1.0.15, Illumina). Contigs were analysed using the Artemis DNA sequence viewer (http://www.sanger.ac.uk/resources/software/artemis/) and annotation tool and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Analysis of whole-genome sequences was performed as described in the relevant chapters (see section 3.2.6 and 4.2.3).

Chapter 3

Oxacillin resistant, PBP2a-positive *S. aureus* isolates that lack *mecA* by conventional *mecA* PCR

3.1 Introduction

MRSA comprise *S. aureus* strains that exhibit phenotypic resistance to methicillin and related β-lactam antibiotics, including cefoxitin and oxacillin. All MRSA isolates carry the *mecA* gene which encodes an alternative penicillin binding protein (PBP) designated PBP2a which can perform all the cell well synthesis functions necessary when the four native PBPs have been inactivated by β-lactam antibiotics. The first report of MRSA was in England in 1961 (Jevons, 1961) and since then it has become a worldwide problem. Traditionally MRSA were considered to be associated predominantly with healthcare-associated (HCA) infections, especially in patients with specific risk factors including prolonged hospital stay and an immunocompromised state. However, in recent years there has been a worldwide increase in community-associated (CA) MRSA infections among otherwise healthy individuals without the usual HCA-risk factors (Boyle-Vavra and Daum, 2008) and in MRSA among animals including livestock (so called livestock-associated (LA) MRSA) and companion animals such as cats and dogs (Weese *et al.,* 2010; Graveland *et al.*, 2011). The spread of CA-MRSA into hospitals has also been reported as well as the zoonotic spread of LA-MRSA and the spread of HCA-MRSA between humans and companion animals (Hidron *et al.,* 2005; Strommenger *et al.,* 2006; Walther *et al.,* 2009; Cuny *et al.,* 2010; Graveland *et al.,* 2011; Jimenez *et al.,* 2012; Loncaric *et al.,* 2013; Sanchini *et al.,* 2013;).

The *mecA* gene is carried on a mobile genetic element called SCC*mec*. Ten SCC*mec* types (I-X) and numerous subtypes have been described to date in MRSA (Ito *et al.*, 2001; Ma *et al.*, 2002; Ito *et al.*, 2004; Boyle-Vavra *et al.*, 2005; Shore *et al.*, 2005; Oliveira *et al.*, 2006; Berglund *et al.*, 2008; IWG-SCC, 2009; Zhang *et al.*, 2009) (Table 3.1). Each SCC*mec* type has a unique combination of the *mec* and cassette chromosome recombinase (*ccr*) gene complex (IWG-SCC, 2009) (Table 3.1). Three classes of the *mec* gene complex (A-C) have been described to date in the published literature, which differ in

Table 3.1. Details of SCC*mec* elements reported to date in MRSA

SCC*mec* type	*ccr* gene complex	*mec* gene complex	Size (kb)	Additional resistance determinants (resistance conferred) & location within SCC*mec*	Reference strain	Reference
I	1 (*ccrA1, ccrB1*)	B (IS*431-mecA-ΔmecR1-IS1272*)	34	n/a	NCTC140442	Ito *et al.*, 2001
II	2 (*ccrA2, ccrB2*)	A (IS*431-mecA-mecR1-mecI*)	53	*aadD* (Kn, Nm, Tb) on p*UB110*	N315	Ito *et al.*, 2001
III	3 (*ccrA3, ccrB3*)	A (IS*431-mecA-mecR1-mecI*)	67	*cadD* (Cd) on ψTn554, *erm*(A) (Er) on Tn*554*, *spc* (Sp) on Tn*554*, *tet*(K) (Te) on p*T181*, *merA* & *merB* (Mc) on p*T181*	85/2082	Ito *et al.*, 2001
IV	2 (*ccrA2, ccrB2*)	B (IS*431-mecA-ΔmecR1*-IS*1272*)	25	n/a	CA05	Ma *et al.*, 2002
V	5 (*ccrC1*)	C2 (IS*431-mecA-ΔmecR1*-IS*431*)[a]	24	n/a	WIS	Ito *et al.*, 2004
VI	4 (*ccrA4, ccrB4*)	B (IS*431-mecA-ΔmecR1*-IS*1272*)	21	n/a	HDE288	Oliveria *et al.*, 2002
VII	5 (*ccrC1*)	C1 (IS*431-mecA-ΔmecR1*-IS*431*)[a]	27	n/a	JCSC6082	Berglund *et al.*, 2008
VIII	4 (*ccrA4, ccrB4*)	A (IS*431-mecA-mecR1-mecI*)	32	n/a	C10682	Zhang *et al.*, 2009
IX	1 (*ccrA1, ccrB1*)	C2 (IS*431-mecA-ΔmecR1*-IS*431*)[a]	44	*cadDX* (Cd), a*rsRBC* (Ar), *arsDARBC* (Ar)	JCSC6943	Li *et al.*, 2011
X	7 (*ccrA1, ccrB6*)	C1 (IS*431-mecA-ΔmecR1*-IS*431*)[a]	51	*cadDX* (Cd), a*rsRBC* (Ar)	JCSC6945	Li *et al.*, 2011

[a] The class C1 and C2 *mec* gene complexes differ in the orientation of the two IS*431* elements i.e. in the class C1 *mec* gene complex both IS*431*s are arranged in the same orientation while in C2 the two IS*431*s are arranged in the opposite orientation.

Abbreviations: n/a, not applicable; Ar, arsenic; Cd, cadmium; Er, erythromycin; Kn, kanamycin; Mc, mercury; Nm, neomycin; Sp, spectinomycin; Tb, tobramycin; Te, tetracycline.

their genetic organisation (Katayama *et al.,* 2001; Lim *et al.,* 2003; Ito *et al.,* 2004; IWG-SCC, 2009) (Table 3.1). Each *mec* complex has an intact copy of *mecA*, one or two copies of insertion sequence IS*431* and complete or truncated *mec* regulatory genes *mecI* and *mecR1* (Table 3.1). The *ccr* genes are involved in site-and orientation-specific integration and excision of SCC*mec* and three distinct *ccr* genes termed *ccrA*, *ccrB*, and *ccrC* have been described (Ito *et al.*, 2001). Four allotypes of *ccrA* (*ccrA1-4*), five allotypes of *ccrB* (*ccrB1-4* and *ccrB6*) and only one of *ccrC* (*ccrC1*) have been reported in MRSA to date (IWG-SCC, 2009).

The IWG-SCC has recommended that complete nucleotide sequencing of novel SCC*mec* elements should be performed for type designation to avoid misclassification of new SCC*mec* types (IWG-SCC, 2009). SCC*mec* subtypes are assigned based on either DNA sequence variation or the presence or absence of integrated mobile genetic elements in the joining or "J" region, located outside the *ccr* and *mec* complexes; i.e. from the right chromosomal junction to the *ccr* genes (J1), the region between the *ccr* and *mec* gene complex (J2) and lastly the region from the *mec* complex to the left chromosomal junction (J3) (IWG SCC, 2009). The mobile genetic elements located in the J regions include integrated transposons and plasmids which often harbour additional resistance determinants (Table 3.1).

Numerous studies have demonstrated that SCC*mec* and *mecA* are not exclusive to MRSA. In fact, SCC*mec* is more abundant and diverse among CoNS than MRSA, with many CoNS harbouring SCC*mec* elements that are non-typeable by conventional SCC*mec* typing indicating that CoNS are a reservoir for SCC*mec* in MRSA (Wisplinghoff *et al.*, 2003; Hanssen *et al.,* 2006; Barbier *et al.*, 2010; Bloemendal *et al.*, 2010; Garza-Gonzalez *et al.,* 2010;; Smyth *et al.*, 2010). Additionally, putative ancestral SCC elements and *mec* genes have been identified in CoNS and related species from animals including *S. sciuri, S. fleuretti* and *Macrococcus caseolyticus* (Wu *et al.,* 1998; Schnellmann *et al.*, 2006;

Tsubakishita *et al.*, 2010a). The *mecA* gene has been found to be ubiquitous among *S. sciuri* and is a potential precursor for *mecA* in MRSA (Couto *et al.*, 1996). The *S. sciuri mecA* gene does not encode methicillin resistance, exhibits 80% nucleotide sequence similarity to *mecA* found in MRSA and is located adjacent to a homologue of the regulatory gene, *mecR2* (previously designated *xylR*) found in the class A *mec* complex (Arede *et al.*, 2012; Couto *et al.*, 1996; Wu *et al.*, 1998). However, the finding of a *mecA* homologue and an adjacent non-*mecA* SCC element on a transposon in *M. caseolyticus* is evidence that *mecA* and SCC*mec* could have originated in another closely related species. The *mecA* homologue from *M. caseolyticus*, designated $mecA_m$, was found as part of a 5.1 kb *mec* gene complex that contained *mecR1, mecI* and *blaZ* (Tsubakishita *et al.*, 2010a). The *mecA, mecR1, mecI* and *blaZ* genes of *M. caseolyticus* exhibited 72%, 53%, 64% and 66% amino acid identity respectively, to the corresponding genes in MRSA. The finding of *blaZ* in association with a *mec* complex, as well as the similiarity between the *mec* (*mecI* and *mecR1*) and *blaZ* (*blaI* and *blaR1*) regulatory genes supports a theory proposed in the 1980's that the *mec* gene complex was the result of a recombination event between *mecA* and the *blaZ* regulatory genes (Song *et al.*, 1987). The presence of *blaZ* in the *M. caseolyticus mec* complex suggests that this could be an ancestral form of the *mec* gene complex found in MRSA (Tsubakishita *et al.*, 2010a). The *mecA* homologue and class A *mec* complex found in *S. fleuretti* could also be the origin of the *mec* complex in MRSA (Tsubakishita *et al.*, 2010b).

Despite the identification of these potential precursors to *mecA* and SCC*mec* in animal CoNS and related species, no ancestral forms of SCC*mec* have been identified in *S. aureus* and the precise steps in the evolution of SCC*mec* have not yet been elucidated. In the present study two human clinical isolates from Ireland that were found to belong to a clonal complex commonly associated with MSSA from animals (CC130) were detected as PBP2a positive by a latex agglutination assay, but lacked *mecA* by conventional *mecA*

PCR. A comprehensive analysis of the whole-genome sequence of one of these isolates resulted in the identification of a novel SCC*mec* element with highly divergent *mecA, mecI, mecR1, blaZ* and *ccr* genes that may represent an ancestral form of SCC*mec* in MRSA.

3.2 Material and Methods

3.2.1 *Staphylococcus aureus* isolates investigated

The isolates and reference strains used are listed in table 3.2. Two *S. aureus* clinical isolates were investigated in this part of the study, M10/0148 and M10/0061 (Table 3.2). M10/0148 was isolated from a 64-year old female in-patient in an acute care hospital in Dublin, Ireland, in February 2010 and M10/0061 was isolated from an 85-year old male in-patient in a regional hospital in the South-East of Ireland, in May 2010. The two hospitals were approximately 160 kilometres apart and there was no obvious epidemiological connection between these patients. There was no information available indicating that the patients had any contact with animal husbandry, domestic animals, raw meat, or other potential sources of zoonotic bacteria. Isolates were identified as *S. aureus* and were inoculated onto Protect beads as described in Chapter 2, sections 2.3.1, and 2.1.1, respectively.

A *S. aureus* isolate (21250_V454, Table 3.2) recovered from a brown rat (*Rattus norvegicus*) in Germany (courtesy of Sebastian Günther, Berlin University) was also included in the study for comparison since it was found to belong to the same clonal lineage and yielded similar DNA microarray hybridisation patterns to the two clinical isolates (Tables 3.4 and 3.5). An arsenic-resistant and arsenic-susceptible reference strain (WBG8404 and RN4220, respectively) were used for antimicicrobial resistance testing (Table 3.2).

3.2.2 Investigation of resistance to antimicrobial agents

Screening for oxacillin/methicillin resistance was performed by culture of isolates on cefoxitin-containing ChromID MRSA selective agar (bioMérieux, Nuertlingen, Germany), as well as by using 30-µg cefoxitin disks, 1-µg and 5-µg oxacillin disks, determination of oxacillin MICs, PBP2a detection and *mecA* PCR as described in Chapter

Table 3.2. *Staphylococcus aureus* isolates investigated as part of the study of oxacillin & cefoxitin resistant *S. aureus* isolates lacking *mecA* and reference strains for arsenic resistance testing.

Isolate no.	Year of isolation	Source	Description of isolate	Reference
M10/0061	2010	Nasal swab from an 85-year old patient in a hospital in south-east Ireland	Oxacillin- and cefoxitin-resistant isolate lacking *mecA*	This study
M10/0148	2010	Nasal swab from a 64-year old patient in a Dublin hospital	Oxacillin- and cefoxitin-resistant isolate lacking *mecA*	This study
21250_V454	2010	Brown rat (Rattus norvegicus) in Germany	MSSA isolate belonging to the same CC as M10/0061 and M10/0148	This study (Courtesy of Sebastian Gunther, Berlin University)
WBG8404	n/a	n/a	Arsenic-resistant reference strain	O'Brien *et al.*, 2004
RN4220	n/a	n/a	Arsenic-susceptible reference strain	Kreiswirth *et al.*, 1983
CA05	n/a	n/a	SCC*mec* IV reference strain	Luedicke *et al.*, 2010

Abbreviations: CC, clonal complex; n/a, not applicable.

2, section 2.4.1. Oxacillin MICs were repeated using broth culture growth from induced colonies that had been exposed to 1 mg/L and 5 mg/L oxacillin. The two clinical isolates underwent antibiogram-resistogram (AR) typing against a panel of 23 antimicrobial agents as described in Chapter 2, section 2.4.2.

The two clinical isolates were also tested for susceptibility to arsenicals using sterile blank antimicrobial susceptibility discs (Oxoid) containing 100 μg, 250 μg and 500 μg of sodium arsenite and disks containing 250 μg, 500 μg, and 1000 μg of sodium arsenate and CLSI methodology with modifications (CLSI, 2006). The arsenic-resistant and arsenic-susceptible *S. aureus* reference strains indicated in Table 3.2 were used as controls. Stock concentrations of sodium arsenite and sodium arsenate (BDH Chemical Ltd., Poole, Dorset, UK) were prepared in Mili-Q water (Millipore) and were added to sterile blank antimicrobial susceptibility discs to yield the desired concentration. Control disks were prepared by adding–Mili-Q water only to blank discs. An inoculum of 0.5 McFarland of each of the four isolates (two clinical isolates and two reference strains) was lawned onto six Mueller Hinton agar plates (Oxoid) (one for each concentration of sodium arsenite and sodium arsenate) using CLSI methodology (CSLI, 2006). Three disks were added to each lawned agar plate, two containing the same concentration of sodium arsenite or sodium arsenate and one containing sterile water as indicated in Figure 3.1. Plates were incubated overnight at 37°C in a static incubator and were examined the following day for inhibition of growth adjacent to disk.

3.2.3 Total genomic DNA isolation

Genomic DNA was extracted from all isolates using the StaphyType DNA microarray kit and the Qiagen DNeasy kit as described in Chapter 2, section 2.5.4.1.

3.2.4 Molecular typing and DNA microarray analysis

3.2.4.1 Multilocus sequence typing

Multilocus sequence typing (MLST) was performed using primers and PCR conditions as previously described (Enright *et al.*, 2000). This involved the DNA sequencing of internal fragments of seven housekeeping genes, namely carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerise (*tpi*) and acetyl coenzyme A acetyltransferase (*yqiL*). Amplimers were sequenced commercially by Geneservice Ltd. (Source Bioscience) and sequences were analysed as described in Chapter 2, section 2.5.2. The sequence of each allele was compared to ST alleles in the MLST website (http://www.mlst.net) and were assigned an allele number. A sequence type (ST) was assigned based on the allelic profile of the seven genes. The assignment of STs to a clonal complex (CC) was undertaken using the rule that 6/7 alleles should be identical to the allelic profile of at least one ST within a CC to be assigned to that CC.

3.2.4.2 DNA microarray analysis

DNA microarray analysis was performed on the isolates using the StaphyType kit (Alere) as described in Chapter 2, section 2.5.4. The genes detected using the array include previously described SCC*mec*-associated gene sequences including *mecA*, its regulatory genes *mecI* and *mecR*1, various allotypes of the *ccr* genes and gene sequences previously identified in the J regions of various SCC*mec* elements. DNA microarray analysis of each isolate was performed on three separate occasions.

3.2.4.3 *spa* typing

The isolates underwent *spa* typing as described in Chapter 2, section 2.3.3.

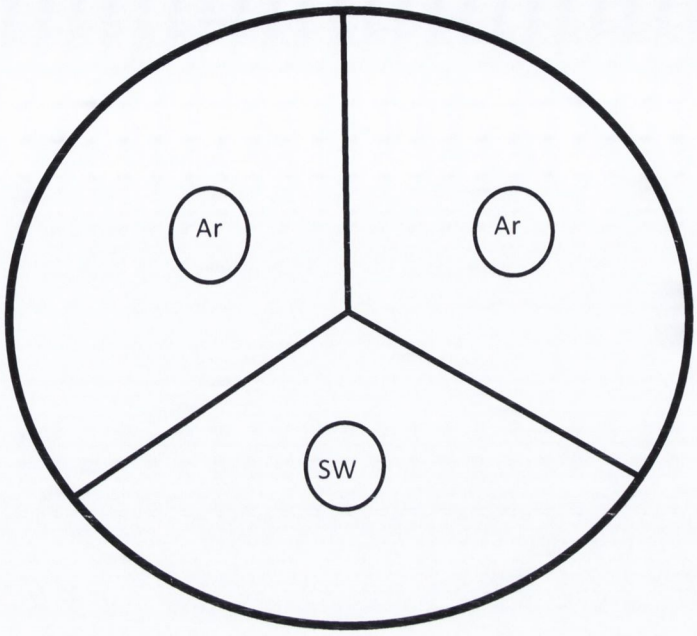


Figure 3.1. Schematic representation of a Mueller Hinton agar plate used for testing isolates for susceptibility to sodium arsenate and sodium arsenite. Four isolates were tested, namely the PBP2a-positive and *mecA*-negative *S. aureus* clinical isolates M10/0061 and M10/0148, the arsenic susceptible *S. aureus* control strain RN4220 and the arsenic resistant *S. aureus* control strain WBG8404. Each isolate was lawned onto six Mueller Hinton agar plates using CSLI methodology (0.5 McFarland) and three disks were placed onto a different section of each plate; one disk containing sterile water (SW) and two of the disks contained the same concentration of sodium arsenite or sodium arsenate (Ar) at one of the following concentrations: 100 μg, 250 μg and 500 μg for sodium arsenite and 250 μg, 500 μg, and 1000 μg for sodium arsenate. Plates were incubated overnight at 37°C in a static incubator and were examined the following day for inhibition of growth.

3.2.5 Whole-genome sequencing of M10/0061

The whole-genome of one clinical MRSA isolate, M10/0061, was sequenced to investigate the location of *ccr* genes identified in both isolates using DNA microarray and to investigate the possible presence of a novel SCC*mec* element. High-throughput *de novo* sequencing was undertaken commercially by Geneservice, reads were assembled into contigs and analysed as described in Chapter 2, section 2.5.5. Any contigs found to carry SCC*mec*-associated gene sequences were analysed further by a collaborator, Dr. Peter Slickers, Alere Techonologies (Germany). Open reading frames (ORFs) were detected using Prokaryotic Dynamic Programming Genefinding Algorithm (Prodigal) (http://prodigal.ornl.gov/) (Hyatt *et al.,* 2010), a gene prediction algorithm designed specifically for prokaryotic sequences. Prodigal was trained with four fully sequenced and annotated *S. aureus* genomes (GenBank accession numbers CP000046.1, BA000017.4, BX571856.1, AJ938182.1). All predicted ORFs were analysed against a local database of all staphylococcal sequences extracted from GenBank using the BLAST standalone software package (version 2.2.24; (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the ORFs were globally aligned with the best matching sequences from the local database and the positions of start and stop codons and potential ribosomal binding sites were checked for consistency. All ORFs were translated into protein sequences and the protein sequences were analysed against the NCBI non-redundant database of protein sequences using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Amino acid identities between two sequences were calculated from global sequence alignments. The sequences were aligned with the needle global alignment program from the EMBOSS (European Molecular Biology Open Software Suite) software package (Rice *et al.,* 2000). Relative identities were calculated from the number of identical amino acids in the alignment with respect to the length of the new sequence from a novel SCC*mec* element identified, termed SCC*mec* XI. These overall amino acid identities were calculated for the

best matching BLAST sequence. In addition, for the *mec* complex genes, amino acid identities were calculated for additional sequences which represented different allotypes or paralogs of the gene concerned. Occurrences of characteristic direct repeats (DRs) of SCC*mec* were detected with the bl2seq program from the NCBI BLAST software package.

3.2.6 PCRs to confirm the presence of novel *mecA* and *ccr* allotypes and SCC*mec* element in M10/0148

Whole-genome sequencing of M10/0061 revealed the presence of novel allotypes of *ccr*, *mecA*, *blaZ* and the *mec* regulatory genes *mecI* and *mecR*1. Primers for *ccrA1B3*$_{M10/0061}$ (ccrA1B3$_{M10/0061}$ F1/R1) and *mecA*$_{M10/0061}$ (mecA$_{M10/0061}$ F1/R1) (Table 3.3) were designed to investigate M10/0148 for the novel allotypes of these genes. Primer pairs were also designed to amplify the entire novel SCC*mec* element in M10/0148 (Table 3.3). DNA fragments were obtained by PCR amplification of chromosomal DNA using either GoTaq DNA polymerase (Promega) for primers ccrA1B3$_{M10/0061}$ F1/R1 and mecA$_{M10/0061}$ F1/R1 or the Expand Long-Template PCR system (Roche) for all other primer pairs according to the manufacturer's instructions. The whole-genome sequenced isolate M10/0061 harbouring the novel *mecA* allotype and MRSA strain CA05 (Luedicke *et al.*, 2010) harbouring SCC*mec* IV and encoding the original *mecA* allotype, were used as positive and negative controls, respectively. PCR products were analysed, purified and sequenced as described in Chapter 2, section 2.5.2.

3.2.7 Nucleotide sequence accession numbers

The nucleotide sequence number of the SCC*mec* element (SCC*mec* XI) and the 3 kb DNA sequence identified adjacent to SCC*mec* XI were deposited in GenBank under accession number FR823292.

Table 3.3. Primers designed and used in the present study for the detection of SCC*mec* XI

Gene or region amplified	Primer pair	Nucleotide sequence (5'-3')	Nucleotide coordinates[a]	Product size
$mecA_{M10/0061}$	$mecA_{M10/0061}$ F1	CCAGATATAGTAGCATTATA	1799-1818	1873 bp
	$mecA_{M10/0061}$ R1	AAAGATGACGATATTGAG	3672-3655	
$ccrA1B3_{M10/0061}$	$ccrB3_{M10/0061}$ F1	CTACTTGAAGTTATCCAATC	10125-10144	3302 bp
	$ccrA1_{M10/0061}$ R1	CACATTACTCGCTGATTTAG	13427-13408	
$orfX$-$mecI_{M10/0061}$	orfX F1	GTGTTAATTGAGCAAGTGTA	418-437	5652 bp
	$mecI_{M10/0061}$ R2	GCATTCTTACATCCGTTTTG	6070-6051	
$mecI_{M10/0061}$-$ccrA1_{M10/0061}$	$mecI_{M10/0061}$ F1	CTATGATATATCAGCGTCAG	56041-56023	6959 bp
	$ccrA1_{M10/0061}$ R1	ACAAGCTCAAGCGATACGGT	12563-12544	
$ccrA1_{M10/0061}$-$ORF_119_{M10/0061}$	$ccrA1_{M10/0061}$ F1	AATAACGTAATGTGCGGTGC	12421-12440	8201 bp
	$ORF_119_{M10/0061}$R1	ACTTAAATCAGGCTTATATCC	20622-20602	
$ORF_119_{M10/0061}$-$arsC_{M10/0061}$	$ORF_119_{M10/0061}$F1	TCTAGTATCTGAAAGGTC	20475-20492	7488 bp
	$arsC_{M10/0061}$ R1	AACCATACGTCAGACTTGA	27963-27945	
$arsC_{M10/0061}$-downstream of DR3	$arsC_{M10/0061}$ F1	GACCACTCTTTACCTGCT	27809-27826	6588 bp
	tnp1S2 R1	AGATCATGGAAAACCGATCA	34397-34378	

[a] Nucleotide coordinates based on the nucleotide sequence of SCC*mec* XI from clinical MRSA strain M10/0061 (Genbank accession number FR823292).

3.3 Results

3.3.1 Identification of two oxacillin and cefoxitin resistant PBP2a-positive *S. aureus* isolates that lack *mecA* by conventional *mecA* PCR

The *S. aureus* isolates M10/0061 and M10/0148 exhibited resistance to cefoxitin and oxacillin and exhibited growth on Chrom ID MRSA selective agar. The two isolates exhibited oxacillin MICs of 1.0 mg/L and 2.0 mg/L, respectively and then both rose to 16 mg/L following induction with 1 mg/L and 5 mg/L oxacillin. Both isolates were positive for the production of PBP2a using the latex agglutination assay. Furthermore, neither isolate yielded an amplimer following PCR for the presence of *mecA*. Both isolates exhibited resistance to ampicillin only from the group of 23 antimicrobial agents tested. Both MRSA isolates exhibited resistance to sodium arsenite and sodium arsenate (Figure 3.2).

The rat MSSA isolate did not grow in the presence of cefoxitin, was negative for the production of PBP2a and was *mecA* negative by PCR.

3.3.2 Molecular typing

Multilocus sequence typing resulted in both MRSA isolates being assigned to two different STs, ST130 and ST1764, within CC130 (Table 3.4). The MSSA isolate was also assigned to a ST within CC130 (ST2024). Both MRSA isolates were assigned to the closely related *spa* types t843 and t373, which differ by the presence of two additional repeat units in t843 (Table 3.4). The MSSA isolate exhibited a novel but related *spa* type, t8403, which differed from *spa* type t373 by the presence of one addition repeat unit and from *spa* type t843 in the deletion of one repeat unit (Table 3.4).

3.3.3 DNA microarray analysis

DNA microarray analysis also confirmed the absence of *mecA* and *mec* regulatory genes in the MSSA and MRSA isolates (Table 3.5). Both MRSA isolates yielded weak and/or variable signals for *ccrB1*, *ccrA3* and *ccrB3* (Table 3.5). No other SCC or SCC*mec*-associated genes were detected in either MRSA isolate (Table 3.5). The two MRSA isolates were assigned to *arg* group III and capsule type 8 (Table 3.5). The multidrug efflux pump *sdrM*, which encodes resistance to norofloxacin, acriflavine, and possibly ethidium bromide (Yamada *et al.*, 2006), was the only resistance gene detected in the two MRSA isolates. Both MRSA isolates lacked the immune invasion complex (IEC) genes, and harboured an untruncated copy of the beta-haemolysin gene *hlb*. The *edinB* gene, encoding the epidermal cell differentiation inhibitor B was also detected as well as the alpha and delta haemolysin genes *hla* and *hld*, respectively (Table 3.5).

The MSSA isolate yielded very similar signals to the MRSA isolates, except that it lacked the weak and/or variable signals for *ccrB1*, *ccrA3* and *ccrB3* (Table 3.5).

3.3.4. Identification of a SCC*mec* element encoding a novel *mec* allotype in MRSA isolates M10/0061.

Whole-genome sequencing of the ST130 MRSA isolates M10/0061 yielded 401 contigs ranging in size from ca. 100 bp to 100,000 bp, 46 of which were >20,000 bp in size. The *ccr* genes were localised to a 90 kb contig that was found to contain sequences encoding a 30 kb SCC*mec* element which was analysed in depth (Figure 3.3). As this study was focused on the 30 kb SCC*mec* element the rest of the 90 kb contig and whole-genome sequence was not analysed. Details of the ORFs identified within SCC*mec* of M10/0061 are shown in Table 3.6.

The SCC*mec* element harboured a novel allotype of the *mecA* gene, $mecA_{M10/0061}$ which had 62% amino acid (aa) identity with *mecA* previously described in MRSA. Novel

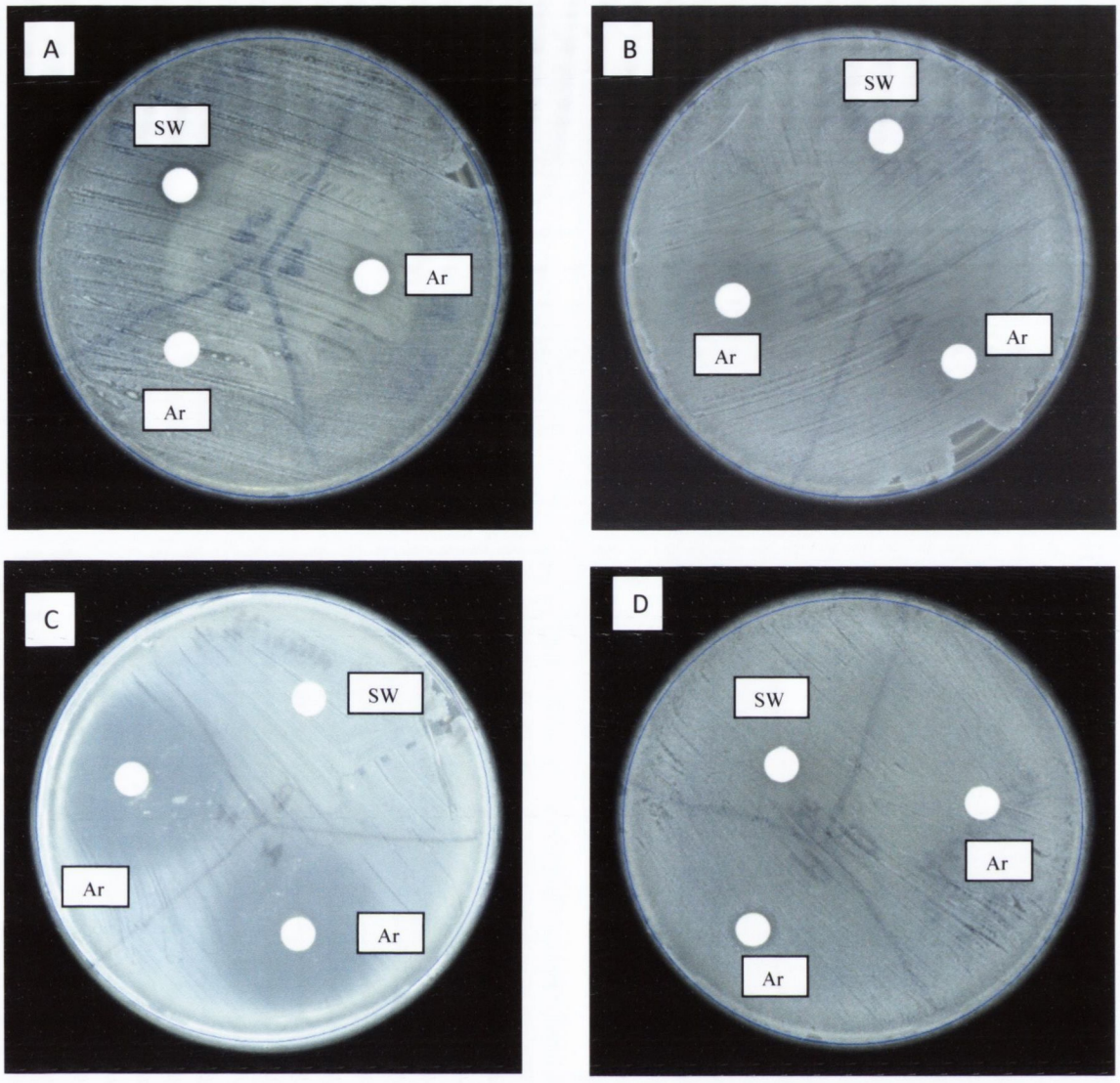


Figure 3.2. Sodium arsenite disc susceptibility testing of *S. aureus*. Mueller Hinton agar plates with two 250 μg sodium arsenite disks (Ar) and one disc containing sterile water (SW) lawned with bacterial suspensions of the PBP2a-positive, *mecA*-negative and aresenic-resistant MRSA isolates M10/0061 (panel A) and M10/0148 (panel B). The arsenic susceptible *S. aureus* control strain RN4220 and the arsenic resistant *S. aureus* control strain WBG 8404 are shown in panels C and D, respectively.

Table 3.4. MLST, *spa* types, AR patterns and oxacillin minimum inhibitory concentrations (MICs) of MRSA isolates M10/0061 and M10/0148 and MSSA isolate 21250_V454

Isolate no.	MLST CC/ST (allelic profile)	*spa* type (repeat succession)	AR pattern[1]	Oxacillin MIC	
				Initial	Induced[2]
M10/0061	CC130/ST13 (6-57-45-2-7-58-52)	t843 (04-82-17-25-17-25-25-16-17)	Ap	1.0 mg/L	16 mg/L
M10/0148	CC130/ST1764 (6-57-45-2-7-193-52)	t373 (04-17-25-17-25-16-17)	Ap	2.0 mg/L	16 mg/L
21250_V454	CC130/ST2024 (6-57-45-2-219-58-52)	t8372 (04-82-17-25-25-25-16-17)	n/d	n/d	n/d

[1]Antibiogram resistogram (AR) pattern determined by AR typing which involves determining the susceptibility of isolates to a panel of 23 antimicrobial agents including amikacin, ampicillin, cadimium nitrate, chloramphenicol, ciprofloxacin, ethidium bromide, erythromycin, fusidic acid, gentamicin, kanamycin, lincomycin, mercurie chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampicin, streptomycin, spectiomycin, sulphonamide, tobramycin, tetracycline, trimethoprim and vancomycin.

[2]Resistance to oxacillin was induced by culturing each isolate in the presence of sub-inhibitory concentrations of oxacillin.

Abbreviations; Ap, Ampicillin; MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type; MIC, minimum inhibitory concentration; n/d, not determined

Table 3.5. DNA microarray[a] hybridisation profiles of MRSA isolates M10/0061 and M10/0148 and MSSA isolate 21250_V454

Isolate		M10/0061	M10/0148	21250_V454
Gene class	**Genes**			
Species markers	*katA, coa, nuc, spa*	**Pos**	**Pos**	**Pos**
***agr* group**	*agr* group III	**Pos**	**Pos**	**Pos**
SCC*mec*-associated markers	*mecA*	Neg	Neg	Neg
	delta mecR	Neg	Neg	Neg
	mecR/I	Neg	Neg	Neg
	ccrA-1	Neg	Neg	Neg
	ccrB-1	**Variable**	**Variable**	Neg
	Q9XB68-dcs	Neg	Neg	Neg
	ccrA/B-2	Neg	Neg	Neg
	kdp-SCC locus	Neg	Neg	Neg
	ccrA-3	**Variable**	**Variable**	Neg
	ccrB-3	**Variable**	**Variable**	Neg
	ccrC, ccrA/B4	Neg	Neg	Neg
Virulence-associated genes	*tst1*	Neg	Neg	Neg
	sea, seb, see, seh	Neg	Neg	Neg
	sec/l, sed/j/r	Neg	Neg	Neg
	seg/i/m/n/o/u	Neg	Neg	Neg
	sek/q	Neg	Neg	Neg
	lukF/S-PV	Neg	Neg	Neg
	lukF-PV (P83)/lukM	Neg	Neg	**Pos**
	hla/hld	**Pos**	**Pos**	**Pos**
	hlb	**Pos**	**Pos**	**Pos**
	un-truncated hlb	**Pos**	**Pos**	**Pos**
	sak/chp/scn	Neg	Neg	Neg
	etA/B/D	Neg	Neg	Neg
	edinA/C	Neg	Neg	Neg
	edinB	**Pos**	**Pos**	**Pos**
	arcA/B/C/D	Neg	Neg	Neg
Antimicrobial resistance genes	*blaZ*	Neg	Neg	Neg
	blaI/R1	Neg	Neg	Neg
	erm(A), *erm*(B)	Neg	Neg	Neg
	erm(C)	Neg	Neg	Neg
	lnu(A)	Neg	Neg	Neg
	msr(A), *mph*(C)	Neg	Neg	Neg
	aacA-aphD	Neg	Neg	Neg
	aadD	Neg	Neg	Neg
	aphA3/sat	Neg	Neg	Neg
	dfrS1	Neg	Neg	Neg
	far1	Neg	Neg	Neg

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Table 3.5 continued. DNA microarray[a] hybridisation profiles of MRSA isolates M10/0061 and M10/0148 and MSSA isolate 21250_V454

	Isolate	M10/0061	M10/0148	21250_V454
Gene class	**Genes**			
Antimicrobial resistance genes	*mupA*	Neg	Neg	Neg
	tet(K)	Neg	Neg	Neg
	tet(M)	Neg	Neg	Neg
	cat	Neg	Neg	Neg
	cfr	Neg	Neg	Neg
	fexA	Neg	Neg	Neg
	fosB	Neg	Neg	Neg
	sdrM	**Pos**	**Pos**	**Pos**
Capsule type	capsule type 8	**Pos**	**Pos**	**Pos**

[a] The StaphyType kit (Alere Technologies) was used for DNA microarray analysis. The table shows only the clinically relevant markers including exotoxins and resistance genes and does not represent the complete hybridisation profile of 334 probes on the array.

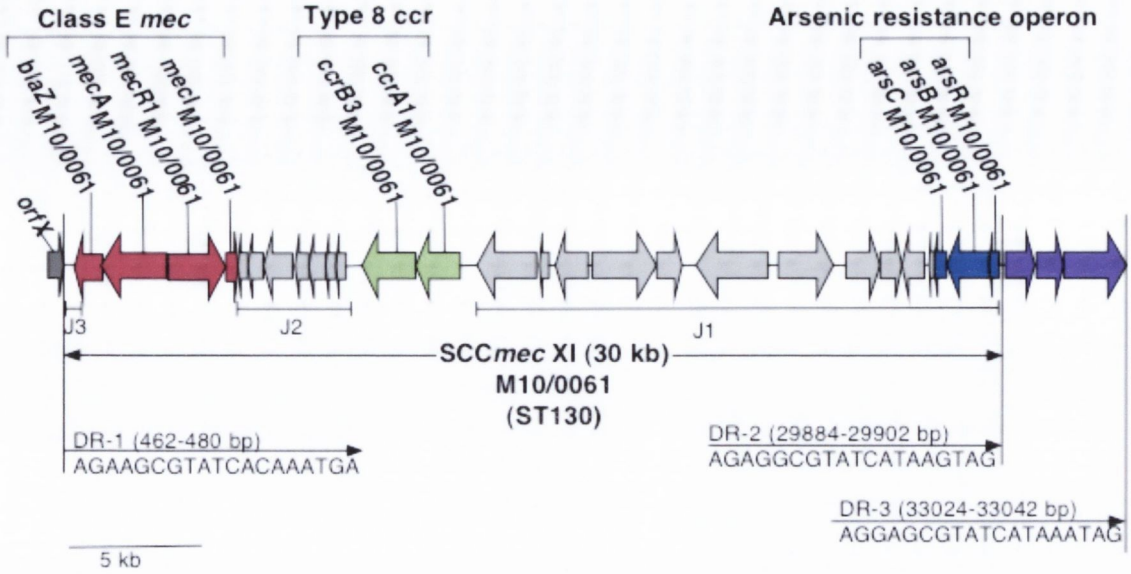

Figure 3.3. Schematic diagram showing the genetic organisation of the SCC*mec* element designated SCC*mec* XI and the adjacent 3 kb downstream region in the ST130 MRSA isolate M10/0061 (GenBank accession number FR823292). SCC*mec* XI is bordered by direct repeat (DR) sequences DR-1 and DR-2 and the 3-kb downstream region is bordered by DR-2 and DR-3. The *mec* complex, *ccr* complex and arsenic resistance operon genes are shown in red, green and blue, respectively. The three ORFs identified in the adjacent 3 kb downstream region are shown in purple. A full list of ORFs encoded by the SCC*mec* element and adjacent regions is shown in Table 3.5. The structure of the SCC*mec* elements was determined by high-throughput whole-genome sequencing of M10/0061.

Table 3.6. Details of ORFs identified in SCC*mec* XI[a] of ST130 MRSA isolate M10/0061

ORF/DR	Nucleotide coordinates[b]	Comparative ORF (GenBank accession number)[c]	Protein accession number	% amino acid identity[d]	Gene product/DR nucleotide sequence (5'-3')
ORF_100 (*orfX*)	1-480	*orfX* MRSA strain MW2 (BA000033.2)	BAA82243.1	99%	23S rRNA methyltransferase (formerly known as open reading frame X conserved hypothetical protein)[e]
DR-1	462-480	n/a	n/a	n/a	AGAAGCGTATCACAAATGA
ORF_101 ($blaZ_{M10/0061}$)	796-1647 (complement)	*blaZ S. aureus* plasmid SAP015A (GQ900380.1)	ADA61365	67%	β-lactamase protein BlaZ
		blaZm Macrococcus caseolyticus JCSC7096 (AB498756.1)	BAI83352.1	44%	
		blaZ Staphylococcus saprophyticus ATCC 15305 (NC_007350)	BAE19331.1	49%	
ORF_102 ($mecA_{M10/0061}$)	1741-3738 (complement)	*mecA Staphylococcus kloosii* CSCA8 (AM048808.2)	CAJ15584.2	63%	Penicillin binding protein 2a
		mecA Staphylococcus vitulinus CSWY15 (AM048811.2)	CAJ15587.2	63%	
		mecA MRSA N315 (D86934)			
		mecA Staphylococcus sciuri ATCC29062 (AB547234.1)	BAA82220.1	62%	
			BAJ15061.1	62%	
		mecA M. caseolyticus JCSC7096 (AB498756.1)	BAI83351.1	21%	
ORF_103 ($mecR1_{M10/0061}$)	3839-5593	*mecR1* MRSA N315 (D86934)	BAA82219.2	45%	Signal transducer protein MecR1
		mecR1 M. caseolyticus JCSC7096 (AB498756.1)	BAI83350.1	35%	Beta-lactamase regulatory protein BlaR1
		blaR1 MRSA252 (BX571856.1)			
		blaR1 Staphylococcus haemolyticus plasmid NVH96 (AJ302698)	CAG40821.1	38%	
			CAC16666.1	37%	
ORF_104 ($mecI_{M10/0061}$)	5590-5964	*mecI* MRSA N315 (D86934)	BAA82218.1	66%	Methicillin resistance regulatory protein MecI
		mecIm M. caseolyticus JCSC7096 (AB498756.1)	BAI83349.1	40%	Beta-lactamase regulatory protein BlaI
		blaI MRSA N315 plasmid pN315 (NC_003140)	BAB43881.1	52%	
		blaR1 MRSA252 (BX571856.1)	CAG40820.1	51%	
ORF_105	6220-6522 (complement)	ORF MCCL_plsB0028 *M. caseolyticus* JCSC5402 plasmid pMCCL2 (AP009486.1)	BAH18706.1	49%	Hypothetical protein
ORF_106	6542-7009 (complement)	ORF 21617-22813 *Clostridium sp.* M62/1C_sp_M621-1.0.1 (ACFX02000052.1)	EFE10767.1	21%	Transposase

Continued overleaf

Table 3.6 continued. Details of ORFs identified in SCC*mec* XI[a] of ST130 MRSA isolate M10/0061

ORF/DR	Nucleotide coordinates[b]	Comparative ORF (GenBank accession number)[c]	Protein accession number	% amino acid identity[d]	Gene product/DR nucleotide sequence (5'-3')
ORF _107	7026-7910 (complement)	ORF HMPREF0783_1004 *S. aureus* ATCC BAA-39 (AEEK01000038.1)	EFM07337.1	90%	Hypothetical protein
ORF _108	8108-8527 (complement)	**ORF 12873-13292 *S. saprophyticus* SCC*mec* strain TSU 33 (AB353724.1)**	BAG24384.1	91%	Hypothetical protein
ORF _109	8548-9054 (complement)	ORF CZ120 MRSA SRI*mec*-III and SCC*mec*-III region, strain 85/2082 (AB038513.1)	BAC53831.1	88%	Hypothetical protein
ORF _110	9070-9381 (complement)	ORF8 MRSA SCC*mec*ZH47 (AM292304.1)	CAL22885.1	82%	Hypothetical protein
ORF _111	9477-9815 (complement)	0RF23 MRSA BK16691 transposon Tn6072 (GU235985.1)	ADC53390.1	92%	Hypothetical protein
ORF _112 (*ccrB-3*$_{M10/0061}$)	10290-11918 (complement)	*ccrB3* MRSA 85/2082(AB014436.1)	BAA88755.1	93%	Cassette chromosome recombinase B3
ORF _113 (*ccrA-1*$_{M10/0061}$)	11939-13288 (complement)	*ccrA S. saprophyticus* SCC*mec* strain TSU 33 (AB353724.1)	BAG24390.1	86%	Cassette chromosome recombinase A1
ORF _115	15584-15877 (complement)	ORF no. C16 SCC*mec* IV MRSA JCSC6670 (AB425824.1)	BAG84216.1	62%	Hypothetical protein
ORF_116	16060-17052 (complement)	ORF11 SCC*mec* *Staphylococcus hominis* strain GIFU12263 (AB063171.1)	BAB83482.1	64%	Hypothetical protein
ORF _117	17171-19102	ORF FP2_13010 (FP929045.1) *Faecalibacterium prausnitzii* L2/6	CBK98818.1	32%	DNA helicase protein
ORF_118	19196-19975	ORF HMPREF0789_1672 *Staphylococcus epidermidis* BCM-HMP0060 (ACHE01000062.1)	EES57797.1	48%	Hypothetical protein
ORF_119	20388-22553 (complement)	*geh1 S. epidermidis* (AF053006.1)	AAC38597.1	39%	Lipase precursor protein

Continued overleaf

Table 3.6 continued. Details of ORFs identified in SCC*mec* XI[a] of ST130 MRSA isolate M10/0061

ORF/DR	Nucleotide coordinates[b]	Comparative ORF (GenBank accession number)[c]	Protein accession number	% amino acid identity[d]	Gene product/DR nucleotide sequence (5'-3')
ORF_120	22852-24501	ORF no. SH0213 *S. haemolyticus* strain JCSC1435 (AP006716.1)	BAE03522.1	75%	FAD-binding dehydrogenase
		ORF ACJB01000028.1 *S. epidermidis* M23864:W1 contig00029 (ACJB01000028.1)	EES41411.1	75%	
ORF_121	24867-25916	**ORF no. SSP0088 *S. saprophyticus* strain ATCC15305 (AP008934.1)**	BAE17233.1	84%	ABC transporter permease protein LolE
ORF_122	25934-26602	OFR no. SSP0089 *S. saprophyticus* strain ATCC15305 (AP008934.1)	BAE17234.1	89%	ABC transporter ATP-binding protein
ORF_123	26743-27423 (complement)	ORF STACA0001_1525 *Staphylococcus capitis* SK14 (ACFR01000014.1)	EEE48440.1	93%	Flavin reductase like domain protein
		ORF GG730274.1:513166-513858 *S. aureus* C160 (GG730274.1)	EFC01991.1	93%	
ORF_124	27544-27714 (complement)	ORF STAWA0001_0106 *Staphylococcus warneri* L37603 (ACPZ01000067.1)	EEQ79095.1	41%	DUF1837 superfamily protein
		ORF GL545278.1:540491-541666 *Staphylococcus caprae* C87 (GL545278.1)	EFS16465.1	41%	
ORF_125 ($arsC_{M10/0061}$)	27751-28143 (complement)	*arsC S. capitis* SK14 (ACFR01000004.1)	EEE49308.1	92%	Arsenate reductase
		arsC S. haemolyticus JCSC1435 (AP006716.1)	BAE03410.1	92%	
		arsC MRSA252 (BX571856.1)	CAG39708.1	91%	
ORF_126 ($arsB_{M10/0061}$)	28161-29450 (complement)	*arsB Staphylococcus xylosus* plasmid pSX267 (M80565.1)	AAA27588.1	93%	Arsenic efflux pump protein
		arsB MRSA 252 (BX571856.1)	CAG39707.1	93%	
ORF_127 ($asrR_{M10/0061}$)	29450-29764 (complement)	*asrR S. aureus* IMCJ1379 plasmid pSA1379 (AB255366.1)	BAE92849.1	79%	Arsenic resistance operon repressor protein
DR-2	29884-29902	n/a	n/a	n/a	AGAGGCGTATCATAAGTAG

Continued overleaf

Table 3.6 continued. Details of ORFs identified in SCC*mec* XI[a] of ST130 MRSA isolate M10/0061

ORF/DR	Nucleotide coordinates[b]	Comparative ORF (GenBank accession number)[c]	Protein accession number	% amino acid identity[d]	Gene product/DR nucleotide sequence (5'-3')
ORF_128	29941-30855	ORF HMPREF0432_00311 *Gemella moribillum* M424 (ACRX01000004.1)	EFV36237.1	52%	Hypothetical protein
ORF _129	30855-31700	ORF Sez_1540 *Streptococcus equi* subsp. *zooepidemicus* MGCS1056 (ACG62873.1)	ACG62873.1	53%	DNA helicase protein
ORF _130	31697-32962	ORF Sez_1540 *Streptococcus equi* subsp. *zooepidemicus* MGCS1056 (ACG62873.1)	ACG62873.1	39%	DNA helicase protein
DR-3	33024-33042	n/a	n/a	n/a	AGGAGCGTATCATAAATAG

[a] The final three ORFs listed in the table (ORF_128, ORF_129 and ORF_130) are located downstream of SCC*mec* XI in M10/0061 and are not part of the SCC*mec* element. However, the region harbouring these three ORFs is flanked by direct repeat (DR) sequences and is considered to be a remnant of a SCC element and is therefore included in the table.

[b] Nucleotide coordinates based on the nucleotide sequence of SCC*mec* XI of M10/0061 (GenBank accession number FR823292).

[c] For all ORFs identified within M10/0061, apart from the *mec* complex genes, only the top hit identified using BLAST is shown.

[d] Amino acid identities between two sequences were calculated from global sequence alignments. The sequences were aligned with the needle global alignment program from the EMBOSS software package (Rice *et al.*, 2000). Relative identities were calculated from the number of identical amino acids in the alignment with respect to the length of the new sequence from SCC*mec*XI. These overall amino acid identities were calculated for the best matching BLAST sequence and for some other staphylococcal sequences which represent different allotypes or paralogs of that gene.

[e] *orfX* has been annotated as a 23S rRNA methyltransferase (http://www.uniprot.org/uniprot/P0C1V0). n/a, not applicable

allotypes of the *mecA* regulatory genes were identified upstream of $mecA_{M10/0061}$. These genes had 66% and 45% aa identity with previously described MRSA *mecI* and *mecR1* genes, respectively. A novel allotype of the gene *blaZ,* encoding β-lactamase resistance, was found downstream of $mecA_{M10/0061}$ and this exhibited 67% aa identity with *blaZ* genes previously identified in *S. aureus.* A novel combination of *ccr* genes were also identified in this SCC*mec* element and consisted of *ccrA1* and *ccrB3*, which had 86% and 93% aa identity to previously described *ccrA1* and *ccrB3* genes, respectively.

The SCC*mec* element was integrated at the same nucleotide position within *orfX* in M10/0061 as all other SCC*mec* elements described to date in MRSA. The SCC*mec* element was also flanked by DR sequences (DR-1 and DR-2, Figure. 3.3) characteristic of SCC*mec* elements. A third DR (DR-3, Figure 3.3) was identified 3 kb downstream of DR-2 in the novel element. There were three ORFs identified in this 3 kb region and they had very weak similarity (39-53% aa identity) with ORFs from non-staphylococcal species (Table 3.6).

There were no ORFs identified in the J3 regions (DNA sequence between *orfX* and *blaZ*). The region between the *mec* complex and the *ccr* genes (J2 region) contained seven ORFs. For six of the seven ORFs the highest similarities ranged from 49-91% to hypothetical proteins of *S. aureus, M. caseolyticus* and *Staphylococcus saprophyticus* (Table 3.6). For the remaining ORF in the J2 region the highest similarity (21%) was to a transposase of *Clostridium sp.* (Table 3.6). Fourteen ORFs were identified within the J1 region (between the *ccr* genes and DR-2). Three of these ORFs had 79-93% aa identity to the arsenic resistance operon genes *arsC, arsB*, and *arsR* (Table 3.6). For 2/14 ORFs (ORF_115 and ORF_116) the highest similarity was to ORFs encoding hypothetical proteins described in SCC*mec* elements, one in MRSA and one in *Staphylococcus hominis* (Table 3.6). Of the nine remaining ORFs, eight exhibited aa identities ranging from 39–89% to ORFs from CoNS including a cassette chromosome helicase protein, a lipase

precursor protein, a FAD-binding dehydrogenase, two ABC transporter proteins, a flavin reductase protein, a major facilitator superfamily protein and a hypothetical protein (Table 3.6). The remaining ORF (ORF_117) exhibited weak similarity (32% aa identity) with a DNA helicase in a non staphylococcal species (Table 3.6).

3.3.5. Designation of the SCC*mec* element of M10/0061 as SCC*mec* XI.

At the time of this study, the IWG-SCC website listed a SCC*mec* type, designed SCC*mec* XI, from MRSA isolate LGA251 with a similar genetic organisation within the *mec* and *ccr* complexes as the SCC*mec* element identitfied in the present study in M10/0061. A search of the published literature and GenBank failed to yield descriptions of SCC*mec* XI. LGA251 was recovered from a bovine source and had been assigned to ST425 (http://www.sanger.as.uk/pathogens). The whole-genome sequence of LGA251 was determined by the Wellcome Trust Sanger Institute (Cambridge, United Kingdom) and is available on the Sangar Institute website but at the time this work was being undertaken it was not available in GenBank and was not published. Because of this, permission was sought and obtained from the Sanger Institute to compare the SCC*mec* element identified in the human clinical MRSA isolate M10/0061 in the present study with that of SCC*mec* XI first identified by the Sanger Institute in bovine MRSA isolate LGA251. The comparative analysis showed that the SCC*mec* elements in both isolates exhibited 99.9% DNA sequence identity. The SCC*mec* element from M10/0061 was thus designated SCC*mec* XI. SCC*mec* XI has a class E *mec* complex ($blaZ_{M10/0061}$-$mecA_{M10/0061}$-$mecR1_{M10/0061}$-$mecI_{M10/0061}$) and type 8 *ccr* complex (*ccrA1B3*).

No sequence similarity to the 3 kb region downstream of DR-2 in SCC*mec* $XI_{M10/0061}$ was found in SCC*mec* XI_{LGA251}.

3.3.6. Detection of SCC*mec* XI in M10/0148.

The presence of *ccrA1B3*$_{M10/0061}$ and *mecA*$_{M10/0061}$ as well as *mecI*$_{M10/0061}$, *mecR1*$_{M10/0061}$ and *blaZ*$_{M10/0061\ 0061}$ were all confirmed in the second clinical CC130 MRSA isolate M10/0148 by PCR and sequencing. Each of these genes exhibited 99-100% DNA sequence identity with the corresponding genes identified in M10/0061. The presence of the entire SCC*mec* XI element and the 3 kb region between DR-2 and DR-3 was also confirmed in M10/0148 using PCR and 10 primer pairs (Table 3.3). M10/0148 yielded amplimers of the expected size using all primer pairs.

3.4 Discussion

The accurate identification of MRSA is essential to ensure appropriate isolation and treatment of patients as failure to do so can have potentially serious consequences for patients and place significant extra demands on healthcare resources. The identification of two CC130 MRSA isolates that could not be identified as MRSA using conventioal *mecA* PCR and DNA microarray profiling is alarming. Two Irish CC130 MRSA isolates recovered from separate patients in two different hospitals in Ireland were found to harbour a 30 kb SCC*mec* element that had highly divergent *mecA*, *mecI, mecR1, blaZ* and *ccr* genes. This element was designated SCC*mec* XI by the IWG-SCC (http://www.sccmec.org/Pages/SCC_TypesEN.html). The ORFs within this SCC*mec* element exhibited 21-93% aa identity to previously described ORFs in GenBank indicating that this SCC*mec* is distantly related to previously described SCC*mec* elements and may possibly represent an ancestral form of SCC*mec* in MRSA.

The SCC*mec* elements that have been described to date have all carried *mec* gene complexes that are almost identical, but the *mecA, mecR1* and *mecI* genes in SCC*mec* XI exhibited only 62%, 45% and 66% aa identity, respectively, with the corresponding genes previously described in MRSA isolates. Homologues of *mecA* have also been identified in *Staphylococcus kloosii* (Schnellmann *et al.,* 2006), *S. sciuri* (Cunto *et al.*, 1996), *M. caseolyticus* (Tsubakishita *et al.*, 2010a) and *S. fleuretti* (Tsubakishita *et al.*, 2010b) but the *mec gene* found in the two CC130 isolates exhibited only 21-63% aa identity to these. A *blaZ* gene, usually found as part of the *bla* operon (*blaI, blaR1* and *blaZ)*, was identified within SCC*mec* XI upstream of $mecA_{M10/0061}$ and the only previous report of a *mec* complex with *blaZ* was in a transposons in *M. caseolyticus* with the same genetic organisation of the *mec* complex identified in the present study. Therefore, the *mec* gene complex (*blaZ-mecA-mecR1-mecI*) identified in the present study may have evolved from

the *mec* gene complex in *M. caseolyticus* (Tsubakishita *et al.*, 2010). The combination of *ccr* genes identified within SCC*mec* XI was also different to those previously described and was assigned a novel *ccr* type combination designated type 8. The very significant differences between SCC*mec* XI and other SCC*mec* types together with the similarity in genetic organisation of the SCC*mec* XI *mec* complex relative to *M. caseolyticus* suggest that SCC*mec* XI may have originated in another species. This is also further evidence of how interspecies transfer of SCC*mec* that may play a role in the emergence of novel MRSA strains.

The J regions of the SCC*mec* XI also differed significantly from previously described SCC*mec* elements. The J3 region was much smaller (316 bp) than J3 regions in other SCC*mec* elements (I-X) but the J2 and J1 regions were more comparable in size to previously described SCC*mec* elements. The 21 ORFs identified (excluding the *ccr* and *mec* complex genes) exhibited only 21%-93% similarity to previously described ORFs. Of these 21, only four had high similarity (62 to 88%) to genes associated with SCC*mec* elements in MRSA or CoNS. The remaining 17 ORFs exhibited similarities to non-SCC*mec* DNA sequences in *S. aureus,* CoNS and also weak similarity to non-staphylococcal species. Three of these ORFs were part of an arsenic resistance operon (79-93% aa identity), that consisted of *arsR*, encoding a transcriptional repressor, *arsB*, encoding an arsenite efflux pump and *arsC*, encoding an arsenate reductase. Arsenic occurs naturally in the environment and is commonly found in the earth's crust. It is also associated with agriculture and industry, due to the use of pesticides, water processing and mining (Paez-Espino *et al.*, 2009). Arsenic resistance genes have been identified previously in chromosomal and plasmid locations in many bacterial species, including *S. aureus*, CoNS, *Escherichia coli,* and *Pseudomonas fluorescens* (Paez-Espino *et al.*, 2009). Arsenic resistance operons have also be found associated with SCC*mec* IX and SCC*mec* X and both types are animal associated SCC*mec* types (Li *et al.*, 2011).

Simultaneous to the findings of the present study being reported another publication identified SCC*mec* XI among MRSA from patients in England, Scotland and Denmark and among bovine milk samples in England (Garcia-Alvarez *et al.,* 2011). Subsequently there have been numerous reports of $mecA_{M10/0061}$ and SCC*mec* XI across Europe among humans, livestock and domestic and wild animals (Cuny *et al.*, 2011; Laurent *et al.,* 2012; Paterson *et al.*, 2012; Petersen *et al.,* 2012; Pichon *et al.,* 2012; Robb *et al.,* 2012; Sabat *et a*l., 2012; Stegger *et al.*, 2012; Walther *et al.*, 2012; Basset *et al.,* 2013; Eriksson *et al.,* 2013; Lorcaric *et al.,* 2013; Medhus *et al.,* 2013; Monecke *et al.,* 2013; Unnerstad *et al.,* 2013; Vandendriesosche *et al.,* 2013) (Table 3.7). The isolates predominantly belonged to CC130, but other animal-associated lineages have also been reported with $mecA_{M10/0061}$ (Table 3.7).

Prior to the identification of $mecA_{M10/0061}$, clonal complex 130 had been reported only in MSSA isolates and only in animals, including cows and rats (Jorgensen *et al.,* 2005, Sung *et al.,* 2008, van de Giessen *et al.,* 2009). While there is no evidence in the present study of a link between animals and the patients carrying CC130 MRSA and SCC*mec* XI, the absence of lysogenic β-toxin-converting bacteriophages in the two isolates also suggests an animal origin as this feature is characteristic of *S. aureus* from animals. Subsequent studies have also provided evidence to support the possibility of the zoonotic transmission of these strains. Peterson *et al.* (2012) found an epidemiological link between cattle and humans in which MRSA with this novel *mec* gene were identified. These isolates underwent whole-genome sequencing and only a small number of single nucleotide polymorphisms (SNP) differences were identified between the animal and humans isolates (Harrison *et al.*, 2013). Many studies have reported a low prevalence of $mecA_{M10/0061}$ among animals, and most of the reports have been from small animals, predominantly wildlife e.g. rats, hedgehogs and rabbits (Table 3.7) indicating that they

Table. 3.7 Details of *mecA*$_{M10/0061}$-positive MRSA isolates reported to date from animals and humans

Country	Investigation period	No. of isolates investigated	No. of *mecC* positive isolates	Source (*n*)	CC/ST	*spa* types	Reference
Ireland	2010	2	2	Human (2)	CC130/ST130 & ST1764	t843, t373	Shore *et al.*, 2011
England	1975-2011	4329	30	Human (15), cow milk samples (15)	CC130, CC425, CC705, CC1943	t529, t843, t742, t978, t1535, t1736, t6220, t6292, t6293, t6360, t6386	Garcia-Alvarez *et al.*, 2011
Scotland	1975-2011	3389	12	Human (12)	CC130, CC1945	t843, t1535, t7485, t7946, t4947	Garcia-Alvarez *et al.*, 2011
Denmark	1975-2011	3389	24	Human (24)	CC130	t843, t1535	Garcia-Alvarez *et al.*, 2011
Germany	2006-2011	12691	11	Human (11)	CC130	t843, t1736, t1773	Cuny *et al.*, 2011
Belgium	1993-2011	52	5	Rat (4), rabbit (1)	CC425, ST2273	t208, t742	Paterson *et al.*, 2012
Scotland	1993-2011	52	3	Chaffinch (1), seal (1), dog (1)	CC130, ST1764, ST1254	t6293, t6594, t7914	Paterson *et al.*, 2012
Denmark	1993-2011	52	3	Sheep (3)	CC130	t843	Paterson *et al.*, 2012
Germany	Not Stated	18	18	Human (18)	CC130, ST599	t843, t978, t1535, t1773, t7189, t7189	Sabat *et al.*, 2012
Denmark	1960-2011	12200	112	Human (112)	CC130, CC2361	t373, t528, t843, t978, t1046, t1532, t1535, t1736, t1773, t2345, t3218, t3256, t3391, t3570, t5970, t6620, t8835, t9395, t9397,	Peterson *et al.*, 2012,
Germany	2008-2011	10	10	Cat (7), dog (2), guinea pig (1)	CC130, ST599	t278, t843, t1694, t1773, t10006, t10009, t10033	Walther *et al.*, 2012
France	2007-2008	3	3	Human (1), cow milk samples (2)	CC130	t843	Laurent *et al.*, 2012
England	2011	2263	10	Human (10)	Not stated	t843, t742, t6292, t6293, t6386, t9376, t9605	Pichon *et al.*, 2012
Scotland	1995-2011	3	3	Chaffinch (3)	Not stated	t6293	Robb *et al.*, 2012
Denmark	2011	203	12	Human (12)	CC130, CC1943	t843, Others not stated	Stegger *et al.*, 2012

Continued overleaf

Table. 3.7 continued. Details of *mecA*$_{M10/0061}$-positive MRSA isolates reported to date from animals and humans

Country	Investigation period	No. of Isolates investigated	No. of *mecC* positive isolates	Source (n)	CC/ST	*spa* Types	Reference
Norway	2012	1	1	Cat (1)	ST2497	t6902	Medhus *et al.*, 2013
Sweden	2010-2011	311	4	Bovine milk (4)	CC130, CC425	t525, t9111	Unnerstad *et al.*, 2013
Denmark	2011-2012	85	2	Sheep (2)	ST130	t843	Eriksson *et al.*, 2013
Germany	2010-2012	6	5	Otter (1), hare (3), hedgehog (1)	ST130, ST2620	t843, t10513, t3256, t4335	Lorcaric *et al.*, 2013
Switzerland	2005-2012	8000	1	Human (1)	ST130	t11150	Basset *et al.*, 2013
Belgium	2009-2011	344	3	Cattle (3)	ST130, ST2508	t1736, t9925	Vandendriesosche *et al.*, 2013
Sweden	2003 & 2011	2	2	Hedgehog (2)	ST130	t843, t5771	Monecke *et al.*, 2013

Abbreviations: CC, clonal complex; ST, sequence type; *n*, number of isolates

may be a reservoir for *mecA*$_{M10/0061}$. However, detailed systematic studies of wildlife are required to determine this.

Peterson *et al.* (2012) also reported that SCC*mec* XI MRSA are mainly associated with SSTIs and CA-MRSA and that patients carrying SCC*mec* XI MRSA are slightly older than traditional CA-MRSA patients and predominantly in rural areas (Laurent *et al.,* 2012; Peterson *et al.,* 2012; Pichon *et al.*, 2012; Basset *et al.,* 2013). In contrast, the patients from whom the two *mecA*$_{M10/0061}$ MRSA isolates were recovered in the present study were 64 and 85 years of age, respectively and are therefore older than patients in other studies and they were asymptomatic carriers. Of the two Irish CC130 isolates, one of the isolates was recovered from a rural hospital and one from an urban tertiary referral hospital.

Recently an allotype of *mecA*$_{M10/0061}$ was identified in *Staphylococcus xylosus* (Harrison *et al.,* 2013). This allotype exhibited 93.5% nucleotide sequence identity to *mecA*$_{M10/0061}$, and 69.9% identity to *mecA.* The isolate did not exhibit resistance to oxacillin and cefoxitin, due to a frame shift mutation near the 5' end of the *mec* gene that encodes a truncated protein. This *mecA* allotype was found as part of a class E *mec* complex (*mecI-mecR1-mecA*$_{M10/0061}$*-blaZ*) on a 29.4 kb SCC*mec* remnant that was integrated into *orfX* in this *S. xylosus* isolate. No *ccr* genes were identified. The *mecI, mecR1* and *blaZ* found in this strain shared 91.1%, 90.0% and 90.9% nucleotide identity, respectively, with the genes found in SCC*mec* XI. Interestingly, the arsenic resistance genes, *arsR, arsB,* and *arsC*, were also found in this isolate, but were found to be associated with a transposon located elsewhere on the chromosome. This *mec* gene and arsenic operon could be a precursor to the *mecA*$_{M10/0061}$ and SCC*mec* XI found in the present study.

The relatively small size of SCC*mec* XI (30 kb) suggests that it has the potential to become widespread among staphylococci. The prevalence of *mecA*$_{M10/0061}$ among human MRSA isolates in Europe has been reported as ranging from 0.2-6% (Cuny *et al.*, 2011;

Pichon *et al.*, 2012; Stegger *et al.*, 2012; Peterson et *al.*, 2013). Denmark has reported the highest prevalence rate among human isolates (5.9%) (Steger *et al.*, 2012) while Switzerland has only one confirmed case (Basset *et al.*, 2013). However, MRSA strains harbouring SCC*mec* XI may be even more widespread than is currently known because of the problems associated with detecting $mecA_{M10/0061}$. They often exhibit low-level resistance or even susceptibility to oxacillin (Garcia-Alvarez *et al.*, 2011; Paterson *et al.*, 2012; Peterson *et al.*, 2012; Pichon *et al.*, 2012; Robb *et al.*, 2012; Sabat *et al.*, 2012; Unnerstad *et al.*, 2012; Walther *et al.*, 2012; Lunatic *et al.*, 2013; Medhau *et al.*, 2013; Monecke *et al.*, 2013). Commercial kits for the production of PBP2a can give variable results (Cuny *et al.*, 2011; Garcia-Alvarez *et al.*, 2011; Basset *et al.*, 2013; Monecke *et al.*, 2013) and this could be due to the differences in the PBP2a protein encoded by *mecA* and the new *mec* gene (Kim *et al.*, 2012). Chromogenic media for the detection of MRSA has also been shown not to be reliable for the detection of SCC*mec* XI MRSA (Cuny *et al.*, 2011; Basset *et al.*, 2013) and an evaluation of chromogenic media for detecting SCC*mec* XI MRSA is needed so that they do not go undetected. Several rapid MRSA assays also fail to detect SCC*mec* XI MRSA (Laurent *et al.*, 2012; Sabat *et al.*, 2012; Basset *et al.*, 2013). Collaborators in Germany tested the two Irish SCC*mec* XI MRSA using the GeneXpert real-time PCR assay for the rapid detection of MRSA and both isolates were identified as MSSA (data not shown). New multiplex PCRs have been developed to detect this new *mec* gene, together with detection of *mecA*, *spa* and *pvl* (Cuny *et al.*, 2011; Pinchon *et al.*, 2012; Stegger *et al.*, 2012; Becker *et al.*, 2013). Since the completion of the present study only one additional MRSA isolate from a human has been found to carry SCC*mec* XI in Ireland (Grainne Brennan, NMRSARL, personal communication). Whether the low numbers of SCC*mec* XI MRSA reported in Ireland is due to a low prevalence or to problems associated with the detection of these isolates remains to be determined. Systematic studies of MRSA from humans and animals, particularly in rural areas of

Ireland are required in order to determine the true prevalence of MRSA carrying SCC*mec* XI in Ireland.

A recent publication by the IWG-SCC indicated that any *mec* gene homologue exhibiting less than 70% nucleotide sequence identity to any previously described *mec* gene should be designated a new *mec* gene type. This has resulted in the redesignation of $mecA_{M10/0061}$ as *mecC* (Ito *et al.*, 2012) and the *mec* allotype reported in *M. caseolyticus* as *mecB* (Ito *et al.*, 2012). In addition, *mec* genes exhibiting 70 to 95% nucleotide sequence identity to an existing *mec* gene, are classified as *mec* gene allotypes and are assigned a numerical designation after the appropriate gene name e.g. *mecA1* (*S. sciuri*), *mecA2* (*S. vitulinus*), share 79% and 91% identity, respectively, to *mecA* and *mecC1* (*S. xylosus*) 93.5% identity to *mecC* (Cunto *et al.*, 1996; Schnellman *et al.*, 2006; Harrison *et al.*, 2013). A *mec* genes exhibiting ≥95% nucleotide sequence identity to a *mec* gene allotype are assigned to that allotype but, using this nomenclature no designation is assigned to differentiate these. A recent study performed by Monecke *et al.*, (2012) revealed that there are 32 *mecA* alleles in Genbank all with >95% identity to *mecA* in *S. aurues* and CoNS. Isolates carrying these *mecA* alleles exhibitd varied resistance levels to β-lactams, and the alleles that did not confer β-lactam resistance were mainly found in CoNS (Monecke *et al.*, 2012).

The identification of the *mecC* gene in MRSA that is not detected by *mecA* PCR, a DNA microarray, and the GeneXpert real-time PCR assay is alarming. The problems with the detection of *mecC* MRSA by chromogenic media and rapid detection assays could mean there are more *mecC* MRSA undetected in Ireland. With regard to infection prevention and therapy, isolates harbouring this SCC*mec* element should be treated as MRSA even though they are identified by PCR as *mecA*-negative. Therefore, isolates in which unexplained resistance phenotypes are detected, such as methicillin resistance in apparently *mecA*-negative *S. aureus*, should undergo PCR for *mecC* and should be

submitted to specialised laboratories for detailed analysis. Routine procedures for MRSA screening need to be re-evaluated and phenotypic approaches such as the use of chromogenic media should not be abandoned. The very significant differences between SCC*mec* XI and previously described SCC*mec* elements suggests that SCC*mec* XI may have originated in another species or genus and is further evidence to support the theory that interspecies transfer of SCC*mec* may play a role in the emergence of novel MRSA strains. The associated of these strains with an animal source also highlight the increasing importance of zoonotic transfer of MRSA.

Chapter 4

Staphylococcus epidermidis ccrAB4 and additional SCC-CI associated genes in MRSA

4.1 Introduction

Staphylococcal cassette chromosome *mec* (SCC*mec*) elements are mobile genetic elements that carry the *mec* gene complex encoding resistance to almost all beta lactam antibiotics and the *ccr* genes necessary for precise integration and excision of the cassette from the staphylococcal chromosome. However, a range of atypical SCC*mec* elements have also been described in *S. aureus* and other staphylococci including (i) SCC elements that lack *mecA* but carry the *ccr* genes, (ii) pseudo SCC*mec* (ψSCC*mec*) elements that carry a *mec* gene complex but lack *ccr,* and (iii) pseudo SCC (ψSCC) elements that lack *ccr* and *mec* genes (Shore and Coleman, 2013). Many of these elements harbour additional resistance genes and in some instances virulence genes. Examples include, SCC*Hg* encoding resistance to mercury (Chen *et al.*, 2010), SCC*476* (SCC*fus*) encoding resistance to fusidic acid (Holden *et al.,* 2004), capsular polysaccharide encoded by SCC*cap1* (Luong *et al.*, 2002) and the *arc* genes enocding an arginine deiminase pathway carried by ACME (Diep *et al.,* 2006; Shore *et al.*, 2011; Bartels *et al.,* 2011). All of these elements share several common features including insertion into the same chromosomal site (*orfX*), flanking direct repeat sequences (DRs) and the use of the SCC-associated *ccr* genes for mobility (IWG, 2009).

Multiple SCC*mec* and atypical SCC*mec* elements can accumulate in the staphylococcal chromosome to form SCC composite islands (CIs) which consist of two or more SCC, SCC*mec* or pseudo SCC/SCC*mec* elements differentiated by multiple DRs flanking the individual elements (Table 4.1). A small number of SCC-CIs ranging in size from 45 to 67 kb have been described in *S. aureus* (Table 4.1); e.g. a 46 kb CI that consisted of an SCC*mec* element (SCC*mec* IVh) and two pseudo SCC elements (ΔJ1 SCC*mec* I and ψSCC*arc*) reported previously in Irish ST22-MRSA-IV isolates (Shore *et al.*, 2011). Just two SCC-CIs have been fully sequenced in CoNS, one from *S.*

Table 4.1 Examples of fully sequenced SCC composite islands (CIs) identified in staphylococci

Element type (size)	Associated staphylococcal species	Elements within CI (size)	Characteristic genes			Reference
			ccr type	*mec* complex genes	Additional antibiotic resistance or virulence genes	
ψSCC*mec*-SCC-SCC (51 kb)	*S. aureus*	ψSCC$mec_{M10/0171}$ (16 kb)	None	IS*431*, *mecA* & *ΔmecR1* & IS*431*	*fusC, copB, copC*	Kinnevey *et al.*, 2013
		$SCC_{M10/0171}$ (18 kb)	*ccrAB4*	None	None	
		SCC_{CRISPR} (17 kb)	*ccrC*	None	CRISPR	
ψSCC- ψSCC-SCC*mec* (44.5 kb)	*S. aureus*	ΔACME II (ψSCC*arc*) (12 kb)	None	None	*arc* operon	Shore *et al.*, 2011b
		ΔJ1 SCC*mec* I (9.5 kb)	None	None	None	
		SCC*mec* IVh (23 kb)	*ccrAB2*	IS*431*, *mecA*, *ΔmecR1* & IS*1272*	None	
SCC- ψSCC-SCC*mec* (50 kb)	*S. aureus*	SCC_{MI} (14 kb)	*ccrAB4*	None	None	Bartels *et al.*, 2011
		ψSCC*arc* (12 kb)	None	None	*arc* cluster	
		SCC*mec* IVa (24 kb)	*ccrAB2*	IS*431*, *mecA*, *ΔmecR1* & IS*1272*	None	
SCC*mec*-SCC (67 kb)	*S. aureus*	SCC*mec* III (35 kb)	*ccrAB3*	IS*431*, *mecA*, *mecR1* & *mecI*	*cadA, cadC. tet(K)*	Ito *et al.*, 2001
		SCC*Hg* (32 kb)	*ccrC*	None	*merA* & *merB*	

Continue overleaf

Table 4.1 continued. Examples of fully sequenced SCC composite islands (CIs) identified in staphylococci

Element type (Size)	Associated staphylococcal species	Elements within CI (size)	Characteristic genes			Reference
			ccr type	*mec* complex genes	Additional antibiotic resistance or virulence genes	
ψSCC-ψSCC-SCC-ψSCC-SCC*mec*-ψSCC-SCC*mec*- ψSCC (91 kb)	*S. haemolyticus*	ψSCC*h1*[a]	None	None	*kdp*	Takeuchi *et al.*, 2005
		ψSCC*h2*[a]	None	None	None	
		SCC*h1435*[a]	*ccrC*	None	None	
		ψSCC*h3*[a]	None	None	None	
		ψSCCmec_{h14}[a]	None	*mecA* & *ΔmecR1*	*cadD, cadX, arsC, arsB, arsR*	
		ψSCC*h4*[a]	None	None	None	
SCC- ψSCC-SCC (91 kb)	*S. epidermidis*	SCC_{pbp4} (19 kb)	*ccrAB2*	None	*pbp4, tagF*	Mongkolrattanothai *et al.*, 2004
		ACME II (34 kb)	None	None	*copA, arc* operon	
		$SCC_{ATCC12228}$ (38 kb)	*ccrAB4*	None	*copA*, mercury & cadmium resistance gene cluster	

[a] The size of the individual elements of the *S. heamolyticus* CI could not be determined, as the size of each element was not reported by Takeuchi *et al.*, 2005 and the direct repeat flanking elements are not shown in the Genbank entry (accession number AP006716).
Adapted from Shore & Coleman, 2013.

haemolyticus and one from *S. epidermidis* (Monkolrattanothai *et al.*, 2004; Takeuchi *et al.*, 2005) (Table 4.1). The SCC-CI found in *S. haemolyticus* JCSC1435 comprises six elements, including four pseudo SCCs, one pseudo SCC*mec* and one SCC element (Takeuchi *et al.*, 2005). The SCC-CI found in *S. epidermidis* ATCC 12228 is made up of three SCC elements, two carrying *ccrAB* genes and one containing the ACME-*arc* genes (Figure 4.1). The first of these SCC elements (SCC_{pbp4}) is 19 kb in size and consists of *ccrAB2* as well as the *pbp4* and *tagF* genes encoding a penicillin binding protein and a teichoic acid synthesis protein. The second SCC element ($SCC_{ATCC12228}$) is 38 kb in size and carries *ccrAB4* and gene clusters encoding resistance to the heavy metals cadmium and mercury (Monkolrattanothai *et al.*, 2004). The third SCC element (ACME) is 34 kb in size and carries the *arc* gene cluster and *copA*, the latter gene encoding a copper-transporting ATPase.

A study by Shore *et al.* (2008) identified that the majority of (a) ST8-MRSA-IIA-IIE MRSA that predominated in Irish hospitals in the 1990s, (b) ST8-MRSA-IVE/IVF found only in Northern Ireland in 1999, and (c) some GrMSSA isolates from patients in Irish hospitals, harboured the *S. epidermidis* ATCC 12228 SCC-CI *ccrAB4* gene and that in the MRSA isolates investigated this was located outside of the SCC*mec* element. This *ccrAB4* gene also showed 91% DNA sequence homology to *ccrAB4* found in SCC*mec* VI (Oliveria *et al.*, 2006), but 100% DNA sequence identity to the *ccrAB4* found in SCC*mec* VIII (Zhang *et al.*, 2009). Sequencing of the region between *ccrAB4* and the left SCC*mec* junction in one representative ST8-MRSA-IIE isolate (AR13.1/3330.2) revealed that this region exhibited 98.5% DNA sequence identity to the region between *ccrAB4* and SCC_{pbp4} in SCC-CI of *S. epidermidis* ATCC 12228 (Shore *et al.*, 2008; Monkolrattanothai *et al.*, 2004).

The complex organisation of many of these elements and CIs can cause problems for rapid MRSA detection assays that target the *orfX* integration site because a MSSA isolate harbouring an SCC element(s) but no *mecA* gene within *orfX* may be wrongly identified as a MRSA (Huletsky *et al.*, 2004; Shore *et al.*, 2008; Blanc *et al.*, 2011). Some rapid MRSA detection assays target the DNA sequence in the chromosomal *orfX*-SCC*mec* junction site (Rossney *et al.*, 2008), so the insertion of a SCC or pseudo SCC element or CI into this region in MSSA could lead to false-positive identification as MRSA using rapid MRSA detection methods. In addition, the presence of uncharacterised CIs in MRSA can compromise SCC*mec* typing, which is routinely used for genotyping MRSA. For example, if an isolate harbours multiple *ccr* genes within a CI, SCC*mec* typing may detect the multiple *ccr* genes but it will be not possible to determine from the SCC*mec* typing results which is *ccr* genes are associated with the SCC*mec* element without further detailed investigations. Therefore, for effective tracking and epidemiological typing of MRSA, and in order to enhance our understanding of the evolution of MRSA, it is essential that the genetic organisation of SCC-CIs are determined, ideally by sequencing of the entire element, so that detection of these elements can be incorporated into SCC*mec* typing and rapid MRSA assays.

The purpose of this part of the present study was to extend the work of Shore *et al.*, (2008) by comprehensively characterising the region surrounding *ccrAB4* in Irish MRSA isolates and to determine the similarity between any CIs identified and the *S. epidermidis* SCC-CI element originally described in *S. epidermidis* ATCC 12228.

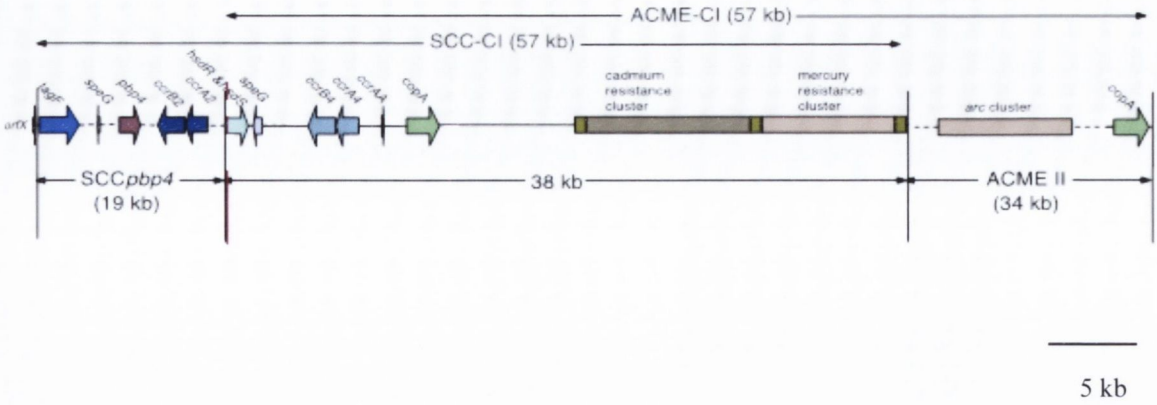

Figure. 4.1. Schematic diagram showing the genomic organisation of the ACME and SCC-CI previously reported in *S. epidermidis* ATCC 12228 (NC004461) (Zhang *et al.*, 2003).

4.2 Material and Methods

4.2.1 *S. aureus* isolates investigated

Eighty-five *S. aureus* isolates were investigated by PCR for the presence of the allelic variant of *ccrAB4* that was initially identified in a SCC-CI element in *S. epidermidis* strain ATCC 12228 (GenBank accession number BK0001539) (Table 4.2). This included 54 ST8-MRSA-IIA-IIE and 20 ST8-MRSA-IVE-IVF isolates recovered from patients in Irish hospitals between 1989 and 2002 (Shore *et al.*, 2005) (Table 4.2). These isolates were selected for investigation because a preliminary study identified the *S. epidermidis ccrAB4* genes in isolates representative of these genotypes (Shore *et al.*, 2008). Nine additional ST8-MRSA-IIA-IIE isolates were also investigated, including two (M07/0318 and M08/0462) that were identified as part of a study of MRSA on four wards of one Dublin hospital during two six-week study periods in 2007 and 2008 (Shore *et al.*, 2010) and seven collected from either patients with BSIs or from healthy carriers as described in Chapter 6, section 6.2.1 (Table 4.2). Two non-ST8-MRSA-IIA-IIE/IVE-IVF isolates (M08/0222 (ST22-MRSA-IV) and M03/0169.2 (ST94-MRSA-IV)) were also included in this part of the study because they were found to yield signals for *ccrB4* by DNA microarray profiling which is indicative of the presence of *S. epidermidis ccrAB4* (Table 4.2). As well as undergoing PCR for *S. epidermidis ccrAB4* genes, 48/92 isolates also underwent DNA microarray analysis (see Chapter 2, section 2.5.4) as part of ongoing studies in this laboratory, which includes detection of *ccrAB4* and ACME (Table 4.2).

4.2.2 *ccrAB4* and SCC-CI PCRs

The *S. epidermidis ccrAB4* gene was amplified using previously described PCR conditions and primers (Shore *et al.*, 2008) (Table 4.3). Additional PCRs were performed on one isolate, AR13.1/3330.2, in order to try to characterise the entire SCC element surrounding *ccrAB4* using the primers and conditions listed in Table 4.3 and either GoTaq

DNA polymerase (Promega) or the Expand Long Template PCR System (Roche), as described in Chapter 2, section 2.5.2. The *S. epidermidis* strain ATCC 12228 was used as a positive control for *ccrAB4* and SCC-CI related PCRs.

4.2.3 Whole-genome sequencing

One isolate, AR13.1/3330.2, underwent whole-genome sequencing as it was representative of the most common genotype that harboured *S. epidermidis ccrAB4* (ST8-MRSA-IIA-IIE, *spa* type t190) and that previously underwent nucleotide sequencing of the SCC*mec* element and the region between SCC*mec* and *ccrAB4* (Shore *et al.*, 2005 and 2008).

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 40X. The reads were assembled into contigs using a Velvet *de novo* genome assembler (version 1.0.15; Illumina). Contigs were analysed using the Artemis DNA sequence viewer and annotation tool (Rutherford K, *et al.*, 2000) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Contigs that contained SCC-associated DNA sequences were identified by using BioNumerics (version 5.1) and DNA strider (version 1.3f11) software. Any gaps found between the SCC-related contigs were closed by primer walking based on the surrounding contigs, and using the Expand long-template PCR system (Roche). PCR amplimers were sequenced by Geneservice (Chapter 2, section 2.5.2) and analysed using the DNA strider and NCBI BLAST software packages.

4.2.4 Confirmation of the genetic organisation and location of *ccrAB4*

Using four overlapping primers pairs (Table 4.4) based on the whole-genome sequencing of AR13.1/3330.2, the location and genetic organisation of the *ccrAB4* region

Table 4.2. Details of the 85 *Staphylococcus aureus* isolates investigated for the presence of the *Staphylococcus epidermidis* SCC-CI *ccrAB4* gene

Source	ST-SCC*mec* [a]	Year of isolation	Isolate no.[b]	AR type (AR pattern)[c]	*ccrAB4* by PCR	PFT[d]	*spa* type[e]	Reference
Patients in Irish hospital between 1989 and 2002	**ST8-MRSA-IIB**	**1989**	**0.1345**	**05.5 (ApCdErFdKnMcNmPmaSmSpTbTe)**	**Pos**	**00002**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIA	1993	0132	13 (Ak^{1}ApCdCpEbErGnKnLnMcNmPmaSmSpTb)	Pos	00002	t190	Shore *et al.*, 2005
	ST8-MRSA-IIA	1993	0055	13 (Ak^{1}ApCdCpEbErGnKnLnMcNmPmaSmSpTb)	Pos	00072	t190	Shore *et al.*, 2005
	ST8-MRSA-IIA	**1993**	**0236**	**13.1(Ak^{1}ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00002**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIA	**1993**	**0066**	**13.1(Ak^{1}ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00002**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIC	**1993**	**0246**	**14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)**	**Pos**	**00002**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIA	1993	0298	14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)	Pos	00002	t190	Shore *et al.*, 2005
	ST8-MRSA-IIA	1993	0225	14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)	Pos	00002	t190	Shore *et al.*, 2005
	ST8-MRSA-IIA	**1993**	**0078**	**14.2 (Ak^{1}ApCdCpEbErGnKnMcMpNmPmaSmSpTb)**	**Pos**	**00013**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1998**	**2179**	**13.1 (Ak^{1}ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00026**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIA	**1998**	**2141**	**13.1 (Ak^{1}ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00001**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1998**	**2142**	**13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00015**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1998	2113	13 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Neg	00027	t190	Shore *et al.*, 2005
	ST8-MRSA-IIC	**1998**	**2027**	**14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)**	**Pos**	**00020**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIE	**1998**	**2175**	**14.2 (Ak^{1}ApCdCpEbErGnKnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00022**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIC	**1998**	**2188**	**14.4 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)**	**Pos**	**00048**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIC	**1998**	**2080**	**14.4 (Ak^{1}ApCdCpEbErFd^{1}GnKnMcMpNmPmaSmSpTbTp)**	**Pos**	**00040**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1999**	**3144**	**13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00022**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1999**	**3442**	**New03 (ApCdCpEbErGnKnLnMcMp^{1}PmaSpTb)**	**Pos**	**00027**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1999**	**3686**	**New03 (ApCdCpEbErGnKnLnMcMp^{1}PmaSpTb)**	**Pos**	**00002**	**t2196**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1999	3137	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00049	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3008	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00025	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3053	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00027	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	**1999**	**3114**	**New01 (Ak^{1}ApCdCpEbErGnKnLnMcMp^{1}PmaSpTb)**	**Pos**	**00052**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1999	3698	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00023	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3635.2	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00080	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3243.2	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00017	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3596	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00051	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3017	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00027	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3439	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00051	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	**1999**	**3317.2**	**13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00071**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1999	3636.2	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00080	t190	Shore *et al.*, 2005
	ST8-MRSA-IIE	**1999**	**3330.2**	**13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00002**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1999	3258	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00002	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3453	13.1 (ApCdCpEbErGnKnLnMcMp^{1}NmPmaSmSpTb)	Pos	00004	t190	Shore *et al.*, 2005
	ST8-MRSA-IIC	1999	3045	14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)	Neg	00176	t190	Shore *et al.*, 2005
	ST8-MRSA-IIC	1999	3146	14 (Ak^{1}ApCdCpEbErGnKnMcNmPmaSmSpTb)	Pos	00083	t190	Shore *et al.*, 2005
	ST8-MRSA-IIE	**1999**	**3225**	**14.2 (Ak^{1}ApCdCpEbErGnKnMcMp^{1}NmPmaSmSpTb)**	**Pos**	**00124**	**t190**	**Shore *et al.*, 2005**

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Table 4.2 continued. Details of the 85 *Staphylococcus aureus* isolates investigated for the presence of the *Staphylococcus epidermidis* SCC-CI *ccrAB4* gene.

Source	ST-SCC*mec* [a]	Year of isolation	Isolate no.[b]	AR type (AR pattern)[c]	*ccrAB4* by PCR	PFT[d]	*spa* type[e]	Reference
Patients in Irish hospitals between 1989 and 2002	**ST8-MRSA-IID**	**1999**	**3352**	**14.2 (Ak^{l}ApCdCpEbErGnKnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00017**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**1999**	**3132**	**14.2 (ApCdCpEbErGnKnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00055**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIC	**1999**	**3369**	**14.4 (Ak^{l}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)**	**Pos**	**00085**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIC	1999	3600	14.4 (Ak^{l}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)	Pos	00085	t190	Shore *et al.*, 2005
	ST8-MRSA-IIC	1999	3685	14.4 (Ak^{l}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)	Pos	00001	t190	Shore *et al.*, 2005
	ST8-MRSA-IIC	**1999**	**3411**	**14.4(Ak^{l}ApCdCpEbErFd^{l}GnKnMcMpNmPmaSmSp TbTp)**	**Pos**	**00040**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	1999	3176.1	14.4 (Ak^{l}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)	Pos	00002	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	1999	3448	14.4 (Ak^{l}ApCdCpEbErGnKnMcNmPmaSmSpTbTp)	Pos	00146	t190	Shore *et al.*, 2005
	ST8-MRSA-IID	**2002**	**E1140**	**13.1 (ApCdCpEbErGnKnLnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00098**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**2002**	**E1168**	**13.1 (ApCdCpEbErGnKnLnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00099**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**2002**	**E1154**	**13.1(ApCdCpEbErFdGnKnLnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00079**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIE	**2002**	**E1134**	**14.2 (ApCdCpEbErGnKnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**00103**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIE	**2002**	**E1119**	**14.4(ApCdCpEbErGnKnMcMpNmPmaSmSpTbTp)**	**Neg**	**00071**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IIE	**2002**	**E1121**	**14.4(ApCdCpEbErGnKnMcMpNmPmaSmSpTbTp)**	**Pos**	**00070**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**2002**	**E1038**	**New03 (ApCdCpEbErGnKnLnMcMp^{m}PmaSpTb)**	**Neg**	**00052**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IID	**2002**	**E0908**	**New03 (ApCdCpEbErGnKnLnMcMp^{m}PmaSpTb)**	**Neg**	**00074**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3623	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00143	t190	Shore *et al.*, 2005
	ST8-MRSA-IVF	**1999**	**3246**	**43 (ApCd^{l}CpErKnNmSmSpTp)**	**Pos**	**00140**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3310	43 (ApCd^{l}CpErKnNmSmSpTp)	Pos	00146	t190	Shore *et al.*, 2005
	ST8-MRSA-IVF	**1999**	**3402**	**43 (ApCd^{l}CpErKnNmSmSpTp)**	**Pos**	**00017**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3488	43 (ApCd^{l}CpErKnNmSmSpTp)	Pos	00146	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3635.1	43 (ApCd^{l}CpErKnNmSmSpTp)	Pos	00151	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3331	43 (ApCd^{l}CpErKnNmSmSpTp)	Pos	00067	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	**1999**	**3322**	**43 (ApCd^{l}CpErKnNmSmSpTp)**	**Pos**	**00150**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3317.1	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00141	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3316	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00143	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3625	43 (ApCd^{l}CpErKnNmSmSpTp)	Pos	00150	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	**1999**	**3330.1**	**43 (ApCd^{l}CpErKnNmSmSpTeTp)**	**Pos**	**00067**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3337	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00070	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3665	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00079	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3243.1	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00138	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	1999	3328	43 (ApCd^{l}CpErKnNmSmSpTeTp)	Pos	00125	t190	Shore *et al.*, 2005
	ST8-MRSA-IVF	**1999**	**3636.1**	**43 (ApCdCpEbErKnNmSmSpTp)**	**Pos**	**00140**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3176.2	43 (ApCpErKnNmSmSpTp)	Pos	00142	t190	Shore *et al.*, 2005
	ST8-MRSA-IVE	**1999**	**3487**	**43 (ApCpErKnNmSmSpTeTp)**	**Pos**	**00149**	**t190**	**Shore *et al.*, 2005**
	ST8-MRSA-IVE	1999	3094	43 (ApCpErKnNmSmSpTeTp)	Pos	00136	t190	Shore *et al.*, 2005

Continued overleaf

Table 4.2 continued. Details of the 85 *Staphylococcus aureus* isolates investigated for the presence of the *Staphylococcus epidermidis* SCC-CI *ccrAB4* gene.

Source	ST-SCC*mec* [a]	Year of isolation	Isolate no.[b]	AR type (AR pattern)[c]	*ccrAB4* by PCR	PFT[d]	*spa* type[e]	Reference
Patient and environmental sites in four wards in an Irish hospital in 2007 and 2008	ST8-MRSA-IIE	2007	M07/0318 (E)	New03 (ApCdEbErGnKnLnMcMp^{m}PmaSpTb)	Pos	00041	t037	Shore *et al.*, 2008
	ST8-MRSA-IID	**2008**	**M08/0462**	**AR14.4 (ApCdCpEbErGnKnMcNmPmaSmSpTbTp)**	**Pos**	**01156**	**t190**	**Shore *et al.*, 2010**
Patients with bloodstream infection in Irish hospitals	**ST8-MRSA-IIA**	**2006**	**E3017**	**13.1 (ApCdCpEbErGnKnLnMcNmPmaSmTbSp)**	**Pos**	**00002**	**t190**	**Grundmann *et al.*, 2010**
	ST8-MRSA-IIE	**2006**	**E3110**	**14.1 (ApCdCpEbErGnKnMcNmPmaSmSpTbTp)**	**Pos**	**00055**	**t190**	**Grundmann *et al.* ,2010**
	ST8-MRSA-IIE	**2006**	**E3150**	**14.1 (ApCdCpEbErGnKnMcNmPma SmSpTb)**	**Pos**	**00071**	**t190**	**Grundmann *et al.*, 2010**
	ST8-MRSA-IIC	**2006**	**E3166**	**14.1 (ApCdCpEbErGnKnMcNmPmaSmSpTbTp)**	**Pos**	**00071**	**t190**	**Grundmann *et al.*, 2010**
Healthcare staff nasal carriage in one Irish hospital	**ST8-MRSA-IID**	**2003**	**M02/0047**	**13.1 (ApCdCpEbErGnKnLnMcMp^{l}NmPmaSmSpTb)**	**Pos**	**n/d**	**t190**	**This Study**
	ST8-MRSA-IID	**2003**	**M04/0287**	**New03 (ApCdEbErGnKnLnMcMp^{l}PmaSpTb)**	**Pos**	**n/d**	**t190**	**This Study**
	ST8-MRSA-IID	**2003**	**M04/0295**	**New03 (ApCdEbErGnKnLnMcMp^{l}PmaSpTb)**	**Pos**	**n/d**	**t190**	**This Study**
Patient and environmental sites in four wards in an Irish hospital in2007 and 2008	**ST22-MRSA-IVh**	**2008**	**M08/0222 (E)**	**06.5 (ApCdCpEr)**	**Pos**	**00080**	**t190**	**Shore *et al.*, 2010**
Patients in Irish hospitals between 1989 and 2002	**ST94-MRSA-IVg**	**2003**	**M03/0169.2**	**Unf (Ak^{l}ApCdGnKnMcNmSmTbTe)**	**Pos**	**99940**	**t032**	**Shore *et al.*, 2005**

[a] The 8 isolates highlighted in blue were selected as representatives of each ST and SCC*mec* type combination identified as harbouring the *S. epidermidis ccrAB4* gene and were further investigated for the presence of a novel SCC element.

[b] The 47 isolates highlighted in bold also underwent DNA microarray analysis as part of a previous study (Brennan, 2013) using the StaphyType Kit (Alere Technologies GmbH, Jena, Germany). The DNA microarray includes detection of the *S. aureus ccrAB4* and the results obtained corresponded to those obtained by PCR amplification of *ccrAB4*; i.e. isolates that were negative for *ccrAB4* by PCR were also negative for *ccrAB4* by DNA microarray profiling and isolates that were positive for *ccrAB4* by PCR yielded positive or ambiguous results for *ccrAB4* by DNA microarry profiling.

[c] The antibiogram-resistogram (AR) pattern of each isolate was determined by AR typing which involves determining an isolates susceptibility to a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadimium nitrate (Cd), chloramphenicol, ciprofloxacin (Cp), ethidium bromide (Eb), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride (Mc), mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate (Pma), rifampicin, streptomycin (Sm), spectiomycin (Sp), sulphonamide (Su), tobramycin (Tb), tetracycline (Te), trimethoprim (Tp) and Vancomycin.

[d] Each pulsed-field gel electrophoresis (PFGE) profile was assigned a 5-digit pulsed field type (PFT) and rPFTs that differ by ≤ 6 bands are assigned blocks of 1000 numbers starting at 00001. Therefore, a particular series of related PFTs are in the 00001-00999 group and the next group of related PFTs are in the group 01000-01999.

[e] *spa* typing was performed as part of a previous study (Brennan, 2013).

Abbreviations; n/d, not determined; Unf, unfamiliar AR pattern or variant of a known AR pattern; PFT, pulsed-field type as determined by PFGE; I, intermediate resistance; E, environmental isolate (all other isolates are from patients).

Table 4.3. Primers used to amplify the *S. epidermidis ccrAB4* gene and the entire *S. epidermidis* SCC-CI in MRSA isolate AR13.1/3330.2

Primer application	Primer pair	Nucleotide sequence (5'-3')[a]	Thermal cycling conditions	Nucleotide coordinates[b]	Region amplified	Expected amplimer size
ccrAB4	α4.3 β4.3	**AGCGTATGAATCAAAA** **CGATGACAAATTAAAAT**	94°C for 2 min; 30 cycles of 94°C for 2 min, 48°C for 1 min, and 72°C for 2 min and a final extension for 10 min at 72°C	27155-27170 27907-27891	*ccrAB4*	752 bp
SCC-CI amplification	SCC-CI F3 α4.3	**GCGATAATCGGTTCCTCATT** **CGATGACAAATTAAAAT**	94°C for 2 min; 30 cycles of 94°C for 2 min, 55°C for 1 min, and 72°C for 1.5 min and a final extension for 10 min at 72°C	26957-26976 27907-27891	*ccrAB4* to within the previously sequenced 7-kb region between SCC*mec* IID and SCC-CI	950 bp
	SCC-CI F1 SCC-CI R1	GCTTCTTCGAATCTGCAAATC ATCACGACGGTCCAACGTG	92°C for 2 min; 10 cycles of 92°C for 10 s, 48°C for 30 s, and 68°C for 9 min; 20 cycles of 92°C for 10 s, 48°C for 30 s, and 68°C for 10 min.	27566-27587 34372-34352	*ccrAB4* to upstream of the cadmium resistance operon (*CadR*)	6806 bp
	SCC-CI F2 SCC-CI R2	CCTTATTGTATCAGTAATATC CAAAGCCAGTACTGCAGAG	92°C for 2 min; 10 cycles of 92°C for 10 s, 50°C for 30 s, and 68°C for 9 min; 20 cycles of 92°C for 10 s, 50°C for 30 s, and 68°C for 10 min	34216-34236 40393-40375	Upstream of *CadR* to within *CadR*	6177 bp
	SCC-CI F4 SCC-CI R4	GCTATGAAACACATGAGGAT TCTTTGATTGTACGTGTGAGC	92°C for 2 min; 10 cycles of 92°C for 10 s, 45°C for 30 s, and 68°C for 9 min; 20 cycles of 92°C for 10 s, 45°C for 30 s, and 68°C for 10 min.	40140-40159 48078-48058	*CadR* to upstream of the mercury resistance operon (*MerR*)	7938 bp

Continued overleaf

Table 4.3 continued. Primers used to amplify the *S. epidermidis ccrAB4* gene and the entire *S. epidermidis* SCC-CI in MRSA isolate AR13.1/3330.2

Primer application	Primer pair	Nucleotide sequence (5'-3')[a]	Thermal cycling conditions	Nucleotide coordinates[b]	Region amplified	Expected amplimer size
	SCC-CI F7 SCC-CI R7	GTACATCCGCACTCGAATTA AAAAACCGCATCATTTATGA	92°C for 2 min; 10 cycles of 92°C for 10 s, 47°C for 30 s, and 68°C for 9 min; 20 cycles of 92°C for 10 s, 47°C for 30 s, and 68°C for 10 min.	53495-53514 57474-57455	Within *MerR* to end of SCC-CI	3979 bp
	SCC-CI F8 SCC-CI R8	**CTTGTGTTTGTTGCTGTATT** **CTTCAACCGTTGCTTCTCCG**	94°C for 2 min; 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; and a final extension for 10 min at 72°C	40274-40293 41076-41057	Internal region of *CadR*	802 bp
	merAB-F1 merAB-R1	**CCATTCAAGGCATGACATG** **GTTCAAAGGCATCTTCAACG**	94°C for 2 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min; and a final extension for 10 min at 72°C	52802-52820 55084-55065	Internal region of *MerR*	2282 bp

[a] PCRs using primers highlighted in bold were performed using GoTaq DNA polymerase (Promega) (as described in Chapter two, section 2.5.2). PCR using all other primer pairs were performed using the Expand long template system (Roche) (as described in Chapter 2, section 2.5.2).

[b] Nucleotide coordinates based on the nucleotide sequence SCC-CI from *S. epidermidis* strain ATCC 12228 (GenBank accession number BK0001539).

Table 4.4. Novel PCR primers used to characterise novel composite islands

Gene or region amplified	Primer pair	Nucleotide sequence (5'-3')	Nucleotide coordinates	Product Size
J1 SCC*mec* IIE to the end of the *ccrAB4*-carrying element	J1_IIE_F3	CAAGTTATCGGATAATGAA	184-166[a]	4820 bp
	Contig 101 R2	GCCAAGCAATTATCATTATG	4636-4617[b]	
	Contig 101 F2	CCTCGTCTTGAATTTCTTTC	4598-4617[b]	2607 bp
	ccrA4R5	CAATATCGTTGAACAATCAC	7205-7186[b]	
	ccrA4F5	CTTCATTCCTTTCAGGAGTT	7159-7178[b]	2401 bp
	M1_R2	AACATGCTTAACGTGAAGTG	9560-9541[b]	
	M1_F2	TAGGAGCACACTTACACAAG	9498-9517[b]	4435 bp
	M1_R4	CAGCGTATCGTTTTATACTG	13933-13914[b]	
J1 SCC*mec* IVc and IVE to within adjacent *ccrAB4*-carrying element	J1_IVc_F2	GACAGTATTCCAACAGTCAA	84-65[c]	4720 bp
	Contig 101 R2	GCCAAGCAATTATCATTATG	4636-4617[b]	
J1 SCC*mec* IVb and IVF to within adjacent *ccrAB4*-carrying element	J1_IVb_F2	ACTTAAGTAGTAGCTAACAG	172-153[d]	4808 bp
	Contig 101 R2	GCCAAGCAATTATCATTATG	4636-4617[b]	
J1 SCC*mec* IVg to within adjacent *ccrAB4*-carrying element	J1_IVg_F1	CGTGATAGCGACACAATAC	133-115[e]	4769 bp
	Contig 101 R2	GCCAAGCAATTATCATTATG	4636-4617[b]	
SCC_{MI}-ACME	ccrAB4-F1	AGTCGGTTCAAGCGATAGCA	8282-82487[f]	8109 bp
	arc-R1	GCTCCCAAAAGTTGAAGCTG	16391-16371[f]	
ACME-SCC*mec* IV	arcA-F1	GTTTGAGCAAATTTGTCATG	21547-21566[f]	9523 bp
	dcs-R1	CTAAATCATAGCCATGACCG	37670-37689[g]	

Nucleotide coordinates based on the nucleotide sequences of [a]SCC*mec* IIE (GenBank accession number AJ810120.1), [b]SCC_{MI} of AR13.1/3330.2 (HE858191.1), [c]SCC*mec* IVc (AB096217.1), [d]SCC*mec* IVb (AB063173.1), [e]SCC*mec* IVg (DQ106887.1), [f]SCC_{MI}-SCC_{arc}-SCC*mec* (HM030720) and [g] SCC*mec* type I (Accession no. AB033763).

on a SCC element adjacent to SCC*mec* in AR13.1/3330.2 was confirmed. PCRs were performed using GoTaq (Promega) or the Expand Long-Template PCR system (Roche) as described in Chapter 2 (section 2.5.2). The sizes of amplicons obtained were compared to the expected size of amplicons based on the whole-genome sequence.

Among the MRSA, one isolate representative of each ST and SCC*mec* type combination identified as harbouring the *S. epidermidis ccrAB4* gene was chosen for further investigation of the presence of the SCC element including ST8-MRSA-IIA (AR13/0132), ST8-MRSA-IIB (AR05/1345), ST8-MRSA-IIC (AR14/0246), ST8-MRSA-IID (AR13/3698), ST8-MRSA-IVE (AR43/3330.1), ST8-MRSA-IVF (AR43/3246), ST94-MRSA-IVg (M03/0169.2) and ST22-MRSA-IVh (M08/0222). For the amplification of the *ccrAB4*-SCC element, in all except the ST22-MRSA-IV isolate, the primers were as described in Table 4.4 for AR13.1/3330.2; i.e. the primers used to amplify the J1 region of SCC*mec* IIE to the end of the *ccrAB4*-carrying element, except that primers J1_IVc_F2, J1_IVb_F2 and J1_IVg_F1 were used instead of primer J1_IIE_F3 for isolates harbouring SCC*mec* types IVE, IVF and IVg, respectively (Table 4.4). The ST22-MRSA-IV isolate, M08/0222, was also found to harbour ACME using the DNA microarray and two sets of primers (ccrAB4-F1 & arc-R1 and arcA-F1 & dcs-R1) were designed to confirm the presence of an SCC_{M1}-SCC_{arc}-SCC*mec* element that was identified in the Danish ST8 MRSA isolate M1 (*spa* type t024) during the course of the present study (Bartels *et al.*, 2011) (Table 4.4). All PCR products were visualised by agarose gel electrophoresis and the sizes of amplicons obtained were compared to the expected size of amplicons based on the whole-genome sequence.

4.2.5 Nucleotide sequence accession numbers

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

4.3 Results

4.3.1 Detection of *ccrAB4* in MRSA and GrMSSA by PCR and DNA microarray analysis

The majority (80/85; 94%) of isolates investigated were positive for *S. epidermidis ccrAB4* by PCR (Table 4.2). The *ccrAB4*-positive isolates included 92% (58/63) of the ST8-MRSA-IIA-IIE isolates, all ST8-MRSA-IVE/IVF isolates (n = 20), the ST22-MRSA-IVh isolate, and the ST94-MRSA-IVg isolate (Table 4.2). Fifty-five percent of these isolates (47/85) were also investigated by DNA microarray profiling and all yielded positive or ambiguous signals for *ccrA4* and/or *ccrB4* (Table 4.2). All but one isolate, M08/0222 (ST22-MRSA-IVh), investigated using the DNA microarray were negative for the ACME-*arc* genes.

4.3.2 Detection of a novel SCC*mec*-SCC composite island in ST8-MRSA-IIE isolate AR13.1/3330.2.

One isolate, AR13.1/3330.2 (ST8-MRSA-IIE, t190), representative of the most common genotype that harboured *S. epidermidis ccrAB4*, underwent detailed PCR amplification and sequencing in order to comprehensively characterise the entire SCC element surrounding *ccrAB4*. Shore *et al.* (2008) previously identified that this isolate harboured a 5.5 kb DNA region, located between *ccrAB4* and the left SCC*mec* junction, with 98.5% DNA sequence identity to the region between *ccrAB4 and* SCC_{pbp4} in SCC-CI of *S. epidermidis* (Figure 4.2). Primers were designed based on the *S. epidermidis* SCC-CI element to determine how much of the SCC-CI was present in AR13.1/3330.2 (Table 4.3 and Figure 4.2). Amplification of the region from *ccrAB4* to upstream of the cadmium resistance operon (*cadR*) resulted in an amplimer of the expected size in AR13.1/3330.2 (Figure 4.2). However, repeated attempts to amplify the remainder of the SCC-CI element in AR13.1/33302 failed to yield any amplimers, while the positive control *S. epidermidis*

strain ATCC 12228 yielded the expected size amplimers with each primer pair. Since no amplimers were obtained for AR13.1/3330.2, the isolate underwent whole-genome sequencing in order to determine if a novel SCC element was present in this isolate.

Whole-genome sequencing of AR13.1/3330.2 yielded 344 contigs ranging in size from ca. 113 bp to 137 kb. Three contigs were identified as containing SCC-associated DNA sequences (13 kb, 11 kb and 764 bp in size). The gaps between each of the contigs were closed by designing primers based on the surrounding contigs and using long-range PCR amplification and sequencing. The order of the contigs were also confirmed by PCR. The analysis revealed a 14-kb SCC element that is located downstream and adjacent to SCC*mec* IIE (Figure 4.3). This element showed 99% DNA sequence identity to SCC_{MI} (Figure 4.3) that was identified in the Danish ST8-MRSA M1 (*spa* type t024) by other researchers during the course of the present study (Bartels *et al.*, 2011). In MRSA strain M1, the SCC_{MI} element is part of a large composite island. It is located next to *orfX* and upstream of a truncated ACME element and SCC*mec* IVa (Figure 4.3). In contrast, in AR13/3330.2 the SCC*mec* IIE element was located adjacent to *orfX*, whereas SCC_{MI} was located downstream of SCC*mec* IIE (Figure. 4.3). The SCC_{MI} element comprises 18 ORFs that have been described previously (Bartels *et al.*, 2011), most significantly those encoding *ccrAB4*, a truncated copy of *ccrA* and a gene encoding a copper-transporting ATPase (*copA*) (Figure 4.3). SCC_{MI} also has a similar genetic organisation to (i) the region between SCC_{pbp} and IS*431* in SCC-CI in the *S. epidermidis* strain ATCC 12228 (99% DNA sequence identity) but is significantly smaller in size and is lacking the mercury and cadmium resistance genes and (ii) a region downstream of *mecA* in SCC*mec* VIII (99% DNA sequence identity). The composite island (CI) detected in AR13.1/33302 was designated SCC*mec* IIE-SCC_{MI} (Figure 4.3).

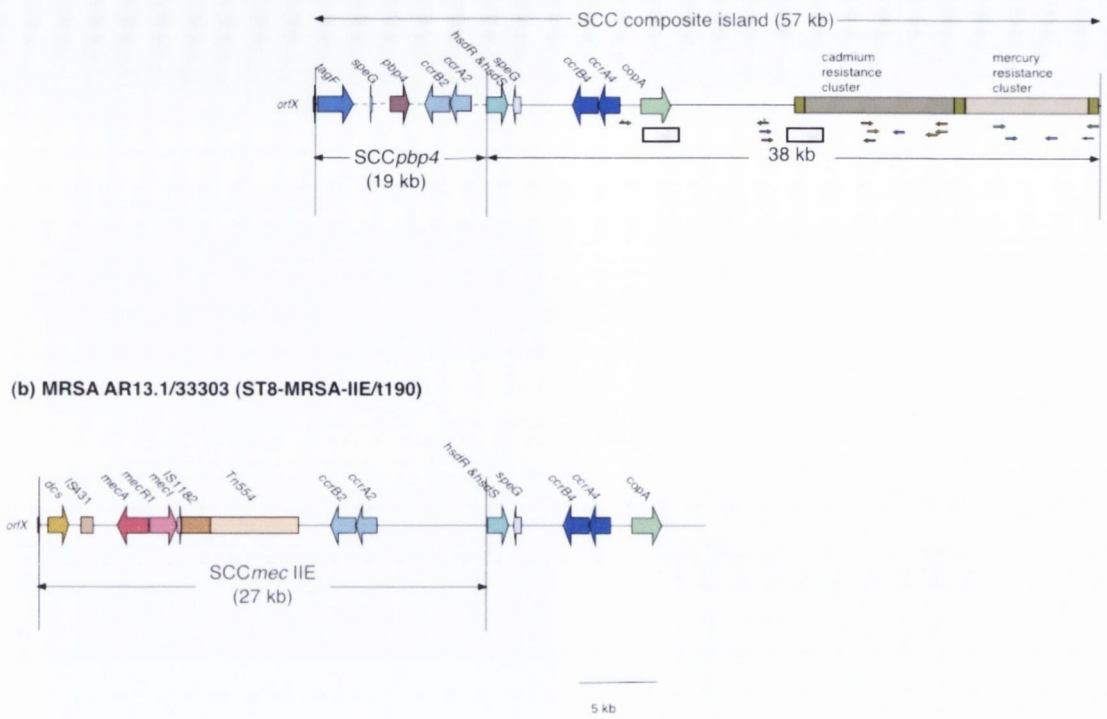

Figure 4.2. Schematic diagram showing the genetic organisation of (a) the 57 kb SCC composite island (SCC-CI) from *S. epidermidis* strain ATCC 12228 (GenBank accession number BK001539) and (b) SCC*mec* IIE from ST8-MRSA-IIE isolate AR13.1/3330.2 (Genbank accession number AJ810120), the previously described region from SCC*mec* IIE to *ccrAB4* in this isolate (Shore *et al.*, 2008) and the region upstream of *ccrAB4* identified in the present study. A previous study found that the region from SCC*mec* IIE to *ccrAB4* in MRSA isolate AR13.1/3330.2 exhibited 98.5% to the region between SCC*pbp4* and *ccrAB4* in SCC-CI from *S. epidermidis* (Shore *et al.*, 2008). In the present study PCRs were performed on AR13.1/3330.2 with primer pairs (indicated with coloured arrows in panel (a)) based on the DNA sequence of SCC-CI from *S. epidermidis* to determine if the entire SCC-CI element was present in this MRSA isolate. The primer pair (indicated in boxes), which amplifies from *ccrAB4* to the region between *ccrAB4* and the cadmium resistance cluster, was the only primer pair which yielded an amplimer in AR13.1/3330.2. Whole-genome sequencing of AR13.1/3330.2 was performed in order to determine the genetic organisation of the entire CI in this MRSA isolate which was designated SCC*mec* IIE-SCC$_{MI}$.

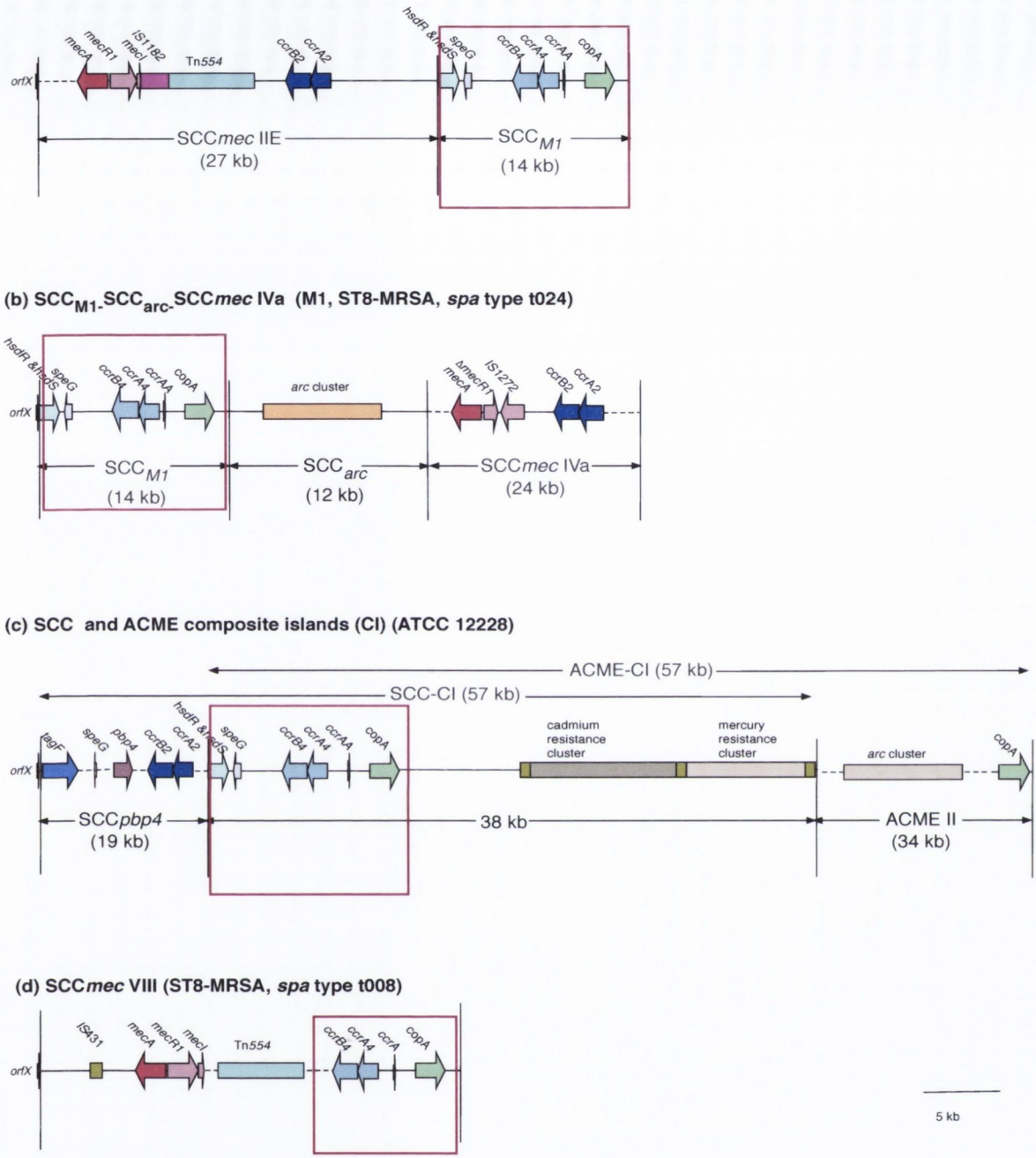

Figure. 4.3. Schematic diagram showing the genomic organisation of (a) the novel SCC*mec*-SCC composite island (CI) SCC*mec* IIE-SCC$_{MI}$ identified in the ST8-MRSA *spa* type t190 isolate AR13.1/3330.2 (Genbank accession number HE858191.1), (b) the SCC$_{MI}$-SCC$_{arc}$-SCC*mec* element previously reported in ST8-MRSA *spa* type t024 isolate M1 (HM030720) (Bartels *et al.*, 2011), and also identified in an ST22-MRSA-IVh isolate, M08/0222, in the present study by PCR, (c) the ACME- and SCC-CI previously reported in *S. epidermidis* ATCC 12228 (NC004461) (Zhang *et al.*, 2003) and (d) SCC*mec* VIII previously described in ST8-MRSA *spa* type t008 isolate C10682 (FJ390057) (Zhang *et al.*, 2009). The structure of the novel SCC*mec* IIE-SCC$_{MI}$ element was determined by high-throughput whole-genome sequencing of AR13.1/3330.2 and was confirmed using primers spanning the SCC*mec*/SCC region. The areas surrounded by the red square indicate the regions of high similarity between SCC$_{MI}$ identified in isolate AR13.1/3330.2 and the other SCC*mec*/SCC elements.

4.3.3 Confirmation of the presence of SCC_{M1} in representative CC8 isolates harbouring the *S. epidermidis ccrAB4* genes.

The presence of the SCC_{M1} element and its location downstream of SCC*mec* was investigated by PCR in seven additional isolates representative of other ST and SCC*mec* type combinations within CC8 identified as harbouring the *S. epidermidis ccrAB4*. Five of the seven isolates, including those representative of ST8-MRSA-IIA, ST8-MRSA-IIB, ST8-MRSA-IIC, ST8-MRSA-IID and ST94-MRSA-IVg, yielded amplicons of the expected size for SCC_{M1} and its location was downstream of SCC*mec* as found in isolate AR13.1/3330.2 (Figure 4.3). These SCC*mec*-SCC CIs were assigned the following designations: SCC*mec* IIA-SCC_{M1}, SCC*mec* IIB-SCC_{M1}, SCC*mec* IIC-SCC_{M1}, SCC*mec* IID-SCC_{M1} and SCC*mec* IVg-SCC_{M1}, respectively. For the remaining two isolates (ST8-MRSA-IVE and ST8-MRSA-IVF) the primer pairs located within the SCC_{M1} element (primer pairs contig101-F2 & ccrA4-R5, ccrA4-F5 & M1-R2 and M1-F2 & M1-R4, Table 4.3) yielded amplicons of the expected size but those amplifying from the respective SCC*mec* elements to within the SCC_{M1} element (primer pairs J1-IVc-F2 & contig101-R2 and J1-IVb-F2 & contig101-R2, Table 4.3) failed to yield any amplicons. These findings indicated that while the SCC_{M1} element is present in these ST8-MRSA isolates harbouring SCC*mec* IVE and IVF, further investigations are required to determine the genetic organisation of the SCC*mec* and SCC_{M1} elements in these isolates.

4.3.4 Investigation of the genetic organisation of ACME and *ccrAB4* in a ST22-MRSA-IVh isolate.

The ST22-MRSA-IVh isolate M08/0222 yielded the expected size amplimers using primers designed to amplify from the SCC_{M1} element containing *ccrAB4* to the truncated ACME element and from ACME to the J3 region of SCC*mec* IV, as was reported previously in the Danish ST8-MRSA strain M1 (Figure 4.3). The only difference between

these two CIs is the presence of SCC*mec* IVa in strain M1 and IVh in M08/0222. The CI in the latter isolate was designated SCC_{M1}-SCC_{arc}-SCC*mec* IVh.

4.4 Discussion

In the present study PCR and whole-genome sequencing were used to characterise nine novel SCC*mec*-SCC-CIs, ranging in size from 37-54 kb in MRSA isolates belonging to two distinct pandemic lineages, CC8 and CC22. Each CI consisted of a SCC*mec* II or IV element (IIA-IIE, IVE/IVF or IVg in CC8 isolates and IVh in a CC22 isolate) and an adjacent SCC_{MI} element harbouring *ccrAB4*, with one isolate (ST22-MRSA-IVh) also harbouring a truncated ACME element (SCC*arc*). The SCC_{MI} element was reported by other researchers during the course of the present study in an ST8-MRSA-IVa *spa* type t024 strain M1 in Denmark, as part of a CI with SCC*arc* and SCC*mec* IVa (Bartels *et al.*, 2011). While the CI identified in the ST22 isolate was very similar to the SCC_{MI}-SCC*arc*-SCC*mec* IVa element in strain M1 it was found in association with a different SCC*mec* IV subtype (IVh) and this is the first report of such a large CI in a CC22 isolate. In addition the ST22 SCC_{MI}-SCC*arc*-SCC*mec* IVh element identified in the present study harboured the same ACME and SCC*mec* element as a CI previously identified in Irish ST22-MRSA-IVh isolates (Shore *et al.,* 2011) but it lacked the pseudo SCC*mec* I element and instead carried the *ccrAB4* element SCC_{MI}. In contrast, the CC8 isolates carried SCC_{MI} and a SCC*mec* element but lacked ACME and the CIs had a different genetic organisation to that in strain M1. The similarities between SCC_{MI} and the SCC-CI in *S. epidermidis* and the presence of SCC_{MI} in distinct MRSA strains as identified in the present study is further evidence of horizontal transfer and recombination of SCC elements between MRSA strains (within CC8 and between CC8 and CC22) and staphylococcal species (*S. epidermidis* and *S. aureus*).

A previous study found evidence of SCC_{MI} as part of a SCC*mec*-SCC remnant in a ST8-MSSA isolate and of a possible SCC_{MI} element in ST8 and ST5 MSSA (Shore *et al.*, 2008). Six GrMSSA isolates were found to harbour *ccrAB4* with 100% homology to SCC-CI from *S. epidermidis*. DNA sequencing of one isolate revealed that *ccrAB4* was located

5.5 kb upstream of a SCC*mec* IID remnant and the region from *ccrAB4* to the SCC*mec* IID remnant had 98.5% DNA sequence homology to the region from *ccrAB4* to SCC_{pbp4} in the SCC-CI from *S. epidermidis* (Shore *et al.*, 2008). While further PCRs are required to clarify the presence of the entire SCC_{MI} element in these MSSA isolates it is possible that these represent precursors of the MRSA with SCC*mec*-SCC_{MI} CIs prior to acqusition of SCC*mec* or they may have evolved from the MRSA strains following loss of SCC*mec*.

For two of the nine MRSA strains found to carry SCC*mec*-SCC_{MI} CIs (ST8-MRSA-IVE and ST8-MRSA-IVF) it was not possible to determine the location of SCC_{MI} in relation to SCC*mec*. It may be that in these isolates SCC*mec* IVE/IVF and SCC_{MI} have a different genetic organisation to the CIs in ST8-MRSA-IIA-IIE isolates. A previous study suggested that due to their similar genetic background i.e. ST8 and *spa* type t190, that isolates carrying SCC*mec* IVE/IVF may have emerged from those with SCC*mec* IIA-IIE following SCC*mec* recombination events (Shore *et al.*, 2005). Such recombination events may also have resulted in genomic rearrangements of the SCC*mec* and SCC_{MI} elements. Another possibility is that additional SCC elements are located between SCC*mec* IVE/IVF and SCC_{MI} in these isolates. Further investigations are required to determine the precise genetic organisation of the CI in ST8-MRSA isolates carrying SCC*mec* IVE/IVF.

The identification of SCC_{MI} in six distinct clones of MSSA and MRSA (and three lineages: CC8, CC22 and CC5) in this and previous studies (Shore *et al.*, 2008; Bartels *et al.*, 2011) suggests that at least six possible independent acquisitions of SCC_{MI} may have occurred and that it may be more widespread than is currently realised. The majority of SCC_{MI} isolates (both MRSA and MSSA) identified to date belong to CC8. Clonal complex 8 is one of the largest MRSA lineages consisting of a substantial number of MRSA strains with an extensive diversity in SCC*mec* (Enright *et al.*, 2002; Shore *et al.*, 2005; Monecke *et al.*, 2011). In the 1990's the ST8-MRSA-IIA-IIE and *spa* type t190 isolates that harboured this SCC*mec*/SCC-CI element predominated in Irish hospitals, but have since

been replaced by ST22-MRSA-IV (Shore *et al.,* 2010), and are now only reported sporadically. The decline of ST8-MRSA-IIA-IIE and the fact that just one ST22-MRSA-IVh isolate was found to harbour this CI may be due, at least in part, to a fitness cost associated with harbouring such a large genetic element. Only 7% (6/84) of ST8-MRSA-IIA-IIE isolates were found not to carry *ccrAB4.* These six isolates may have never harboured *ccrAB4*, but it is also possible that they have lost *ccrAB4*. It would be interesting to investigate if remnants of the SCC_{MI} element are present in these ST8-MRSA-IIA-IIE isolates lacking *ccrAB4.*

How widespread SCC-CIs are among *S. aureus* and CoNS has yet to be determined, due to the small number of CIs that have been fully sequenced (Table 4.1). However, evidence to date suggests that they may be more widespread, particularly among CoNS than is currently recognised. Several studies have highlighted the presence of multiple *ccr* genes in CoNS on elements that have not been fully sequenced (Ruppe *et al.*, 2009; Bouchami *et al.,* 2011; Zong *et al.,* 2011). Data from another section of the present study (Chapter 5) has revealed that 82% (46/56) of CoNS isolates investigated harboured multiple *ccr* genes (with and without *mecA*), suggesting that these CoNS may carry SCC/SCC*mec*-CIs. Complete nucleotide sequencing of these possible novel CIs is required in order to determine the genetic organisation of these complex SCC*mec* elements and CIs. Advances in whole-genome sequencing will allow us to gain a better understanding of the prevalence of these complex elements. The cost of high-throughput whole-genome sequence has significantly fallen in recent years and this will in the future represent a viable method for detailed characterisation of novel CIs.

Detailed characterisation of CIs by complete nucleotide sequencing in both MRSA and MSSA is essential for accurate SCC*mec* typing and rapid detection of MRSA. Strains carrying *ccrAB4* in addition to *ccrAB2* and a *mec* complex, as was identified in the present study, could be classified as the wrong SCC*mec* type. While it remains to be determined if

the CC5 and CC8 MSSA identified by Shore *et al.* (2008) carry the complete SCC_{MI} element, it is clear that the presence of SCC elements within *orfX* in MSSA can result in their misclassification as MRSA using some rapid detection assays (Huletsky *et al.,* 2004; Shore *et al.*, 2008; Blanc *et al.,* 2011).

The results of this study highlight the complex genetic organisation of SCC*mec* and SCC elements in staphylococci and that horizontal transfer and recombination events between SCC and SCC*mec* elements in different staphylococcal species and different strains are constantly occurring resulting in novel composite islands. This level of diversity of CIs in MRSA has not been reported previously and complete nucleotide sequencing of novel SCC-CIs is essential for accurate MRSA detection and tracking.

Chapter 5

Investigation of SCC and SCC*mec* elements, antimicrobial resistance and ACME among Irish clinical isolates of coagulase-negative staphylococci

5.1 Introduction

Coagulase-negative staphylococci are part of the normal microbial flora of human and animal skin and mucous membranes. They are also opportunistic pathogens that can cause a range of infections including endocarditis, septicaemia and infections associated with indwelling and implanted devices particularly in immunocompromised patients (Piette *et al.*, 2009). Over 30 species of CoNS have been described to date, with *S. epidermidis* being the most clinically relevant and being predominantly associated with implanted and indwelling device-related infections. Other CoNS species that have been implicated in clinical infections include *S. haemolyticus, S. hominis, S. saprophyticus, S. capitis and S. lugdunensis*.

Antimicrobial resistance is common among CoNS and they appear to be a reservoir for antimicrobial resistance genes in *S. aureus* (Zong *et al.*, 2011). For example, evidence from a range of studies has revealed that methicillin resistance is more common among CoNS, especially *S. epidermidis*, than *S. aureus* (Monkolrattanotahi *et al.*, 2004). In addition, CoNS harbour a more diverse range of SCC*mec* and SCC elements than MRSA as well as putative ancestral SCC elements and *mec* genes (Couto *et al.*, 1996; Wu *et al.*, 1998; Schnellmann *et al.*, 2006; Miragaia *et al.*, 2007; Tsubakishita *et al.*, 2010; Harrison *et al.*, 2013a). Coagulase-negative staphylococci often carry non-typeable SCC*mec* elements with possible novel *ccr* and *mec* gene complexes or unusual combinations of these genes (Ruppe *et al.*, 2009; Bouchami *et al.*, 2011; Zong *et al.*, 2011; Lebeaux *et al.*, 2011). Additionally, several studies provided evidence indicating that certain CoNS species may be a reservoir for particular SCC*mec* elements or genes (Wisplinghoff *et al.*, 2003; Hanssen *et al.*, 2006; Barbier *et al.*, 2010; Bloemendaal *et al.*, 2010; Garza-Gonzalez *et al.*, 2010a; Smyth *et al.*, 2011) (Table 5.1). For example *S. epidermidis* isolates are predominantly associated with SCC*mec* IV (Wisplighoff *et al*, 2003; Zhang *et al.*, 2008; Garza-Gonzalez, *et al.*, 2010b, Barbier *et al.*, 2010; Bloemendaal *et al.*, 2010), *S.*

Table 5.1. Examples of SCC*mec* types and genes previously identified among selected coagulase-negative staphylococcal species

Staphylococcal species	SCC*mec* type/genes[a]	References
Staphylococcus epidermidis	**Known SCC*mec* types**	
	IV (class B *mec* & *ccrAB2*)	Wisplighoff *et al*, 2003; Machado *et al.*, 2007; Miragaia *et al.*, 2007; Fessler *et al.*, 2008; Zhang *et al.*, 2008; Garza-Gonzalez, *et al.*, 2010; Barbier *et al.*, 2010; Zong *et al.*, 2011
	I (class B *mec* & *ccrAB1*)	Wisplighoff *et al*, 2003; Machado *et al.*, 2007; Zong *et al.*, 2011
	II (class A *mec* & *ccrAB2)*	Wisplighoff *et al*, 2003; Barbier *et al.*, 2010; Zong *et al.*, 2011
	III (class A *mec* & *ccrAB3*)	Wisplighoff *et al*, 2003; Machado *et al.*, 2007; Miragaia *et al.*, 2007; Garza-Gonzalez, *et al.*, 2010; Zong *et al.*, 2011
	V (class C *mec* & *ccrC*)	Barbier *et al.*, 2010; Zong *et al.*, 2011
	VIII (class A *mec* & *ccrAB4*)	Barbier *et al.*, 2010; Zong *et al.*, 2011
	Non-typeable SCCmec types	
	Class A *mec, ccrC*	Miragaia *et al.*, 2007
	Class C *mec, ccrAB2*	Miragaia *et al.*, 2007
	Class B *mec, ccrAB3*	Miragaia *et al.*, 2007
	Class A *mec, ccrNT*	Barbier *et al.*, 2010; Garza-Gonzalez, *et al.*, 2010
	Class A *mec, ccrAB2, ccrAB4*	Barbier *et al.*, 2010
	Class B *mec, ccrNT*	Garza-Gonzalez, *et al.*, 2010
	Class B *mec, ccrAB1, ccrAB4*	Barbier *et al.*, 2010
	Class C *mec, ccrAB2*	Barbier *et al.*, 2010
	Class C *mec, ccrAB4*	Barbier *et al.*, 2010
	Class C *mec, ccrAB2, ccrC*	Zong *et al.*, 2011
	Class NT *mec, ccrAB2*	Garza-Gonzalez, *et al.*, 2010
Staphylococcus haemolyticus	**Known SCC*mec* types**	
	V (class C *mec* & *ccrC*)	Fessler *et al.*, 2008; Barbier *et al.*, 2010; Garza-Gonzalez *et al.*, 2010; Bouchami *et al.*, 2012
	I (class B *mec* & *ccrAB1*)	Machado *et al.*, 2007
	II (class A *mec* & *ccrAB2*)	Machado *et al.*, 2007; Garza-Gonzalez *et al.*, 2010
	Non-typeable SCC*mec* types	
	Class A *mec, ccrAB4*	Bouchami *et al.*, 2012
	Class B *mec, ccrNT*	Barbier *et al.*, 2010
	Class C *mec, ccrAB1*	Bouchami *et al.*, 2012
	Class C *mec, ccrAB4*	Macado *et al.*, 2007; Bouchami *et al.*, 2012
	Class C *mec, ccrAB1, ccrC*	Barbier *et al.*, 2010

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Table 5.1 continued. Examples of SCC*mec* types and genes previously identified among selected coagulase-negative staphylococcal species

Staphylococcal species	SCC*mec* type/genes[a]	Referance
Staphylococcus haemolyticus	**Non-typeable SCC*mec* types**	
	Class C *mec, ccrAB1, ccrC*	Barbier *et al.*, 2010
	Class C *mec, ccrAB2, ccrC*	Barbier *et al.*, 2010
	Class C *mec, ccrAB4*	Zong *et al.*, 2011
	Class NT *mec, ccrAB1*	Garza-Gonzalez *et al.*, 2010; Bouchami *et al.*, 2012
	Class NT *mec, ccrC*	Garza-Gonzalez *et al.*, 2010; Bouchami *et al.*, 2012
	Class NT *mec, ccrAB3*	Garza-Gonzalez *et al.*, 2010
	Class NT *mec, ccrAB4*	Bouchami *et al.*, 2012
Staphylococcus hominis	**Known SCC*mec* types**	
	III (class A *mec* & *ccrAB3*)	Mendoza-Olazaran *et al.*, 2012
	VI (class B *mec* & *ccrAB4*)	Barbier *et al.*, 2010; Bouchami *et al.*, 2011
	VIII (class A *mec* & *ccrAB4*)	Barbier *et al.*, 2010; Bouchami *et al.*, 2011
	Non-typeable SCC*mec* types	
	Class A *mec, ccrAB1*	Barbier *et al.*, 2010; Bouchami *et al.*, 2011; Mendoza-Olazaran *et al.*, 2012
	Class A *mec, ccrNT*	Barbier *et al.*, 2010; Bouchami *et al.*, 2011; Mendoza-Olazaran *et al.*, 2012
	Class A *mec, ccrAB1, ccrAB4*	Barbier *et al.*, 2010; Bouchami *et al.*, 2011
	Class A *mec, ccrAB1, ccrC*	Bouchami *et al.*, 2011; Mendoza-Olazaran *et al.*, 2012
	Class A *mec, ccrAB4, ccrC*	Bouchami *et al.*, 2011
	Class A *mec, ccrC*	Mendoza-Olazaran *et al.*, 2012
	Class A *mec, ccrA5B3*	Zong *et al.*, 2011
	Class B *mec, ccrNT*	Mendoza-Olazaran *et al.*, 2012
	Class C *mec, ccrAB1, ccrAB2*	Barbier *et al.*, 2010
Staphylcoccus capitis	**Known SCC*mec* types**	
	I (class B *mec* & *ccrAB1*)	Machado *et al.*, 2007
	III (class A *mec* & *ccrAB3*)	Machado *et al.*, 2007
	VI (class B *mec* & *ccrAB3*)	Fessler *et al.*, 2008
Staphylococcus lentus	**Known SCC*mec* types**	
	III (class A mec & ccrAB3)	Zhang *et al.*, 2008
Staphylococcus saprophyticus	**Know SCC*mec* types**	
	III (class A *mec* & *ccrAB3*)	Fessler *et al.*, 2008; Zhang *et al.*, 2009
	Non-typeable SCC*mec* types	
	Class A *mec, ccrA1B4*	Zong *et al.*, 2010

Continued overleaf

Table 5.1 continued. Examples of SCC*mec* types and genes previously identified among selected coagulase-negative staphylococcal species

Staphylococcal species	SCC*mec* type/genes[a]	Referance
Staphylococcus warneri	**Known SCC*mec* types**	
	III (class A *mec* & *ccrAB3*)	Zong *et al.*, 2011
	IV (class B *mec* & *ccrAB2*)	Zong *et al.*, 2011
Staphylococcus cohnii	**Non-typeable SCC*mec* types**	
	Class A *mec, ccrA5B3*	Zong *et al.*, 2011
	Class A *mec, ccrAB1, ccrC*	Zong *et al.*, 2011
	Class A *mec, ccrC*	Barbier *et al.*, 2010

[a]The most common SCC*mec* type identified among *S. epidermidis, S. haemolyticus and S. hominis* isolates to date are highlighted in bold. Common SCC*mec* type could not be assigned to any of the other staphylococcal species because of the small number of studies that have been reported to date.
Abbreviations; NT, non typeable.

haemoylticus with SCC*mec* V or components thereof i.e. class C *mec* or *ccrC* (Fessler *et al.,* 2010; Garza-Gonzalez *et al.*, 2010a; Barros *et al.*, 2011; Bouchami *et al.*, 2012) and *S. hominis* with class A *mec, ccrAB1* and *ccrAB4* (Bouchami *et al.*, 2011; Zhang *et al.*, 2013). Bouchami *et al.* (2011) also found evidence to suggest that *S. hominis* may be a reservoir for SCC elements and while these lack *mecA*, they often carry alternative antimicrobial resistance or virulence genes and may represent SCC*mec* precursors. However, apart from *S. epidermidis* the number of isolates of other CoNS species investigated in detail for SCC*mec* and SCC elements is somewhat limited and questions still remain about the role that specific CoNS species may play in the evolution of SCC and SCC*mec* elements.

In addition to methicillin resistance, resistance to other antimicrobial agents including macrolides, glycopeptides, lincosamides, trimethoprim, tetracycline, linezolid, fusidic acid and quaternary ammonium compounds has also been reported to be more common among CoNS, mainly *S. epidermidis,* than *S. aureus* (Koksal *et al.*, 2009; Bouchami *et al.,* 2011; LeBouter *et al.,* 2011; Zhang *et al.*, 2011; Mendes *et al.,* 2012; Cabrara-Contreras *et al.*, 2013). Genes encoding resistance to many of these antimicrobial agents in CoNS are located on MGEs and similar genes have been identified in *S. aureus* indicating that horizontal transfer of these genes can occur (Malachowa and DeLeo, 2010). However, very few studies have investigated both the antimicrobial resistance phenotype and the presence or absence of specific antimicrobial resistance genes in CoNS. Studies that have been undertaken have been restricted to either single CoNS species (LeBouter *et al.*, 2011) or to a limited number of antimicrobial agents or resistance genes (Bouchami *et al.*, 2011; Zhang *et al.*, 2011).

While often more resistant to antimicrobial agents, CoNS are generally less virulent that *S. aureus* because they harbour less virulence genes, but the ability of CoNS to produce biofilm is considered a key virulence factor that plays a major role in CoNS

infection of implanted and indwelling devices (von Eiff *et al.*, 2002). In addition, during the last decade ACME, which can harbour the *arc* and *opp* gene operons, encoding an arginine deiminase pathway and ABC transporter, respectively, were recently identified in staphylococci (Diep *et al.*, 2006; Diep *et al.*, 2008; Miragaia *et al.*, 2009; Montogomery *et al.*, 2009). ACME has been identified in a small number of MRSA clones (predominantly ST8-MRSA-IV/USA300) but has been found to be more prevalent among CoNS and has been identified in *S. epidermidis, S. capitis* and *S. haemolyticus* (Diep *et al.*, 2006; Pi *et al.*, 2009). Though the precise function(s) of ACME has not yet been determined, animal model studies have shown that it improves fitness and enhances the ability of *S. aureus* to colonise the skin and mucous membranes (Diep *et al.*, 2008; Montogomery *et al.*, 2009) and evidence suggests that ACME facilitates staphylococcal colonisation rather than infection by playing a role in maintaining a pH balance on the human skin (Diep *et al.*, 2006; Du *et al.*, 2013).

In Ireland, CoNS are the third most common cause of BSIs in hospitals (Burns *et al.*, 2012) but despite this, little is known about the molecular epidemiology and population structure of CoNS from patients in Irish hospitals. The high prevalence of CoNS associated with nosocomial infections has also been reported from other countries (Grohnskopf *et al.*, 2001; Wisplinghoff *et al.*, 2003; Hidron *et al.,* 2008). PFGE has for many years been considered the gold standard for molecular epidemiological typing of the two most clinically relevant CoNS species, *S. epidermidis* and *S. haemolyticus* (Muldrew *et al.*, 2008; Kristof *et al.*, 2011). While PFGE is more suitable for outbreak investigations, in recent years MLST schemes have been developed for typing of *S. epidermidis* and *S. haemolyticus* and offer more reproducibility and the potential for investigating the population structure of these staphylococcal species (Wang *et al.*, 2003; Wisplinghoff *et al.*, 2003; Thomas *et al.*, 2007; Miragaia *et al.*, 2007; Miragaia *et al.,* 2008; Voronina *et al.,* 2011; Cavanagh *et al.,* 2012). One MLST scheme in particular has been found to be

effective for typing of *S. epidermidis* isolates and has revealed that *S. epidermidis* has an epidemic population structure where recombination and the frequent transfer of MGEs contributes significantly to clonal expansion (Miragaia *et al.*, 2007). In contrast, *S. aureus* has a highly clonal population structure due to clonal diversification occurring predominantly via point mutations. However, despite a high level of genetic diversity and a large numbers of STs within *S. epidermidis*, one lineage, CC2, predominates among *S. epidermidis* isolates worldwide (Winderstom, 2010; Miagaia *et al.*, 2007; Mendes *et al.*, 2012; Du *et al.*, 2013). To date no consensus MLST scheme has been developed for *S. haemolyticus.* One scheme recently proposed by Cavanagh *et al.* (2012) revealed a high level of conservation in core *S. haemolyticus* genome genes and questions remain as to the usefulness of this MLST scheme for investigating the population structure of *S. haemolyticus*. The study by Cavanagh *et al.* (2012) revealed that the majority of *S. haemolyticus* isolates recovered from around the world over a long time period were assigned to just one main lineage and the usefulness of this MLST scheme for molecular typing of *S. haemolyticus* requires further investigation.

Due to the increasing importance of CoNS as a cause of HCAIs, their resistance to multiple antimicrobial agents and unusual virulence traits and the fact that they appear to be a reservoir for SCC*mec*, antimicrobial resistance genes and ACME in *S. aureus*, it is essential that their population structure and molecular characteristics are better understood. In addition, due to recent studies that have highlighted that particular CoNS species may be reservoirs for particular SCC*mec* elements or genes, it is essential that individual CoNS species are accurately identified and investigated. Therefore, the aim of this part of the present study was to characterise the SCC*mec* and SCC elements, antimicrobial resistance genes and ACME among clinical CoNS isolates recovered from patients in Irish hospitals. A total of 53 CoNS isolates underwent 16S ribosomal DNA gene (rDNA) sequencing and were identified as *S. epidermidis, S. hominis, S. haemolyticus, S. capitis,* and *S. warneri*

and underwent antimicrobial susceptibility testing and DNA microarray analysis to detect an extensive range of SCC*mec* and SCC associated genes, ACME and antimicrobial resistance genes. Isolates representative of the two most clinically relevant species, *S. epidermidis* and *S. haemolyticus*, also underwent MLST.

5.2 Material and Methods

5.2.1 Coagulase-negative staphylococcal (CoNS) isolates investigated

Fifty-three CoNS isolates were investigated. This included (i) 10 CoNS isolates recovered between February 2004 and May 2006 in a large acute hospital in Dublin from external ventricular devices (EVDs) in patients with device-related meningitis or from non-EVD cerebrospinal fluid specimens (Stevens *et al.*, 2008) and (ii) 43 CoNS isolates originally reported as contaminants in clinical specimens or recovered from BSIs in patients in another Dublin hospital between 2010 and 2011.

5.2.2 Antimicrobial susceptibility testing

All CoNS isolates underwent antimicrobial susceptibility testing by determining their resistance to a panel of 23 antimicrobial agents as described in Chapter 2, section 2.4.2. The CoNS isolates also underwent cefoxitin susceptibility testing using 30 μg cefoxitin discs as described in Chapter 2, section 2.4.1.

5.2.3 Whole-genomic DNA isolation

Whole-genomic DNA was extracted from all CoNS isolates for use in 16S rDNA sequencing, MLST and DNA microarray profiling using the lysis buffers and solutions supplied with the StaphyType DNA microarray and the Qiagen DNeasy kit, as described in Chapter 2, section 2.5.4.1.

5.2.4 Species identification of CoNS isolates

Species identification of all CoNS isolates was performed by PCR amplification and sequencing of the 16S rRNA gene (rDNA) using previously published primers (Singh *et al.*, 2003) (Table 5.2). The following thermal cycling conditions were used for PCR: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 10 s,

and a final elongation step at 72°C for 10 min. The resulting amplimers were sequenced commercially by Genservice (Chapter 2, section 2.5.2) and DNA sequences were analysed using the BioNumerics software package (version 5.1). Homology searches were performed using BLAST software (http://ncbi.nih.gov/BLAST).

5.2.5 Molecular typing

5.2.5.1 Multilocus sequence typing of *S. epidermidis* isolates

Multilocus sequence typing (MLST) was performed on 17 *S. epidermidis* isolates using the *S. epidermidis* MLST scheme and primers reported by Thomas *et al.,* 2007 with the following thermal cycling conditions: 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, and a final elongation step at 72°C for 10 min (Table 5.2). This involved sequencing the internal fragments of seven housekeeping genes encoding carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), an ABC transporter (*gtr*), DNA mismatching repair protein (*mutS*), pyrimidine operon regulatory protein (*pyrA*), triosephosphate isomerase (*tpi*) and acetyl coenzyme A acetyltransferase (*yqiL*). All amplimers were sequenced commercially by Geneservice Ltd. (Source Bioscience) as described in Chapter 2, section 2.5.2 and sequences were analysed using the BioNumerics software package (version 5.1). For each isolate, the sequence of each allele was compared to a consensus sequence of the corresponding allele on the *S. epidermidis* MLST website (http://sepidermidis.mlst.net/) and were assigned an allele number based on any single-nucleotide polymorphisms (SNPs). The *S. epidermidis* MLST website was then able to assign a sequence type (ST) based on the allelic profile of the seven genes. Sequence types were assigned to a clonal complex (CC) if the sequences of 5/7 alleles were the same.

5.2.5.2 Multilocus sequence typing of *S. haemolyitcus* isolates

Mutlilocus sequence typing was performed on seven *S. haemolyticus* isolates. Currently there is no on-line MLST database for *S. haemolyticus* and therefore a scheme

Table 5.2. Primers designed and used for 16S rRNA gene (rDNA) sequencing of CoNS, multilocus sequence typing (MLST) of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* and *mec* gene amplification

Primer purpose	Gene amplified	Primer pair	Nucleotide sequence (5'-3')	Product size	Reference sequence			Reference
					Coordinates	Analysed segment start and end points[c]	Size of sequenced fragment	
Species identification	16s rDNA	**533-F** **142-R**	AGAGTTTGATYMTGGCTCAG CGGYTACCTTGTTACGAC	950 bp	n/a	n/a	n/a	Singh *et al.*, 2003 Singh *et al.*, 2003
Staphylococcus epidermidis	*arcC*	**arcC-F** **arcC-R**	TGTGATGAGCACGCTACCGTTAG TCCAAGTAAACCCATCGGTCTG	507 bp	2398372-2398350[a] 2397865-2397886[a]	S TTCTACA E TAATCCT	456 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
MLST	*aroE*	**aroE-F** **aroE-R**	CATTGGATTACCTCTTTGTTCAGC CAAGCGAAATCTGTTGGGG	457 bp	1198976-1198998[a] 1199433-1199415[a]	S GCGGTAA E AGAAGCT	420 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
	gtr	**gtr-F** **gtr-R**	CAGCCAATTCTTTTATGACTTTT GTGATTAAAGGTATTGATTTGAAT	507 bp	2021318-2021340[a] 2021825-2021803[a]	S CCAATTC E CAACTTCAC	438 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
	mutsS	**mutS-F3** **mutS-R3**	GATATAAGAATAAGGGTTGTGAA GTAATCGTCTCAGTTATCATGTT	607 bp	874113-874135[a] 874720-874698[a]	S TTTGAAAT E TCATAT	421 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
	pyrA	**pyr-F2** **pyr-R4**	GTTACTAATACTTTTGCTGTTT GTAGAATGTAAAGAGACTAAAATGAA	805 bp	762032-762051[a] 762882-762857[a]	S CGTACAA E ACGAGA	428 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
	tpi	**tpi-F2** **tpi-R2**	ATCCAATTAGACGCTTTAGTAAC TTAATGATGCGCCACCTACA	591 bp	449127-449149[a] 449718-449699[a]	S AATTGGA E TCAAGAG	424 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
	yqiL	**yqiL-F2** **yqiL-R2**	CACGCATAGTATTAGCTGAAG CTAATGCCTTCATCTTGAGAAATAA	657 bp	26292-26312[a] 26949-26925[a]	S TATTTAA E ATGGGT	416 bp	Thomas *et al.*, 2007 Thomas *et al.*, 2007
Staphylococcus haemolyticus	*ABC*	**SHABC-F** **SHABC-R**	GAGACGATTCAGCTAAGCAA CGCCTTTCATTAGGCCATTA	632 bp	176932-176951[b] 177564-177545[b]	S ATTTAAT E GGTAAAG	561 bp	Cavanagh *et al.*, 2012 Cavanagh *et al.*, 2012
MLST	*arcC*	**SHarc-F** **SHarc-R**	AGTGACTCAAGTTGAA AATCTTACCATCTAGG	588 bp	259319-259334[b] 259907-259892[b]	S TGGCTTA E GTGTATT	483 bp	Cavanagh *et al.*, 2012 Cavanagh *et al.*, 2012
	cfx	**SHcfx-F** **SHcfx-R**	GAAGCACAAATTGATGGTCTGC TCTGCCCCATTATCAACACA	499 bp	1752457-1752436[b] 1751958-1751977[b]	S ATTTAATT E GGTAAAG	431 bp	Cavanagh *et al.*, 2012 Cavanagh *et al.*, 2012

Continued overleaf

Table 5.2 continued. Primers designed and used for 16S rRNA gene (rDNA) sequencing of CoNS and multilocus sequence typing (MLST) of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*

Primer purpose	Gene amplified	Primer pair	Nucleotide sequence (5'-3')	Product size	Reference sequence			Reference
					Coordinates	Analysed segment start and end points[c]	Size of sequenced fragment	
Staphylococcus haemolyticus	*leuB*	**SHleuB-F**	AGCCATAGATTCGCATGGTGT	617 bp	982823-982812[b]	S GATTTTC	518 bp	Cavanagh *et al.*, 2012
		SHleuB-R	CCTAATGAACCTGGAATGGTAG		982206-982227[b]	E GTCAGCA		Cavanagh *et al.*, 2012
MLST	*hemH*	**SHhemH-F**	CTGATCGTCAAGCTGAAGCAT	504 bp	1137358-1137378[b]	S AATCAAG	422 bp	Cavanagh *et al.*, 2012
		SHhemH-R	GTACCTGTGTGACCCTCAGA		1137862-1137843[b]	E CTTCAAA		Cavanagh *et al.*, 2012
	SH 1200	**SH1200-F**	CGGTAATGTAACACACGCAGT	483 bp	1229454-1229434[b]	S ACAGAGTA	394 bp	Cavanagh *et al.*, 2012
		SH1200-R	ACCAGGCTTGTCACCATGA		1228971-1228989[b]	E AAACTAA		This study
	SH 1431	**SH1431-F**	TCAGACCAACAATTCCCACC	618 bp	1456411-1456430[b]	S GACGTCA	545 bp	This study
		SH1431-R	CTTTAGCGTCACGATGGTCG		1457029-1457010[b]	E TCAAAACA GAAA		Cavanagh *et al.*, 2012

The DNA sequence of [a] *S. epidermidis* RP62A (Genbank accession number CP000029.1) and [b] *S. haemolyticus* JCSC1435 (Genbank accession number AP006716.1) was used as the reference sequence for MLST. The coordinates represent the location of each primer sequence within the DNA sequence of RP62A and JCSC1435.

[c] The start and end points represent the start (S) and end (E) sequences used for allele identification.

Abbreviations: n/a, not applicable.

was designed based on a previous study (Cavanagh *et al.,* 2012). The genes selected for the MLST scheme were *arc* (carbamate kinase), SH 1200 (Ser A: D-3-phosphoglycerate dehydrogenase), *hemH* (ferrochelatase), *leuB* (3-isopropymalate dehydrogenase), SH 1431 (cell surface elastin binding protein), *cfxE* (Ribulose 5-phosphate epimerase), and ribose ABC (transport efflux pump) and all but two primers (SH1220-R and SH1431-F) were as described by Cavanagh *et al.* (2012) (Table 5.2). Thermal cycling conditions for the amplification of all target gene sequences were as follows: 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 50°C for 30 s and 72 °C for 30 s, and a final elongation for 72°C for 5 min. Amplimers were sequenced commercially by Geneservice Ltd. (Source Bioscience) as described in Chapter 2, section 2.5.2 and sequences were analysed using the BioNumerics software package (version 5.1). Each sequence of each allele was aligned with a reference sequence for each gene from the whole genome sequencing of *S. haemolyticus* JCSC1435 (Genbank accession number AP006716.1) using Mega5 software (Tamura *et al.,* 2011) and any SNPs were identified. Once the seven genes were analysed for each isolate each distinct sequence of the same gene was assigned a distinct allelic number and the allelic profile of the seven genes were used to define STs. As there was no central database and no consensus MLST scheme for *S. haemolyticus* MLST data, it was not possible to compare *S. haemolyticus* STs in this study to those reported in other studies.

5.2.6 DNA microarray profiling

DNA microarray profiling was performed on all CoNS isolates using the StaphyType kit (Alere) which can detect a range of staphylococcal genes including SCC*mec*, antimicrobial resistance and virulence-associated genes. The DNA microarray was designed for profiling of *S. aureus*, however as CoNS carry many genes found in *S. aureus,* many of these on MGEs, the DNA microarray can be used to profile CoNS isolates. The DNA microarray profiling was performed as described in Chapter 2, section 2.5.4.

5.3 Results

5.3.1 Species identification and methicillin resistance testing

The 53 CoNS isolates investigated were found to belong to five CoNS species. The majority were identified as *S. epidermidis* (30/53, 56.6%), followed by *S. hominis* (10/53, 18.8%), *S. haemolyticus* (7/53, 13.2%), *S. capitis* (4/53, 7.5%), and *S. warneri* (2/53, 3.7%) (Table 5.2). The majority of all the CoNS isolates (39/53, 73.5%) were methicillin resistant and included methicillin-resistant *S. haemolyticus* (MRSH, 6/7, 85.7%), methicillin-resistant *S. epidermidis* (MRSE, 24/30, 80%), methicillin-resistant *S. hominis* (MRSHo, 8/10, 80%) and methicillin-resistant *S. warneri* (MRSW, 1/2, 50%) (Table 5.3).

5.3.2 Multilocus sequence typing of *S. epidermidis* and *S. haemolyticus*

Twenty-four isolates representative of the two most clinically relevant species identified, namely *S. epidermidis* and *S. haemolyticus*, were subjected to MLST. This included 17/30 *S. epidermidis* isolates, 15 of which were subsequently found to be MRSE and two were found to be methicillin-susceptible *S. epidermidis* (MSSE) and 7/7 *S. haemolyticus* isolates identified, six of which were subsequently found to be MRSH and one which was a methicillin-susceptible *S. haemolyticus* (MSSH).

The *S. epidermidis* isolates were assigned to ten STs with ST2 predominating (5/17, 29.4%), followed by ST35, ST87 and ST83, with the latter three being assigned to two isolates each (Table 5.4). The remaining six *S. epidermidis* STs (ST6, ST85, ST81, ST357, ST539 and ST540) were each represented by a single isolate (Table 5.4). Seventy-one percent of the *S. epidermidis* isolates subjected to MLST (12/17) were assigned to a single clonal complex (CC2) that included ST2, ST35 (a single-locus variant (SLV) of ST2), ST6 (a double locus variant (DLV) of ST2), ST85 (a DLV of ST2), and ST87 (a DLV of ST2) and the remaining were deemed singletons (Table 5.4).

Table 5.3. Species identified and methicillin resistance phenotypes of the 53 coagulase-negative staphylococcal isolates investigated

Staphylococcal species[a]	Total no. of isolates	No. of methicillin resistant isolates[b]
Staphylococcus epidermidis	30	24
Staphylococcus hominis	10	8
Staphylococcus haemolyticus	7	6
Staphylococcus capitis	4	0
Staphylococcus warneri	2	1

[a] Staphylococcal species were identified by 16S rDNA sequencing (Singh *et al.*, 2003).
[b] Methicillin resistance was determined using 30 μg cefoxitin disks (Oxoid) (Rossney *et al.*, 2007).

Table 5.4. Multilocus sequencing typing (MLST) of *S. epidermidis* and *S. haemolyticus* isolates and SCC*mec*/SCC-associated genes detected

		MLST[a]		
Methicillin resistance phenotype & staphylococcal species (*n*)	**CC**	**ST (*n*)**	**Allelic profile[b]**	**SCC/SCC*mec* types/genes (*n*)[c]**
Methicillin resistant *S. epidermidis* (15)	CC2	ST2 (5)	7-1-2-2-4-1-1	IV (3), II (2)
		ST35 (2)	2-1-2-2-4-1-1	IV + *ccrAB4* (1) IV + *ccrAB1* (1)
		ST87 (2)	7-1-1-2-2-1-1	IV
		ST6 (1)	1-1-2-2-2-1-1	IV, + *kdp, ccrA3, ccrB4* (1)
		ST85 (1)	1-1-2-2-1-1-1	IV (1)
	S	ST81 (1)	2-17-1-1-2-1-1	IV + *ccrC* (1)
	S	ST83 (2)	1-2-1-2-1-1-10	IV +*ccrC* (1), IV + *ccrAB4* (1)
	S	ST539 (1)	28-1-47-5-7-5-1	IV (1)
Methicillin susceptible *S. epidermidis* (2)	S	ST540 (1)	1-1-2-6-2-42-33	*ccrAB2* (1)
	S	ST357 (1)	1-1-1-1-4-1-7	None detected (1)
Methicillin resistant *S. haemolyticus* (6)	CC1	ST1 (4)	2-1-1-1-1-1-1	*mecA, ccrB4* (4)
		ST7 (1)	2-2-1-1-1-1-1	*mecA, ccrAA* (1)
		ST8 (1)	2-1-1-1-1-2-1	*mecA, ccrB4* (1)
Methicillin susceptible *S. haemolyticus* (1)	CC1	ST9 (1)	2-1-1-1-3-1-1	*ccrAA, ccrA4* (1)

[a]Seventeen *S. epidermidis* and seven *S. haemolyticus* isolates were subjected to MLST. The *S. epidermidis* isolates were subjected to the MLST scheme developed by Thomas *et al.*, (2007) and allele sequences were compared to an online database (http://sepidermidis.mlst.net/). The *S. haemolyticus* isolates were subjected to a MLST scheme based on a previous study (Cavanagh *et al.*, 2012) and since there currently is no online database for *S. haemolyticus* MLST the isolate profiles were only compared against each other.

[b]For each isolate the MLST allelic profile was used to define the sequence type (ST) and was assigned based on the sequence of the seven housekeeping genes. For *S. epidermidis* the seven MLST genes were, *arcC, aroE, gtr, mutS, pyrR,tpi, yqiL*, and for *S. haemolyticus* the seven MLST genes were, *ribose* ABC, *arc, cfx, leuB, hemH, SH1200, SH1431.*

[c]SCC/SCC*mec* types and genes were detected using the StaphyType DNA microarray (Alere). Where possible SCC*mec* types were assigned and are listed above, or if not possible, the SCC*mec*/SCC associated genes detected are listed.

Abbreviations: *n*, number of isolates; CC, clonal complex; ST, sequence type; S, singleton.

The seven *S. haemolyticus* isolates subjected to MLST were assigned to four STs (Table 5.3), with only one ST, ST1, being represented by >1 isolate (Table 5.4). The remaining STs identified among the *S. haemolyticus* isolates were either SLVs or DLVs of ST1 and were all assigned to *S. haemolyticus* CC1.

5.3.3. SCC*mec* and SCC-associated genes detected among CoNS

Using the StaphyType DNA microarray 88.7% (47/53) of CoNS isolates investigated were found to harbour various combinations of *mec*, *ccr* and J region-associated genes, 80.9% (38/47) of which harboured *mecA* (Table 5.5).

5.3.3.1 SCC*mec*

Of the 38 *mecA*-positive CoNS isolates detected, all were phenotypically resistant to methicillin.

Among the *mecA*-positive isolates 17/38 (44.7%) were assigned to previously described SCC*mec* types, either SCC*mec* IV (13/38, 34.2%), SCC*mec* III (2/38, 5.3%) or SCC*mec* VIII (1/38, 2.6%) (Table 5.5). All but two of these isolates were *S. epidermidis*, with one being *S. hominis* (SCC*mec* VIII) and one being *S. warnerii* (SCC*mec* IV) (Table 5.5). The remaining *mecA*-positive isolates were assigned to (i) possible novel SCC*mec* IV subtypes due to the presence of a combination of *ccr* and *mec* genes indicating the presence of SCC*mec* IV but with additional SCC*mec*-associated genes not previously reported in SCC*mec* IV subtypes, or (ii) possible novel SCC*mec* types due to the detection of novel combinations of *ccr* and *mec* genes that could not be assigned to previously described SCC*mec* types and are described in detail below (Table 5.5).

5.3.3.1.1 Possible novel SCCmec IV subtypes

Four possible novel SCC*mec* IV subtypes were identified among 6/38 (15.8%) of *mecA*-positive isolates (Table 5.5). These four subtypes were all detected in MRSE isolates

and carried class B *mec* and *ccrAB2* indicative of SCC*mec* IV but also harboured additional *ccr* genes, including *ccrC* (three isolates), *ccrAB1* (one isolate), *ccrAB4* (one isolate) or *ccrAB3* (one isolate) and in two isolates they lacked *dcs* which is commonly, but not always, found in the J3 region of SCC*mec* IV (Table 5.5). All six isolates with possible novel SCC*mec* IV subtypes underwent MLST and were assigned to four STs, three of which belonged to CC2 and one to CC59 (Table 5.4).

5.3.3.1.2 Possible novel SCCmec types

Eight possible novel SCC*mec* elements with novel combinations of *ccr* and *mec* genes were identified among 15/38 (39.4%) of *mecA*-positive isolates (Table 5.5). These possible novel SCC*mec* types were tentatively designated novel SCC*mec* types 1-8 and were identified in MRSE (three isolates), MRSH (six isolates) and MRSHo (six isolates). Only one possible novel SCC*mec* type (novel SCC*mec* type 1: class A *mec* and *ccrAB1* only) was found in two different species of CoNS (MRSE and MRSHo) (Table 5.5). However, three additional possible novel SCC*mec* elements (novel types 6-8 in five isolates) also consisted of class A *mec* with *ccrAB1* genes, which is a type combination not associated with any previously reported SCC*mec* type, but also harboured various combinations of additional *ccr* genes including *ccrB4* and/or *ccrA4* and *ccrB3* (Table 5.5). These possible novel elements consisting predominanly of class A *mec* and *ccrAB1* accounted for the majority (3/4) of possible novel SCC*mec* elements in MRSHo isolates (Table 5.5). The remaining MRSE isolates carried (i) a class A *mec* complex with *ccrAB3* and *ccrC* which indicates the possible presence of SCC*mec* III but *ccrB4* was also detected (novel SCC*mec* type 2) or (ii) a class B *mec* complex but no *ccr* genes (novel SCC*mec* type 3) (Table 5.5). The MRSH isolates (n = 6) harboured *mecA* but no additional *mec* complex genes but two different combinations of *ccr* genes were detected including *ccrB4*

Table 5.5 Species, SCC and SCC*mec* associated genes, antimicrobial susceptibility patterns and antimicrobial resistance genes detected among the 53 coagulase-negative staphylococcal isolates investigated

Species (*n*)[a]	SCC/SCC*mec* type (*n*)[b]	SCC/SCC*mec* genes (*n*)[b,c]	Antimicrobial resistance pattern (*n*)[c,d]	Antimicrobial resistance genes (*n*)[b,c]
Staphylococcus epidermidis (30)	IV (13)	Class B *mec* (*mecA, ΔmecR1*), *dcs, ccrAB2,* ACME (1)	Ap, Cp (9), Er (12), Eb (8), Fd (12), Gn (10), Kn (10), Ln (3), Mp (1), Rf (1), Sp (2), Su (11), Tb (10), Te (1), Tp (10)	*blaZ, erm*(A) (2), *erm*(C) (3), *msr*(A) (9), *mpb*(C) (1), *vat*(B) (2), *vga*(A) (2), *aacA-aphD* (9), *dfrS1* (12), *fusB* (12), *fusC* (1), *mupA* (1), *tet*(K) (1), *qacA* (12), *qacC* (2)
	IV (*-dcs*) + *ccrC* (3)	Class B *mec* (*mecA, ΔmecR1*), *ccrAB2, ccrC,* ACME (2)	Ap, Cp (1), Er, Eb (1), Fd (2), Gn (1), Kn (1), Mp (2), Pma (1), Su (1), Te (1), Tb (1), Tp (1)	*blaZ, erm*(C) (1), *msr*(A) (2), *mpb*(C) (1), *aacA-aphD* (1), *dfrS1* (2), *fusB* (2), *mupA* (2), *tet*(K) (1), *qacA* (2), *qacC* (2)
	IV (*-dcs*) + *ccrAB4* (1)	Class B *mec* (*meccA, ΔmecR1*), *ccrAB2, ccrAB4*	Ap, Cp, Fd, Mp, Su, Tp	*blaZ, dfrS1, fusB, mupA, qacA*
	IV + *ccrAB1*(1)	Class B *mec* (*mecA, ΔmecR1*), *dcs, ccrAB2,ccrAB1,* ACME	Ap, Cp, Er, Kn, Nm, Tb, Tp	*blaZ, msr*(A), *mpb*(C), *fusB, qacA*
	IV + *kdp, ccrAB3, ccrAB4* (1)	Class B *mec* (*mecA, ΔmecR1*), *dcs, kdp, ccrAB2, ccrAB4,* ACME	Ak, Ap, Er, Gn, Kn, Ln, Su, Tb	*blaZ, erm*(C), *aacA-aphD, dfrS1, mupA, qacA*
	III (2)	Class A *mec* (*mecA, mecR1, mecI*), *dcs, ccrAB3*	Ak, Ap, Cd, Cp, Er, Gn, Kn, Mc, Mp, Nm, Pma, Rf, Su, Tb, Tp	*blaZ, erm*(A) (1), *aacA-aphD, aadD, dfrS1, qacA*
	Novel SCC*mec* type 1 (1)	Class A *mec (mecA, mecR1, mecI), ccrAB1*	Ap, Cd, Er, Fd, Tp	*blaZ, msr*(A), *mpb*(C), *dfrS1, fusB, fusC, qacA*
	Novel SCC*mec* type 2 (1)	Class A *mec* (*mecA, mecR1, mecI*), *dcs, ccrAB3, ccrC, ccrB4*	Ap, Cd, Cp, Er, Eb, Fd, Gn, Kn, Ln, Pma, Rf, Su, Tb, Tp	*blaZ, erm*(C), *aacA-aphD, dfrS1, fusB, qacA*
	Novel SCC*mec* type 3 (1)	Class B *mec (mecA, ΔmecR1)*	Ap, Eb, Fd, Su, Tp	*blaZ, dfrS1, fusC, qacA*

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Table 5.5 continued. Species, SCC and SCC*mec* associated genes, antimicrobial susceptibility patterns and antimicrobial resistance genes detected among 53 coagulase-negative staphylococcal isolates

Species (*n*)[a]	SCC/SCC*mec* type (*n*)[b]	SCC/SCC*mec* genes (*n*)[b,c]	Antimicrobial resistance pattern (*n*)[c,d]	Antimicrobial resistance genes (*n*)[b,c]
	Novel SCC type 1 (3)	*ccrAB2*	Ap, Er (2), Ln (2), Mp (2), Su (1)	*blaZ*, *msr*(A) (2), *mpb*(C) (2), *vga* (2), *mupA* (2)
	N/A (3)	None detected	Ap, Cp (1), Er (2), Fd (2), Mp (1), Su (1), Te (1), Tp (1)	*blaZ*, *erm*(C) (2), *dfrS1* (2), *fusB* (2), *mupA* (1), *tet*(K) (1) *qacA*, *qacC* (1)
Staphylococcus haemolyticus (7)	Novel SCC*mec* type 4 (5)	*mecA, ccrB4*	Ap, Cp (4), Er, Gn (4), Kn, Ln (1), Rf (1), Su, Tb (4), Tp	*blaZ, erm*(C) (1), *msr*(A), *mpb*(C), *aacA-aphD, qacA* (4)
	Novel SCC*mec* type 5 (1)	*mecA, ccrAA, ccrC*	Ap, Cp, Er, Fd, Kn, Nm, Su, Tp	*blaZ, erm*(C), *msr*(A), *mpb*(C), *aacA-aphD, aadD, aphA3* & *sat, fusC, qacA*
	Novel SCC type 2 (1)	*ccrAA, ccrA4*	Susceptible	*qacC*
Staphylococcus hominis (10)	VIII (1)	Class A *mec* (*mecA, mecR1, mecI*), *ccrAB4*	Ap, Cl, Er, Kn, Mc, Sp, Te, Tb, Tp	*blaZ, erm*(C), *aadD, tet*(K), *fosB*-plasmid
	Novel SCC*mec* type 6 (3)	Class A *mec* (*mecA, mecR1, mecI) ccrAB1, ccrAB4*	Ap, Cd (1), Cp (1), Er, Fd (2), Kn, Su, Te (1), Tb (1), Tp	*blaZ, erm*(C), *aacA-aphD, aadD* (1), *fusC* (2), *tet(K)* (1), *qacA*
	Novel SCC*mec* type 1 (1)	Class A *mec* (*mecA, mecR1, mecI), ccrAB1*	Ap, Cd, Er, Fd, Su, Tp	*blaZ, erm*(C), *fusC, qacA*
	Novel SCC*mec* type 7 (1)	Class A *mec* (*mecA, mecR1, mecI), ccrAB1, ccrB3, ccrAB4*	Ap, Er, Fd, Mc, Tp	*blaZ, erm*(C), *fusC, fosB*-plasmid
	Novel SCC*mec* type 8 (1)	Class A *mec* (*mecA, mecR1, mecI), ccrAB1, ccrA4*	Ap, Cd, Cp, Er, Fd, Kn, Ln, Su, Tb, Tp	*blaZ, erm*(C), *aacA-aphD, aadD, fusC, qacA*

Continued overleaf

Table 5.5 continued. Species, SCC and SCC*mec* associated genes, antimicrobial susceptibility patterns and antimicrobial resistance genes detected among 53 coagulase-negative staphylococcal isolates

Species (*n*)[a]	SCC/SCC*mec* type (*n*)	SCC/SCC*mec* genes[b]	Antimicrobial resistance pattern (*n*)[c,d]	Antimicrobial resistance genes (*n*)[b,c]
	Novel SCC type 3 (1)	*ccrAB1*	Ap, Er, Fd	*blaZ, erm*(C), *fusC, qacC*
	Novel SCC type 4 (1)	*ccrAB4*	Ap, Er, Ln, Mp	*blaZ, erm*(C), *qacA*
	N/A (1)	None detected	Ap, Cd, Fd, Ln	*blaZ, lun*(A), *fusB, qacA*
Staphylococcus capitis (4)	Novel SCC type 1 (3)	*ccrAB2*	Susceptible (2), Eb (1)	None detected (2), qacA (1)
	N/A (1)	ACME (1)	Ap	*blaZ, qacA*
Staphylococcus warneri (2)	IV (1)	Class B *mec* (*mecA, ΔmecR1*), *dcs, ccrAB2*	Ap, Eb, Gn, Kn, Tb	*blaZ, aacA-aphD, qacA*
	N/A (1)	None detected	Ap, Er, Mc	*blaZ*

[a] Species identification were determined by 16S rDNA sequencing (Singh *et al.*, 2003).

[b] SCC/SCC*mec* genes and antimicrobial resistance genes were detected using the StaphyType DNA microarray (Alere) and SCC*mec* types were assigned following manual inspection of DNA microarray profiles for each isolate.

[c] The number of isolates positive for a specific gene or resistance phenotype are shown in parenthesis only when all isolates within a particular species and SCC*mec* type were not positive for that gene or resistance phenotype.

[d] Antimicrobial resistance patterns were determined by testing the susceptibility of isolates to a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadimium nitrate (Cd), chloramphenicol (Cl), ciprofloxacin (Cp), ethidium bromide (Eb), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride (Mc), mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate, rifampicin (Rf), streptomycin, spectiomycin (Sp), sulphonamide (Su), tobramycin (Tb), tetracycline (Te), trimethoprim (Tp) and vancoymcin. Isolates that were susceptible to all antimicrobial agents tested are indicated as susceptible. Abbreviations; N/A, not applicable i.e. no SCC/SCC*mec* genes detected so no SCC*mec* or SCC type name was assigned for these isolates.

(novel SCC*mec* type 4) in five isolates and *ccrC* and *ccrAA* (novel SCC*mec* type 5) in one isolate (Table 5.5).

5.3.3.2 SCC-associated genes detected among CoNS

Four possible novel SCC elements that harboured *ccr* genes but no *mecA* or *mec* regulatory genes were identified among 9/53 (16.7%) of the CoNS isolates. These possible novel SCC elements were tentatively designated novel SCC types 1-4 and were identified in *S. epidermidis* (three isolates), *S. haemolyticus* (one isolates) *S. hominis* (two isolates), and *S. capitis* isolates (three isolates) (Table 5.5). The majority of these isolates (6/9, 66.7%), belonged to novel SCC type 1 and consisted of *ccrAB2* only and was identified in all *S. epidermidis* (n=3) and all *S. capitis* (n=3) isolates carrying SCC elements (Table 5.5). The remaining three possible novel SCC types were each represented by a single isolate and consisted of *ccrAA* and *ccrA4* (novel SCC type 2 in *S. haemolyticus*), *ccrAB1* only (novel SCC type 3 in *S. hominis*) or *ccrAB4* only (novel SCC types 4 *S. hominis*). All isolate carrying a possible novel SCC element were methicillin susceptible.

5.3.3.3 ACME

The ACME-*arc* gene cluster was identified in 6/53 (11.3%) of the CoNS isolates investigated, the majority of which were MRSE (5/6, 83.3%) and harboured SCC*mec* IV or possible novel SCC*mec* IV subtypes (Table 5.5). One methicillin-susceptible *S. capitis* isolate carried ACME but no other SCC*mec* or SCC-associated genes were detected (Table 5.5).

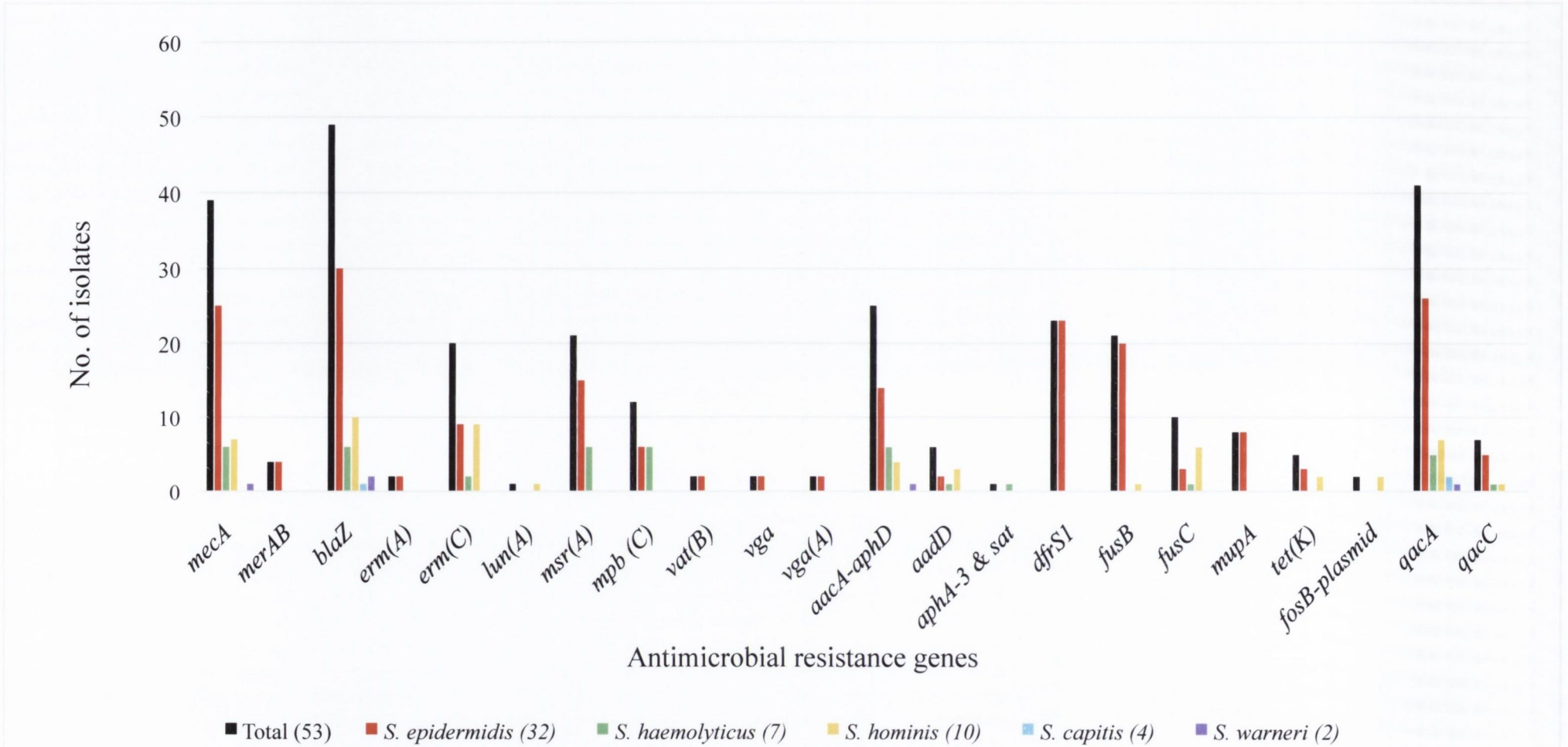
5.3.4 Antimicrobial resistance phenotype and genes detected

The majority of isolates (51/53, 96.2%) were found to harbour at least one of the antimicrobial resistance genes detected using the DNA microarray and to exhibit resistance to one or more of the antimicrobial agents tested (Table 5.5). The two isolates in which no

antimicrobial resistance genes were detected were both *S. capitis* and both isolates were susceptible to all antimicrobial agents tested (Table 5.5). One *S. haemoylticus* isolate was susceptible to all antimicrobial agents tested, but the *qacC* gene, encoding resistance to quaternary ammonium compounds (including ethidium bromide, with which the isolate was tested for resistance to but was found to be susceptible) was detected using the DNA microarray (Table 5.5). Other discrepancies between the antimicrobial resistance phenotype and the presence or absence of a particular gene were noted including (i) the presence of *qacA* (28 isolates), *aacA-aphD* (5 isolate), *qacC* (5 isolates), *dfrS1* (3 isolates), *merAB* (1 isolates), *msr*(A) (1 isolate), *aadD* (1 isolate) and *mupA* (1 isolate) without the associated resistant phenotype, and (ii) resistance to trimethoprim (16 isolates), mercuric chloride (3 isolates), erythromycin (2 isolates), mupirocin (3 isolate), kanamycin (2 isolates), lincomycin (1 isolates), gentamicin (1 isolate), neomycin (1 isolate) and chloramphenicol (1 isolate) with no associated gene detected using the DNA microarray (Table 5.5).

The most common antimicrobial resistance gene detected was the beta-lactamase resistance gene *blaZ* (49/53, 92.5%), followed by *qacA* (41/53, 77.4%), *mecA* (39/53, 73.6%), *aacA-aphD* (25/53, 47.2%) (encoding resistance to gentamicin, kanamycin and tobramycin), *dfrS1* (trimethoprim resistance gene) (23/53, 43.4%), *msr*(A) (21/53, 39.6%) (macrolide resistance gene), *fusB* (21/53, 39.6%) (fusidic acid resistance gene), *erm*(C) (20/53, 37.7%) (macrolide resistance gene) and *mph*(C) (12/53, 22.6%) (macrolide resistance gene) (Figure 5.1a). Other resistance genes detected included *fusC* (10/53, 18.9%) (fusidic acid resistance gene), *mupA* (8/53, 15.1%) (mupirocin resistance gene), *qacC* (7/53, 13.2%) (encoding resistance to quaternary ammonium compounds), *aadD* (6/53, 11.3%) (encoding resistance to amikacin, kanamycin, neomycin and tobramycin), *tet*(K) (5/53, 9.4%) (tetracycline resistance genes) and *merAB* (4/53, 7.5%) (mercury resistance gene) (Figure 5.1a). Four genes were detected in just two isolates each i.e.

(a)


60
50
40
30
20
10
0
No. of isolates
mecA
merAB
blaZ
erm(A)
erm(C)
lun(A)
msr(A)
mpb (C)
vat(B)
vga
vga(A)
aacA-aphD
aadD
aphA-3 & sat
dfrS1
fusB
fusC
mupA
tet(K)
fosB-plasmid
qacA
qacC
Antimicrobial resistance genes
Total (53)
S. epidermidis (32)
S. haemolyticus (7)
S. hominis (10)
S. capitis (4)
S. warneri (2)

(b)

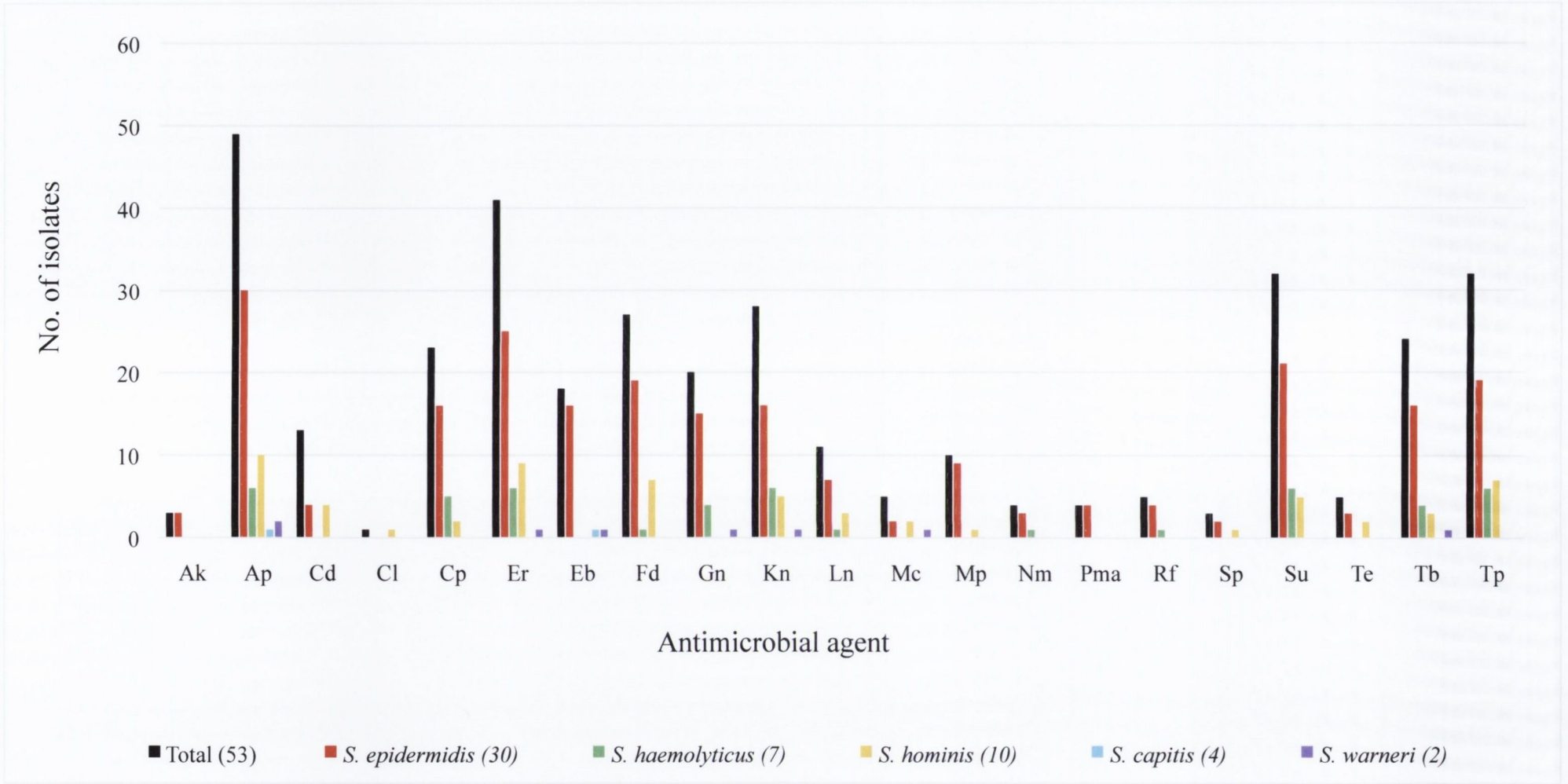

60
50
40
30
20
10
0
No. of isolates
Ak
Ap
Cd
Cl
Cp
Er
Eb
Fd
Gn
Kn
Ln
Mc
Mp
Nm
Pma
Rf
Sp
Su
Te
Tb
Tp
Antimicrobial agent
Total (53)
S. epidermidis (30)
S. haemolyticus (7)
S. hominis (10)
S. capitis (4)
S. warneri (2)

Figure 5.1. Antimicrobial resistance genes (panel a) and phenotypic antimicrobial resistance (panel b) detected among the 53 CoNS isolates investigated. The antimicrobial resistance genes were detected using the StaphyType DNA microarray (Alere). All isolates lacked *erm*(B), *mef*(A), *vat*(A), *vgb, tet*(M), *fexA*, *fosB*, *cfr* and *sdrM*, therefore these genes are not listed in panel (a). The antimicrobial resistance phenotype was determined by investigating the resitance of each isolate to a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadimium nitrate (Cd), chloramphenicol (Cl), ciprofloxacin (Cp), ethidium bromide (Eb), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride (Mc), mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate (Pma), rifampicin (Rf), streptomycin (Sm), spectiomycin (Sp), sulphonamide, tobramycin (Tb), tetracycline (Te), trimethoprim (Tp) and vancomycin. All isolates were susceptible to vancomycin and sulphonamide so the results for these antimicrobial agent are not shown in panel (b).

erm(A), *vat*(B), *vga,* and *fosB*-plasmid. The following were identified in a single isolate each; *lun*(A) and *aphA3 & sat* (Figure 5.1a).

The largest number of antimicrobial resistance genes and resistance to the greatest number of antimicrobial agents were detected among the *S. epidermidis* isolates with 19 of the 31 resistance genes detected by the DNA microarray identified among these isolates. Furthermore, from the panel of 23 antimicrobial agents tested, resistance was seen to all but three (chloramphenicol, sulphonamide and vancomycin) of the antimicrobial agents tested (Figure 5.1a and b). This was followed by *S. hominis* (12 antimicrobial resistance genes detected by the array and phenotypic resistance to 14 antimicrobial agents determined), *S. haemoylticus* (11 antimicrobial resistance genes detected by the array and phenotypic resistance to 14 antimicrobial agents determined), *S. warneri* (four antimicrobial resistance genes detected by the array and phenotypic resistance to seven antimicrobial agents phenotypic resistance determined) and *S. capitis* (two antimicrobial resistance genes detected by the array and phenotypic resistance to two antimicrobial agents determined) (Figures 5.1a and b). Nine antimicrobial resistance genes were detected in signal species of CoNS, with *dfrS1* (23/30), *mupA* (8/30), *vga* (4/30), *merAB* (4/30), *erm*(A) (2/30), and *vat*(B) (2/30) being found only in *S. epidermidis*, *lun*(A) (1/10) and *fosB*-plasmid (2/10) being found only in *S. hominis* and *aphA3 & sat* (1/7) being found in *S. haemolyticus* (Figure 5.1b).

5.3.4.1 Multiresistant CoNS

More than half of the isolates (32/53, 60.4%) exhibited resistance to at least three classes of commonly used antimicrobial agents including fusidic acid, fluoroquinolones, aminoglycosides, macrolides/lincosamides, tetracyclines and mupirocin and were classified as multiresistant. The majority of multiresistant isolates belonged to *S. epidermidis* (22/32, 68.8%) but multiresistant *S. haemolyticus* (5/32, 15.6%) and *S.*

hominis (5/32, 15.6%), were also identified. No *S. capitis or S. warneri* isolates exhibited multiresistance.

Fifteen different mulitiresistance phenotypes were identified among the isolates, with only four combinations being found in multiple species of CoNS (Figure 5.2). The most common multiresistant phenotype was combined resistance to the fluoroquinolone ciprofloxacin (Cp), aminoglycosides (Ag), macrolides/lincosamides (Er/Ln) and fusidic acid (Fd), which was detected in 9/33 (27.3%) of isolates including seven *S. epidermidis* (7/22, 31.8%), one *S. hominis* (1/5, 20%) and one *S. haemolyticus* (1/5, 20%) isolates (Figure 5.2). The second most common multiresistant phenotype included resistance to Cp, Ag and Er/Ln and was detected in 18.2% of isolates (6/33), including four *S. heamolyticus* (4/5, 80%), a *S. epidermidis* (1/22, 4.5%) and a *S. hominis* (1/5, 20%) isolate (Figure 5.2).

Thirteen different multiresistant phenotypes were identified among the *S. epidermidis* isolates, with ten combinations being unique to *S. epidermidis* (Figure 5.2). Five different multiresistant phenotypes were identified among the *S. hominis* isolates, with two being unique to that species (Figure 5.2). Among the *S. haemolyticus* isolates just two multiresistant phenotypes were identified, both of which were also found among *S. epidermidis* isolates and *S. hominis* isolates (Figure 5.2).

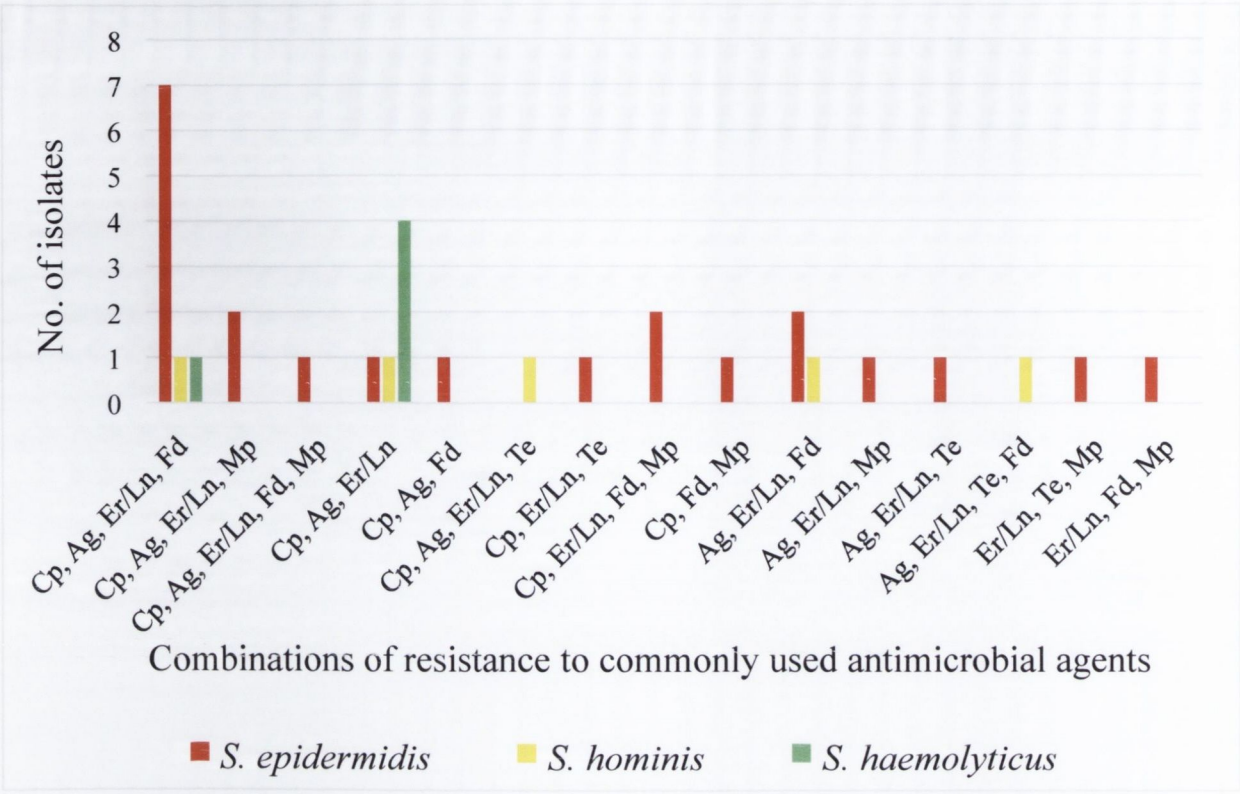


Figure 5.2. Multiresistance phenotypes identified among each species of the multiresistant CoNS isolates investigated. Multiresistance was determined based on resistance to three of more classes of commonly used antimicrobial agents i.e. fusidic acid (Fd), fluoroquinolones (ciprofloxacin, Cp), aminoglycosides (Ag), macrolides/lincosamides (Er/Ln), tetracyclines (Te) and mupirocin (Mp) and 32/53 isolates tested were determined to be multiresistant. Fifteen different multiresistant phenotypes were identified among three species of CoNS (*S. epidermidis, S. hominis* and *S. haemolyticus*).

5.4 Discussion

CoNS are a significant cause of nosocomial infections, particularly among immunocompromised patients. They share their primary ecological niche (i.e. animal and human skin and mucous membranes) with *S. aureus* and represent a reservoir for SCC*mec* and antimicrobial resistance genes in *S. aureus*. It is therefore crucial that in order to control and prevent the spread of CoNS in our hospitals and to provide further insights into the evolution of resistance in *S. aureus* that we have a detailed understanding of the molecular characteristics of CoNS. The results of the present study have highlighted the extensive reservoir of SCC*mec* and SCC genes that exists in CoNS from Irish hospitals with 89% of CoNS isolates investigated (n = 47) carrying SCC*mec* or SCC-associated genes and 81% of these carrying *mecA*. These isolates were originally recovered from infection or reported as contaminants and were not selected on the basis of being methicillin resistante. This result is agreeing with the high rate of methicillin resistance that has been seen in previous studies. Extensive genetic diversity was identified in the SCC and SCC*mec* elements of these isolates with only 45% of isolates harbouring *mecA* being assigned to previously identified SCC*mec* types, predominantly SCC*mec* IV. The remaining 55% of *mecA* positive CoNS isolates were assigned to four possible novel SCC*mec* IV subtypes (16%) and nine novel SCC*mec* types (39%).

Several other studies have also highlighted the diversity of SCC*mec* elements among CoNS species. For example, a study by Ruppe *et al.* (2009) of CoNS recovered from outpatients in four different countries, revealed that 44% of the CoNS harboured SCC*mec* elements that could not be assigned to any of the known SCC*mec* types. There were 15 distinct combinations of *ccr* and *mec* genes identified, two of which were also identified in the present study, namely SCC*mec* type IV with *ccrC* and novel SCC*mec* type 1 (Class A *mec* & *ccrAB1*). Similarly, a study by Zong *et al.* (2011) found that 37% of

clinical CoNS isolates from China were assigned to five possible novel SCC*mec* types, none of which were identified in the present study. A study undertaken by Bouchami *et al.* (2011) revealed that 27% of clinical CoNS could not be assigned to a SCC*mec* type and six novel combinations of *ccr* and *mec* genes were identified, two of which were also identified among isolates in the present study i.e. new1 (class A *mec* & *ccrAB1*) and NT1 (class A *mec*, *ccrAB1* & *ccrAB4*) which were designated novel SCC*mec* types 1 and 7, respectively, in the present study.

The large number of SCC elements identified among the CoNS isolates was surprising. Four different combinations of SCC genes were identified among 17% of the CoNS isolates investigated. One of the possible novel SCC elements identified in a *S. hominis* isolate in the present study, novel SCC type 3 (*ccrAB1*), has been reported previously by Bouchami *et al.* (2011) in two *S. hominis* isolates. SCCs have been shown to harbour additional virulence or antimicrobial resistance genes (Ito *et al.*, 2001; Holden *et al.*, 2004; Monkolrattanothai *et al.*, 2004) but more detailed characterisation of the genetic organisation of these SCC elements is required in order to identify these among the isolates in the present study. Interestingly, in the present study the *S. hominis* isolate carrying *ccrAB1* also harboured *fusC*, which may be indicative of the presence of SCC*fus* (Holden *et al.*, 2004). These results highlight the need for detailed characterisation of the genetic organisation of the novel SCC*mec* and SCC elements in CoNS.

Despite the extensive diversity detected within SCC*mec* in the CoNS isolates investigated, for some of the CoNS species the results supported the theory that certain SCC*mec* types may have originated in particular CoNS species. For example in the present study SCC*mec* IV predominated among *S. epidermidis* isolates and the majority of *S. hominis* isolates carried Class A *mec* and *ccrAB1* with additional *ccr* genes and in both cases these results were similar to previous studies. However, in contrast to previous

studies which have shown that *S. haemolyticus* predominantly carry SCC*mec* V, or the SCC*mec* type V *ccrC* or class C *mec* gene complex individually (Fessler *et al.*, 2010; Barbier *et al.*, 2010; Garza-Gonzalez *et al.*, 2010; Bouchami *et al.*, 2012), for the majority of the *S. haemolyticus* isolates in the present study the *mec* complex type could not be identified as all isolates carried *mecA* only with no other *mec* complex genes detected. The *ccrA4/B4* genes predominated in the *S. haemolyticus* isolates with just one isolate carrying *ccr* genes indicative of class C *mec* (*ccrAA* and *ccrC*). These findings highlight the need for additional studies of SCC*mec* within CoNS species, particularly *S. haemolyticus*, in order to identify predominant SCC*mec* types and genes in order to provide insights into the epidemiology of *S. haemolyticus* and the SCC*mec* reservoirs that exist among CoNS.

ACME was detected in 11% of isolates in the present study. This is surprisingly low compared to other studies of CoNS where a much higher prevalence rate of ACME has been reported, particularly among *S. epidermidis* (Diep *et al.*, 2006; Miragaia *et al.*, 2009; Du *et al.*, 2013). While the majority of ACME-positive isolates were *S. epidermidis* (83.3%), just 16.7% of the *S. epidermidis* isolates in the present study carried ACME. Recently, Du *et al.* (2013) found that ACME was more common among MSSE than MRSE but this was not seen in the present study where ACME was only detected in MRSE. However, it is important to note that the number of MSSE isolates investigated in the present study was low. Du *et al.* (2013) also found that 48% of clinical *S. haemolyticus* isolates carried ACME, while in the present study no *S. haemolyticus* isolates carried ACME. Whether this is due to the low numbers of *S. haemolyticus* isolates identified in the present study or to a truly low prevalence of ACME among *S. haemolyticus* from hospitalised patients requires further studies of additional *S. haemolyticus* isolates. Interestingly, Du *et al.*, also reported a higher rate of carriage of ACME among colonising rather than invasive *S. epidermidis* isolates (88.9% versus 54.8%, respectively). This correlates with animal model studies that have shown that ACME plays a role in

colonisation rather than infection (Montgomery *et al.*, 2009). Therefore, the low prevalence of ACME among isolates in the present study may indicate a greater propensity of these isolates for infection rather than colonisation. It should be noted that some ACME-positive isolates may not have been detected using the DNA microarray system used in the present study because it only detects the ACME-*arc* genes but ACME elements carrying the ACME-*opp* genes only (ACME type III) have also been reported (Miragaia *et al.*, 2009; Du *et al.*, 2013). However, the majority of CoNS reported to date harbour ACME-*arc* genes with (ACME type I) or without (ACME type II) ACME-*opp* genes (Deip *et al.*, 2006; Diep *et al.*, 2008; Miragaia *et al.*, 2009; Du *et al.*, 2013).

An extensive range of antimicrobial resistance genes were also detected among the CoNS isolates investigated with most isolates harbouring at least one antimicrobial resistance gene with an average of four genes per isolate. *Staphylococcus epidermidis* isolates carried the greatest number of resistance genes and accounted for the highest number of multiresistant isolates (65.6%), but multiresistant *S. hominis* and *S. haemolyticus* isolates were also detected. The high prevalence of *qacA* (75%), encoding resistance to antiseptics, identified among CoNS in the present study has also been reported previously (Zhang *et al.*, 2011). Resistance to some commonly used antimicrobial agents in particular fusidic acid, trimethoprim, and macrolides/lincosamides, was also high among CoNS isolates in the present study (58.5%, 39.6% and 37.5%, respectively).

While 39.6%, 9.4% and 58.5% of the CoNS isolates investigated in the present study carried genes encoding resistance to trimethoprim, tetracycline and fusidic acid, respectively, a study by Koksal *et al.* (2006) found a higher rate of trimpethoprim (53%) and tetracycline resistance (53%) among CoNS, mainly *S. epidermids* (43%), *S. heamolyticus* (11.5%) and *S. hominis* (9.5%), from patients with BSIs but a lower rate of fusidic acid resistance (20%). LeBouter *et al.* (2011) found a higher prevalence of genes encoding resistance to macrolides, lincosamides and streptogramin$_B$ compounds, including

msr(A) (73%) and *lnu*(A) (3%), but a lower prevalence of *erm*(C) (15%) among clinical methicillin-resistant *S. saprophyticus* isolates from urine specimens than were identified among the CoNS in the present study (*msr*(A) (39.6%), *erm*(C) (37.5%) and *lnu*(A) (1.8%)). However, while the LeBouter study examined *S. saprophyticus* isolates only, isolates of this species was not included in the present study. The majority of the antimicrobial resistance genes identified among the CoNS in the present study have been identified previously in *S. aureus* isolates, and similar to SCC*mec*, their high prevalence among CoNS suggests that they may be a reservoir for these genes in *S. aureus*.

A number of discrepancies were noted between the antimicrobial resistance phenotype and the presence or absence of a relevant antimicrobial resistance gene in the present study and this may be due to (i) the presence of alternative resistance genes in these isolates that are not detected using the current version of the DNA microarray system e.g. alternative trimethoprim resistance genes: *dfrK, dfrA, dfrD* and *dfrG*, (ii) sequence variation within the CoNS resistance genes that may result in these genes not being detected if this affects the array primer or probe binding sites, (iii) the presence of a resistance gene but with mutations that affect its expression e.g. *dfrS1* or *mupA* gene and (iv) the antimicrobial agent breakpoints used for susceptibility testing in the present study were originally developed for *S. aureus* and not CoNS (Rossney *et al.*, 2007). PCR and sequence analysis of the antimicrobial resistance genes found among these CoNS isolates would help clarify at least some of these discrepancies.

Molecular typing using MLST of the *S. epidermidis* and *S. haemolyticus* isolates investigated in the present revealed insights into the population structure of these species among hospitalised patients in Ireland. Similar to previous MLST studies, the majority of *S. epidermidis* isolates investigated were assigned to a range of STs within a single CC, CC2, which is the most predominant nosocomial *S. epidermidis* lineage reported to date (Miragaia *et al.*, 2007; Miragaia *et al.*, 2008; Mendes *et al.*, 2012; Cherifi *et al.*, 2013; Du

et al., 2013; Hellmark *et al.*, 2013). Furthermore, while all *S. haemolyticus* isolates in the present study were also assigned to a single CC by MLST it was not possible to compare the results to other studies as there is currently no curated on-line database for *S. haemolyticus* MLST. Cavanagh *et al.* (2012) also reported that the majority of *S. haemolyticus* isolates were assigned to one main lineage although it is not possible to determine if it is the same lineage as isolates in the present study. Cavanagh *et al.* (2012) indicated that the MLST scheme that they developed, and which was used in the present study, was not discriminatory enough because many globally-distinct isolates were assigned to the same lineage and that alternative alleles should be investigated in order to improve the discriminatory power of this MLST scheme. PFGE has been considered the gold standard for typing of *S. epidermidis* and *S. haemolyticus* and it should be used in outbreak situations, but there is a need for a more reproducible method of molecular typing, especially for *S. haemolyticus*, to allow population structures to be examined. The use of whole-genome sequencing for typing of CoNS could be a possibility. A study undertaken by Harris *et al.* (2012) used whole-genome sequencing to type isolates from an outbreak of MRSA, but the relative lack of published whole-genomes of CoNS isolates could cause difficulties as there may not be sufficient sequences to use as backbones to scaffold the new sequences against and also to compare SNPs too.

In the present study, high-throughput DNA microarray analysis of CoNS from BSIs provided valuable insights into the antimicrobial and virulence-associated genes harboured by CoNS and the diversity of their SCC and SCC*mec* elements. It also provided further evidence that CoNS are a reservoir not only for SCC and SCC*mec* elements, but also possibly for antimicrobial resistance genes in *S. aureus*. Comparative DNA sequence analysis of these SCC*mec*, antimicrobial genes and ACME in CoNS and *S. aureus* and of the possible MGEs on which they are located will provide further evidence to support this theory. The results of the present study also highlighted the need for whole-genome

sequencing of clinically relevant CoNS species to allow for the optimisation of typing systems for the analysis of CoNS populations.

Chapter 6

Comparative molecular analysis of MRSA and MSSA from bloodstream infections and healthy carriers and MRSA from patients, environmental sources and healthcare workers

6.1 Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) has been endemic in Irish hospitals for almost four decades (Hone *et al.*, 1974; Coleman *et al.*, 1985; Mulvey *et al.*, 1986; Carroll *et al.*, 1989; Humphreys *et al.*, 1990; Rossney *et al.*, 2002; Shore *et al.*, 2005; Rossney *et al.*, 2006; Shore *et al.*, 2010). Since 1999 the prevalence of MRSA among *S. aureus* isolates from bloodstream infections (BSIs) in Ireland has been monitored by EARS-Net. The annual prevalence rate of MRSA among *S. aureus* isolates from BSIs in Ireland reached 42% in 2006, the highest prevalence rate reported to date in Ireland and at the time this was one of the highest prevalence rates of MRSA in Europe (EARS-Net, 2006). However, between 2006 and 2012, the prevalence rate of MRSA among *S. aureus* isolates from BSIs in Ireland decreased 1.8-fold, to 22.8% (EARS-Net, 2013). In contrast, during the same time period the prevalence of MSSA among *S. aureus* isolates from BSIs in Ireland increased 1.3-fold (EARS-Net, 2013).

Despite the increasing prevalence of MSSA among *S. aureus* isolates from BSIs, there is only limited typing data on MSSA isolates from Ireland. A European-wide study was undertaken during 2006 and 2007 to investigate the *spa* types of *S. aureus* isolates, both MRSA and MSSA, from patients with BSIs in hospitals throughout Europe (Grundmann *et al.*, 2009). The first five MRSA and MSSA isolates from individual patients with BSIs in 22 Irish hospitals during the six-month study period from September 2006 to February 2007 were included in the study resulting in 85 MSSA and 84 MRSA isolates being investigated (Grundmann *et al.*, 2009). The study revealed that the MSSA isolates recovered from BSIs were more genetically diverse than the MRSA isolates (Grundmann *et al.*, 2009). A total of 55 and 26 *spa* types were identified among the MSSA and MRSA isolates recovered from patients in Irish hospitals with BSIs, respectively (Grundmann *et al.*, 2009). Another study examined MSSA isolates recovered from the anterior nares of students attending an Irish University between 1999 and 2005 and

investigated the isolates using multiple locus variable number tandem repeat analysis (MLVA), MLST and PCR for the detection of 16 enterotoxin and enterotoxin-like genes and the toxic shock syndrome toxin-1 gene (Collery *et al.*, 2008). Similar to the MSSA isolates from BSIs, extensive genetic diversity was identified among MSSA isolates from the anterior nares of healthy carriers (Collery *et al.*, 2008). The Collery *et al.* (2008) study also revealed that all MSSA isolates investigated harboured at least one enterotoxin or enterotoxin-like gene. Since nasal carriage of *S. aureus* is a known risk factor for invasive *S. aureus* infections (von Eiff *et al.*, 2001; Wertheim *et al.*, 2005) a detailed comparative molecular analysis of MSSA isolates from healthy carriers and BSIs is warranted. Previous studies outside of Ireland have focused on comparing MRSA and MSSA isolates from infections or MSSA isolates from infections or healthy carriers but there have been no studies with detailed molecular analysis of *S. aureus* isolates from both BSIs and healthy carriers (Hallin *et al.*, 2007; Argudin *et al.*, 2009; Megavard *et al.*, 2010; Lozano *et al.*, 2012; Argudin *et al.*, 2013; Jimenez *et al.*, 2013). Since the Grundmann *et al.* (2009) and Collery *et al.* (2008) studies used different typing methods to investigate MSSA isolates from BSIs and from healthy carriers, comparison of the MSSA isolates from these two studies is difficult. Furthermore, neither study examined the MSSA isolates for the presence of antimicrobial resistance genes and only the study of MSSA isolates from healthy carriers (Collery *et al.*, 2008) investigated the presence of virulence genes, albeit a limited number. Lastly, many different studies have shown that MRSA arise from MSSA through the acquisition of SCC*mec* (Enright *et al.*, 2002; Hallin *et al.*, 2007; Argudin *et al.*, 2009), again highlighting the importance of detailed molecular characterisation and comparative analysis of MSSA and MRSA isolates.

Staphylococcus aureus isolates can harbour and express a wide range of virulence genes including genes encoding toxins that contribute to its ability to cause disease. The staphylococcal superantigens, which include the enterotoxins and the toxic shock

syndrome toxin (TSST-1), cause non-specific activation of T-cells resulting in a massive release of cytokines from the host macrophages and T cells. Toxic shock syndrome is characterised by a high fever, rash, hypotension, and the involvement of multiple organ systems and other tissues (i.e. gastrointestinal tract, muscular, renal, hepatic and mucous membranes) and can result in shock and multiple organ failure which can be fatal (Fraser *et al.*, 2008; Dinges *et al.,* 2000). The TSST-1 toxin is encoded by the *tst* gene and is encoded on several different pathogenicity islands (Kreiswirth *et al.,* 1989; Lindsay *et al.*, 1998; Ubeda *et al.,* 2003). A number of studies have shown that a high percentage (30-47%) of *S. aureus* isolates carried in the nares of healthy individuals harbour *tst* (Megevand *et al.*, 2010; Lozano *et al.*, 2011; Argudin *et al.*, 2013). However, there are very few studies that have investigated the expression levels of *tst* among *S. aureus* isolates (Chini *et al.*, 2007; Smyth *et al.,* 2010; Qiu *et al.*, 2010; Qiu *et al.*, 2011). A study performed by Chini *et al.* (2007) investigated *tst* expression levels among clinical *S. aureus* isolates and revealed slightly higher expression levels of *tst* among isolates from skin and soft tissue infections compared to invasive infections, but the isolates were MRSA and no *S. aureus* isolates recovered from healthy carriers were investigated.

It is widely known that MRSA can survive in various environmental reservoirs, especially those close to patients such as beds and lockers (Wilson *et al.,* 2007; Sherlock *et al.,* 2008; Stiefel *et al.*, 2011). It is also known that healthcare workers can play a key role in the transmission of MRSA via their hands (Creamer *et al.*, 2010). Studies performed in the USA using PFGE have revealed indistinguishable strains of MRSA from patients, environmental sites and healthcare workers (Schweizer *et al.*, 2012). While a recent Irish study investigated the genetic relatedness of isolates recovered from patients and environmental sites using *spa*, *dru* and PFGE typing (Shore *et al.,* 2010), there have been no comparative studies that examined the genetic relatedness of isolates from patients,

environmental sites and hand carriage isolates from healthcare workers to show evidence of transmission between the three groups in a hospital setting.

Since 2002 a single clone, ST22-MRSA-IV, has predominated in Irish hospitals, accounting for 70-80% of all MRSA isolates from BSIs each year (NMRSARL, 2007; Shore *et al.,* 2010). Previous studies have highlighted the difficulties associated with differentiating between highly clonal ST22-MRSA-IV isolates in an endemic setting using conventional typing methods such as PFGE, *spa* and *dru* typing and how the combination of these three methods can enhance discrimination (Simpson's Index of Diversity (SID) 96.53%) (Shore *et al.*, 2010). However, combining these three typing methods is time consuming, data analysis can be difficult, particularly for PFGE, and these methods provide no information regarding the virulence or resistance gene content of isolates. In contrast, while the use of DNA microarray profiling combined with *spa* typing has a slightly lower discriminatory power (SID 91.10%) for differentiating ST22-MRSA-IV isolates than the combination of PFGE, *spa* and *dru* typing, it has a similar SID to *spa* and PFGE typing combined (SID 91.00%) and it has been shown to provide informative and accurate differentiation of ST22-MRSA-IV isolates while also detecting a range of clinically relevant virulence and resistance genes (Brennan, 2013).

Due to the increasing prevalence and limited genetic information available in relation to the molecular characteristics and population structure of MSSA from patients with BSIs and from healthy carriers in Ireland, the first aim of this part of the present study was to perform a comparative molecular analysis of MSSA isolates recovered from both healthy carriers and from BSIs using *spa* typing and DNA microarray profiling and to compare the results to those from MRSA isolates recovered from similar groups i.e. healthy carriers and BSIs. Because the results of this study revealed that *tst* was more common among MSSA from healthy carriers than from patients with BSIs this study also

aimed to compare the *tst*-expression levels among MSSAs from both groups. The final aim of this part of the present study was to investigate the genetic relatedness of MRSA isolates recovered from the hands of healthcare workers, from patients and from environmental sites in one Dublin hospital using *spa* typing and DNA microarray profiling and to assess the role that each of these sources play in the transmission of *S. aureus* in hospitals.

6.2 Material and Methods

6.2.1 *Staphylococcus aureus* isolates investigated

6.2.1.1 MRSA and MSSA isolates from bloodstream infections

Eighty-three MRSA and 85 MSSA isolates recovered from patients with bloodstream infections (BSIs) in Irish hospitals were investigated (Tables 6.1 and 6.2). The isolates were recovered between 1st September 2006 and 28th of February 2007 as part of a European *spa* typing study and had previously underwent antimicrobial susceptibility testing and *spa* typing and the MRSA isolates had also previously been typed by PFGE (Brennan, 2009; Grundman *et al.,* 2009) (Tables 6.1 and 6.2).

6.2.1.2 MRSA and MSSA isolates recovered from healthy carriers

Fifty-three MRSA and 98 MSSA isolates recovered from healthy carriers were also investigated (Tables 6.1 and 6.2). The 53 MRSA isolates were recovered from the anterior nares of healthcare workers (NHCWs) in a Dublin hospital between 2002 and 2009 (Table 6.1) and the 98 MSSA isolates were recovered from the anterior nares of undergraduate students attending a Dublin University between 1994-2005 (Collery *et al.*, 2008) and 2009-2013 (Table 6.2).

6.2.1.3 MRSA isolates recovered from healthcare workers and from patient and environmental sites

Thirty-eight MRSA isolates recovered from the hands of healthcare workers (HHCWs), 82 MRSA isolates recovered from patients and 62 MRSA isolates recovered from environmental sites were also investigated (Table 6.3). The 38 isolates from the HHCWs were recovered in a large acute Dublin hospital between February and September 2009 (Creamer *et al.*, 2010). The patient and environmental isolates were recovered in the same large acute Dublin hospital in 2009 (Table 6.3). The ward and sample location for

Table 6.1. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from bloodstream infections and the nares of healthcare workers

MRSA isolate group	Year(s) of isolation	Isolate no.	AR type (AR pattern)[a]	PFT[b]	*spa* type[c]	Reference
Bloodstream infections (BSIs) (n = 83)	2006-2007	E2995/SpaR001	AR06.1 (ApCp)	01024	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2996/SpaR002	AR06.3 (ApCd)	01059	t022	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3008/SpaR003	AR06.5 (ApCdCpErFd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3043/SpaR004	AR06.6 (ApCpEr)	01042	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3093/SpaR005	AR06.5 (ApCdCpEr)	01042	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3063/Spar006	AR07.3 (ApCpErFdKnNmSpTb)	03027	t463	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3064/SpaR007	Unf (AkApCdClCpErGnKnRf SmSpTbTe)	99048	t030	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3065/SpaR008	Unf (ApCdCpErGnKnMpTb)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3066/SpaR009	NT (ApCpErFdLn)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3067/SpaR010	AR06.3 (ApCpCd)	01039	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3036/SpaR011	AR13 (ApCdErGnKnLnNmSmSpTb)	00111	t2942	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3046/SpaR012	AR06.5 (ApCpCdFd)	01024	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3069/SpaR013	AR06.1 (Ap)	01039	t022	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3085/SpaR014	AR06.5 (ApCpCdEr)	01024	t1214	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3090/SpaR015	AR07.4 (ApCpErKnLnNm SpTb)	03027	t045	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3000/SpaR016	AR06.5 (ApCdCpErPlr)	01024	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3018/SpaR017	AR06.5 (ApCdCpErFd)	01039	t785	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3017/SpaR018	AR13.1 (ApCdCpEbErGnKnLnMcNm PmaSmSpTb)	00002	t190	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3019/SpaR019	AR06.5 (ApCdCpErFd)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3038/SpaR020	AR06.5 (ApCdCpEr)	01125	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3262/SpaR021	AR06.6 (ApCpEr)	01039	t885	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3119/SpaR022	AR06.5 (ApCdCpEr)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3120/SpaR023	AR06.5 (ApCdCpErFd)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3140/SpaR024	AR06.5 (ApCdCpEr)	01018	t022	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3193/SpaR025	AR06.5 (ApCdCpErFd)	01042	t1802	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3005/SpaR026	AR06.3 (ApCdCp)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3029/SpaR027	AR06.6 (ApCpEr)	01001	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3028/SpaR028	AR06.3 (ApCdCp)	01088	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3117/SpaR029	AR06.5 (ApCdCpEr)	01042	t1292	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3168/SpaR030	AR06.5 (ApCdCpE)	01039	t1214	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2992/SpaR031	AR06.5 (ApCdCpEr)	01017	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3156/SpaR032	AR06.1 (ApCp)	01039	t885	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3206/SpaR033	AR06.1 (ApCp)	01018	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3230/SpaR034	AR06.6 (ApCpErFd)	01120	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3020/SpaR035	AR06.1 (ApCp)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3027/SpaR036	AR06.6 (ApCpEr)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010

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Table 6.1 continued. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from bloodstream infections and the nares of healthcare workers

MRSA isolate group	Year(s) of isolation	Isolate no.	AR type (AR pattern)[a]	PFT[b]	*spa* type[c]	Reference
Bloodstream infections (BSIs) (n = 83)	2006-2007	E3049/SpaR037	AR06.5 (ApCdCpEr)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3152/SpaR039	AR06.1 (ApCp)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3114/SpaR040	AR06.5 (ApCdCpEr)	01042	t2945	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3110/SpaR041	AR14.1 (ApCdCpEbErGnKnMcNm PmaSmSpTbTp)	00055	t190	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3111/SpaR042	AR06.5 (ApCpEr	01024	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3165/SpaR043	AR06.5 (ApCdCpEr)	01039	t025	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3163/SpaR044	AR06.5 (ApCdCpEr)	01122	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2999/SpaR045	AR06.3 (ApCdCpFd)	01022	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2998/SpaR046	NT (ApFd)	99542	t878	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3022/SpaR047	AR06.6 (ApCpErFd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3023/SpaR048	AR06.6 (ApCpErFd)	01039	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3026/SpaR049	AR06.5 (ApCdCpErFd)	01018	t020	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3086/SpaR050	AR06.5 (ApCpCd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3286/SpaR051	AR06.3 (ApCdCpEr)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2994/SpaR052	AR06.5 (ApCdCpErFd)	01021	t747	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3121/SpaR053	AR06.5 (ApCdErFdPlr)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3122/SpaR054	AR06.6 (ApErFd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3125/SpaR055	AR06.5 (ApCdCpEr)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3126/SpaR056	AR06.5 (ApCdCpEr)	01039	t2945	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3127/SpaR057	AR06.5 (ApCdCpEr)	01018	t718	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3033/SpaR058	AR06.1 (ApCpFdPlr)	01076	t1802	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3032/SpaR059	AR06 (ApCdCpErFdTe)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3047/SpaR060	AR06.6 (ApCpEr)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3048/SpaR061	AR06.3 (ApCdC)	01039	t020	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3089/SpaR062	AR06.5 (ApCdCpClEr)	01072	t2951	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3188/SpaR063	AR06 (ApCdCpErMp)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3189/SpaR064	AR06.6 (ApCpEr)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3186/SpaR065	AR06.5 (ApCdCpEr)	01018	t1865	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3190/SpaR066	NT (ApCdCpErFd)	99513	t727	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3187/SpaR067	AR06.5 (ApCdCpEr)	01039	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3104/SpaR068	06.1 (ApCp)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3150/SpaR069	AR14.1 (ApCdCpEbErGnKnMcNm PmaSpTbTP)	00071	t190	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3166/SpaR070	AR14.2 (ApCdCpEbErGnKnMcNm PmaSmSpTb)	00071	t190	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3194/SpaR071	AR06.6 (ApCpEr)	01039	t025	Brennan, 2009; Grundmann *et al.*, 2010

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Table 6.1 continued. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from bloodstream infections and the nares of healthcare workers

MRSA isolate group	Year of isolation	Isolate no.	AR type (AR pattern)[a]	PFT[b]	*spa* type[c]	Reference
Bloodstream infections (BSIs) (n = 83)	2006-2007	E3024/SpaR073	AR06 (ApCpErFdMpTp)	01024	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3071/SpaE074	AR06.6 (ApCpEr)	01018	t578	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3072/SpaR075	AR06.6 (ApCpEr)	01039	t025	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	SpaR076	NT (ApCdErFdLnSp)	99523	t045	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3237/SpaR077	AR06.5 (ApCdCpEr)	01088	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3272/SpaR078	AR06.5 (ApCdCpEr)	01130	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3398/SpaR079	AR06.6 (ApCpErFd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3399/SpaR080	AR06.6 (ApCpErFd)	01018	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E2997/SpaR081	AR06.5 (ApCdCpEr)	01039	t515	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3006/SpaR082	Unf (ApCpErGnKnLnNmSuTbTeTp)	n/d	t064	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3035/SpaR083	AR06.1 (ApCp)	01018	t2231	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3042/SpaR084	AR06.3 (ApCpCd)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
	2006-2007	E3084/SpaR085	AR06.5 (ApCdCpErRf)	01039	t032	Brennan, 2009; Grundmann *et al.*, 2010
Nares of healthcare workers (NHCWs) (n = 53)	2002-2009	M00/0033	AR06.5 (ApCdCpEr)	n/d	t032	This study
	2002-2009	M02/0047	AR13.1 (ApCdCpEbErGnKnLnMcNmPmaSmSpTb)	n/d	t190	This study
	2002-2009	M02/0075	AR06.5 (ApCdCpEr)	n/d	t032	This study
	2002-2009	M02/0076	AR06.5 (ApCdCpEr)	n/d	t032	This study
	2002-2009	M02/0077	AR06.6 (ApCpEr)	n/d	t032	This study
	2002-2009	M03/0054	AR06.3 (ApCdCp)	n/d	t032	This study
	2002-2009	M03/0055	AR06.6 (ApCpEr)	n/d	t032	This study
	2002-2009	M03/0094	AR44 (AkApCdCpEbErGnKnLnMcNmPmaSmSpSuTbTeTp)	n/d	t037	This study
	2002-2009	M03/0097	AR07 (ApCdCpErKnLnMnSpTb)	n/d	t018	This study
	2002-2009	M04/0222	AR06.5 (ApCdCpEr)	n/d	t718	This study
	2002-2009	M04/0273.1y	AR06.6 (ApEr)	n/d	t1378	This study
	2002-2009	M04/0287	New03 (ApCdCpEbErGnKnLnMcPmaSpTb)	n/d	t190	This study
	2002-2009	M04/0291	AR06 (ApCpErTe)	n/d	t032	This study
	2002-2009	M04/0292	AR06.1 (ApCp)	n/d	t8684	This study
	2002-2009	M04/0294	AR06.1 (ApCp)	n/d	t032	This study
	2002-2009	M04/0295	New03 (ApCdCpEbErGnKnLnMcPmaSpTb)	n/d	t190	This study
	2002-2009	M04/0298.2y	AR06.6 (ApErFd)	n/d	t022	This study
	2002-2009	M05/0195	AR06.5 (ApCdCpEr)	n/d	t032	This study
	2002-2009	M05/0196	AR06.5 (ApCdCpEr)	n/d	t032	This study
	2002-2009	M06/0100	AR06.3 (ApCdCpFd)	n/d	t030	This study
	2002-2009	M06/0370	AR06.6 (ApCpEr)	n/d	t030	This study
	2002-2009	M07/0161	NT (Ap)	n/d	t002	This study
	2002-2009	M07/0213	AR06.3 (ApCdCp)	n/d	t020	This study
	2002-2009	M07/0369	AR06.6 (ApCpErFd)	n/d	t515	This study
	2002-2009	M07/0402	AR06.5 (ApCdCpEr)	n/d	t022	This study
	2002-2009	M08/0142	AR06.5 (ApCdCpEr)	n/d	t515	This study
	2002-2009	M08/0145	AR06.3 (ApCdCp)	n/d	t032	This study
	2002-2009	M08/0153	Unf (ApCdTeTp)	n/d	t318	This study
	2002-2009	M08/0158	NT (ApErFd)	n/d	t002	This study
	2002-2009	M08/0177	AR06.5 (ApCdCpEr)	n/d	t2645	This study
	2002-2009	M08/0207	AR06.6 (ApCpErFd)	n/d	t032	This study
	2002-2009	M08/0208	AR06.6 (ApCpEr)	n/d	t515	This study
	2002-2009	M08/0209	AR06.6 (ApCpErFd)	n/d	t032	This study
	2002-2009	M08/0210	AR06.1 (ApCp)	n/d	t515	This study
	2002-2009	M08/0211	NT (ApCdCpErFdLn)	n/d	t032	This study
	2002-2009	M08/0212	AR06.6 (ApCpErFd)	n/d	t032	This study
	2002-2009	M08/0213	AR06.1 (ApCp)	n/d	t515	This study
	2002-2009	M08/0214	AR06.3 (ApCdCp)	n/d	t032	This study
	2002-2009	M08/0215	AR06.1 (ApCp)	n/d	t515	This study

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Table 6.1 continued. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from bloodstream infections and the nares of healthcare workers

MRSA isolate group	Year of isolation	Isolate no.	AR type (AR pattern)[a]	PFT[b]	*spa* type[c]	Reference
Nares of healthcare workers (NHCWs) ($n = 53$)	2002-2009	M08/0216	AR06.6 (ApCpErFd)	n/d	t032	This study
	2002-2009	M08/0217	AR06.6 (ApCpEr)	n/d	t032	This study
	2002-2009	M08/0302	AR06.5 (ApCdCpErFd)	n/d	t032	This study
	2002-2009	M09/0022	AR06.5 (ApCdCpErFd)	n/d	t032	This study
	2002-2009	M09/0023	AR06.5 (ApCdCpErFd)	n/d	t515	This study
	2002-2009	M09/0025	AR06.3 (ApCdCp)	n/d	t3444	This study
	2002-2009	M09/0100	NT (ApCpErLn)	n/d	t611	This study
	2002-2009	M09/0101	AR06.6 (ApCpEr)	n/d	t515	This study
	2002-2009	M09/0152	06.1 (ApCp)	n/d	t032	This study
	2002-2009	M09/0153	NT (ApCp)	n/d	t008	This study
	2002-2009	M09/0236	AR06.1 (ApCp)	n/d	t032	This study
	2002-2009	M09/0237	NT (ApCdCpFd)	n/d	t727	This study
	2002-2009	M09/0239	AR06.6 (ApCpErFd)	n/d	t032	This study
	2002-2009	M09/0264	AR06.5 (ApCdCpEr)	n/d	t032	This study

[a]The antibiogram resistogram (AR) type and pattern was determined by AR typing (Rossney *et al.,* 2007) which involves determining the susceptibility of isolates to a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadmium nitrate (Cd), chloramphenicol (Cl), ciprofloxacin (Cp), ethidium bromide (EB), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercurie chloride (Mc), mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate (Pma), rifampicin (Rf), streptomycin (Sm), spectiomycin (Sp), sulphonamide (Su), tobramycin (Tb), tetracycline (Te), trimethoprim (Tp) and vancomycin. The AR typing of all MRSA isolates from patients with BSIs was performed as part of a previous study (Brennan, 2009).

[b]Pulsed-field gel electrophoresis (PFGE) profiles were assigned 5-digit pulsed field type (PFT) numbers as described previously (Rossney *et al.*, 2006) i.e. related PFGE patterns were assigned blocks of 1000 numbers starting at 00001, so those sharing ≤ 7 bands were assigned to a particular series of related PFTs e.g. 00001-00999 or 01000-01999. Sporadic patterns are assigned numbers within the series 99,000-99,999 in blocks of 100 numbers so that numbers are reserved for each sporadically occurring pattern to cater for future variants of such a pattern. PFGE typing of all MRSA isolates from patients with BSIs was performed as part of a previous study (Brennan, 2009).

[c]The MRSA isolates recovered from bloodstream infections underwent *spa* typing as part of a European *spa* typing (Grundmann *et al.*, 2010). All other isolates were *spa* typed as part of the present study.

Abbreviations; n/d, not determined; Unf, unfamiliar AR pattern or variant of a known AR pattern; NT, non-typeable AR type pattern indicative of AR06 but urease positive; PFT, pulsed-field type as determined by PFGE.

Table 6.2. Methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates recovered from bloodstream infections and the nares of healthy carriers

MSSA isolate group	Year of isolation	Isolate no.	AR pattern[a]	*spa* type[b]	Reference
Bloodstream infections (BSIs) (n = 85)	2006-2007	SpaS001	Fd	t088	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS002	Susceptible	t160	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS003	SuTp	t2939	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS004	ApCd	t166	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS005	ApFd	t127	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS007	Ap	t279	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS008	Ap	t2941	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS009	ApCd	t1414	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS010	ApCd	t382	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS011	ApCd	t078	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS012	ApCd	t223	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS013	Ap	t015	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS014	Ap	t230	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS015	Ap	t548	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS016	Ap	t331	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS017	ApLnNnTbTe	t091	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS018	ApCd	t2944	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS019	ApCd	t012	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS020	ApCd	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS021	ApCd	t089	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS022	Ap	t548	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS023	Ap	t179	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS024	Ap	t026	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS025	Ap	t026	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS026	ApCd	t012	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS027	ApCd	t382	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS028	ApFdTe	t008	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS029	ApSu	t071	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS030	ApErFd	t088	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS031	ApErFd	t065	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS032	ApCd	t2787	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS033	ApCd	t166	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS034	Ap	t2094	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS035	ApMpTb	t362	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS036	Susceptible	t065	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS037	Ap	t078	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS038	ApCd	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS039	Ap	t005	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS040	ApCd	t012	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS041	Ap	t018	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS042	ApCpErKnLnNmSpTb	t002	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS043	Ap	t005	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS044	Ap	t2143	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS045	Ap	t091	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS046	ApErFdLn	t1391	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS047	ApCpGnKnSuTb	t2658	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS048	Ap	t2360	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS049	ApFd	t008	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS050	ApCp	t2948	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS051	ApCd	t089	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS052	Ap	t002	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS053	ApCd	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS054	Ap	t772	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS055	ApErFdSp	t127	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS056	ApCd	t2950	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS057	ApCd	t2938	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS058	ApFd	t171	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS059	Ap	t230	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS060	Ap	t349	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS061	ApErFdSp	t1321	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS062	CdErSp	t338	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS063	AkApGnKnLnMpNmTb	t024	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS064	Ap	t2940	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS065	Ap	t084	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS066	Ap	t382	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS068	ApER	t209	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS069	Ap	t084	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS071	ApCd	t1827	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS072	ApCd	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS073	ApCd	t166	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS074	ApFd	t171	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS075	Ap	t548	Brennan, 2009; Grundmann *et al* 2010

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Table 6.2 continued. Methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates recovered from bloodstream infections and the nares of healthy carriers

MSSA isolate group	Year of isolation	Isolate no.	AR pattern[a]	*spa* type[b]	Reference
Bloodstream Infection (BSIs) (n = 85)	2006-2007	SpaS076	Ap	t216	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS077	Susceptible	t127	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS078	Ap	t449	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS079	Susceptible	t2943	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS080	Ap	t2946	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS081	ApCd	t012	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS082	Cd	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS083	ApCdER	t209	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS084	ApTe	t2947	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS085	ApCd	t399	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS086	Susceptible	t2949	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS087	Susceptible	t021	Brennan, 2009; Grundmann *et al* 2010
	2006-2007	SpaS088	Susceptible	t088	Brennan, 2009; Grundmann *et al* 2010
Student nasal carriage (Healthy carriers, HCs) (n = 98)	1995	MC08	n/d	t852	Collery *et al.*, 2008
	1995	MC26	n/d	t018	Collery *et al.*, 2008
	1995	MC40	n/d	t364	Collery *et al.*, 2008
	1995	MC43	n/d	t207	Collery *et al.*, 2008
	1995	MC44	n/d	t084	Collery *et al.*, 2008
	1995	MC45	n/d	t8682	Collery *et al.*, 2008
	1997	MC14	n/d	t230	Collery *et al.*, 2008
	1997	MC17	n/d	t012	Collery *et al.*, 2008
	1997	MC31	n/d	t209	Collery *et al.*, 2008
	1997	MC35	n/d	t3914	Collery *et al.*, 2008
	1997	MC37	n/d	t005	Collery *et al.*, 2008
	2000	MC18	n/d	t3964	Collery *et al.*, 2008
	2000	MC19	n/d	t275	Collery *et al.*, 2008
	2000	MC20	n/d	t230	Collery *et al.*, 2008
	2000	MC22	n/d	t8685	Collery *et al.*, 2008
	2000	MC23	n/d	t160	Collery *et al.*, 2008
	2000	MC24	n/d	t275	Collery *et al.*, 2008
	2000	MC36	n/d	t019	Collery *et al.*, 2008
	2002	MC25	n/d	t408	Collery *et al.*, 2008
	2002	MC13	n/d	t084	Collery *et al.*, 2008
	2002	MC39	n/d	t548	Collery *et al.*, 2008
	2003	MC02	n/d	t8681	Collery *et al.*, 2008
	2003	MC28	n/d	t085	Collery *et al.*, 2008
	2003	MC41	n/d	t002	Collery *et al.*, 2008
	2004	MC03	n/d	t089	Collery *et al.*, 2008
	2004	MC06	n/d	t015	Collery *et al.*, 2008
	2004	MC07	n/d	t089	Collery *et al.*, 2008
	2004	MC27	n/d	t1067	Collery *et al.*, 2008
	2004	MC30	n/d	t002	Collery *et al.*, 2008
	2004	MC32	n/d	t7785	Collery *et al.*, 2008
	2004	MC33	n/d	t3914	Collery *et al.*, 2008
	2004	MC34	n/d	t160	Collery *et al.*, 2008
	2004	MC38	n/d	t002	Collery *et al.*, 2008
	2004	MC42	n/d	t8683	Collery *et al.*, 2008
	2004	MC48	n/d	t701	Collery *et al.*, 2008
	2011	ED01	n/d	t382	This study
	2011	ED02	n/d	t5598	This study
	2011	ED03	n/d	t166	This study
	2011	ED04	n/d	t451	This study
	2011	ED05	n/d	t018	This study
	2011	ED06	n/d	t166	This study
	2011	ED07	n/d	t342	This study
	2011	ED08	n/d	t012	This study
	2011	ED09	n/d	t133	This study
	2011	ED10	n/d	t089	This study
	2011	ED11	n/d	t065	This study
	2011	ED12	n/d	t3258	This study
	2011	ED13	n/d	t127	This study
	2011	ED14	n/d	t342	This study
	2011	ED15	n/d	t056	This study
	2011	ED16	n/d	t2275	This study
	2011	ED17	n/d	t1250	This study
	2011	ED18	n/d	t9567	This study
	2011	ED19	n/d	t002	This study

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Table 6.2 continued. Methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates recovered from bloodstream infections and the nares of healthy carriers

MSSA isolate group	Year of isolation	Isolate no.	AR pattern[a]	*spa* type[b]	Reference
Student nasal carriage (Healthy carriers, HCs) ($n = 98$)	2011	ED20	n/d	t012	This study
	2011	ED21	n/d	t012	This study
	2011	ED22	n/d	t032	This study
	2011	M11/0171	n/d	t022	This study
	2011	OB02	n/d	t2923	This study
	2011	OB03	n/d	t2923	This study
	2012	ED23	n/d	t11988	This study
	2012	ED24	n/d	t408	This study
	2012	ED25	n/d	t002	This study
	2012	ED26	n/d	t012	This study
	2012	ED27	n/d	t338	This study
	2012	ED28	n/d	t339	This study
	2012	ED29	n/d	t774	This study
	2012	ED30	n/d	t127	This study
	2012	ED31	n/d	t085	This study
	2012	ED32	n/d	t320	This study
	2012	ED33	n/d	t021	This study
	2012	ED34	n/d	t021	This study
	2012	ED35	n/d	t084	This study
	2012	ED36	n/d	t1574	This study
	2012	ED37	n/d	t9050	This study
	2012	ED38	n/d	t015	This study
	2012	ED39	n/d	t189	This study
	2012	ED40	n/d	t159	This study
	2012	ED41	n/d	t166	This study
	2012	ED42	n/d	t2049	This study
	2012	ED43	n/d	t050	This study
	2012	ED44	n/d	t1510	This study
	2012	ED45	n/d	t383	This study
	2012	ED46	n/d	t012	This study
	2012	ED47	n/d	t1491	This study
	2012	ED48	n/d	t233	This study
	2012	ED49	n/d	t316	This study
	2012	ED50	n/d	t330	This study
	2013	ED51	n/d	t216	This study
	2013	ED52	n/d	t156	This study
	2013	ED53	n/d	t230	This study
	2013	ED54	n/d	t11655	This study
	2013	ED55	n/d	t002	This study
	2013	ED56	n/d	t805	This study
	2013	ED57	n/d	t822	This study

[a]Antibiogram resistogram (AR) patterns were determined by AR typing (Rossney *et al.*, 2007) which involves determining the susceptibility of isolates to a panel of 23 antimicrobial agents including amikacin (Ak), ampicillin (Ap), cadmium nitrate (Cd), chloramphenicol, ciprofloxacin (Cp), ethidium bromide, erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Kn), lincomycin (Ln), mercuric chloride, mupirocin (Mp), neomycin (Nm), phenyl mercuric acetate, rifampicin, streptomycin, spectiomycin (Sp), sulphonamide (Su), tobramycin (Tb), tetracycline (Te), trimethoprim and vancomycin. Isolates that were susceptible to all antimicrobial agents tested are indicated as "susceptible". The AR typing of all MSSA isolates from patients with BSIs was performed as part of a previous study (Brennan, 2009).

[b]The MSSA isolates recovered from bloodstream infections underwent *spa* typing as part of a European *spa* typing study (Grundmann *et al.*, 2010). All other isolates were *spa* typed as part of the present study.

Abbreviations; n/d, not determined

Table 6.3 Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from the hands of healthcare workers, patients and environmental sites in nine wards in one Dublin hospital in 2009

MRSA isolate group	Isolate no.[a]	Ward	Source	*spa* type[b]	Reference
Hands of healthcare workers (HHCWs) ($n = 38$)	07382	D	n/a	t032	Creamer *et al.*2010
	07486	H	n/a	t032	Creamer *et al.*2010
	07489	H	n/a	t032	Creamer *et al.*2010
	07506	H	n/a	t032	Creamer *et al.*2010
	07525	D	n/a	t8679	Creamer *et al.*2010
	07547	I	n/a	t032	Creamer *et al.*2010
	07610	B	n/a	t032	Creamer *et al.*2010
	07649	I	n/a	t032	Creamer *et al.*2010
	07696	C	n/a	t032	Creamer *et al.*2010
	07706	C	n/a	t032	Creamer *et al.*2010
	07789	E	n/a	t3185	Creamer *et al.*2010
	07791	E	n/a	t032	Creamer *et al.*2010
	07804	E	n/a	t032	Creamer *et al.*2010
	07806	E	n/a	t032	Creamer *et al.*2010
	07809	E	n/a	t3185	Creamer *et al.*2010
	07813	E	n/a	t032	Creamer *et al.*2010
	07814	E	n/a	t032	Creamer *et al.*2010
	07821	E	n/a	t3185	Creamer *et al.*2010
	07833	E	n/a	t032	Creamer *et al.*2010
	07838	E	n/a	t032	Creamer *et al.*2010
	10029	F	n/a	t032	Creamer *et al.*2010
	10042	F	n/a	t032	Creamer *et al.*2010
	10049	F	n/a	t032	Creamer *et al.*2010
	10054	H	n/a	t022	Creamer *et al.*2010
	10061	H	n/a	t515	Creamer *et al.*2010
	10074	H	n/a	t515	Creamer *et al.*2010
	10108	D	n/a	t3459	Creamer *et al.*2010
	10124	D	n/a	t557	Creamer *et al.*2010
	10148	C	n/a	t032	Creamer *et al.*2010
	10183	A	n/a	t032	Creamer *et al.*2010
	10212	G	n/a	t515	Creamer *et al.*2010
	10228	H	n/a	t032	Creamer *et al.*2010
	10235	H	n/a	t022	Creamer *et al.*2010
	10323	I	n/a	t379	Creamer *et al.*2010
	10326	I	n/a	t032	Creamer *et al.*2010
	10337	D	n/a	t1214	Creamer *et al.*2010
	10380	B	n/a	t1214	Creamer *et al.*2010
	10447	A	n/a	t032	Creamer *et al.*2010
Environmental sites ($n = 61$)	07899	C	Air	t628	This study
	08406	B	Air	t032	This study
	08409	B	Air	t032	This study
	08824	E	Air	t032	This study
	09957	C	Air	t557	This study
	09889	C	Not stated	t032	This study
	11261	B	Air	t032	This study
	11664	A	Air	t515	This study
	11729	G	Air	t557	This study
	11730	G	Air	t557	This study
	11734	G	Air	t557	This study
	11749	G	Mattress	t557	This study
	11759	G	Mattress	t9568	This study
	11799	F	Mattress	t557	This study
	11821	F	Locker	t557	This study
	11831	F	Mattress	t557	This study
	11833	F	Bedframe	t557	This study
	11834	F	Locker	t557	This study
	11836	F	Pillow	t557	This study
	11837	F	Bedframe	t557	This study
	11838	F	Locker	t557	This study
	11843	F	Mattress	t557	This study
	11846	F	Mattress	t557	This study
	11847	F	Mattress	t557	This study
	11848	F	Mattress	t557	This study
	11858	F	Air	t557	This study
	11859	F	Air	t557	This study
	11860	F	Air	t557	This study
	11862	F	Air	t557	This study
	12042	F	Air	t557	This study
	12043	F	Air	t557	This study

Continued overleaf

Table 6.3 continued. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

MRSA isolate group	Isolate no.[a]	Ward	Source	*spa* type[b]	Reference
Environmental sites	12044	F	Air	t557	This study
($n = 61$)	12252	C	Air	t515	This study
	13010	C	Locker	t515	This study
	13018	C	Locker	t515	This study
	13022	C	Locker	t515	This study
	13029	C	Not stated	t515	This study
	13120	G	Not stated	t032	This study
	13149	F	Not stated	t1565	This study
	13172	H	Not stated	t032	This study
	13841	B	Air	t515	This study
	13842	B	Air	t515	This study
	13945	B	Air	t515	This study
	13946	B	Air	t515	This study
	14017	B	Bedframe	t515	This study
	14028	B	Mattress	t515	This study
	14035	B	Locker	t515	This study
	14036	B	Mattress	t515	This study
	14037	B	Bedframe	t515	This study
	14038	B	Pillow	t515	This study
	14047	B	Mattress	t515	This study
	14049	B	Mattress	t515	This study
	14053	B	Air	t515	This study
	14055	B	Air	t515	This study
	14057	B	Air	t515	This study
	14058	B	Air	t515	This study
	14059	B	Air	t515	This study
	14072	A	Not stated	t515	This study
	14818	B	Not stated	t032	This study
	14819	B	Not stated	t032	This study
	14836	B	Not stated	t557	This study
Patients	07878	A	Nasal	t515	This study
($n = 82$)	07901	I	Nasal	t032	This study
	07905	I	Nasal	t032	This study
	07974	H	Groin	t032	This study
	08045	A	Nasal	t885	This study
	08209	A	Nasal	t515	This study
	08289	B	Nasal	t032	This study
	08299	B	Nasal	t1021	This study
	08322	B	Nasal	t032	This study
	08481	C	Groin	t3213	This study
	08484	C	Nasal	t190	This study
	08488	C	Nasal	t032	This study
	08496	D	Nasal	t032	This study
	08498	D	Nasal	t032	This study
	08500	D	Nasal	t1214	This study
	08777	E	Nasal	t032	This study
	08781	E	Nasal	t1214	This study
	09074	I	Nasal	t032	This study
	09212	F	Nasal	t032	This study
	09630	G	Nasal	t032	This study
	09658	G	Nasal	t756	This study
	09835	C	Nasal	t032	This study
	09839	C	Nasal	t515	This study
	09886	C	Groin	t022	This study
	09963	C	Nasal	t190	This study
	10249	H	Nasal	t032	This study
	10468	A	Nasal	t032	This study
	10518	G	Nasal	t032	This study
	10560	G	Nasal	t032	This study
	10585	B	Groin	t628	This study
	10602	B	Nasal	t032	This study
	10624	B	Nasal	t628	This study
	10652	C	Nasal	t3213	This study
	10672	C	Nasal	t032	This study
	10757	I	Nasal	t032	This study
	10767	I	Nasal	t3139	This study
	10773	I	Nasal	t628	This study
	10783	I	Nasal	t032	This study
	10796	I	Nasal	t557	This study

Continued overleaf

Table 6.3 continued. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

MRSA isolate group	Isolate no.[a]	Ward	Source	*spa* type[b]	Reference
Patients	10807	I	Nasal	t628	This study
(n = 82)	10912	H	Nasal	t2945	This study
	10936	H	Nasal	t032	This study
	11090	G	Nasal	t022	This study
	11137	A	Nasal	t515	This study
	11166	A	Nasal	t032	This study
	11168	A	Nasal	t032	This study
	11172	A	Nasal	t032	This study
	11176	A	Nasal	t432	This study
	11184	A	Nasal	t032	This study
	11192	A	Nasal	t515	This study
	11196	A	Nasal	t7983	This study
	11212	B	Nasal	t032	This study
	11248	B	Nasal	t515	This study
	11268	B	Nasal	t190	This study
	11633	A	Nasal	t515	This study
	11672	A	Groin	t032	This study
	12008	F	Groin	t515	This study
	12009	F	Nasal	t515	This study
	12011	F	Nasal	t515	This study
	12013	F	Nasal	t557	This study
	12015	F	Nasal	t557	This study
	12019	F	Nasal	t557	This study
	12049	H	Nasal	t032	This study
	12055	H	Nasal	t032	This study
	12167	B	Nasal	t032	This study
	12323	I	Nasal	t557	This study
	13793	**A**	**Nasal**	**t515**	**This study**
	13794	**A**	**Groin**	**t515**	**This study**
	13811	A	Nasal	t515	This study
	13894	A	Nasal	t032	This study
	13922	A	Nasal	t515	This study
	14303	A	Nasal	t032	This study
	14341	A	Nasal	t718	This study
	14347	A	Nasal	t032	This study
	14409	A	Nasal	t6768	This study
	14447	A	Nasal	t515	This study
	14544	B	Nasal	t515	This study
	14740	**B**	**Nasal**	**t032**	**This study**
	14741	**B**	**Groin**	**t032**	**This study**
	14797	**B**	**Nasal**	**t032**	**This study**
	14798	**B**	**Groin**	**t032**	**This study**
	15020	B	Nasal	t032	This study

[a] Isolate pairs highlighted in bold were recovered from the same patient.
[b] All isolates were *spa* typed as part of the present study.
Abbreviations: n/a, not applicable.

each isolate is listed in Table 6.3. Three wards, which has largest amount of positive MRSA samples during the screening process, were investigated to determine the relatedness of the ST22-MRSA-IV isolates recovered from HHCWs, patients and environmental sites within wards.

6.2.2 Molecular typing

6.2.2.1 Whole-genomic DNA isolation

Whole-genomic DNA for use with DNA microarray profiling and *spa* typing was extracted from all isolates using the lysis buffers and solutions supplied with the StaphyType DNA microarray kit and the Qiagen DNeasy kit as described in Chapter 2, section 2.5.4.1.

6.2.2.2 DNA microarray profiling

DNA microarray profiling was performed on all isolates using the StaphyType kit (Alere) to detect a range of staphylococcal antimicrobial resistance, virulence and SCC*mec*-associated genes and to assign isolates to MLST CC/ST and SCC*mec* types. The DNA microarray profiling was performed as described in Chapter 2, section 2.5.4.

6.2.2.3 *spa* typing

All isolates, except the MRSA and MSSA isolates recovered from BSIs, underwent *spa* typing as described in Chapter 2, section 2.5.3. The BSI isolates previously underwent *spa* typing as part of a European *spa* typing study (Grundman *et al.*, 2009).

6.2.2.4 Assigning isolates to microarray group (MG) combinations

The ST22-MRSA-IV isolates from the HHCWs, patients and environmental sites were assigned to microarray groups (MG) based on their combinations of antimicrobial resistance and virulence-associated genes. A previous study assigned 168 ST22-MRSA-IV

isolates to 13 MGs (MG1-MG13) based on different combinations of 10 variable genes (Brennan, 2013). These variable genes included the antimicrobial resistance genes *blaZ*, *erm*(C), *aacA-aphD, aadD, lnu*(A) and *mupA*, and the virulence genes and gene clusters *sec/sel, egc,* IEC and ACME (Brennan, 2013). Using the criteria described by Brennan (2013) isolates within MGs that differed by one gene only were deemed to be closely related. Isolates within MGs were deemed to be possibly related if they differed by the presence or absence of two or more antimicrobial resistance and/or virulence genes if evidence suggested that the genes that differed were co-located. Isolates within MGs were deemed different if they differed by the presence or absence of two or more antimicrobial and/or virulence gene and if evidence suggested that the genes that differ were not colocated. When assigning MGs the IEC genes were considered to be one gene cluster as they are co-located on a bacteriophage that integrates and disrupts the chromosomal *hlb* gene (van Wammel *et al.,* 2006). The enterotoxin genes *sec* and *sel* were also considered one gene cluster as they are co-located on the pathogenicity island SaPIbov1 (Avena *et al.,* 1697; Baba et al., 2002). The entrotoxin gene cluster (*egc*) genes *seg, sei, sem, sen, seo* and *seu* have previously been shown to be located on one genomic island and were therefore considered as one gene cluster (Malachowa *et al.*, 2010). The antimicrobial resistance genes *aacD-aphD* and *mupA* have also been reported previously to be co-located on the same plasmid (McDougal *et al.,* 2010; Brennan, 2013).

6.2.3 Detection of the toxic shock syndrome toxin (TSST-1) protein

All MRSA and MSSA isolates from the NHCWs, HCs and BSIs that were *tst*-positive by the DNA microarray (Table 6.4) were tested for the production of the TSST-1 protein using a Reverse Passive Latex Agglutination (RPLA) kit (Oxoid) according to the manufacturer's instructions and as outlined in Figure 6.1. The RPLA kit consists of latex-bound antibodies specific to TSST1. Agglutination of these latex particles occurs in the presence of TSST-1 protein (Figure 6.1). A control reagent was provided with the kit and

Table 6.4. Methicillin-susceptible *Staphylococcus aureus* (MSSA) (n = 53) and methicillin-resistant *S. aureus* (MRSA) (n = 4) isolates investigated for TSST-1 production[a] and *tst* expression[a].

MSSA/MRSA isolate group (CC)	Isolate no.[b]	DNA microarray profile[c]	*spa* type
MSSA student nasal carriage (CC30)	ED03	*blaZ, sdrM, fosB, tst, egc,seh*	t166
	ED05	*blaZ, sdrM, fosB, tst, sea,egc,* IEC A	t018
	ED06	*blaZ, sdrM, fosB, tst, egc, she,* IEC B	t166
	ED07	*blaZ, sdrM, fosB, tst, egc,* IEC B	t343
	ED08	***blaZ, erm*(A), *sdrM, fosB, tst, sea, egc,* IEC A**	**t012**
	ED12	***blaZ, sdrM, tet*(M),*fosB, tst, sea, egc, seh***	**t3258**
	ED14	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t342
	ED20	*blaZ, sdrM, fosB, tst, egc,* IEC E	t012
	ED21	*blaZ, sdrM, fosB, fosB*-plasmid, *tst, egc,* IEC B	t012
	ED23	*blaZ, sdrM, fosB, tst, egc,* IEC B	t11988
	ED27	*blaZ,erm*(A), *erm*(C),*tet*(M), *sdrM, fosB, fosB*-plasmid, *tst, egc, seh,* IEC B	t336
	ED28	*blaZ, sdrM, fosB, tst, sec & sel,* IEC B	t399
	ED33	*blaZ, sdrM, fosB, fosB*-plasmid, *tst, egc,* IEC B	t021
	ED34	*blaZ, erm*(C),*tet*(M), *sdrM, fosB, fosB*-plasmid, *tst, egc,* IEC B	t021
	ED41	*blaZ, sdrM, fosB, tst, seb, egc, seh,* IEC E	t166
	ED48	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t233
	ED56	*blaZ, sdrM, fosB, tst, egc,* IEC E	t805
	ED57	*blaZ, sdrM, fosB, tst, egc,* IEC E	t822
	MC02	***blaZ, sdrM, fosB, tst, sec & sel, seo, lukS/F-PV,* IEC B**	**t8681**
	MC07	*blaZ, sdrM, fosB, tst, egc, seh,* IEC B	t089
	MC12	***blaZ, sdrM, fosB, tst, sea, egc,* IEC A**	**t021**
	MC17	*blaZ, sdrM, fosB, tst, egc,* IEC B	t012
	MC19	***blaZ, sdrM, fosB, egc***	**t275**
	MC24	*blaZ, sdrM, fosB, tst, egc*	t275
	MC26	*blaZ, sdrM, fosB, tst, egc,*	t018
	MC27	*blaZ, sdrM, fosB, tst, egc,* IEC E	t1067
	MC32	*blaZ,erm*(A), *sdrM, fosB, tst, sea, egc,* IEC A	t7785
	MC36	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t190
	OB02	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t2923
	OB03	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t2923
MSSA student nasal carriage (CC45)	ED44	*sdrM, tst sec & sel, egc,* IEC E	t1510
MSSA student nasal carriage (CC5)	M11/0171	*sdrM, fosB, tst, sep, sec & secl, egc,* IEC F	t022
	ED55	*msr*(A), *mpb*(C), *srdM, fosB, tst, sep, sed, sej & ser,* IEC F	t002
MSSA bloodstream infections (CC30)	SpaS004	*blaZ, sdrM, fosB, tst, egc, seh,* IEC E	tt166
	SpaS009	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t1414
	SpaS010	***blaZ, sdrM, fosB, tst, sec & sel,* IEC C**	**t382**
	SpaS019	***blaZ, sdrM, fosB, tst, egc,* IEC B**	**t012**
	SpaS020	*blaZ, sdrM, fosB, tst, egc,* IEC B	t021
	SpaS021	*blaZ, sdrM, fosB, tst, egc, she,* IEC E	t089
	SpaS038	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t012
	SpaS040	***blaZ, sdrM, fosB, tst, sea, egc,* IEC A**	**t012**
	SpaS041	*blaZ, sdrM, fosB, tst, sea, egc,* IEC A	t018
	SpaS051	***blaZ, sdrM, fosB, tst, egc, seh,* IEC B**	**t089**
	SpaS062	*blaZ, emr*(A), *sdrM, fosB, tst, sea, egc,* IEC D	t338
	SpaS066	*blaZ, sdrM, fosB, tst, sec & sel,* IEC B	t382
	SpaS071	***blaZ, sdrM, fosB, tst, sec & sel***	**t1827**
	SpaS073	*blaZ, sdrM, fosB, tst, egc, she,* IEC B	t166

Continued overleaf

Table 6.4 continued. Methicillin-susceptible *Staphylococcus aureus* (MSSA) (n = 53) and methicillin-resistant *Staphylococcus aureus* (MRSA) (n = 4) isolates investigated for TSST-1 production[a] and *tst* expression[a].

MSSA/MRSA isolate group (CC)	Isolate no.[b]	DNA microarray profile[c]	*spa* type
MSSA bloodstream infections (CC30)	SpaS081	*blaZ, sdrM, fosB, tst, sea, egc*, IEC A	t012
	SpaS087	*blaZ, sdrM, fosB, tst, egc*, IEC B	t021
MRSA nares of healthcare workers (ST36/39-MRSA-II)	M03/0097	*blaZ, erm*(A), *aadD, sdrM, fosB, tst, egc*, IEC B	t018
MRSA bloodstream infections (CC5-MRSA-II)	SpaR006	*blaZ, erm*(A), *aadD, sdrM, fosB, tst, sea, egc, sed, sej & ser*, IEC A	t463
	SpaR015	*blaZ, erm*(A), *aadD, sdrM, fosB, tst, sep, egc, sed, sej & ser*, IEC F	t045
	SpaR076	*blaZ, erm*(A), *sdrM, fosB, tst, sea, egc, sed, sej & ser*, IEC D	t045
Patients with toxic shock syndrome[d] (CC30-MSSA)	**X91**	***blaZ, fosB, sdrM, tst, egc, sak***	**n/d**
	X111	***blaZ, fosB, sdrM, tst, sea, egc*, IEC A**	**n/d**
	X128	***blaZ, aacA-aphD, dfrS1, fosB, tst egc, seh, sak***	**n/d**
	X185	***blaZ, fosB, sdrM, tst, egc*, IEC B**	**n/d**

[a]Detection of the TSST-1 protein was performed using the Oxoid TSST-1 RPLA kit and the expression levels of *tst* were detected using real-time PCR.

[b]The isolates shown in bold type were investigated for *tst* expression levels using real-time PCR. The isolates highlighted in red type were negative for the production of TSST-1 when tested using the RPLA kit.

[c]The immune evasion complex (IEC) type of each isolate type was determined based on the combination of IEC genes detected by the DNA microarray and 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 E: *sak & scn*; IEC type F: *sep, sak, chp & scn*.

[d]Four CC30-MSSA isolates were obtained from a collaborator in Germany (Dr. Stefan Monecke, Institute for Medical Microbiology and Hygiene, Technical University of Drresden, Germany) from patients with toxic shock syndrome. DNA microarray analysis of these isolates was performed in Dr. Monecke's laboratory.

Abbreviations: n/d, not determined.

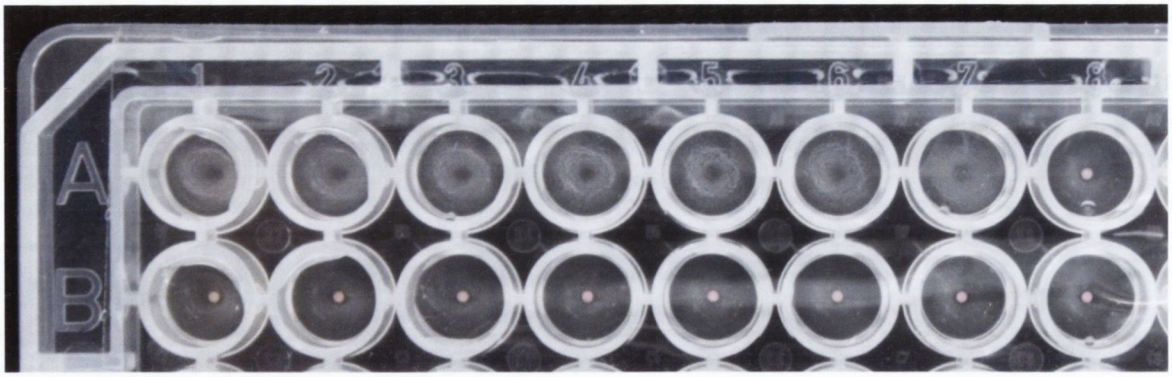

Figure 6.1. Detection of the staphylococcal toxic shock toxin protein TSST-1 using the Reverse Passive Latex Agglutination (RPLA) kit (Oxoid). Each isolate was cultured for 18-24 h at 37°C with shaking at 200 rpm in brain-heart infusion broth. The cultures were then centrifuged at 900 x g for 20 min at 4°C and doubling dilutions of each culture supernatant were made in two rows of a 96-well plate using the diluent supplied with the kit. Dilutions of each culture supernatant were made in wells 1-7 of two rows of the 96-well plate using the diluent supplied with the kit. The final well of each row (well 8) was used as a negative control and contained diluent but no culture supernatant. The TSST-1 test latex suspension was then added to each well of one row for each isolate supernatant (row A) and the latex control reagent was added to the second row for each isolate supernatant (row B). The contents were mixed by gently agitating the plate and the plate was then incubated on a flat surface at room temperature for 24 h after which the plate was examined visually. Positive agglutination of the test latex reagent (denoting the presence of TSST-1 protein in the sample supernatant) was visible as a lattice forming a diffuse layer on the base of the well (row A, wells 1-7). If TSST-1 was absent no such lattice structure could be formed (row B, wells 1-7). Isolates were recorded as TSST-1-positive following positive test latex agglutination, provided that no agglutination was observed in the absence of test latex (row A, well 8) and that no agglutination was observed with the control latex (row B, wells 1-7) or in the absence of control latex (row B, well 8).

consisted of latex particles bound with non-immune rabbit globulins. RPLA assays were performed in a 96 V-well microtitre plates (Thermo Fisher Scientific Inc.). Using the test latex preparation, agglutination of latex particles occurred in the presence of TSST-1 protein (i.e. from culture supernatants) and was visible as a lattice formation forming a diffuse layer on the base of the microtitre plate wells (Figure 6.1). No such lattice structure was formed in the absence of TSST-1 protein (Figure 6.1). The TSST-1-positive *S. aureus* control strain FRI913 was used as a positive control (Monday *et al.*, 1999) and the TSST-1-negative laboratory strain 80CR3 (Stobberingh *et al.*, 1977) was used as a negative control for all TSST-1 RPLA assays.

6.2.4 Quantification of the level of expression of *tst* in selected MSSA isolates using real-time PCR

6.2.4.1 MSSA isolates investigated

The level of expression of the *tst* was investigated in selected *tst*-positive CC30-MSSA isolates from HCs and BSIs (Table 6.4). In addition, four CC30-MSSA isolates, obtained from a collaborator in Germany (Dr. Stefan Monecke, Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Germany) from patients with toxic shock syndrome, were also investigated (Table 6.4).

6.2.4.2 Probe and primer design

Primers and the probe for *tst* and the 16S rDNA gene used as an endogenous control gene were designed and synthesised by Life Technologies (Dun Laoghaire, Co. Dublin) (Table 6.5). The amplification efficiency of the *tst* and 16S rDNA primers and probes were investigated by preparing a 10-fold dilution series of DNA and amplifying each dilution series, in triplicate, separately with both pairs of primers and probes using standard quantitative real-time PCR (qRT-PCR) cycling conditions. The average threshold cycle (Ct) value for each dilution was plotted for both the *tst* and 16S rDNA primers and

probe, and a trendline was added. If the slope of each trendline fell within 10% of each other, the amplification efficiency was considered to be optimal for real-time PCR.

6.2.4.3 RNA isolation

Total RNA was extracted from each isolate using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions and the RNAprotect reagent (Qiagen) which was used to stabilise RNA during the extraction process. Each isolate was grown in (i) 30 ml BHI broth and (ii) 30 ml BHI broth supplemented with 10% foetal bovine serum (Sigma) at 37°C with shaking to the post-exponential growth phase (16-18 h). Two ml of the overnight culture was added to 4 ml of the RNA protect reagent in a 13 ml Falcon tube (Cruinn Diagnostics Ltd.), vortexed for 10 s and incubated at room temperature for 5 min. The samples were then centrifuged at 5,000 x g for 10 min, the supernatant was discarded and the pellet was resuspended in 700 µl RLT buffer (supplied with Qiagen RNeasy mini kit) and 7 µl β-mercaptoethanol (Sigma). The suspension was then transferred to a 2 ml screw cap microtube (Sarstedt Ltd., Wexford, Ireland) filled to the half-way mark with 400-600 µm sterile glass beads (Sigma) and the cells were mechanically disrupted using a ThermoSavant Fastprep FP120 cell disrupter (Savent Instrument Inc. Holbrook, New York) for two x 20 s at a speed of 4.0 m/s. The speed refers to the maximum vertical velocity achieved by a sample tube during reciprocating motion. The samples were then transferred to ice and were maintained at 4°C for the rest of the process. The samples were centrifuged at 16,000 x g for 10 min at 4°C in an Eppendorf centrifuge (model 5417R). The supernatant was transferred to a new 1.5 ml sterile microfuge tube (Eppendorf) and an equal volume of 70% (v/v) ethanol (Sigma) was added to the sample. This was then transferred to a mini-column inside a collection tube (supplied with the Qiagen RNeasy mini kit). The mini-column contains a silica gel membrane that binds RNA and allows cellular debris to wash through. The membrane was washed once with RW1 buffer and twice with RPE buffer (both supplied with Qiagen RNeasy mini kit). The RNA was finally

Table 6.5. Custom designed primers and probes used to investigate the level of expression of the toxic shock toxin gene *tst* among CC30 MSSA isolates using the Taqman assay and real-time PCR

Gene target	Primer/probe	Nucleotide sequence (5'-3')
tst	TSST-F primer	AAAACTGCAAAAGCATCTACAAACGA
	TSST-R primer	CTATTTGTAAAAGTGTCAGACCCACTACT
	TSST-M probe	CCAGTCTAGCAAATCC
16S rDNA (endogenous control)	16SrDNA-F primer	GGTCTGTAACTGACGCTGATGTG
	16SrDNA-R primer	GTGGACTACCAGGGTATCTAATCCT
	16SrDNA-M probe	TCCCCACGCTTTCG

eluted into 50 µl of RNA-free water (supplied with Qiagen RNeasy mini kit) and centrifuged at 8,000 x g for 1 min. The extracted RNA was then stored at -80°C prior to DNase treatment and cDNA synthesis (see sections 6.2.4.4 and 6.2.4.5, respectively). The concentration of total genomic RNA (ng/µl) was measured using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.).

6.2.4.4 DNase treatment

The removal of any remaining DNA from each RNA preparation was performed prior to cDNA synthesis using the TURBO DNA-*free* ™ kit (Life Technologies) according to the manufacturer's instructions. In brief, the 50 µl RNA sample was added to 5 µl 10X Turbo DNase buffer and 1 µl of Turbo DNase (both supplied with TURBO DNA-*free* ™ kit). The samples were incubated at 37°C in a shaking incubator for 30 min. The reaction was then inactivated by the addition of 5 µl of resuspended DNase inactivation buffer (supplied with TURBO DNA-*free* ™ kit) and incubated at room temperature for 2 min, with brief vortexing every 30 s. The samples were then centrifuged at 10,000 x *g* for 90 s and the RNA from each sample was transferred to a new 1.5 ml sterile microfuge tube (Eppendorf) and stored at -80°C.

6.2.4.5 cDNA synthesis

Reverse transcription of RNA to cDNA was performed using the Invitrogen Superscript III reverse transcription kit (Life Technologies) according to the manufacturer's instructions. One µl of each DNase treated RNA sample was added to 1 µl of oligo dT (Promega), 0.5 mM dNTPs (Promega) and 10 µl of sterile water (Sigma). The samples were incubated at 65°C for 5 min, immediately placed on ice for 1 min and 4 µl of 5X first-strand buffer, 5 mM dithiothreitol (DTT), 1µl of RNase Out and 1 µl of Superscript III reverse transcriptase (all supplied with the Superscript III reverse transcription kit) was added to each sample. The samples were then incubated at 50°C for

30-60 min. A final step to inactivate the enzyme was performed by incubating the samples at 70°C for 15 min. The concentration of genomic cDNA (ng/µl) was measured using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.). The cDNA was stored at 4°C until use.

6.2.4.6 Real-Time PCR

Quantitative real-time PCR (qRT-PCR) was performed using cDNA reverse transcribed from each DNase treated RNA sample, the Life Technologies custom Taqman gene expression assay and an ABI 7500 Real-time PCR system (Applied Biosystems) (Figure 6.2). The Taqman assay was performed according to the manufacturer's instructions in a 20 µl volume using 10 µl of TaqMan master mix (Applied Biosystems), 0.9 mM of custom primer (Life Technologies), 0.25 mM of custom probe (Life Technologies) and 50 ng of cDNA. For each isolate examined, separate 16S rDNA and *tst* qRT-PCR reactions were carried out in triplicate on two separate occasions, using the same cDNA, in an Applied Biosystems Microamp Fast Optical 96-well Reaction Plate (Applied Biosystems) in an ABI 7500 FAST Real-time PCR instrument (Applied Biosystems). Applied Biosystems software (version 2.0.6) was used to determine the threshold cycle (Ct) values for each reaction of both *tst* and 16S rDNA. For each isolate investigated, the average of three Ct values was determined separately for each gene and the resulting data was analysed according to Livak and Schmittgen (2001) i.e. the ΔCt values (the average Ct of the endogenous control gene (16S rDNA) subtracted from the average Ct for the gene of interest (*tst*)) were calculated for each isolate, from which the $2^{-\Delta Ct}$ (fold differences) in gene expression were determined.

6.2.5 Statistical analysis

Statistical analysis was performed on the expression levels of *tst* among each group of isolates investigated using a two-sample t-test. A two-tailed *z*-test was used to assess the

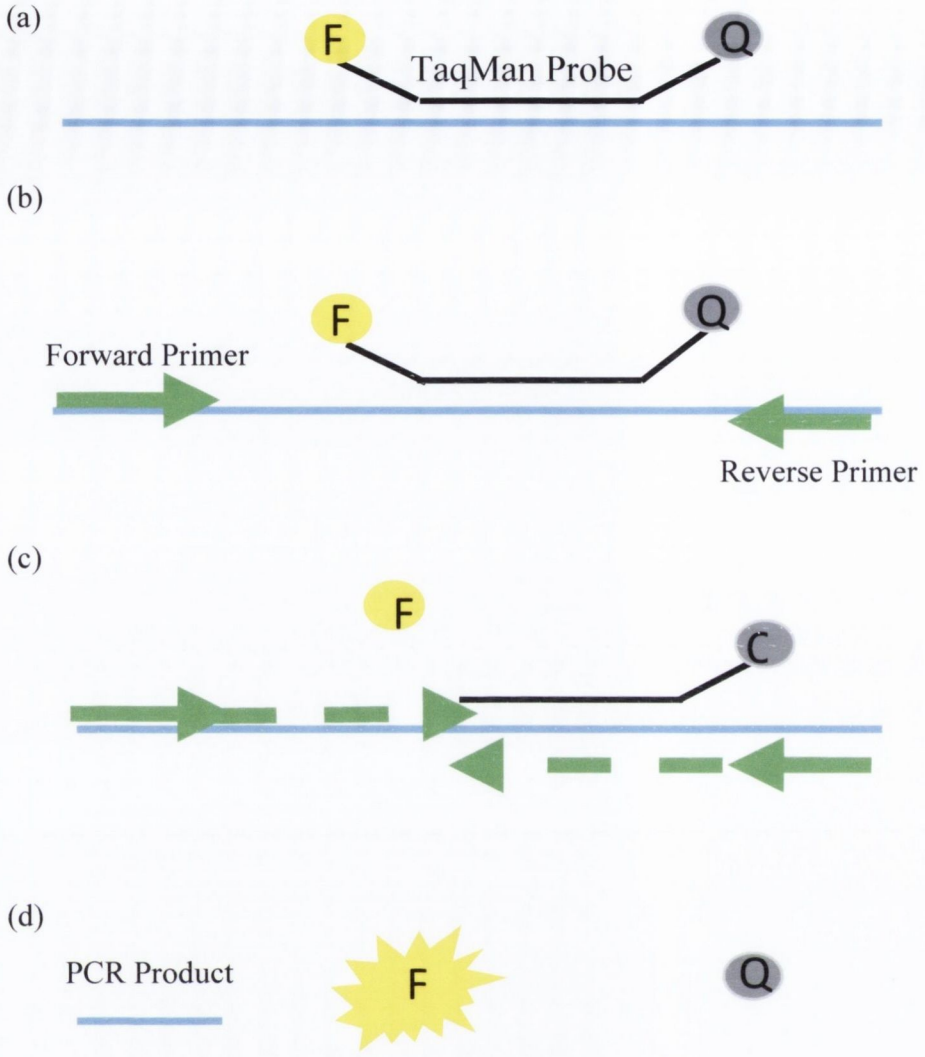

Figure 6.2. Schematic diagram illustrating the principle of the TaqMan assay. The TaqMan assay consists of custom designed primers and probes for *tst* and the endogenous control (16S rDNA). The primers consisted of a sequence of around 25 base pairs that is complementary to the gene. The probe consists of a sequence complementary to an area between the forward and reverse primer, that is flanked on either end by a fluorophore (F), which is a fluorescent chemical that emits light, and a quencher molecule (Q) (panel A). No light is emitted when both the fluorophore and quencher are bound to the probe. The TaqMan probe is designed to anneal to a short DNA sequence (14-16 bps) located between two primer binding sites (panel B). Once the primers anneal to the DNA sequence and the PCR reaction starts the fluorophore is detached from the probe as the PCR reaction proceeds (panel C) and this allows light to be emited (panel D). This light is detected by the real-time PCR machine optics and the level of fluorescence detected is proportional to the amount of amplified target produced during the reaction, i.e. the greater the level of expression of a gene the greater the level of light emitted. The greater the amount of light emitted the lower the threshold cycle (Ct) value will be. This is used to calculate the ΔCt (the average Ct of the endogenous control gene (16S rDNA) subtracted from the average Ct for the gene of interest (*tst*)), which shows the fold difference in expression levels.

significance of the difference between two population proportions. A p-value ≤ 0.05 was considered significant at a significance level of 0.05.

6.3 Results

The present study used *spa* typing and DNA microarray profiling to compare MRSA isolates from bloodstream infections (BSIs) and MRSA recovered as nasal carriage isolates from the nares of healthcare workers (NHCWs). MSSA isolates from bloodstream infections (BSIs) and nasal carriage isolates from the nares of healthy carriers (HCs) were also investigated. The *spa* typing and DNA microarray profiling results for MRSA from BSIs and NHCWs and MSSA isolates from BSIs and HCs are shown in Tables 6.6 and 6.7, respectively. The DNA microarray was used to assign isolates to MLST STs and/or CCs and SCC*mec* types and to detect a range of virulence and resistance genes.

6.3.1 Comparative molecular analysis of MRSA isolates recovered from BSIs and NHCWs and MSSA isolates recovered from BSIs and HCs

6.3.1.1 *spa* typing of MRSA isolates from BSIs and NHCWs

The BSI MRSA isolates were differentiated into 25 *spa* types while the MRSA isolates from NHCWs were differentiated into 17 *spa* types (Table 6.6). Among both groups of isolates *spa* type t032 predominated (BSIs, 38/83, 45.7%; NHCWs, 22/53, 41.5%) followed by t515 (BSIs, 8/83, 9.6%; NHCWs, 8/53, 15.1%) and t190 (BSIs, 4/83, 4.8%; NHCWs, 3/53, 5.7%). Other *spa* types that were found among MRSA isolates from both BSIs and NHCWs included t022, t020, t718, t727 and t2945 (Table 6.6).

BURP analysis assigned 18/25 *spa* types identified among BSI MRSA isolates into two clusters (Figure. 6.3). Cluster 1 consisted of all isolates that were assigned to ST22-MRSA-IV and cluster 2 consisted of only two *spa* types, t190 and t2942, which were assigned to ST8-MRSA-IIA-E & SCC_{MI} (the ST-SCC*mec* type assignments for these isolates are described below in section 6.3.1.2.1) (Figure. 6.3). Of the remaining seven *spa* types, (i) four *spa* types were deemed to be singletons, namely t030 (ST239-MRSA-III),

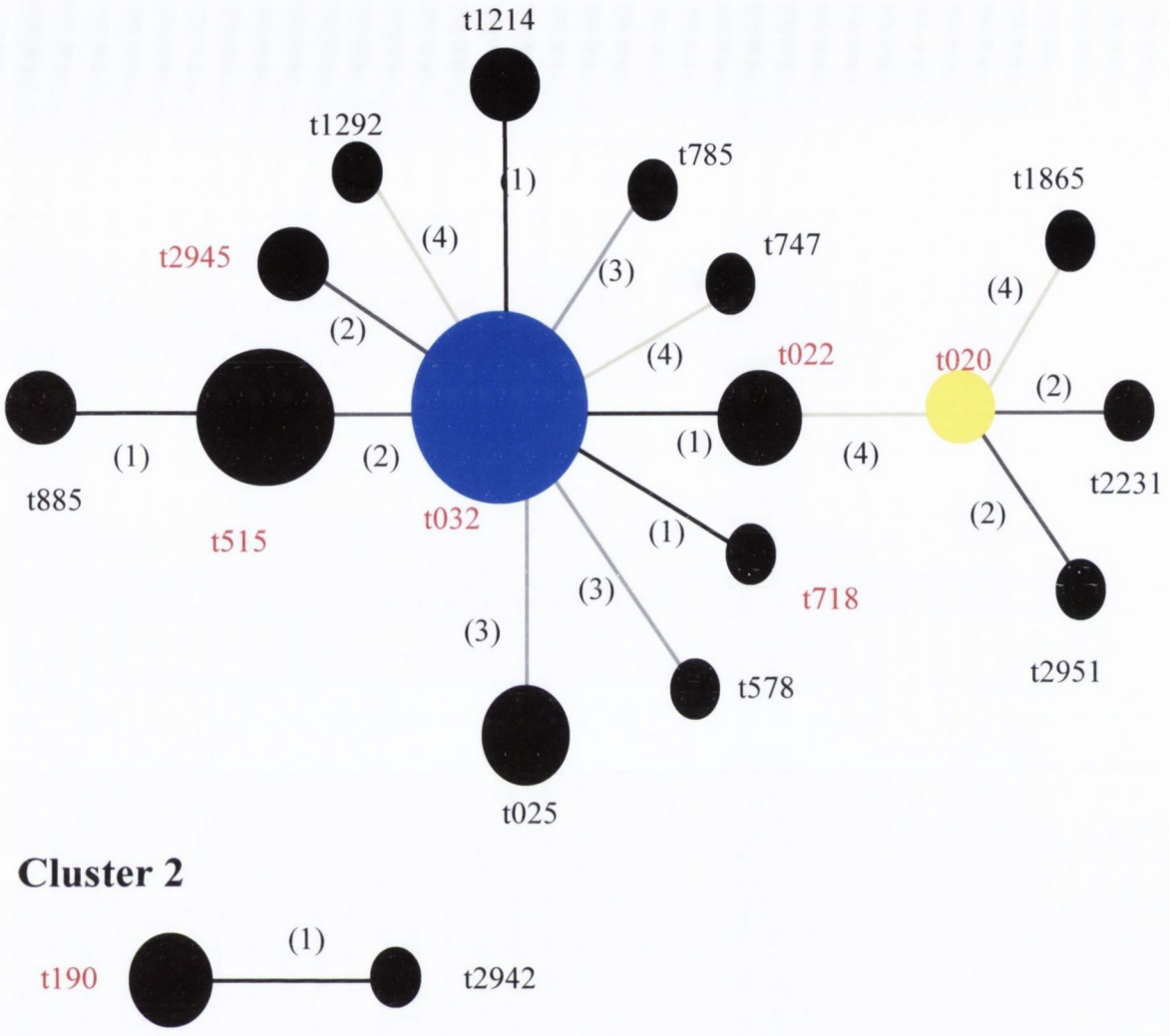

Figure 6.3. BURP analysis of the 25 *spa* types identified among 83 MRSA isolates recovered from patients with bloodstream infections (BSIs) between September 2006 and February 2007. Eighteen of the 25 *spa* types were assigned to two clusters (Cluster 1, *spa*-CC 032 (n = 16) and Cluster 2, (n = 2)) using the BURP algorithm (Mellmann *et al.*, 2007). *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value ≤ 4 where cost accounts for the number of steps of evolution between *spa* types. *spa* types t030, t045, t064, t878 were defined as singletons by BURP as they could not be clustered with any other *spa* type i.e. they had a cost value > 5. *spa* types t463, t727 and t1802 were excluded from BURP analysis as they consisted of four repeat units only. Cost values are shown in parenthesis. For each cluster the group founder is shown in blue and was determined based on the *spa* type that shares the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. The cluster with no founder was not assigned a *spa*-CC name. The founder of any subgroup is shown in yellow. The sizes of the circles reflect the number of isolates assigned to each *spa* type. The exact number of isolates represented by each *spa* type is shown in Table 6.5. *spa* types in red text were also identified among MRSA isolates from the nares of healthcare workers (NHCWs) (Figure 6.4). The excluded *spa* type t727 was also found among MRSA from NHCWs.

t045 (ST5-MRSA-II), t064 (CC8-MRSA-IV) and t878 (ST779 ψSCC*mec*-SCC-SCC_{CRISPR}), (ii) three were excluded from BURP analysis as they only contained four repeat units which are considered to be too short to deduce evolutionary relatedness i.e. t463 (ST5-MRSA-II), t727 (CC45-MRSA-IV) and t1802 (ST22-MRSA-IV) (Figure 6.3).

BURP analysis of the MRSA from the NHCWs resulted in 10/17 *spa* types being assigned to just one cluster (*spa*-CC 032) (Figure 6.4). This cluster consisted of ST22-MRSA-IV isolates only (Figure 6.4). Six *spa* types were deemed to be singletons including the CC8 *spa* types t008 (ST8-MRSA-IV, $n = 1$), t190 (ST8-MRSA-IIA-IIE, IVE/F, $n = 3$) and t037 (ST239-MRSA-III, $n = 1$), the CC22/ST22-MRSA-IV *spa* type t002 ($n = 2$) and the CC30 *spa* types t018 (ST36-MRSA-II, $n = 1$) and t318 ($n = 1$) (Figure 6.4). The CC45 *spa* type t727 was excluded from BURP analysis as it contained only four repeat units.

6.3.1.2. DNA microarray analysis of MRSA isolates from BSI and NHCWs

6.3.1.2.1 Genotype assignment

Using the DNA microarray the MRSA isolates from BSIs and NHCWs were assigned to seven and eight genotypes, respectively (Table 6.6). Among both groups of isolates ST22-MRSA-IV predominated and accounted for 85.5% (71/83) of MRSA isolates from BSIs and 81.1% (43/53) from NHCWs. The ST8-MRSA-IIA-E & SCC_{MI} (BSIs, 5/83, 6%; NHCWs, 3/53, 5.7%) and ST239-MRSA-III (BSIs, 1/83, 1.2%; NHCWs, 1/53, 1.9%) genotypes were also identified among both groups of isolates. Both groups contained additional isolates belonging to the lineages CC5 (BSIs, 3/83, 3.6% (ST5-MRSA-II); NHCWs, 2/53, 3.8% (CC5-MRSA-IV)), CC8-MRSA-IV (BSIs, 1/83, 1.2%; HCWs, 1/53, 1.9%) and CC45-MRSA-IV (BSIs, 1/85, 1.2%; HCs, 1/53, 1.9%). ST779 ψSCC*mec*-SCC-SCC_{CRISPR} was found among MRSA isolates from BSIs only ($n = 1$), while CC30 isolates were unique to NHCWs ($n = 2$) and included ST36/39-MRSA-II and ST30-MRSA-IV.

6.3.1.2.2 Antimicrobial resistance genes

The MRSA isolates from BSIs and NHCWs exhibited similar rates of carriage of the following antimicrobial resistance genes detected using the array (Table 6.6); the β-lactam resistance gene *blaZ* (BSIs, 83/83, 100%; NHCWs, 53/53, 100%): the macrolide resistance genes *erm*(C) (BSIs, 54/83, 65.1%; NHCWs, 29/53, 54.7%), the aminoglycoside genes *aacA-aphD* (BSIs, 8/83, 9.6%; NHCWs, 4/53, 7.5%), a gene encoding resistance to quaternary ammonium compounds (*qacA*) (BSIs, 5/83, 6%; NHCWs, 4/53, 7.5%), the tetracycline resistance genes *tet*(K) (BSIs, 1/83, 1.2%; NHCWs, 2/53, 3.8%), and *tet*(M) (BSIs, 2/83, 2.4%; NHCWs, 1/53, 1.9%), the multidrug efflux pump *sdrM* (BSIs, 12/83, 14.5%; NHCWs, 10/53, 18.9%), the trimethoprim resistance gene *dfrS1* (BSIs, 2/83, 2.4%; NHCWs, 1/53, 1.9%) and the mercury resistance genes *merA* and *merB* (BSIs, 5/83, 6%; HCWs, 5/53, 9.4%). The following genes were found in both groups but their prevalence was slightly higher among the BSIs MRSA isolates, but this was not statistically significant: *aadD* (BSIs, 5/83, 6%; NHCWs, 1/53, 1.9%) (p = 0.25), *aphA3* (BSIs, 5/83, 6%; NHCWs, 2/53, 3.8%) (p = 0.56), and *erm*(A) (BSIs, 9/83, 10.8%; NHCWs, 4/53, 7.5%) (p = 0.52). The high-level mupirocin resistance gene *mupA* (4/83, 4.8%; ST22-MRSA-IV (n = 3), ST8-MRSA-IIA-E +/- SCC_{MI} (n = 1)), the fosfomycin resistance gene *fosB* (2/83, 2.4%, ST22-MRSA-IV) and the fusidic acid resistance genes *fusB* (2/83, 2.4%) and *fusC* (1/83, 1.2%) were identified among BSI MRSA isolates only. A gene encoding resistance to quaternary ammonium compounds (*qacC*) was identified among one ST22-MRSA-IV isolate from NHCWs and was not identified among MRSA from BSIs.

6.3.1.2.3 Virulence-associated genes

Similar rates of carriage of virulence-associated genes were also identified among the MRSA isolates from BSIs and NHCWs (Table 6.6) including *egc* (BSIs 76/83, 92%; NHCWs 48/53, 91%), *tst* (BSIs 3/83, 4%; NHCWs 1/53, 2%), *sea* (BSIs 9/83, 11%;

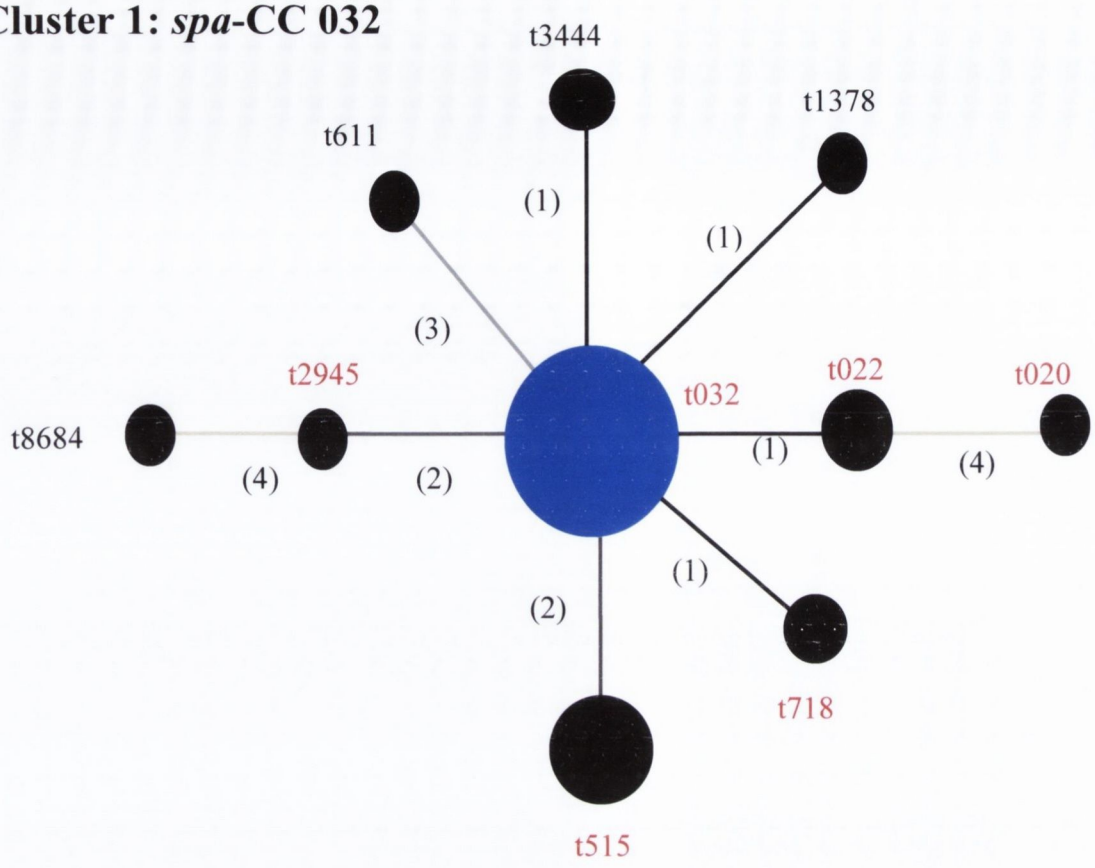

Figure 6.4. BURP analysis of the 17 *spa* types identified among 53 nasal carriage MRSA isolates recovered from healthy healthcare workers in a Dublin hospital between 2002 and 2009. Ten of the 17 *spa* types were assigned to a single cluster (Cluster 1, *spa*-CC 032) using the BURP algorithm (Mellmann *et al.*, 2007). *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value ≤ 4 where cost accounts for the number of steps of evolution between *spa* types. *spa* types t002, t008, t018, t037, t190, t318 were defined as singletons by BURP as they could not be clustered with any other *spa* type i.e. they had a cost value > 5. *spa* type t727 was excluded from BURP analysis as it consisted of four repeat units only. Cost values are shown in parenthesis. For each cluster the group founder is shown in blue and was determined based on the *spa* type that shares the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. The sizes of the circles reflect the number of isolates assigned to each *spa* type. The exact number of isolates represented by each *spa* type is shown in Table 6.5. *spa* types highlighted in red text are *spa* types also identified among MRSA from bloodstream infections (BSIs) (Figure 6.3). The singleton *spa* type t190 and excluded *spa* type t727 were also identified among MRSA from BSIs.

Table 6.6. DNA microarray and *spa* typing results for MRSA isolates investigated in the present study from bloodstream infections and the anterior nares of healthcare workers

Isolate group	Microarray CC/ST-MRSA-SCC*mec* designation (*n*)	*spa* type (*n*)	DNA microarray genes detected			
			***agr*/*cap*/IEC type[a] (% positive isolates, if not 100%)**	**SCC*mec*-associated genes (% positive isolates, if not 100%)**	**Antimicrobial resistance genes (% positive isolates, if not 100%)**	**Virulence-associated genes (% positive isolates, if not 100%)**
Bloodstream infection (BSIs) (*n* = 83)	CC22/ST22-MRSA-IV (*n* = 71)	t032 (*n* = 38), t515 (*n* = 8), t022 (*n* = 4), t025 (*n* = 3), t020 (*n* = 2), t1214 (*n* = 2), t1802 (*n* = 2), t2945 (*n* = 2), t885 (*n* = 2), t1292 (*n* = 1), t1865 (*n* = 1), t2231 (*n* = 1), t2951 (*n* = 1), t578 (*n* = 1), t718 (*n* = 1), t747 (*n* = 1), t785 (*n* = 1)	*agr* I, *cap* 5 IEC B (94%) IEC neg (6%)	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2,ccrC* (1%)	*blaZ, erm*(C) (73%), *aacA-aphD* (1%), *dfrS1* (1%), *mupA* (4%), *tet(K)* (1%), *fosB*-plasmid (3%)	*sec & sel* (48%), *egc*, *sak* (94%), *chp* (94%), *scn* (94%), ACME (1%)
	CC8/ST8-MRSA-IIA-E & SCC_{MI} (*n* = 5)	t190 (*n* = 4), t2942 (*n* = 1)	*agr* I, *cap* 5 IEC D (60%) IEC novel (20%) IEC neg (20%)	*mecA, ΔmecR1, ugpQ, mecI & xylR* (40%), *ccrAB2, ccrB4*	*blaZ, erm(A), aacA-aphD, aadD* (40%), *aphA3& sat, mupA* (20%), *sdrM, fosB, qacA, merA & merB* (80%),	*sea, sak, scn* (60%)
	CC8/ST239-MRSA-III (*n* = 1)	t030 (*n* = 1)	*agr* I, *cap* 8 IEC D	*mecA, ΔmecR1, mecR1,mecI, xylR, ugpQ, dcs, ccrAB3, ccrC*	*blaZ, erm(A), aacA_aphD, tet(M), sdrM, cat*-pMC524, *fosB*	*sea, sek & seq, sak & scn*
	CC5/ST5-MRSA-II (*n* = 3)	t045 (*n* = 2), t463 (*n* = 1)	*agr* II, *cap* 5 IEC A (33%) IEC F (33%) ICE E (33%)	*mecA, ΔmecR1, ugpQ, dcs, kdp, mecI. mecR, xylR, ccrAB2*	*blaZ, erm(A), aadD* (67%), *sdrM,, fosB*	*tst, sea* (67%), *selp* (33%), *sed, sej & ser, egc, sak, chp* (67%), *scn,*
	CC8-MRSA-IV (*n* = 1)	t064 (*n* = 1)	*agr* I, *cap* 5 IEC D	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, aacA-aphD, aadD, dfrS1, fusB, tet(M), sdrM, fosB*	*sea, seb, sek & seq, sak, scn*
	CC45-MRSA-IV (*n* = 1)	t727 (*n* = 1)	*agr* IV, *cap* 8 IEC B	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, erm(C), fusB,, sdrM, merA & merB*	*egc, sak, chp, scn*

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Table 6.6 continued. DNA microarray and *spa* typing results for MRSA isolates investigated in the present study from bloodstream infections and nasal carrier of healthcare workers

Isolate group	Microarray CC/ST-MRSA-SCC*mec* designation (*n*)	*spa* type (*n*)	DNA microarray genes detected			
			***agr*/*cap*/IEC type**[a] **(% positive isolates, if not 100%)**	**SCC*mec*-associated genes (% positive isolates, if not 100%)**	**Antimicrobial resistance genes (% positive isolates, if not 100%)**	**Virulence-associated genes (% positive isolates, if not 100%)**
Bloodstream infection (BSIs) (*n* = 83)	ST779 ψSCC*mec*-SCC-SCC_{CRISPR} (*n* = 1)	t878 (*n* = 1)	*agr* III, *cap* 5 IEC B	*mecA, ugpQ, ccrAA, ccrB4*	*blaZ, fusC, sdrM*	*seb, sak, chp, scn, etD, edinB*
Nares of healthcare workers (NHCWs) (*n* = 53)	CC22/ST22-MRSA-IV (*n* = 43)	t032 (*n* = 26), t515 (*n* = 8), t022 (*n* = 2), t020 (*n* = 1), t611 (*n* = 1), t718 (*n* = 1), t1378 (*n* = 1), t2945 (*n* = 1), t3444 (*n* = 1), t8684 (*n* = 1)	*agr* I, *cap* 5 IEC B (86%) IEC neg (14%)	*mecA, ΔmecR1, ugpQ, dcs, mecR1* (2%), *ccrAB2*	*blaZ, erm*(C) (67%), *tet*(K) (2%), *qacC* (2%)	*sec* & *sel* (47%), egc, *sak* (86%), *chp* (86%), *scn* (86%), ACME (2%)
	CC8/ST8-MRSA-IIA-E & SCC_{M1} (*n* = 3)	t190 (*n* = 3)	*agr* I, *cap* 5 IEC D (67%) IEC neg (33%)	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2, mecI, mecR1, ccrA4*	*blaZ, erm(A), aacA-aphD, aphA3 & sat (33%), sdrM, fosB, qacA, merA & merB*	*sea* (67%), *sak* (67%), *chp* (67%)
	CC8/ST8-MRSA-IV (*n* = 1)	t008 (*n* = 1)	*agr* I, *cap* 5 IEC B	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, sdrM, fosB*	*sek & seq, lukF/S-PV, sak, chp, scn,* ACME
	CC8/ST239-MRSA- III (*n* = 1)	t037 (*n* = 1)	*agr* I, *cap* 8 IEC D	*mecA, ΔmecR1, ugpQ, dcs, mecI, mecR, xylR, ccrAB3, ccrC*	*blaZ, aacA-aphD, aphA3 & sat, tet(M), srdM, fosB, qacA, merA & merB*	*sea, sak, scn*
	CC30/ST36/39-MRSA-II (*n* = 1)	t018 (*n* = 1)	*agr* III, *cap* 8 IEC B	*mecA, ΔmecR1, ugpQ, dcs, kdp, mecI, mecR1, xylR ccrAB2*	*blaZ, erm(A), aadD, sdrM, fosB*	*tst, sea,* egc, *sak, chp, scn*

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Table 6.6 continued. DNA microarray and *spa* typing results for MRSA isolates investigated in the present study from bloodstream infections and nasal carrier of healthy healthcare workers

Isolate group	Microarray CC/ST-MRSA-SCC*mec* designation (*n*)	*spa* type (*n*)	DNA microarray genes detected			
			agr/cap/IEC type[a] (% positive isolates, if not 100%)	SCC*mec*-associated genes (% positive isolates, if not 100%)	Antimicrobial resistance genes (% positive isolates, if not 100%)	Virulence-associated genes (% positive isolates, if not 100%)
Nares of healthcare workers (NHCWs)	CC30/ST30-MRSA-IV (*n* = 1)	t318 (*n* = 1)	*agr* III, *cap* 8 IEC A	*mecA, ΔmecR1, ugpQ, ccrAB2*	*blaZ, dfrS1, tet(K), sdrM,, fosB*	*sea, lukF/S-PV, sak,, scn*
(*n* = 53)	CC5-MRSA-IV (*n* = 2)	t002 (*n* = 2)	*agr* II, *cap* 5 IEC D (50%) IEC F (50%)	*mecA, ΔmecR1, ugpQ, dcs xylR (50%), ccrAB2*	*blaZ, erm(C)* (50%), *fusC* (50%), *sdrM,, fosB*	*sea* (50%),*sed & sej & ser, egc, sak, chp* (50%), *scn*
	CC45-MRSA-IV (*n* = 1)	t727 (*n* = 1)	*agr* I, *cap* 8 IEC neg	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, fusB, sdrM,, merA & merB*	*egc*

[a]*agr*, accessory gene regulatory genes; *cap,* capsule genes; the immune evasion complex (IEC) type of each isolate type was determined based on the combination of 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 D: *sea, chp & scn*; IEC type E: *sak & scn*; IEC type F: *sep, sak, chp & scn*; IEC novel, novel combination of IEC genes *sea* & *sak* only; IEC neg; no IEC genes detected.

NHCWs 5/53, 9%), *sec & sel* (BSIs 35/83, 42%; NHCWs 20/53, 38%), *sed, sej & ser* (BSIs 3/83, 4%; NHCWs 1/53, 2%) and *sek & seq* (BSIs 2/83, 2%; NHCWs 1/53, 2%). The immune evasion cluster genes, *sak* (BSIs 80/83, 96%; NHCWs 45/53, 85%), *chp* (BSIs 72/83, 87%; NHCWs 41/53, 77%), *scn* (BSIs 78/83, 94%; NHCWs 45/53, 85%) and *sep* (BSIs 1/83, 1%; NHCWs 1/53, 2%) were also detected at similar prevalence rates among both groups. The enterotoxin gene *seb* (*n* = 2, ST8-MRSA-IV and ST779 ψSCC*mec*-SCC-SCC_{CRISPR}), the exfoliative toxin gene *etD* and the epidermiolytic toxin gene *edinB* (*n* = 1, ST779 ψSCC*mec*-SCC-SCC_{CRISPR}) were identified among MRSA from BSIs isolates only (Table 6.5). The *lukF/S-PV* genes were detected in two MRSA from NHCWs only (ST30-MRSA-IV and ST8-MRSA-IV). ACME was detected in one BSI isolate (ST22-MRSA-IV) and two NHCW isolates (ST22-MRSA-IV and ST8-MRSA-IV) (Table 6.6).

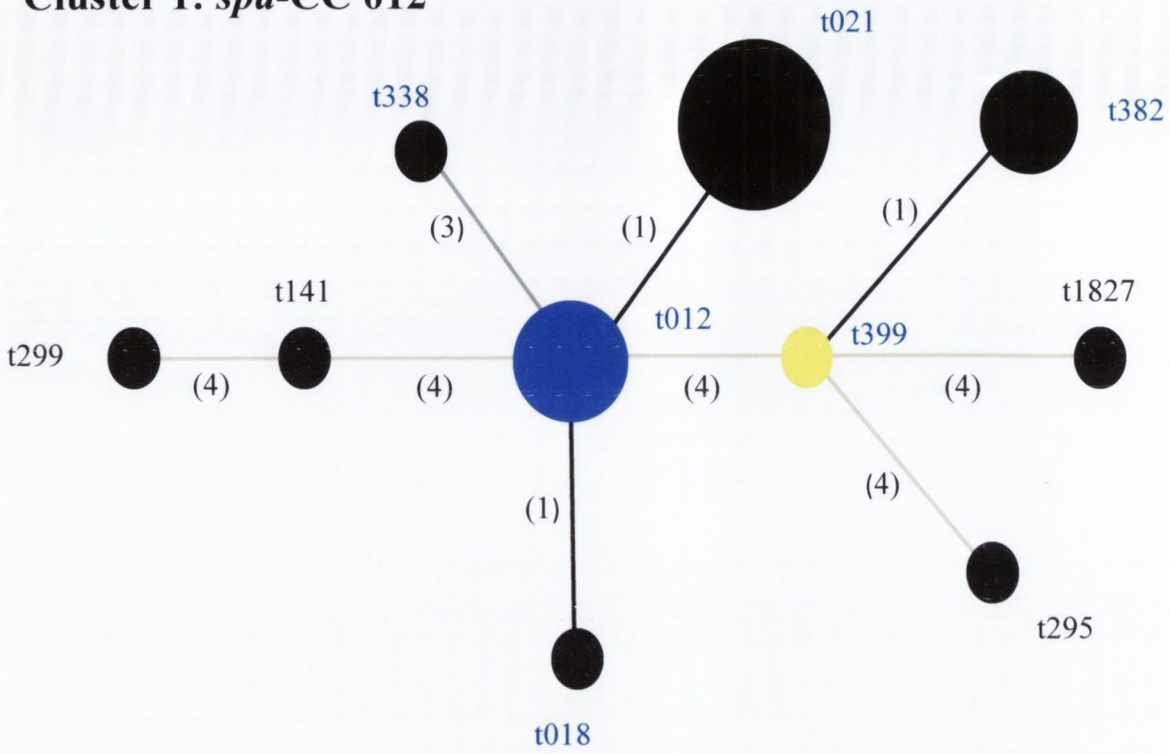
A total of 90.4% of isolates (BSIs, 78/83, 93.9%; NHCWs, 45/53, 84.9%) carried IEC genes and the most common IEC type was B (*sak, chp*, and *scn*) (BSIs, 69/83, 83.1%; NHCWs, 39/53, 73.6%), followed by D (*sea, sak* and *scn*) (BSIs, 6/83, 7.2%; NHCWs, 4/53, 7.5%). IEC type A (*sea, chp, sak*, and *scn*) and F (*sep, chp, sak*, and *scn*) were found in just a single isolate each from both BSIs and NHCWs. Type C (*chp* and *scn*) was only found in one BSI isolate. Two BSI isolates (ST8-MRSA-IIA-E & SCC_{MI}) carried *sea* and *sak* only which is a novel IEC gene combination type. The most common *agr* (accessory gene regulator) type was *agr* I (BSIs 78/83, 93.9%; NHCWs 48/53, 90.6%). The *agr* types II, III and IV were also found in MRSA from BSI (3/83, 3.6%; 1/831.2%, 1/83, 1.2%, respectively), while only *agr* types II and III were found in isolates from NHCWs (2/53, 3.8%; 3/53, 5.7%, respectively). The type 5 *cap* (capsule) genes predominated among both MRSA groups (BSIs 81/83, 97.6%; NHCWs 49/53, 92.5%) with the remaining isolates harbouring *cap* type 8 (BSIs 2/83, 2.4%; NHCWs 4/53, 7.5%).

6.3.1.3. *spa* typing of MSSA isolates from BSIs and HCs

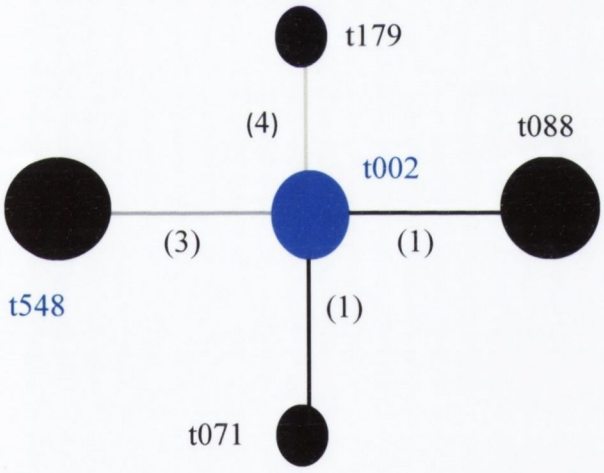
The MSSA isolates from BSIs and HCs were differentiated into 54 and 65 *spa* types, respectively (Table 6.7). The most common *spa* types among the MSSA from BSIs were t021 (6/83, 7.2%), t012 (3/83, 3.6%), t088 (3/83, 3.6%), t127 (3/83, 3.6%), t166 (3/83, 3.6%), t382 (3/83, 3.6%), and t548 (3/83, 3.6%) and among the HCs were t002 (6/98, 6.1%), t012 (6/98, 6.1%), t230 (6/98, 6.1%), t021 (3/98, 3.1%), t084 (3/98, 3.1%), t089 (3/98, 3.1%) and t166 (3/98, 3.1%) (Table 6.7). All other *spa* types were represented by one or two isolates only (Table 6.7). Nineteen of the *spa* types were found in both BSI and HC MSSA isolates while 35 were unique to the BSI isolates and 46 were unique to HC isolates (Figures 6.5 and 6.7).

Among the BSI MSSA isolates 40 of the 54 *spa* types detected were assigned to ten clusters using BURP analysis (Figure 6.5). Cluster 1 (*spa*-CC 012) was the largest cluster with the greatest number of isolates (n = 19) and *spa* types (10 *spa* types). This cluster contained all isolates that were assigned to CC30 by the DNA microarray (section 6.3.1.4.1 and Table 6.6). Cluster 2 (*spa*-CC 002) contained five *spa* types (n = 10 isolates) that were all assigned to CC5 by the DNA microarray. The *spa* types assigned to clusters 3, 4, 6, 7, 8 and 9 belonged to CC22, CC8, CC20, CC45, CC15, CC45 and CC25/28, respectively. Cluster 5 (*spa*-CC 2948) contained *spa* types assigned to CC9 and CC45. The CC9 *spa* type was most likely grouped with the CC45 isolates due to the fact that t209 contained only five repeats, and the closest CC45 *spa* types also contained just five repeats. Twelve *spa* types were deemed to be singletons and included t089 and t166 (ST34), t091 (CC7), t127 (CC1), t160 (CC12), t171 (CC121), t216 (CC59), t2143 and t2940 (CC45), t2360 (CC6), t2938 (CC22), and t2939 (CC25/28). Two *spa* types were excluded as they contained less than four repeats (t026 and t362).

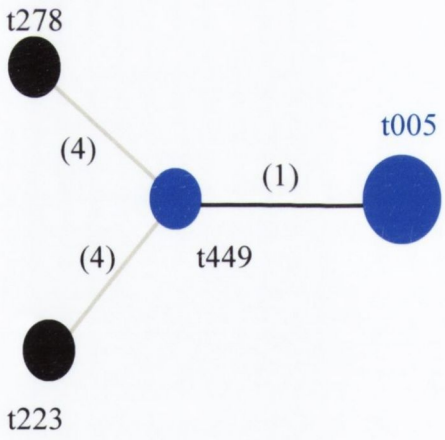
Cluster 1: *spa*-CC 012



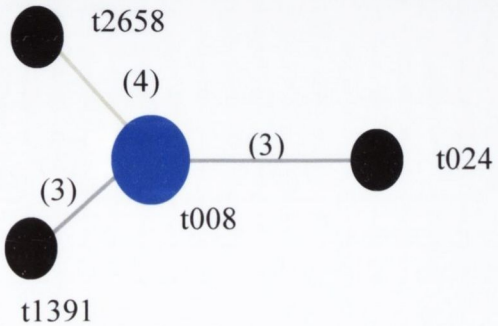
Cluster 2: *spa*-CC 002



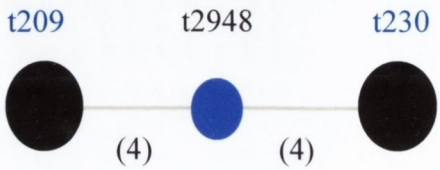
Cluster 3: *spa*-CC 449/005



Cluster 4: *spa*-CC 008



Cluster 5: *spa*-CC 2948



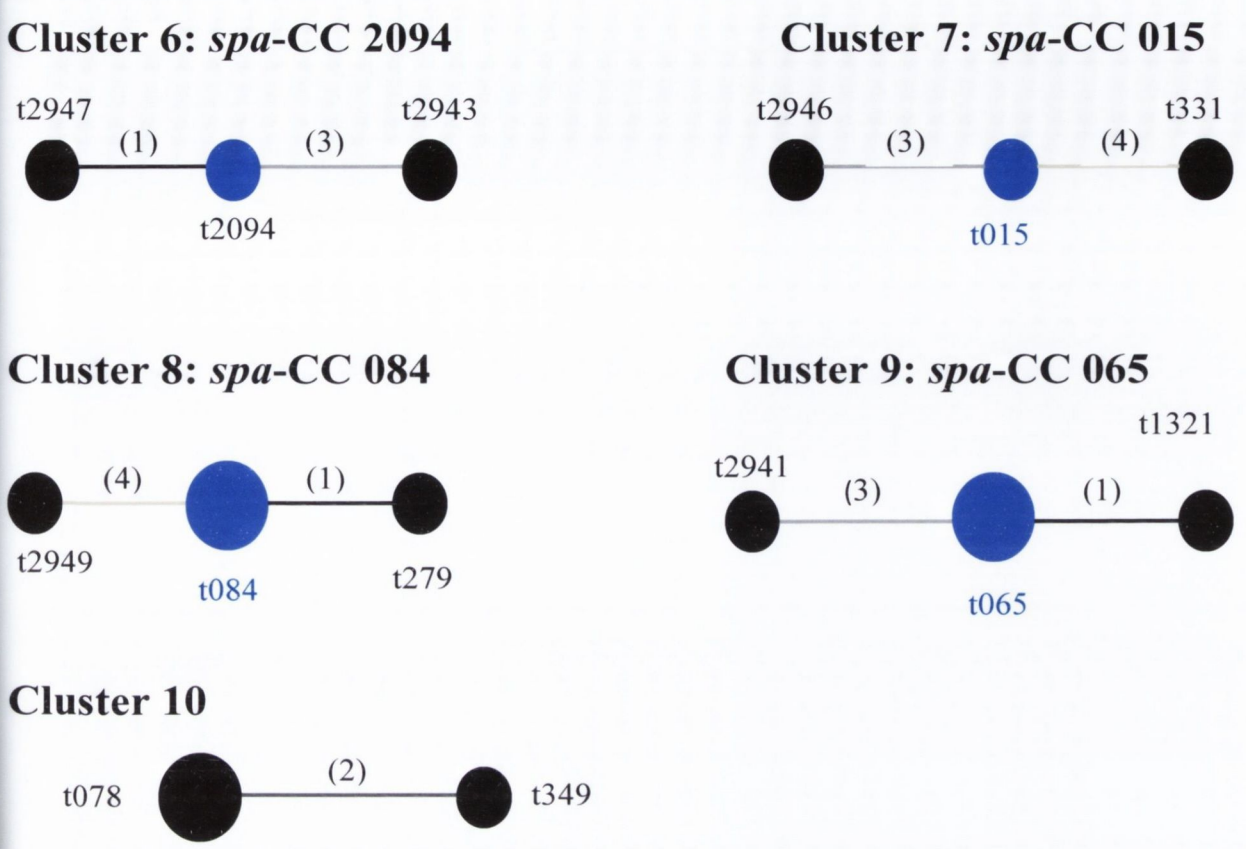


Figure 6.5. BURP analysis of the 53 *spa* types identified among the 83 MSSA isolates collected from BSIs between Septembers 2006 and February 2007. Thirty-eight *spa* types were differentiated into ten clusters using the BURP algorithm (Mellmann *et al.*, 2007). *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value ≤ 4 where cost accounts for the number of steps of evolution between *spa* types. The twelve *spa* types t089, t091, t127, t160, t166, t171, t216, t2143, t2360, t2938, t2939, t2940 were defined as singletons by BURP as they could not be clustered with any other *spa* type i.e. cost value > 5. *spa* types t026 and t362 were excluded from BURP analysis as they consisted of three and two repeat units only, respectively. Cost values are shown in parenthesis. For each cluster the group founder is shown in blue and was determined based on the *spa* type that shares the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. Clusters with no founder were not assigned a *spa*-CC name. The founder of any subgroup is shown in yellow. The sizse of the circles reflects the number of isolates assigned to each *spa* type. The exact number of isolates represented by each *spa* types is shown in Table 6.6. *spa* types highlighted in blue text are *spa* types were also found among MSSA isolates from healthy carriers (HCs) (Figure 6.6). *spa* type t089, t127, t160, t166 and t216 were also found among MSSA from HCs.

The sixty-five *spa* types from the MSSA isolates from HCs were assigned to ten clusters and 17 singletons (Figure 6.6). Cluster 1 (*spa*-CC 012) was the largest and contained 14/65 *spa* types and 25 isolates. These *spa* types all were assigned to CC30 by the DNA microarray. Cluster 2 (*spa*-CC 015) contained 8/65 *spa* types and all but one belonged to CC45. Cluster 3 (*spa*-CC 022) contained five *spa* types and all were found to belong to CC22. Cluster 4 (*spa*-CC 451/701) contained four *spa* types and these were assigned to CC6 (n = 2), CC8 and CC30 by the DNA microarray. This could be because they have a cost value of 4 and BURP may have incorrectly placed them together. Cluster 5, 6 and 7 (*spa*-CCs 065, 160, 084, respectively) had three *spa* types each and within each of these the isolates were assigned to the same CC by the DNA microarray (CC45, CC12 and CC15, respectively). Clusters 8-10 all only contained two *spa* types each and each of these pairs of *spa* types were assigned to the same CC by the DNA microarray. The *spa* types assigned to cluster 8, 9 and 10 belonged to CC59, CC5 and CC30 respectively. Seventeen *spa* types were deemed to be singletons and these included *spa* types t8683 and t11655 (CC45), t089 and t3258 (CC30), t127 and t1491 (CC1), t159 and t408 (CC121), t2049 (CC5), t209 (CC9), t3914 (CC8), t8682 (CC22), t364 (ST182), t5598 (ST291/831), t189 (CC181), t056 (CC101) and t1250 (CC398). Two *spa* type (t233 and t8681), both belonging to CC30, was excluded due to the fact that they only contained four repeat units.

6.3.1.4 DNA microarray analysis of MSSA isolates from BSIs and HCs

6.3.1.4.1 Genotype assignment

The MSSA isolates from BSIs and HCs were assigned to 16 and 19 genotypes, respectively (Table 6.7). Among both groups of isolates CC30 predominated (BSIs, 24/83, 28.9%; HCs, 37/98, 37.8%). Five genotypes were unique to the MSSA isolates from HCs, and each were found in a single isolate each. These included ST182, ST291/813, CC101,

CC118 and CC393. Two genotypes, CC25/28 (4/83, 4.8%) and CC7 (2/83, 2.4%), were found only in MSSA isolates from BSIs only.

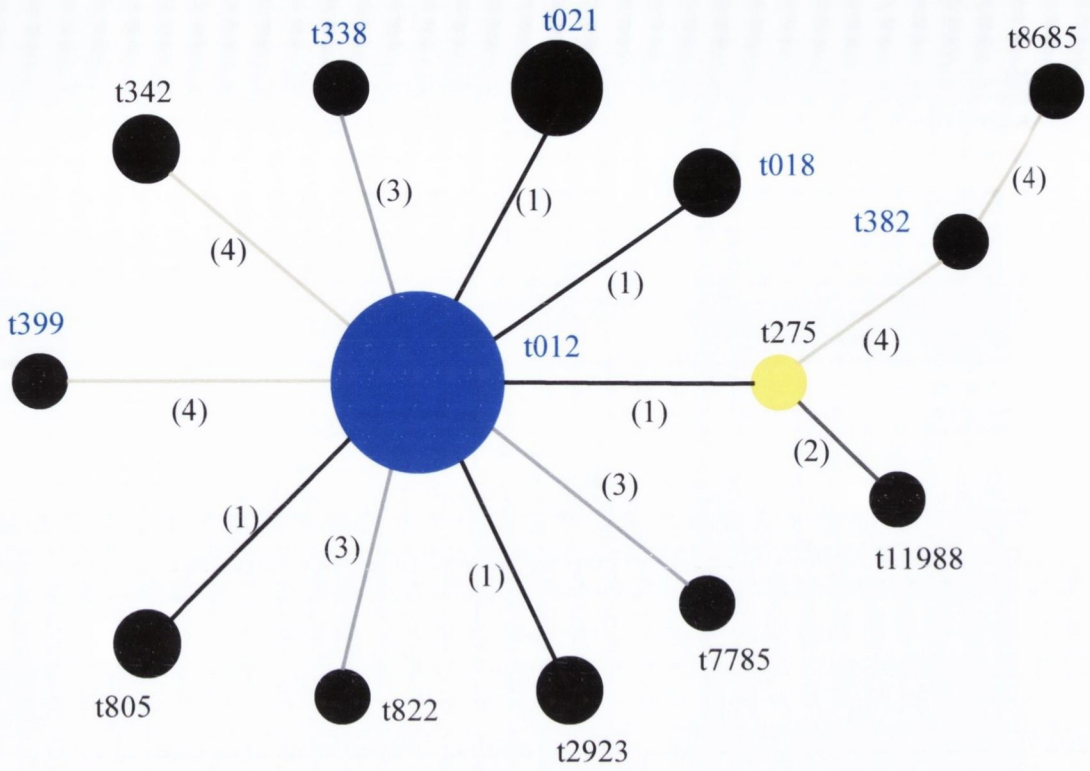
6.3.1.4.2 Antimicrobial resistance genes

The MSSA isolates from BSIs and HCs exhibited similar rates of carriage of the following antimicrobial resistance genes (Table 6.7): *fusB* (BSIs, 4/83, 4.8%; HCs, 4/98, 4.1%), *sdrM* (BSIs, 77/83, 92.8%; HCs, 91/98, 92.9%) and *fosB* (BSIs, 56/83, 67.5%; HCs, 65/98, 66.3%). The prevalence of *blaZ* (BSIs, 76/83, 91.6%; HCs, 71/98, 72.4%), the fusidic acid resistance gene *fusC* (BSIs, 5/83, 6%; HCs, 2/98, 2%), the tetracycline resistance genes *tet*(K) (BSIs, 4/83, 4.8%; HCs, 1/98, 1%) and the macrolide resistance gene *erm*(A) (BSIs, 7/83, 8.4%; HCs, 5/98, 5.1%), were found to be slightly higher in BSI isolates compared to HCs, but only *blaZ* was seen to have statistical significance (p = 0.001). The gene *erm*(C) was found in both groups of isolates but at a slightly higher level among the MSSA isolates from HCs (BSIs, 3/83, 3.6%; HCs, 7/98, 7.1%), but this was not statistically significant (p = 0.30). The genes *aacA-aphD* (2/83, 2.4%; CC8), *aadD* (4/83, 4.8%; CC8, CC5, CC7 and CC45), *mupA* (2/83, 2.4%; CC8 and CC45) and *qacC* (3/35, 3.6%; CC8 (n = 1) and CC45 (n = 2)) were identified in MSSA isolates recovered from BSIs, but not from HCs. The genes *msr*(A) (1/98, 1%; CC5), *mph*(C) (2/98, 2%; CC5, CC59), *vgaA* (3/98, 3.1%; CC5, CC15, CC30), and *tet*(M) (5/98, 5.1%; CC30 (n = 4), CC45 (n = 1)) were identified in MSSA isolates from HCs, but not from BSIs.

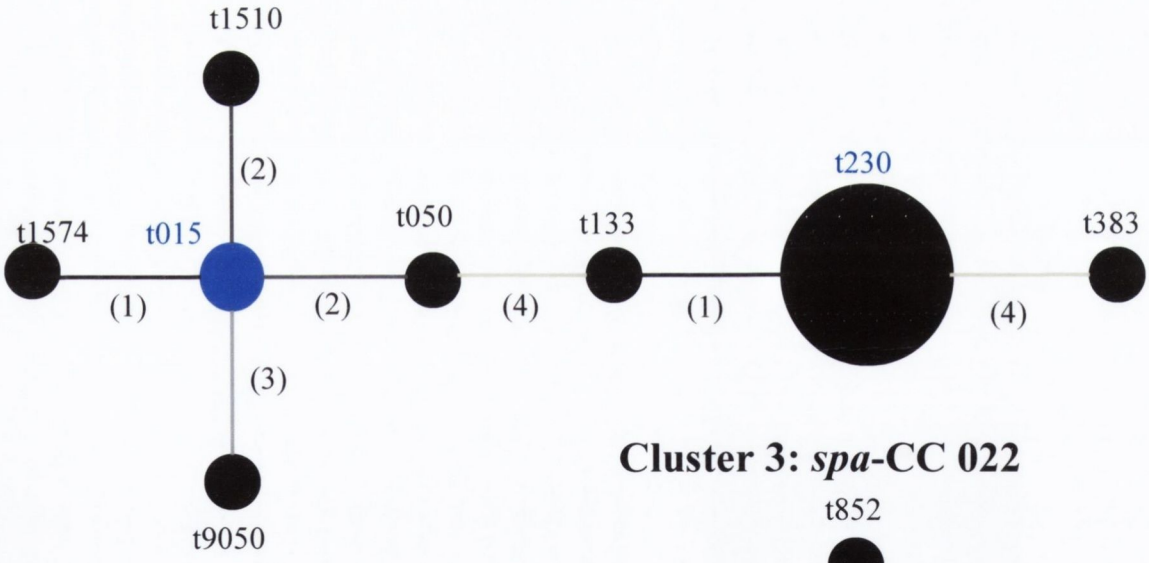
6.3.1.4.3 Virulence-associated genes

Similar rates of carriage of the following virulence-associated genes were detected among the MSSA isolates from BSIs and HCs: *egc* (BSI, 60/83, 72.3%; HCs, 72/98, 73.5%), *sea* (BSI, 16/83, 19.3%; HCs, 22/98, 22.4%), *seh* (BSI, 8/83, 9.6%; HCs, 12/98, 12.2%), *lukF/S*-PV (BSI, 2/83, 2.4%; HCs, 2/98, 2%), *scn* (BSI, 77/83, 92.8%; HCs, 91/98, 92.9%), *sak* (BSI, 73/83, 87.9%; HCs, 84/98, 85.7%), and *chp* (BSI, 58/85, 69.9%; HCs,

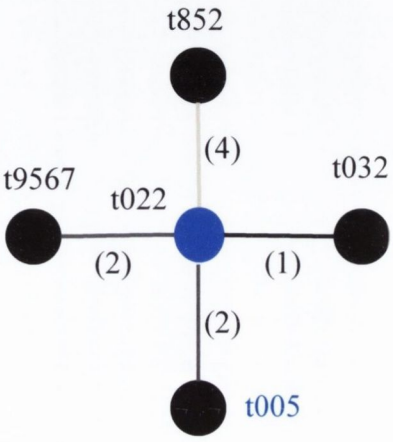
Cluster 1: *spa*-CC 012



Cluster 2: *spa*-CC 015



Cluster 3: *spa*-CC 022



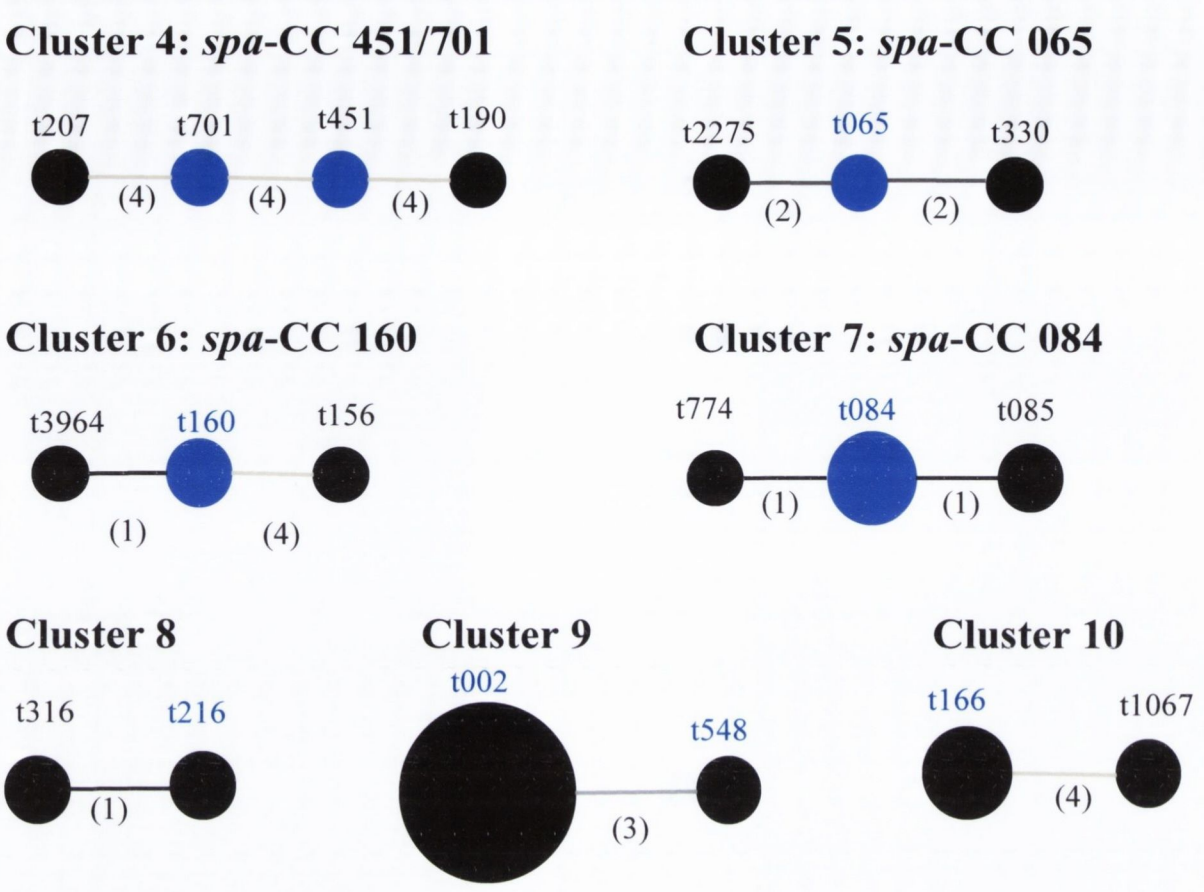


Figure 6.6. BURP analysis of 65 *spa* types identified among 98 nasal carriage MSSA isolates recovered from the nares of students in a Dublin University between 1995-2013. Sixty-five *spa* types were differentiated into ten clusters using the BURP algorithm (Mellmann *et al.*, 2007). *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value ≤ 4 where cost accounts for the number of steps of evolution between *spa* types. The 17 *spa* types t056, t089, t127, t159, t189, t209, t364, t408, t1250, t1491, t2049, t3258, t3914, t5598, t8682, t8683 and t11655 were defined as singletons by BURP as they could not be clustered with any other *spa* type i.e. cost value > 5. *spa* types t233 and t8681 was excluded as they consisted of four repeat units only. Cost values are shown in parenthesis. For each cluster the group founders is shown in blue and was determined based on the *spa* type that shares the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. Clusters with no founder were not assigned a *spa*-CC name. The founder of any subgroup is shown in yellow. The sizes of the circles reflected the number of isolates assigned to each *spa* type. The exact number of isolates represented by each *spa* is shown in Table 6.6. The *spa* types highlighted in blue text are *spa* types also found among the MSSA isolates from BSIs (Figure 6.6). *spa* types t089, t127 and t209 were also found among MSSA from BSIs.

Table 6.7. DNA microarray and *spa* typing results for MSSA isolates investigated in the present study from bloodstream infections and healthy carriers

Isolate group	Microarray CC/ST-MSSA designation (*n*)	*spa* type (*n*)	DNA microarray genes detected		
			***agr*/*cap*/IEC type**[a] **(% positive isolates, if not 100%)**	**Antimicrobial resistance genes (% positive isolates, if not 100%)**	**Virulence-associated genes (% positive isolates, if not 100%)**
Bloodstream infection (BSIs) (*n* = 83)	CC30-MSSA (*n* = 19)	t021 (*n* = 6), t012 (*n* = 3), t382 (*n* = 3), t018 (*n* = 1), t338 (*n* = 1), t399 (*n* = 1), t1414 (*n* = 1), t1827 (*n* = 1), t2944 (*n* = 1), t2950 (*n* = 1)	*agr* III, *cap* 8 IEC B (42%), IEC A (32%), IEC E (11%), ICE C (5%) IEC neg (10%)	*blaZ* (90%), *erm*(A) (5%), *sdrM, fosB*	*tst* (63%), *sea* (37%), sec & *sel* (16%), egc (68%), *lukF/S*-PV (5%), *sak* (89%), *chp* (79%), *scn* (89%)
	CC30/ST34-MSSA (*n* = 5)	t166 (*n* = 3), t089 (*n* = 2)	*agr* III, *cap* 8 IEC B (40%), IEC E (40%), IEC neg (20%)	*blaZ, sdrM, fosB*	*tst* (80%), *egc, sak* (80%), *chp* (40%), *scn* (80%)
	CC45-MSSA (*n* = 15)	t026 (*n* = 2), t065 (*n* = 2), t230 (*n* = 2), t015 (*n* = 1), t331 (*n* = 1), t362 (*n* = 1), t1321 (*n* = 1), t2143 (*n* = 1), t2940 (*n* = 1), t2940 (*n* = 1), t2941 (*n* = 1), t2946 (*n* = 1), t2948 (*n* = 1)	*agr* I, *cap* 8, IEC B	*blaZ* (93%), *erm*(A) (13%), *aadD* (7%), *fusB* (13%), *mupA* (7%), *sdrM, qacC* (7%)	*sec & sel* (33%), *sed & sej & ser* (13%), *egc, sak, chp, scn*
	CC5-MSSA (*n* = 10)	t088 (*n* = 3), t548 (*n* = 3), t002 (*n* = 2), t071 (*n* = 1), t179 (*n* = 1)	*agr* II, *cap* 5 IEC F (40%), IEC D (30%), IEC B (10%), IEC neg (20%)	*blaZ* (90%), *erm*(A) (10%), *erm*(C) (20%), *aadD* (10%), *sdrM, fosB*	*sea* (30%), *sed* (70%), *egc*, *sej* & *ser* (60%), *sak* (80%), *chp* (50%), *scn* (80%), *sep* (30%)
	CC22-MSSA (*n* = 6)	t005 (*n* = 2), t223 (*n* = 1), t449 (*n* = 1), t2787 (*n* = 1), t2938 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ*	*sec & sel* (17%), *egc, sak, chp, scn*
	CC8-MSSA (*n* = 5)	t008 (*n* = 2), t024 (*n* = 1), t1391 (*n* = 1), t2658 (*n* = 1)	*agr* I, *cap* 8 IEC D (40%), IEC B (20%), IEC F (20%), IEC neg (20%)	*blaZ, erm*(C) (20%), *accA-aphD* (40%), *aadD* (20%), *mupA* (20%), *tet*(K) (20%), *sdrM, fosB, qacC* (40%)	*sea* (40%), *seb* (20%), *sek & seq* (40%), *sak, chp* (20%), *scn* (80%), *sep* (20%),

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Table 6.7 continued. DNA microarray and *spa* typing results for MSSA isolates investigated in the present study from bloodstream infections and healthy carriers

Isolate group	Microarray CC/ST-MSSA designation (*n*)	*spa* type (*n*)	DNA microarray genes detected		
			agr/*cap*/IEC type[a] (% positive isolates, if not 100%)	Antimicrobial resistance genes (% positive isolates, if not 100%)	Virulence-associated genes (% positive isolates, if not 100%)
Bloodstream infection (BSIs) (*n* = 83)	CC15-MSSA (*n* = 4)	t084 (*n* = 2), t279 (*n* = 1), t2949 (*n* = 1)	*agr* II, *cap* 8, IEC C	*blaZ* (75%), *tet*(K) (25%), *sdrM, fosB*	*chp, scn*
	CC25/28-MSSA (*n* = 4)	t078 (*n* = 2), t349 (*n* = 1), t2939 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ* (75%), *sdrM, fosB*	*seb* (50%), *sec & sel* (25%), *egc*, *lukF/S*-PV (25%), *sak, chp, scn, etB* (25%), *etD & edinB*
	CC1-MSSA (*n* = 3)	t127 (*n* = 3)	*agr* III, *cap* 5, IEC B	*blaZ* (67%), *erm(A)* (33%), *fusB* (67%), *sdrM*	*sea, seb* (33%), *seh, sek & seq, sak, chp*
	CC20-MSSA (*n* = 3)	t2094 (*n* = 1), t2943 (*n* = 1), t2947 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ, tet*(K) (33%), *sdrM, fosB*	*egc, sak, chp, scn*
	CC7-MSSA (*n* = 2)	t091 (*n* = 2)	*agr* I, *cap* 8, IEC G	*blaZ, aadD* (50%), *tet*(K) (50%), *sdrM*	*sak, scn, sep*
	CC9-MSSA (*n* = 2)	t209 (*n* = 2)	*agr* II, *cap* 5, IEC B	*blaZ, erm*(A), *sdrM, fosB*	*egc, sak, chp, scn, etA* (50%)
	CC121-MSSA (*n* = 2)	t171 (*n* = 2)	*agr* IV, *cap* 8, IEC E	*blaZ, fusB, sdrM, fosB*	*egc, sak, scn, etA* (50%), *etB & edinC* (50%)
	CC6-MSSA (*n* = 1)	t2360 (*n* = 1)	*agr* I, *cap* 8, IEC E	*blaZ, sdrM, fosB*	*sea, sak, scn*
	CC12-MSSA (*n* = 1)	t160 (*n* = 1)	*agr*II, *cap* 8, IEC G	*sdrM, fosB*	*sep, sak, scn*
	CC59-MSSA (*n* = 1)	t216 (*n* = 1)	*agr* I, *cap* 8, IEC A	*blaZ, srdM*	*seb, sek &, seq, sak, chp, scn*
Healthy carriers (HCs) (Student nasal carriage) (*n* = 98)	CC30-MSSA (*n* = 29)	t012 (*n* = 6), t021 (*n* = 3), t018 (*n* = 2), t019 (*n* = 1), t275 (*n* = 2), t342 (*n* = 2), t2923 (*n* = 2), t133 (*n* = 1), t233 (*n* = 1), t338 (*n* = 1), t382 (*n* = 1), t399 (*n* = 1), t805 (*n* = 1) t822 (*n* = 1), t7785 (*n* = 1), t8681 (*n* = 1), t8685 (*n* = 1), t11988 (*n* = 1)	*agr* III, *cap* 8 IEC A (45%), IEC B (34%), IEC C (7%), IEC E (7%), IEC neg (7%)	*blaZ* (97%), *erm*(A) (10%), *erm*(C) (7%), *vga*(A) (3%), *tet*(M) (7%), *sdrM,, fosB, fosB*-plasmid (17%)	*tst* (83%), *sea* (45%), *sec & sel* (10%), *egc* (86%), *sak* (86%), *chp* (79%), *scn* (90%)

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Table 6.7 continued. DNA microarray and *spa* typing results for MSSA isolates investigated in the present study from bloodstream infections and healthy carriers

Isolate group	Microarray CC/ST-MSSA designation (*n*)	*spa* type (*n*)	DNA microarray genes detected		
			***agr*/*cap*/IEC type**[a] **(% positive isolates, if not 100%)**	**Antimicrobial resistance genes (% positive isolates, if not 100%)**	**Virulence-associated genes (% positive isolates, if not 100%)**
Healthy carriers (HCs) (Student nasal carriage) (*n* = 98)	CC30/ST34-MSS (*n* = 8)	t089 (*n* = 3), t166 (*n* = 3),t1067 (*n* = 1), t3258 (*n* = 1)	*agr* III, *cap* 8 IEC E (38%), IEC A (25%), IEC B (13%), IEC neg (24%)	*blaZ*, *erm*(C) (25%), *tet*(M) (25%), *sdrM*, *fosB*	*tst* (75%), *sea* (25%), *seb* (13%), *egc*, *sak* (75%), *chp* (50%), *scn* (75%)
	CC45-MSSA (*n* = 18)	t230 (*n* = 6), t015 (*n* = 2), t050 (*n* = 1), t065 (*n* = 1), t330 (*n* = 1), t383 (*n* = 1), t1510 (*n* = 1), t1574 (*n* = 1), t2275 (*n* = 1), t8683 (*n* = 1), t9050 (*n* = 1), t11655 (*n* = 1)	*agr* I, *cap* 8 IEC B (83%), IEC E (6%) IEC neg (11%)	*blaZ* (61%), *erm*(A) (6%), *erm*(C) (6%), *fusB* (6%), *tet*(M) (6%), *sdrM*, *fosB* (6%)	*tst* (6%), *seb* (6%), *sec* & *sel* (61%), *egc*, *sak* (89%), *chp* (83%), *scn* (89%)
	CC5-MSSA (*n* = 9)	t002 (*n* = 7), t548 (*n* = 1), t2049 (*n* = 1)	*agr* I, *cap* 8 IEC B (78%), IEC F (11%), IEC E (11%)	*blaZ* (22%), *erm*(C) (11%), *msr*(A) (11%), *mph*(C) (11%), *sdrM*, *fosB*	*tst* (22%), *sec* & *sel* (11%), *sed* & *sej* & *ser* (11%), *egc*, *lukF/S*-PV (11%), *sak*, *chp* (88%), *scn*
	CC15-MSSA (*n* = 6)	t084 (*n* = 3), t085 (*n* = 2), t774 (*n* = 1)	*agr* II, *cap* 8 IEC C (83%), IEC B (17%)	*blaZ* (67%), *vga* (33%), *tet*(K) (17%), *sdrM*, *fosB*, *fosB*-plasmid (33%)	*sec* & *sel* (17%), egc (17%), *seg* (17%), *sak* (17%), *chp*, *scn*, *etA* (17%)
	CC22-MSSA (*n* = 5)	t005 (*n* = 1), t032 (*n* = 1), t852 (*n* = 1), t8682 (*n* = 1), t9567 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ* (60%), *erm*(C) (20%), *fusC* (20%),	*seb* (20%), *sec* & *sel* (40%), *egc*, *sak*, *chp*, *scn*
	CC12-MSSA (*n* = 4)	t160 (*n* = 3), t 3694 (*n* = 1)	*agr* II, *cap* 8, IEC G	*sdrM*, *fosB*	*seb* (75%), *sec* & *sel* (25%),*sep*, *sak*, *scn*
	CC1-MSSA (*n* = 3)	t127 (*n* = 2), t1491 (*n* = 1)	*agr* III, *cap* 8, IEC D	*blaZ*, *erm(A)* (33%), *fusC* (33%), *sdrM*, *fosB*-plasmid (33%)	*sea*, *seb* (33%), *sec* & *sel* (33%), *she* (33%),*sek* & *seq* (67%), *sak*, *scn*
	CC121-MSSA (*n* = 3)	t408 (*n* = 2), t159 (*n* = 1)	*agr* IV, *cap* 8, IEC E	*blaZ*, *fusB* (67%), *sdrM*, *fosB*	egc, *sak*, *scn*, *etA* (67%), *etB* & *edinC* (33%)

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Table 6.7 continued. DNA microarray and *spa* typing results for MSSA isolates investigated in the present study from bloodstream infections and healthy carriers

Isolate group	Microarray CC/ST-MSSA designation (*n*)	*spa* type (*n*)	DNA microarray genes detected		
			***agr*/*cap*/IEC type[a] (% positive isolates, if not 100%)**	**Antimicrobial resistance genes (% positive isolates, if not 100%)**	**Virulence-associated genes (% positive isolates, if not 100%)**
Healthy carriers (HCs) (Student nasal carriage) (*n* = 98)	CC6-MSSA (*n* = 2)	t207 (*n* = 1), t701 (*n* = 1)	*agr* I, *cap* 8, IEC D	*blaZ* (50%), *sdrM, fosB*	*sea, seb* (50%), *sak, scn*
	CC8-MSSA (*n* = 2)	t3914 (*n* = 1), t451 (*n* = 1)	*agr* I, *cap* 5, IEC D	*blaZ, sdrM, fosB*	*sea, sak, scn*
	CC59-MSSA (*n* = 2)	t216 (*n* = 1), t316 (*n* = 1)	*agr* I, *cap* 8 IEC B (50%), IEC F (50%)	*blaZ* (50%), *mph(C)* (50%), *fusB* (50%), *sdrM*	*seb* (50%), *sek* (50%), *seq* (50%), *sep* (50%), *sak, chp, scn*
	CC9-MSSA (*n* = 1)	t209 (*n* = 1)	*agr* II, *cap* 5, IEC B	*blaZ, sdrM,, fosB*	egc, *sak, chp, scn, etA*
	CC20-MSSA (*n* = 1)	t3914 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ, sdrM, fosB*	*seb*, egc, *sak, chp, scn,*
	CC101-MSSA (*n* = 1)	t056 (*n* = 1)	*agr* I, *cap* 8, IEC E	*sdrM, fosB*	*sak, scn*
	CC118-MSSA(*n* = 1)	t189 (*n* = 1)	*agr* I, *cap* 8, IEC B	*blaZ, sdrM*	*sak, chp, scn*
	CC398-MSSA (*n* = 1)	t1250 (*n* = 1)	*agr* I, *cap* 5, IEC C	*sdrM*	*chp, scn*
	ST182-MSSA (*n* = 1)	t364 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ, sdrM*	egc, *seh, sak, chp, scn*
	ST291/813-MSSA (*n* = 1)	t5598 (*n* = 1)	*agr* I, *cap* 5, IEC B	*blaZ, sdrM*	*sak, chp, scn, etD & edinB*

[a]*agr*, accessory gene regulatory genes; *cap,* capsule genes; the immune evasion complex (IEC) type of each isolate type was determined based on the combination of 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 C: *chp & scn*; IEC type D: *sea, chp & scn*; IEC type E: *sak & scn*; IEC type F: *sep, sak, chp & scn*; IEC G: *sep, sak & scn*; IEC neg; no IEC genes detected.

69/98, 70.4%). The virulence genes *tst* (HCs, 33/98, 33.8%; BSI, 16/83, 19.3%), *seb* (HCs, 10/98, 10.2%; BSI, 5/83, 6%), *sec* & *sel* (HCs, 20/98, 20.5%; BSI, 10/83, 12.1%) and exfoliative toxin gene *etA* (HCs, 4/98, 4.1%; BSIs, 2/83, 2.4%) were more common among the MSSA isolates from HCs than BSIs (Table 6.7), but only the carriage of the *tst* gene was statistically significant ($p = 0.02$). In contrast, the virulence genes *sed, sej* & *ser* (BSI, 8/83, 9.6%; HCs, 1/98, 1%), *sek* & *seq* (BSI, 6/83, 7.2%; HCs, 3/98, 3.1%), *etD* & *edinB* (BSIs, 4/83, 4.8%; HCs, 1/98, 1%) and *etB* & *edinC* (BSIs 2/85, 2%; HCs 1/99, 1%) were more common among the MSSA from BSIs than HCs (Table 6.7). Of these, only the higher rate of carriage of *sed, sej* & *ser* genes among the BSIs were statistically significant ($p = 0.006$).

The IEC genes were identified in 93.4% (BSIs, 77/83, 92.8%; HCs, 92/98, 93.9%) of isolates and the most common IEC type was IEC type B (*sak, chp, scn*) (BSIs, 36/83, 43.3%; HCs, 52/98, 53.1%), followed by IEC type A (*sea, sak, chp* and *scn*) (BSIs, 7/83, 8.4%; HCs, 14/98, 14.2%) and IEC type C (*chp* and *scn*) (BSIs, 5/83, 6%; HCs, 8/98, 8.1%). The most common *agr* type was *agr* I (BSIs 37/83, 44.5%; HCs 45/98, 45.9%), followed by *agr* III (BSIs 27/83, 32.5%; HCs 39/98, 39.8%), *agr* II (BSIs 17/83, 20.5%; HCs 11/98, 11.2%), and *agr* IV (BSIs 2/83, 2.4%; HCs 3/98, 3.1%). The *cap* type 8 was found to be the most common among both MSSA groups (BSIs 55/83, 66.3%; HCs 86/98, 87.8%) with the remaining isolates harbouring *cap* type 5 (BSIs 28/83, 33.7%; HCs 12/98, 12.2%).

6.3.2. Investigation of TSST-1 production and *tst* expression

6.3.2.1 Comparative analysis of TSST-1 production in MRSA and MSSA isolates from HCs and BSIs

Due to the higher prevalence of *tst* among MSSA (49/181, 27.1%) compared to MRSA (4/136, 2.9%) isolates ($p < 0.05$), all *tst*-positive MRSA and MSSA isolates were

investigated for the production of the TSST-1 protein using a TSST-1-specific RPLA assay (Table 6.4). The majority of the *tst*-positive MSSA isolates (46/49, 93.9%) and 1/4 (25%) *tst*-positive MRSA isolates belonged to CC30 (Table 6.4). The three remaining MRSA isolates belonged to ST5-MRSA-II while the remaining MSSA isolates belonging to CC5 (n = 2) and CC45 (n = 1) (Table 6.4). Production of TSST-1 was detected in all but two CC30-MSSA *tst*-positive isolates, including one HC isolate (ED41) and one BSI isolate (SpaS066) (Table 6.4).

6.3.2.2 Comparative analysis of *tst* expression among MSSA isolates from HCs and BSIs

Due to the higher prevalence of *tst* among MSSA isolates recovered from HCs (33/98, 33.7%) compared to BSIs (16/83, 19.3%) (p = 0.02) and the fact that the majority of *tst*-positive isolates belonged to CC30, the level of expression of *tst* in five *tst*-positive CC30 HC MSSA isolates and five CC30 *tst*-positive BSI MSSA isolates was investigated by qRT- PCR (Table 6.4). These isolates represented a range of CC30 *spa* types and DNA microarray profiles (Table 6.4). Four additional *tst*-positive isolates recovered from patients with toxic shock syndrome (TSS) in a German hospital were also included in this analysis for comparative purposes (Table 6.4).

Overall, the mean *tst* expression level was higher amongst MSSA isolates recovered from BSIs than HCs when cultured in BHI broth in either the presence (2.6-fold higher) or absence (1.7-fold higher) of 10% foetal bovine serum (FBS) (Figure 6.7). However, the observed differences in *tst* expression levels were not statistically significant either in the presence (p = 0.73) or absence (p = 0.68) of 10% FBS when tested using a two-sample Student's t-test.

When MSSA isolates recovered from HCs were cultured in BHI supplemented with 10% FBS the mean level of *tst* expression decreased by 1.7-fold. This difference was not statistically significant (p = 0.73) (Table 6.8). The mean levels of *tst* expression of isolates

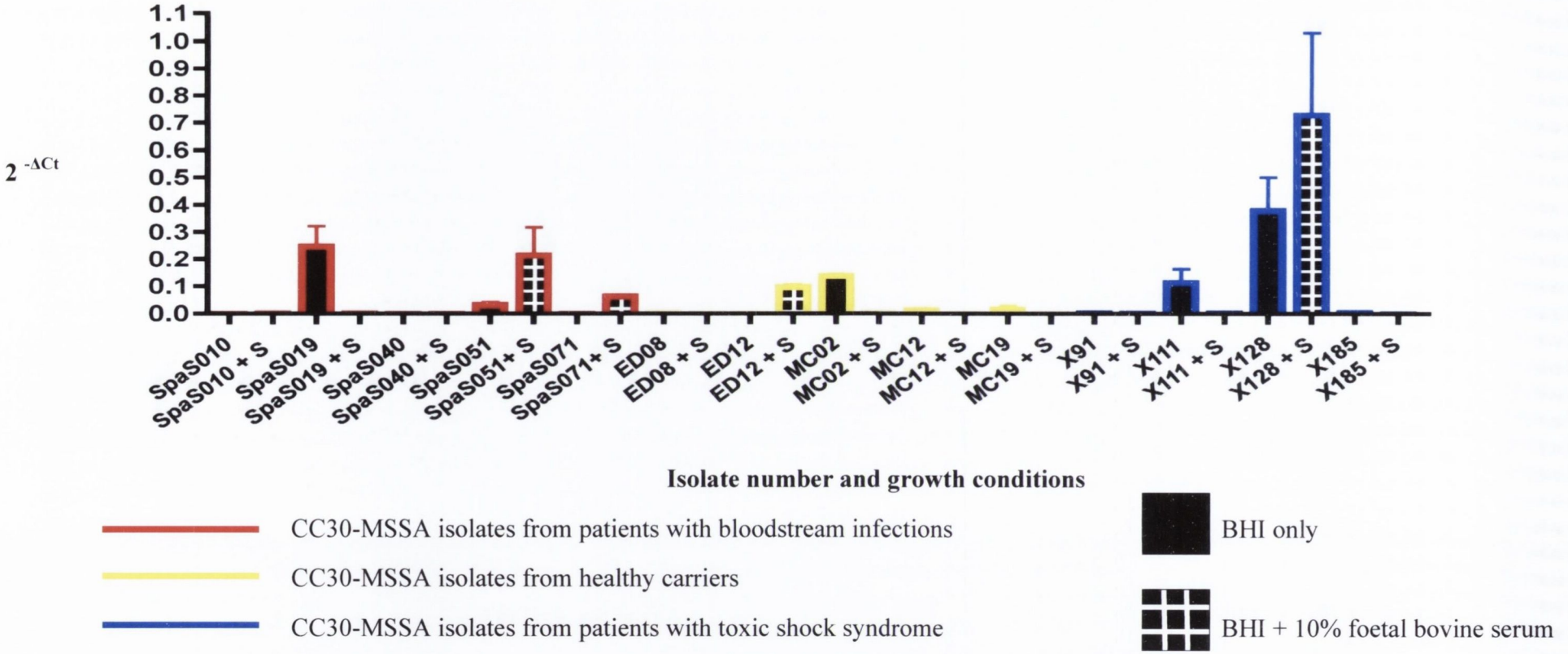


Figure 6.7. Expression levels of *tst* among CC30-MSSA isolates from patients with bloodstream infections ($n = 5$), healthy carriers ($n = 5$) and patients with toxic shock syndrome ($n = 4$) cultured in brain heart infusion broth (BHI) in the presence (+S) and absence of 10% foetal bovine serum. For each isolate investigated, the average of three Ct values was determined separately for each gene and the resulting data were analysed according to Livak and Schmittgen (2001) i.e. the ΔCt value (the average Ct of the endogenous control gene (16S rDNA) was subtracted from the average Ct for the gene of interest (*tst*)) was calculated for each isolate, from which the fold differences in gene expression ($2^{-\Delta Ct}$) was determined.

Table 6.8. Real-time PCR results for *tst* expression by selected CC30 MSSA isolates

Isolate group	*n*	Media used	Mean $2^{-\Delta Ct}$ expression levels[a]	Standard deviation
Bloodstream infections	5	BHI	5.57×10^{-2}	$\pm 1.1 \times 10^{-1}$
	5	BHI + 10% FBS	5.56×10^{-2}	$\pm 9.2 \times 10^{-2}$
Healthy carriers	5	BHI	3.33×10^{-2}	$\pm 5.8 \times 10^{-2}$
	5	BHI + 10% FBS	1.95×10^{-2}	$\pm 4.3 \times 10^{-2}$
Patients with toxic shock syndrome	4	BHI	1.24×10^{-1}	$\pm 1.8 \times 10^{-1}$
	4	BHI + 10% FBS	1.83×10^{-1}	$\pm 3.6 \times 10^{-1}$

[a]ΔCt values were determined for each gene per isolate by subtraction of the average Ct values for the endogenous control 16S rDNA gene from those of the *tst* gene of interest. Following this, the $2^{-\Delta Ct}$ value for each isolate was calculated as described previously to determine the fold difference in the expression levels (Livak *et al.*, 2001).

Abbreviations: BHI, brain heart infusion broth; *n*, number of isolates investigated by quantitative real-time PCR (qRT-PCR) from each group of MSSA isolates; FBS, foetal bovine serum.

recovered from BSIs grown in BHI with 10% FBS remained similar to the levels measured when the isolates were grown in the absence of FBS (Table 6.8). When the MSSA isolates from TSS were cultured in BHI broth supplemented with 10% FBS, there was a 1.5-fold increase in the mean expression level of *tst* compared to the mean *tst* expression when isolates were cultured in BHI alone. This increase was not statistically significant (p = 0.60).

The highest mean *tst* expression levels were observed in MSSA isolates recovered from patients with TSS and cultured in BHI in both the presence and absence of 10% FBS. Under both growth conditions this was not statistically significant when compared to isolates recovered from BSIs (p = 0.45 (BHI only) and p = 0.50 (BHI and 10% FBS)) or HCs (p = 0.33 (BHI only) and p = 0.32 (BHI and 10% FBS)) (Table 6.8 and Figure 6.7).

6.3.3 Comparative molecular analysis of MRSA isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital

Thirty-eight MRSA isolates recovered from the hands of healthcare workers, 82 MRSA isolates from patients and 61 MRSA isolates recovered from environmental sites in one Dublin hospital in 2009 were compared using *spa* typing and DNA microarray profiling (Table 6.9).

6.3.3.1 *spa* typing

The MRSA isolates from the hands of healthcare workers (HHCWs), patients and environmental sites were differentiated into nine, 17 and six *spa* types, respectively (Table 6.9). The most common *spa* type among isolates recovered from the HHCWs was t032 (24/38, 63.2%) followed by t3185 (3/38, 7.9%), t515 (2/38, 7.9%), t557, (2/38, 7.9%), t1214 (2/38, 5.3%) and t022 (2/38, 5.3%). The three remaining *spa* types identified among MRSA isolates from the HHCWs (t379, t3459 and t8679) were each represented by one

isolate only (2.6%). The nine *spa* types identified among MRSA isolates from the HHCWs were assigned to a single cluster, *spa*-CC 032, using the BURP algorithm (Figure. 6.8) and all were assigned to ST22-MRSA-IV by the DNA microarray.

Among the MRSA isolates recovered from patients *spa* type t032 also predominated (39/82, 47.6%), followed by t515 (16/82, 19.5%), t557 (5/82, 6.1%), t628 (4/82, 4.9%), t190 (3/82, 3.7%), t022 (2/82, 2.4%), t1214 (2/82, 2.4%) and t3213 (2/82, 2.4%) (Table 6.9). The remaining *spa* types (t432, t885, t718, t756, t1021, t2945, t3139, t6768 and t7983) were each identified in one isolate only (1.2%). Using BURP analysis 15/17 *spa* types identified among MRSA from patients were assigned to one cluster, *spa*-CC 032 which consisted of 15/16 ST22-MRSA-IV *spa* types identified (Figure 6.8). The ST22-MRSA-IV *spa* type t3213 (n = 2) was excluded from this cluster as well as the ST8-MRSA-IIA-E & SCC_{MI} *spa* type t190 (n = 3) as they differed from other *spa* types identified among the patient MRSA isolates by a cost value >5.

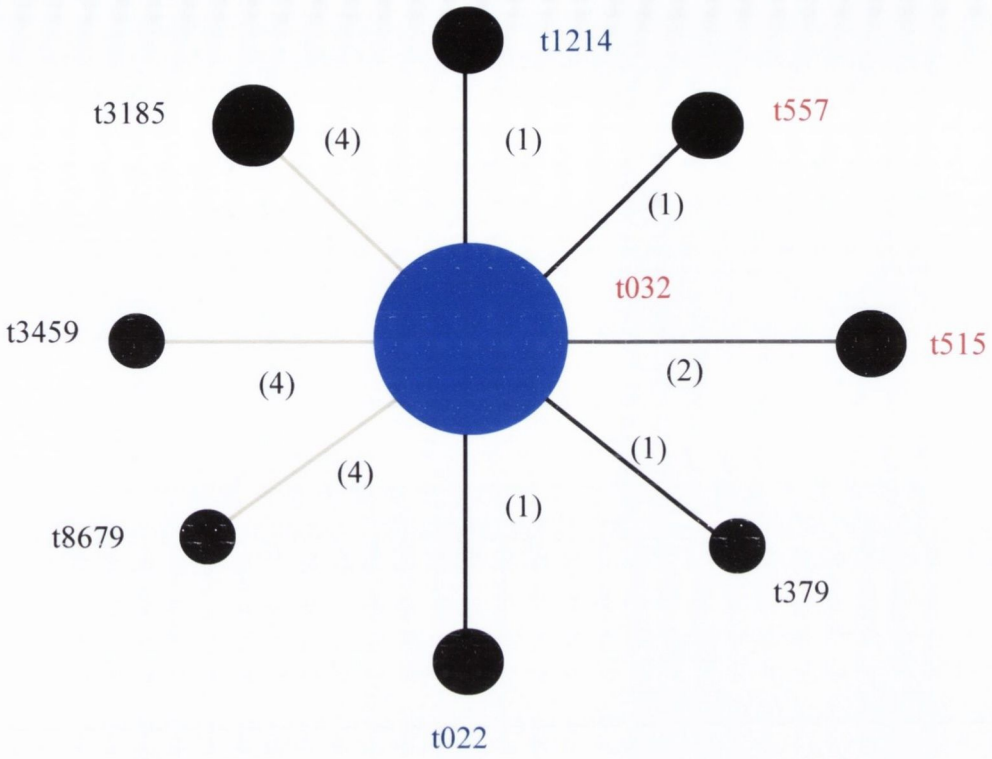
Six *spa* types were identified among MRSA isolates from environmental sites with t557 (25/61, 40.9%) and t515 (24/61, 39.7%) predominating, followed by t032 (9/61, 14.8%), t628 (1/61, 1.6%), t1565 (1/61, 1.6%) and t9568 (1/61, 1.6%) (Table 6.9). Using BURP analysis all six *spa* types identified among this isolate group were assigned to a single cluster, *spa*-CC 032 (Figure. 6.8). All 61 MRSA isolates from environmental sites were assigned to ST22-MRSA-IV by the DNA microarray.

6.3.3.2 DNA microarray profiling

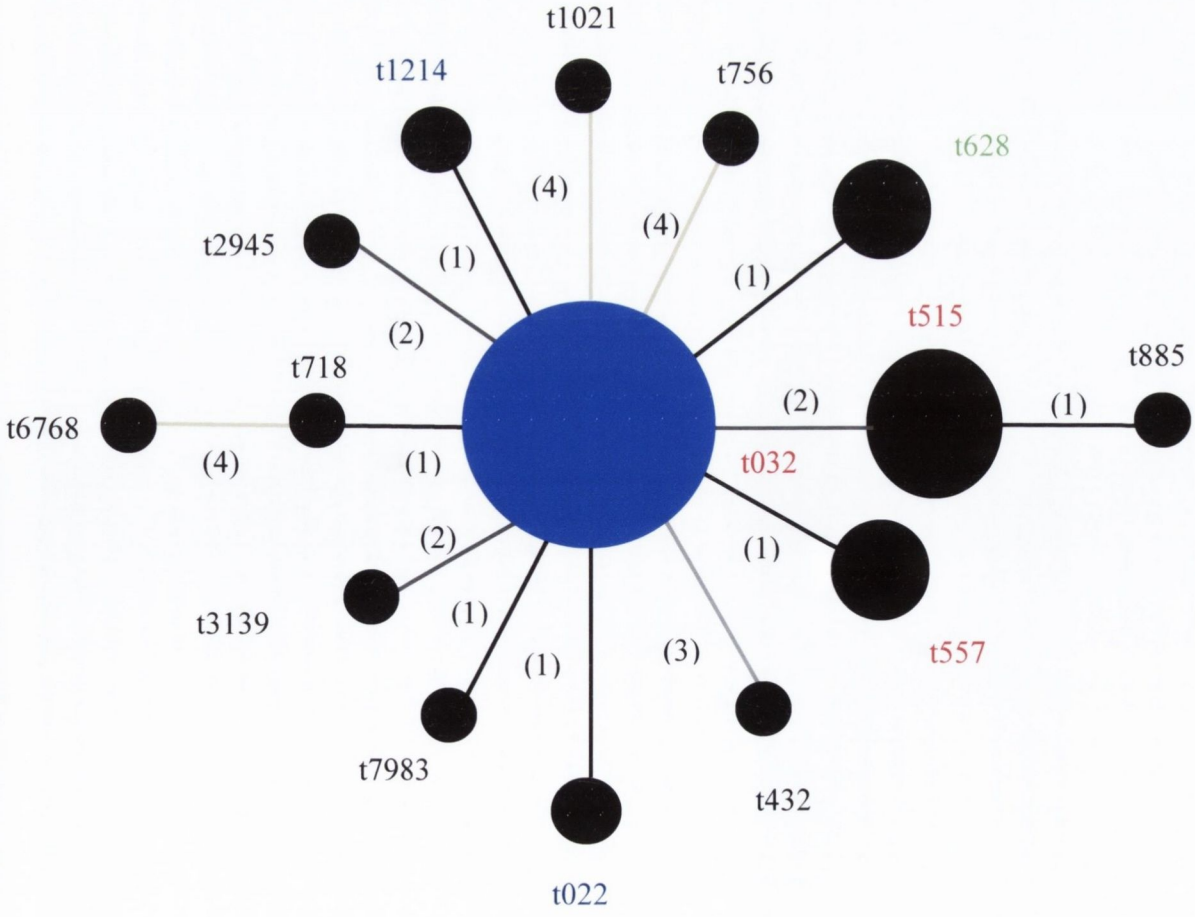
6.3.3.2.1 Genotype assignment

Using the DNA microarray all MRSA isolates from HHCWs (n = 38) and environmental sites (n = 61) and the majority of isolates from patients (79/82) were

(A) Cluster 1: *spa*-CC 032



(B) Cluster 1: *spa*-CC 032



(C) Cluster 1: *spa*-CC 032

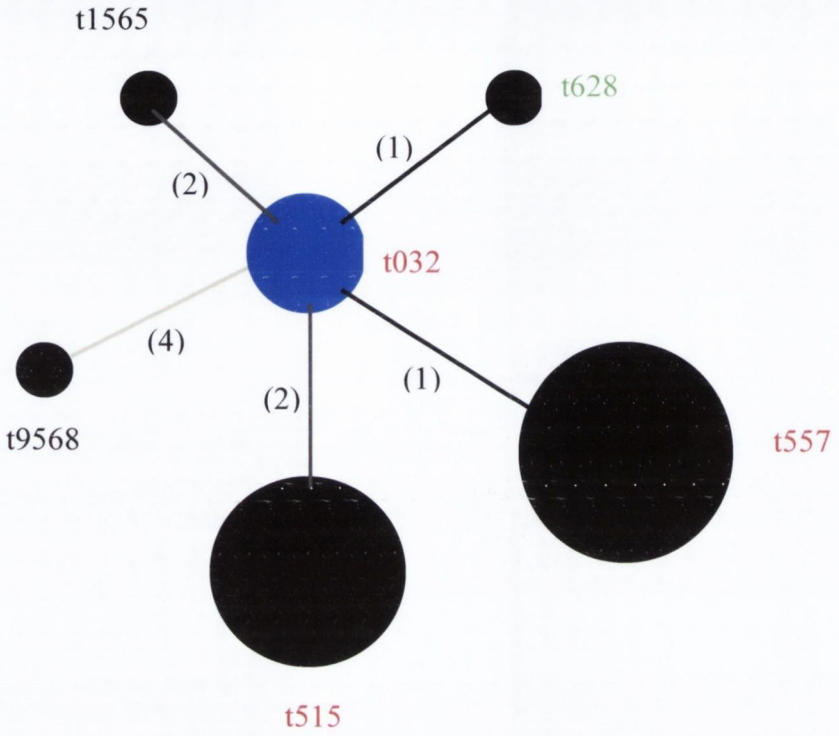


Figure 6.8. BURP analysis of the *spa* types identified among the MRSA isolates recovered from the hands of healthcare workers (HHCWs) (panel A), patients (panel B) and environmental sites (panel C) in one Dublin hospital using the BURP algorithim described in Chapter 2, section 2.5.2.1. The nine *spa* types identified among the 39 MRSA isolates recovered from HHCWs were assigned to one cluster (*spa*-CC 032) (Panel A). Fifteen of the 17 *spa* types identified among from the 82 MRSA isolates recovered from patients were assigned to one cluster (*spa*-CC 032) (Panel B). The two remaining *spa* types identified among MRSA isolates from patients were defined as singletons by BURP (t190 and t3213) as they could not be clustered with any other *spa* type i.e. they had a cost value greater than 5. The six *spa* types identified among MRSA isolates recovered from environmental sites were assigned to one cluster (*spa*-CC 032) (Panel C). *spa* types were clustered together if they contained five or more *spa* repeats and if they had a cost value $\leq$ 4 where cost accounts for the number of steps of evolution between *spa* types. Cost values are shown in parenthesis. The group founder in each case is shown in blue and was determined based on the *spa* type that shared the highest sequence identity with the greatest number of *spa* types within that cluster as determined by the cost values. The size of the circles reflects the number of isolates assigned to each *spa* type. The exact number of isolates represented by each *spa* type in show in Table 6.8. *spa* types in red text were found among MRSA from the HHCWs, patients and environmental sites. *spa* types in blue text were identified found among MRSA from the HHCWs and patients, and *spa* types in green text were identified among MRSA form patients and environmental sites.

assigned to ST22-MRSA-IV (Table 6.9). The three remaining patient MRSA isolates were assigned to ST8-MRSA-IIA-E & SCC_{MI} (Table 6.9).

6.3.3.2.2 Antimicrobial resistance genes

The most common antimicrobial resistance gene detected among the ST22-MRSA-IV isolates from HHCWs, patient and environmental sites was *blaZ* (beta-lactamase resistance), which was detected in all isolates, followed by *erm*(C) (macrolide-lincosamide-streptogramin B resistance) (HHCWs, 28/38, 73.7%; Patients, 52/82, 63.4%; Environmental sites, 55/61, 90.2%). The higher rate of carriage of *erm*(C) detected among MRSA isolates from environmental sites was statistically significant (MRSA from environmental sites versus MRSA from HHCWs, $p = 0.03$; MRSA from environmental sites versus MRSA from patients, $p = 0.0002$). The *aacA-aphD* (resistance to amikcain, gentamicin, kanamycin and tobramycin) and *mupA* (high-level mupirocin resistance) genes were identified among ST22-MRSA-IV isolates from HHCWs (7/38, 18.4%), patients (4/82, 4.9%) and environmental sites (2/61, 3.3%) but were more common among the MRSA isolates from HHCWs and this was statistically significant (MRSA from HHCWs versus MRSA from patietns, $p = 0.02$; MRSA form HHCWs versus MRSA from environmental sites sites, $p = 0.01$). The *vga* gene (streptogramin A, lincosamide, pleuromutilin resistance) was identified in one ST22-MRSA-IV isolate from a patient and one from an environmental site, but not in any MRSA from HHCWs. The gene *aphA3* (kanamycin, neomycin and tobramycin resistance) and *fosB*-plasmid (fosfomycin resistance) were detected in ST22-MRSA-IV recovered from environmental sites only (*aphA3*, 6/61, 9.8%; *fosB*, 1/61, 1.6%). The tetracycline resistance gene *tet*(K) and the macrolide resistance gene *erm*(A) were detected in one ST22-MRSA-IV isolate from HHCW (1/39, 3%) and one from a patient (1/82, 1.2%), respectively (Table 6.9).

The three ST8-MRSA-IIA-E & SCC_{MI} isolates from patients harboured more resistance genes than the ST22-MRSA-IV isolates with all isolates carrying *blaZ*, *erm*(A), *aacA-aphD*, *aphA3*, the general efflux pump *sdrM*, *fosB,* the mercury resistance genes *merA* & *merB* and quaternary ammonium compounds and antiseptics resistance gene *qacA*. One ST8-MRSA-IIA-E & SCC_{MI} isolate also harboured an additional aminoglycoside resistance gene *aadD* (amikacin, kanamycin, neomycin and tobramycin resistance) (Table 6.9).

6.3.3.2.3 Virulence-associated genes

All ST22-MRSA-IV isolates harboured *egc* and 60.5% (23/38), 53.7% (44/82) and 78.7% (48/61) of isolates from HHCWs, patients and environmental sites, respectively, also harboured *sec* and *sel* (Table 6.9). The higher rates of carriage of *sec* and *sel* detected among the MRSA isolates from environmental sites was statistically significant (MRSA from environmental sites versus MRSA from HHCWs, $p = 0.05$; MRSA from environmental sites versus MRSA from patients, $p = 0.002$). One ST22-MRSA-IV isolate recovered from a patient harboured ACME (Table 6.8). The IEC genes were detected in 92.1% (164/178) of ST22-MRSA-IV isolates (HHCWs 30/38, 78.9%; Patients, 78/79, 98.7%; Environmental sites, 56/61, 91.8%) and IEC type B was the only IEC type identified (*sak, chp* and *scn*). The higher rate of carriage of IEC type B detected among MRSA isolates from patients was statistically significant when compared to the MRSA isolates from HHCWs ($p = 0.006$), but was not statistically significant when compared to the MRSA isolates from environmental sites ($p = 0.44$). The *agr* type I and *cap* type 5 were detected among all ST22-MRSA-IV isolates.

Two of the three ST8-MRSA-IIA-E & SCC_{MI} isolates from patients harboured the enterotoxin A gene (*sea*) and all three isolates carried IEC genes. Two isolates were

Table 6.9. DNA Microarray and *spa* typing results for the 181 MRSA isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

Isolate group (*n*)	Microarray CC/ST-MRSA-SCC*mec* designation (*n*)	*spa* type (*n*)	DNA microarray genes detected			
			agr/*cap*/IEC type[a] (% positive isolates, if not 100%)	SCC*mec*-associated genes (% positive isolates, if not 100%)	Antimicrobial resistance genes (% positive isolates, if not 100%)	Virulence-associated genes (% positive isolates, if not 100%)
Hands of healthcare workers (HHCWs) (*n* = 38)	ST22-MRSA-IV (*n* = 38)	t032 (*n* = 24), t3185 (*n* = 3), t022 (*n* = 2), t515 (*n* = 2), t557 (*n* = 2), t1214 (*n* = 2), t379 (*n* = 1), t3459 (*n* = 1), t8679 (*n* = 1)	*agr* I, *cap* 5 IEC B (79%), IEC neg (21%)	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, erm*(C) (74%), *aacA-aphD & mupA* (18%), *tet(K)* (3%)	*sec & sel* (58%), *egc, sak* (79%), *chp* (79%), *scn* (79%)
Patients (*n* = 82)	ST22-MRSA-IV (*n* = 79)	t032 (*n* = 39), t515 (*n* = 16), t557 (*n* = 5), t628 (*n* = 4), t022 (*n* = 2), t1214 (*n* = 2), t3213 (*n* = 2), t432 (*n* = 1), t718 (*n* = 1), t756 (*n* = 1), t885 (*n* = 1), t1021 (*n* = 1), t2945 (*n* = 1), t3139 (*n* = 1), t6768 (*n* = 1), t7983 (*n* = 1)	*agr* I, *cap* 5 IEC B (95%), IEC neg (5%)	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, erm*(A) (3%), *erm*(C) (66%), *vga* (1%), *aacA-aphD & mupA* (5%)	*sec & sel* (55%), *egc*, *sak* (95%), *chp* (95%), *scn* (95%), ACME (1%)
	ST8-MRSA-IIA-E & SCC_{M1} (*n* = 3)	t190 (*n* = 3)	*agr* I, *cap* 5 IEC D (67%) IEC B (33%)	*mecA, ΔmecR1, ugpQ, dcs, mecI* (67%), *mecR, xylR* (67%), *ccrAB2, ccrB4*	*blaZ, erm*(A), *aacA-aphD, aadD (33%), aphA3 & sat, sdrM, fosB, qacA, merA & merB,*	*sea* (67%), *sak, chp* (33%), *scn*
Environmental sites (*n* = 61)	ST22-MRSA-IV (*n* = 61)	t557(*n* = 25), t515 (*n* = 24), t032 (*n* = 9), t682 (*n* = 1), t1565 (*n* = 1), t9568 (*n* = 1)	*agr* I, *cap* 5 IEC B (92%), IEC neg (8%)	*mecA, ΔmecR1, ugpQ, dcs, ccrAB2*	*blaZ, erm*(C) (90%), *vga* (2%), *aacA-aphD & mupA* (3%), *aphA3* (10%) *sat* (3%), f*osB*-plasmid (3%)	*sec & sel* (79%), *egc, sak* (92%), *chp* (92%), *scn* (92%)

[a]*agr*, accessory gene regulatory genes; *cap,* capsule genes; the immune evasion complex (IEC) type of each isolate type was determined based on the combination of IEC genes detected as described by van Wamel *et al.*,2006.; IEC type B: *sak, chp & scn*; IEC type D: *sea, chp & scn.*; IEC neg; no IEC genes detected

assigned to IEC type D (*sea, sak* and *scn*) and one to IEC type B (*sak, chp,* and *scn*). All three ST8-MRSA-IIA-E & SCC_{MI} isolates were assigned to *agr* type I and *cap* type 5.

6.3.3.3. Combined *spa* and DNA microarray profiling of ST22-MRSA-IV isolates

In order to investigate the relatedness of ST22-MRSA-IV isolates from HHCWs, patients and environmental sites, the *spa* type and DNA microarray profile of each isolate was combined. Firstly, each DNA microarray profile was assigned a microarray group (MG) number (Table 6.10 and section 6.3.3.3.1). Then the number of gene differences between each MG was determined (Table 6.11) as well as the number of gene differences between the MGs within each ST22-MRSA-IV *spa* type (Table 6.12 and section 6.3.3.3.2). Based on this, the relatedness of MRSA isolates from the HHCWs, patients and environmental sites assigned to each MG within each *spa* type represented by >1 MG was determined (Table 6.13 and section 6.3.3.3.3).

6.3.3.3.1 Microarray groups

Analysis of the DNA microarray profiles of the 178 ST22-MRSA-IV isolates identified from HHCWs, patients and environmental sites resulted in the identification of 12 variable genes or gene clusters among the isolates including the antimicrobial resistance genes *erm*(A), *erm*(C), *aacA-aphD & mupA, aadD, aphA3 & sat, fosB, tet*(K), *sdrM* and *qacA* and the virulence-associated genes *sec/sel,* ACME and the IEC genes (Table 6.10). A microarray group (MG) number was assigned to each different combination of these genes resulting in 19 MGs (Table 6.10), nine of which have been described in a previous study (MGs 1, 2, 4-6, 8, 9, 11 &12; Brennan, 2013) and nine (MGs 14-22) which were assigned in the present study (Table 6.10). The most common MG identified among the ST22-MRSA-IV isolates was MG11 (*blaZ, erm*(C), *sec/sel, egc,* IEC type B), which accounted for 39.9% of isolates (71/178) (Table 6.10). MG11 was also the most common MG among each group of isolates accounting for 23.7% (9/38), 30.5% (25/82) and 60.7% (37/61) of

isolates from the HHCWs, patients and environmental sites, respectively. The second most common microarray group was MG6 which accounted for 19.1% (34/178) of isolates (HHCWs, 7/38, 18.4%; P, 19/82, 23.2%; E, 8/61, 13.1%) (Table 6.10). The antimicrobial resistance and virulence-associated genes and gene cluster differences between each MG are shown in Table 6.11. The MGs identified differed by between one and six gene or gene cluster differences.

6.3.3.3.2 ST22-MRSA-IV spa types and microarray groups combined

Of the 22 ST22-MRSA-IV *spa* types identified, eight were represented by >1 isolate and of these seven were assigned to more than one MG and accounted for 87.1% (155/178) of isolates (Table 6.12). The most common *spa* types identified among the ST22-MRSA-IV isolates, t032 (40.4%, 72/178), t515 (24.2%, 43/178) and t557 (17.4%, 31/178) were divided into 14, five and six MGs, respectively (Table 6.12). For each *spa* type represented by more than one MG, the number of gene/gene cluster differences between the MGs were analysed and interpreted as described in section 6.2.2.4 i.e MGs were deemed to be (i) closely related if they differed by the presence or absence of one antimicrobial resistance or virulence gene, (ii) possibly related if they differed by the presence or absence of two or more genes that previous studies had shown to be co-located and (iii) different if they differed by the presence or absence of two or more genes.

Sixty-six pairwise combinations of MGs were identified within *spa* type t032, 12, three and 51 of which were deemed to be closely related, possibly related and different, respectively (Table 6.13). For five of the six other *spa* types represented by >1 MG, the pairwise combinations were deemed to be either closely related or different, while the remaining *spa* type (t3213) consisted of closely related MGs only (Table 6.13).

Table 6.10 Characteristic antimicrobial resistance and virulence genes and *spa* types among each microarray group (MG) identified among the 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

MG (*n*)[a]	Characteristic antimicrobial resistance and virulence genes[b]	*spa* type (*n*)
MG1 (2)	*blaZ, egc*	t032 (2)
MG2 (21)	*blaZ, egc*, IEC type B	t032 (12), t515 (2), t557 (1), t756 (1), t1021 (1), t1214 (1), t3185 (1), t3139 (1), t3213 (1)
MG4 (6)	*blaZ, erm*(C), *egc*	t032 (4), t515 (2)
MG5 (16)	*blaZ, sec & sel, egc,* IEC type B	t032 (7), t628 (5), t002 (2), t557 (1), t718 (1)
MG6 (34)	*blaZ, erm*(C), *egc,* IEC type B	t515 (13), t032 (11), t1214 (2), t022 (1), t379 (1), t885 (1), t1565 (1), t2945 (1), t3185 (1), t3213 (1), t7983 (1)
MG8 (1)	*blaZ, erm*(C), *egc,* IEC type B, ACME	t6768 (1)
MG9 (3)	*blaZ, erm*(C), *sec & sel, egc*	t032 (3)
MG11 (71)	*blaZ, erm*(C), *sec &sel, egc,* IEC type B	t515 (25), t557 (23), t032 (18), t022 (1), t432 (1), t3185 (1), t3459 (1), t8679 (1)
MG12 (6)	*blaZ, erm*(C), *aacA-aphD, mupA, sec & sel, egc,* IEC type B	t032 (6)
MG14 (1)	*blaZ, aacA-aphD, mupA, sec & sel, egc*, IEC type B	t032 (1)
MG15 (2)	*blaZ, erm*(A), *sec & sel, egc,* IEC type B	t032 (2)
MG16 (6)	*blaZ, erm*(C), *aacA-aphD, mupA, sec & sel, egc*	t032 (5), t9568 (1)
MG17 (1)	*blaZ, sec & sel, egc*, IEC type C	t032 (1)
MG18 (1)	*blaZ, erm*(C), *aphA3 & sat, sec & sel, egc,* IEC type B	t557 (1)
MG19 (1)	*blaZ, erm*(C), *fosB*-plasmid, *sec & sel, egc,* IEC type B	t515 (1), t557 (1)
MG20 (1)	*blaZ, erm*(C), *tet*(K), *egc,* IEC type B	t1214 (1)
MG21 (1)	*blaZ, erm*(C), *aphA3*, *fosB*-plasmid, *sec & sel, egc,* IEC type B	t557 (1)
MG22 (4)	*blaZ. aphA3, sec & sel, egc,* IEC type B	t557 (4)

[a] Each ST22-MRSA-IV MRSA isolate was assigned a microarray group (MG) based on the different combination of antimicrobial resistance and virulence-associated genes identified using the StaphyType DNA microarray (Alere). MGs numbers in bold text were assigned during a previous study (Brennan, 2013). MGs 3, 7, 10 and 13 detected during that study were not identified among isolates in the present study and so were excluded. The remaining MGs (MG14-22) were assigned during the present study.

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

Table 6.11a Matrix showing the antimicrobial resistance and virulence gene differences between microarray groups 1-12 identified among 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

Microarray Group (MG)[a]	MG1	MG2	MG4	MG5	MG6	MG8	MG9	MG11	MG12
MG2	IEC type B (1)	.....	.....	.....	.....	.....	.....	.....	.....
MG4	*emr*(C) (1)	*erm*(C), IEC type B (2)	.....	.....	.....	.....	.....	.....	.....
MG5	*sec/sel*, IEC type B (2)	*sec/sel* (1)	*erm*(C), *sec/sel*, IEC type B (2)	.....	.....	.....	.....	.....	.....
MG6	*emr*(C), IEC type B (2)	*erm*(C) (1)	IEC type B (1)	*erm*(C), *sec/sel* (2)	.....	.....	.....	.....	.....
MG8	*emr*(C), IEC type B, ACME (3)	*erm*(C), ACME (2)	IEC type B, ACME (2)	*erm*(C), *sec/sel*, ACME (3)	ACME (1)	.....			
MG9	*emr*(C), *sec/sel* (2)	*erm*(C), *sec/sel*, IEC type B (3)	*sec/sel* (1)	*erm*(C), IEC type B (2)	*sec/sel*, IEC type B (2)	*sec/sel*, IEC type B, ACME (3)	.....	.....	.....
MG11	*emr*(C), *sec/sel*, IEC type B (3)	*erm*(C), *sec/sel* (2)	*sec/sel*, IEC type B (2)	*erm*(C) (1)	*sec/sel* (1)	*sec/sel*, ACME (2)	IEC type B (1)	.....	.....
MG12	*erm*(C), *aacA-aphD*, *mupA*, *sec/sel*, IEC type B (5)	*erm*(C), *aacA-aphD*, *mupA*, *sec/sel* (4)	*aacA-aphD*, *mupA*, *sec/sel*, IEC Type B (4)	*erm*(C), *aacA-aphD*, *mupA* (3)	*aacA-aphD*, *mupA*, *sec/sel* (3)	*aacA-aphD*, *mupA*, *sec/sel* , ACME (4)	*aacA-aphD*, *mupA*, IEC type B (3)	*aacA-aphD*, *mupA* (2)	.....
MG14	*aacA-aphD*, *mupA*, *sec/sel*, IEC type B (4)	*aacA-aphD*, *mupA*, *sec/sel* (3)	*erm*(C), *aacA-aphD*, *mupA*, *sec/sel*, IEC type B (5)	*aacA-aphD*, *mupA* (2)	*erm(C)*, *aacA-aphD*, *mupA*, *sec/sel* (4)	*erm(C)*, *aacA-aphD*, *mupA*, *sec/sel*, ACME (5)	*erm(C)*, *aacA-aphD*, *mupA*, IEC type B (4)	*erm(C)*, *aacA-aphD*, *mupA* (3)	*erm*(C) (1)
MG15	*erm*(A), *sec/sel*, IEC type B (3)	*erm*(A), *sec/sel* (2)	*erm*(A), *erm*(C), *sec/sel*, IEC type B (4)	*erm*(A) (1)	*erm*(A), *erm*(C), *sec/sel* (3)	*erm*(A), *erm*(C), *sec/sel*, ACME (3)	*erm*(A), *erm*(C), IEC type B (3)	*erm*(A), *erm*(C) (2)	*erm*(A), *erm*(C), *aacA-aphD*, *mupA* (4)

Continued overleaf

Table 6.11a continued. Matrix showing the antimicrobial resistance and virulence gene differences between microarray groups 1-12 identified among 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

Microarray Group (MG)[a]	MG1	MG2	MG4	MG5	MG6	MG8	MG9	MG11	MG12
MG16	*erm*(C), *aacA-aphD, mupA, sec/sel* (4)	*erm*(C), *aacA-aphD, mupA, sec/sel,* IEC type B (5)	*aacA-aphD, mupA, sec/sel* (3)	*erm*(C), *aacA-aphD, mupA,* IEC type B (4)	*aacA-aphD, mupA, sec/sel* IEC type B (3)	*aacA-aphD, mupA, sec/sel* IEC type B, ACME (4)	*aacA-aphD, mupA* (2)	*aacA-aphD, mupA,* IEC type B (3)	IEC type B (1)
MG17	*sec/sel,* IEC type C (2)	*sec/sel,* IEC type C, IEC type B (3)	*emr*(C), *sec/sel,* IEC type C (3)	IEC type C, IEC type B (2)	*erm*(C), *sec/sel,* IEC type C, IEC type B (4)	*erm*(C), *sec/sel,* IEC type C, IEC type B, ACME (5)	*erm*(C), IEC type C (2)	*erm*(C), IEC type B, IEC type C (3)	*erm*(C), *aacA-aphD, mupA,* IEC type B, IEC type C (5)
MG18	*erm*(C), *aphA3/sat, sec/sel,* IEC type B (4)	*erm*(C), *aphA3/sat, seec/sel* (3)	*aphA3/sat, sec/sel,* IEC type B (3)	*erm*(C), *aphA3/sat* (2)	*aphA3/sat, sec/sel* (2)	*aphA3/sat, sec/sel,* ACME (3)	*aphA3/sat,* IEC type B (2)	*aphA3/sat* (1)	*aacA-aphD, aphA3/sat, mupA* (2)
MG19	*erm*(C), *fosB*-plasmid *sec/sel,* IEC type B (4)	*erm*(C), *fosB*-plasmid, *sec/sel* (3)	*fosB*-plasmid, *sec/sel,* IEC type B (3)	*erm*(C), *fosB*-plasmid (2)	*fosB*-plasmid, *sec/sel,* (2)	*fosB*-plasmid, *sec/sel,* ACME (3)	*fosB*-plasmid, IEC type B (2)	*fosB*-plasmid, (1)	*aacA-aphD, mupA, fosB*-plasmid (3)
MG20	*erm*(C), *tet*(K), IEC type B (3)	*erm*(C), *tet*(K) (2)	*tet*(K), IEC type B (2)	*erm*(C), *tet*(K), *sec/sel* (3)	*tet*(K) (1)	*tet*(K), ACME (2)	*tet*(K), *sec/sel,* IEC type B (3)	*tet*(K), *sec/sel* (2)	*aacA-aphD, mupA, tet*(K), *sec/sel* (4)
MG21	*erm*(C), *aphA3, fosB*-plasmid, *sec/sel,* IEC type B (5)	*erm*(C), *aphA3, fosB*-plasmid, *sec/sel* (4)	*aphA3, fosB*-plasmid, *sec/sel,* IEC type B (4)	*erm*(C), *aphA3, fosB*-plasmid (3)	*aphA3, fosB*-plasmid, *sec/sel,* (3)	*aphA3, fosB*-plasmid, *sec/sel,* ACME (4)	*aphA3, fosB*-plasmid, IEC type B (3)	*aphA3, fosB*-plasmid (2)	*aacA-aphD, mupA, aphA3, fosB*-plasmid (4)

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Table 6.11a continued. Matrix showing the antimicrobial resistance and virulence gene differences between microarray groups 1-12 identified among 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

Microarray Group (MG)[a]	MG1	MG2	MG4	MG5	MG6	MG8	MG9	MG11	MG12
MG22	*aphA3/sat*, *sec/sel*, IEC type B (3)	*aphA3/sat*, *sec/sel*, (2)	*erm*(C), *aphA3/sat*, *sec/sel*, IEC type B (4)	*aphA3/sat* (1)	*erm(C)*, *aphA3/sat*, *sec/sel*, (3)	*erm(C)*, *aphA3/sat*, *sec/sel*, ACME (4)	*erm*(C), *aphA3/sat*, IEC type B (3)	*erm*(C), *aphA3/sat* (2)	*erm(C)*, *aacA-aphD*, *mupA*, *aphA3/sat* (4)
MG23	*aphA3 sec/sel*, IEC type B (3)	*aphA3 sec/sel*, (2)	*erm*(C), *aphA3 sec/sel*, IEC type B (4)	*aphA3* (1)	*erm(C)*, *aphA3 sec/sel*, (3)	*erm(C)*, *aphA3 sec/sel*, ACME (4)	*erm*(C), *aphA3*, IEC type B (3)	*erm*(C), *aphA3* (2)	*erm(C)*, *aacA-aphD*, *mupA*, *aphA3* (4)

Table 6.11b Matrix showing the antimicrobial resistance and virulence gene differences between microarray groups 14-22 identified among 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in one Dublin hospital in 2009

Microarray Group (MG)[a]	MG14	MG15	MG16	MG17	MG18	MG19	MG20	MG21
MG15	*erm*(A), *aacA-aphD, mupA* (3)	.....	.....	.....	.....	.....	.....	.....
MG16	*erm*(C), IEC type B (2)	*erm*(A), *erm*(C), *aacA-aphD, mupA*, IEC type B (5)	.....	.....	.....	.....	.....	.....
MG17	*aacA-aphD, mupA*, IEC type B, IEC type C (3)	*erm*(A), IEC type B, IEC type C (3)	*erm*(C), *aacA-aphD, mupA*, ICE type C (4)	.....	.....	.....	.....	.....
MG18	*erm*(C), *aacA-aphD, mupA, aphA/sat* (4)	*erm*(A), *erm*(C), *aphA3, seb* (4)	*aacA-aphD, mupA, aphA3/sat*, IEC type B (4)	*erm(C), aphA/sat,*, IEC type B, IEC type C (4)	.....	.....	.....	.....
MG19	*erm*(C), *aacA-aphD, mupA, fosB*-plasmid (4)	*erm*(A), *erm*(C), *fosB*-plasmid (3)	*aacA-aphD, mupA , fosB-plasmid,* IEC type B (4)	*erm*(C), *fosB-plasmid,* IEC type B, IEC type C (4)	*aphA3/sat, fosB*-plasmid (2)	.....	.....	.....
MG20	*erm*(C), *aacA-aphD, mupA, tet*(K), *sec/sel* (5)	*erm*(A), *erm*(C), *tet* (K) (3)	*aacA-aphD, mupA , tet*(K), *sec/sel*, IEC type B (5)	*erm*(C), *tet*(K), *sec/sel*, IEC type B, IEC type C (5)	*aphA3/sat, tet*(K), *sec/sel* (3)	*tet*(K), *fosB*-plasmid, *sec/sel* (3)	.....	.....
MG21	*aacA-aphD, mupA, aphA3, fosB*-plasmid (4)	*erm*(A), *erm*(C), *aphA3, fosB*-plasmid (4)	*aacA-aphD, mupA, aphA3, fosB-plasmid,* IEC type B (5)	*erm*(C), *aphA3, fosB-plasmid,* IEC type B, IEC type C (5)	*sat, fosB*-plasmid (2)	*aphA3* (1)	*aphA3, fosB*-plasmid, *tet*(K), *sec/sel* (4)	.....
MG22	*aacA-aphD, mupA, aphA3/sat* (3)	*erm*(A), *aphA3/sat* (2)	*erm(C), aacA-aphD, mupA, aphA3/sat,* IEC type B (5)	*aphA3,* IEC type B, IEC type C (3)	*erm*(C) (1)	*erm*(C),*aphA3/sat, fosB*-plasmid (3)	*erm*(C), *aphA3/sat, tet*(K), *sec/sel* (4)	*sat, fosB*-plasmid (2)

[a]Each ST22-MRSA-IV isolate was assigned a microarray group (MG) number based on the different combinations of antimicrobial resistance and virulence genes identified. Numbers in parenthesis refer to the numbers of gene differences between each MG.
Abbreviations: IEC, immune evasion complex. (The IEC of each isolate type was determined based on the combination of IEC genes detected and as described by van Wamel *et al.*,2006. IEC type B: *sak, chp & scn*; IEC type D: *sea, chp & scn*.)

Table 6.12. The different microarray groups (MGs) identified among each *spa* type for the 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients, environmental sites in one Dublin hospital in 2009

***spa* type (*n*)**	**Source (*n*)**	**MG (*n*)**
t032	Patients (39)	MG11 (12), MG2 (10), MG5 (4), MG6 (4), MG12 (3), MG15 (2), MG1 (1), MG9 (1), MG16 (1), MG17 (1)
	HHCWs (24)	MG6 (5), MG4 (4), MG11 (4), MG5 (3), MG12 (3), MG16 (3), MG9 (1), MG14 (1)
	Environmental sites (9)	MG2 (2), MG6 (2), MG11 (2), MG1 (1), MG9 (1), MG16 (1)
t557	Environmental sites (25)	MG11 (18), MG22 (4), MG2 (1), MG18 (1) MG19 (1)
	Patients (5)	MG11 (4). MG5 (1)
	HHCW (1)	MG11 (1)
t515	Environmental sites (24)	MG11 (17), MG6 (5), MG4 (1), MG19 (1)
	Patients (16)	MG6 (8), MG11 (7), MG4 (1)
	HHCWs (3)	MG2 (2), MG11 (1)
t002	Patients (2)	MG5 (2)
	HHCWs (2)	MG6 (1), MG11 (1)
t628	Patients (4)	MG5 (4)
	Environmental sites (1)	MG5 (1)
t1241	Patients (2)	MG6 (2)
	HHCWs (2)	MG2 (1), MG20 (1)
t379	HHCW (1)	MG6 (1)
t432	Patient (1)	MG11 (1)
t718	Patient (1)	MG5 (1)
t756	Patient (1)	MG2 (1)
t885	Patient (1)	MG6 (1)
t1021	Patient (1)	MG2 (1)
t1565	Environmental site (1)	MG6 (1)
t2945	Patient (1)	MG6 (1)
t3139	Patient (1)	MG2 (1)
t3185	HHCW (3)	MG2 (1), MG6 (1), MG11 (1)
t3213	Patient (2)	MG2 (1), MG6 (1)

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Table 6.12 continued. The different microarray groups (MGs) identified among each *spa* type for the 178 ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients, environmental sites in one Dublin hospital in 2009

***spa* type**	**Source (*n*)**	**MG (*n*)**
t3459	HHCW (1)	MG11 (1)
t6768	Patient (1)	MG8 (1)
t7983	Patient (1)	MG6 (1)
t8679	HCW (1)	MG11 (1)
t9568	Environmental site (1)	MG16 (1)

Abbreviations: HHCWs, hands of healthcare workers; *n*, number of isolates.

Table 6.13. The relatedness of MRSA isolates within each microarray group (MG) for *spa* types represented by more than one MG

***spa* type (*n*)**	**Relatedness[a]**		
	Closely related[a]	**Possibly related[a]**	**Different[a]**
t032 (*n* = 72)	MG1 & MG2	MG5 & MG14	MG1 & MG5
	MG1 & MG4	MG9 & MG16	MG1 & MG6
	MG2 & MG5	MG11 & MG12	MG1 & MG9
	MG2 & MG6		MG1 & MG11
	MG4 & MG6		MG1 & MG12
	MG4 & MG9		MG1 & MG14
	MG5 & MG11		MG1 & MG15
	MG5 & MG15		MG1 & MG16
	MG6 & MG11		MG1 & MG17
	MG9 & MG11		MG2 & MG4
	MG12 & MG14		MG2 & MG9
	MG12 & MG16		MG2 & MG11
			MG2 & MG12
			MG2 & MG14
			MG2 & MG15
			MG2 & MG16
			MG2 & MG17
			MG4 & MG5
			MG4 & MG11
			MG4 & MG12
			MG4 & MG14
			MG4 & MG15
			MG4 & MG16
			MG4 & MG17
			MG5 & MG6
			MG5 & MG9
			MG5 & MG12
			MG5 & MG16
			MG5 & MG17
			MG6 & MG9
			MG6 & MG12
			MG6 & MG14
			MG6 & MG15
			MG6 & MG16
			MG6 & MG17
			MG9 & MG12
			MG9 & MG14
			MG9 & MG15
			MG9 & MG17
			MG11 & MG14
			MG11 & MG15
			MG11 & MG16
			MG11 & MG17
			MG12 & MG15
			MG12 & MG17

Continued overleaf

Table 6.13 continued. The relatedness of MRSA isolates within each microarray group (MG) for *spa* types represented by more than one MG

***spa* type (*n*)**	**Relatedness[a]**		
	Closely related[a]	**Possibly related[a]**	**Different[a]**
t032 (*n* = 72)			MG14 & MG15 MG14 & MG16 MG14 & MG17 MG15 & MG16 MG15 & MG17 MG16 & MG17
t515 (*n* = 43)	MG2 & MG6 MG4 & MG6 MG6 & MG11 MG11 & MG19		MG2 & MG4 MG2 & MG11 MG2 & MG19 MG4 & MG11 MG4 & MG19 MG6 & MG19
t557 (*n* = 31)	MG2 & MG5 MG5 & MG11 MG5 & MG22 MG11 & MG18 MG18 & MG21 MG18 & MG22		MG2 & MG11 MG2 & MG18 MG2 & MG21 MG2 & MG22 MG5 & MG18 MG5 & MG21 MG11 & MG21 MG11 & MG22 MG21 & MG22
t002 (*n* = 4)	MG5 & MG11 MG6 & MG11		MG5 & MG6
t1214 (*n* = 4)	MG2 & MG6 MG6 & MG20		MG2 & MG20
t3185 (*n* = 3)	MG2 & MG6 MG6 & MG11		MG2 & MG11
t3213 (*n* = 2)	MG2 & MG6		

[a]MGs were deemed to be closely related if they differed by the presence or absence of one antimicrobial resistance or virulence gene only. MGs were deemed to be possibly related if they differed by the presence or absence of two or more antimicrobial and/or virulence genes if previous studies had shown that the genes were co-located. MGs were deemed unrelated if they differed by the presence or absence of two or more antimicrobial and/or virulence genes and if there was no evidence to suggest colocation of the variable genes.

6.3.3.3.3 Determination of the relatedness of the ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites using spa types and DNA microarray groups

Among the 22 *spa* types identified among the 178 ST22-MRSA-IV isolates recovered from the HHCWs, patients and environmental sites six *spa* types were represented by isolates belonging to more than one of these MRSA isolate groups i.e. three *spa* types (*spa* types t032, t557 and t515) consisted of MRSA isolates from all three groups (HHCWs, patient and environmental sites), two consisted of isolates from patients and the HHCWs and one consisted of isolates from patients and environmental sites (Table 6.12). The isolates represented by these six *spa* types account for 89.3% (159/178) of the ST22-MRSA-IV isolates investigated.

The 72 *spa* type t032 MRSA isolates were assigned to 12 MGs, 10 of which were represented by >1 isolate (Table 6.12). Three of the t032 MGs (MG11, MG6 and MG9) were identified among all three MRSA isolate groups (HHCWs, patients and environmental sites), represented 44.4% of t032 isolates (32/72) and were deemed to be indistinguishable (Table 6.12). There were 66 possible pairwise combinations identified among the MGs assigned to the 72 t032 MRSA isolates (Table 6.13). Using criteria described in section 6.2.2.4, 13 of these pairwise combinations were deemed to be closely related, three were deemed to be possibly related and 51 were deemed to be different (Table 6.13).

Of these 31 t557 MRSA isolates, 23 (7.2%) were assigned to MG11 and were deemed to be indistinguishable (Table 6.12). The *spa*-MG t557-MG11 predominantly consisted of environmental isolates (n = 18) but patient (n = 4) and one isolate from HHCW were also identified (Table 6.12). The remaining eight t557 isolates were seven environmental MRSA isolates and one patients MRSA isolate and were assigned to five

MGs (Table 6.12). Six pairwise combinations of these MGs were deemed to be closely related and nine pairwise combinations were deemed to be different (Table 6.13).

Among the 43 *spa* type t515 MRSA isolates from the HHCWs, patients and environmental sites, 25 were deemed to be indistinguishable (MG11) (Table 6.12). Again, the *spa*-MG t515-MG11 predominantly consisted of environmental isolates (n = 17) but isolates from patients (n = 7) and HHCWs (n = 1) were also assigned to this group. The remaining 18 t515 MRSA isolates were assigned to four MGs (MG2, MG4, MG6, and MG19). Four pairwise combinations of these MGs were deemed to be closely related and six were deemed to be different (Table 6.13).

Four MRSA isolates recovered from patients and one from an environmental site were identified as *spa* type t628 and all isolates were assigned to the same MG (MG5) and were therefore considered to be indistinguishable (Table 6.12). Two *spa* types (t002 and t1214) were represented by MRSA isolates recovered from the HHCWs and patients only (Table 6.12). The four MRSA isolates assigned to t002 were differentiated into three MGs. Two pairwise combinations of the t002 MGs were deemed to be closely related (MG5 & MG11, and MG6 & MG11) while one MG pair was deemed to be different (MG5 & MG6) (Tables 6.12 and 6.13). The four MRSA isolates assigned to t1214 were differentiated into three MGs (Table 6.12). Two pairwise combinations of these MGs were deemed to be closely related (MG2 & MG6, and MG6 & MG20) while one MG pairwise combination (MG2 & MG20) was deemed to be different (Tables 6.12 and 6.13).

6.3.3.3.4 Interpreting the spa-MG data in the context of available epidemiological data for three wards in a Dublin hospital

Ward A consisted of 23.6 % (42/178) of the ST22-MRSA-IV isolates investigated. The isolates were assigned to seven *spa* types and eight microarray groups (Table 6.14). Of the seven *spa* types only two *spa* types, t032 and t515, were represented by >1 MRSA

Table 6.14. *spa* type and microarray groups of ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in three wards in one Dublin hospital in 2009

Ward	*spa* type (*n*)	Source (*n*)	MGs (*n*)
A (*n* = 42)	t515 (*n* = 26)	Environmental sites (*n* =18)	MG11 (*n* = 17) MG19 (*n* = 1)
		Patients (*n* = 7)	MG6 (*n* = 4) MG11 (*n* = 3)
		HHCW (*n* = 1)	MG4 (*n* = 1)
	t032 (*n* = 11)	Patients (*n* = 10)	MG2 (*n* = 2) MG4 (*n* = 1) MG11 (*n* = 6) MG17 (*n* = 1)
		HHCW (*n* = 1)	MG5 (*n* = 1)
	t432 (*n* = 1)	Patients (*n* = 1)	MG11 (*n* = 1)
	t717 (*n* = 1)	Patients (*n* = 1)	MG5 (*n* = 1)
	t885 (*n* = 1)	Patients (*n* = 1)	MG6 (*n* = 1)
	t6768 (*n* = 1)	Patients (*n* = 1)	MG8 (*n* = 1)
	t7983 (*n* = 1)	Patients (*n* = 1)	MG6 (*n* = 1)
B (*n* = 24)	t032 (*n* = 16)	Patients (*n* = 11)	MG2 (*n* = 4) MG5 (*n* = 2) MG6 (*n* = 4) MG11 (*n* = 1)
		Environmental sites (*n* = 5)	MG 1 (*n* = 1) MG2 (*n* = 1) MG6 (*n* = 1) MG11 (*n* = 1)
		HHCW (*n* = 1)	MG5 (*n* = 1)
	t515 (*n* = 4)	Patients (*n* = 3)	MG11 (*n* = 3)
		Environmental sites (*n* = 1)	MG11 (*n* = 1)
	t557 (*n* = 1)	Environmental sites (*n* = 1)	MG2 (*n* = 1)
	t628 (*n* = 1)	Patients (*n* = 1)	MG5 (*n* = 1)
	t1021 (*n* = 1)	Patients (*n* = 1)	MG2 (*n* = 1)

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Table 6.14 continued. *spa* type and microarray groups of ST22-MRSA-IV isolates recovered from the hands of healthcare workers, patients and environmental sites in three wards in one Dublin hospital in 2009

Ward (n)	*spa* type (*n*)	Source (*n*)	MGs (*n*)
B ($n = 24$)	t1212 ($n = 1$)	HHCW ($n = 1$)	MG2 ($n = 1$)
F ($n = 30$)	t557 ($n = 22$)	Environmental sites ($n = 19$)	MG11 ($n = 15$) MG22 ($n = 4$)
		Patients ($n = 3$)	MG11 ($n = 3$)
	t032 ($n = 4$)	HHCWs ($n = 3$)	MG5 ($n = 1$) MG16 ($n = 2$)
		Patients ($n = 1$)	MG11 ($n = 1$)
	t515 ($n = 3$)	Patients ($n = 3$)	MG6 ($n = 3$)
	t1565 ($n = 1$)	Environmental sites ($n = 1$)	MG6 ($n = 1$)

Abbreviations; HHCW, Hands of healthcare workers; MG, microarray group.

isolate (Table 6.14). Among the 11 t032 isolates, ten were recovered from patients and one from HHCW and were assigned to five MGs (Table 6.14). Of the 10 pairwise combinations of these MGs, two were deemed closely related (MG2 & MG5 and MG5 & MG11), while the remaining eight were deemed different (Table 6.13). Therefore, the one t032 isolate from HHCW (MG5) was deemed to be closely related to patient isolates within MG2 and MG11. There were two MRSA isolates recovered from two different sites from the same patient in Ward A. These isolates were both being assigned to t032-MG11 and deemed to be indistinguishable.

Also within Ward A, isolates from all three MRSA groups (P, E and HHCW) were identified among the isolates assigned to t515 (n = 23) (Table 6.14). The 23 t515 isolates were assigned to four MGs (MG4, MG6, MG11, and MG19) and of the six possible pairwise combinations of these MGs, three were deemed to be closely related (MG4 & MG6, MG6 & MG11 and MG11 & MG19) and three (MG4 & MG11, MG4 & MG19 and MG6 & MG19) were deemed to be different (Table 6.13). Therefore MRSA isolates recovered from patients and HHCWs were deemed to be closely related and MRSA isolates recovered from patients and environmental sites were deemed to be closely related. In addition, indistinguishable isolates belonging to the *spa*-MG combination, t515-MG11, were identified from both patient and environmental sites (Table 6.14).

Ward B consisted of 14.6% (26/178) of the ST22-MRSA-IV isolates recovered from HHCWs, patients and environmental sites. The isolates were assigned to six *spa* types and of these only two *spa* types (t032 and t515) were represented by >1 MRSA isolate (Table 6.14). The 18 t032 MRSA isolates recovered from HHCWs, patients and environmental sites and were assigned to five MGs. Among these MGS ten pairwise combinations of these MGs, isolates within five were deemed to be closely related (MG1 & MG2, MG2 & MG5, MG2 & MG6, MG5 & MG11 and MG6 & MG11) and five were

different (MG1 & MG5, MG1 & MG6, MG1 & MG11, MG2 & MG11 and MG5 & MG6) (Table 6.13). The pairwise combinations revealed that there were closely related strains among MRSA recovered from patients and environmental sites, HHCWs and patients and HHCWs and environmental sites. There was also one HHCW isolate (t032-MG5) was indistinguishable from t032-MG5 isolates from patients. There were patient and environmental isolates within t032-MG6, t032-MG11 and t032-MG2 that were deemed to be indistinguishable. There were two pairs of isolates recovered from the same patient in Ward B, and all four isolates were assigned to *spa* type t032. The pair of isolates from each patient were indistinguishable, with both sets being assigned to MG2. Also within Ward B four ST22-MRSA-IV isolates exhibited *spa* type t515 and they were all indistinguishable (MG11) and were represented by MRSA isolates recovered from patients (n = 3) and environmental sites (n = 1) (Table 6.14).

Ward F consisted of 16.9% (30/178) of the ST22-MRSA-IV isolates recovered from HHCWs, patients and environmental sites and were assigned to four different *spa* types, with all but one (t1565) represented by more than one isolate (Table 6.14). These MRSA isolates were also assigned to five MG (MG5, MG6, MG11, MG16 and MG22). Four MRSA isolates from HHCWs (n = 3) and patients (n = 1) were assigned to t032 and to three MGs (Table 6.14). Two pairwise combinations of these MGs were deemed to be closely related (MG5 & MG11 and MG6 & MG11) indicating that the patient and HHCW isolates were closely related while one pairwise combination, identified among the HHCWs only was deemed to be different (MG5 & MG6) (Table 6.13). Also in Ward F, 22 MRSA isolates recovered from patients (n = 3) and environmental sites (n = 19) exhibited *spa* type t557 and were assigned to two MGs, MG11 and MG22. These two MGs were deemed to be different (Table 6.13), but the *spa*-MG combination t557-MG11 was identified among MRSA isolates from both patients and environmental sites that were therefore deemed to be indistinguishable (Table 6.14). Three MRSA isolates recovered

from patients were assigned to *spa* type t515, and all were assigned to the same MG (MG6) (Table 6.14).

6.4 Discussion

6.4.1 Comparative molecular analysis of MRSA and MSSA isolates from bloodstream infections and healthy carriers

The results of this study have revealed several insights into the epidemiology, population structure and molecular characteristics of MSSA and MRSA from BSIs and HCs. Firstly, as expected, the MSSA isolates exhibited more diversity in terms of *spa* types and MLST CCs/STs than the MRSA isolates. While the MRSA isolates were assigned to six CCs and 34 *spa* types, the MSSA isolates were assigned to 33 CCs and 100 *spa* types. Other studies have also highlighted that MSSA are more diverse than MRSA (Argudin *et al.*, 2009; Lim *et al.*, 2012; Jimenez *et al.,* 2013), which may reflect the fact that only some MSSA clones can successfully acquire and retain SCC*mec* and become MRSA. Among the MSSA isolates CC30 and CC45 predominated and accounted for 61/181 (33.7%) and 33/181 (18.2%) of MSSA isolates, respectively. Similar to MRSA, different MSSA clones appear to predominate in different regions. For example, CC30 and CC45 MSSA have also been identified as the predominant MSSA clones among MSSA nasal carriage isolates in some other countries including Switzerland and Spain (Megavand *et al.,* 2010; Larano *et al.*, 2011; Argudin *et al.*, 2013). However, while MSSA isolates belonging to these clones were also identified among nasal carriage and infection MSSA from Spain, Tunisia and Nigeria, MSSA belonging to CC5 and CC1 predominated in these regions (Argudin *et al.*, 2009; Kechrid *et al.*, 2010; Shittu *et al.*, 2011).

ST22-MRSA-IV, the pandemic nosocomial MRSA clone endemic in Irish hospitals, accounted for the majority of MRSA isolates (114/136, 83.8%). Many of the other MRSA clones identified are also generally considered to be healthcare-associated (HCA). Firstly, ST239-MRSA-III, a pandemic multidrug resistant nosocomial MRSA clone that predominates in hospitals in Asia, China, Turkey and South America (Harris *et*

al., 2010) that has only been detected sporadically in Ireland in recent years (Kinnevey *et al.*, 2014, in press). It was the predominant MRSA clone in Irish hospitals in the late 1980s and early 1990s and was associated with an outbreak in an Irish hospital in 2004 (Epi Insight, 2003; Shore *et al.*, 2005). Secondly, ST8-MRSA-IIA-IIE & SCC_{MI}, a MRSA clone that predominated in Irish hospitals in the 1990s (Shore *et al.*, 2012). Thirdly, ST36-MRSA-II (EMRSA-16), which predominated in Irish hospitals in the late 1990s (Rossney *et al.*, 2006). Fourthly, ST5-MRSA-II and ST45-MRSA-IV that have only been recovered sporadically from Irish hospitals but have predominated in other countries (Monecke *et al.*, 2011) and finally ST779- ψSCC*mec*-SCC-SCC_{CRISPR}, that has recently been reported as an emerging nosocomial MRSA clone in Ireland (Kinnevey *et al.*, 2013). Of the remaining MRSA isolates, only four were assigned to genotypes commonly associated with community-associated (CA) MRSA including CC5-MRSA-IV, CC30-MRSA-IV and ST8-MRSA-IV/t008 (USA300) and one isolate was assigned to a lineage previously associated with horses (CC8-MRSA-IV/t064) (Walther *et al.*, 2009; Abbott *et al.*, 2010). However, accurate designation of isolates as HCA, CA or livestock-associated would require detailed epidemiological information which was not available for the isolates investigated in this study.

Analysis of genotyping data of the MSSA and MRSA isolates in the present study provided further evidence of the ability of similar *S. aureus* isolates to colonise the nares and to cause BSIs. Seventy percent of the MRSA isolates from BSIs and HCs exhibited the same CC/ST-SCC*mec* and *spa* type, with 66.7% of these belonging to ST22-MRSA-IV/t032. Among the MSSA isolates from HCs and BSIs, 46.4% exhibited the same CC/ST and *spa* type. These results highlight the importance of effective nasal decolonisation in reducing invasive *S. aureus* infections, both MRSA and MSSA.

Another key finding of the present study was the identification of similar lineages among both MRSA and MSSA indicating the possible local emergence of MRSA from MSSA by the acquisition of SCC*mec* or of MSSA from MRSA by the loss of SCC*mec*. However, while CC22, CC8, CC30, CC5 and CC45 MRSA and MSSA isolates were identified, only 27.3% (72/264) of these isolates shared the same *spa* type, and the majority of these isolates were CC22/t032 MRSA (n = 64), with only one CC22/*spa* type t032 MSSA identified, indicating a low frequency of potential emergence of MRSA from MSSA or MSSA from MRSA.

Unsurprisingly, the present study also revealed that the MSSA isolates carried less resistance genes than MRSA. This is in part due to the presence of SCC*mec* elements in MRSA that often harbour integrated plasmids and transposons with additional resistance determinants including genes encoding resistance to aminglycosides, macrolides, mercury, and tetracycline (IWG-SCC, 2009). However, some MSSA isolates, particularly those belonging to CCs also identified among MRSA, did in some instances harbour multiple resistance genes indicating that these lineages may have a greater ability to acquire and maintain resistance determinants compared to other MSSA lineages.

In contrast, virulence genes were found to be more common among the MSSA than the MRSA, in particular, the superantigen genes *tst* and *sea*, as well as the *pvl* genes *lukF/S*, highlighting the virulence potential of MSSA isolates. In fact, *sea, tst* and *pvl* were also more common among MSSA from HCs than BSIs. Previous studies have also found a high rate of carriage of *tst* among MSSA from HCs (Argudin *et al.*, 2012; Megavand *et al.*, 2010; Lazano *et al.*, 2012), and a higher rate of carriage of *tst* and *pvl* among MSSA compared to MRSA isolates (Hallin *et al.*, 2007; Sila *et al.*, 2009). In the majority of studies, the high rate of carriage of *tst* was also predominantly associated with CC30 MSSA (Diep *et al.*, 2006; Durand *et al.*, 2006). However, there is conflicting data

regarding the prevalence of the enterotoxin gene *sea* among MSSA compared to MRSA. One study reported a lower prevalence rate of *sea* among MSSA compared to MRSA isolates (Sila *et al.*, 2009), another study reported a higher rate of carriage of *sea* (76.7%) among MSSA than in the present study (27.1%) (Argudin *et al.*, 2013), while other studies reported similar rates of carriage of *sea* among MSSA as was identified in present study (Kim *et al.*, 2012; Kamarchei *et al.*, 2013).

6.4.1.1 Identification of lower levels of *tst* expression among MSSA from HCs compared to BSIs and TSS

Although the rate of carriage of some toxin genes has also been shown to be high among MSSA in other studies, very few studies have investigated whether the associated proteins are produced or have compared the levels of expression of the toxin genes. The *tst* and *sea* genes encode superantigens that can cause non-specific T-cell stimulation and can cause multiple organ failure, and *pvl* is a bicomponent toxin that causes pore formation in leukocyte plasma membranes or mitochondrial membranes resulting in leukocyte lysis or apoptosis. For MSSA isolates to persist as colonisers in the nasal passage without causing disease but with a high rate of carriage of particular toxin genes, it is possible that these genes are not expressed or are expressed at very low levels. Interestingly, all MRSA and all but two MSSA isolates (HCs (n = 1) and BSIs (n = 1)) in the present study that carried *tst* were found to produce TSST-1. While the reason for the lack of production of TSST-1 in these two isolates was not investigated further it is possible that it is due to mutations in TSST-1. Additionally, the present study also revealed that the overall mean *tst* expression levels were lower among CC30 MSSA isolates from HCs than from patients with BSIs and TSS. However, the results were not statistically significant making interpretation of the results difficult. Low levels of expression of *tst* among *S. aureus* in the nares could play a role in *S. aureus* nasal colonisation by allowing it to evade the immune system while

higher levels of *tst* expression may play a role in systemic infection. Further studies are needed to clarify the role of *tst* expression in MSSA colonisation and should include expression analysis of additional *tst*-positive MSSA isolates from infections and HCs as well as careful consideration of alternative growth conditions to detect *tst* expression as several studies have shown that multiple factors including pH, glucose, carbon dioxide, nitrogen and oxygen levels and magnesium concentrations affect *tst* expression (Todd *et al.,* 1987; Ross *et al.,* 2000; Yarwood *et al.,* 2000; Stevens *et al.*, 2007; Audrey *et al.*, 2010). Furthermore, as *tst* expression is regulated by the *agr* system, the addition of an analysis of the expression of *agr* as well as *tst* would provide further insights into the role of *tst* expression in MSSA. Alternative endogenous control genes to 16S rDNA should also be considered for use in expression studies including *gyrA, gmk, proC* and 23S rDNA, as it is not yet clear what is the best endogenous control gene for expression analysis in *S. aureus*. Similar studies should also be performed to further investigate the expression of *sea* and *pvl* among MSSA from HCs compared to infections.

6.4.2 Comparative molecular analysis of MRSA isolates recovered from healthcare workers, patients and environmental sites

Using *spa* typing and DNA microarray analysis, the present study revealed the presence of indistinguishable MRSA isolates from the HHCWs, hospitalised patients and hospital environmental sites. Among all groups of isolates ST22-MRSA-IV predominated and accounted for the majority of isolates (98.3%). The most common *spa* type among the MRSA isolates from HHCWs and patients was t032, while *spa* type t557 predominated among those recovered from environmental sites isolates, followed by t032. Shore *et al.* (2010) also found that *spa* type t032 predominated among ST22-MRSA-IV isolates recovered from patient and environmental sites in the same Irish hospital during 2007 and 2008, but no t557 isolates were identified among the environmental isolates in that study.

In many studies of ST22-MRSA-IV isolates reported to date, t032 is the predominant *spa* type, highlighting the need for enhanced discrimination of isolates of this *spa* type. While 39.6% of the MRSA isolates from patient, environmental and the HHCWs in the present study were assigned to *spa* type t032 (72/182), these isolates were differentiated into 14 different MGs based on different combinations of virulence and antimicrobial resistance genes detected using the DNA microarray. Although some of these t032 MGs only differed by the presence or absence of a single virulence or antimicrobial resistance gene, most of which are located on MGEs which can potentially be lost and gained during a single genetic event and therefore have to be considered to be closely related. Other MGs within t032 differed by two or more genes and in many cases were considered to be distinct. A previous study demonstrated the stability of these genes used for MG designation following repetitive sub-culturing, freeze-thawing and growth at elevated temperatures (Brennan, 2013).

The presence of indistinguishable, closely and possibly related MRSA isolates among MRSA from the HHCWs, patients and environmental sites is evidence of the transmission of MRSA strains between these groups. Previous studies have shown that contaminated environmental sites and colonised healthcare workers can be reservoirs for the transmission of MRSA to hospitalised patients (Hardy *et al.*, 2006; Wilson *et al.*, 2007; Stiefel *et al.,* 2011; Schweizer *et al.*, 2012). In the present study, when isolates from three individual wards were analysed further no indistinguishable isolates were identified among all three MRSA groups. However, within these wards indistinguishable isolates were identified among MRSA from the HHCWs and from patients, among MRSA from patients and environmental sites and among MRSA from the HHCWs and environmental sites. While the lack of additional epidemiological data hampered further interpretation of this data, these findings have provided convincing genotypic evidence for the transmission of MRSA between patients, healthcare workers and the environment.

Apart from ST22-MRSA-IV, the only other genotype identified among the MRSA isolates was ST8-MRSA-IIA-E & SCC_{MI} which was identified among MRSA isolates from patients only (3/82). These isolates were indistinguishable using *spa* typing, but using the DNA microarray they differed by the addition of an antimicrobial resistance gene (*aadD*) in one isolate and two different IEC types among the isolates. ST8-MRSA-IIA-E +/- SCC_{MI}, *spa* type t190 was the predominate MRSA strain in Irish hospitals in the 1990's (Shore *et al.*, 2005; Shore *et al.*, 2012) but it is now only recovered sporadically and is mainly associated with long-term care and chronically ill patients (Shore *et al.*, 2008). The fact that ST8 isolates were only found among MRSA isolates recovered from patients suggests that ST22 has a greater propensity for environmental contamination than ST8 isolates but due to the small number of isolates of this clone identified, a larger study would be needed to determine this more convincingly.

6.4.3 Concluding remarks

In conclusion, this study has revealed diverse genotypes, virulence and resistance genes among MSSA and similar MRSA and MSSA strains among patients with BSIs and among HCs. However, unique MRSA and MSSA strains were also identified among BSIs and HCs suggesting that some MRSA and MSSA strains may not be associated with both nasal carriage and BSIs. This difference was more notable with MSSA isolates, probably due to the fact that the HC group were healthy students and not healthcare workers as it was for the MRSA HC group. In order to fully understand why some *S. aureus* strains cause BSIs and others are predominantly associated with colonisation, and why some can be colonisers and also cause BSIs requires investigation of additional isolates, in particular a healthy population MRSA group not linked with healthcare facilities, as well as an investigation into virulence gene expression among isolates from different patient groups and an analysis of host factors.

The presence of indistinguishable MRSA isolates among patients, environmental sites and healthcare workers is a cause for concern and further highlights the importance of enhanced hand hygiene and environmental decontamination in the prevention of spread and control of MRSA. The combination of *spa* typing and DNA microarray analysis provided in depth discrimination of ST22-MRSA-IV isolates and also demonstrated the usefulness of the DNA microarray for detailed analysis of the virulence and antimicrobial resistance potential of MRSA and MSSA isolates.

Chapter 7

General Discussion

7.1 General Discussion

Staphylococcus aureus is the most pathogenic of the staphylococcal species and it is by far the most frequently isolated staphylococcal species recovered from cases of infection. Its ability to colonise and establish infection are directly linked with its ability to express an extensive range of virulence factors. Furthermore, both *S. aureus* and CoNS often exhibit resistance to multiple antimicrobial agents, including methicillin and almost all beta-lactam antibiotics, the most commonly used class of antimicrobial agents. Methicillin resistance in staphylococci is encoded by the *mec* gene encoded on SCC*mec* and a considerable body of evidence suggests that this MGE originated in CoNS and that specific species of CoNS may have contributed specific SCC*mec* genes to MRSA. A detailed understanding of SCC*mec* in MRSA and CoNS is vital to enhance our understanding of the emergence of MRSA and its evolution but also to ensure accurate typing and tracking of isolates to reduce transmission events and infections.

7.1.1 SCC*mec* among staphylococci in Ireland

7.1.1.1 Identification and characterisation of a novel *mec* gene, *mecC*, and SCC*mec* type XI in MRSA

The present study identified for the first time a highly divergent and novel *mec* gene, designated *mecC*, located on a novel SCC*mec* element designated SCC*mec* XI in two CC130 MRSA clinical isolates, a lineage previously only associated with animals. These findings suggested the possible zoonotic spread of the CC130 isolates from animals to humans. Interestingly, since its initial identification in the present study, *mecC*-positive MRSA has been recovered from both humans and animals in several other European countries and predominantly among animal-associated *S. aureus* lineages (Cuny *et al.*, 2011; Paterson *et al.*, 2012; Petersen *et al.*, 2012; Pichon *et al.*, 2012; Robb *et al.*, 2012; Sabat *et al.*, 2012; Stegger *et al.*, 2012; Walther *et al.*, 2012; Basset *et al.*, 2013; Eriksson

et al., 2013; Lorcaric *et al.,* 2013; Medhus *et al.,* 2013; Monecke *et al.,* 2013; Vandendriesosche *et al.,* 2013). The prevalence of *mecC* MRSA in Europe among human isolates varies from 0.2% up to 6% (Cuny *et al.*, 2011; Pichon *et al.,* 2012; Stegger *et al.*, 2012; Basset *et al.,* 2013; Peterson et *al.,* 2013) and a rate of 2.15% was recently reported among milk samples in England and Wales (Patterson *et al.*, 2013). In Ireland, only one additional human *mecC*-positive CC130 MRSA (*spa* type t12399) isolate recovered from an invasive infection has been identified to date (Grainne Brennan, NMRSARL, personal communication), indicating a very low prevalence of *mecC* MRSA in Ireland. However, additional studies are required to determine the true prevalence of *mecC* MRSA among animals and humans in Ireland and elsewhere and to determine the potential threat posed by these organisms.

The fact that *mecC* MRSA has mainly been associated with animal lineages strongly suggests a possible animal origin. The emergence of *mecC* MRSA in animals has been mainly associated with dairy cows and small mammals, such as rats and hedgehogs (Garcia-Alvarez *et al.,* 2011; Paterson *et al.,* 2012; Walter *et al.,* 2012: Monecke *et al.,* 2013). This could be associated with the widespread use of antibiotics to treat mastitis in dairy cows. *mecC*-positive MRSA could then be transferred to small animals that frequent farmlands. A study in 2009 reported that rats living on pig farms harboured *S. aureus* strains also isolated from pigs, including CC130-MSSA, suggesting the rats are a possible source of transmission of *S. aureus* among the pigs (van de Giessen *et al.*, 2009). *mecC*-positive MRSA could also be transferred to humans by direct contact with colonised cows or contaminated milk or milking machinery, as previous studies have shown the transfer of MRSA form cows to humans (Jubasz-Kaszanyi *et al.*, 2007; Antoci *et al.*, 2013).

Although *mecC* exhibited only 62% aa identity to *mecA,* the similar genetic organisation of the *mec* complex to that in *M. caseolyticus* indicates that it may have originally emerged from this animal commensal and may represent the first identification

of an ancestral form of *mec* in MRSA. The recent identification of a *mecC* allotype, *mecC1*, in *S. xylosus* that exhibited 93.5% nucleotide sequence identity to *mecC,* may represent another possible precursor to *mecC* in MRSA (Harrison *et al.,* 2013) and other possible precursors to *mecC* and *mecA* may exist among MRSA and CoNS. There has been a number of *mecA* allotypes identified in staphylococci by Monecke *et al.* (2012) and several were found not to confer resistance to methicillin and β-lactam antibiotics. This could indicate an alternative function for the *mecA* gene in some CoNS species, possibly cell wall metabolism.

The present study also highlighted the problems associated with detection of *mecC*-positive MRSA. Not only is *mecC* not detected using conventional *mecA* PCR, but the production of PBP2a by *mecC*-positive MRSA is not detected using all commercially available kits. Furthermore, some chromogenic media are unreliable in detecting *mecC* MRSA isolates and *mecC*-positive MRSA tend to have lower oxacillin MICs than *mecA*-positive MRSA. Since many diagnostic laboratories rely on chromogenic media and phenotypic testing, *mecC*-positive MRSA isolates may not always be detected. Further studies into the development of reliable chromogenic media for the detection of *mecC*-positive MRSA are required. Although *mecC*-positive MRSA have not been found to harbour any resistance determinants apart from *mecC* and the arsenic resistance operon, and the major virulence associated gene detected among *mecC*-positive isolates is *etD2*, they could potentially acquire additional resistance and virulence determinants encoded by MGEs. For example, Sabat *et al.* (2012) recently reported an *mecC*-positive MRSA isolate carrying *tst*. The acquisition of additional virulence and/or antimicrobial resistance genes by *mecC*-positive MRSA coupled with the difficulties associated with their detection could pose a serious threat to public health in the future.

Whether the prevalence of *mecC* MRSA in Ireland is truly very low remains to be seen but it is possible that *mecC*-positive isolates are not being detected. In Denmark

mecC-positive MRSA have been identified among isolates recovered as far back as the 1970s (Stegger *et al.*, 2012). The increased detection of *mecC*-positive MRSA in recent years in some European countries may reflect a true increase in its prevalence or more likely it is due to improved detection systems especially the use of *mecC*-specific PCRs. Furthermore, recent advances in whole-genome sequencing technology have facilitated the easier detection and characterisation of these strains.

7.1.1.2 Identification of a novel SCC*mec*-SCC-CI among MRSA and evidence of horizontal transfer of SCC, SCC*mec* and antimicrobial resistance genes between staphylococci

The detection of novel SCC*mec*-SCC-CIs in Irish ST8-MRSA-IIA-E & IVE/F isolates, and the similarities between these and a SCC-CI from *S. epidermidis* is further evidence of the horizontal transfer and recombination of SCC elements among staphylococci. Complete nucleotide sequencing of these complex elements is essential, with several studies revealing the possible presence of CIs, but only a handful actually undertaking detailed characterisation of these elements by nucleotide sequencing (Ito *et al.*, 2001; Monkolrattanothai *et al.*, 2004; Takeuchi *et al.*, 2005; Bartels *et al.*, 2011; Shore *et al.*, 2011b; Kinnevey *et al.*, 2013). In the 1990's ST8-MRSA-IIA-IIE and *spa* type t190 isolates predominated in Irish hospitals, and are now only reported sporadically. Previous studies have revealed that the carriage of large SCC*mec* elements is associated with a reduced fitness cost in MRSA (Ender *et al.*, 2004; Knight *et al.*, 2013) and the carriage of this large SCC*mec*-SCC-CI by ST8-MRSA-IIA-IIE and *spa* type t190 isolates may have contributed to their decline. It is also interesting to note that only one ST22-MRSA-IV isolate, which is the current predominant MRSA clone in Irish hospitals, was found in the present study to carry a SCC*mec*-SCC-CI. A recent study by Knight *et al.* (2013) demonstrated that the carriage of the SCC*mec* IV elements by CC22 isolates is not associated with a fitness cost. A comparative study of the growth rate of ST8-MRSA-IIA-

E isolates with and without the SCC_{MI} and with ST22-MRSA-IV isolates could provide evidence in support of the theory of an increased fitness cost associated with the carriage of SCC_{MI}.

The identification in the present study of SCC*mec* elements previously described in MRSA as well as several potentially novel SCC and SCC*mec* elements among CoNS from hospitalised patients is further evidence that CoNS are a reservoir for SCC and SCC*mec* in *S. aureus* and for their horizontal transfer between staphylococci. Several studies have found evidence to indicate that SCC*mec* originated in CoNS (Wu *et al.*, 1998; Schnellmann *et al.*, 2006; Tsubakishita *et al.*, 2010) and the identification of such an abundance and diverse range of SCC*mec* elements in CoNS in the present study supports this theory. Detailed characterisation of the SCC*mec* and SCC elements harboured by these CoNS isolates is warranted. However, as more SCC*mec* elements are characterised from CoNS clear guidance on the nomenclature to be used for describing these elements will be required. The current nomenclature system recommended for SCC*mec* elements was originally developed for MRSA (IWG-SCC, 2009) but, in many instances, the same nomenclature is used for CoNS. Ideally, the same SCC*mec* nomenclature should be used for MRSA and CoNS to allow for ease of comparison. Lastly, it is of paramount importance that SCC*mec* elements in individual CoNS species are systematically investigated combined with more detailed investigation of their population structure (see section 7.1.2.3)

In addition to *mecA*, the CoNS isolates investigated in the present study were found to carry a large number of antimicrobial resistance genes also commonly found in *S. aureus*. This suggests that horizontal transfer of many of these antimicrobial resistance genes occurs between these staphylococcal species and that CoNS are again a reservoir for these genes in *S. aureus*. However, further studies are required to confirm this including a

comparative molecular analysis of the MGEs carrying the antimicrobial resistance genes in CoNS with those in *S. aureus*.

7.1.2 Insights into the molecular characteristics of staphylococci in Ireland

The present study also revealed that detailed molecular characterisation of MRSA, MSSA and CoNS can be achieved using DNA microarray profiling and that this can be enhanced using *spa* typing for *S. aureus* and MLST for CoNS.

7.1.2.1 Enhanced discrimination of the endemic ST22-MRSA-IV clone and identification of similar ST22-MRSA-IV isolates among patients, environmental sites and healthcare workers

The problems posed by typing of isolates of the highly clonal ST22-MRSA-IV strain that is endemic in Irish hospitals have been well documented previously. The present study revealed the usefulness of *spa* typing combined with DNA microarray profiling for enhancing discrimination of ST22-MRSA-IV isolates. Many ST22-MRSA-IV isolates with the same *spa* type were further differentiated using the DNA microarray. However, there is a need for further studies of additional ST22-MRSA-IV isolates with detailed epidemiological information to confirm the usefulness of this combined typing approach. Recent studies have revealed the potential usefulness of whole-genome sequencing for tracking and typing ST22-MRSA-IV (Holden *et al.*, 2012; Holmes *et al.*, 2013). For example, Holmes *et al.*, (2013) recently developed a whole-genome sequence SNP typing assay and the application of this method for typing of 17 ST22-MRSA-IV isolates yielded a higher discriminatory power than *spa* typing and a similar discriminatory power to PFGE. Additionally, this SNP-typing assay is more rapid than PFGE and results were obtained within two days. The authors suggested that this approach could be adapted for use with any system that can identify SNPs, making interlaboratory comparison of results easier than PFGE (Holmes *et al.*, 2013). However, there are still difficulties associated

with the application of whole-genome sequencing for routine typing. Koser *et al.* (2012) reported difficulties in imposing thresholds for determining SNP differences between MRSA isolates in an outbreak situation and highlighted the need for protocols to be developed to address this (Koser *et al.*, 2012). While whole-genome sequencing is still relatively expensive compared to some other typing methods, many laboratories would not be able to type large amount of isolates using this method. Also, the data analysis is quite complex, requires detailed bioinformatic analysis, expertise using sophisticated software and expensive computer hardware. There is a need for automated data reporting systems to be developed that will use whole genome sequence data to provide information such as the number of SNP differences between isolates and the significance of these as well as important typing information such as the CC/ST, *spa* and SCC*mec* type of isolates, and the virulence and antimicrobial resistance genes that they possess. Until this is widely available and is cost effective it is unlikely that whole-genome sequencing will be widely used for routine typing.

The use of *spa* typing and DNA microarray profiling also revealed the presence of similar MRSA strains among patients with BSIs and from HCs and the predominance of ST22-MRSA-IV among both groups. Since the MRSA from HCs were carriage isolates from the nares of healthcare workers (HCWs) this also highlights the role that colonisation of HCWs can play in the transmission of MRSA to vulnerable patients and the need for routine screening of HCWs to prevent and control the spread of MRSA. The role of HCWs in MRSA transmission was recently highlighted in the spread of ST772-MRSA-V in a neonatal intensive care unit in an Irish Maternity Hospital (Brennan *et al.*, 2012). However, it should be noted that screening of HCWs in not mandatory in Ireland but should be considered. The MRSA decolonisation process is relatively easy and involves the use of a mupirocin ointment for decolonisation of the nose and a chlorhexidine body wash and mouthwash to decolonise other body sites. The screening of HCWs family members for

MRSA colonisation should also be considered, as the study by Brennan *et al.* (2012) revealed that even though a female HCW was successfully decolonised of MRSA, she became recolonised from her colonised child who acted a reservoir. This led to a subsequent episode of the spread of MRSA from the HCW to babies in the neonatal intensive care unit referred to above.

In another part of the present study similar strains of ST22-MRSA-IV were also identified among patients, the hands of HCWs and environmental sites using *spa* typing and DNA microarray profiling. This highlights the need for thorough and effective environmental decontamination, as patients and HCWs can contaminate environmental surfaces and the air. It also highlights the need for increased compliance with bacteriologically effective handwashing among healthcare workers. A recent report revealed low compliance with handwashing guidelines among HCWs in many Irish hospitals (HIQA, 2013), which contributes significantly to the spread of MRSA.

7.1.2.2. MSSA are more genetically diverse and potentially more virulent than MRSA

A much greater diversity of genotypes was identified among the MSSA isolates investigated from BSIs and HCs compared to MRSA. A very small number of similar lineages were identified among MRSA and MSSA isolates, and although this could indicate the possible local emergence of MRSA from MSSA or *vice versa*, it appears to be a rare event. Unsurprisingly, the MRSA isolates were found to carry more resistance genes than MSSA, but the MSSA isolates harboured more virulence genes, including the superantigen genes *tst* and *sea* and the *pvl* genes *lukF/S*. Additionally, it was also noted than among the MSSA isolates, the isolates recovered from HCs had twice as high a rate of carriage of *tst* compared to those from BSIs. Interestingly, while not statistically significant, the expression levels of *tst* were lower among the MSSA from HCs than among MSSA from BSIs and both of these were lower than the corresponding *tst*

expression levels by MSSA isolates from patients with toxic shock syndrome. While additional studies are required to further investigate the role of *tst* expression as well *sea* and *pvl* expression in MSSA virulence, the increased prevalence of these viruence genes among MSSA is worrying, particularly due to the increasing prevalence of MSSA BSIs in Ireland (EARS-Net, 2013).

7.1.2.3 Insights into the population structure of *S. epidermidis* and *S. haemolyticus* isolates among patients in Irish hospitals

The present study revealed that the majority of the *S. epidermidis* clinical isolates investigated belonged to the predominant global nosocomial lineage CC2. Similarly, the *S. haemolyticus* isolates were also assigned to a single CC. However, a study by Cavanagh *et al.* (2012) revealed the low discriminatory power of the *S. haemolyticus* MLST scheme used in the present study. In that study international *S. haemolyticus* clones were all assigned to a single lineage due to a high level of genetic conservation in the core *S. haemolyticus* genome genes. While PFGE could have been used in the present study for typing of *S. epidermidis* and *S. haemolyticus* isolates, it is best suited to outbreak situations and the high discriminatory power of PFGE would have masked the relatedness of these isolates. Alternative typing schemes need to be developed to allow for accurate typing of *S. haemolyticus*. This probably should include the development of more discriminatory alternative MLST schemes for *S. haemolyticus* and other DNA-sequencing based typing methods. Furthermore. it is also likely that the current MLST scheme for *S. epidermidis* could be improved. This could be facilitated by the determination of whole genome sequences of a wide variety of *S. epidermidis* as currently only a limited number of whole genome sequences are available for this species.

7.1.3 Concluding remarks

MRSA, MSSA and CoNS have become well-established as a cause of infection in healthcare facilities around the world. Accurate identification and tracking of staphylococcal isolates is essential to prevent and control their spread. The identification of indistinguishable strains among MRSA isolates recovered from patients, environmental sites and healthcare workers, but also among MRSA isolates from BSIs and from HCs, highlights the need for continual surveillance and strict infection control practices in Irish hospitals. The emergence of a novel *mec* gene in MRSA that may not be detected with commonly used MRSA detection methods highlights the need for optimised screening methods for these novel MRSA strains and ongoing surveillance and vigilance in this area.

This study has provided evidence for the horizontal transfer of SCC, SCC*mec* and antimicrobial resistance genes between staphylococcal species and the need for complete nucleotide sequencing to determine the genetic organisation of SCC and SCC*mec* elements among MRSA and CoNS. Ongoing surveillance of MRSA, MSSA and CoNS is warranted and currently DNA microarray profiling combined with DNA sequencing based typing methods allow for high-throughout analysis of isolates of these species. Further reductions in the cost of whole-genome sequencing and improvements in data handling and analysis will in the future allow for even more detailed, rapid and accurate typing of staphylococci.

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Antimicrobial Agents and Chemotherapy

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

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

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

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

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

Whole-genome sequencing. The whole genome of one MRSA isolate (AR13.1/3330.2) representative of the most frequently occurring ST-SCC*mec* type identified as harboring the *S. epidermidis ccrAB4* genes was sequenced to further investigate the location and adjacent sequences of these genes. High-throughput *de novo* sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, United Kingdom) using the Illumina Genome Analyzer system (Illumina HiSeq 2000 platform; Illumina, Essex, United Kingdom). The average coverage across the genome was 40×. The reads were assembled into contigs using a Velvet *de novo* genome assembler (version 1.0.15; Illumina). Contigs were analyzed using the Artemis DNA sequence viewer and annotation tool (42) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (1). Contigs identified as containing SCC-associated DNA sequences were aligned using the BioNumerics (version 5.1) (Applied Maths, Ghent, Belgium) and DNA Strider (version 1.3f11) (CEA Saclay, Gif-sur-Yvette, France) software packages. Any gaps identified between the SCC-related contigs in the whole-genome sequence of AR13.1/3330.2 were closed by primer walking using PCR with primers based on the surrounding contigs and the Expand long-template PCR system (Roche Diagnostics Ltd., West Sussex, United Kingdom) followed by amplimer sequencing by Geneservice (SourceBioscience, Guinness Enterprise Centre, Dublin). Data were analyzed, and overlapping sequences were assembled using the BLAST, Bionumerics, and DNA Strider software packages.

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

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

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

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

RESULTS

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

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

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

(Continued on following page)

TABLE 1 (Continued)

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

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

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

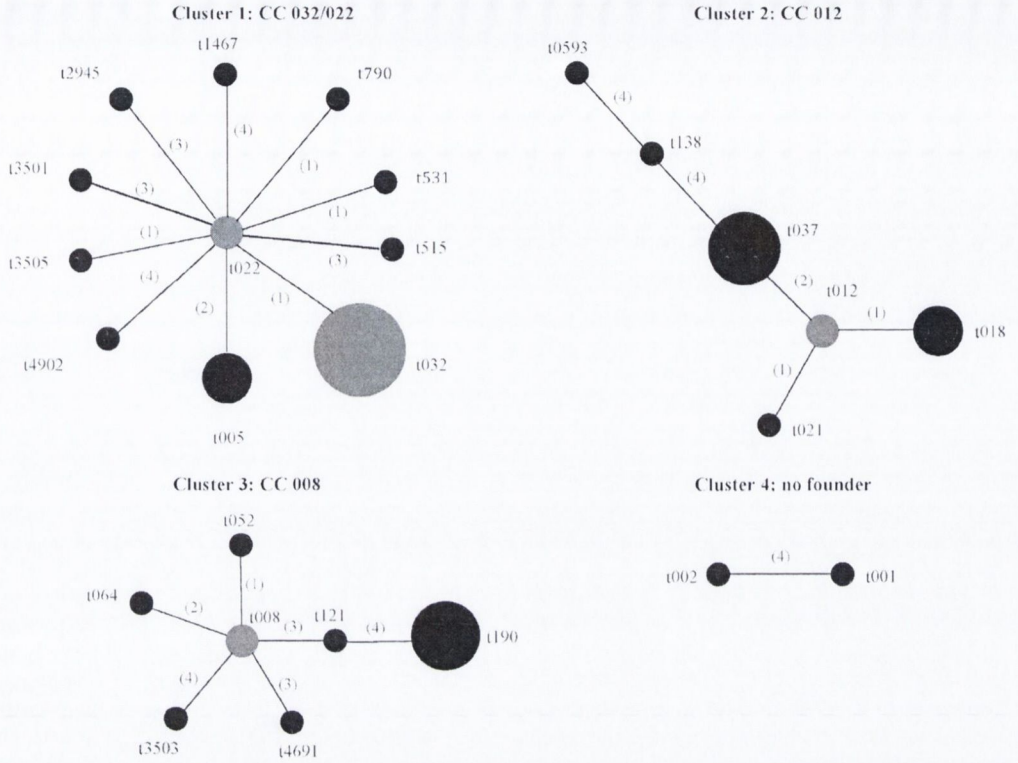

FIG 1 Based Upon Repeat Pattern (BURP) analysis of the *spa* types identified among 175 MRSA isolates representative of the different antibiogram-resistogram, sequence type, and SCC*mec* type combinations recovered from patients in Irish hospitals between 1971 and 2004. Twenty-six of the 35 *spa* types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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(Continued on following page)

TABLE 2 (Continued)

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

[a] The StaphyType kit (Alere Technologies) was used for DNA microarray analysis. *n*, number of isolates; *agr*, genes encoding accessory gene regulators; *cap*, genes encoding capsular proteins; CC, clonal complex; ST, sequence type; MSCRAMM, microbial surface components recognizing adhesive matrix molecules; ....., no genes detected.

[b] Immune evasion cluster (IEC) type as defined by van Wamel et al. (54): A = *sea*, *sak*, *chp*, and *scn*; B = *sak*, *chp*, and *scn*; C = *chp* and *scn*; D = *sea*, *sak*, and *scn*; E = *sak* and *scn*; F = *sep*, *sak*, *chp*, and *scn*; G = *sep*, *sak*, and *scn*; NT, novel IEC type consisting of *sak* and *sea* only; Neg (negative), no IEC genes detected.

[c] *seg* and *sei* were not detected in one and two ST5-MRSA-II isolates, respectively, found to carry the enterotoxin gene cluster (*egc*) genes seg/*sei*, *sem*, *sen*, *seo*, and *seu*.

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

(Continued on following page)

TABLE 3 (Continued)

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

[a] Variations in expression of phenotypic resistance to amikacin are not considered to be discrepant results because the reduction in amikacin susceptibility encoded by *aacA-aphD* may be very slight (27).

[b] QAC, quaternary ammonium compound. *qacA* encodes resistance to QACs, divalent cations and intercalating dyes (such as ethidium bromide); *qacC* encodes resistance to QAC and intercalating dyes.

[c] One of the three ampicillin resistant isolates exhibited intermediate resistance to ampicillin.

[d] Numbers in square brackets indicate numbers of isolates exhibiting resistance to lincomycin.

[e] Two of 29 tetracycline-resistant isolates exhibited intermediate resistance, and no tetracycline resistance genes were detected.

[f] AMI, amikacin; GEN, gentamicin; KAN, kanamycin; MER, mercuric chloride; NEO, neomycin; PMA, phenylmercuric acetate; TOB, tobramycin.

[g] The numbers in parentheses refer to the total number of isolates for which the antimicrobial phenotype correlated with the genotype as determined by array profiling over the total number of isolates investigated.

[h] ....., no phenotypic resistance detected to the relevant aminoglycoside antimicrobial.

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

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

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

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

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

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

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

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

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

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

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

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Detection of Staphylococcal Cassette Chromosome *mec* Type XI Carrying Highly Divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* Genes in Human Clinical Isolates of Clonal Complex 130 Methicillin-Resistant *Staphylococcus aureus*[▽]†

Anna C. Shore,[1] Emily C. Deasy,[1] Peter Slickers,[2] Grainne Brennan,[3] Brian O'Connell,[3,4] Stefan Monecke,[5] Ralf Ehricht,[2] and David C. Coleman[1]*

Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin, Ireland[1]; Alere Technologies GmbH, Jena, Germany[2]; National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland[3]; Department of Clinical Microbiology, University of Dublin, Trinity College Dublin, St. James's Hospital, James's St., Dublin 8, Ireland[4]; and Institute for Medical Microbiology and Hygiene, Faculty of Medicine Carl Gustav Carus, Technical University of Dresden, Dresden, Germany[5]

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Methicillin resistance in staphylococci is mediated by penicillin binding protein 2a (PBP 2a), encoded by *mecA* on mobile staphylococcal cassette chromosome *mec* (SCC*mec*) elements. In this study, two clonal complex 130 (CC130) methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients in Irish hospitals were identified that were phenotypically PBP 2a positive but lacked *mecA* by conventional PCR and by DNA microarray screening. The isolates were identified as methicillin-susceptible *S. aureus* using the GeneXpert real-time PCR assay. Whole-genome sequencing of one isolate (M10/0061) revealed a 30-kb SCC*mec* element encoding a class E *mec* complex with highly divergent *blaZ-mecA-mecR1-mecI*, a type 8 cassette chromosome recombinase (*ccr*) complex consisting of *ccrA1-ccrB3*, an arsenic resistance operon, and flanking direct repeats (DRs). The SCC*mec* element was almost identical to that of SCC*mec* type XI (SCC*mec* XI) identified by the Sanger Institute in sequence type 425 bovine MRSA strain LGA251 listed on the website of the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements. The open reading frames (ORFs) identified within SCC*mec* XI of M10/0061 exhibited 21 to 93% amino acid identity to ORFs in GenBank. A third DR was identified ca. 3 kb downstream of SCC*mec* XI, indicating the presence of a possible SCC remnant. SCC*mec* XI was also identified in the second CC130 MRSA isolate by PCR and sequencing. The CC130 MRSA isolates may be of animal origin as previously reported CC130 *S. aureus* strains were predominantly from bovine sources. The highly divergent nature of SCC*mec* XI relative to other SCC*mec* elements indicates that it may have originated in another taxon.

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are *S. aureus* isolates that exhibit phenotypic resistance to methicillin and related β-lactam antibiotics, including cefoxitin and oxacillin. The first report of MRSA was in England in 1961 (24), and since then MRSA has become a major nosocomial problem. Today MRSA is one of the most important antibiotic-resistant pathogens in hospitals and communities around the world. There is also an increasing awareness of the significance of MRSA in animals and of the zoonotic potential of MRSA (69).

The success of *S. aureus* is partly due to its ability to acquire mobile genetic elements encoding virulence and antimicrobial resistance genes from other *S. aureus* isolates and from coagulase-negative staphylococcal (CoNS) species (12, 17, 21, 33, 46, 57, 58, 66). One such group of mobile genetic elements are the staphylococcal cassette chromosome *mec* (SCC*mec*) elements, which harbor the methicillin resistance gene *mecA* (22). Existing MRSA clones have arisen following the acquisition and integration of SCC*mec* into a specific site (the integration site sequence [ISS]) within an open reading frame (ORF) in the *S. aureus* chromosome designated *orfX* (21, 22). Until recently, the function of *orfX* was unknown, but in recent months it has been annotated as a 23S rRNA methyltransferase (http://www.uniprot.org/uniprot/P0C1V0). SCC*mec* elements are characterized by the presence of flanking direct repeat (DR) sequences that are generated at both ends of the integrated SCC*mec* element (22). The *mecA* gene encodes the additional penicillin binding protein (PBP) 2a (in addition to PBP A to PBP D or PBP 1 to PBP 4) with a low affinity for β-lactam antibiotics, and the expression of *mecA* in MRSA allows cell wall synthesis to continue, despite inactivation of native PBPs by β-lactams (21, 48). The *mecA* gene forms part of the *mec* gene complex in SCC*mec* elements. Four classes of the *mec* gene complex (A to D), which differ in their genetic organization, have been described in the published literature (21, 26). Each has an intact copy of *mecA*, a copy of insertion sequence IS*431*, and, when present, complete or truncated *mec* regula-

* Corresponding author. Mailing address: Microbiology Research Unit, Division of Oral Biosciences, School of Dental Science, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin 2, Ireland. Phone: 353 1 612 7276. Fax: 353 1 612 7295. E-mail: david.coleman@dental.tcd.ie.

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tory genes *mecI* and *mecR1*. The cassette chromosome recombinase (*ccr*) genes form another essential component of SCC*mec* that are involved in site-specific integration and excision of SCC*mec* (21, 27). To date, five *ccr* gene complexes (*ccrA1B1*, *ccrA2B2*, *ccrA3B3*, *ccrA4B4*, and *ccrC*) and eight SCC*mec* types (I to VIII) have been described in MRSA in the published literature, with the latter characterized by the presence of different combinations of *ccr* and *mec* complex genes and with subtypes defined by variations in the junkyard (J) regions (7, 11, 21–23, 31, 45, 56, 75). Furthermore, some additional *mec*, *ccr*, and SCC*mec* types that have not yet been described in the published literature are listed on the SCC*mec* website curated by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC; http://www.sccmec.org/Pages/SCC_TypesEN.html). Because of the extensive diversity that has been observed in the genetic organization of SCC*mec* elements, the IWG-SCC was established to standardize criteria for the classification of SCC*mec* elements. Advice on appropriate nomenclature for novel SCC*mec* types and subtypes is available from the IWG-SCC, and it is recommended that the entire nucleotide sequence of all novel SCC*mec* elements be determined prior to SCC*mec* type designation (21).

SCC*mec* elements are not exclusive to MRSA and have also been reported in several CoNS species, many of which form part of the normal flora of healthy human and animal skin and mucous membranes and are frequently associated with opportunistic human and veterinary infections (9, 15, 18, 53, 62, 66, 70, 76). The presence of very similar SCC*mec* elements in MRSA and CoNS strongly suggests horizontal transfer of SCC*mec* (6, 8, 10, 17, 18, 61, 70). While the mechanism(s) and the exact route of transfer of SCC*mec* are presently unknown, the higher frequency and diversity of SCC*mec* elements in CoNS suggest that CoNS are potentially a reservoir of SCC*mec* elements that may facilitate the emergence of new MRSA clones (15, 16). In addition, several studies have identified *mecA* gene homologs with and without *mec* complex genes, as well as SCC elements without *mecA* but with *ccr* genes, as individual genetic components in CoNS and in the closely related species *Macrococcus caseolyticus* (40, 55, 65, 66, 73, 74). Thus, some researchers have speculated that such genetic elements may be the evolutionary precursors of SCC*mec* in CoNS and MRSA (66, 72).

In Ireland, MRSA has been endemic in hospitals since the 1980s, and different strains have predominated at different time periods (56). For the last decade, sequence type 22-MRSA-SCC*mec* type IV (ST22-MRSA-IV) isolates have dominated, and since 2003 they have accounted for 80% of MRSA isolates recovered from patients in Irish hospitals (51, 59). In line with trends worldwide, MRSA isolates harboring the Panton-Valentine leukocidin gene have emerged as community-acquired pathogens in Ireland and MRSA has also been reported among animals (1, 2, 32, 41, 52).

In the present study, we report on the identification of human clinical MRSA isolates from Ireland belonging to clonal complex 130 (CC130) that are detected as PBP 2a positive using two different latex agglutination assays but lack *mecA*, as detected by conventional *mecA* PCR. These MRSA isolates were detected as methicillin-susceptible *S. aureus* (MSSA) using the GeneXpert real-time PCR assay, which targets SCC*mec* and *mecA*, as well as the *S. aureus* protein A gene *spa* and the ISS, for the rapid detection of MRSA in clinical specimens (71). A comprehensive analysis of these isolates resulted in the identification of a novel SCC*mec* element with highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes.

MATERIALS AND METHODS

***Staphylococcus aureus* isolates investigated.** Two clinical MRSA isolates (M10/0148 and M10/0061) were investigated in the present study. M10/0148 was isolated from a 64-year-old female inpatient in an acute care hospital in Dublin, Ireland, in February 2010, and M10/0061 was isolated from an 85-year-old male inpatient in a regional hospital in the southeast of Ireland in May 2010. There was no obvious epidemiological connection between these patients, and the two hospitals are approximately 160 kilometers apart. There was no information available indicating that the patients had had any contact with animal husbandry, domestic animals, raw meat, or other potential sources of zoonotic bacteria. Isolates were identified as *S. aureus* by detection of staphylocoagulase production (50), were inoculated onto Protect beads (Technical Service Consultants Limited, Heywood, United Kingdom), and stored at −70°C prior to subsequent detailed analysis.

A MSSA isolate (21250_V454) recovered from a brown rat (*Rattus norvegicus*) in Germany (courtesy of Sebastian Günther, Berlin University) was also included in the study for comparison since it was found to belong to the same clonal lineage and yielded DNA microarray hybridization patterns (see below) similar to those of the two clinical isolates.

Investigation of resistance to antimicrobial agents. Screening for oxacillin/methicillin resistance was performed by culture of isolates on cefoxitin-containing ChromID MRSA selective agar (bioMérieux, Nuertlingen, Germany). The isolates were tested for methicillin resistance using 30-μg cefoxitin disks (Oxoid, Basingstoke, Hampshire, United Kingdom) on BBL Mueller-Hinton II agar (Becton Dickinson and Company, Sparks, MD) using the Clinical and Laboratory Standards Institute (CLSI) methodology (13). The MRSA isolates were further tested against oxacillin (1-μg and 5-μg disks; Oxoid) on Columbia blood agar (CBA; Oxoid) at 30°C. Oxacillin MICs were determined using the Etest system (bioMérieux) according to the manufacturer's instructions, and oxacillin MIC determinations were repeated using induced colonies that had been exposed to oxacillin. Additionally, susceptibility tests for the MRSA isolates were performed using the Vitek-2 system according to the manufacturer's instructions (Gram-positive bacteria susceptibility test card; bioMérieux). Both the MSSA isolate and the MRSA isolates were tested for PBP 2a production using a Mastalex MRSA kit (Mast Diagnostics, Bootle, United Kingdom), an MRSA-Screen latex agglutination assay (Innogenetics, Gent, Belgium), and a Clearview Exact PBP 2a kit (Alere, Cologne, Germany). All isolates were also tested for the presence of *mecA* by PCR using previously described primers (44) and novel primers to amplify the entire *mecA* gene (Table 1), with MRSA strains N315 (29) and ATCC 43300 as positive controls and MSSA strains MSSA476 (19, 29) and ATCC 25923 as negative controls. The MRSA isolates underwent antibiogram-resistogram (AR) typing as described previously to determine their resistance to a panel of 23 antimicrobial agents (52). The AR typing panel consists of the following antimicrobial agents: 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 (52). The MRSA isolates were tested for susceptibility to arsenicals using 100-, 250-, and 500-μg sodium arsenite disks and 250-, 500-, and 1,000-μg sodium arsenate disks and the CLSI methodology (13). The arsenic-resistant *S. aureus* reference strain WGB8404 (43) and the arsenic-susceptible *S. aureus* reference strain RN4220 (28) were used as controls. The MRSA isolates were also tested for susceptibility to sodium arsenite by broth microdilution. For these experiments, the arsenic-susceptible *S. aureus* strain NCTC8325 (42) and the arsenic-resistant strain WGB8404 were used as controls. Briefly, doubling dilutions of sodium arsenite (range, 10 to 10,000 μg/ml) were prepared in nutrient broth (Oxoid) supplemented with 2% (wt/vol) glucose. Inocula were prepared from cultures grown on CBA and adjusted to a density corresponding to a 0.5 McFarland standard (bioMérieux), and 20 μl was added to the wells of 96-well microtiter plates containing 80 μl of the respective sodium arsenite dilutions. Following overnight incubation at 37°C, plates were examined visually for growth.

Investigation of isolates using the GeneXpert real-time PCR assay for the rapid detection of MRSA in clinical specimens. The MRSA isolates M10/0148 and M10/0061 were both tested using the Xpert MRSA/SA SSTI assay on a

TABLE 1. Novel PCR primers used in the study

Gene or region amplified	Primer pair	Nucleotide sequence (5′–3′)	Nucleotide coordinates
mecA	mecA_fw	ACCTTCTACACCTCCATATCAC	24320–24341[a]
	mecA_rv	CGTTACGGATTGCTTCACTG	26452–26433[a]
pbp1	Pbp1_fw	CGATAATGTAAAGGTAGTGCGTAG	828245–828222[b]
	Pbp1_rv	CCCTTATTTAATGTAGCCATTCTTG	825960–825984[b]
pbp2	Pbp2_fw	GATGAAAGTGAGGACCGCG	477649–477631[b]
	Pbp2_rv	ATAACTGAGGATTTTATGTTGAGTGG	475419–475444[b]
pbp3	Pbp3_fw	GAAAGACTTTGAATAGAGGTAGGTAG	332226–332251[b]
	Pbp3_rv	TGCGCTACACAATCGTCAG	334356–334338[b]
pbp4	Pbp4_fw	TTGGAAAAGGGAAGATTAACGC	308863–308884[c]
	Pbp4_rv	GCAACTTGTCCGTTTTTAGTATG	310208–310186[c]
$ccrA1B3_{M10/0061}$	$ccrB3_{M10/0061}$ F1	CTACTTGAAGTTATCCAATC	10125–10144[d]
	$ccrA1_{M10/0061}$ R1	CACATTACTCGCTGATTTAG	13427–13408[d]
$mecA_{M10/0061}$	$mecA_{M10/0061}$ F1	CCAGATATAGTAGCATTATA	1799–1818[d]
	$mecA_{M10/0061}$ R1	AAAGATGACGATATTGAG	3672–3655[d]
orfX-$mecI_{M10/0061}$	orfX F1	GTGTTAATTGAGCAAGTGTA	418–437[d]
	$mecI_{M10/0061}$ R2	GCATTCTTACATCCGTTTTG	6070–6051[d]
$mecI_{M10/0061}$-$ccrA1_{M10/0061}$	$mecI_{M10/0061}$ F1	CTATGATATATCAGCGTCAG	56041–56023[d]
	$ccrA1_{M10/0061}$ R1	ACAAGCTCAAGCGATACGGT	12563–12544[d]
$ccrA1_{M10/0061}$-$ORF_119_{M10/0061}$	$ccrA1_{M10/0061}$ F1	AATAACGTAATGTGCGGTGC	12421–12440[d]
	$ORF_119_{M10/0061}$ R1	ACTTAAATCAGGCTTATATCC	20622–20602[d]
$ORF_119_{M10/0061}$-$arsC_{M10/0061}$	$ORF_119_{M10/0061}$ F1	TCTAGTATCTGAAAGGTC	20475–20492[d]
	$arsC_{M10/0061}$ R1	AACCATACGTCAGACTTGA	27963–27945[d]
$arsC_{M10/0061}$-downstream of DR3	$arsC_{M10/0061}$ F1	GACCACTCTTTACCTGCT	27809–27826[d]
	tnp1S2 R1	AGATCATGGAAAACCGATCA	34397–34378[d]

[a] Nucleotide coordinates based on the nucleotide sequence of SCC*mec* III (GenBank accession number AB037671.1).
[b] Nucleotide coordinates based on the nucleotide sequence of *S. aureus* strain MN8 contig 215 (GenBank accession number ACJA02000004.1).
[c] Nucleotide coordinates based on the nucleotide sequence of *S. aureus* strain MN8 contig 215 (GenBank accession number ACJA02000003.1).
[d] Nucleotide coordinates based on the nucleotide sequence of SCC*mec* XI of M10/0061 (GenBank accession number FR823292).

GeneXpert DX system (version 1.2) real-time PCR platform (Cepheid, Sunnyvale, CA), according to the manufacturer's instructions, to determine if the MRSA isolates would be detected as MRSA using this system, which targets SCC*mec*-associated and *spa* DNA sequences. The MSSA isolate 21250_V454 was also tested.

Total genomic DNA isolation. Genomic DNA was extracted from all isolates by enzymatic lysis using the buffers and solutions provided with the StaphyType DNA microarray kit (described below) and the Qiagen DNeasy kit (Crawley, West Sussex, United Kingdom) as described previously (35).

Molecular typing. The isolates underwent *spa* typing and multilocus sequence typing (MLST) as described previously (14, 60).

DNA microarray analysis. The isolates were subjected to DNA microarray analysis using the StaphyType kit (Alere Technologies GmbH), which detects 334 *S. aureus* gene sequences and alleles, including species-specific, antimicrobial resistance- and virulence-associated genes, and typing markers (35). The genes detected using the array include previously described SCC*mec*-associated gene sequences, including *mecA*, its regulatory genes *mecI* and *mecR1*, various allotypes of the *ccr* genes, and gene sequences previously identified in the J regions of various SCC*mec* elements. The DNA microarray procedures have been described previously and were performed according to the manufacturer's instructions (35). DNA microarray analysis of each isolate was performed on three separate occasions, and two different labeling approaches were employed. In one, amplification and labeling were directed by target-specific primers (35). In the second, the complete genome was labeled using randomized oligonucleotides instead of the site-specific labeling as in the first method (37, 39). The latter protocol proved to be useful for the detection of mutated or divergent target genes, e.g., genes from strains that are not fully represented by the published *S. aureus* genome sequences due to the different stringency of the overall labeling and hybridization process.

PCR and sequencing of the genes encoding the native *S. aureus* PBPs 1 to 4. The *S. aureus* genes encoding PBPs 1 to 4 were amplified and sequenced using *Taq* polymerase (Genaxxon BioScience GmbH, Biberach, Germany), the primers PBP 1 to PBP 4 (Table 1), and the following conditions: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 2 min, with a final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis in 1% (wt/vol) agarose gels, ethidium bromide staining, and visualization under UV light. Bands were extracted from agarose gels, and DNA was purified using a Qiaex II gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing was performed using the PCR primers and BigDye Terminator cycle sequencing kit (version 1.1; Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. Sequence analysis was performed using a 3130 genetic analyzer (Applied Biosystems).

Whole-genome sequencing of M10/0061. The whole genome of one MRSA isolate, M10/0061, was sequenced to investigate the location of the *ccr* genes identified in both isolates using the DNA microarray and to investigate the possible presence of a novel SCC*mec* element. High-throughput *de novo* sequencing was undertaken commercially by Geneservice (Source BioScience plc, Nottingham, United Kingdom) using an Illumina genome analyzer system (HiSeq 2000 platform; Essex, United Kingdom). The average coverage across the genome was 40 times. 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 (54) and BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (3). ORFs were predicted using prodigal (http://prodigal.ornl.gov/) (20), a gene prediction algorithm designed specifically for prokaryotic sequences. Prodigal was trained with four fully sequenced and annotated *S. aureus* genomes (GenBank accession numbers CP000046.1, BA000017.4, BX571856.1, and AJ938182.1). All predicted ORFs were analyzed against a local database of all staphylococcal sequences extracted from GenBank using the BLAST standalone software package (version 2.2.24; NCBI). The sequences of the ORFs were globally aligned with the best-matching sequences from the local database, and the positions of start and stop codons and potential ribosomal binding sites were checked for consistency. All ORFs were translated into protein sequences, and the protein sequences were analyzed against the NCBI nonredundant database of protein sequences with the BLAST program. Amino acid identities between two sequences were calculated from global sequence alignments. The sequences were aligned with the needle global alignment program from the EMBOSS software package (49). Relative identities were calculated from the number of identical amino acids in the alignment with respect to the length of the new sequence from SCC*mec* type XI (SCC*mec* XI). These overall amino acid identities were calculated for the best-matching BLAST sequence. In addition, for the *mec* complex genes, amino acid identities were calculated for additional sequences which represented different allotypes or paralogs of the gene concerned. Occurrences of the characteristic DRs of SCC*mec* were detected with the bl2seq program from the NCBI BLAST software package.

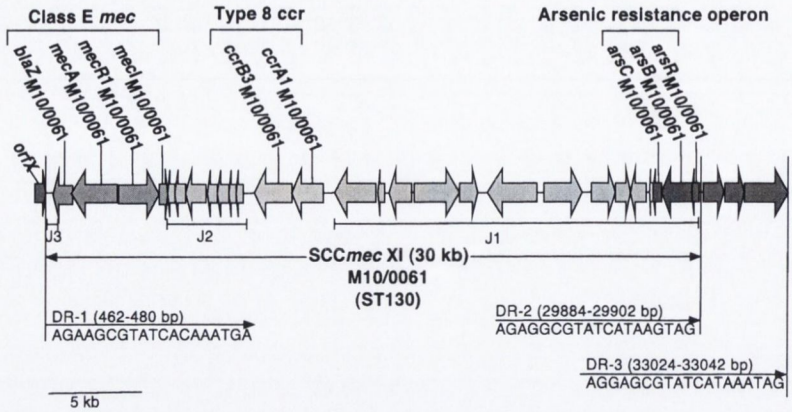

FIG. 1. Schematic diagram showing the genetic organization of the SCC*mec* element designated SCC*mec* XI and the adjacent 3-kb downstream region in the ST130 MRSA isolate M10/0061 (GenBank accession number FR823292). SCC*mec* XI is bordered by DR sequences DR-1 and DR-2, and the 3-kb downstream region is bordered by DR-2 and DR-3. The *mec* complex, *ccr* complex, and arsenic resistance operon genes are shown in red, green, and blue, respectively. The three ORFs identified in the adjacent 3 kb downstream region are shown in purple. A full list of ORFs encoded by the SCC*mec* element and adjacent region is provided in Table S1 in the supplemental material. The structure of the SCC*mec* element was determined by high-throughput whole-genome sequencing of M10/0061.

PCRs to confirm the presence of novel *mecA* and *ccr* allotypes and SCC*mec* element in M10/0148. Having determined the presence of novel allotypes of *ccr*, *mecA*, *blaZ*, and the *mec* regulatory genes *mecI* and *mecR1* in M10/0061 by analysis of its whole-genome sequence, primer pairs $ccrB3_{M10/0061}$ F1/$ccrA1_{M10/0061}$ R1 and $mecA_{M10/0061}$ F1/$mecA_{M10/0061}$ R1 were designed to investigate M10/0148 for the presence of $ccrA1B3_{M10/0061}$ and $mecA_{M10/0061}$, respectively (Table 1). In addition, primer pair orfX F1/$mecI_{M10/0061}$ R2 was used to amplify and sequence the complete *mec* gene complex in M10/0148, and four additional primer pairs were used to amplify the remainder of the novel SCC*mec* element (between DRs 1 and 2) and the 3-kb region identified between the second and third DRs in M10/0061 in M10/0148 (Table 1 and Fig. 1). DNA fragments were obtained by PCR amplification of chromosomal DNA using either GoTaq DNA polymerase (Promega Corporation, Madison, WI) for primers $ccrB3_{M10/0061}$ F1/$ccrA1_{M10/0061}$ R1 and $mecA_{M10/0061}$ F1/$mecA_{M10/0061}$ R1 or the Expand Long-Template PCR system (Roche Diagnostics Ltd., Lewes, East Sussex, United Kingdom) for all other primer pairs according to the manufacturers' instructions. The whole-genome-sequenced isolate M10/0061 harboring the novel *mecA* allotype and MRSA strain CA05 (31) harboring SCC*mec* IV and encoding the original *mecA* allotype were used as positive and negative controls, respectively. PCR products were visualized by agarose gel electrophoresis, gel red staining (Biotium, Hayward, CA), and visualization under UV light. PCR products were purified using a Genelute PCR cleanup kit (Sigma-Aldrich Chemical Co., Tallaght, Dublin, Ireland). DNA sequencing was performed commercially by Geneservice (Source Bioscience, St. James's Hospital, Dublin, Ireland). Sequence analysis was performed using the BLAST, BioNumerics (version 5.1; Applied Maths, Ghent, Belgium), and DNA Strider software packages.

Nucleotide sequence accession numbers. The nucleotide sequence of the SCC*mec* element (SCC*mec* XI) and the 3-kb DNA sequence identified adjacent to SCC*mec* XI were deposited in GenBank under the accession number FR823292. The nucleotide sequences of PBPs 1, 2, 3, and 4 identified in M10/0061 by whole-genome sequencing have been deposited in GenBank under the accession numbers FR823293, FR823294, FR823295, and FR823296, respectively.

RESULTS

Identification of two PBP 2a-positive clinical MRSA isolates that lack *mecA* by conventional *mecA* PCR. The *S. aureus* isolates M10/0061 and M10/0148 exhibited resistance to cefoxitin (30-μg cefoxitin disks) and oxacillin (1-μg and 5-μg disks), exhibited growth on ChromID MRSA selective agar, and were identified by the Vitek-2 system as MRSA. The two isolates exhibited oxacillin MICs of 1.0 mg/liter and 2.0 mg/liter, respectively, and for both isolates, these rose to 16 mg/liter when the colonies were induced (i.e., cultured in the presence of oxacillin) prior to oxacillin MIC determination. Both isolates were positive for production of PBP 2a using the Mastalex and Clearview latex agglutination assays but were PBP 2a negative using the Innogenetics PBP 2a assay. Furthermore, neither isolate yielded any amplicons following PCR using *mecA* primers, while the positive control MRSA strains N315 and ATCC 43300 yielded amplicons corresponding to the amplification of *mecA*. AR typing revealed that the isolates exhibited resistance to ampicillin only out of the 23 antimicrobials tested in the AR typing panel. Both MRSA isolates exhibited resistance to sodium arsenite and sodium arsenate.

The MSSA isolate did not grow in the presence of cefoxitin, was negative for the production of PBP 2a using the Mastalex, Innogenetics, and Clearview latex agglutination assays, and was *mecA* negative by both *mecA* PCR assays.

GeneXpert. The two MRSA isolates, M10/0061 and M10/0148, were identified as *S. aureus* but not as MRSA using the GeneXpert MRSA real-time PCR assay. Identical results were obtained for the rat MSSA isolate 21250_V454.

Molecular typing. The two MRSA isolates exhibited two different STs, ST130 and ST1764, following MLST analysis, with both belonging to CC130 (Table 2), respectively. ST1764 is a single-locus variant of ST130 that differs by only 1 nucleotide base within the *tpi* gene. The MSSA isolate was also assigned to CC130 (Table 2). Using *spa* typing, the MRSA isolates exhibited two closely related *spa* types (t843 and t373) (Table 2), which differ by the presence of two additional repeat units in t843. The MSSA isolate exhibited a novel but related *spa* type t8403 which differed from *spa* type t843 in the deletion of one repeat unit and from t373 in the presence of one additional repeat unit.

DNA microarray analysis. The DNA microarray results for the two MRSA isolates and the MSSA isolate are shown in Table 2. DNA microarray analysis confirmed the absence of *mecA* in the MSSA and MRSA isolates. No *mec* regulatory genes were detected. The MSSA and MRSA isolates yielded

TABLE 2. MLST, *spa* types, and DNA microarray hybridization profiles of MRSA isolates M10/0061 and M10/0148 and MSSA isolate 21250_V454

Gene class and gene(s)	DNA microarray[a] hybridization profile		
	M10/0061	M10/0148	21250_V454
Species markers *katA*, *coa*, *nuc*, *spa*	Pos	Pos	Pos
agr group III	Pos	Pos	Pos
SCC*mec*-associated markers			
mecA	Neg	Neg	Neg
Δ*mecR1*	Neg	Neg	Neg
mecR1-mecI	Neg	Neg	Neg
ccrA1	Neg	Neg	Neg
ccrB1	Variable	Variable	Neg
Q9XB68-*dcs*	Neg	Neg	Neg
ccrA2-ccrB2	Neg	Neg	Neg
kdp-SCC locus	Neg	Neg	Neg
ccrA3	Variable	Variable	Neg
ccrB3	Variable	Variable	Neg
ccrC, *ccrA4-ccrB4*	Neg	Neg	Neg
Antimicrobial resistance genes			
blaZ	Neg	Neg	Neg
blaI-blaR1	Neg	Neg	Neg
erm(A), *erm*(B)	Neg	Neg	Neg
erm(C)	Neg	Neg	Neg
lnu(A)	Neg	Neg	Neg
msr(A), *mph*(C)	Neg	Neg	Neg
aacA-aphD	Neg	Neg	Neg
aadD	Neg	Neg	Neg
aphA3-sat	Neg	Neg	Neg
dfrS1	Neg	Neg	Neg
far1	Neg	Neg	Neg
mupA	Neg	Neg	Neg
tet(K)	Neg	Neg	Neg
tet(M)	Neg	Neg	Neg
cat	Neg	Neg	Neg
cfr	Neg	Neg	Neg
fexA	Neg	Neg	Neg
fosB	Neg	Neg	Neg
tet efflux	Pos	Pos	Pos
Virulence-associated genes			
tst1	Neg	Neg	Neg
sea, *seb*, *see*, *seh*	Neg	Neg	Neg
sec-sel, *sed-sej-ser*	Neg	Neg	Neg
seg-sei-sem-sen-seo-seu	Neg	Neg	Neg
sek-seq	Neg	Neg	Neg
lukF-PV–lukS-PV	Neg	Neg	Neg
lukF-PV (P83), *lukM*	Neg	Neg	Pos
hla, *hld*	Pos	Pos	Pos
hlb	Pos	Pos	Pos
Untruncated *hlb*	Pos	Pos	Pos
sak-chp-scn	Neg	Neg	Neg
etA, *etB*, *etD*	Neg	Neg	Neg
edinA, *edinB*	Neg	Neg	Neg
edinB	Pos	Pos	Pos
arcA-arcB-arcC-arcD	Neg	Neg	Neg
Capsule type 8	Pos	Pos	Pos

[a] The StaphyType Kit (Alere Technologies) was used for DNA microarray analysis. The table shows only the clinically relevant markers, including exotoxins and resistance genes, and does not represent the complete hybridization profile of 334 probes on the array. Full data sets are available upon request. Isolate M10/0061 was CC130/ST130 and had the MLST allelic profile 6-57-45-2-7-58-52, *spa* type t843, and *spa* repeat succession 04-82-17-25-17-25-25-16-17. Isolate M10/0148 was CC130/ST1764 and had the MLST allelic profile 6-57-45-2-7-193-52, *spa* type t373, and *spa* repeat succession 04-17-25-17-25-16-17. Isolate 21250_V454 was CC130/ST2024 and had the MLST allelic profile 6-57-45-2-219-58-52, *spa* type t8403, and *spa* repeat succession 04-82-17-25-25-25-16-17. Pos, positive; Neg, negative; Variable, MRSA isolates M10/0061 and M10/0148 exhibited weak and/or variable signals for *ccrB1*, *ccrA3*, and *ccrB3* in the primer-directed protocol but yielded positive signals for *ccrB1* and *ccrB3* in the random protocol outlined in the Materials and Methods.

very similar DNA microarray signals, except that the MRSA isolates exhibited weak and/or variable signals for *ccrB1*, *ccrA3*, and *ccrB3* in the primer-directed protocol, while the random protocol yielded signals for *ccrB1* and *ccrB3* (Table 2). This suggested the possible presence of a novel *ccr* allotype in the MRSA isolates. No other known SCC or SCC*mec*-associated genes were detected in MRSA isolates M10/0061 and M10/0148 (Table 2).

DNA and amino acid sequence analysis of the genes encoding PBPs 1 to 4. Comparative analysis of the DNA sequences of the genes encoding PBPs 1 to 4 from CC130 MRSA isolates M10/0061 and M10/0148 and the CC130 MSSA isolate revealed that each gene in the MRSA isolates exhibited 100% DNA sequence identity to each other and to the corresponding genes in the MSSA isolate. The PBP sequences identified in M10/0061 by whole-genome sequencing were submitted to GenBank.

Identification of an SCC*mec* element encoding a novel *mecA* allotype in MRSA isolate M10/0061. Whole-genome sequencing of the ST130 MRSA isolate M10/0061 yielded 401 contigs ranging in size from ca. 100 bp to 100 kb, and 46 of these were >20 kb in size. The *ccr* genes were localized to a 90-kb contig that was found to contain sequences encoding a 30-kb SCC*mec* element which was analyzed in depth (Fig. 1). Because the present study focused on the 30-kb SCC*mec* element and immediately adjacent ORFs, other ORFs encoded by the 90-kb contig or other contigs are not discussed further here. Details of the ORFs identified within SCC*mec* of M10/0061 are described in Table S1 in the supplemental material. The SCC*mec* element harbored a novel allotype of the *mecA* gene designated $mecA_{M10/0061}$, the predicted protein from which exhibited 62% amino acid (aa) identity with MecA proteins previously described in MRSA and 63% aa identity with MecA from *Staphylococcus kloosii* and *Staphylococcus vitulinus*. Novel allotypes of the *mec* regulatory genes *mecI* and *mecR1* were also identified upstream of $mecA_{M10/0061}$, and they encoded proteins that exhibited 66% and 45% aa identities with proteins encoded by previously described MRSA *mecI* and *mecR1* genes, respectively (Fig. 1). A novel allotype of the β-lactamase resistance gene *blaZ* was identified downstream of *mecA* but still within the SCC*mec* element and exhibited 67% aa identity with the previously known *S. aureus blaZ* gene. The *ccrAB* genes within the SCC*mec* element from M10/0061 consisted of *ccrA1* and *ccrB3* genes and exhibited 86% and 93% aa identities to previously described *ccrA1* and *ccrB3* genes in the GenBank database, respectively.

The SCC*mec* element in M10/0061 was integrated at the same nucleotide position within *orfX* in M10/0061 as all other SCC*mec* elements described in MRSA isolates to date and was flanked by DR sequences (DR-1 and DR-2) that are characteristic of SCC*mec* elements (Fig. 1). A third DR (DR-3; Fig. 1) was identified outside the SCC*mec* region ca. 3 kb downstream of DR-2 in the novel SCC*mec* element. Three ORFs were identified within this 3-kb, region but they exhibited only weak similarity (39 to 53% aa identity) with ORFs in nonstaphylococcal species (Fig. 1; see Table S1 in the supplemental material).

No ORFs were identified in the 316-bp DNA sequence identified between *orfX* and *blaZ* in the SCC*mec* element from M10/0061; i.e., no ORFs were identified in the J3 region. The

J2 region (between the *mec* gene complex and the *ccr* genes) of the SCC*mec* element was ca. 4.5 kb and contained seven ORFs. For 6/7 ORFs, the highest similarities (49 to 91%) were to hypothetical proteins of *S. aureus* (2 of which were SCC*mec* related), *M. caseolyticus*, and *Staphylococcus saprophyticus*. For one ORF in the J2 region, the highest similarity was to a transposase of *Clostridium* spp., but these ORFs exhibited just 21% aa identity. The J1 region (between the *ccr* genes and DR-2) of the SCC*mec* element was ca. 16.5 kb, and 14 ORFs were identified. The highest similarities for any ORFs within the J1 region were of three ORFs (79 to 93% aa identity) to the arsenic resistance operon genes *arsC*, *arsB*, and *arsR*. For 2/14 ORFs (ORF_115 and ORF_16) in the J1 region, the highest similarity was to ORFs encoding hypothetical proteins described in SCC*mec* elements, one in MRSA and one in *Staphylococcus hominis*. Of the nine remaining ORFs within the J1 region, eight exhibited amino acid identities ranging from 39 to 89% to ORFs from CoNS, including a cassette chromosome helicase protein, a lipase precursor protein, a FAD-binding dehydrogenase, two ABC transporter proteins, a flavin reductase protein, a major facilitator superfamily protein, and a hypothetical protein. The remaining ORF (ORF_117) exhibited weak similarity (32% aa identity) with a DNA helicase in a nonstaphylococcal species.

Designation of the SCC*mec* element of M10/0061 as SCC*mec* XI. The website of the IWG-SCC (http://www.sccmec.org/Pages/SCC_TypesEN.html) lists a SCC*mec* type, designated SCC*mec* XI, from MRSA isolate LGA251 with a genetic organization within the *mec* and *ccr* complex genes similar to that of the SCC*mec* element identified in the present study in M10/0061. A search of the published literature and GenBank failed to yield descriptions of SCC*mec* XI. MRSA isolate LGA251 was originally recovered from a bovine source and has previously been assigned to ST425 (34) (http://www.sanger.as.uk/pathogens). The whole-genome sequence of LGA251 has been determined by the Wellcome Trust Sanger Institute (Cambridge, United Kingdom) and is available on the Sanger Institute website (http://www.sanger.as.uk/pathogens). Because the whole-genome sequence of LGA251 is not currently available in GenBank and has not yet been published, permission was obtained from the Sanger Institute to compare the SCC*mec* element identified in the human clinical MRSA isolate M10/0061 in the present study with that of SCC*mec* XI first identified by the Sanger Institute in bovine MRSA isolate LGA251. This comparative analysis revealed that both SCC*mec* elements exhibited 99.9% DNA sequence identity. Thus, we have designated the SCC*mec* type of M10/0061 XI (http://www.sccmec.org/Pages/SCC_TypesEN.html). Similarly, we have designated the *mec* complex of M10/0061, consisting of divergent *blaZ*, *mecA*, *mecR1*, and *mecI* genes, class E *mec*, reflecting the corresponding IWG-SCC designation for LGA251 (Fig. 1). The *ccrA1B3* gene combination of M10/0061 was designated type 8 *ccr* complex, again reflecting the corresponding IWG-SCC designation for LGA251 (Fig. 1).

No sequence similarity to the 3-kb region downstream of DR-2 in SCC*mec* $XI_{M10/0061}$ (Fig. 1) was identified downstream of SCC*mec* XI in LGA251.

Detection of SCC*mec* XI in M10/0148. PCR and sequencing revealed the presence of $ccrA1B3_{M10/0061}$ and $mecA_{M10/0061}$ as well as $mecI_{M10/0061}$, $mecR1_{M10/0061}$, and $blaZ_{M10/0061}$ in the second clinical CC130 MRSA isolate, M10/0148. Each of these genes exhibited 99 to 100% DNA sequence identity with the corresponding genes identified in M10/0061. The presence of the entire SCC*mec* XI element and the 3-kb region between DR-2 and DR-3 was also confirmed in M10/0148 using PCR and 10 primer pairs. M10/0148 yielded amplicons of the expected size using all primer pairs.

DISCUSSION

Methicillin-resistant *S. aureus* continues to be a significant problem in many hospitals worldwide, with potentially serious consequences for patients and significant extra demands on health care resources. Correct identification of MRSA is essential to ensure appropriate isolation and treatment of patients. Here we report on two MRSA isolates recovered from patients in Irish hospitals that exhibited phenotypic resistance to methicillin but were identified as MSSA using *mecA* PCR, a DNA microarray, the GeneXpert rapid real-time PCR assay, and a PBP latex agglutination assay. These two CC130 MRSA isolates harbored a 30-kb SCC*mec* element, designated SCC*mec* XI, that had several striking features, including highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes, and that was almost identical to an unpublished SCC*mec* element, designated SCC*mec* XI on the IWG-SCC website (http://www.sccmec.org/Pages/SCC_TypesEN.html). Each ORF identified within SCC*mec* element XI exhibited 21 to 93% aa identity to previously described ORFs in GenBank (see Table S1 in the supplemental material), indicating that this SCC*mec* element is distantly related to previously described SCC*mec* elements and may possibly represent an ancestral form of SCC*mec* in MRSA.

In all SCC*mec* elements that have been described previously in the literature, the *mec* complex genes (*mecA*, *mecI*, and *mecR1*) are almost identical. In contrast, the *mecA*, *mecR1*, and *mecI* genes identified in the CC130 MRSA isolates in the present study were highly divergent, exhibiting only 62%, 45%, and 66% aa identities, respectively, with the corresponding genes in GenBank. In addition, while *mecA* homologs have been described previously in *Staphylococcus kloosii*, *Staphylococcus sciuri*, *Staphylococcus vitulinus*, and *M. caseolyticus* (55, 65, 66, 73), the *mecA* gene in the CC130 isolates was also clearly distinct from each of these genes, exhibiting only 21 to 63% aa identity. The combination of *ccr* genes identified within SCC*mec* XI was also different from those previously described and was assigned a novel *ccr* type combination designated type 8.

Another unusual feature of the *mec* complex identified in SCC*mec* XI was the presence of *blaZ* upstream of *mecA*. The *blaZ* gene encodes a β-lactamase and is generally found as part of the *bla* operon (*blaI*, *blaR1*, and *blaZ*), which is widespread among Gram-positive bacteria. Apart from the *mec* complex identified in SCC*mec* XI in CC130 MRSA isolates in the present study and in bovine isolate LGA251 on the IWG-SCC website, the only previous report of a *mec* complex with *blaZ* was from *M. caseolyticus*, where a transposon harboring a *mec* gene complex with the same genetic organization as that in SCC*mec* XI (*blaZ*, *mecA*, *mecR1*, and *mecI*) was integrated downstream of *orfX* and was flanked by DRs (65). A non-*mecA* SCC element was integrated next to the *mec* transposon in *M.*

caseolyticus and was separated from *mecA* by DRs. It has been speculated previously that the *mec* gene complex was generated following integration of *mecA* into the *bla* operon and that the *blaZ-mecA-mecR1-mecI* complex in *M. caseolyticus* may represent the ancestral form of the *mec* gene complex in staphylococci (4, 63, 65). The *mec* complex of SCC*mec* XI may represent a putative ancestral *mec* gene complex in MRSA.

The J regions of SCC*mec* XI also differed significantly from those of previously described SCC*mec* elements. The J3 region of SCC*mec* XI was just 316 bp in size, which is substantially smaller than the J3 regions identified in SCC*mec* I to VIII. The J2 and J1 regions were more comparable in size to those of previously described SCC*mec* elements, but the 21 ORFs identified (excluding the *ccr* and *mec* complex genes) exhibited similarities to previously described ORFs ranging from only 21% to 93%. In addition, for only 4/21 ORFs the highest similarities (62 to 88%) were to genes previously identified in SCC*mec* elements in MRSA or CoNS. The remaining 17 ORFs exhibited similarities to non-SCC*mec* DNA sequences in *S. aureus* and CoNS and in some cases weak similarity to the sequences in nonstaphylococcal species. Of particular interest was the identification of genes with similarity (79 to 93% aa identity) to those of an arsenic resistance operon, consisting of *arsR*, a transcriptional repressor; *arsB*, an arsenite efflux pump; and *arsC*, an arsenate reductase. Both isolates exhibited resistance to sodium arsenite and sodium arsenate. Arsenic occurs naturally in the environment and is commonly found in the earth's crust; but it can also originate from agriculture and industry as a result of the use of pesticides, waste processing, and mining, and arsenic in drinking water is a problem in many parts of the world (47). Arsenic resistance genes have been identified in chromosomal and plasmid locations in many bacterial species, including *S. aureus*, CoNS, *Escherichia coli*, and *Pseudomonas fluorescens* (47). While genes encoding resistance to other heavy metals such as cadmium and mercury have been reported previously in SCC elements, including SCC*mer* in MRSA (22) and the SCC composite island in *S. epidermidis* strain ATCC 12228 (40), previously reported arsenic resistance genes in staphylococci have been plasmid located (47) and have not been associated with SCC*mec*. The very significant differences between SCC*mec* XI and previously described SCC*mec* elements suggest that SCC*mec* XI may have originated in another species or genus and are further evidence to support the theory that interspecies transfer of SCC*mec* may play a role in the emergence of novel MRSA strains.

It has been suggested previously that smaller SCC*mec* elements are more competitive than larger mobile genetic elements and that they may be more readily transferred between staphylococci (21, 31). The relatively small size of SCC*mec* XI (30 kb) suggests that it has the potential to become widespread among *S. aureus* and other staphylococci. In fact, MRSA strains harboring this novel SCC*mec* element may be more widespread than is currently known. First, this study identified SCC*mec* XI in CC130 MRSA clinical isolates from humans, and the Sanger Institute has also identified its presence in the ST425 bovine MRSA isolate LGA251 (http://www.sanger.as.uk/pathogens). This suggests that SCC*mec* XI has been horizontally transferred between two distinct *S. aureus* clones. However, the 3-kb region that was identified downstream of SCC*mec* in the CC130 isolates and that was flanked by DRs characteristic of SCC elements is absent in LGA251. This suggests that in the CC130 isolates SCC*mec* XI may have integrated adjacent to an existing SCC/SCC*mec* element that was subsequently partially lost, leaving behind this 3-kb SCC remnant. The weak similarity between the ORFs in this 3-kb region to unrelated nonstaphylococcal species suggests that another taxon may be the source of this possible SCC remnant. In addition, the CC130 MRSA isolates in the present study were not detected as MRSA using different *mecA* PCRs, the DNA microarray genotyping approach, or the GeneXpert MRSA assay, and therefore, there may be more MRSA strains harboring this SCC*mec* element that have not yet been detected. While confirmation of this hypothesis requires detailed molecular characterization of strains from human and animal sources, inclusion of the DNA sequences of these highly variant *mec* complex and *ccr* genes into the design of *mecA* PCR, DNA microarray, and rapid real-time PCR as well as antibody-based assays will aid the future detection of MRSA strains harboring this novel SCC*mec* element.

To the best of our knowledge, all CC130 isolates that have been reported to date have been MSSA and have mainly been reported from animals, including cows and rats (25, 64, 67). While in the present study there was no evidence of any association between the patients from whom the CC130 MRSA isolates were recovered and any animals, it is interesting to speculate that the patients may have acquired these MRSA strains from animal sources. First, it is very difficult to completely rule out a link between patient and animal sources, particularly livestock, pets, and raw meat products. The fact that Ireland has a large agriculture industry, particularly dairy and beef farming, coupled with the fact that the lineages (CC130 and ST425) in which SCC*mec* XI was identified have previously been found to be mainly associated with cattle, indicates that the CC130 MRSA isolates in the present study may have been acquired from bovine sources. Interestingly, the ST425 isolate LGA251 reported on the Sanger Institute website was recovered from a bovine source (http://www.sanger.as.uk/pathogens). In addition, the DNA microarray revealed the absence of β-toxin-converting bacteriophages in the two CC130 MRSA isolates. Previous studies have shown that β-toxin-converting bacteriophages are rare among *S. aureus* isolates from bovine sources (36) but common among those from humans (30, 38, 39). The zoonotic spread of MRSA has been reported previously, and this may contribute to the burden of MRSA strains among humans (68, 69).

It has been reported previously that mutations in several *S. aureus* genes, including *pbp4*, can result in high-level β-lactam resistance (5). However, comparative DNA sequence analysis of the PBP genes from the two CC130 MRSA isolates and the CC130 MSSA isolate revealed that there were no differences in the PBP gene sequences in the MRSA isolates and the MSSA isolate (which also lacked *mecA* and the SCC*mec* cassette), indicating that it is the novel *mecA* gene and not alterations in PBPs that is responsible for the methicillin resistance phenotype in these isolates. Further studies involving controlled expression of the novel *mecA* gene in methicillin-susceptible *S. aureus* laboratory strains are under way in order to provide further evidence that the novel *mecA* gene identified in CC130 MRSA is responsible for the methicillin resistance phenotype in these isolates.

The identification of a *mecA* gene in MRSA that is not detected by *mecA* PCR, a DNA microarray, the GeneXpert rapid real-time PCR assay, or a PBP latex agglutination assay is alarming. Thus, with regard to infection prevention and therapy, isolates harboring this SCC*mec* element should be treated as MRSA, even though they are identified by PCR to be *mecA* negative. Therefore, isolates in which unexplained resistance phenotypes are detected, such as methicillin resistance in apparently *mecA*-negative *S. aureus* isolates, require further characterization and should be submitted to specialized laboratories until updated routine assays are available. Routine procedures for MRSA screening need to be reevaluated, and phenotypic approaches such as the use of chromogenic media should not be abandoned.

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ADDENDUM IN PROOF

Following submission of our study for peer-review evaluation, we became aware that García-Álvarez et al. had submitted a paper for publication describing the identification and characterization of SCC*mec* type XI in the bovine ST425 MRSA isolate LGA251 (L. García-Álvarez et al., Lancet Infect. Dis., 2011. [Epub ahead of print.] doi:10.1016/S1473-3099(11)70126-8). This study was the first to identify the SCC*mec* XI element, the nomenclature for which was approved by the IWG-SCC.

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Air and surface contamination patterns of meticillin-resistant *Staphylococcus aureus* on eight acute hospital wards

E. Creamer [a], A.C. Shore [b,c], E.C. Deasy [b], S. Galvin [a], A. Dolan [a], N. Walley [a], S. McHugh [d], D. Fitzgerald-Hughes [a], D.J. Sullivan [b], R. Cunney [e,f], D.C. Coleman [c], H. Humphreys [a,g,*]

[a] *Department of Clinical Microbiology, Education and Research Centre, Royal College of Surgeons in Ireland, Dublin, Ireland*
[b] *Microbiology Research Unit, Division of Oral Biosciences, School of Dental Science and Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Ireland*
[c] *Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St James's Hospital, Dublin, Ireland*
[d] *Department of Surgery, The Royal College of Surgeons in Ireland and Beaumont Hospital, Dublin, Ireland*
[e] *Health Protection Surveillance Centre, Dublin, Ireland*
[f] *Department of Microbiology, Children's University Hospital, Dublin, Ireland*
[g] *Department of Microbiology, Beaumont Hospital, Dublin, Ireland*

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SUMMARY

Background: Meticillin-resistant *Staphylococcus aureus* (MRSA) can be recovered from hospital air and from environmental surfaces. This poses a potential risk of transmission to patients.

Aim: To investigate associations between MRSA isolates recovered from air and environmental surfaces with those from patients when undertaking extensive patient and environmental sampling.

Methods: This was a prospective observational study of patients and their environment in eight wards of a 700-bed tertiary care hospital during 2010 and 2011. Sampling of patients, air and surfaces was carried out on all ward bays, with more extended environmental sampling in ward high-dependency bays and at particular times of the day. The genetic relatedness of isolates was determined by DNA microarray profiling and *spa* typing.

Findings: MRSA was recovered from 30/706 (4.3%) patients and from 19/132 (14.4%) air samples. On 9/132 (6.8%) occasions both patient and air samples yielded MRSA. In 32 high-dependency bays, MRSA was recovered from 12/161 (7.4%) patients, 8/32 (25%) air samples, and 21/644 (3.3%) environmental surface samples. On 10/132 (7.6%) occasions, MRSA was isolated from air in the absence of MRSA-positive patients. Patient demographic data combined with *spa* typing and DNA microarray profiling revealed four likely transmission clusters, where patient and environmental isolates were deemed to be very closely related.

* Corresponding author. Address: Department of Clinical Microbiology, RCSI Education and Research Centre, Beaumont Hospital, PO Box 9063, Beaumont Road, Dublin 9, Ireland. Tel.: +353 1 8093710/3708; fax: +353 1 8092871.
E-mail address: hhumphreys@rcsi.ie (H. Humphreys).

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Conclusion: Air sampling yielded MRSA on frequent occasions, especially in high-dependency bays. Environmental and air sampling combined with patient demographic data, *spa* typing and DNA microarray profiling indicated the presence of clusters that were not otherwise apparent.

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Introduction

Numerous studies have shown that the hospital environment is frequently contaminated with potential pathogens that pose a risk of cross-transmission to patients.[1,2] Meticillin-resistant *Staphylococcus aureus* (MRSA) can survive for long periods on environmental surfaces, and may be transmitted to patients via healthcare workers' hands or the environment.[3,4] Studies on MRSA in the environment have mostly related to outbreaks, intensive care units (ICUs), or isolation rooms with MRSA patients, rather than in routine ward areas over a period of time.[5–9] There is relatively little information on the dispersal of airborne MRSA and this may be an often underestimated method of transmission that results in clusters or outbreaks. It is not clear what impact MRSA in the environment and in air may have on patient acquisition and cross-transmission, and many studies involving the sampling of air in the vicinity of patients have been once-off investigations. Previous studies have strongly suggested or confirmed the transmission of bacteria by air, especially when carriers of *S. aureus* have viral infection.[10,11] However, the extent to which MRSA may be recovered from the air and how commonly this occurs in an acute hospital outside of an outbreak are unclear.

The current study describes the pattern of MRSA recovered from air and surfaces on a number of wards where MRSA is endemic over a period of time, and investigates patient and environmental links using *spa* typing and DNA microarray profiling of isolates.

Methods

Patients

The study was conducted on patients and their environment on eight wards, four general surgical and four general medical, in a 700-bed tertiary referral acute care hospital during 2010–2011. The study was undertaken as part of a larger study of MRSA that was approved by the Hospital Ethics (Medical Research) Committee. Patient and environmental sampling were conducted on a scheduled basis, and were not in response to an outbreak investigation. Bed occupancy was ~100%.

Sampling was carried out accordingly in three ways. First, sampling was conducted on 132 occasions on eight wards between March 2010 and February 2011. From March to June 2010, eight wards were sampled successively; three wards were sampled once, and five were sampled twice. From September 2010 to February 2011, the patients and the environment of four wards were each sampled for 4 weeks consecutively. Patient screening involved the taking of swabs from the nose, groin and non-intact skin/wound if available ($n = 706$), with one air sample taken in each ward bay.

Second, on 32 occasions, extended environmental sampling was conducted in the immediate area of patients in two high-dependency bays (HDBs) on eight wards, where more vulnerable and sicker patients are cared for. Patients were screened for MRSA on study wards as previously described.[12,13] At-risk patients were those as defined in national guidelines, i.e. patients known to be previously MRSA positive, patients transferred from another hospital or a long-term care facility, patients with chronic ulcers or urinary catheters, and patients who had been hospitalized within the last 18 months.[1] Environmental sampling involved surface sampling of each patient's mattress, pillow, bed rail or bed frame and locker. In addition, a settle-plate was placed on each patient's locker and one air sample was collected at the window-ledge of each ward bay.

Third, air sampling was conducted on HDBs at different times over a 24 h period: 07:30–09:00, 09:30–11:30, 14:00–16:00, 17:00–19:00, and 20:00–22:00 h, but without simultaneous patient sampling. However, during this study phase, routine ward screening revealed no MRSA-positive patients in the HDBs during these periods of sampling. No area sampled had any form of artificial ventilation. Cleaning was performed daily by the cleaning services, using water and detergent. A hypochlorite, 1000 ppm available chlorine, was used for MRSA and other infected/contaminated patients. Terminal decontamination of the bed and bed area after a patient's discharge of the bed and bed area was performed by a designated cleaning team.

Air and environmental surface sampling

Samples of air (1000 L) were taken using an impact air sampler (AES Chemunex Air Sampler, Department SAV, Rue Maryse Bastié–Ker Lann, 35172 BRUZ cedex, France) with MRSA*Select* chromogenic agar (CA) plates (Bio-Rad Life Science Group, Marnes La Coquette, France) which was placed on the ledges of outer wall windows in ward bays. CA settle plates were also placed on patients' lockers for 1 h. Neutralizing buffer swabs (Technical Services Consultants, Heywood, UK) were used to sample mattresses, pillows, bed rail or bed frames and patient lockers. An area, 10 × 10 cm, of each surface was sampled. In addition, mattresses were assessed by sweeping a CA plate over the surface of the mattress. Most wards consisted of 35 beds, each with one four-bedded and one six-bedded HDB, three other six-bedded bays, one two-bedded bay and five single rooms (Figure 1a). HDBs mainly comprised of four-bedded (wards A) or six-bedded (wards B) ward bays, but one ward had a five-bedded bay. The environmental sampling sites were those immediately associated with the patient's bed area and are shown in Figure 1b. All environmental swabs were enriched in tryptone soy broth with 6% (w/v) NaCl (TB05S-100, Cruinn Diagnostics, Dublin, Ireland) and incubated at 37 °C for 18 h. These were subsequently subcultured on to CA and incubated along with settle plates and sweep plates for 24 h at 37 °C. Presumptive MRSA was confirmed by coagulase and clumping factor production using Staphaurex Plus (Remel, Dartford, UK) and meticillin resistance determination using

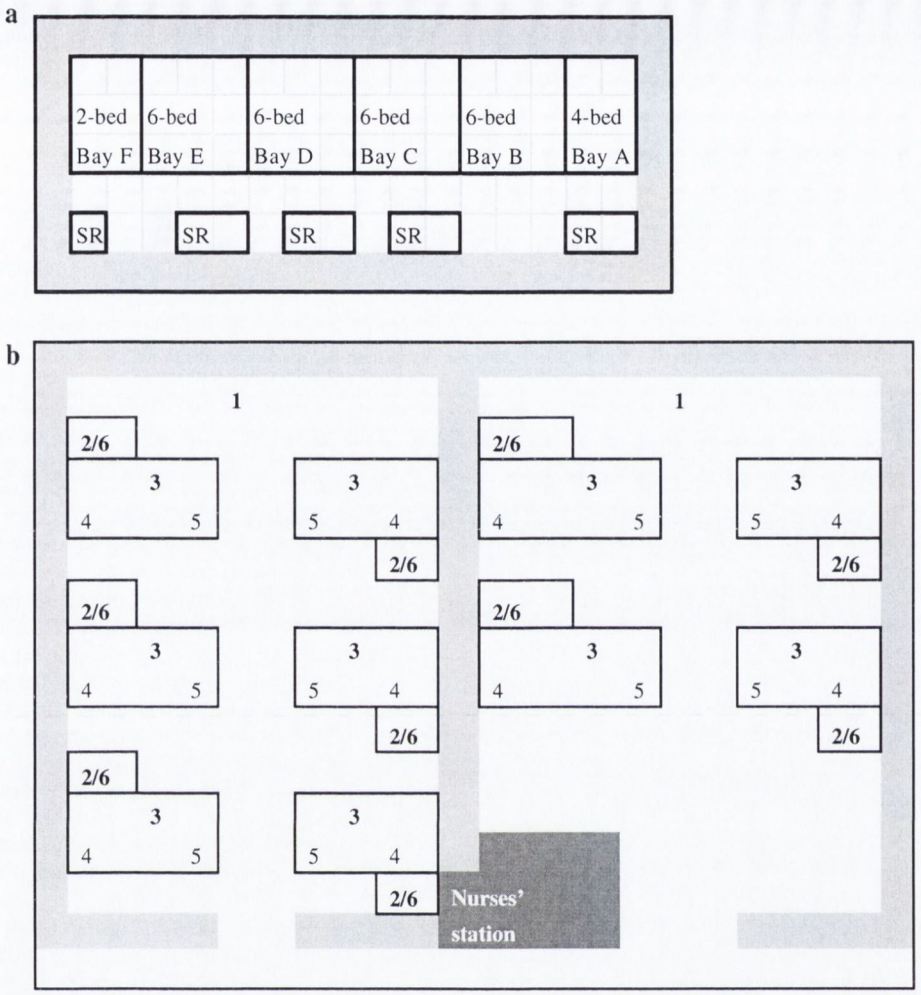

Figure 1. (a) Schematic layout of ward bays in a typical 35-bed ward. SR, single room. (b) Schematic layout of environmental sampling sites in typical high-dependency bays (ward bay A, four-bed; ward bay B, six-bed). 1, air sampling; 2, air settle-plate; 3, mattress; 4, pillow; 5, bed rail/bed frame; 6, locker.

oxacillin minimum inhibitory concentration (MIC) evaluator strips (Oxoid, Basingstoke, UK) and detection of resistance with cefoxitin, 30 μg discs (Oxoid).

Molecular typing

spa typing was carried out on one isolate per MRSA-positive patient and/or the patients' environment in the HDBs using the European Network of Laboratories for Sequence Based Typing of Microbiol Pathogens and amplimer sequencing was performed by Source BioScience (Guinness Enterprise Centre, Dublin, Ireland). Where two sampling methods (e.g. swab and sweep-plate) were MRSA-positive from mattress sampling, only one MRSA isolate was included in the analysis. DNA microarray profiling was performed using the StaphyType Kit (Alere, Jena, Germany) on all except two of the isolates that underwent *spa* typing; these two isolates were not available for DNA microarray profiling. The StaphyType Kit detects 334 *S. aureus* gene sequences and alleles including typing and species-specific markers as well as virulence-associated and antimicrobial resistance genes. DNA microarray procedures were performed according to the manufacturer's instructions.[14]

Statistical analysis

Statistical analysis was performed using Epi Info 6 (version 6.04c; Centers for Disease Control and Prevention, Atlanta, GA, USA) and odds ratios were calculated. The Mantel–Haenszel chi-square method was used to assess the significance of the difference between proportions.

Results

Patient and air sampling

Overall, 30/706 (4.3%) patients and 19/132 (14.4%) air samples yielded MRSA (Table I). Two of the 30 MRSA-positive patients had infections; the remainder were colonized only. On 9/132 (6.8%) sampling occasions both patient and air

samples were MRSA positive, but on 10/132 (7.6%) occasions, air samples yielded MRSA but no MRSA-positive patients were identified. When four wards were sampled consecutively for a 4-week period, MRSA was isolated on week 1 (five patients, two air samples), week 2 (four patients, two air samples), week 3 (one patient, no air samples), and week 4 (two patients, one air sample). Three of the five air samples were linked to an MRSA-positive patient in a specific ward bay.

Environmental sampling on 32 occasions in high-dependency bays

MRSA was recovered from 9/85 (10.6%) male and 3/76 (4%) female patients on nine environmental sampling occasions resulting in 12/161 (7.4%) positive patients. MRSA was recovered from 8/32 (25%) air samples, from 12/161 (7.5%) settle plates and from 21/644 (3.3%) surface environmental sites. On five occasions, MRSA was isolated from two patients in a ward bay. Ten of 161 (6.2%) mattresses, 2/161 (1.2%) pillows, 3/161 (1.9%) bed rail/frames and 6/161 (3.7%) bedside lockers yielded MRSA. MRSA was isolated from at least one sample (patient or environment) on 16/32 (50%) occasions. On 2/32 (6.3%) occasions, MRSA was recovered from one or more patients as well as from air samples, settle plates and one or more environmental surface sites. For three of 12 MRSA-positive patients, MRSA was recovered from the patient's mattress, plus the locker for one patient, from the mattress and locker for another patient, and from the mattress, bed frame and pillow of another patient. Two of these MRSA-positive patients were associated with extensive contamination (ward 2 in June 2010 and ward 7 in January 2011). Patient risk factors for the 12 MRSA patients included previous MRSA (1/12), transfer from another hospital (1/12), the presence of a urinary catheter (3/12) and admission within the previous 18 months (7/12). MRSA was isolated from nasal swabs in 10 patients, a groin swab in one and both nasal and groin in one patient. One of 12 patients with nasal/groin swab MRSA positive was associated with extensive MRSA environmental contamination in January 2011.

Air sampling over a 24 h period

Thirteen of 128 (10%) air samples in HDBs yielded MRSA: 7/32 (22%) between 07:30 and 09:00; 3/32 (9%) between 09:30 and 11:30; 1/32 (3%) between 14:00 and 16:00; 0/16 between 17:00 and 19:00; and 2/16 (13%) between 20:00 and 22:00. Significantly more MRSA-positive samples were recovered between 07:30 and 09:00 compared with other times [i.e. 7/32 (22%) versus 6/96 (6%); odds ratio: 4.20; 95% confidence interval: 1.13–15.81; $P = 0.01$].

Molecular epidemiological typing

spa typing was conducted on 42/53 (79%) MRSA isolates arising from extended patient and environmental screening in HBDs; 10 patient isolates, five air samples, nine settle-plate isolates and 18 environmental surface isolates. Four different *spa* types were identified among patient and environmental isolates including t515 (19 isolates), t557 (19 isolates), t032 (four isolates) and t022 (one isolate). DNA microarray profiling was performed on 40 of the isolates that were *spa*-typed. The array assigned all isolates to ST22-MRSA-IV and revealed that all harboured the beta-lactamase resistance gene *blaZ*, the enterotoxin gene cluster *egc* and the immune evasion complex (IEC) genes *sak*, *chp* and *scn*. Limited variability was seen among the isolates in relation to the carriage of the macrolide resistance gene *erm*(C) (37/40, 92.5%) and the co-located enterotoxin genes *sec* and *sel* (36/40, 90%). The DNA microarray profiles of all *spa* type t557 isolates were indistinguishable [*blaZ*, *erm*(C), *sec* and *sel*, *egc* and IEC type B, $N = 20$]. Two array profiles were identified among t515 isolates [*blaZ*, *erm*(C), *sec* and *sel*, *egc* and IEC B, $N = 12$; *blaZ*, *erm*(C), *egc* and IEC B, $N = 3$], whereas three were identified among t032 isolates (*blaZ*, *egc*, IEC B, $N = 1$; *blaZ*, *sec* and *sel*, *egc*, IEC B, $N = 1$; *blaZ*, *ermC*, *sec* and *sel*, *egc*, IEC B, $N = 2$).

Patient demographic data combined with *spa* typing and DNA microarray profiling suggested four clusters, where closely related MRSA was isolated from one or more patients and their environment or from two or more patients without any environmental sites positive for MRSA (Table II). The first of these was on 6th November 2010 and involved 19 *spa* type t557 isolates from three patients with 16 environmental sites in two bays of ward 2; these isolates all yielded indistinguishable DNA microarray profiles. The second *spa* typing cluster was recovered on 14th June 2010 and involved two *spa* type t032 isolates from two patients in two ward bays of ward 3; these isolates differed by one gene combination only using the DNA

Table I
Isolation of MRSA from patients and air on 132 sampling occasions

Ward bay	Sampling occasions	Patients	Patient $MRSA^+$ Air $MRSA^{+,a}$	Patient $MRSA^+$ Air $MRSA^{-,b}$	Air $MRSA^+$ Patient $MRSA^{-,c}$	Air $MRSA^{+,d}$
A	29	8/116	2/29	4/29	2/29	4/29
B	29	9/174	3/29	4/29	3/29	6/29
C	22	3/132	1/22	2/22	1/22	2/22
D	29	6/174	1/29	4/29	4/29	5/29
E	16	3/96	2/16	1/16	0/16	2/16
F	7	1/14	0/7	1/7	0/7	0/7
All wards	132	30/706 (4.2%)	9/132 (6.8%)	16/132 (12.1%)	10/132 (7.6%)	19/132 (14.4%)

[a] Among patients who were $MRSA^+$ the number of air sampling $MRSA^+$.
[b] Among patients who were $MRSA^+$ the number of air sampling $MRSA^-$.
[c] Among air sampling that was $MRSA^+$ the number of patients $MRSA^-$.
[d] Number of air $MRSA^+$ in each ward bay.

Table II
The distribution of MRSA recovered from patients, air, settle plates and environmental surfaces on 32 occasions of high-dependency bays with *spa* typing of 42/52 isolates and DNA microarray profiling of 40/52 isolates

Date	Ward [a]	Ward Bay	Patients	Air sampling	Air settle plates	Surface environmental[b]
			(n/total)	*(n/total)*	*(n/total)*	*(n/total)*
08/06/10	1	Bay A	0/4	0/1	1/4	0/16
08/06/10	1	Bay B	0/6	0/1	0/6	2/24
11/06/10	2	Bay A	3/6	1/1	3/6	10/24
11/06/10	2	Bay B	0/6	1/1	1/6[c]	1/24
14/06/10	3	Bay A	1/4 [d]	0/1	0/4	0/16
14/06/10	3	Bay B	1/5	0/1	0/5	0/20
16/06/10	4	Bay A	0/4	0/1	0/4	0/16
16/06/10	4	Bay B	0/6	0/1	0/6	0/24
23/06/10	5	Bay A	0/4	0/1	0/4	1/16
23/06/10	5	Bay B	0/6	1/6	1/6	2/24 [c]
28/06/10	6	Bay A	1/4	0/1	0/4	0/16
28/06/10	6	Bay B	0/6	0/1	0/6	0/24
20/09/10	1	Bay A	0/4	0/1	0/4	0/16
20/09/10	1	Bay B	0/6	0/1	0/6	0/24
27/09/10	1	Bay A	0/4	0/1	0/4	0/16
27/09/10	1	Bay B	0/6	0/1	0/6	0/24
04/10/10	1	Bay A	0/4	0/1	0/4	0/16
04/10/10	1	Bay B	0/6	0/1	0/6	0/24
18/10/10	5	Bay A	0/4	0/1	0/4	0/16
18/10/10	5	Bay B	0/6	0/1	0/6	0/24
26/10/10	5	Bay A	1/4	1/1	0/4	0/16
26/10/10	5	Bay B	2/6 [e]	1/1	0/6	0/24
08/11/10	5	Bay A	0/4	0/1	0/4	0/16
08/11/10	5	Bay B	0/6	0/1	0/6	0/24
04/01/11	7	Bay A	0/4	1/1	1/4	0/16
04/01/11	7	Bay B	1/6	1/1	5/6	5/24
10/01/11	7	Bay A	0/4	0/1	0/4	0/16
10/01/11	7	Bay B	0/6	1/1	0/6	0/24
31/01/11	8	Bay A	0/4	0/1	0/4	0/16
31/01/11	8	Bay B	1/6	0/1	0/6	0/24
21/02/11	8	Bay A	0/4	0/1	0/4	0/16
21/02/11	8	Bay B	1/6	0/1	0/6	0/24
Total	8 wards	32 Bays	12/161(7.5%)	8/32(25%)	12/161(7.5%)	21/644(3.3%)

[a] Wards 1-8

[b] Mattress, pillow, bedrail/frame or locker.

[c] One isolate not *spa* typed.

[d] Using DNA microarray, one t032 *spa* type isolate lacked the *sec* & *sel* gene cluster

[e] Total 2/6 isolates MRSA-positive, one t515 *spa* type, one t022

Cell shading indicates the combined *spa* types and DNA microarray profiles of isolates within the four clusters;

spa type t557; DNA microarray profile *blaZ*, *erm*(C), *sec* & *sel*, egc and IEC B
spa type t515, DNA microarray profile *blaZ*, *erm* (C), *egc* and IEC B
spa type t032, DNA microarray profile *blaZ*, *erm* (C), *sec* & *sel*, *egc* and IEC B

microarray and are considered to be closely related. The next *spa* typing cluster was recovered on 26th October 2010 and again involved two *spa* type t032 isolates, one recovered from a patient and the other from an environmental site of ward bay A on ward 5; these isolates exhibited indistinguishable DNA microarray profiles. The final *spa* typing cluster involved 12 *spa* type t515 isolates recovered on 1st April 2011 and involved one patient and 11 environmental isolates on ward A; again these isolates exhibited indistinguishable DNA microarray profiles. Three of four other environmental isolates yielded indistinguishable *spa* (t515) and microarray pattern profiles, on 23rd June 2011, in two bays of ward 5. However, no positive MRSA patients were identified as being epidemiologically associated with these isolates.

Discussion

MRSA is endemic in most Irish hospitals and this study was part of a programme of research to determine whether current methods of screening underestimate the extent of MRSA colonization and environmental contamination. Through a combination of admission screening of all patients (not just those in at-risk categories such as patients being transferred from another hospital), follow-up screening while inpatients, air and environmental sampling, and the prospective collection of detailed demographic data and molecular typing, we have endeavored to gain a deeper understanding of the extent of MRSA colonization and transmission. Results showed that some patients are MRSA-positive even though not in an at-risk category and who would not normally be routinely screened; that transmission can occur but remain undetected if solely relying on routine screening; and that the method of environmental sampling influences the density of MRSA recovered from the healthcare environment.[12,13,15]

In this study, MRSA was mainly isolated from the air in ward bays where MRSA-positive patients were identified (air sampling sites outlined in Figure 1b). However, on 7.6% of 132 sampling occasions, MRSA was detected from air samples when no known MRSA-positive patients were identified, possibly because of unidentified MRSA-positive individuals in the vicinity, recently discharged MRSA-positive patients, or environmental contamination. When patient screening and air sampling were conducted on four wards over a consecutive 4-week period, more MRSA-positive patients and environmental samples were identified during the first 2 weeks than during weeks 3–4. This may suggest that the early identification of MRSA patients arising from this study and their subsequent isolation may have contributed to fewer MRSA-positive samples during the last 2 weeks of screening.

In HDBs, MRSA was recovered on 50% of occasions from either patients or their environment, probably due to the greater risk of these more vulnerable patients having MRSA. Apart from the presence of a urinary catheter in 3/12 patients, no other MRSA risk factors were associated with environmental contamination. Skin scales can travel significant distances especially when associated with extensive activity.[9,16–18] We assessed particle counts and particle size variation on one occasion (data not shown), finding no correlation with bacteriological sampling results.

Active air sampling by its very nature draws air from a wide area, which makes it difficult to associate environmental isolates with specific patients. In order to more accurately measure the dispersal of MRSA from individual patients we conducted environmental sampling of bed spaces. For three of 12 MRSA-positive patients, MRSA was recovered from the patients' mattresses as well as other environmental sites surrounding the patient's bed. Two of these patients were associated with extensive environmental contamination on ward 2 on 11th June 2010 and on ward 7 on 4th January 2011. Although not conclusive, these patients may have been index patients for outbreaks. The greater likelihood of recovering MRSA from air samples compared with settle plates and environmental surfaces is possibly related to the increased sensitivity of this method of sampling due to the increased volume of air sampled (i.e. 1000 L).[19] Seven of 12 (75%) MRSA-positive settle plates were related to two clusters involving four patients, suggesting that MRSA may be dispersed in the air from the immediate vicinity of MRSA-positive patients to other ward areas.

Rates of recovery of MRSA are higher during ward activities, and at different times during the day – before, during and after cleaning.[6,18] We recovered significantly higher rates of MRSA in air samples early in the morning, i.e. between 07:30 and 09:00 compared with other times. Sampling at this time, before routine ward cleaning, would generally be regarded as a low activity time, i.e. less movement of patients and staff.[20–22] Air samples positive for MRSA declined during the day when cleaning, ward rounds and other activities were taking place, with less MRSA being isolated during the afternoon/evening, similar to a study on two respiratory wards that showed higher levels of particulate matter in the morning during ward activity – i.e. bed making, nebulization – peaking again around lunch time and reducing during times of low activity.[12] More frequent recovery of MRSA from surfaces in ICUs has been reported between 08:00 and 09:00 than during the rest of the day.[6] Aerial dispersal of *Clostridium difficile* was found to be higher during the distribution of meals.[23] However, patients may shed more skin scales during the night when sleeping, and this may explain our finding of more MRSA between 07:30 and 09:00, but some ward activity may have been taking place before 07:30.[6,23]

Effective cleaning and the decontamination of environmental surfaces have been associated with reduced MRSA in the patient's environment, but cleaning may not always be effective.[24] In the present study, mattresses were the items of equipment from which MRSA was most frequently isolated and these may be a significant reservoir in the environment. Patients may acquire MRSA from a previous MRSA-positive patient if decontamination of that clinical area before patient admission is inadequate.[25] The surface of patients' lockers was also contaminated, possibly from aerial transmission, but also due to direct patient contact and indirect contact with MRSA-positive patients via healthcare staff combined with inadequate cleaning.

The use of *spa* typing as a standalone typing method for differentiating between highly clonal ST22-MRSA-IV isolates has been shown to be inadequate.[26] In the present study *spa* typing was combined with DNA microarray profiling to attempt to enhance discrimination of the ST22-MRSA-IV isolates based on the presence or absence of virulence-associated and antimicrobial resistance genes. This combined molecular typing approach together with epidemiological data identified four clusters, three of which were on different wards involving patients and the environment. In addition, three of four isolates that were linked epidemiologically and isolated from the

environment were indistinguishable but these were not associated with MRSA-positive patients. Whereas it is important not to rely on *spa* typing alone for typing of MRSA particularly in an endemic setting, in the present study DNA microarray profiling did not result in isolates clustered based on *spa* typing being further differentiated. However, the DNA microarray permits identification of characteristic resistance and virulence genes among isolates, as we have demonstrated for a collection of isolates causing MRSA bloodstream infection.[27]

Patterns of air dispersal of *S. aureus* are not generally well understood in the healthcare environment or elsewhere. A recent study of livestock-associated MRSA traced the spread of MRSA in the vicinity of pig barns, and *spa* typing indicated that MRSA spread was influenced by the season and the wind direction.[28] The clinical areas sampled in our study were naturally ventilated and therefore air flow would have been influenced by open doors and windows and the movement of patients and staff.

The limitations of this study include taking samples mainly in HDBs and not as frequently throughout the rest of the ward, as this would have more accurately determined the full extent of MRSA dispersal. We sampled mainly nasal and groin patient sites but not other sites such as throat, sputum, urine, etc. which may be associated with significant additional air dispersal. Because of logistical and resource reasons, active air sampling was conducted at one position only, i.e. the outer window of each ward bay and not beside each patient in all ward bays; thus, our sampling possibly missed MRSA dispersion from patients further away from the window. Sampling was conducted during the day and early night periods, but not between 22:00 and 07:30, when patients were asleep and there was less activity on the ward. Broth enrichment was not used for patient samples and healthcare staff were not screened; both may have underestimated the true number of MRSA-positive carriers on the ward and may explain the presence of MRSA in the air without known MRSA-positive patients in the immediate vicinity. One study found that the average limit of detection for direct culture and broth enrichment in an *in vitro* study was about 750 and 40 cfu/mL, respectively.[29] Hence broth enrichment significantly enhances the sensitivity of MRSA detection but with a longer time to confirmation of MRSA status.

In conclusion, MRSA was recovered from either patients or their environment on 50% of sampling occasions with patterns suggesting dispersal from patients to the surrounding air. This suggests that aerial transmission from patient to patient may be common. While not conclusive, patients appear to shed MRSA more frequently early in the morning or during the night. The use of computational fluid dynamics might assist in the accurate prediction of the spatial distribution of MRSA, as shown in a recent study which investigated the use of a physical partition between patients and the location of patients with respect to the air supply.[30] However, further prospective studies of patients in different settings are required, for example in single rooms and in multiple bed settings, during different periods of activity such as the changing of bed linen, and under natural and artificially ventilated facilities.

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Conflict of interest statement

H.H. has received research support from Steris Corporation, Inov8 Science, Pfizer and Cepheid in recent years. He has also recently received lecture and other fees from Novartis, Astellas and AstraZeneca. No conflict of interest is reported by the other co-authors.

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of infections in animals and humans. The methicillin resistance gene *mecA* is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) mobile element. Composite islands (CIs) consisting of two or more SCC*mec*, SCC or SCC-like elements also occur in MRSA and characterisation of these is essential for accurate typing and tracking of MRSA. Coagulase-negative staphylococci (CoNS) are thought to be a reservoir for SCC*mec* and other genes in *S. aureus*. CoNS and methicillin-susceptible *S. aureus* (MSSA) are leading causes of healthcare-associated infections (HCAIs), but detailed molecular characterisation of both is lacking from Ireland. CoNS often harbour the arginine catabolic mobile element (ACME) that enhances survival on skin. Over the last decade one highly-clonal MRSA lineage, ST22-MRSA-IV, has predominated making tracking of MRSA in Irish hospitals very difficult. The high-throughput StaphyType DNA microarray offers an alternative typing method for *S. aureus* by assigning isolates to a multilocus sequence type (MLST) and/or clonal complex (CC) and also detects a wide range of virulence, resistance and SCC*mec*/SCC genes.

The five aims of the present study were as follows:

- To investigate two methicillin resistant clinical *S. aureus* isolates that lacked *mecA* by conventional PCR for the possible presence of a novel *mec* gene.
- To investigate the genetic organisation of a possible novel CI among MRSA from Irish hospitals.
- To investigate the SCC, resistance genes and ACME in CoNS from patients in Irish hospitals by microarray profiling and to perform molecular typing of the two most clinically-relevant species identified including *S. epidermidis* and *S. haemolyticus*.
- To perform a comparative molecular analysis of MSSA and MRSA isolates from bloodstream infections (BSIs) and healthy carriers (HCs).
- To investigate the combination of microarray profiling and *spa* typing to improve discrimination of ST22-MRSA-IV isolates from patients, environmental sites and healthcare workers in one Dublin hospital.

The two *mecA*-negative MRSA isolates were identified as CC130-MRSA and whole-genome sequencing of isolate M10/0061 identified a novel *mec* gene, *mecC*, with 62% amino acid identity to *mecA* and a novel 30 kb SCC*mec* element designated SCC*mec* XI, which consisted of a novel *mec* (class E *mec*: *blaZ-mecC-mecR1-mecI*) and *ccr* (type 8: *ccrA1B3*) complex. SCC*mec* XI was also identified in the second CC130 MRSA isolate (M10/0148) by PCR and sequencing. The highly divergent nature of SCC*mec* XI relative to other SCC*mec* elements indicates that it may have originated in another taxon.

A previous study identified the *S. epidermidis ccrAB4* gene in ST8-MRSA-IIA-IIE and IVE/IVF (*spa* type t190) from Irish hospitals. In the present study whole-genome sequencing of an ST8-MRSA-IIE isolate (AR13.1/3330.2) identified a 14 kb *ccrAB4*-SCC element downstream and adjacent to SCC*mec* IIE. The SCC exhibited 99% DNA identity to SCC_{MI} previously identified as part of a CI in Danish ST8 MRSA (*spa* type t008) isolates and was similar to the region between SCC_{pbp} and IS*431* in SCC-CI in *S. epidermidis* ATCC12228. Eighty-five isolates were tested for the *S. epidermidis ccrAB4* gene by PCR including 83 ST8-MRSA-IIA-IIE and IVE-IVF isolates and one isolate each of ST94-MRSA-IV and ST22-MRSA-IV that yielded signals for *ccrAB4* by microarray profiling and 94% of isolates were positive. Long-range PCRs were used to investigate one isolate representative of each of these *ccrAB4*-positive genotypes for SCC_{MI} and its location downstream of SCC*mec* was confirmed for all except the ST8-MRSA-IVE/IVF isolates. The ST22-MRSA-IV isolate harboured ACME in addition to SCC_{MI}. In total nine novel SCC*mec*-SCC CIs were identified.

Fifty-three CoNS isolates from patients in Irish hospitals including *S. epidermidis* (30), *S. hominis* (10), *S. haemolyticus* (7), *S. capitis* (4) and *S. warneri* (2) were subjected to microarray profiling which identified SCC and SCC*mec*-associated genes in 88.7% (47/53) of isolates and 71.7% (38/53) harboured *mecA*. Previously identified SCC*mec* types were found in 34.1% of SCC*mec*/SCC-positive isolates, while 65.9% harboured possible novel SCC and SCC*mec* types and subtypes. SCC*mec* IV predominated among *S. epidermidis*, class A *mec* and *ccrAB1* among *S. hominis* and *ccrAB4* among *S. haemolyticus*. Each isolate harboured at least one antimicrobial resistance gene. ACME was detected in 11.3% of isolates. MLST assigned the majority of *S. epidermidis* to the predominant nosocomial lineage, CC2, and all *S. haemolyticus* isolates were assigned to CC1. These results provide further evidence that specific CoNS species are a reservoir for SCC*mec* genes as well as antimicrobial resistance genes and ACME in *S. aureus*.

A total of 83 MRSA and 85 MSSA from bloodstream infections (BSIs) and 53 MRSA and 98 MSSA from healthy carriers (HCs) were investigated by *spa* typing and microarray profiling. MRSA were assigned to six CCs and 34 *spa* types and MSSA to 33 CCs and 100 *spa* types. ST22-MRSA-IV predominated among both groups of MRSA isolates (83.8%) and CC30-MSSA (33.7%) and CC45-MSSA (18.2%) among the MSSA. Similar genotypes were detected among MRSA from BSIs and HCs and among MSSA from BSIs and HCs. Unsurprisingly MRSA harboured more antimicrobial resistance genes than the MSSA isolates, but MSSA isolates harboured more virulence genes.

Analysis of 178 MRSA from the hands of healthcare workers (HHCWs) (n = 38), patients (n = 82) and environmental sites (n = 61) in one Irish hospital was undertaken by *spa* typing and microarray profiling. ST22-MRSA-IV predominated (98.3%). The *spa* type t032 predominated among isolates from HHCWs (63.2%) and patients (47.6%), but t557 predominated among environmental isolates (40.9%). Microarray profiling identified 12 variable virulence and resistance genes among ST22-MRSA-IV isolates and each combination of variable genes was assigned a microarray group (MG) number, yielding 19 MGs. Isolates with the *spa* type, t032, were assigned to 14 MGs. Among the 66 possible pairwise combinations of these 14 MGs, isolates from 12 were deemed to be closely related (differed by one gene/gene cluster), three were deemed to be possibly related (differed by two genes/gene clusters) and 51 were deemed to be different (differed by $\geq$ 3 more genes). Analysis of *spa* type/MG combinations exhibited by isolates from three wards identified similar ST22-MRSA-IV strains from patients, environmental sites and HHCWs. The combination of *spa* typing and microarray profiling enhanced the discrimination of ST22-MRSA-IV isolates.

This study has revealed unprecedented diversity among SCC*mec*, SCC and CIs among staphylococci with the identification of a novel *mec* gene and SCC*mec* element and several novel CIs in MRSA and a large range of SCC*mec* and SCC genes among CoNS. Additionally, the higher virulence potential of MSSA compared to MRSA was revealed as well as the usefulness of array profiling and *spa* typing for characterisation of MRSA, MSSA and CoNS.