The molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* at a London teaching hospital

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1 ABSTRACT

New community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) strains have emerged worldwide. These strains have the capacity to cause infections in healthy individuals in community settings, but are beginning to cause infections in hospitals. Since the original epidemiological definition of CA-MRSA is no longer appropriate, this thesis uses a molecular definition.

Most hospital MRSA are ciprofloxacin-resistant so I used ciprofloxacin susceptibility (Cip-S) as a screening marker to determine whether putative CA-MRSA were present at Guy's & St. Thomas' Hospital (GSTT) from 2000-2006, but had not been identified due to the volume of hospital MRSA identified in the clinical laboratory. Due to the poor sensitivity of Cip-S and other screening markers for the identification of CA-MRSA, I studied all MRSA identified during the first six months of universal admission screening to investigate the prevalence and circulating strain types of CA-MRSA at GSTT. Finally, I performed detailed molecular analysis of a sub-set of CA-MRSA isolates to investigate variation in the Panton-Valentine leukocidin (PVL) genes and their associated bacteriophages.

Cip-S MRSA were present in increasing numbers during 2000-2006 and had characteristics consistent with published accounts of CA-MRSA. The overall prevalence of MRSA among patient admission screens was 1.6%; colonisation rates were higher in medical specialties (2.4%) and lower in surgical specialties (1.2%). CA-MRSA strains accounted for up to 25% of MRSA in certain specialties. A wide variety of PVL-positive and PVL-negative clones were identified among CA-MRSA. The most common type was a PVL-negative sequence type (ST)-1 clone associated with injecting drug users / homeless patients. Sequence variation in the PVL genes tended to vary according to lineage and correlated with the PVL-encoding bacteriophage.

CA-MRSA strains are present at low frequency at GSTT but their prevalence is increasing. Future studies should further define the epidemiology of CA-MRSA in order to develop effective control strategies.

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2.3 List of commonly used abbreviations and acronyms

A&E = accident and emergency
AMS = antimicrobial susceptibility
AOR = adjusted odds ratio
BLAST = basic local alignment search tool
BURP = based upon repeat patterns
BURST = based upon related sequence types
CA-MRSA = community-associated MRSA
CC = clonal complex
CI = confidence interval
Cip-R = ciprofloxacin-resistant
Cip-S = ciprofloxacin-susceptible
DI = discriminatory index
ED = emergency department
EMRSA = epidemic MRSA
GSTT = Guy's and St. Thomas' Hospital
HA-MRSA = healthcare-associated MRSA
HIV = human immunodeficiency virus
ICU = intensive care unit
IDU = injecting drug user
MGE = mobile genetic element
MIC = minimum inhibitory concentration
MLST = multilocus sequence typing
MSSA = methicillin-susceptible *Staphylococcus aureus*
nmMRSA = non-multiresistant MRSA
NICU = neonatal intensive care unit
OR = odds ratio
PFGE = pulsed field gel electrophoresis
Phage = bacteriophage
PVL = Panton-Valentine leukocidin
SCC*mec* = staphylococcal cassette chromosome *mec*
SNP = single nucleotide polymorphism
SSTI = skin and soft tissue infection

3 ACKNOWLEDGEMENTS

I wish to acknowledge Professor Gary French (King's College London / Guy's and St. Thomas' NHS Foundation Trust) and Dr Kevin Shannon (Guy's and St. Thomas' NHS Foundation Trust) for supervising the project; Professor Peter Hawkey (Heartlands Hospital / University of Birmingham), Dr Peter Wilson (University College London Hospital / University College London) and Dr Robert Vaughan (King's College London) for their invaluable input on my PhD steering committee; Dr Teruyo Ito (Juntendo University, Tokyo, Japan) for the provision of reference strains; Dr Angela Kearns, Dr Matthew Ellington, Mark Ganner and Eve Boakes at the Health Protection Agency *Staphylococcus* reference laboratory for the provision of reference strains; Dr Angela Kearns and Dr Matthew Ellington for the use of their facilities for Pulsed Field Gel Electrophoresis and Clondiag ArrayTube hybridisations and for providing useful comments on my methods section relating to these techniques; Professor Andy Grieve (King's College London Statistical Advisory Service) for statistical advice; Dahir Mohamed (Guy's and St. Thomas' NHS Foundation Trust) for laboratory assistance; Dr Bryn Williams and Dr Trent Herdman (Guy's and St. Thomas' NHS Foundation Trust) for the provision and analysis of databases; Dr Stefan Monecke (Dresden University, Germany) for analysis of Clondiag ArrayTube pictures, technical support, confirmatory analysis of selected isolates and for providing useful comments on my methods section for this technique and Dr Stephanie Dancer (NHS Lanarkshire) and Dr Alan Johnson (Health Protection Agency) for their constructive comments during the examination of this thesis.

The project was funded by a grant from the Royal Commission for the Exhibition of 1851 (London), King's College London and Bioquell (UK) Ltd, at which I have been employed part-time throughout the project.

4 EXECUTIVE SUMMARY

New community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains have emerged worldwide in recent years. CA-MRSA appear to have emerged *de novo* through the acquisition of *mecA* by community strains of methicillin-susceptible *S. aureus* (MSSA). Unlike healthcare-associated (HA)-MRSA, CA-MRSA are often susceptible to non-β-lactam antimicrobials, can produce primary skin sepsis and affect young, previously healthy individuals without healthcare contact. Some strains of CA-MRSA produce the Panton-Valentine Leukocidin (PVL) and this may enhance their virulence.

The original epidemiological definition of CA-MRSA based on whether patients present with MRSA in a hospital or community setting is no longer appropriate. This is because CA-MRSA strains can occur in patient groups (such as intravenous drug users, IDUs) who have repeated healthcare contact and CA-MRSA strains are now causing hospital infections. Further, some CA-MRSA strains have developed multidrug-resistance and many (especially in Europe) do not produce PVL. Therefore, the most useful definition of CA-MRSA at the current time is one based on genotyping and analysis of the staphylococcal cassette chromosome *mec* (SCC*mec*) to infer the likely origin of the MRSA. This thesis uses this molecular definition of CA-MRSA.

CA-MRSA are common in the USA but reported rarely in the UK. CA-MRSA are usually ciprofloxacin-susceptible (Cip-S) whereas most HA-MRSA are resistant. Ciprofloxacin susceptibility can therefore be used as a preliminary screen for CA-MRSA.

I conducted a retrospective analysis of all Cip-S MRSA reported at Guy's & St. Thomas' Hospital (GSTT) from 2000-2006 to investigate whether CA-MRSA were present. I then studied prospective collections of MRSA to examine the utility of antimicrobial susceptibility (AMS) algorithms for the presumptive identification of CA-MRSA and to determine their prevalence

and molecular epidemiology during the first six months of universal admission screening for MRSA at GSTT. All MRSA were characterised by *spa* typing and SCC*mec* allotyping, and selected isolates were typed by pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and an oligonucleotide array to detect a range of regulatory, virulence and resistance genes. Cluster analysis was performed on *spa* types and PFGE profiles. Variation in the Panton-Valentine leukocidin (PVL) genes *lukSF-PV* and their associated bacteriophages was investigated in a selection of CA-MRSA isolates.

Cip-S MRSA were identified during 2000-2006 and had characteristics consistent with published accounts of CA-MRSA. There were increases in the number (from 49 to 102) and proportion (from 4% to 13%) of MRSA that were Cip-S, and in the proportion of Cip-S MRSA isolates that were PVL-positive (from 12% during 2000-2004 to 40% during 2005-2006). AMS algorithms had poor sensitivity for the identification of CA-MRSA strain types; ciprofloxacin susceptibility had a sensitivity of 63%.

The overall prevalence of all MRSA types among patient admission screens was 1.6%; colonisation rates were higher in medical specialties (2.4%), particularly critical care (5.1%), and lower in surgical specialties (1.2%), which included many elective surgical patients. CA-MRSA strains accounted for approximately 15% of all MRSA screens and up to 25% in certain specialties, for example in A&E and pre-admission clinics where patients are primarily from the community.

CA-MRSA were generally associated with younger patients, certain community-based groups (such as IDUs/homeless), presentation in community settings or in hospital specialties in which MRSA have historically been uncommon, resistance to fewer classes of antimicrobial agents, SCC*mec* types IV and V and carriage of PVL. The CA-MRSA isolates showed considerable genetic diversity. Whereas two clones, multilocus sequence type (ST) 22 (EMRSA-15) and ST36 (EMRSA-16) dominated the HA-MRSA strain collection, a wide variety of PVL-positive

and PVL-negative clones were identified among CA-MRSA. The most common CA-MRSA type was a PVL-negative ST1 clone associated with IDUs / homeless patients. Singleton PVL-positive and PVL-negative *spa* lineages were common among the CA-MRSA isolates but rare among the HA-MRSA isolates; these likely represent recent acquisitions of *mecA* by MSSA clones. Previously reported PVL-positive CA-MRSA clones occurred at low frequency, including ST80-IV (European clone), ST8-IV (USA300), ST1-IV (USA400), ST59-IV or V and ST30-IV (SWP).

Seven single nucleotide polymorphisms were identified in *lukSF-PV*, which tended to vary according to lineage and correlated with the PVL-encoding phage. Despite intra-lineage variations in SCC*mec* type and toxin gene profile, particularly in the ST8, 80 and 88 isolates, there was a consistent association between PVL gene sequence and specific phages. Therefore, it seems that the PVL sequence and phage vary with the clone, even within the same lineage. This supports the model that PVL-encoding phages firstly infected MSSA, some clones of which subsequently acquired SCC*mec,* giving rise to PVL-positive CA-MRSA.

CA-MRSA strains are present at low frequency at GSTT but their prevalence is increasing. It seems likely that CA-MRSA will continue to emerge in London and elsewhere in the UK. Future studies should further define the epidemiology of CA-MRSA in order to develop effective control strategies.

5 INTRODUCTION

5.1 *Staphylococcus aureus*: microbiology and pathogenicity

Staphylococcus aureus is a member of the Micrococcaceae and is a Gram-positive coccus with a characteristic “bunch of grapes” appearance under the microscope.[1] *S. aureus* was first discovered through the investigation of surgical pus by Alexander Ogston in 1880.[2] *S. aureus* is named ‘*Staphylococcus*’ after its microscopic appearance based on the Greek staphylé (a ‘‘bunch of grapes’’) and *‘aureus’* (which means “golden” in Latin) after the golden yellow colour of the first pure isolates identified by Rosenbach.[3,4] *S. aureus* can be both a commensal and pathogen of humans and certain animal species.[4,5]

S. aureus is identified in the laboratory by its characteristic Gram-stain, the production of the coagulase enzyme and certain biochemical properties, chiefly the production of catalase and fermentation of mannitol. The coagulase enzyme is a virulence factor in *S. aureus* that can be cell-bound or free (extracellular).[6] A test for coagulase is crucial for the differentiation of *S. aureus* from coagulase-negative staphylococci such as *S. epidemidis*, which are common skin commensals.

5.1.1 Colonisation

S. aureus can be a commensal and pathogen of humans and certain animal species.[4,7] The primary ecological niche of *S. aureus* in humans is the anterior nares and it seems that most infections are endogenous.[1,4,8-10] *S. aureus* colonises other body sites including the axillae, perineum, throat, digestive tract and vagina, usually at lower frequency than the nose.[4,9] The nose is thought to be the primary niche for *S. aureus* colonisation because decolonisation of the nose results in decolonisation of other body sites.[4,9] The role of colonisation at other body sites is not as well understood.[4,9]

Approximately 20% of individuals are persistent *S. aureus* nasal carriers, 30% are intermittent carriers and 50% are non-carriers; children tend to have higher rates of carriage than adults.[4,9] Persistent carriers tend to have a higher bacterial load and a higher risk for endogenous infection.[4,9] Several factors determine the extent to which *S. aureus* is shed from colonised patients, including the site of colonisation and host factors; for example, infection with respiratory viruses can induce a “cloud” super-shedder state.[4,9,11]

Several factors determine whether an individual is colonised with *S. aureus*. *S. aureus* colonisation can be initiated via various routes, including contact with colonised individuals or contaminated surfaces and air.[9,12] Host risk factors include age, ethnicity, diabetes, obesity, other underlying medical conditions, exposure to antimicrobial agents and compromised immune systems.[9,13,14] Other host factors affecting the interaction with *S. aureus* include the presence or absence of various receptors and most likely the efficiency of the competent immune system.[9,15] Environmental factors include hospitalisation, colonised family members and crowded housing.[9] Bacterial factors include strain variation of virulence factors required for adherence to mucin, which appears to be the critical host surface, and immune-modulators that suppress the host immune response.[9] Interaction with other *S. aureus* and other bacteria are also important in determining whether an individual is colonised with *S. aureus*, and may have therapeutic applications.[4,16]

Decolonisation of *S. aureus* can be achieved through topical use of antimicrobial agents, often mupirocin, to decolonise the nose combined with antiseptic washes, often with chlorhexidine, to decolonise skin sites.[17,18] However, mupirocin and disinfectant resistance are increasing, topical antimicrobial therapy may be required for the removal of throat colonisation, and relapse following apparently successful decolonisation is common.[17,19]

5.1.2 Infection

Although the commensal state is more common, *S. aureus* causes a wide spectrum of diseases ranging from superficial skin and soft tissue infections (SSTI) to invasive, life threatening infections.[1,9] A portal of entry into the body is required for *S. aureus* to cause a disease, such as a breach in the skin or contact with mucous membranes.[1] As with the initiation of colonisation, several factors increase the risk of infection, including host factors, such as underlying medical conditions and immune-suppression, environmental factors, such as the insertion of foreign bodies, and bacterial strain-specific factors, such as the possession of virulence factors.[1,9,20] Quorum sensing plays a role in the transition from colonisation to infection.[21-23] *S. aureus* constitutively produce an octapeptide pheromone, for which all staphylococci have the appropriate receptor, allowing bacteria to detect the density of their own species, and modulate their gene expression accordingly.[24] If cell density becomes high and nutrients become limiting, the expression of virulence factors to initiate an infection will provide more space and nutrients at a deeper or different body site.[21] However, it is currently not clear whether host, environmental or bacterial virulence factors are more important for the transition from colonisation to infection.[4,25,26]

S. aureus diseases include SSTIs such as abscesses, boils, carbuncles, furuncles, scalded skin syndrome, cellulitis and impetigo; infections of mucous membranes such as sinusitis and styes; toxin mediated disease such as diarrhoea, emesis and toxic shock syndrome; urinary tract infection; and invasive diseases such as osteomyelitis, pneumonia, septic arthritis, endocarditis, bloodstream infection and sepsis.[1,9]

In order to cause invasive disease, *S. aureus* must adhere to and invade epithelial cells and survive the host immune response once phagocytosed into invaded cells.[1,4,9] Invaded epithelial cells then provide a focus for invasive infections.[1]

5.1.3 Genome and population structure

The genome of *S. aureus* is circular and ranges from 2800-2900kbp.[5,27] Comparative genomic analysis of sequenced *S. aureus* genomes indicates that approximately 75% is core genome, which is conserved between lineages and the remaining 25% is accessory genome, which is variable between lineages.[5,27,28]

There are three possible routes for the evolution of the *S. aureus* genome: mutation, recombination and horizontal gene transfer.[27,29] Recombination is rare in *S. aureus* and it seems that evolution of the core genome occurs mainly through point mutation.[5,30] Mobile genetic elements (MGEs) in the accessory genome are transferred horizontally, but MGEs are not freely transferred between lineages.[5,31]

The core genome is split into core regions that are always conserved and core variable regions that are usually conserved.[5,26] The core genome includes those genes that are essential for the replication and division of *S. aureus*, the 'housekeeping' genes.[5,26] Multilocus sequence typing (MLST) is a method which sequences seven housekeeping genes and compares the sequence of each gene to an online database to generate an allelic profile.[32] Related MLST allelic profiles are grouped together into clonal clusters (CCs) using the Based Upon Related Sequence Type (BURST) algorithm.[33] MLST and microarray analysis of *S. aureus* has determined that almost 90% of all *S. aureus* are grouped into 10 or 11 MLST CCs, and that these CCs each contain a unique combination of surface-associated and regulatory genes.[26,34]

5.1.3.1 Mobile genetic elements

Table 5-1, p.21 summarises the MGEs that have been identified in *S. aureus.*[35-37]

Table 5-1. Mobile genetic elements in *S. aureus*.

Mobile genetic element	Associated genes
Bacteriophages	Virulence determinants
Pathogenicity islands	Virulence determinants
Genomic islands	Virulence determinants
Staphylococcal cassette chromosomes (SCC)	Resistance genes
Plasmids	Resistance genes
Transposons	Resistance genes

Horizontal gene transfer can occur by one of three mechanisms in bacteria: transformation (although *S. aureus* lacks the necessary genes), conjugation (uncommon in *S. aureus*) or bacteriophage (phage) transduction.[28,37] Bacteriophage transduction is the key method for the transfer of MGEs in *S. aureus*.[28,38] Phage transduction occurs by one of two mechanisms: general transduction and phage conversion.[28,38] In general transduction, generic phages, such as Φ11, can deliver up to 45kp of DNA but do not enter lytic or lysogenic cycles. General transduction is probably responsible for the majority of MGE transfer in *S. aureus*. Phage conversion occurs when a phage enters a new cell and is integrated site-specifically into the host genome due to the activity of the phage integrase genes. The phage then enters either a lysogenic cycle (‘a prophage’), where the phage is replicated as part of the genome, or a lytic cycle, where phage multiplies and multiple phage copies are released. Induction, the conversation from a lysogenic to a lytic cycle, usually occurs in response to stress at which stage phage genes may be hyper-produced.[28,39,40]

Several barriers to horizontal gene transfer exist: restriction modification, phage immunity where a strain with a lysogenised phage is resistant to infection with a related phage, and host factors, such as the lack of an attachment site.[38,40] The *sau1* restriction modification system described by Waldron and Lindsay[31] seems the most likely explanation for the

restriction of the transfer of MGEs between lineages. Restriction enzymes digest DNA at specific sequences. Modification enzymes modify the bacterium's own DNA, preventing restriction digestion of its own DNA. Thus, restriction modification prevents the horizontal transfer of MGE from "foreign" bacteria.[28,41] The 10 dominant lineages of *S. aureus*[26] all have a different variant of the *sau1hsdS* gene, which controls the specificity of the restriction modification system.[31]

S. aureus contains several prophages, which often carry genes encoding virulence factors such as the Panton-Valentine leukocidin (PVL, encoded by *lukS-PV* and *lukF-PV*), staphylokinase A thrombolytic enzyme (*sak*), chemotaxis inhibitory protein (*chp*), enterotoxin A (*sea*) and the epidermolytic toxins.[5,28,42] A remarkable feature of phages is their conserved mosaic structure.[5,43] Phages can be classified into six functional categories: DNA replication, integration, packaging, head, tail and lysis regions. Lindsay and Holden[37] proposed classifying *S. aureus* phages on the basis of the sequence of their conserved integrase genes, although recombination may make this proposed nomenclature system unsafe.[5]

S. aureus also contains several pathogenicity islands (SaPIs).[28,35] SaPIs are similar to phages and share a conserved mosaic structure. Unlike phages, SaPIs require the use of a 'helper phage' to mediate horizontal gene transfer via general transduction. SaPIs encode a number of virulence genes including superantigens, toxic shock syndrome toxin-1 (*tst*) and enterotoxins B and C (*seb, sec*).

Nearly all *S. aureus* have stable regions of the genome, designated genomic islands *v*Saα and *v*Saβ, which are thought to have arisen through horizontal gene transfer.[5,28] Genomic islands often contain virulence genes such as exotoxin (*set*), lipoproteins and serine protease homologues.

Staphylococcal cassette chromosomes (SCCs) insert site-specifically into the genome close to the origin of replication by site-specific recombinase

genes encoded on the SCC.[27,44] The method of horizontal transfer of SCCs is unknown because they are too large to fit inside general transduction phages, perhaps explaining their low frequency of transfer.[28] SCC elements are associated with antimicrobial resistance genes; SCC*mec*, which encodes the methicillin resistance gene *mec*A, is the best described SCC[45,46] but others do exist such as SCC*far*, encoding fusidic acid resistance.[47]

S. aureus carry three classes of plasmid, classified according to their size and ability to conjugate.[28,37] Classes I and II are always transferred by generalised transduction but class III plasmids can be transferred by conjugation because they encode the required *tra* genes.[28] *S. aureus* plasmids encode a variety of resistance genes including those for tetracycline, kanamycin, bleomycin, aminoglycosides, β-lactams, heavy metals and antiseptics.[28] Once inside the recipient cell, plasmids can be integrated into the genome or remain separate from it.

S. aureus carry transposons, which are MGEs of variable size containing a transposase gene that catalyses excision, replication and integration.[28,37] *S. aureus* transposons are associated with resistance genes including those for erythromycin, β-lactams and tetracycline among others.[28] Transposons are most commonly transferred by integration into a plasmid in the host cell, which is then transferred into a recipient cell; some transposons encode the *tra* genes necessary for conjugation, but they cannot replicate independently.[28]

5.1.4 Virulence factors

S. aureus encodes a host of virulence factors, in contrast to the much less virulent coagulase-negative staphylococci.[1,5] Virulence factors in *S. aureus* are summarised in Table 5-2, p.25.

Surface proteins are essential for the virulence of *S. aureus* because they facilitate the attachment of *S. aureus* to host cells.[1,48,49] Surface expressed

proteins involved in the attachment of *S. aureus* to host cells are collectively known as microbial surface components recognising adhesive matrix molecules (MSCRAMMs).[48,49] MSCRAMMs are often membrane spanning proteins with a conserved structure and include protein A (encoded by the *spa* gene), which plays a role in biofilm formation and immune evasion, and clumping factors, which bind fibrinogen and fibronectin binding proteins.[48,49] Surface proteins are up-regulated during exponential growth and down-regulated during stationary phase when cell adhesion has occurred and other virulence determinants are required.[1,25]

Once *S. aureus* have attached themselves to host cells, they begin to expresses a wide range of excreted protein superantigens and cytotoxins, enzymes and capsular polysaccharide (Table 5-2, p.25).[52-55] Superantigens are potent virulence factors that bind to major histocompatibility complex class II and T-cell receptors to induce an overwhelming and damaging host immune response.[54,56] Superantigens produced by *S. aureus* include enterotoxins, exotoxins and toxic shock syndrome toxins (TSSTs), which are responsible for *S. aureus* toxin-mediated disease such as toxic shock syndrome, food poisoning and scalded skin syndrome.[54]

Cytotoxins produced by *S. aureus* include two-component pore-forming toxins and haemolysins.[52,53] The two-component pore-forming toxins include PVL and γ-haemolysin.[52,53] These toxins both lyse polymorphonuclear neutrophils (PMN) and γ-haemolysins also lyse erythrocytes.[52,53] The haemolysins include α-, β- and δ-haemolysin.[52] The α-haemolysin lyses erythrocytes and is dermonecrotic and neurotoxic.[52] The toxin is secreted and integrates into the host cell membrane where a pore is formed, resulting in cytotoxicity. β-haemolysin lyses erythrocytes and δ-haemolysin results in damage to the host cell membrane *in vitro*, but the role of these haemolysins in *S. aureus* pathogenesis is not well understood.[52]

Table 5-2. Virulence factors and their regulation in *S. aureus*.

Adapted from Novick 2003,[50] Cheung *et al.* 2004,[25] Bronner *et al.* 2004[51] and Feng *et al.* 2008.[5]

0 = no effect; + = upregulated; - = downregulated; ? = unclear.

Gene	Location	Product	Activity/function	Action of regulatory genes						
				agr	*sae*	*rot*	*sarA*	*sarS*	*sarT*	*tst*
Surface proteins										
spa	Genomic	Protein A	Anti-immune, anti-PMN	–	?	+	–	+		
can	Genomic	Collagen binding protein	Collagen binding	0			–			
fnbA	Genomic	Fibronectin binding protein A	Fibronectin binding	–			+			
fnbB	Genomic	Fibronectin binding protein B	Fibronectin binding	–			+			
clfA	Genomic	Clumping factor A	Fibrinogen binding	0						
clfB	Genomic	Clumping factor B	Fibrinogen binding	0		+	+			
Capsular polysaccharides										
cap5	Genomic	Polysaccharide capsule type 5	Antiphagocytosis	+			+			
cap8	Genomic	Polysaccharide capsule type 8	Antiphagocytosis	+						

Gene	Location	Product	Activity/function	Action of regulatory genes						
				agr	*sae*	*rot*	*sarA*	*sarS*	*sarT*	*tst*
Superantigens										
sea	Phage	Enterotoxin A	Food poisoning, TSS[a]	0						0
seb	SaPI3	Enterotoxin B	Food poisoning, TSS	+			+			–
sec	SaPI4	Enterotoxin C	Food poisoning, TSS	+						
sed	Plasmid	Enterotoxin D	Food poisoning, TSS	+						
eta	Phage	Exfoliatin A	Scalded skin syndrome	+						
etb	Plasmid	Exfoliatin B	Scalded skin syndrome	+						
tst	SaPI1	Toxic shock toxin-1	TSS	+			+			–
set8	*v*Saα	Exotoxin		+						
set9	*v*Saα	Exotoxin					+			
Cytotoxins										
hla	Genomic	α-Haemolysin	Haemolysin, cytotoxin	+	+	–	+	–	–	–
hlb	Genomic	β-Haemolysin	Haemolysin, cytotoxin	+	+	–	+			
hld	Genomic	δ-haemolysin	Haemolysin, cytotoxin	+	0		+	+	–	0
hlg	Genomic	γ-Haemolysin	Haemolysin, cytotoxin	+		–	+			
lukS/F	PVL phage	PVL	Leukolysis	+		–				–
lukE/D	*v*Saα	Leukocidin	Leukolysis	+						

Gene	Location	Product	Activity/function	Action of regulatory genes						
				agr	*sae*	*rot*	*sarA*	*sarS*	*sarT*	*tst*
Enzymes										
splA–F	*v*Saβ	Serine protease-like	Putative protease	+		–	+			
ssp	Genomic	V8 protease	Spreading factor	+	0		–	0		–
aur	Genomic	Metalloprotease (aureolysin)	Processing enzyme?	+			–			
sspB	Genomic	Cysteine protease	Processing enzyme?	+		–	–			
scp	Genomic	Staphopain (protease II)	Spreading, nutrition	+			–			
geh	Genomic	Glycerol ester hydrolase	Spreading, nutrition	+	0	–	?			–
lip	Genomic	Lipase (butyryl esterase)	Spreading, nutrition	+	0		–			
fme	Genomic	Fatty acid modifying enzyme	Fatty acid esterification	+			+			
plc	Genomic	PI-phospholipase C	Membrane hydrolysis	+						
nuc	Genomic	Nuclease	Nutrition	+	+					
hys	Genomic	Hyaluronidase	Spreading factor	?						
coa	Genomic	Coagulase	Clotting, clot digestion		+	+	+			
sak	Phage	Staphylokinase	Plasminogen activator	+	0					
femA/B	Genomic	Factors essential of methicillin	Methicillin resistance	+						

[a] TSS = toxin shock syndrome.

S. aureus produces a wide range of enzymes, which can contribute to virulence by, for example, lysing host tissues and suppressing the immune system.[56,57] Key enzymes include coagulase, which converts fibrinogen to fibrin and staphylokinase, which activates plasminogen to form plasmin, which disrupts fibrin clots that would usually localise an infection.[57]

Together, these virulence determinants facilitate adhesion, intracellular survival, immune evasion, suppression and/or hyper-activation of the host immune system and lysis of host cells and tissues.[5,56]

5.1.5 Regulation of virulence gene expression

The regulation of the expression of virulence genes in *S. aureus* is a complex process involving multiple interlinked genes and systems.[25,50,51] Genes and systems regulating virulence gene expression are summarised in Table 5-3, p.29 and the effect of these systems on individual virulence determinants is summarised in Table 5-2, p.25.

The network of global regulators controlling the expression of virulence determinants in *S. aureus* includes several two component sensor regulators (TCSRs). These regulatory systems facilitate altered expression of virulence determinants in response to various environmental factors such as culture density, pH and availability of nutrients and gasses.[50,51] TCSRs include a membrane bound sensor histidine kinase, a response regulator and other proteins. In response to specific environmental stimuli, the sensor histidine kinase autophosphorylates and begins a phosphorylation cascade, resulting in the binding of the response regulator to a DNA promoter and the up- or down-regulation of gene expression.[50,51]

Table 5-3. Global regulators of virulence gene expression.

Adapted from Novick 2003.[50]

Gene(s)	Name	Type	Function	Genes regulated
agr	Accessory gene regulator	TCSR[a]	Growth dependent autoinduction	Multiple genes
sae	*S. aureus* exoprotein expression	TCSR	Autoinduction	Extracellular protein genes
srr	Staphylococcal respiratory response	TCSR	Energy metabolism	Indirect down-regulation of virulence genes
arl	Autolysis-related locus	TCSR	Growth dependent autolysis induction	Indirect down-regulation of virulence genes
lyt	-	TCSR	Inhibition of cell wall synthesis; autolysis	Indirect down-regulation of murein hydrolases
*svr*A	Staphylococcal virulence regulator	Membrane protein	Regulates *agr* expression	Indirect, via *agr*
σ^B	Sigma factor B	Sigma factor	Activate genes in late exponential phase	Multiple genes
sarA	Staphylococcal accessory regulator	Transcription factor	*agr* dependent and independent regulation of virulence factors	Multiple genes

Gene(s)	Name	Type	Function	Genes regulated
sarS	Staphylococcal accessory regulator	Transcription factor	*agr* independent regulation of virulence factors	*spa, hla, hld*
sarT	Staphylococcal accessory regulator	Transcription factor	*agr* independent regulation of virulence factors	*hla, hld*
rot	Repressor of toxin	Transcription factor	*agr* independent regulation of virulence factors	Multiple genes

[a] TCSR = two component sensor-regulator.

5.1.5.1 agr

The accessory gene regulator (*agr*) is a TCSR global regulator of the expression of virulence genes in *S. aureus*.[25,51] The locus includes five genes, *agrA,B,C,D* and *hld,* and two promoters, P2 and P3. AgrA is a response regulator; AgrB is a pore forming protein; AgrC is a membrane spanning sensor histidine kinase; and AgrD is an autoinducing peptide. The density of AgrD determines the threshold for activation of the effector protein of the locus, RNAIII. Activation of the *agr* locus is dependent on cell density detected by quorum sensing: in a culture of *S. aureus*, AgrD produced by all the cells accumulates until the required activation density is achieved.[22,23] The *agr* locus is controlled by a complex network of global regulators, including other TCSRs and various transcription factors (Table 5-3, p.29), which result in *agr* mediated up-regulation of the expression of exoproteins and down-regulation of the expression of surface proteins in the post-exponential growth phase.[25,51] Therefore, it is the density dependent quorum sensing regulation of the *agr* locus that determines the up- and down-regulation of many genes involved in *S. aureus* virulence.[22,23]

Four types of the *agr* locus have been described in *S. aureus*, types I, II, III and IV.[51] *S. aureus* from different *agr* groups compete because the autoinducing peptide from one *agr* group is inhibitory to the *agr* locus in *S. aureus* from a different *agr* group.[51]

Other TCSRs also have a direct or indirect effect on virulence gene expression Table 5-3, p.29.

5.1.5.2 Other global regulators

Staphylococcal accessory regulator (SarA) is a transcription factor that is expressed from the *sarA* locus during all growth phases.[51] SarA directly binds DNA in the promoter region of target genes, including the *agr* locus, regulating the expression of more than 100 genes (Table 5-2, p.25).[25]

SarA up-regulates several cell-wall associated adhesion proteins and is probably primarily responsible for the up-regulation of these MSCRAMMs in the exponential growth phase.[25,51] Although the expression of *sarA* peaks during the late exponential phase, post-translational regulation probably explains the constant level of SarA throughout all growth phases.[51]

Several SarA homologues have been described, including SarS, SarT, SarU and Rot, which act as transcription factors regulating the expression of virulence genes (Table 5-3, p.29).

Sigma factors are also involved in global regulation of gene expression in *S. aureus*. One group of sigma factors, σ^A, control the expression of housekeeping genes and another group, σ^B, regulate gene expression in response to environmental stimuli.[50] σ^B is active in the late exponential phase and is responsible for the regulation of many virulence genes.

Sub-lethal exposure to antimicrobial agents can also regulate toxin production.[21] For example, sub-lethal exposure to oxacillin enhances the production of PVL whereas clindamycin, linezolid, and fusidic acid reduce it.[58,59] Similar findings have been reported for the staphylococcal α-toxin.[60] The finding that sub-lethal exposure to β-lactam antibiotics increases toxin production in *S. aureus* suggests that inappropriate therapy for β-lactam therapy for MRSA may increase its virulence.

Finally, there is evidence that expressed toxins themselves can act as global regulators of gene expression. For example, TSST-1 and enterotoxin B appear to down-regulate the expression of other toxins.[50,51] Labandeira-Rey *et al.* found that the expression of PVL induced global changes in the transcription of several genes encoding secreted and cell-wall-anchored proteins, including *spa*, in a mouse model of pneumonia.[61] However, a more recent study found that a point mutation in the *agr* locus was responsible for the alterations in global gene expression attributed to PVL by Labandeira-Rey *et al.*[61,62]

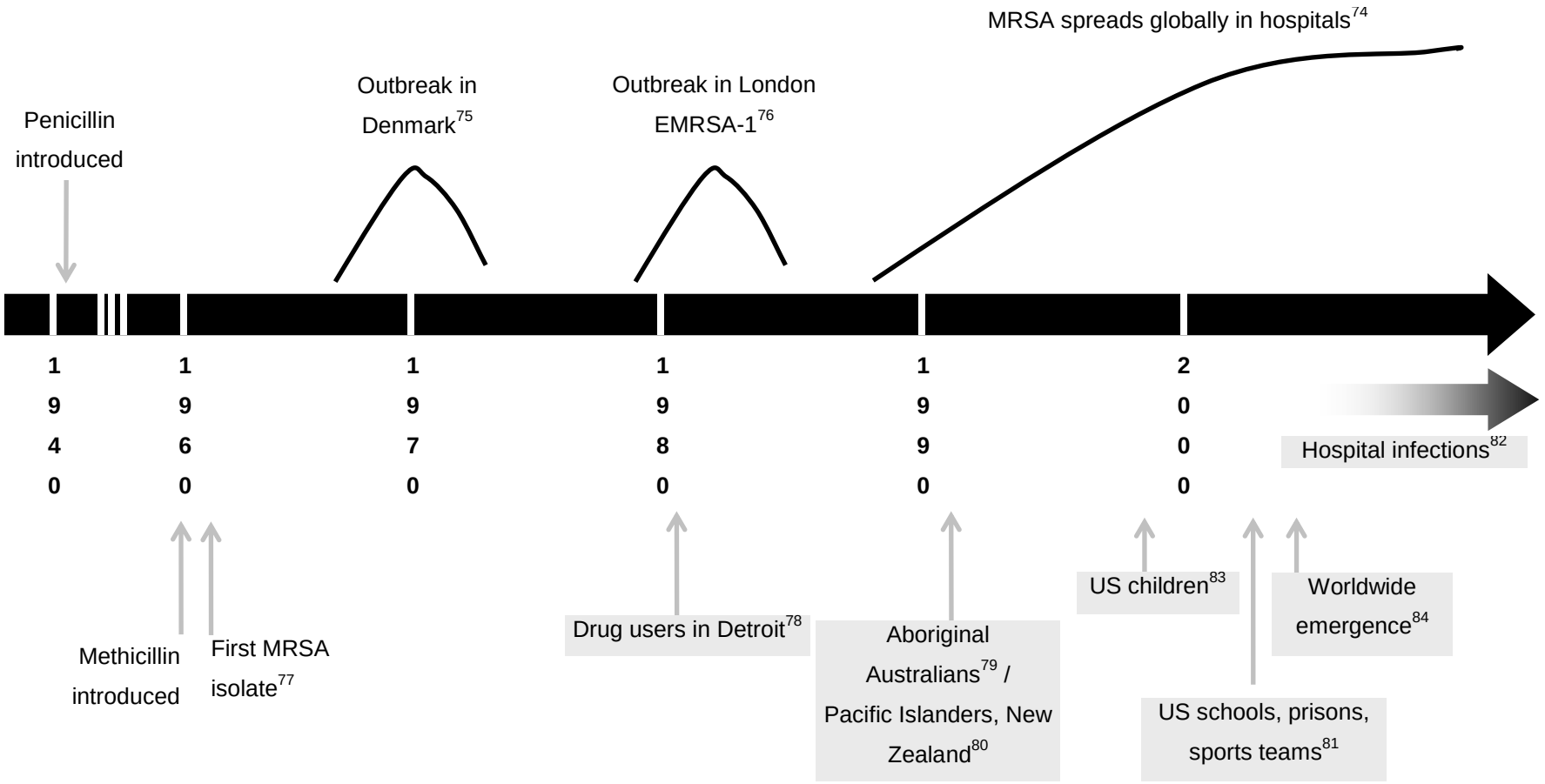
5.2 Penicillin and penicillin resistance

Serious infections with *S. aureus* were a significant cause of mortality prior to the discovery of antibiotics: in a study of one hundred infected patients in the pre-antibiotic era, *S. aureus* bacteraemia had a mortality of 82%.[63] Penicillin was first discovered by Alexander Fleming in 1929 and was introduced as a therapeutic agent in the 1940s following development by Howard Florey and Ernst Chain, reducing mortality from *S. aureus* bacteraemia to <30% (Figure 5-1, p.34).[21,64,65]

Penicillin and other β-lactam antibiotics inhibit cell wall synthesis by interrupting the enzymatic action of Penicillin-Binding Proteins (PBPs), which are necessary for the cross-linkage of peptidoglycan through transpeptidase, transglycosylase and carboxypeptidase activity.[66,67] Resistance to penicillin emerged shortly after its introduction and spread rapidly among staphylococci due to the phage transfer of plasmids carrying the *blaZ* gene encoding penicillinase.[66] Penicillinase was first discovered in *Escherichia coli* before penicillin was introduced as a therapeutic agent.[68] Penicillinase and other β-lactamase enzymes hydrolyse the β-lactam ring of β-lactam antimicrobial agents.[69] The *blaZ* gene is transcribed from the *blaZ* operon, which also includes the regulatory genes *blaR* and *blaI,* producing inducible expression of *blaZ* in response to penicillin.[70]

By the late 1950s the majority of *S. aureus* in hospitals were penicillin-resistant, and the mortality from *S. aureus* bacteraemia returned to approximately 50%.[21,71,72] Shortly thereafter, penicillin-resistant *S. aureus* began to be seen in the community and prevalence rates then rose steadily.[71,72] One strain, *S. aureus* phage type 80/81, emerged as a common cause of staphylococcal infection both in the community and in hospitals.[73] The phage type 80/81 strain waned following the introduction of methicillin in the 1960s, but other strains of penicillin-resistant *S. aureus* emerged so that by the 1970s, penicillin-resistance rates in both hospital and community isolates were 70-85%.[71]

Figure 5-1. Timeline of milestones in the epidemiology of MRSA.



Not to scale. Grey shaded boxes refer to community-associated MRSA.

5.3 Methicillin resistance

5.3.1 Molecular mechanisms of methicillin resistance

Methicillin, a semi-synthetic derivative of penicillin, was developed as a β-lactamase-stable β-lactam agent to treat penicillinase-producing *S. aureus*.[66] Methicillin was first introduced in 1961 and resulted in a reduction in the mortality of *S. aureus* bacteraemia to <30% - a similar impact to the introduction of penicillin 20 years earlier.[21] However, the first methicillin-resistant *S. aureus* (MRSA) were reported shortly after the introduction of methicillin (Figure 5-1, p.34).[77]

High-level methicillin resistance is conferred by expression of the *mec*A gene, which encodes a modified PBP (PBP2a or PBP2').[85] This has a lower affinity for methicillin and thus facilitates cell wall synthesis in the presence of methicillin and all other β-lactam antibiotics.[85]

*5.3.1.1 SCC*mec

The *mec*A gene is encoded by a staphylococcal cassette chromosome, SCC*mec*.[27] The region contains terminal and direct repeats, the cassette chromosome recombinase complex (*ccr*) responsible for the mobility of SCC*mec*, varying amounts of DNA of an unknown function termed "junkyard" (J) DNA, and the *mec* gene complex, which consists of *mec*A, *mec*I and *mec*R1.[44,86,87] Each SCC*mec* cassette has three J regions and a conserved composition: J3-*mec*-J2-*ccr*-J1. SCC*mec* probably originated in coagulase-negative staphylococci and integrates site-specifically into the *S. aureus* genome through *ccr* genes.[44,86,88] *mec*A is the gene encoding PBP2a and *mecI* and *mec*R1 are regulatory genes that control the transcription of *mec*A; *mec*R1 is a slow inducer of *mec*A, whereas *mec*I is a strong repressor of *mec*A.[89] In the presence of a β-lactam antibiotic, MecRI is produced; this cleaves MecI and *mecA* is transcribed.[29,66] Certain SCC*mec* types contain insertion sequences for other resistance genes, especially in successful multi-resistant hospital MRSA clones.[27,87]

The nature of the SCC*mec* region varies with the ecological niche of the MRSA. Hospital strains usually have larger SCC*mec* regions (I-III) containing multiple antimicrobial resistance genes in addition to *mec*A, whereas successful community usually have smaller SCC*mec* regions (IV or V) without additional antimicrobial resistance genes.[29] These variations provide fitness advantages in different environments.[90-92] SCC*mec* VI has only been described in isolates in Portugal,[93] SCC*mec* VII, formerly SCC*mec* V_T, has been described in CA-MRSA from Taiwan,[94] and SCC*mec* VIII has been reported from a Canadian epidemic healthcare-associated clone.[95]

SCC*mec* types I-VIII (including variants) have been described and are summarised in Table 5-4, p.37.[29,87,96-98]

Characterisation of the SCC*mec* region is a useful molecular typing method because the genetic element is well conserved.[45,46,98] However, a number of studies have reported non-typeable strains and new SCC*mec* types continue to be reported, suggesting the presence of novel SCC*mec* types or that the structure of SCC*mec* may be more heterogeneous than assumed so far.[93,94,96,100,101]

In vitro methicillin resistance can also be exhibited through the hyper-production of β-lactamase or through the possession of other modified PBPs[66,102] and there is evidence that this is clinically significant.[103,104]

Table 5-4. The defining characteristics of SCC*mec* types I-VIII.

SCC*mec* type	Size[a] / kb	*mec* gene complex[b]	*ccr* gene complex[c]	Resistance genes[d]	Association[e]
I	34	Class B	Type 1	*mecA*	H
II	60	Class A	Type 2	*mecA, aadD, ermK*	H
III	67	Class A	Type 3	*mecA, aadD, tetK*	H
IV	21-24	Class A[f]	Type 2	*mecA*	C/H
V	28	Class C	Type 5	*mecA*	C
VI	21	Class B	Type 4	*mecA*	Sporadic
VII	36	Class C	Type 5	*mecA*	C
VIII	31	Class A	Type 4	*mecA, aad9, ermA*	H

a. The amount of DNA with no known function, known as “Junkyard” DNA, affects the size of the SCC*mec* region.

b. Class A: IS*431-mec*A-*mec*R1-*mec*I; class B: IS*431-mec*A-Δ*mec*R1-IS*1272*; class C: IS*431-mec*A-Δ*mec*R1-IS*431.*

c. Type 1: *ccrB1-ccrA1*; type 2: *ccrB2-ccrA2*; type 3: *ccrB3-ccrA3*; type 4: *ccrB4-ccrA4*; type 5: *ccrC.*

d. MRSA with the same SCC*mec* type may carry variable antimicrobial resistance genes; *aadD* encodes resistance to tobramycin and kanamycin; *aad9* encodes resistance to streptomycin/spectinomycin; *ermK* and *ermA* encode resistance to marcolide-lincosamine-streptogramin antibiotics; *tetK* encodes resistance to tetracycline.

e. H = healthcare-associated MRSA, C = community-associated MRSA. Not all MRSA with SCC*mec* type IV are CA-MRSA. For example, EMRSA-15 is SCC*mec* type IV.[99]

f. SCC*mec* IVd has the Class B *mec* gene complex.

5.3.2 Laboratory testing methods

The detection of methicillin resistance *in vitro* has been problematic.[105] Early MRSA isolates expressed resistance to methicillin and other β-lactam antibiotics heterogeneously or not at all, even though *mec*A was present.[106] Heterogeneous resistance is a phenotypic phenomenon in which the degree of resistance in a single population of cells varies, making the laboratory detection of methicillin resistance difficult.[85] Epidemic hospital strains do not usually exhibit heterogeneous resistance and usually have higher methicillin minimum inhibitory concentrations (MIC) so the detection of methicillin resistance in the clinical laboratory in these strains is easier.[74,107]

Changes in the testing conditions can improve detection of MRSA.[6,85] Conditions that improve detection include the use of certain types of media (specifically Columbia or Mueller-Hinton agar), addition of 2% NaCl, incubation at a lower temperature (30°C) for a longer period (48 hours) and a heavy inoculum (0.5 McFarland Index).[6] The exact mechanisms by which these test conditions promote the expression of methicillin resistance in *S. aureus* remain largely unknown.[85]

5.4 Typing of *S. aureus*

Many different phenotypic and genotypic methods have been used to type *S. aureus.*[108,109] Those that have been widely adopted are compared in Table 5-5, p.40.

5.4.1 Phenotypic methods

5.4.1.1 Antibiogram

S. aureus can be compared by their susceptibility to a range of antimicrobial agents. This is a simple, useful method because

antibiograms can be determined relatively inexpensively and are usually completed for clinical reasons and therefore require no additional resources.[108] Methods have been standardised and the recent introduction of automated testing methods has improved the accuracy of reporting.[110,111] However, antibiograms lack resolution because of variable expression and transfer of resistance genes. The major use of antibiograms is to identify isolates of interest for more detailed microbiological analysis, or for the generation of antimicrobial-based algorithms to predict genotype.[112,113] Furthermore, specific resistance patterns can be useful for the first detection of an outbreak or for investigation of cross-transmission during outbreaks. Changes in common resistance patterns over time can signal changes in the epidemiology of *S. aureus*.[99,114-116]

5.4.1.2 Phage typing

Phage typing uses the narrow host range of lytic phages to provide a profile of phage susceptibility.[108] The phage set used to challenge *S. aureus* was standardised in the 1970s and phage typing remained the mainstay of *S. aureus* typing until the advent of molecular methods.[108] A variation on phage typing is reverse-phage typing, where mitotoxin C is used to induce lysogenised phages in the test strain which are then introduced onto a lawn of various test strains.[119] This method may be useful for strains that are non-typable by standard phage typing sets.[119] Phage typing is limited by poor inter-laboratory reproducibility and lack of discriminatory power compared with molecular methods and has been almost completely superseded. However, phage typing only requires overnight incubation so provides rapid epidemiological information and may be useful in resource limited countries that cannot afford the facilities required for molecular methods.

Table 5-5. Laboratory methods to type *S. aureus*.

Name	Type	Target	Discriminatory power	Reproducibility (intra/inter lab)	Portability of results	Time[a]	Cost	Technical demand
Antibiogram	Phenotypic	Antimicrobial susceptibility profile	Poor	Poor	Good	1 day	Low	Low
Phage typing	Phenotypic	Phage susceptibility profile	Poor	Poor	Good	1 day	Low	Moderate
Rep-PCR	Genotypic; gel-based	Repetitive sequences in the genome	Moderate	Poor[b]	Poor[b]	1 day	Low[c]	Moderate
PFGE	Genotypic; gel-based	Chromosomal digestion with a rare cutting restriction enzyme	Excellent	Good	Moderate	3 days	Moderate	High
VNTR/MLVA[d]	Genotypic; gel-based	PCR amplification and sizing of tandem repeats	Variable[e]	Good	Good	1 day	Low	Moderate
MLST	Genomic; sequence-based	Sequencing of seven housekeeping genes	Moderate	Excellent	Excellent	2 days	High	Moderate
spa typing	Genomic; sequence-based	Sequencing of the protein A *spa* gene	Good	Excellent	Excellent	2 days	Moderate	Moderate
Microarrays	Genomic; sequence-based	Hybridisation of genomic DNA with oligonucleotide probes	Excellent	Excellent	Excellent	3 days	High	High
SNP detection	Genomic; gel or sequence-based	Identification of known highly discriminatory SNPs	Variable[e]	Excellent	Moderate	1 day	Low	Moderate

[a] From a pure culture to results.

[b] Recent attempts to standardise the visualisation of Rep-PCR band patterns in the Biomerieux Diversilab™ system have improved reproducibility and portability.[117,118]

[c] The Diversilab system increases the cost of Rep-PCR due to the need to purchase proprietary equipment and reagents.

[d] VNTR/MLVA = variable number of tandem repeats / multilocus VNTR assay.

[e] Depends on the method used.

5.4.2 Genotypic methods

5.4.2.1 Pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis is a whole chromosome method, which has been the "gold standard" for *S. aureus* typing for a decade, but has been largely superseded in recent years by sequence-based methods.[108,109] In PFGE, genomic DNA is digested using an infrequently cutting restriction enzyme, typically *sma*1, and run through an agarose gel with pulses of current that allow separation of large fragments.[120] PFGE has excellent discriminatory power and has proven useful for outbreak investigation and longitudinal typing studies.[108,109] Pulsotypes can be digitalised and compared objectively using computer software.[121] However, despite standardisation of methods and interpretational criteria, PFGE suffers from moderate inter-laboratory reproducibility and limited portability of results.[120,122,123] Furthermore, although almost all *S. aureus* can be typed by PFGE, an emerging community MRSA clone associated with livestock, ST398, cannot be typed by standard PFGE protocols.[124]

5.4.2.2 Repetitive element PCR (Rep-PCR)

Rep-PCR uses primers designed to anneal to sequences known to repeat at different locations and frequencies throughout the genome.[89] Several different Rep-PCR schemes for *S. aureus* have been published, which have shown reasonably good discriminatory power.[125,126] However, Rep-PCR methods are limited by poor inter-laboratory reproducibility and poor portability of gel-based profiles. A recent commercial Rep-PCR platform for *S. aureus* and other micro-organisms, Diversilab™ (Bio-Merieux), has an electronic output, which improves reproducibility and portability.[117,118] The Diversilab system has excellent reproducibility but moderate discrimination and currently lacks standardised interpretation guidelines.[109]

5.4.2.3 Variable number of tandem repeat (VNTR) methods

The *S. aureus* genome contains a number of tandem repeat regions. VNTR-based methods use PCR to amplify tandem repeats at a single locus (single locus VNTR) or multiple loci (multiple locus VNTR assay, MLVA) and the PCR products are run through an agarose gel or a sequencer to size the fragments.[109] This results in a profile in the form of a numeric code. Several different MLVA methods have been published; for example, staphylococcal interspersed repeat units (SIRU) typing,[127] a CDC method,[128] and a recent Dutch method.[129] MLVA methods have good discriminatory power and are relatively rapid compared with sequence-based systems.[127-129] However, they have less discriminatory power and there are currently no internationally accepted protocols or interpretation criteria, which limits their portability.[109]

Sequence-based methods have several advantages over gel-based methods, including objectivity and high inter-laboratory reproducibility.[108,109,130]

5.4.2.4 Multilocus sequence typing (MLST)

MLST has become the “gold standard” for longitudinal and evolutionary studies of *S. aureus.*[109] The method involves sequencing of seven housekeeping genes that are necessary for the function of the cell and are conserved in all *S. aureus* strains.[32] The sequences of the seven genes are compared with an online database (www.mlst.net) and given an arbitrary numerical value; the seven housekeeping gene numbers provide a seven digit allelic profile. This profile is synchronised with the MLST server and can be related to other MLST types using the BURST algorithm (www.eburst.mlst.net).[33] BURST identifies unique genotypes, attempts to identify a founder for each group of related genotypes in a related clonal cluster (CC) and predicts descent from the founding genotype. MLST is relatively expensive due to the need to sequence seven genes. It assesses core genome and is therefore important for investigation of the

global or historical evolution of *S. aureus* strains, but it lacks discriminatory power for short-term epidemiological studies where the accessory genome can show rapid variation.[109]

5.4.2.5 spa *typing*

spa typing is a single-locus sequence-based genotypic typing method for *S. aureus* that was first proposed in 1996.[131] The method compares the sequence, number and length of tandem repeats in the 3' coding region of the protein A gene, *spa*.[130,132]

The sequence of the *spa* gene for each isolate is compared with an online database to assign a *spa* type. There are currently two systems for the nomenclature of *spa* types, one commonly used in Europe (www.spaserver.ridom.de)[121,130,132] and one commonly used in the USA.[133,134]

spa type relatedness can be analysed by applying the Based Upon Repeat Patterns (BURP) algorithm.[135] BURP is a sequence alignment tool for comparing 'edit operations' to measure the relatedness of different *spa* types using a novel Excisions Duplications Substitutions and Indels [Insertions/Deletions] (EDSI) model.[135] BURP assigns an evolutionary cost to each EDSI operation, which is used as a measure of evolutionary distance and hence *spa* type relatedness.

Despite being a single locus method, *spa* typing currently has good discriminatory power and correlates well with MLST and PFGE, even for longitudinal studies.[27,121,134]

5.4.2.6 Microarrays and single nucleotide polymorphism (SNP) detection

DNA microarrays consist of oligonucleotide probes fixed to a solid matrix onto which genomic DNA is hybridised under stringent conditions.[136] The probe-target hybridisation is visualised using a fluorescent or other

marker. By using a high density array of a large number of oligonucleotides with varying sequences, the complimentary sequence of the bacterial DNA can be assessed. Microarrays offer a truly whole genome approach to strain typing and can detect single nucleotide polymorphisms (SNPs). Microarray analysis of *S. aureus* has provided insights into the clonal structure and is extremely discriminatory.[26] However, the method is low throughput and expensive and cannot be considered as a candidate for routine typing at present.[109]

An alternative to high-density microarrays is using either low density arrays or PCR-based methods to identify small sets of SNPs that are known to be discriminatory.[137-140] Several different SNP detection methods have been published but no standardised method has emerged. The portability of results is therefore currently limited. However, it is likely that a standardised set of SNPs will emerge in the near future and SNP detection is likely then to become a standard rapid genotyping method.[109]

5.4.2.7 Other methods

The identification of various virulence genes is a useful method to characterise *S. aureus*, and this may be achieved through a series of multiplex PCR reactions.[101] An alternative to multiple PCR reactions is an oligonucleotide array containing a limited number of probes for genes of known epidemiological significance. For example, the Clondiag™ platform has been shown to be a useful rapid method for the simultaneous detection of regulatory, virulence, resistance and other important genes in *S. aureus*.[141,142]

PCR allotyping of the SCC*mec* cassette is a useful method for determining the genetic background of MRSA, particularly when used in combination with MLST.[27,45] MRSA clones may then be designated as MLS type plus SCC*mec* type, for example, ST36-II and ST8-IV. This combined method has the advantage that the MLS type identifies part of the stable core genome and SCC*mec* type part of the variable accessory genome.

Several novel methods that can be used for typing *S. aureus* have emerged recently. On optical method based on Ramen spectroscopy concurred with PFGE in a recent study.[143] A method using electrospray ionisation-mass spectrometry (PCR/ESI-MS) analysis of PCR products from the seven MLST housekeeping genes has produced results concurring with PFGE and Rep-PCR.[144,145]

5.5 Epidemiology and control of healthcare-associated MRSA

5.5.1 Clinical features, infection control and treatment

The spectrum of disease caused by MRSA is similar to that of MSSA. In general, clinical outcomes are worse in infections with MRSA, but there is debate over whether this is because patients with MRSA infections tend to have more host risk factors than those with MSSA or whether there is any difference in virulence between these two organism groups.[146-150] β-lactams (commonly flucloxacillin in the UK) used alone or in combination with other agents, are generally the most effective antimicrobials for the treatment of MSSA infections; since these cannot be used for MRSA, the worse outcomes associated with MRSA infections may be result, in part, from the need to treat with less effective antibiotics.[146-150] Indeed, antimicrobial resistance is generally associated with worse outcomes and may therefore be regarded as a virulence factor.[151] Some studies which have controlled for underlying patient condition have provided evidence that MRSA are more virulent than MSSA but others have concluded there is no difference.[146-150] Regardless of the underlying virulence of the micro-organism, which is strain-specific, MRSA infections often have a poor outcome due to inappropriate or delayed treatment resulting from either delayed methicillin-susceptibility testing or wrong initial choice of antibiotic.[152,153]

The risk factors for MRSA colonisation and subsequent infection are well established and include exposure to healthcare facilities, surgical

procedures, indwelling devices, underlying medical conditions such as diabetes and HIV, previous MRSA episodes, older age and prior antimicrobial use.[154,155]

MRSA are usually transmitted in hospitals via the hands of healthcare workers.[18,156] Other transmission routes also contribute to transmission including contaminated medical equipment or surfaces and possibly contaminated air.[12] Infection control interventions centre on these known transmission routes and include screening and isolation of affected patients, hand hygiene before and after patient contact, the use of gloves and gowns for treating affected patients, disinfection of surfaces and equipment and decolonisation of colonised patients.[18,156] The infection control methods applied vary according to national and local guidelines, and the range of MRSA prevalence – particularly in Europe – suggest that some control strategies are more successful than others.[157]

Several classes of antimicrobial agent are active against *S. aureus*, but *S. aureus* has developed resistance mechanisms to them all meaning that isolates can be resistant to multiple classes simultaneously (Table 5-6, p.48).[158] MRSA in hospitals are typically resistant to multiple classes including fluoroquinolones and macrolides in addition to β-lactams, but are usually susceptible to glycopeptides and newer antimicrobial agents such as daptomycin and linezolid.[158,159]

Table 5-6. Anti-staphylococcal antimicrobial agents and resistance mechanisms.

Adapted from Lowy 2003.[158]

Antimicrobial class	Mechanism of action	Antimicrobial agents	Resistance gene(s)	Mechanism of resistance
β-lactam	Inhibition of cell wall synthesis	Penicillin	*blaZ*	Enzymatic destruction of agent
		Methicillin	*mecA*	Modified penicillin binding protein (PBP2a) with reduced affinity for agent
Glycopeptides	Inhibition of cell wall synthesis	Vancomycin, Teicoplanin	*vanA*	Cell wall precursor (D-Ala-D-Lac) with reduced affinity for agent
			Unknown	Trapping of agent in the cell wall with an altered peptidoglycan
Quinolones	Inhibition of DNA replication	Ciprofloxacin	*parC, gyrA, gyrB*	Mutations in the quinolone resistance-determining region reduce affinity for the agent
Aminoglycosides	Inhibition of protein synthesis	Gentamicin, neomycin, kanamycin	*aac, aph, aadD*	Enzymatic acetylation or phosphorylation of the agent
Trimethoprim-sulfamethoxazole (TMP-SMZ)	Inhibition of folate synthesis	Sulphonamide	*sulA*	Overproduction of p-aminobenzoicacid by enzyme

Antimicrobial class	Mechanism of action	Antimicrobial agents	Resistance gene(s)	Mechanism of resistance
		Trimethoprim	*dfrB*	Reduced affinity for dihydrofolate reductase
Oxazolidinones	Inhibition of protein synthesis	Linezolid	*rrn*	Mutations in domain V of 23S rRNA C component of the 50S ribosome.
Streptogramins (Synercid = Quinupristin + dalfopristin)	Inhibition of protein synthesis	Quinupristin	*ermA, ermB, ermC*	Enzymatic reduction in 23S ribosomal binding via ribosomal methylases
		Dalfopristin	*vatA, vatB*	Enzymatic modification of the agent via acetyltransferases
		Pristinamycin[160]	*vatA, vatB*	Enzymatic modification of the agent via acetyltransferases
			vgaA, vgaB	Efflux pump to remove agent from the cell
			vgbA, vgbB	Enzymatic lysis of the agent
Macrolides[161]	Inhibition of protein synthesis	Erythromycin	*ermA, ermB, ermC*	Enzymatic reduction in 23S ribosomal binding via ribosomal methylases
			msaA, msrB	Efflux pump to remove agent from the cell
			ereA, ereB	Enzymatic cleavage of agent via esterases
Lincosamides[161]	Inhibition of protein synthesis	Clindamycin	*ermA, ermB, ermC*	Enzymatic reduction in 23S ribosomal binding via ribosomal methylases

Antimicrobial class	Mechanism of action	Antimicrobial agents	Resistance gene(s)	Mechanism of resistance
Fusidic acid[162]	Inhibition of protein synthesis	Fusidic acid	*fusA*	Mutations in *fusA* resulting in target modification
Tetracyclines[163]	Inhibition of protein synthesis	Tetracycline	*tetA(K)/tetA(L)*	Efflux pump to remove agent from the cell
			tetA(M)	Ribosomal protection
Rifampicin[164]	Inhibition of protein synthesis	Rifampicin	*rpoB*	Mutations in *rpoB* resulting in target modification
Mupirocin[165]	Inhibition of protein synthesis	Mupirocin	*mupA*	Alternate isoleucyl-tRNA synthetase enzyme results in high-level resistance
			ileS	Mutations in *ileS* result in low-level resistance
Lipopeptides[166]	Cell membrane rupture	Daptomycin	-	Unknown
Oxazolidinones[167]	Inhibition of protein synthesis	Linezolid	-	Mutations in 23S RNA genes

The glycopeptides are the mainstay for the successful treatment of multi-resistant MRSA.[158,159] Resistance to the glycopeptides, which include vancomycin and teicoplanin, has emerged since the first report of MRSA with reduced susceptibility to vancomycin in Japan in 1996.[168,169] Vancomycin-intermediate *S. aureus* (VISA) are defined as those isolates with MICs from 4-8 mg/L; heterogeneous VISA (hVISA) strains appear to be susceptible to vancomycin but contain a subpopulation of cells with reduced susceptibility to vancomycin (MICs ≥ 4 mg/L).[170,171] Reports of high-level vancomycin-resistant MRSA (VRSA) (MIC ≥16mg/L), conferred by the *van*A gene, are rare and most reports have come from the USA since 2002.[172,173] A worrying recent development is reports of gradual increase in vancomycin MIC below the breakpoint for VISA, so called 'MIC creep'.[171,174-176]

A number of antimicrobials are available to treat MRSA with reduced susceptibility to the glycopepetides including quinupristin-dalfopristin (Synercid), linezolid and daptomycin but resistance to these agents has already been reported.[158,166,167]

5.5.2 Global dissemination

Over the past 50 years, MRSA have spread globally (Figure 5-1, p.34).[72,74,157] causing, until recently, predominantly hospital- or healthcare-associated outbreaks and infections. However, this spread has not been uniform. Since the first reports of MRSA in the early 1960s,[77] MRSA spread to countries in Europe and certain successful epidemic clones emerged in the 1970s.[74] For example, in Denmark a small number of successful phage types emerged in the late 1960s and early 1970s so that the proportion of *S. aureus* isolates resistant to methicillin rose to 15% between 1967 and 1971 but then decreased to 0.2% by 1984.[75] The decline in MRSA in Denmark in the 1970s also occurred in other European countries for reasons that are poorly understood; improvements in infection control, changes in antimicrobial usage or the reduction in specific transmissible phage types could have contributed.[74,177]

MRSA emerged later and less rapidly in the USA.[72] The prevalence of MRSA increased progressively since the early 1980s, although MRSA were isolated in the USA before 1980.[178]

There was an apparent change in the epidemiology of MRSA in the UK in the early 1980s with the emergence of an epidemic strain of epidemic MRSA (EMRSA-1) in London which spread to a number of other hospitals in southeast England.[76,107] At least 16 other EMRSA strains have been described but two strains, EMRSA 15 and 16, predominate in the UK.[179-181] The concurrent emergence of EMRSA also occurred in other countries such as Australia.[182]

Since the early 1990s most countries around the world have witnessed a progressive increase in the rate of methicillin resistance among *S. aureus.*[157,183] Notable exceptions to this rise in prevalence of MRSA have been in the Netherlands and the Nordic countries where strict antimicrobial prescribing and aggressive infection control measures, known as the "Search and Destroy" strategy have been applied.[184-186]

Currently, the rate of methicillin resistance among blood isolates of *S. aureus* (mostly HA-MRSA) in European countries currently ranges from <1% in Norway, Sweden and Denmark and <5% in The Netherlands, to >40% in Greece and the UK and >50% in Malta.[187] The large range of MRSA rates in Europe is partially explained by the various control strategies employed by different countries but it also seems that certain strains of MRSA come to pre-eminence and occasionally fade away for reasons that are poorly understood.[73-75,183]

Outside of Europe, the rate of methicillin resistance among *S. aureus* isolates in intensive care units in the USA was more than 50% in 2002 and MRSA accounted for between 30-60% of bloodstream and surgical-site infections.[157,183,188] Other countries are even worse affected by MRSA; the rate of methicillin resistance in a small sample of *S. aureus* isolates from

China was in excess of 80% in 1998-1999 and this is apparently increasing.[189,190] More than 50% of *S. aureus* bloodstream isolates were MRSA in recent reports from Columbia, Iraq, Hong Kong, Singapore, Japan and South Korea reviewed by Grundmann *et al*.[183]

In recent years, several countries have reported reductions in the prevalence of MRSA. Recent data from the UK suggests that enhanced infection control programmes have caused a substantial reduction in the national incidence of MRSA bacteraemias.[187,191] Local data from Guy's and St. Thomas' Hospital (GSTT) demonstrates a sharp reduction in the acquisition of MRSA infection and colonisation in cardiothoracic patients.[192] Furthermore, the incidence of MRSA bacteraemia has fallen at GSTT by 85% from 165/10,000 bed days in 2003/4 to 25/10,000 bed days in 2008/9 (data from www.hpa.org.uk/topic/infectiousdieases). The rate of MRSA among *S. aureus* is falling in other European countries too: the year-on-year change in the proportion of invasive *S. aureus* isolates resistant to methicillin fell in eight countries, increased in eight countries and did not change significantly in the remaining 14 countries included in the 2007 European Antimicrobial Resistance Surveillance System report in contrast to previous reports of increases in most European countries.[187] A recent report from 38 French hospitals shows a significant decrease in the incidence of MRSA cases (from 0.86 to 0.56 per 1000 hospital days) and the proportion of *S. aureus* isolates resistant to methicillin (from 41 to 27%) from 1993-2007 through the implementation of enhanced infection control measures.[193] Several hospitals in the USA have also reported recent success in the reduction of MRSA through enhanced infection control.[194-196] Taken together, these reports suggest that HA-MRSA can be controlled through the rigorous implementation of infection control programmes, even in areas where MRSA is endemic, and this is supported by mathematical modelling.[197]

Despite the global scale of the problems caused by MRSA, it appears that only a small number of major clones have been involved. These have

been identified by MLST and BURST analysis of MLST datasets and SCC*mec* types as forming five predominant clonal clusters (Table 5-7).[29,34]

Table 5-7. The predominant HA-MRSA clonal clusters (CCs).

MLST CC	MLST-SCC*mec*	Name(s)
CC5	ST5-II	New York/Japan, USA100
	ST5-I	EMRSA-3
	ST5-IV	Paediatric, USA800
CC8	ST250-I	Archaic
	ST8-IV	EMRSA-2, EMRSA-6, USA500
	ST8-II	Irish-1
	ST239-III	Brazilian/Portuguese, EMRSA-1, AUS-2/3
	ST247-I	Iberian
CC22	ST22-IV	EMRSA-15
CC30	ST36-II	EMRSA-16, USA200
CC45	ST45-IV	Berlin
	ST45-II	USA600

5.6 Emergence of community-associated MRSA

Despite the global spread of HA-MRSA in hospitals and other healthcare facilities, MRSA have historically failed to spread in the healthy population.[72,183,198] MRSA colonisation can persist for months or years[4,199] and, until recently, MRSA infections presenting outside of hospitals were caused by MRSA strains acquired during prior hospital or healthcare contact.[200-202] It appears that the fitness cost of SCC*mec* was too high for HA-MRSA to succeed as community pathogens.[91,92] True community-associated MRSA (CA-MRSA), caused by strains distinct from HA-MRSA, were first reported as causing an outbreak of skin and soft tissue infection among drug users in Detroit, USA, in the early 1980s (Figure 5-1, p.34).[78] CA-MRSA infections in patients without prior healthcare contact began to emerge in the early 1990s in Western Australia[79] and New Zealand[80] and in American children in the late 1990s.[83,114,203] It had become clear by the late 1990s that the epidemiology of MRSA was changing due to the

emergence of CA-MRSA,[71,114] highlighted by the death of four previously healthy children in Minnesota and North Dakota in 1999.[203]

5.6.1 Characteristics of CA-MRSA

Community strains of MSSA appear to have acquired mobile SCC*mec* cassettes resulting in the generation of CA-MRSA clones.[29,34] While HA-MRSA strains cause infection in hospitalised, compromised, elderly patients, often those with a history of surgery or indwelling devices, CA-MRSA, like community strains of MSSA, affect younger, healthy people and can spread readily in community settings and hospitals.[72,204] The characteristics that distinguish CA-MRSA from HA-MRSA are summarised in Table 5-8, p.56.

5.6.2 Definition of CA-MRSA

CA-MRSA were first identified as MRSA causing infection in previously healthy young patients without prior healthcare contact. The strains are classically susceptible to most non-β-lactam antimicrobial agents, carry PVL genes and are of SCC*mec* types IV or V (Table 5-8, p.56).[29,84,204,205] However, as CA-MRSA have emerged and evolved, defining CA-MRSA has become more difficult.[206]

Table 5-8. Clinical, microbiological and genetic features of CA-MRSA.

Clinical features
Affected patients are less likely to have had healthcare contact[83,204]
Can affect healthy individuals of all ages[115,207]
Characterised by primary SSTIs occurring in patients with no initial skin wound, especially abscesses[84,198]
Can cause life threatening invasive infections such as bacteraemia and necrotizing pneumonia[207-209]
Occasionally fatal in previously healthy paediatric patients and young adults[203,210]
Apparent association with non-nasal sites of colonisation[211,212]
Recurrent SSTIs[213-215]
Transmission within family groups[216-219]
Microbiological features
Faster growth rate and competitive advantage with HA-MRSA *in vitro*[90,92,220]
Less resistant to non-β-lactam antimicrobial classes[84,220]
Low-level / heterogeneous expression of methicillin resistance[220-222]
Genetic features
Usually SCC*mec* IV or V[27,220]
Epidemiological association with PVL carriage[84,220]
Distinct and diverse MLST types and CCs[27,223]

A purely epidemiological definition of CA-MRSA is now unhelpful because patients with MRSA colonisation or infection originating in hospitals may first present with MRSA in the community or at hospital readmission. Several studies have found that MRSA bacteraemia diagnosed in the first 24 hours of hospital admission, which may be considered as “community-acquired MRSA”, is often caused by nosocomial strains of MRSA from a previous healthcare contact.[202,224] An epidemiological definition is further limited by the emergence of CA-MRSA clones as an increasingly common cause of healthcare-acquired infection.[82,225,226] Finally, some patients

(such as injecting drug users, IDUs) with true CA-MRSA may have a history of repeated, but unrelated, healthcare contact.[227,228]

Molecular definitions are confounded by the lack of a stable genetic marker for CA-MRSA strains. Not all successful CA-MRSA clones carry PVL[229-231] and the SCC*mec* region is variable and cannot always be classified into known types, resulting in new types, sub-types and confusion over nomenclature.[27,232] Furthermore, the most common HA-MRSA clone in the UK, ST22 EMRSA-15, is SCC*mec* IV[99,101] as is the ST5 paediatric clone.[72]

Defining CA-MRSA based on their classical susceptibility to non-β-lactam antimicrobial agents is limited by the emergence of multidrug-resistance in CA-MRSA, particularly in areas of high prevalence where CA-MRSA clones have frequent contact with healthcare facilities.[233-236] A particular problem of using antimicrobial susceptibility as a phenotypic marker of CA-MRSA in the UK is that the most common cause of HA-MRSA, EMRSA-15, is typically resistant to few non β-lactam antimicrobials.[99]

Therefore, a combination of a genotypic method such as MLST, *spa* or PFGE, together with SCC*mec* analysis to infer the likely origin of the MRSA, remains the most useful definition of CA-MRSA at the current time. This thesis therefore defines CA-MRSA as an MRSA isolate that has emerged as the result of *mecA* acquisition by a community strain of MSSA. These isolates may colonise or infect patients in the community or in healthcare settings and in many cases it is not possible to determine where the acquisition occurred. Thus, CA-MRSA strain types can be classified as either healthcare- or community-acquired using epidemiological criteria.

5.6.3 PVL and its controversial role in disease

The Panton-Valentine leukocidin toxin (PVL) was first described in 1932 and is a two-component pore-forming cytotoxin that was initially thought to

be associated with SSTIs.[207,237,238] PVL is carried by approximately 2% of *S. aureus* and is encoded by two co-transcribed genes, *lukS-PV* and *lukF-PV*, located on lysogenised phages.[101,239] Six PVL-encoding phages have been described in *S. aureus* with two distinct morphologies: icosahedral head type (ΦPVL and Φ108PVL) or elongated head type (ΦSLT, ΦSa2mw, ΦSa2958 and ΦSa2usa).[40,43,240] Although the sequence of the PVL genes is well-conserved, single nucleotide polymorphisms (SNPs) in the PVL genes tend to vary according to lineage and two variants of the PVL proteins have been described.[94,241-244]

The role of PVL in *S. aureus* disease is the subject of much recent research and debate. PVL causes dose-dependent lysis or apoptosis of human polymorphonuclear leukocytes (PMNs) *in vitro*, is expressed at toxic levels in human skin abscesses and seems to elicit a specific immune response following human infection, suggesting a direct role in pathogenesis.[53,245-247] However, despite compelling epidemiological and some clinical evidence linking PVL with diseases caused by CA-MRSA,[84,207,238,248] animal models have yielded seemingly contradictory findings.[61,249,250] Further, some clinical evidence suggests that PVL is not the prime determinant for the severity of infection.[251,252]

PVL is not a direct virulence factor in various murine models using PVL knockout strains of USA300 and USA400.[250,253-256] However, a laboratory strain lysogenised with a PVL bacteriophage produced necrotising pneumonia in mice[61] and a PVL vaccine protects mice against skin and lung infections.[257] Mouse neutrophils are relatively insensitive to PVL compared with human neutrophils so rabbits may be a better animal model.[249] *In vivo* studies have associated PVL with increased severity in osteomyelitis,[258] dermonecrosis,[259,260] and a transient contribution to bacteraemia[249] in rabbits.

Recent studies have suggested that variation in the expressed level of PVL may explain why animal studies using PVL knockouts have yielded contradictory findings.[261] PVL is not uniformly expressed by different CA-

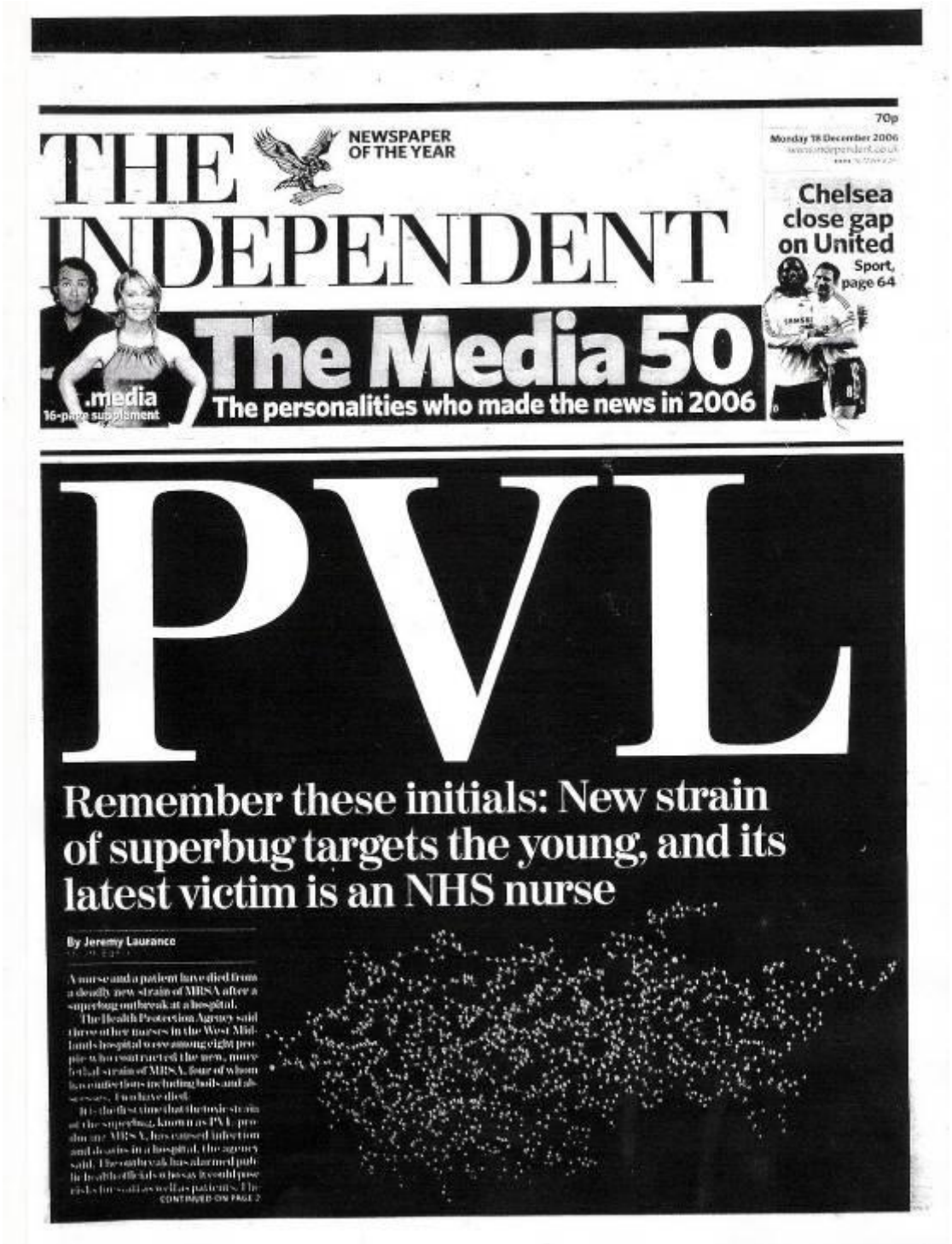
MRSA strains.[262] Genes encoded by lysogenised phage can be co-regulated as part of the host genome or self-regulated when the phage is induced and enters a lytic cycle in response to stress when phage genes may be hyper-produced.[39] Several factors influence the transcription of PVL, including the composition of the growth medium used *in vitro*,[59,263] exposure to sub-lethal concentrations of certain antimicrobial agents,[58,59] the activity of global regulators of gene expression,[263] the phage life cycle[264] and the host background.[264] However, the expression of PVL transcripts does not always correlate with the expression of PVL proteins, suggesting post-transcriptional regulation.[39,264] Further, the USA300 PVL-encoding phage is defective and cannot be induced, suggesting that phage induction is not an important regulatory pathway for toxin expression in CA-MRSA.[261,264] Nonetheless, one recent study of a mouse model of skin infection found that strains producing high levels of PVL resulted in larger skin abscesses, higher bacterial burdens, and more tissue inflammation than did strains producing lower levels of PVL, suggesting that the expression of PVL may be crucial in disease caused by CA-MRSA.[265]

Several recent studies have proposed novel alternatives to PVL as the prime virulence determinant in CA-MRSA. The sequenced genome of a USA300 strain identified several novel proteins, including the Arginine Catabolic Mobile Element (ACME),[240] which may play a role in virulence.[266] A study by Wang *et al.* proposed that the upregulated expression of novel cytolytic peptides, Protein Soluble Modulins (PSMs), could explain the virulence of CA-MRSA[267] and another study proposes synergy between one PSM and PVL.[268] These studies suggest that genetic differences rather than the presence or absence of PVL explain the success of MRSA originating in the community.[269] Therefore, it is not clear whether and to what extent PVL plays a role in the pathogenesis of human infection.

Despite the uncertainty concerning the role of PVL as a virulence factor in disease caused by CA-MRSA, PVL has captured the imagination of the

UK media, as illustrated by The Independent Newspaper headline following the death of a healthcare worker in the UK caused by PVL-positive CA-MRSA in 2006 (Figure 5-2).

Figure 5-2. The Independent front page, 18th December 2006.



5.7 Epidemiology and control of CA-MRSA

CA-MRSA tend to affect certain community groups such as IDUs,[78,227,270-272] indigenous peoples,[79,273] prisoners and those of low socioeconomic status,[274-278] soldiers,[279,280] men who have sex with men,[234] contact sport participants,[281-283] household contacts[216-219] and children.[83,284]

As the epidemic of CA-MRSA has evolved, specific risk factors related to these high risk groups have emerged.[285] These risk factors have been derived largely from outbreaks and form the basis of the Centers for Diseases Prevention and Control (CDC) "5 Cs of CA-MRSA transmission": contact, cleanliness, compromised skin integrity, contaminated objects and crowded living conditions.[285] Prior antimicrobial therapy also appears to be a risk factor for CA-MRSA,[282,286] and has been proposed as a "6th C", namely, "capsules".[285]

Risk factors for CA-MRSA outside of outbreaks are more difficult to identify but seem to include the presence of children at home, home contacts with CA-MRSA SSTIs, lack of hospital contact, recent travel to areas of high CA-MRSA prevalence, injecting drug use, alcoholism, HIV and crowded housing.[287-292] It is likely that CA-MRSA risk factors will reflect the characteristics of local community populations in terms of cultural, behavioural and socio-economic factors.

An emerging feature of the epidemiology of CA-MRSA is non-nasal sites of colonisation and infection without colonisation at recognised carriage sites. Most patients with HA-MRSA infection also have nasal colonisation.[8,9] Colleo *et al.* performed the largest reported study on multiple site screening, examining 403 HA-MRSA-colonised patients: nasal swabs identified 79% of patients colonised at any site; combined swabs from nose and throat, nose and perineum, and nose, throat and perineum identified 86%, 93% and 98% respectively.[293] In contrast, Yang *et al.* found that only 37% of 65 patients with a CA-MRSA infection were colonised at any site, and only 25% were colonised in the nose.[212]

Similarly, another study found that only 22% of IDUs were colonised in the nose whereas 52% were colonised or infected elsewhere.[289] MRSA nasal colonisation was not a significant risk factor for the development of SSTIs in children whereas previous SSTIs and SSTIs in household contacts were.[214] Furthermore, a recent study suggests that exclusive throat carriage of MRSA occurs most frequently in the community in younger people.[294] In contrast to these reports, another study found that nasal colonisation with CA-MRSA was significantly associated with an increased risk of infection.[295] Nevertheless, several reports on CA-MRSA have noted infection in the absence of nasal colonisation, the possibility of homosexual and heterosexual transmission and contamination of fomites, all of which suggest transmission independent of nasal colonisation.[285] It is possible that CA-MRSA are more virulent than HA-MRSA and have the ability to cause infection without first initiating carrier status.

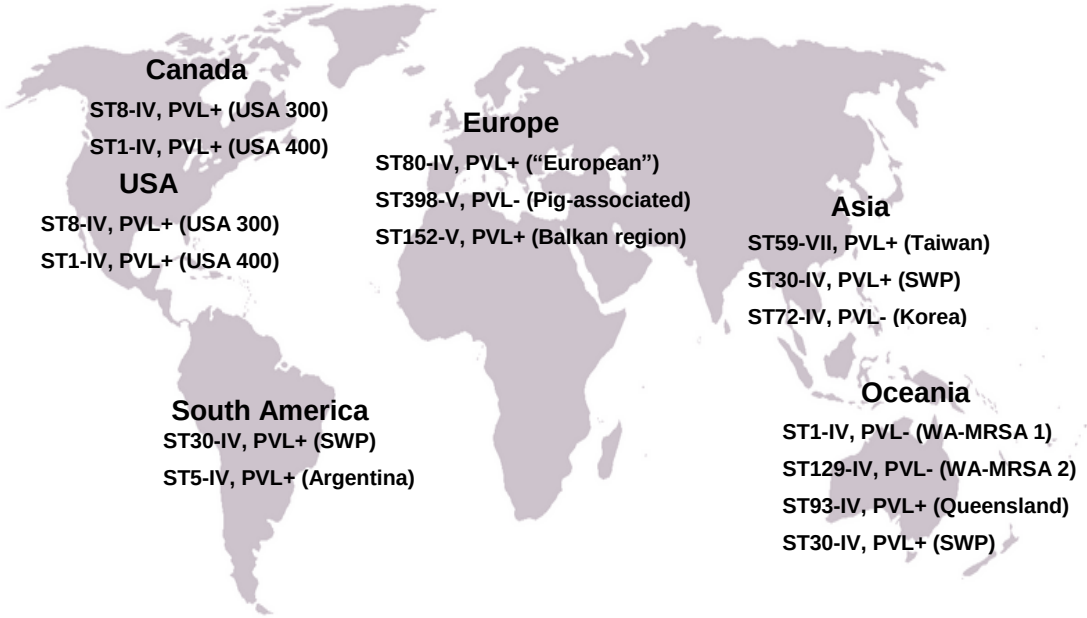
This phenomenon may not be restricted to CA-MRSA. Nasal colonisation was not a risk factor for MSSA infection in a study from New York despite finding five other risk factors: international travel, sports participation, surgery, antibiotic use and towel sharing.[296] Similarly, only 5% of patients with CA-MSSA infection were colonised at any site in the study by Yang *et al.*[212] These data challenge the body of literature showing that approximately 80% of individuals with *S. aureus* SSTIs also have nasal colonisation.[9]

5.7.1 The global molecular epidemiology of CA-MRSA

The most useful molecular methods to differentiate CA-MRSA from HA-MRSA are MLST, *spa* or PFGE combined with SCC*mec* type and PVL status.[297,298] These methods can be combined to characterise CA-MRSA clones circulating in the community and to compare clones internationally. CA-MRSA have been reported from many parts of the world including Europe, North and South America, Australia, Asia and Africa.[72,183,299] As with HA-MRSA, successful clones of CA-MRSA tend to be associated with geographical locations for reasons that are not well understood but

probably relate to socioeconomic factors, antimicrobial prescription and control policies (Figure 5-3).[29,84,299]

Figure 5-3. Global distribution of predominant successful clones of CA-MRSA.



In order to review the literature regarding the global molecular epidemiology of CA-MRSA, PUBMED searches were performed for "community MRSA + [all countries]". Additionally, PUBMED searches were performed for "community MRSA + [all US states]". Relevant articles from the bibliographies of articles identified by PUBMED searches were also included. Only papers written in English were included.

5.7.1.1 USA

The first report of MRSA in the community in the USA came from a community-based outbreak in Detroit, Michigan in the early 1980s.[78,300,301] At the same time, MRSA infections were noticed to be presenting on admission to hospitals in the same geographical area; 66% of 32 isolates shared a single phage type and transmission within the hospital also ocurred.[300] The outbreak caused the proportion of community-acquired *S. aureus* infections resistant to methicillin at a Detroit hospital to rise from 3% in 1980 to 38% in 1981.[301] However, drug use, previous antimicrobial therapy and previous hospital exposure were common risk factors in these

cases, suggesting repeated introduction of the outbreak strain into the hospital rather than widespread nosocomial transmission. Subsequent molecular analysis has identified the outbreak strains as PVL-negative ST74-IV, which has not since become established as a major HA- or CA-MRSA clone.[302]

Reports continued of CA-MRSA among community groups in which close contact, poor hygiene, skin abrasions and sharing of personal items are common, for example, children without traditional risk factors for MRSA,[83,203,303,304] military camps,[305-308] prisons[278,309-311] and sports teams.[81,213,282,312]

Over time, CA-MRSA, in particular USA300, has emerged in those in the community with no recognised CA-MRSA risk factors, suggesting that the CA-MRSA epidemic in the USA has moved to become endemic in the general population. USA300 was responsible for the majority of *S. aureus* skin and soft-tissue infections presenting to emergency departments (EDs) in several states in 2004.[313] It now appears that USA300 is also emerging as a successful cause of healthcare-acquired infection.[314,315]

Data from various national studies support the increase of CA-MRSA in the USA in recent years. One study found that ambulatory care visits to EDs, outpatient clinics and primary care facilities for SSTI increased by 50% from 32.1 to 48.1 visits per 1000 population from 1997-2008.[316] The largest increases were in “safety-net” hospitals (which provide care for uninsured patients), black patients and those under 18, all of which are risk factors for CA-MRSA.[276,277] In another study, ambulatory care outpatient and ED visits increased by 59% and 31%, respectively, in 2003/4 compared with 1992/3.[317] Similarly, ED visits for SSTI increased from 1% of visits in 1993 to 3% of visits in 2005, with the prescription of antibiotics active against MRSA increasing significantly during this period.[318] National studies of the Active Bacterial Core (ABC) network of invasive *S. aureus* disease also implicate CA-MRSA as a substantial cause of invasive disease.[319] For example, USA300 caused 31% of 1984

invasive MRSA isolates in 2005/6; 55% of these were fluoroquinolone resistant.[320]

The first cases of CA-MRSA in the USA were caused by USA400 (ST1-IV, PVL-positive), which includes the MW2 strain that has been sequenced in full.[115,123,321,322] However, USA300 (ST8-IV, PVL-positive) now dominates the CA-MRSA picture in most regions of the USA.[323] Why USA300 is so successful as a community and, increasingly, a hospital pathogen remains to be elucidated, but is the subject of intensive study.[240,269,324,325] Although the USA300 lineage contains some novel genetic markers, such as ACME,[240,323,325] the latest data suggest that modulated gene expression affecting core genome encoded virulence factors may explain the success of USA300.[324]

5.7.1.2 Europe

The molecular epidemiology of CA-MRSA in Europe is summarised in my recent review paper in the *Lancet Infectious Diseases* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**).

In contrast to the dominance of USA300 in the USA, CA-MRSA in Europe is characterised by clonal diversity.[299] The commonest European CA-MRSA isolate is PVL-positive ST80-IV, the so-called ‘European clone’, which has a characteristic antimicrobial susceptibility pattern of resistance to fusidic acid, tetracycline and kanamycin and variable resistance to ciprofloxacin.[84,299,326] This clone may have emerged originally in the Mediterranean, Middle East or North Africa because many of the first patients isolated with this clone in Europe had travel histories to these regions[327-329] and ST80-IV is predominant in Tunisia and Algeria.[330-332] However, regardless of where it originated, this clone currently dominates the CA-MRSA picture in Europe so it is commonly termed the European clone.

Other common international clones such as USA400, SWP and ST59-V have been also reported in Europe (Figure 5-3, p.63).[299,326] In addition, several clones appear to have originated in Europe, such as the ST398-V pig-associated clone, which was reported first from the Netherlands[333-336] and Denmark,[336,337] a PVL-positive ST152-V clone in the Balkan region,[338,339] a Swedish ST150 clone with a novel SCC*mec* type,[340] PVL-negative clones causing infections in injecting drug users in Switzerland (ST45)[271] and the UK (ST1)[227,228] and a ST377-V clone in Greece.[341] Several of these clones, such as the European clone and, increasingly, the ST398-V pig-associated clone, have become globally disseminated while others currently remain localised within Europe.[299,342]

The emergence of the ST398-V pig- or livestock-associated clone is a feature of MRSA epidemiology in the Europe. In 2003, MRSA colonisation was detected in individuals associated with pig farming.[343] The colonising strain was subsequently identified as ST398-V and unusually non-typeable by PFGE with *sma*1;[334,335] it was found to colonise a high proportion of pigs and pig farmers and has the capacity to cause human infections.[124,336] The ST398-V clone recently caused an outbreak in a Dutch hospital affecting nine individuals, including five staff[344] and is now responsible for more than 20% of human MRSA infection in the Netherlands.[333]

Although there is no formal system in place to monitor CA-MRSA in Europe, it appears that the variation in the prevalence of HA-MRSA is not evident for CA-MRSA.[187] For example, the prevalence of HA-MRSA in the Nordic countries and the Netherlands is very low but CA-MRSA infections have emerged despite strict national antimicrobial restriction and infection control policies.[345] Unusual MRSA strains first emerged in the mid to late 1990s in these countries.[346-348] The European clone is currently the predominant CA-MRSA type in the Netherlands and the Nordic countries, although USA300 is emerging.[326,328,349,350]

The prevalence of CA-MRSA in most European countries appears to be low at present, although PVL-positive CA-MRSA accounted for 45% of healthcare-acquired MRSA infections during 2001-3 at hospitals in south-West and central Greece,[351] and hospital outbreaks of CA-MRSA strains have occurred.[352-357] This suggests that CA-MRSA strains could emerge as a more frequent cause of healthcare-acquired infection in European hospitals in the future.

5.7.1.3 The United Kingdom

HA-MRSA is common in the UK[187] but reports of CA-MRSA have been infrequent, although they are increasing. Unusual community strains of MRSA were first identified in IDUs in 2003.[358] More recent reports suggest that an ST1, PVL-negative CA-MRSA clone is circulating among IDUs and homeless people in the UK.[227,228] In 2005, the national *Staphylococcus* reference laboratory for England and Wales reported that only 100 diverse CA-MRSA isolates had been referred in the previous three years, accounting for just 0.005% of all referred MRSA isolates.[359] There have been several sporadic reports of CA-MRSA from other UK laboratories,[101,252,360] including two hospital outbreaks in 2006, one caused by the PVL-negative ST1-IV clone[357] and one by ST30-IV (SWP).[354] The most recent report of 275 PVL-positive isolates referred to the Health Protection Agency in 2005-2006 found that all isolates belonged to recognised CA-MRSA lineages: the European clone accounted for 32% of the isolates, 25% were ST8 (USA300-like) and 18% were the SWP clone.[112] A single ST93 (Queensland clone) isolate was identified and a subsequent study has reported a further 10 cases in the UK, mainly imported from Australia.[361] A recent study using ciprofloxacin susceptibility as a screening marker for CA-MRSA in a Yorkshire found that 24% of isolates were PVL-positive; 24% of these were PVL-positive ST8 and 12% were the European clone.[362]

A recent study indicates that the prevalence of CA-MRSA in the UK may be increasing. This retrospective analysis of the UK General Practice

Research Database reported that the prevalence of epidemiologically-defined presumptive CA-MRSA increased 46% from 332 cases in 2000 to 484 cases in 2004.[363]

5.7.1.4 Australia and New Zealand

Some of the first CA-MRSA to be reported occurred in previously healthy individuals with no recognised risk factors in Australian Aboriginal communities in Western Australia and the Northern Territory in the 1980s and 1990s.[364,365] Despite the emergence of MRSA in the community, hospitals in Western Australia have remained relatively free from HA-MRSA.[79,366] In contrast, HA-MRSA had established themselves in Eastern state hospitals by the late 1970s.[367]

There are substantial differences in the molecular epidemiology of CA-MRSA between states.[231] The differences in the national distribution of CA-MRSA are highlighted in a recent study of 100 consecutive outpatient *S. aureus* isolates from 30 laboratories spread throughout all Australian states and territories.[368] The overall prevalence of MRSA among 2979 *S. aureus* isolates was 16%, ranging from >20% in New South Wales and the Australian Capital Territory to approximately 12% in the other states and territories. The proportion of MRSA that were HA-MRSA clones ranged from 11% in Western Australia to 57% in Victoria/Tasmania. Among the CA-MRSA strains, the Queensland clone (ST93-IV, PVL-positive) was predominant in New South Wales/Australian Capital Territory (56%) and Queensland/Northern Territory (30%), while WA-MRSA-1 (ST1- IV, PVL-negative) accounted for approximately half of the isolates in Western Australia (49%) and South Australia (56%).

CA-MRSA appears to account for a considerable burden of disease with a recent international study reporting that CA-MRSA strains may be responsible for more than a quarter of MRSA bacteraemia in Australia and New Zealand, based on antimicrobial resistance patterns.[369]

The epidemiology of MRSA in New Zealand is largely unknown because most of the results from the National Reference Laboratory are published in local publications that are not readily available outside of New Zealand.[80,370] In 1992-1993 patients who were from, or had recently travelled to Western Samoa, began to be identified with MRSA that had a unique phage pattern, which has since become known as the South West Pacific (SWP) clone. Over the next five years, the prevalence of the SWP clone (predominantly affecting Pacific Islanders) increased more than 10-fold to account for 78% of all MRSA isolates in New Zealand by 1998. However, this decreased to 30% in 2003 due to the emergence of EMRSA-15 in hospitals. By 2005, MRSA affected 165 per 100,000 New Zealanders, although in Auckland, the most populous city in New Zealand by some margin, the incidence was 450 per 100,000.

5.7.1.5 Asia

Most of the reports of CA-MRSA in Asia have come from Taiwan.[371] As in the USA, MRSA were first reported in paediatric patients.[372-374] Genotypic analysis of CA-MRSA in Taiwan has identified two closely related multiresistant clones, both derived from ST59.[236] One is not always PVL-positive and carries SCC*mec* IV[288,375] and the other is PVL-positive and carries a novel SCC*mec* type VII (previously V_T), which contains a variant *ccr* C gene (*ccr*C2).[94,236] A detailed study found that PVL-positive ST59 isolates from Taiwan exhibit unique genetic characteristics, including a unique PVL gene sequence.[94]

The prevalence of MRSA among *S. aureus* isolates in Taiwan is high: for example, in one study MRSA accounted for 74% of 80 *S. aureus* infections, 36% of which occurred in children without recognised risk factors.[376] There is a suggestion that community clones of MRSA are emerging as a significant cause of healthcare-acquired infection in Taiwan. For example, a study of 257 bacteraemia isolates, comprising the first 10% of all bacteraemias from one hospital in Taipei from 1995 to

2006, saw a clonal replacement of HA-MRSA ST239-III with CA-MRSA ST59 isolates.[374]

The prevalence and molecular epidemiology of CA-MRSA elsewhere in Asia is poorly described. There is evidence of a novel PVL-negative ST72-IV clone in Korea, which seems to be associated with both healthcare- and community-acquired infections.[377,378] SWP appears to be the predominant CA-MRSA type in Hong Kong, China and in Singapore, although other clones do appear.[218,379]

5.7.1.6 Other regions

Studies investigating the molecular epidemiology of CA-MRSA in other parts of the world are rare.

USA400 was apparently imported from the USA into neighbouring Canadian provinces in the late 1990s.[380,381] Outbreaks of CA-MRSA have since occurred in high-risk communities groups. For example, USA300 (also known as Canadian MRSA-10) caused an outbreak affecting 5.5% of the homeless, IDUs and individuals with a history of imprisonment in Calgary, Alberta.[382] Recent studies have suggested an increase in the prevalence of CA-MRSA in certain parts of Canada. For example, population-based analysis of *S. aureus* bacteraemia in the Calgary Health Region from 2000-2006 identified a decreasing rate of MSSA bacteraemia but an increasing rate of MRSA bacteraemia, especially with CA-MRSA.[383]

USA300 appears to be established as a cause of CA-MRSA infections in some South American countries,[384-387] although other clones have also been reported, including a SWP replacing traditional HA-MRSA clones as a common cause of healthcare-acquired infection in Uruguay[388] and the emergence of a PVL-positive ST5-IV clone in Argentina.[415]

SWP and the European clone have been reported from Kuwait,[389] and there is evidence that the European clone has been imported into Denmark from Middle Eastern Families.[327]

Several studies suggest that PVL-positive MRSA are a common cause of healthcare and community-acquired infection in some African countries. For example, a study of all 72 MRSA isolates reported at a hospital in Tunisia in 2003-2004 found that 92% were SCC*mec* IV and 99% were PVL positive.[331] A selection of these isolates and additional isolates from 2005 were typed and found to be the European clone.[332] A study from Algeria investigated 61 randomly selected isolates among 204 isolates reported from 2003-2004, which represented 33% of reported MRSA.[330] The European clone accounted for 72% of the isolates and all but one of the PVL-positive isolates. Also, PVL-positive ST88-IV accounted for 45% of MRSA from a Nigerian hospital in 2007.

5.7.2 Prevalence of CA-MRSA and clonal distribution

In most countries, the main burden of MRSA disease continues to be HA-MRSA, but CA-MRSA are now emerging. Surveillance data are limited, but CA-MRSA rates appear to vary considerably around the world.

Accurate ascertainment of the prevalence of CA-MRSA is difficult for several reasons. Firstly, most people affected by CA-MRSA are colonised and not infected so are likely to remain undetected. Secondly, CA-MRSA colonisation of other body sites in the absence of nasal colonisation may be common, so surveys of nasal colonisation rates will underestimate true prevalence.[212,294] Thirdly, even when infections are present, patients are often treated in community or outpatient settings where cultures for *S. aureus* may not be done and/or organisms not identified to strain level. Fourthly, since CA-MRSA are being increasingly isolated in patients with healthcare-contact and are gaining multidrug resistance, they may be misclassified as HA-MRSA.[82] Finally, I only included English language

papers in this review, which may have underestimated the prevalence of CA-MRSA in certain countries.

Despite the limited surveillance data, certain conclusions can be made. The prevalence of CA-MRSA is particularly high in the USA, where CA-MRSA now dominates both hospital and community *S. aureus* infections in certain cities. European CA-MRSA prevalence rates are low but increasing. Strikingly, CA-MRSA have appeared in the Nordic countries and the Netherlands where HA-MRSA rates remain extremely low; in these countries CA-MRSA now appears to be more common than HA-MRSA and is threatening their longstanding success with MRSA control.[328,350] In the Netherlands and the Nordic countries where CA-MRSA have caused much concern, follow-up and investigation of family contacts has increased ascertainment and demonstrated community spread.[327,349] Certain Asian countries seem to be particularly affected, for example Taiwan, where prevalence appears to be high and increasing. Reports of CA-MRSA from most of South America, Asia, Africa and the Middle East are too sporadic to make any firm conclusions.

As with HA-MRSA, the molecular types of CA-MRSA exhibit considerable geographical variation (Figure 5-3, p.63). The USA is dominated by a single successful clone, PVL-positive USA300, but CA-MRSA from most other parts of the world are characterised by clonal diversity.[72,299,323]

In contrast to the predominance of USA300 in the USA, there are many different CA-MRSA clones circulating in Europe.[84,299,326,390] These tend to vary geographically but the PVL-positive European clone is widespread.[84,299] USA300 does occur in Europe and has been reported from several countries but it has not, so far, spread widely. In several reports it is clear that USA300, the European clone and other unusual CA-MRSA types have been introduced into specific areas by immigrants or international travelers.[386,391,392] In the pig-rearing countries of Denmark and the Netherlands the pig-associated ST398-V clone is a particular

emerging problem, which is increasing as a cause of human infections and has already been disseminated internationally.[124,337,342,344]

The SWP clone remains an important cause of CA-MRSA disease globally, but predominantly in the Asia-Pacific region.[80,218,231,379,388] Molecular analysis of the SWP clone suggests that it is descended from the phage type 80/81 MSSA clone that was circulating widely among hospitals in the 1950s.[73]

In Australia, considerable inter-state variation in the types of CA-MRSA is evident.[231,368] The PVL-negative ST1-IV (WA-MRSA-1) predominates in WA whereas the ST93-IV QLD clone predominates CA-MRSA in the Eastern states.

CA-MRSA in several countries seems to be dominated by clones that have arisen locally and not spread widely, for example, PVL-negative ST1-IV in Western Australia,[231,368] ST152-V in the Balkan region in Europe,[338,339] PVL-negative ST45-IV in Israel,[393,394] ST59-IV or VII in Taiwan,[236] ST72-IV in Korea,[378] and ST5-IV in Argentina.[395]

The reasons for the differences in the global molecular epidemiology of CA-MRSA are not well understood. Environmental factors and patient demographics (in particular ethnicity and associated host factors) and socioeconomic factors, are likely involved but have not yet been properly investigated. It is obvious that international travel has been involved in global spread, but this has been rather limited. In a similar way, the much more prevalent and established HA-MRSA clones also remain largely localised. For example, two clones, ST22-IV (EMRSA-15) and ST36-II (EMRSA-16) dominate HA-MRSA in the UK whereas ST5-II (USA100) predominates in the USA.[181,319] Molecular analysis of successful CA-MRSA clones such as SWP and USA300, have identified unique genetic determinants that may well contribute to their success, but do not explain differences in their global distribution.[73,269,323] It seems, therefore, that CA-MRSA have emerged spontaneously by the transfer of SCC*mec* to local

methicillin-susceptible community strains of *S. aureus* in many geographical areas. Those new CA-MRSA strains that have the ability to spread do so locally and then begin to disseminate by international travel. Successful clones may then gradually disseminate widely over time, as USA300 has throughout the USA, the SWP has throughout the Asia-Pacific region and, to a lesser extent, the European clone has throughout Europe. The beginnings of international spread of other clones, such as the ST398 pig-associated clone from Europe, suggests that some other strains may become disseminated globally in the future. However, as with HA-MRSA, the reasons why some clones are more successful than others remain to be elucidated.

5.7.3 Prevalence of colonisation

Despite the global emergence of CA-MRSA, prospective studies of colonisation with MRSA in the community usually identify low rates of carriage, typically 1-3%, in contrast to higher rates of carriage by patients admitted to hospital, typically 3-10%.[155,396-400] I conducted a literature review to determine rates of colonisation in community-based surveys and on admission to hospital. The results of the survey are shown in Appendix 9-1, p.205. The aggregate prevalence of MRSA was 2.6% (1659) of 63564 community-based screens and 5.2% (6552) of 127124 hospital admission screens. The aggregate prevalence of *S. aureus* was 27.1% (14269) of 52657 community-based screens and 21.3% (1814) of 8498 hospital admission screens. However, it is important to note that these aggregates are across different community-based groups ranging from healthily individuals of all ages to special high-risk groups (for example, injecting drug users) and the aggregates of hospital admission screens are across all specialties; so these results should be interpreted with caution.

The majority of studies investigating the prevalence of MRSA in the community rely solely on nasal colonisation; this may be a substantial underestimate of true prevalence in the order of 50% or more.[212] Notwithstanding this crucial limitation, the prevalence of colonisation can

be very high in certain high risk groups: for example, 27% of homeless adults in Columbus, USA,[290] 18% of injecting drug users in Vancouver, Canada,[401] 17% of school children in Pokhara, Nepal,[402] 16% of newly arrested men in Baltimore[403] and 15% of school children in Australian indigenous communities.[404]

Several studies suggest a general increase in the prevalence of CA-MRSA in the USA. For example, the prevalence of MRSA nasal colonisation among a large sample of healthy Americans increased from 0.8% in 2001-2 to 1.5% in 2003-4.[155] Also, the prevalence of MRSA nasal colonisation of healthy children in Nashville, Tennessee rose from less than 1% in 2001 to more than 9% in 2004.[405,406] The general increase in prevalence of CA-MRSA in the USA is supported by the remarkable finding that USA300 strain types were found to be responsible for the majority of *S. aureus* skin and soft-tissue infections presenting to EDs in several states in 2004.[313]

5.7.4 CA-MRSA as a cause of healthcare-acquired infection

One of the hallmarks of the first reports of CA-MRSA was infections in individuals without healthcare contact.[83] However, as the epidemic has evolved, CA-MRSA have begun to emerge as a cause of healthcare-acquired infection.[82,407] This is discussed in a letter that I had published in the *Lancet Infectious Diseases* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**).

Nosocomial outbreaks of CA-MRSA have been reported since 2003 from North America,[216,408-413] Germany,[355] Israel,[394] Switzerland,[217] Greece[352] and the UK,[353,354,357] mostly affecting specialties where the prevalence of HA-MRSA is low, such as paediatrics and obstetrics (Table 5-9, p.76).

Table 5-9. CA-MRSA outbreaks in healthcare settings.

PUBMED search: “Community MRSA healthcare outbreak”. Two PUBMED searches were performed: “MRSA colonisation community” and “MRSA colonisation admission”. Relevant articles from the bibliographies of articles identified by PUBMED searches were also included. Only papers written in English were included.

Ref	Year	Location	Setting	n[a]	Types of Infection (n)	Strain	PVL[b]	Antimicrobial resistance	Comment
411	2003	New York, USA	Maternity	8	Postpartum SSTI (8)[c]	USA300	+	Erythromycin (8/8)	Outbreak strain indistinguishable from USA400 (MW2)
408	2004	Houston, USA	NICU[d]	6	BSI (6)[e]	-	NR	Erythromycin (6/6), Clindamycin (1/6)	All isolates closely related to local CA-MRSA strains by rep-PCR
410	2005	New York, USA	Nursery / Maternity	8	SSTI (8)	USA400	+	None	Outbreak strain indistinguishable or closely related to USA400 (MW2)
394	2005	Ramat-Gan, Israel	NICU	15	BSI (9), sputum (4), nasal colonisation (4)	ST45-IV	-	None	PFGE pattern closely related to an ST45 MSSA and ST45-MRSA-IV identified in the local community
355	2005	Regensbury, Germany	10 healthcare facilities	75	Among patients: colonisation (38) and infection (14)	ST22-IV	+	Fusidic acid	Fifty-two patients, 21 HCWs[f] and 2 others were affected
355	2005	Regensbury, Germany	NICU	8	Among patients: colonisation (3), infection (2)	ST80-IV	+	Fusidic acid	Five patients and three HCWs were affected
409	2006	Chicago,	Nursery	7	SSTI (7)	USA300	NR	NR	5/6 patient isolates and 2/2 isolates from HCW

Ref	Year	Location	Setting	n[a]	Types of Infection (n)	Strain	PVL[b]	Antimicrobial resistance	Comment
		USA							were USA300-0114
409	2006	Los Angeles, USA	Nursery	11	SSTI (7)	USA300	NR	NR	All seven isolates USA300-0114
217	2006	Geneva, Switzerland	NICU	5	Colonisation (5)	ST5-IV	+	Fusidic acid	Mother of the index case was infected with the outbreak strain. Two family members of case patients developed CA-MRSA infection
357	2006	Birmingham, UK	NICU	5	Colonisation (3), sepsis (1), respiratory distress syndrome (1)	ST1-IV	-	Fusidic acid, Clindamycin, Erythromycin	One of the colonised individuals was a HCW; the outbreak strain was indistinguishable from Western Australia MRSA-1 (WA-MRSA-1)
354	2006	West Midlands, UK	Hospital-wide	8	Colonisation (4), infection (4)	ST30-IV	+	None	A previously healthy healthcare worker died of MRSA sepsis
414	2006	Baltimore, USA	Outpatient clinic	2	SSTI (2)	-	+	Erythromycin	Two further HCWs were colonised, but apparently with a distinct strain; 19% of 36 environmental cultures grew MRSA
413	2007	Toronto, Canada	Maternity	45	Babies (35), Mothers (7)	USA300	+	Clindamycin, Erythromycin, Ciprofloxacin	8% of the babies screened during the outbreak were MRSA-positive; no staff tested positive during the outbreak; the outbreak strain was Canadian MRSA-10 (USA300)-related
216	2008	San Antonio, USA	NICU	4	Infection (3), Colonisation (1)	USA300	+	Erythromycin	0.6% of 676 admissions over 18 months were MRSA-positive; three HCWs and two of their children developed MRSA infections

Ref	Year	Location	Setting	n[a]	Types of Infection (n)	Strain	PVL[b]	Antimicrobial resistance	Comment
353	2009	Aberdeen, Scotland	NICU	8	SSTI (8)	ST5-IV	-	Fusidic acid	One mother was infected; all 8 isolates had exfoliative toxin A (*eta*) (epidermolytic toxin)
412	2009	Rhode Island, USA	Security guards	5	SSTI (5)	USA300	+	NR	Four of five cases were traced to restraining one particular patient by a case-control study
352	2009	Athens, Greece	HCW, long term care facility	8	SSTI (8)	ST80-IV	+	NR	MRSA affected 10% of the practice nurses in the facility; being a practice nurse was significantly associated with MRSA in a case-control study

[a] Number of patients involved.

[b] Panton-Valentine leukocidin.

[c] Skin and soft tissue infection.

[d] Neonatal intensive care unit.

[e] Bloodstream infection.

[f] Healthcare workers.

Single strains spreading by cross infection have been responsible for most outbreaks, although six blood stream infections in patients in a Houston NICU may have been due to repeated introduction of local CA-MRSA strains.[408] Healthcare workers have been involved in several of the outbreaks[355,357,409] and in some, hospital workers have been the focus of the epidemics.[352,354,412,414] In one 2006 outbreak affecting healthcare workers in the West Midlands in the UK, a previously healthy healthcare worker died.[354] In several of the outbreaks, family members of affected babies or staff have become infected.[216,217] Although most of the outbreak strains were PVL-positive, the Israeli and two UK outbreaks were caused by PVL-negative strains, demonstrating that CA-MRSA do not need PVL to cause nosocomial infections.[353,357,394]

The control measures applied to outbreaks of CA-MRSA strains in healthcare settings have usually been similar to those implemented for HA-MRSA strains. These include contact isolation of affected patients, screening other patients on the unit for asymptomatic carriage that could be contributing to transmission, educational reinforcement of standard infection control procedures such as hand hygiene, screening staff members for colonization, closure of the unit to new admissions, swabbing of environmental surfaces and equipment and improved environmental cleaning and disinfection.[99,415-419] However, certain features of outbreaks of CA-MRSA strains in healthcare facilities require new control measures. For example, outbreaks have occurred where hospital workers have been the source and/or victims of infection and presumably transmission.[352,354,355,412] Although healthcare workers are often colonised during outbreaks of HA-MRSA strains, they rarely become infected.[420] Therefore, infected healthcare workers need to be managed appropriately in order to control outbreaks of CA-MRSA effectively.

USA300 is an increasingly common community pathogen in the USA with a parallel tendency to cause nosocomial infections after entry into hospitals. Initial reports of USA300 in healthcare settings involved outbreaks in newborn babies and post-operative prosthetic joint

infections.[409,421] Other studies noted that USA300 was responsible for a noticeable proportion of bacteraemias classified as healthcare-acquired.[422,423] In one study, previous hospitalisation was a risk factor for community-onset MRSA infections, 99% of which were caused by USA300.[424]

It appears that USA300 is beginning to supplant traditional HA-MRSA strains as a common cause of hospital infections in Birmingham, Alabama,[425] Chicago, Illinois,[226] Denver, Colorado[315] and San Francisco, California.[426] It seems likely that USA300 has emerged as a significant cause of healthcare-acquired infections elsewhere in the USA. Indeed, national data from the CDC ABC surveillance system identified USA300 as a cause of 16% of hospital onset infections and 22% of healthcare-acquired, community onset infections.[319]

Although most of the literature on the emergence of CA-MRSA strains as a cause of healthcare-acquired infection comes from the USA where the prevalence of CA-MRSA is high, CA-MRSA have caused nosocomial infections in other countries too. In Greece, PVL-positive CA-MRSA accounted for 45% of healthcare-acquired MRSA infections at several hospitals during 2001-3.[351] In Denmark, the incidence of CA-MRSA isolates nationally increased tenfold from 1999-2006 and exceeded that of HA-MRSA isolates in 2006 (2.81 vs. 1.34 per 100,000 inhabitants).[328] The SWP clone is replacing traditional nosocomial strains in one hospital in Uruguay.[388] In Korea, 24% healthcare-acquired bacteraemias in 2007 were caused by the predominant ST72-IV CA-MRSA clone.[378] Although reports from Africa are rare, the European clone appears to account for the majority of healthcare-acquired MRSA in Tunisia and Algeria.[330,332] Several recent mathematical models support the supplanting of HA-MRSA strains by CA-MRSA strains due to an expanding reservoir of MRSA in the community.[427,428]

Studies of CA-MRSA as a cause of epidemiologically defined healthcare-hospitalised with previous MRSA episodes, which may have been

community-acquired, are often classified as healthcare-acquired.[315,425] Furthermore, MRSA infections that manifest after two or three days hospitalisation may have originated from colonisation acquired in the community prior to admission. However, hospital studies demonstrating the supplanting of traditional HA-MRSA clones provide compelling evidence that CA-MRSA strains are being transmitted and acquired inside healthcare facilities.[226,315,388]

The movement of CA-MRSA into hospitals presents several challenges. First, CA-MRSA have the ability to cause infections in previously healthy individuals in the absence of the selective pressure of antimicrobial agents. This puts a wider group of hospitalised patients, healthcare workers and their community contacts potentially at risk of MRSA infection, as demonstrated by CA-MRSA infections in paediatric and obstetric patients,[217,355,357,394,408-411] abscess formation and occasionally severe infection in healthcare workers[354,355] and skin infections in family contacts.[216,217] This resembles the situation with the pandemic phage type 80/81 MSSA, which was a common cause of infection in children and young adults in hospitals and the community in the 1950s, and has been proposed as a progenitor of the SWP clone.[73] However, CA-MRSA strains that have supplanted traditional HA-MRSA strains do not seem to be associated with widespread endemic infection of healthcare workers.

Second, an increased prevalence of PVL-producing CA-MRSA strains in hospitals may increase the severity of nosocomial MRSA infections. However, emerging data suggest that CA-MRSA strains behave like HA-MRSA strains when inside hospitals. A study by Davis *et al.* from Detroit, Michigan, investigated the clinical features and molecular epidemiology of 100 consecutive isolates classified as healthcare-acquired.[429] Fifty-three were USA300 and the remaining 46 were HA-MRSA strain types. The disease profile was similar for both groups of isolates. Similarly, a study from the same research group investigating the clinical features of USA300 strain types causing healthcare- or community-acquired infections found that USA300 causing healthcare-acquired infection were

more likely to cause invasive infections and less likely to cause the uncomplicated SSTIs that have characterised USA300 in community settings.[314] Benoit *et al.* found that healthcare-onset infections caused by CA-MRSA strains were more likely to cause non-skin diseases and to occur in older patients than community-onset infections caused by CA-MRSA strains.[388]

Third, the exposure of CA-MRSA to nosocomial antibiotic pressure will encourage the emergence of multiple-resistance. Even if CA-MRSA strains in healthcare environments behave more like HA-MRSA in terms of the infections they cause, the non-multiresistant phenotype associated with CA-MRSA strains in the community is likely to change with continued exposure to nosocomial antibiotic selection pressure. Ominously, in one US study, USA300 strains classified as healthcare-acquired were significantly more likely to be ciprofloxacin-resistant than USA300 classified as community-acquired.[430] Also, a case of USA300 with intermediate susceptibility to vancomycin and reduced susceptibility to daptomycin was reported in Chicago in 2007.[431]

Fourth, the terminology surrounding CA-MRSA is confused by MRSA originating the community causing healthcare-acquired infection, which is often termed 'healthcare-associated'.[225] Future terminology may need to define true CA-MRSA as MRSA originating in the community.

Finally, the control of MRSA in hospitals will be further hampered by the constant re-introductions of CA-MRSA from an expanding community reservoir.

5.7.5 Controlling CA-MRSA

The emergence of CA-MRSA has produced new infection control challenges that must be urgently addressed to prevent sporadic infections in Europe and other parts of the world from becoming endemic, as in the

USA. The current limited data on how the epidemiology of CA-MRSA differs from HA-MRSA hampers control efforts.[432]

The control of pig-associated CA-MRSA is obviously a special issue that is being addressed by countries with major pig-farming industries, such as the Netherlands and Denmark.[334] Otherwise, control measures are hampered by an incomplete understanding of the epidemiology of CA-MRSA. For example, non-nasal sites of colonisation and uncertainty surrounding transmission routes makes identification of carriers and the development of a community-based infection control strategies difficult.[285] One recent study showed that community-based decolonisation can be effective in an area where colonisation was sporadic,[287] but this may not be feasible in areas of higher prevalence. Indeed, there are several examples of failure to decolonise individuals with CA-MRSA[285,344,433] and the latest CDC guidelines for the control of CA-MRSA in the USA do not recommend contract tracing or decolonisation with topical antimicrobial agents.[434]

Unlike HA-MRSA, CA-MRSA spreads successfully in the community and transmission among household contacts or within community subsets such as IDUs has been a feature of several reports.[228,234,272,273,282,327,328,349,435] CA-MRSA also cause nosocomial outbreaks, affecting younger and less compromised patients than HA-MRSA and involving previously spared groups such as paediatrics and healthcare workers (Table 5-9, p.76).[82,225,407] There is thus an urgent need to clarify the prevalence and epidemiology of CA-MRSA and to develop systems for the identification and control of these organisms in the community, in hospitals and other healthcare facilities, and at the community-hospital interface.

5.8 Aims, objectives and hypotheses

At the commencement of the project in 2005, the molecular epidemiology of CA-MRSA in the UK was poorly described. The only report available

other than outbreaks and case reports was a handful of referred cases from the Health Protection Agency (HPA) national reference laboratory.[359] My initial hypothesis therefore was that CA-MRSA have emerged at GSTT but that this has been masked by the volume of HA-MRSA identified in the clinical laboratory. In order to test this hypothesis, I conducted a retrospective study of stored MRSA using ciprofloxacin susceptibility as a screening marker because the majority of HA-MRSA are ciprofloxacin-resistant and most CA-MRSA that had been reported in the UK were ciprofloxacin-susceptible.[359]

Following the identification of a substantial and apparently increasing presence of CA-MRSA at GSTT, my aim was to better define the prevalence of CA-MRSA. I first tested the hypothesis that antimicrobial-susceptibility based algorithms can be used as an accurate screening marker for the presumptive identification of CA-MRSA in a collection of predominantly HA-MRSA isolates. Then I conducted a prospective study of MRSA identified on admission screens, hypothesising that CA-MRSA strains account for a considerable proportion of MRSA colonising patients admitted to GSTT.

Finally, detailed analysis of PVL-positive MRSA clinical isolates identified at GSTT allowed me to test the hypothesis that polymorphisms in the PVL genes vary with the PVL-encoding phage.

6 METHODS

I conducted all of the practical work, unless otherwise stated.

6.1 Reference strains

The name, origin and use of reference strains used in the project are summarised in Table 6-1, p.86.

6.2 Identification, culture and storage of *S. aureus*

Throughout the project, *S. aureus* was cultured from nutrient agar slopes that were saved from pure cultures by the routine microbiology laboratory and stored at room temperature or from *S. aureus* frozen at -70°C in glycerol broth. Therefore, *S. aureus* required confirmation rather than definitive identification. The standard methods used to confirm *S. aureus* were coagulase production and mannitol fermentation. Gram staining was used to confirm the identity of isolates with equivocal results. *S. aureus* isolates were cultured on blood agar (Oxoid, Basingstoke, UK) unless otherwise stated.

A Pastorex™ Staph-Plus (Bio-Rad, Hemel Hempstead, UK) latex-agglutination test was used for the rapid identification of bound coagulase, as instructed by the manufacturer. A tube coagulase test was used to detect both bound and free coagulase for isolates that were negative by the latex-agglutination test. Fresh human plasma (Octapharma Ltd., Coventry, UK) was diluted 1/10 in sterile saline (Oxoid, Basingstoke, UK) and 1mL added to 1mL tryptone water (Oxoid, Basingstoke, UK) in a plastic bijou bottle. Five colonies from a BA plate were emulsified in the mixture and incubated for four hours at 37°C. Positive and negative controls were incubated with each batch (Table 6-1, p.86). Any degree of clot formation in the test tubes was considered a positive result.

Table 6-1. Reference strains.

Name	Use	Origin
S. aureus NCTC 6571	Positive control for methicillin resistance	NCTC[a]
S. aureus NCTC 8532	Positive control for tube coagulase	NCTC
S. epidermidis NCTC 4276	Negative control for tube coagulase	NCTC
S. aureus BAA-44	SCC*mec* I control	ATCC[b]
S. aureus NCTC 10442	SCC*mec* I control	NCTC
S. aureus 'N315'	SCC*mec* II control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus NCTC 11939 (EMRSA-1)	SCC*mec* III control	NCTC
S. aureus '85/2082'	SCC*mec* III control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'JCSC 4788'	SCC*mec* IVc control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'JCSC 4469'	SCC*mec* IVd control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'WIS'	SCC*mec* V control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus '81/108'	Φ108PVL control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'JCSC2958'	ΦSa2958 control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'RN4220'	ΦSLT control	Dr Teruyo Ito (Juntendo University, Tokyo, Japan)
S. aureus 'ST30-108'	Φ108PVL control	Eve Boakes (Health Protection Agency, London)
S. aureus 'ST22-PVL'	ΦPVL control	Eve Boakes (Health Protection Agency, London)
S. aureus 'PH1'	*t127* strain for PVL phage analysis	Dr Angela Kearns (Health Protection Agency, London)
S. aureus 'PH2'	*t127* strain for PVL phage analysis	Dr Angela Kearns (Health Protection Agency, London)
S. aureus 'WA-MRSA-1 (ST1, PVL-)'	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)
S. aureus 'USA400 (ST1, PVL+)'	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)
S. aureus 'ST5, PVL-'	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)
S. aureus 'USA300 (ST8, PVL+)'	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)

Name	Use	Origin
S. aureus ‘EMRSA-15 (ST22, PVL-)’	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)
S. aureus ‘ST59, PVL+’	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)
S. aureus ‘ST80, PVL+’	PFGE type strain	Dr Angela Kearns (Health Protection Agency, London)

[a] National collection of type culture.

[b] American type culture collection.

To test for mannitol fermentation, mannitol salt agar (MSA, Oxoid, Basingstoke, UK) was inoculated with a single streak of a pure colony and incubated at 37°C overnight. The presence of yellow coloured colonies after overnight incubation was considered a positive result.

To conduct a Gram-stain, a part of a single colony from a BA plate was emulsified in a drop of sterile water on a microscope slide and allowed to air dry. The smear was heat fixed by passing the slide through the flame of a Bunsen burner three times and allowed to cool before staining. The slide was placed on a staining rack, flooded with crystal violet for 30s then rinsed with tap water; flooded with iodine for 30s then rinsed with tap water; flooded with acetone for 5s then rinsed with tap water; and flooded with saffranin for 60s then rinsed with tap water and allowed to air dry. Stained slides were examined using light microscopy: Gram-positive organisms appear purple and Gram-negative organisms appear pink. *S. aureus* cells are Gram-positive cocci with a characteristic “bunch of grapes” appearance.

6.3 Antimicrobial susceptibility testing

6.3.1 BSAC disc diffusion

S. aureus isolates were tested for susceptibility to the following agents using the British Society for Antimicrobial Chemotherapy disc diffusion method:[110] penicillin, gentamicin, neomycin, vancomycin, erythromycin, fusidic acid, tetracycline, linezolid, rifampicin, mupirocin, trimethoprim and ciprofloxacin. Isosensitest agar (ISA, Oxoid, Basingstoke, UK) was used for all antimicrobial agents. *S. aureus* NCTC 6571 was included with each batch of tested organisms as a control.

An inoculum to result in semi-confluent growth after overnight incubation was obtained by emulsifying five colonies of *S. aureus* in 5mL of distilled water. A dry cotton swab was dipped into the suspension and spread over the surface of an ISA plate using a rotary plater. A semi-automated disc

dispenser was used to place discs impregnated with the appropriate concentration of antimicrobial agent onto the surface of the plate. Inoculated plates were incubated for 18-24h and zones of inhibition were measured. *S. aureus* isolates were considered resistant if zones of inhibition were less than or equal to those set out in (Table 6-2, p.89).

The addition of salt to the testing medium and incubation at 30°C rather than 37°C improves the expression of methicillin resistance.[6] Therefore, methicillin susceptibility was tested separately on Columbia agar with 2% NaCl (Oxoid, Basingstoke, UK). A cotton tipped swab was dipped into the suspension described above and used to inoculate one quarter of a CSA plate. Sterile forceps were used to place a methicillin discs in the centre of the inoculum. CSA plates were incubated for 18-24h at 30°C and zones of inhibition were measured. *S. aureus* isolates were considered methicillin-resistant if zones of inhibition were ≤14mm (Table 6-2).

Table 6-2. Zone diameter breakpoints.

Antimicrobial agent	Disc content (µg)	Zone of inhibition (diameter, mm) for resistance
Penicillin	25	≤25
Gentamicin	10	≤19
Vancomycin	5	≤11
Erythromycin	5	≤19
Fusidic acid	10	≤29
Tetracycline	10	≤19
Linezolid	10	≤19
Rifampicin	2	≤29
Neomycin	10	≤16
Mupirocin	5	≤21
Trimethoprim	5	≤19
Ciprofloxacin	1	≤13
Methicillin	5	≤14

6.3.2 Oxacillin minimum inhibitory concentration

6.3.2.1 BSAC agar dilution

To determine the oxacillin MIC by the BSAC agar dilution method,[110,436] plates containing doubling concentrations of oxacillin from 0.125-128 mg/L were prepared using molten Muller-Hinton agar (Oxoid, Basingstoke, UK) with the addition of 2% NaCl. To prepare the inoculum, four colonies of each test organism were incubated overnight at 37°C in 3mL isosensitest broth (ISB, Oxoid, Basingstoke, UK). Ten microlitres of the overnight broth were transferred into 600µL of ISB in the loading well of a 38-well multipoint inoculator. *S. aureus* NCTC 6571 was tested with each batch as a positive control. The multipoint inoculator was used to inoculate each test strain onto each agar dilution plate, including a control plate with no oxacillin. All plates were incubated at 30°C for 24h and the MIC was determined by which plate concentration inhibited the growth of each test strain; organisms were considered oxacillin-resistant if growth was evident on the plate containing ≥4mg/L.

6.3.2.2 Etest®

The Etest (AB BIODISK, Solna, Sweden) consists of a plastic strip (5x57mm) impregnated with a concentration gradient of the test antimicrobial agent and calibrated with a visual MIC scale in µg/mL. The Etest strip is placed onto the centre of an inoculated agar plate and results in a symmetrical inhibition ellipse; the MIC is the point where the edge of the inhibition ellipse intersects the strip.

To determine the oxacillin MIC by Etest, overnight cultures of *S. aureus* on BA were suspended in sterile saline to a 0.5 McFarland standard. A dry cotton swab was dipped into the suspension and spread over the surface of an ISA plate using a rotary plater. An Etest oxacillin strip was laid in the centre of the ISA plate using sterile forceps and incubated at 37°C for 24h.

S. aureus isolates were considered oxacillin resistant if the MIC was ≥4mg/L.

6.3.3 D test for inducible clindamycin resistance

Two primary mechanisms result in resistance to macrolides (including erythromycin), lincosamide (including clindamycin), and Group B Streptogramin (MLSB) antimicrobials in staphylococci: a macrolide efflux pump system does not confer resistance to lincosamides such as clindamycin whereas modification of the drug binding site on the ribosome mediated an *erm* gene methylation of the 23S rRNA binding site does confer resistance to lincosamides.[437,438] *erm* mediated resistance can be expressed either constitutively (MLSBc) or when induced (MLSBi). MLSBc requires additional mutational changes of the binding site resulting in constitutive expression of an *erm* gene. Exposure of an MLSBi strain to a suitable macrolide inducer, such as erythromycin, results in the expression of the *erm* gene and resistance to all MLSB antimicrobials.

The D test is used to determine whether an organism has inducible clindamycin-resistance (MLSBi).[439] Erythromycin and clindamycin discs are placed on a lawn of bacteria and if there is a flattening of the zone of inhibition around the clindamycin disc on the edge facing the erythromycin disc (a “D” shaped zone) after overnight incubation, the organism is considered to have MLSBi.

To conduct the D test for inducible clindamycin resistance in strains that were resistant to erythromycin but apparently susceptible to clindamycin, overnight cultures of *S. aureus* on BA were suspended in sterile saline to a 0.5 McFarland standard and diluted 1/10 in sterile distilled water. A dry cotton swab was dipped into the suspension and spread over the surface of an ISA plate using a rotary plater. Discs containing erythromycin (5µg) and clindamycin (2µg) were placed approximately 20mm apart using sterile forceps. Organisms with D shaped zones of clearing around the

clindamycin disc after incubation at 37°C for 24h were considered to have MLSBi.

6.3.4 Automated broth microdilution (Vitek)

The Vitek 2 uses automated broth microdilution to provide a quantitative MIC for the following antimicrobial agents: benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, teicoplanin, vancomycin, tetracycline, nitrofurantoin, fusidic acid, chloramphenicol, rifampicin and mupirocin (testing card: AST-P555, bioMérieux, Basingstoke, UK). The card includes a cefoxicitn screen to confirm MRSA if the oxacillin MIC is above the breakpoint for resistance. The automated system includes the 'Advanced Expert System' (AES), which compares observed MIC results and the organism identification with a database of expected MIC distributions and makes adjustments accordingly. For example, MRSA that are resistant to erythromycin but have an MIC below the breakpoint for clindamycin resistance are assumed by AES to have inducible clindamycin resistance.

To prepare inocula for Vitek 2, overnight *S. aureus* cultures on BA were suspended in 3mL sterile saline to a 0.5 McFarland standard (acceptable range 0.5-0.63) using a spectrophotometer (bioMérieux, Basingstoke, UK) and loaded into the Vitek 2 machine according to the manufacturer's instructions.

6.4 DNA extraction

DNA was extracted using the ChargeSwitch® gDNA mini-bacteria kit (Invitrogen Ltd., Paisley, UK). The ChargeSwitch system works by using magnetic beads to bind the DNA following lysis of the cells. In low pH conditions, the ChargeSwitch beads have a positive charge that binds the negatively charged nucleic acid backbone. The charge on the surface of the beads is neutralised by raising the pH in a low salt elution buffer to elute the DNA from the beads.

A single colony of *S. aureus* on a BA plate was inoculated into a Brain-Heart Infusion (BHI) broth. Following overnight incubation 37°C overnight, 0.5mL of the broth was used and the ChargeSwitch procedure was followed as per the manufacturer's instructions. DNA was eluted in 200µL volumes, which were stored at -20°C in 50µL aliquots.

6.5 Polymerase chain reaction (PCR)

All PCRs were performed on a Dyad™ DNA Engine Peltier Thermal Cycler (Bio-Rad, Hemel Hempstead, UK). PCRs were conducted using Platinum® Taq DNA polymerase and buffer unless otherwise stated (Invitrogen, Paisley, UK). Massrular™ (Fermantas, York, UK) molecular weight markers were used unless otherwise stated. Positive, negative and template controls were included wherever possible. Template controls were *S. aureus* DNA known to be negative for the target fragment(s) to ensure that no non-specific primer annealing occurred. PCR products were run through 1.5% agarose gels containing 0.4mg/L ethidium bromide at 5V/cm for 60 minutes unless otherwise stated, and visualised and photographed using a UV transilluminator with a fixed camera attached (Mini-darkroom GDS 8000 / camera kit, UVP, Upland, CA, USA).

6.6 DNA sequencing

Dye-terminator sequencing was used to sequence DNA.[440] DNA fragments were amplified by PCR and quantified by comparing the size of the fragment and the intensity of the band with the molecular weight marker using the UV transilluminator software (Mini-darkroom GDS 8000 / camera kit, UVP, Upland, CA, USA). The PCR product was purified from PCR reagents using ChargeSwitch®-Pro PCR Clean-up Kit (Invitrogen Ltd., Paisley, UK). The system uses purification columns containing a membrane that is positively charged at low pH to bind the negatively charged DNA and neutral at pH 8.5–9.0 to elute PCR products.

Sequencing reactions were prepared using a GenomeLab DTCS Quick Start Kit (Beckman Coulter, High Wycombe, UK). Sequencing reactions contained 1 x Mastermix, 0.4µM sequencing primer and 10-15ng template DNA in 10µL reaction volumes. Sequencing reactions were cleaned up from sequencing reaction reagents using an Agencourt® CleanSEQ® Dye Terminator Removal Kit (Beckman Coulter, High Wycombe, UK). The system uses beads that are positively charged at low pH to bind the negatively charged sequenced product and neutral at higher pH to elute sequenced products. Sequences were determined using the Beckman-Coulter SEQ™ 8000 sequencer. In the later stages of the project, DNA sequencing of PCR products was conducted by GATC (GATC Biotech Ltd., Cambridge, UK). Sequences were analysed using Sequencer 4.9 (Gene Codes, Ann Arbor, MI, USA). Related DNA sequences were searched using the Basic Local Alignment Search Tool (BLAST) available at www.blast.ncbi.nlm.nih.gov/Blast.

6.7 SCC*mec* allotyping

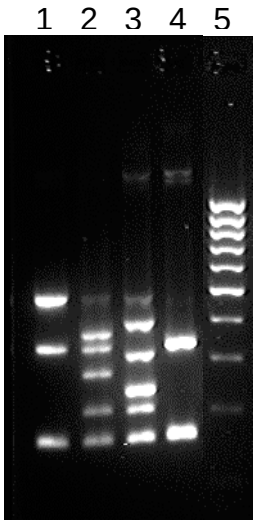
6.7.1 Method 1: Oliveira and de Lencastre

Oliveira and de Lencastre developed a multiplex PCR based method to differentiate structural variants of SCC*mec.*[45] The method includes eight primer pairs (loci A-H) plus primers for *mec*A. The criteria for the development of the assay were to amplify at least one fragment upstream and downstream of *mec*A for SCC*mec* types I, II, III and IV. This was achieved for all but SCC*mec* IV, for which a specific upstream region could not be identified. The assay also included primer pairs to differentiate IA from I and IIIA from III. The name, amplicon size, specificity and final concentrations of primers used for the Oliveira and de Lencastre assay are summarised in Table 6-3, p.96.

PCR reaction mixtures contained a primer mixture with the final concentrations indicated in Table 6-3 (p.96), 1x amplification buffer, 0.2mM each dNTP, 1.5mM $MgCl_2$, 1U DNA polymerase and 2µL of

extracted DNA in 25µL reaction volumes. PCR conditions were: 94°C for 30s; 30 cycles of 94° C for 30s, 53°C for 30s, and 72°C for 1 min; 72°C for 4min; then hold at 4°C. Representative band patterns for the Oliveira and de Lencastre assay are shown in Figure 6-1.

Figure 6-1. Representative SCC*mec* bands with the method of Oliveira and de Lencastre.



Well 1 = SCC*mec* I (NCTC 10442); well 2 = SCC*mec* II (N315); well 3 = SCC*mec* III (85/2082); well 4 = SCC*mec* IV (JCSC 4788); well 5 = representative molecular weight marker, 100-1000bp.

Table 6-3. Primers for the Oliveira and de Lencastre SCC*mec* assay.

Locus	Name	Sequence (5'-3')	Amplicon size / bp	Specificity	Final concentration / µM
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	I	0.4
	CIF2 R2	ATTTACCACAAGGACTACCAGC			0.4
B	KDP F1	AATCATCTGCCATTGGTGATGC	284	II	0.2
	KDP R1	CGAATGAAGTGAAAGAAAGTGG			0.2
C	MECI P2	ATCAAGACTTGCATTCAGGC	209	II,III	0.4
	MECI P3	GCGGTTTCAATTCACTTGTC			0.4
D	DCS F2	CATCCTATGATAGCTTGGTC	342	I, II, IV	0.8
	DCS R1	CTAAATCATAGCCATGACCG			0.8
E	RIF4 F3	GTGATTGTTCGAGATATGTGG	243	III	0.2
	RIF4 F9	CGCTTTATCTGTATCTATCGC			0.2
F	RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	III	0.4
	RIF5 R13	GTCACAGTAATTCCATCAATGC			0.4
G	IS431 P4	CAGGTCTCTTCAGATCTACG	381	IA	0.8
	Pub110 R1	GAGCCATAAACACCAATAGCC			0.4

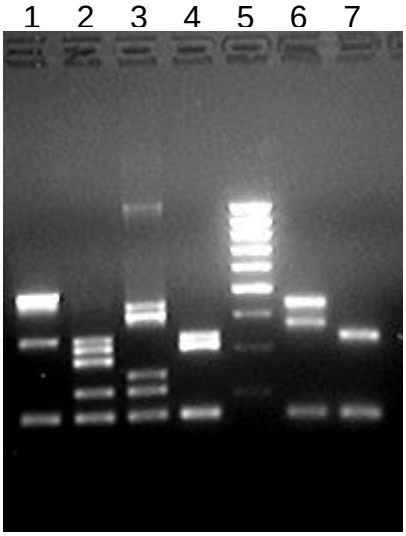
Locus	Name	Sequence (5’-3’)	Amplicon size / bp	Specificity	Final concentration / µM
H	IS431 P4	CAGGTCTCTTCAGATCTACG	303	III (not IIIA)	0.8
	pT181 R1	GAAGAATGGGGAAAGCTTCAC			0.4
*mec*A	MECA P4	TCCAGATTACAACTTCACCAGG	162	*mec*A	0.8
	MECA P7	CCACTTCATATCTTGTAACG			0.8

6.7.2 Method 2: Milheirico *et al.*

In 2007, Milheirico *et al.* published an update of the Oliveria and de Lencastre assay to better characterise the SCC*mec* IV element and to include the SCC*mec* V element.[46] Primers to differentiate types IA from I and IIIA from III were excluded and new primers were added for the detection of *ccr*B2 (specific for SCC*mec* types II and IV), *ccr*C (specific for SCC*mec* type V), the SCC*mec* type III J1 region, and the SCC*mec* type V J1 region (Table 6-4, p.99).

PCR reaction mixtures contained a primer mixture with the final concentrations indicated in Table 6-4 (p.99), 1x amplification buffer, 0.04mM each dNTP, 1.5mM $MgCl_2$, 1U DNA polymerase and 2µL of extracted DNA in 25µL reaction volumes. PCR conditions were: 94°C for 30s; 30 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 1 min; 72°C for 4min; then hold at 4°C. Representative band patterns for the Milheirico *et al.* assay are shown in Figure 6-2.

Figure 6-2. Representative SCC*mec* bands with the method of Milheirico *et al.*



Well 1 = SCC*mec* I (NCTC 10442); well 2 = SCC*mec* II (N315); well 3 = SCC*mec* III (85/2082); well 4 = SCC*mec* IV (JCSC 4788); well 5 = molecular weight marker, 100-1000bp; well 6 = SCC*mec* V (WIS); well 7 = SCC*mec* VI (clinical isolate).

Table 6-4. Primers used for the Milheirico *et al.* SCC*mec* assay.

Locus	Name	Sequence (5'-3')	Amplicon size / bp	Specificity (SCC*mec* type, region)	Final concentration / µM
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	I, J1 region	0.4
	CIF2 R2	ATTTACCACAAGGACTACCAGC			0.4
B	CCRC F2	GTACTCGTTACAATGTTTGG	449	V, *ccr* complex	0.8
	CCRC R2	ATAATGGCTTCATGCTTACC			0.8
C	RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	III, J3 region	0.4
	RIF5 R13	GTCACAGTAATTCCATCAATGC			0.4
D	SCCMEC V J1 F	TTCTCCATTCTTGTTCATCC	377	V, J1 region	0.4
	SCCMEC V J1 R	AGAGACTACTGACTTAAGTGG			0.4
E	DCS F2	CATCCTATGATAGCTTGGTC	342	I, II, IV, and VI, J3 region	0.8
	DCS R1	CTAAATCATAGCCATGACCG			0.8
F	CCRB2 F2	AGTTTCTCAGAATTCGAACG	311	II and IV, *ccr* complex	0.8
	CCRB2 R2	CCGATATAGAAWGGGTTAGC			0.8

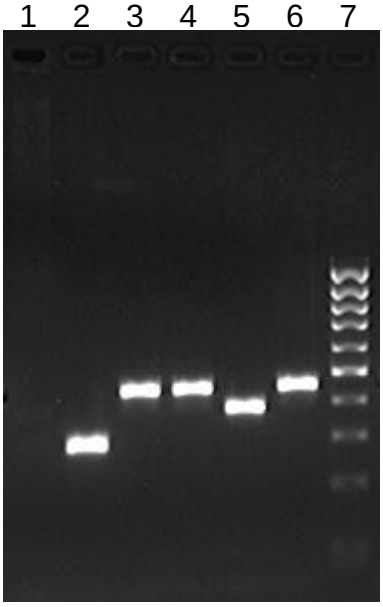
Locus	Name	Sequence (5’-3’)	Amplicon size / bp	Specificity (SCC*mec* type, region)	Final concentration / µM
G	KDP F1	AATCATCTGCCATTGGTGATGC	284	II, J1 region	0.2
	KDP R1	CGAATGAAGTGAAAGAAAGTGG			0.2
H	SCCMEC III J1 F	CATTTGTGAAACACAGTACG	243	III, J1 region	0.4
	SCCMEC III J1 R	GTTATTGAGACTCCTAAAGC			0.4
I	MECI P2	ATCAAGACTTGCATTCAGGC	209	II and III, *mec* complex	0.8
	MECI P3	GCGGTTTCAATTCACTTGTC			0.8
J	*mec*A P4	TCCAGATTACAACTTCACCAGG	162	*mec*A	0.8
	*mec*A P7	CCACTTCATATCTTGTAACG			0.8

6.7.3 SCC*mec* IV sub-typing

Based on differences in the J1 region, SCC*mec* IV can be sub-typed into several structural variants.[29] SCC*mec* IV isolates were sub-typed into elements IVa through IVd using a method described by Holmes *et al.*[101] with primers from Huletsky *et al.* and Okuma *et al.* (Table 6-5, p.102).[202,611] A more recent multiplex PCR method for sub-typing SCC*mec* IV was not used during the project.[441]

PCR reaction mixtures contained a primer mixture with 0.2µM of each primer (Table 6-5, p.102), 1x amplification buffer, 0.2mM each dNTP, 2.5mM $MgCl_2$, 1.25U DNA polymerase and 2µL of extracted DNA in 25µL reaction volumes. PCR conditions were: 94°C for 30s; 30 cycles of 94°C for 30s, 60°C for 1 min, and 72°C for 2 min; 72°C for 4min; then hold at 4°C. Representative band patterns for the SCC*mec* IV sub-typing assay are shown in Figure 6-3.

Figure 6-3. Representative bands from SCC*mec* IV sub-typing.



Well 1 = negative control; well 2 = IVd (clinical isolate); wells 3, 4 and 6 = IVa (clinical isolates); well 5 = IVc (clinical isolate); well 7 = molecular weight marker, 100-1000bp. No IVb clinical isolates were identified and a reference for IVb was requested but not sent.

Table 6-5. Primers used for SCC*mec* IV sub-typing.

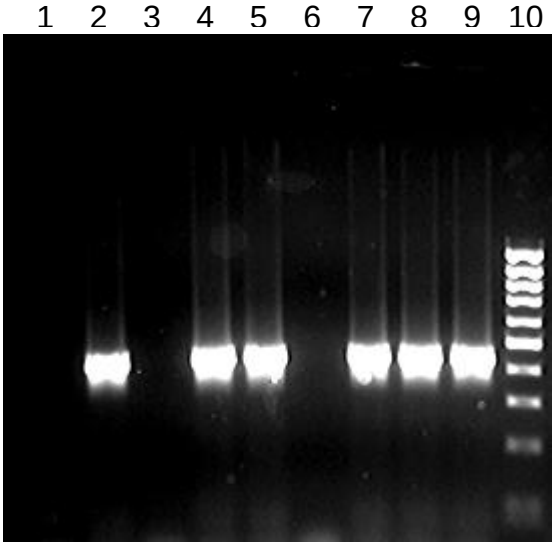
Name	Sequence (5'-3')	Amplicon size / bp	Specificity (SCC*mec,* region)	Final concentration / µM	Reference
4a1	TTTGAATGCCCTCCATGAATAAAAT	450	IVa, J1 region	0.2	Okuma *et al*. 2002[220]
4a2	AGAAAAGATAGAAGTTCGAAAGA			0.2	
4b1	AGTACATTTTATCTTTGCGTA	1000	IVb, J1 region	0.2	Okuma *et al*. 2002[220]
4b2	AGTCATCTTCAATATCGAGAAAGTA			0.2	
mecIVc70	TGGGGTATTTTTATCTTCAACTC	392	IVc, J1 region	0.2	Huletsky *et al*. 2004[442]
mecIVc1079	TGGGATTTTAAAGCAGAATATCA			0.2	
mecIVd26	ACGGGAGATTAGGAGATGTTAT	302	IVd, J1 region	0.2	Huletsky *et al*. 2004[442]
mecIVd307	CAGCCATCAATTTTGTTTCACC			0.2	

6.8 PVL

6.8.1 PCR to detect the PVL genes

A PCR assay that amplifies a 433bp fragment spanning the junction between *luk*S and *luk*F was used to test whether organisms encoded PVL.[238] PCR reaction mixtures contained a 0.2mM final concentration of primers luk-PV-1 (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3') and luk-PV-2 (5'-GCATCAASTGTATTGGATAGCAAAAGC-3'), 1x amplification buffer, 0.2mM each dNTP, 1.5mM $MgCl_2$, 1U DNA polymerase and 2µL of extracted DNA in 25µL reaction volumes. PCR conditions were: 94°C for 30s; 30 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 1 min; then hold at 4°C. Representative bands from the PVL assay described by Lina *et al.* are shown in Figure 6-4.

Figure 6-4. Representative bands from the Lina *et al.* PVL assay.



Well 1 = negative control; well 2 = positive control (clinical isolate); well 3 = template control (PVL-negative clinical isolate); wells 4-9 = clinical isolates; well 10 = molecular weight marker, 100-1000bp.

6.8.2 Sequencing the PVL genes

Primers to amplify and DNA sequence the 1918bp *lukSF-PV* genes were designed based on the genome sequence of USA300 (Table 6-6).[240]

Table 6-6. Primers used to amplify and sequence *lukSF-PV.*

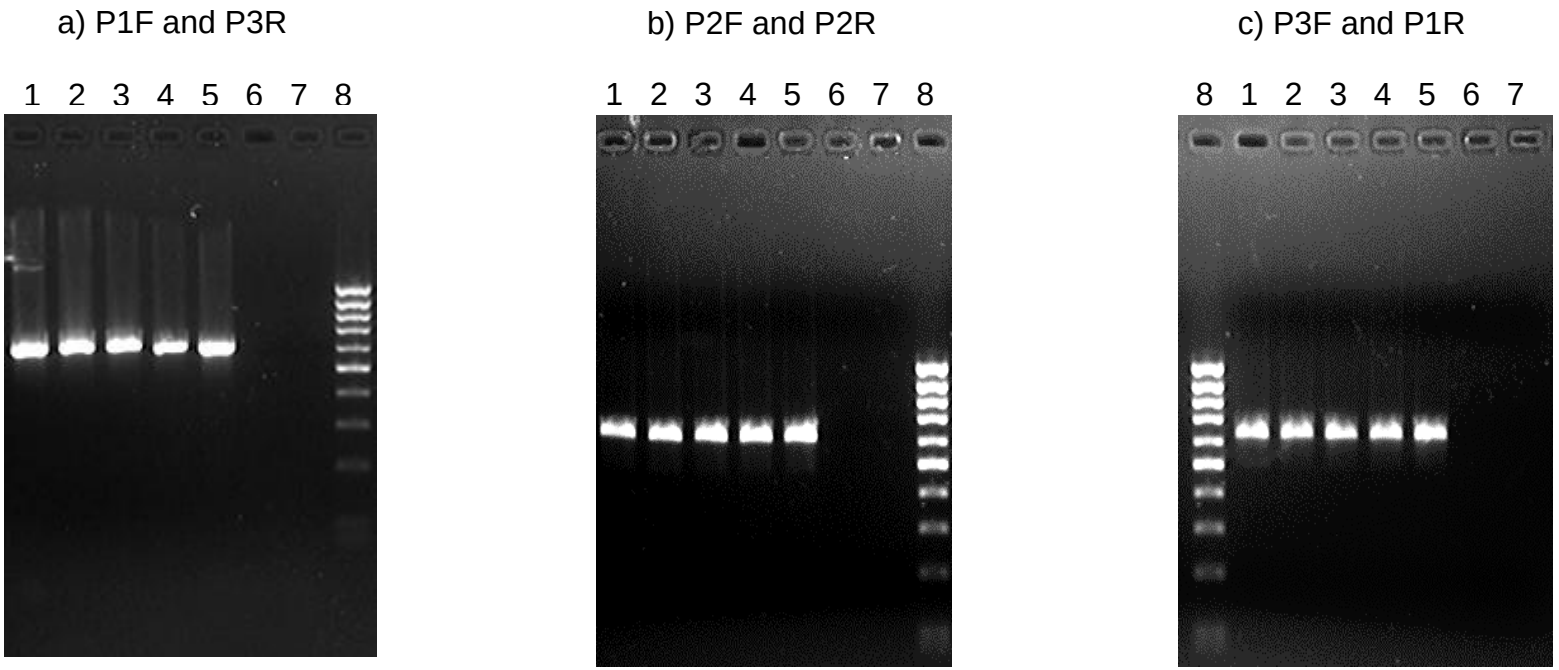
Name	Sequence (5'-3')	Location of primers in *lukSF-PV*
P1F	GTATGCAAAAAAAGACTATTAG	1-22
P1R	GAAAAAAATCCTATGAGCTAA	1897-1918
P2F	TAGTCAAAATCCGAGAGAC	635-654
P2R	TTTATTGGGGTTCTAAGTAC	1238-1258
P3F	GACTCAGTAAACGTTGTAG	1285-1304
P3R	GTAAAATGTCTGGACATGA	586-605

Primers P1F and P1R were forward and reverse primers that were used to amplify the entire fragment; these primers were used along with P2F, P2R, P3F and P3R as sequencing primers to sequence the PCR product in fragments. Online software was used to predict annealing temperatures and confirm that each primer would not be self-complementary (http://www.basic.northwestern.edu/biotools/oligocalc.html).

6.8.2.1 Validation of designed primers

To ensure that all primers were functioning correctly, PCR cycles were performed using adjacent primer pairs (P1F and P3R, P2F and P2R and P3F and P1R). PCR reaction mixtures contained 0.3µM of each primer, 1x AccuPrime™ *Pfx* Reaction mix, 1U AccuPrime™ *Pfx* DNA polymerase and 2µL of extracted DNA in 50µL reaction volumes (Invitrogen Ltd., Paisley, UK). PCR conditions were: 94°C for 2 min, 35 cycles of 95°C for 15s, 55°C for 30s, and 68°C for 2 min; then hold at 4°C. The bands amplified in PCR reactions to validate the primers are shown in Figure 6-5, p.105.

Figure 6-5. Bands from PCR cycles to validate *lukSF-PV* sequencing primers.

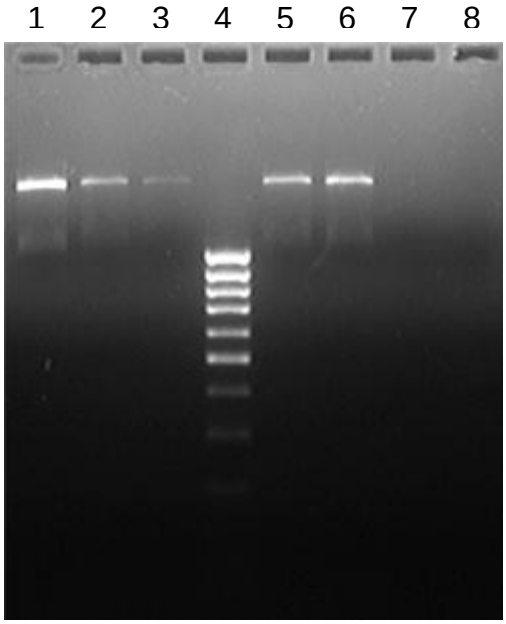


Wells 1-5 = clinical isolates; well 6 = negative control; well 7 = template control (PVL-negative clinical isolate); well 8 = molecular weight marker, 100-1000bp.

6.8.2.2 Amplification and sequencing of lukSF-PV

For amplification of the PVL genes, PCR reaction mixtures contained a final concentration of 0.3µM for primers P1F and P1R (Table 6-6, p.104), 1x AccuPrime™ *Pfx* Reaction mix, 1U AccuPrime™ *Pfx* DNA polymerase and 2µL of extracted DNA in 50µL reaction volumes (Invitrogen Ltd., Paisley, UK). PCR conditions were: 94°C for 2 min, 35 cycles of 95°C for 15s, 55°C for 30s, and 68°C for 2 min; then hold at 4°C. PCR products were DNA sequenced using each of the primers described in Table 6-6 (p. p.104). Representative bands for the amplification of the 1918bp *lukSF-PV* fragment are shown in Figure 6-6.

Figure 6-6. Representative bands from the amplification of *lukSF-PV.*



Wells 1-3 and 5-6 = clinical isolates; well 4 = molecular weight marker, 100-1000bp; well 7 = negative control; well 8 = template control (extracted DNA from a PVL-negative clinical isolate).

6.8.3 PCR strategy to identify the PVL-encoding phage

Eight PCRs were performed to determine the PVL-encoding phage, using primers described by Ma *et al*.[43] (Table 6-7, p.107).

Table 6-7. Primers used to determine the PVL-encoding phage in the assay described by Ma *et al*.

PCR#	Specificity	Name	Sequence (5'-3')	Amplicon size (bp)	Template phage	Location of primers in phage
PCR1	Φ108PVL and ΦPVL	portal-1F	ACACGTGATAAAACAGGAGAA	569	Φ108PVL	21069–21089
		portal-1R	TCTAAATTAGCATCCGTGATAC			21637–21616
		tail-1R	ATAATTGGGATAGCAACGCAA	489		31237–31257
		tail-1F	CTTGATTAGACTCAACCAAACT			31725–31704
PCR2	Φ2958, ΦSLT, ΦSa2MW	portal-2F	GATGGCTAGTTTGCCCTTGA	656	Φ2958	23005–23024
		portal-2R	CTGAGGGCAATTGAAAAACG			23660–23641
		tail-2F	CATAGCGCTAATGTCGCAAA	468		30040–30059
		tail-2R	AGCCTCCATTGTTTGTTTGG			30507–30488
PCR3	Linkage between Φ108PVL and ΦPVL, and *lukSF-PV*	lukSR1	ACGAAGTAGCAATAGGAGTGA	10,497	Φ108PVL	42326–42306
		teil-ico-F	AGATTTAGAAGAGGAGGCACGA			31830–31851
PCR4	Linkage between Φ2958, ΦSLT, ΦSa2MW, and *lukSF-PV*	lukSR1	ACGAAGTAGCAATAGGAGTGA	9,483	Φ2958	44861–44841
		teilE-F2	ATTGATTCAAACTGTTTCTTCTCAGGA[a]			35351–35378
PCR5	Φ108PVL and ΦPVL	Iint-F2	ATGTTTTCGAGTTTTTGAGTTAG	-	Φ108PVL ΦPVL	393–415, 24310–24332
	Φ108PVL	108-aR	TCAAATCCGTAATCACTCATTCT	4,340	Φ108PVL	4732–4710
	ΦPVL	PVL-aR	TTCACTAACTAAACCTATCATTGT	1,411	ΦPVL	25720–25697

PCR#	Specificity	Name	Sequence (5'-3')	Amplicon size (bp)	Template phage	Location of primers in phage
PCR6	Φ2958	Int-F2	ATGTTTTCGAGTTTTTGAGTTAG	2,238	Φ2958	989–1011
		2958-aR	TGGTAATCAACCATTCACTTATGA			3226–3203
PCR7	ΦSa2MW	Int-F2	ATGTTTTCGAGTTTTTGAGTTAG	4,065	ΦSa2MW	1574920–1574898
		MW2-aR	TAAGTTCCTGGTGTCATTCCTAAT			1570856–1570879
PCR8	ΦSLT	Int-F2	ATGTTTTCGAGTTTTTGAGTTAG	8,770	ΦSLT	123–145
		SLT-aR	TCTTACCAAATGCAACACAACGAAT			8892–8868

[a] The sequence for primer teilE-F2 for PCR4 was not included by Ma *et al*.[43] but was provided by Dr Teruyo Ito (Juntendo University, Tokyo, Japan).

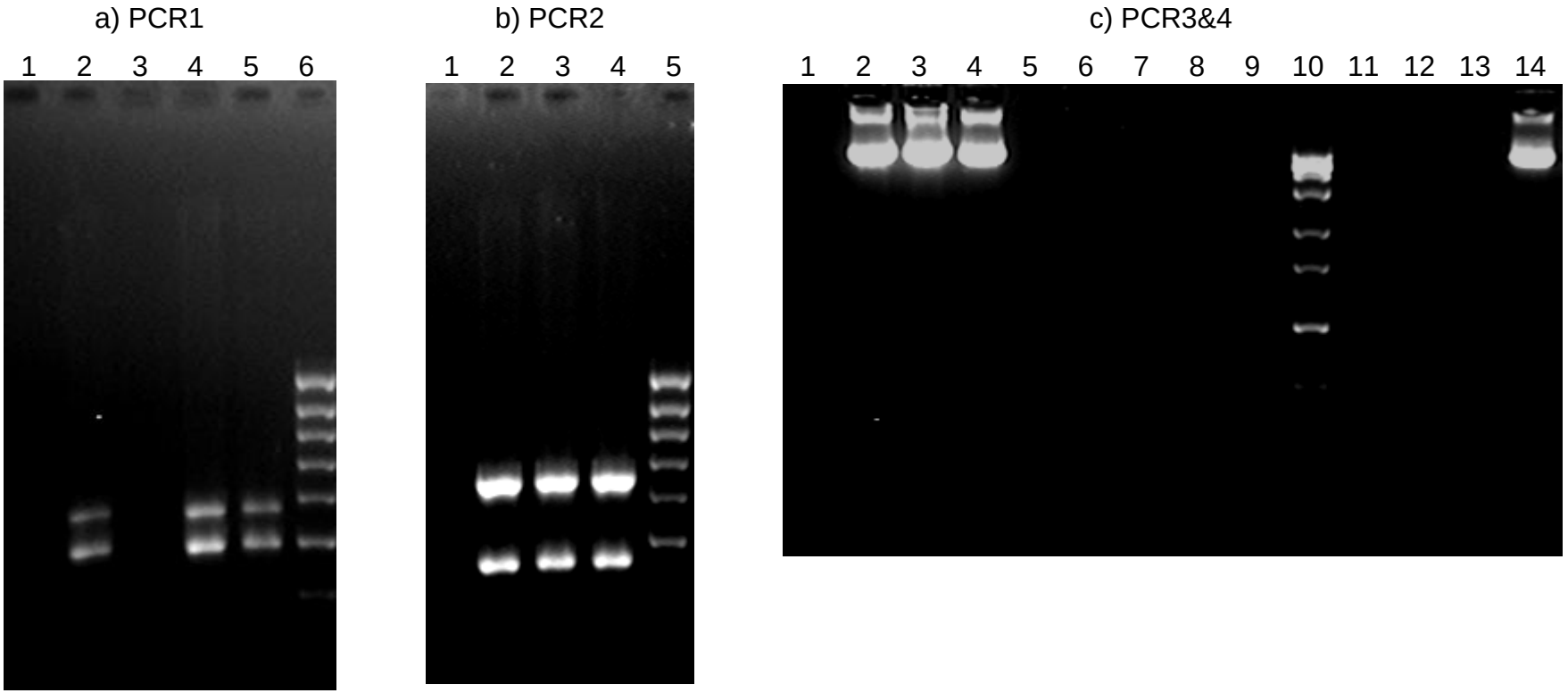
The assay is designed to be used iteratively. PCRs 1 and 2 determine whether the bacteriophage is icosahedral or elongated head type, respectively. If PCR1 is positive, PCR3 is conducted to identify the linkage between the head region and the PVL genes whereas if PCR2 is positive, PCR4 is conducted to detect the linkage. Then, PCR5 is conducted if PCRs 1 and 3 are positive whereas PCRs 6-8 are conducted if PCRs 2 and 4 are positive. However, due to some isolates being positive for both PCRs 1 and 2 and failure to detect the linkage between the head region and the PVL genes in certain isolates, all eight PCR reactions were conducted for all strains.

PCR reactions 1, 2, 5, 6 and 7 contained 0.2µM of each primer, 1x amplification buffer, 0.4mM each dNTP, 1.5mM $MgCl_2$, 4U DNA polymerase and 2µL of extracted DNA in 50µL reaction volumes. PCR conditions were: 94°C for 4 min, 30 cycles of 95°C for 60s, 50°C for 60s and 72°C for 4.5 min; then hold at 4°C.

PCR reactions 3, 4 and 8 contained 0.4µM of each primer, 1x LongRange PCR buffer, 0.5mM each dNTP, 2U LongRange PCR enzyme mix and 2µL of extracted DNA in 50µL reaction volumes (QIAGEN, UK). PCR conditions were: 93°C for 3 min followed by 35 cycles of 93°C for 15s, 55°C for 30s and 68°C for 10.5 min; then hold at 4°C. Representative bands from the PVL bacteriophage PCR reactions are shown in Figure 6-7, p.110.

Figure 6-7. Representative bands from the PVL bacteriophage PCR reactions.

(See Table 6-1, p.86 for the origin of the reference strains.)

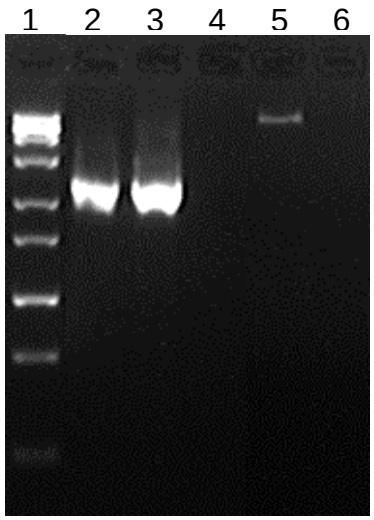


a) PCR1: well 1 = negative control; wells 2 = Φ108PVL positive control ('ST30-108'); wells 3-5 = clinical isolates; well 6 = molecular marker, 100-1000bp.

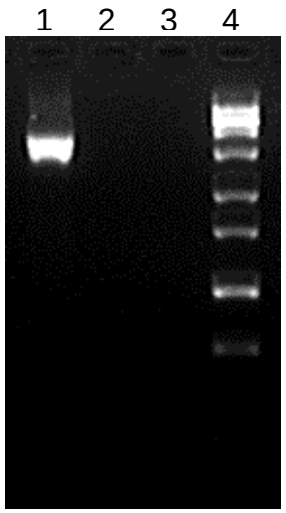
b) PCR2: well 1 = negative control; wells 2 = ΦSa2958 positive control ('JCSC2958'); wells 3-4 = clinical isolates; well 5 = molecular marker, 100-1000bp.

c) PCR3&4: well 1 = PCR3 negative control; wells 2 = Φ108PVL positive control ('ST30-108'); wells 3-9 = clinical isolates; well 10 = molecular weight marker, 100-10000bp; well 11 = PCR4 negative control; wells 12-13 = clinical isolates; well 14 = ΦSa2958 positive control ('JCSC2958').

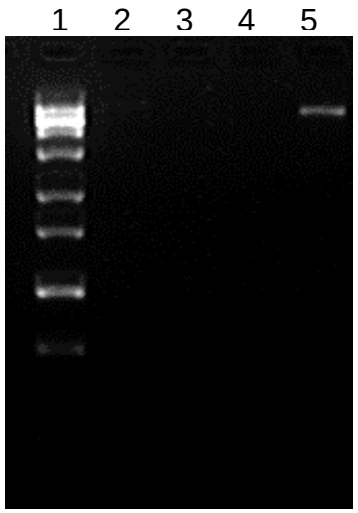
d) PCR5



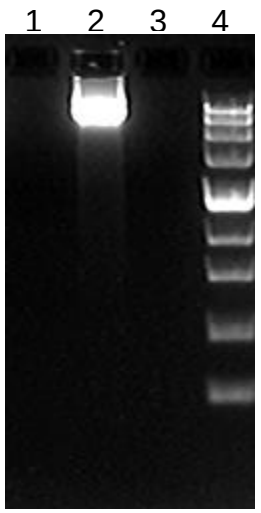
e) PCR6



f) PCR7



g) PCR8



d) PCR5: well 1 = molecular weight marker, 100-10000bp; well 2 = ΦPVL positive control ('ST22-PVL'); wells 3-4 = clinical isolates; well 5 = Φ108PVL positive control ('ST30-108'); well 6 = negative control.

e) PCR6: well 1 = ΦSa2958 positive control ('JCSC2958'); well 2 = clinical isolate; well 3 = negative control; well 4 = molecular weight marker, 100-10000bp.

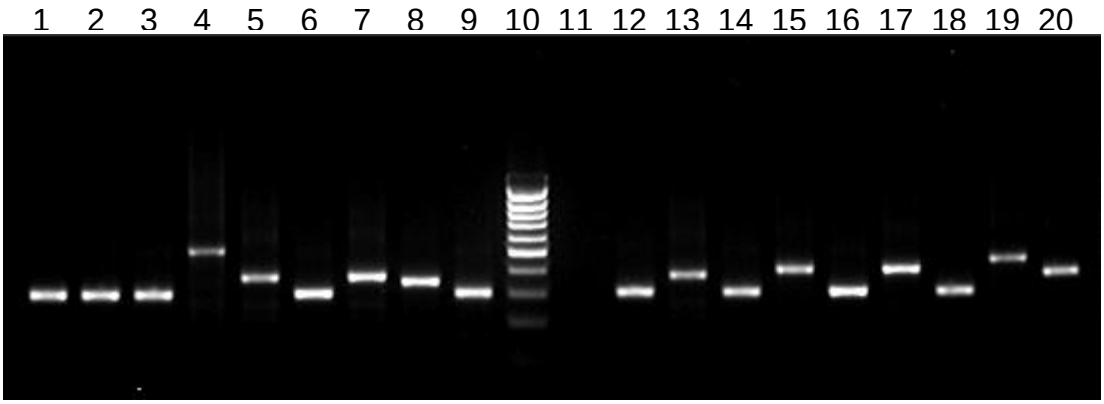
f) PCR7: well 1 = molecular weight marker, 100-10000bp; well 2 = negative control; wells 3-4 = clinical isolates; well 5 = ΦSa2958 positive control ('JCSC2958').

g) PCR8: well 1 = negative control; well 2 = ΦSLT control ('RN4220'); well 3 = clinical isolate; well 4 = molecular weight marker, 100-10000bp.

6.9 *spa* typing

spa typing was conducted according to the method of Aires de Souza *et al.*[130] PCR reaction mixtures contained a 0.4µM final concentration of primers spa-1113f (5'-TAAAGACGATCCTTCGGTGAGC-3') and spa-1514r (5'-CAGCAGTAGTGCCGTTTGCTT-3'), 1x amplification buffer, 0.2mM each dNTP, 1.5mM $MgCl_2$, 1.5U DNA polymerase and 2µL of extracted DNA in 25µL reaction volumes. PCR conditions were: 94°C for 30s; 35 cycles of 94°C for 45s, 60°C for 45s, and 72°C for 1.5 min; then hold at 4°C. PCR products were sequenced using the forward (spa-1113f) and reverse (spa-1514r) primers. Representative bands from *spa* PCR reactions are shown in Figure 6-8.

Figure 6-8. Representative bands from *spa* PCR reactions.



Wells 1-9, 12-20 = clinical isolates; well 10 = molecular weight marker, 100-1000bp; well 11 = negative control.

Sequence data were analysed using Ridom Staphtype v1.2.51 (Ridom GmbH, Würzburg, Germany).[132] The software includes a sequence editor, a database, the ability to synchronise with the Ridom *spa* server (www.spaserver.ridom.de) to automatically assign *spa* types and BURP clustering to assign *spa* types in a given collection to a *spa* clonal complex with a proposed founder *spa* type.

6.10 MLST

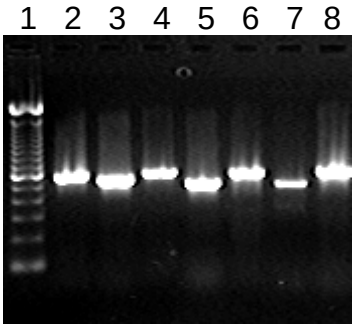
MLST was conducted according to the method of Enright *et al.* using the primers listed in Table 6-8.[32]

Table 6-8. MLST primers.

Gene	Primer	Sequence (5'-3')
arcC	arcC-Up	TTGATTCACCAGCGCGTATTGTC
	arcC-Dn	AGGTATCTGCTTCAATCAGCG
aroE	aroE-Up	ATCGGAAATCCTATTTCACATTC
	aroE-Dn	GGTGTTGTATTAATAACGATATC
glpF	glpF-Up	CTAGGAACTGCAATCTTAATCC
	glpF-Dn	TGGTAAAATCGCATGTCCAATTC
gmk	gmk-Up	ATCGTTTTATCGGGACCATC
	gmk-Dn	TCATTAACTACAACGTAATCGTA
pta	pta-Up	GTTAAAATCGTATTACCTGAAGG
	pta-Dn	GACCCTTTTGTTGAAAAGCTTAA
tpi	tpi-Up	TCGTTCATTCTGAACGTCGTGAA
	tpi-Dn	TTTGCACCTTCTAACAATTGTAC
yqiL	yqiL-Up	CAGCATACAGGACACCTATTGGC
	yqiL-Dn	CGTTGAGGAATCGATACTGGAAC

Seven PCR reactions were conducted for each isolate, one for each locus. Reaction mixtures contained a 1.5µM final concentration of the primer pair (Table 6-8), 1x amplification buffer, 0.2mM each dNTP, 1.5mM $MgCl_2$, 1U DNA polymerase and 2µL of extracted DNA in 50µL reaction volumes. PCR conditions were: 94°C for 30s; 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; 72°C for 5 min; then hold at 4°C. PCR products from each reaction were sequenced using the forward and reverse primer pairs listed in Table 6-8. Representative bands from *spa* PCR reactions are shown in Figure 6-9, p.114.

Figure 6-9. Representative MLST PCR bands for *S. aureus* NCTC 11939.



MLST PCR reactions from *S. aureus* NCTC 11939. Well 1 = 100-10000bp molecular weight marker (Genplot, QIAGEN, UK); well 2 = *arc*; well 3 = *aroE*; well 4 = *glpF*; well 5 = *gmk*; well 6 = *pta*; well 7 = *tpi*; well 8 = *yqiL.*

Sequences for each allele of each isolate were compared with the reference sequence for *S. aureus* ST1, downloaded from www.mlst.net. Analysed sequences were submitted to www.mlst.net to assign an MLST type for each isolate.

6.11 PFGE

PFGE was conducted at the HPA *Staphylococcus* Reference Laboratory. PFGE was conducted according to the HARMONY protocol, with minor adaptations following a HPA *Staphylococcus* Reference Laboratory protocol.[120] The solutions and reagents for PFGE are summarised in Table 6-9. p.115.

In order to prepare agar plugs seeded with a known quantity of *S. aureus* cells, *S. aureus* isolates were grown overnight on nutrient agar (Oxoid) at 37°C. Colonies were suspended in SE buffer to a turbidity of 4-4.5 McFarland units with thorough vortexing. An equal volume (300-500µL) of bacterial suspension was mixed with 2% low gelling agarose (Sigma Aldritch, Gillingham, UK) in SE buffer at 50-56°C, dispensed into a PFGE mould and kept at 4°C until the agarose had set.

In order to lyse the *S. aureus* cells and release genomic DNA, agarose plugs were removed and placed in 3mL of the first lysis buffer for four

hours at 37°C with gentle shaking. After the incubation period, the lysis buffer was drained and replaced with 3mL of the alkaline lysis buffer and incubated at 56°C overnight. Following overnight incubation, plugs were washed three times using 3mL TE buffer for 30 min at 4°C. Following the third wash step, 2mL of TE buffer was added.

Genomic DNA was then digested using the rare-cutting restriction endonuclease *sma1*. A small portion (approximately 2mm) of each plug was cut with a scalpel and placed in a 0.5mL plastic Eppendorf tube. Tubes were covered with approximately 100µL of the enzyme reaction buffer (Sigma) and stored at 4°C for 30 min. After equilibration, 20U of *Sma1* were added and the mixture was mixed gently and incubated at 30°C for at least four hours.

Table 6-9. Solutions for PFGE.

Solution	Ingredients (concentration)
SE buffer, pH 7.5	NaCl (75mM), EDTA (25mM)
First lysis buffer, pH 7.5	Tris (6mM), EDTA (100mM), NaCl (1M), Brij 58 (0.5% w/v), sodium deoxycholate (0.2% w/v), N-Lauroyl sarcosine (0.5%), $MgCl_2$ (1mM), lysostaphin (1µ/mL), lysozyme (500µ/mL)
Alkaline lysis buffer, pH 9.5	N-Lauroyl sarcosine (1% w/v), EDTA (0.5M), proteinase K (1.2µl/mL)
TE buffer, pH 7.5	Tris (10mM), EDTA (10mM)
TBE buffer	Tris (44.5mM), boric acid (44.5mM), EDTA (1mM)

The electrophoresis was conducted on a CHEF-DR II system (Bio-Rad, Hemel Hempstead, UK). A 1.2% molecular grade agarose gel (Sigma) was prepared using 0.5x TBE. Digested plugs were carefully inserted into the wells along with molecular weight markers (Sigma). The wells were sealed using molten agarose at 56°C. The gel was placed into the electrophoresis apparatus and covered with pre-cooled 0.5x TBE, the lid was placed over the electrophoresis tank and electrical connections

secured. The parameters were ramp pulse times from one to 80 seconds for 30 hours. The running temperature was set at 12°C.

The gel was post-stained for one hour in a 1 mg/L ethidium bromide solution, de-stained for one hour using distilled water and viewed under a UV transilluminator.

6.12 Oligonucleotide array (Clondiag)

An oligonucleotide array (Clondiag® ArrayTube™) was used for the simultaneous detection of a range of toxin and antimicrobial resistance genes and species specific markers.[141,142] The ArrayTube system works by linear amplification and biotin labelling of extracted genomic DNA using a multiplex PCR reaction including 128 primers. The labelled DNA is then hybridised to probe sequences on an array chip. Once hybridisation is complete, a digital photograph is taken and the intensity of hybridisation for each probe is measured by the ArrayTube software. The hybridisation pattern is adjusted for intensity using control spots and compared with a database containing hybridisation patterns for all sequenced strains to determine positive and negative reactions. This database was generated by creating a local database of all primer and probe sequences which were analysed against all available *S. aureus* genome sequences to produce all possible hybridisations of primers and probes, allowing up to five mismatches per primer.

The gene targets and species specific markers included in the ArrayTube are listed in Table 6-10, p.117.

Table 6-10. Gene targets and species specific markers in the Clondiag ArrayTube.

Adapted from Monecke *et al.* (2006)[142]

Class	Target gene	Gene function / allelic variant
Genus marker	*23SrRNA*	23S ribosomal RNA gene
Species-specific markers	*coa*	Coagulase
	femA	Gene involved in peptidoglycan synthesis
	gapA	Glyceraldehyde 3-phosphate dehydrogenase
	katA	Catalase
	spa	Protein A
	sarA	*S. aureus* virulence factor regulator
	sbi	IgG-binding protein
agr-typing	*agrB*	agrB-I, II, III, IV
	agrC	agrC-I, II, III, IV
	agrD	agrD-I, II, III, IV
Antimicrobial resistance genes	*mecA*	Methicillin resistance
	blaZ	β-lactamase
	ermA, ermC	Erythromycin resistance and inducible or constitutive clindamycin resistance
	linA	Clindamycin / lincomycin resistance
	msrA	Macrolide resistance
	vatA, B, vga, vgaA, vgb	Streptogramin resistance genes
	aacA-aphD	Gentamicin / tobramycin resistance
	aadD	Neomycin / tobramycin resistance
	aphA-3	Neomycin resistance

Class	Target gene	Gene function / allelic variant
	sat	Streptothricin resistance
	dfrA	Trimethoprim resistance
	far1	Fusidic acid resistance
	mupR	Mupirocin resistance
	tetK, tetM	Tetracycline resistance
	vanA, B, Z	Enterococcal genes involved in glycopeptide resistance
Superantigenic toxins	*tst1*	Toxic shock syndrome toxin
	entA	Enterotoxin A
	seB, C, D, E, G, H, I, J, K, L, M, N, O, Q, R, U, Y	Enterotoxins
	Ubiquitous enterotoxin homologue	
	Enterotoxin-like ORF CM14	
γ-haemolysin and bicomponent leukocidins	*lukF, S*	γ-haemolysin
	hlgA	γ-haemolysin
	lukF-PV	PVL, F subunit
	lukS-PV	PVL, S subunit
	lukF-PV-P83	Bovine bicomponent leukocidin, F subunit
	lukM	Bovine bicomponent leukocidin, S subunit
	lukD, E	LukD/E leukocidin
Leukocidin/ haemolysin toxin family proteins	*Putative leukocidin F subunit*	
	Putative leukocidin S subunit	
Other haemolysins	*hl*	Unnamed haemolysin
	hla, b, d	Haemolysin-α / β / δ
	hl-III	

Class	Target gene	Gene function / allelic variant
Other virulence factors	*etA, B, C, D*	Epidermolytic toxins
	splA, B	Serine protease-like exoprotein A / B
	edinA, B, C	Epidermal differentiation inhibitor genes
	sak	Staphylokinase
Staphylococcal exotoxin-like proteins	*set1,2,3,4,5,6,7,8,9, 11,21,B, C*	

PCR reaction mixtures for the linear amplification and labelling of genomic DNA contained a 0.5µM final concentration of primer mix (Clondiag), 1x Therminator™ amplification buffer (New England BioLabs, Hitchin, Hertfordshire), 1mM dACG TP, 0.65mM dTTP, 0.07mM biotin-16-2'-dUTP, 0.4U Therminator polymerase and 2µL of extracted DNA in 10µL reaction volumes. PCR conditions were: 96°C for 5 min; 50 cycles of 62°C for 20s, 72°C for 40s, and 96°C for 60s; then hold at 4°C.

Hybridisation was conducted at the HPA *Staphylococcus* Reference Laboratory. The buffers used are summarised in Table 6-11, p.120.

In order to condition the ArrayTubes for hybridisation, 500µL of distilled water was added to each ArrayTube and incubated at 55°C for 5 min at 550 rpm on a thermomixer block (Eppendorf® Thermomixer R, Sigma-Aldrich). The water was removed and the 500µL of 3DNA buffer was added to each ArrayTube and incubated at 30°C for 5 min at 550 rpm after which the buffer was removed.

Table 6-11. Hybridisation buffers used for the Clondiag ArrayTube.

Name	Ingredients (concentration)
6xSSPE with 0.005% Triton	6xSSPE = 0.15M NaCl, 0.01M NaH_2PO_4 (sodium phosphate), 0.001 M EDTA, pH 7.0
Wash buffer 1 (2xSSC plus 0.01% Triton)	2xSSC = 0.3M NaCl, 0.03M $Na_3C_6H_5O_7$ (sodium citrate)
Wash buffer 2 (2xSSC)	-
Wash buffer 3 (0.2xSSC)	-
3DNA buffer	1xSSC = 0.15M NaCl, 0,015M $Na_3C_6H_5O_7$ (sodium citrate); Na2HPO4 (250 mM); sodium dodecyl sulphate, SDS (4,5%); EDTA (1 mM)

For hybridisation, 10µL of biotin labelled PCR product was mixed with 100µL 3DNA buffer, heated at 95°C for 5 min, spun down for 5 sec and chilled on ice for 2 min before transfer into the conditioned ArrayTube, which was incubated at 50°C for 60 min at 550rpm.

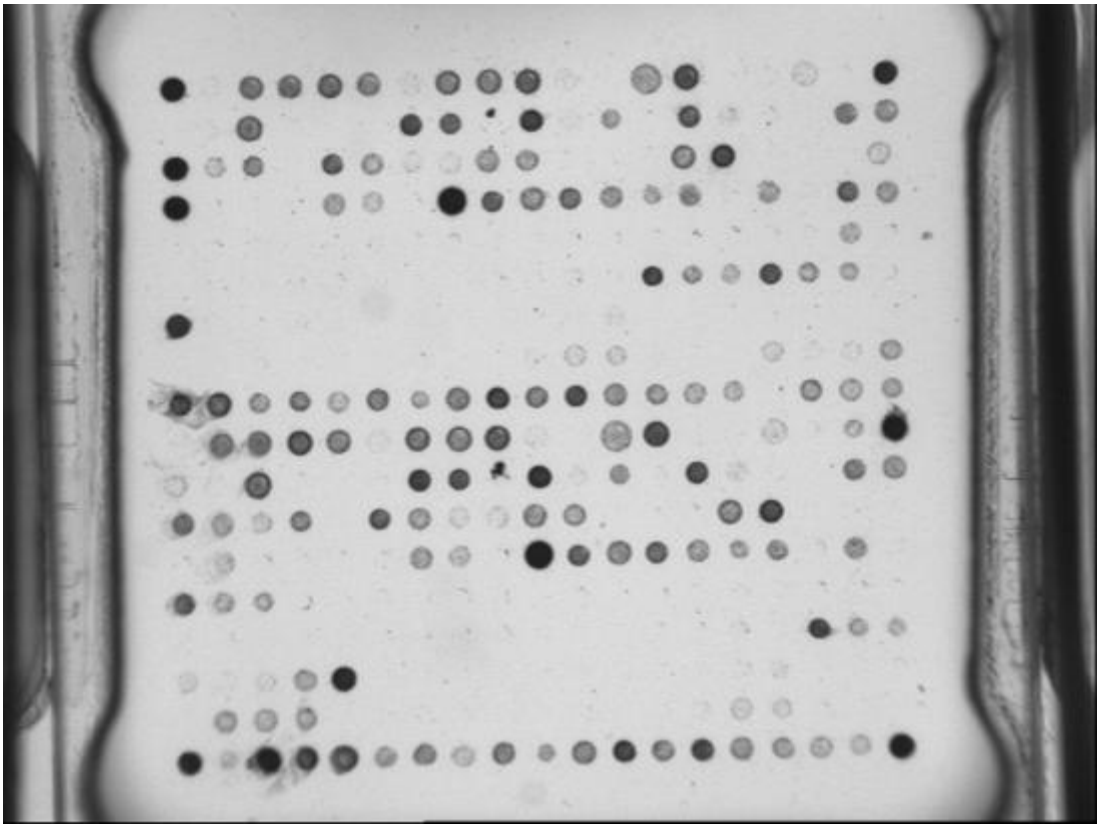
The ArrayTube was washed with 500µL wash buffer 1 incubated at 30°C for 5 min at 550rpm, 500µL wash buffer 2 incubated at 20°C for 5 min at 550rpm and 500µL wash buffer 3 incubated at 20°C for 5 min at 550rpm. Following the washing steps, 100µL of blocking solution (skimmed milk powder) was added and incubated at 30°C for 15 min at 550rpm.

To conjugate the biotin labelled DNA hybridised to the probes, 100µL of a 1:100 dilution of streptavidin horse raddish peroxidase (final concentration, 0.05mg/L) (Thermo Fisher Scientific, Loughborough, UK) in 6xSSPE plus 0.005% Triton was added to the ArrayTube and incubated at 30°C for 15 min at 550 rpm. The ArrayTube was washed with 500µL wash buffer 1 incubated at 30°C for 5 min at 550rpm, 500µL wash buffer 2 incubated at 20°C for 5 min at 550rpm and 500µL wash buffer 3 incubated at 20°C for 5 min at 550rpm.

The final wash buffer was discarded and 100µL of a chromogenic substrate, 1xTMB (3,3´,5,5´-tetramethylbenzidine) (Thermo Fisher Scientific) was added. The ArrayTube was transferred immediately into the

Array-tube reader and photographed after 10, 30 and 60 minutes. The optimal picture in which spots were clearly visible but high intensity spots had not begun to merge was sent to Dr Stefan Monecke (Dresden University, Germany) for analysis because the HPA did not have the necessary software. A representative ArrayTube picture is shown in Figure 6-10.

Figure 6-10. Representative Clondiag ArrayTube picture.
Each spot represents a probe for a target gene (excluding the four corner dots, which are hybridisation controls). Dark spots occur when biotin-labelled amplified DNA hybridises with the probe.



6.13 Statistical software

Various statistical software packages were used during the project. Descriptive statistics, including frequency, mean, median and range, and regression analyses were performed using Microsoft Excel 2003 (Microsoft Corporation, USA) or SPSS v17.0 (SPSS Inc., Chicago, USA). Inferential statistics including the Chi-square, Mann-Whitney and Kruskal-Wallace

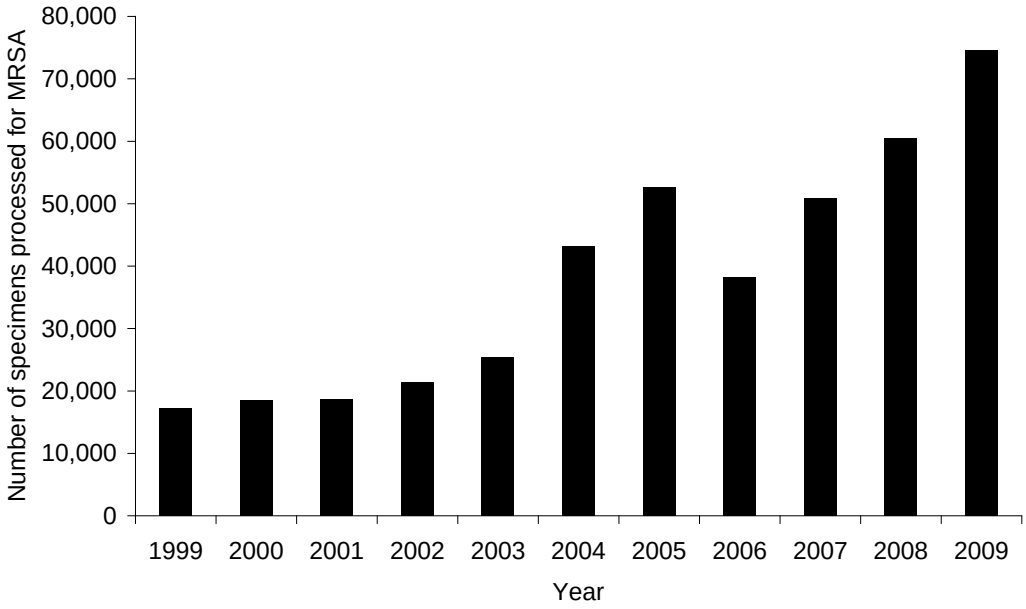
tests were performed using either GraphPad Prism version 5.00 (GraphPad Software, San Diego California USA) or SPSS v17. p values <0.05 were considered statistically significant.

6.14 Retrospective study: the molecular epidemiology of ciprofloxacin-susceptible MRSA at GSTT, 2000-2006

In order to test the hypothesis that CA-MRSA have emerged at GSTT but that their emergence has been masked by the volume of HA-MRSA identified in the clinical laboratory, I conducted a retrospective analysis of stored strains of MRSA to determine whether the collection contained true CA-MRSA. The great majority of HA-MRSA in the UK are ciprofloxacin-resistant epidemic EMRSA-15 or EMRSA-16.[181] In contrast, the handful of CA-MRSA reported at the commencement of the project in the UK were susceptible to most non-β-lactams, including ciprofloxacin;[359] indeed, ciprofloxacin susceptibility has been proposed as a phenotypic marker of CA-MRSA in the UK.[354,359] Therefore, I used ciprofloxacin susceptibility as a screening marker to select isolates likely to be CA-MRSA and employed a variety of molecular methods combined with epidemiological data to further characterise these strains in the collection.

GSTT is a 1200-bed teaching and tertiary referral acute hospital trust located on two sites (St. Thomas' and Guy's) in central London. It has a wide range of specialities, including adult, paediatric and neonatal medicine, surgery and intensive care; cardiology and respiratory medicine; renal, bone marrow and liver transplantation and obstetrics and gynaecology. The hospital is a referral centre for these specialities, nationally and internationally. The number of specimens received for MRSA has increased from 17,000 in 1999 to 75000 in 2009, mainly due to the increases in the number of patients screened for carriage in the Trust (Figure 5-1, p.123).

Figure 6-11. The number of specimens processed for MRSA by the microbiology laboratory, 1999-2009.



The GSTT microbiology laboratory processes specimens from inpatients in both hospitals, outpatients, General Practitioners (GPs) and other healthcare facilities in the London Borough of Lambeth, which has a local catchment population of 280,000. GSTT has a collection of MRSA isolates dating back to the 1990s including isolates from hospital patients and submitted isolates from GPs and other healthcare facilities; one isolate from each MRSA-positive patient is stored. All isolates are stored at room temperature on nutrient agar slopes with the exception of blood isolates, which are stored frozen in glycerol broth at -70°C.

6.14.1 Selection of isolates

I analysed epidemiological data from all patients who yielded MRSA isolates (excluding screening cultures for colonisation only) from 2000-2006. Given the changes in the number of patients screened for MRSA over the study period and the reported clinical association of CA-MRSA with skin and soft tissue infections, we restricted our selection to infected sites.[84,204] In some patients MRSA were isolated from multiple culture sites

but a single representative isolate was analysed for typing and microbiological characteristics.

6.14.2 Epidemiological classifications

The primary aim of this work was to establish whether the new, genetically distinct, MRSA strains that have emerged in the community elsewhere (CA-MRSA) had also appeared at GSTT. All MRSA isolates were also classified epidemiologically to determine whether these CA-MRSA strains were likely to be community- rather than healthcare-acquired. GSTT laboratory records of hospital isolates contain information on patient age, culture site, ward, medical specialty and antimicrobial susceptibility. In addition, since 2003, the hospital Infection Control Team (ICT) has prospectively classified each new inpatient MRSA episode as 'hospital-acquired' or 'present on admission'. The 'present on admission' group is a broad category including patients with and without known healthcare contact. However, this classification was recorded for all for all MRSA episodes so it allowed me to compare the frequency of 'hospital acquired', 'present of admission' and 'previous positives' in the Cip-R and Cip-S groups (results in Table 7-1, p.135).

Additional patient data were downloaded from the hospital patient administration system for each Cip-S MRSA isolate, including patient demographics, admission and discharge dates, and codes for medical specialty, diagnosis, procedure and health-related groups. This allowed me to make a more accurate epidemiological classification of 'healthcare-acquired' or 'community-acquired' for patients with Cip-S MRSA according to the following criteria (results in Table 7-2, p.139). Patients were classified epidemiologically as healthcare-acquired if their MRSA-positive specimen was collected >48 hours after hospital admission or less than 12 months after a previous inpatient stay. Infections in patients with previous MRSA episodes (or with regular day care, for example haematology and renal patients), were also classified as healthcare-acquired. Patients were classified as community-acquired if they had had no inpatient stay in the

previous 12 months and their first MRSA-positive specimen was collected in the community or within 48 hours of hospital admission.

6.14.3 Analysis of surviving MRSA

Four hundred and fifty-eight (6.4%) of the 7146 unique patient MRSA isolates reported during 2000-2006 were Cip-S. One hundred and ninety-four (42.2%) of the 458 reported Cip-S MRSA isolates were recovered from storage. One hundred and sixty-three (35.6%) of the slopes were missing and MRSA was not recovered from 101 (22.1%) of the slopes. The fact than approximately one third of the slopes were missing was due to the heavy workload of the clinical laboratory staff meaning that they did not have time to inoculate the slopes for storage. Confirmed *S. aureus* isolates were tested for AMS by the BSAC disc diffusion method (section 6.3.1, p.88) and the methicillin MIC was determined by the BSAC agar dilution method (section 6.3.2, p.90). DNA was extracted (section 6.4, p.92) and all isolates were tested for SCC*mec* type using the method described by Oliveria and de Lencastre (section 6.7.1, p.94), SCC*mec* IV sub-type (section 6.7.3, p.101), PVL (section 6.8.1, p.103) and *spa* type (section 6.9, p.112). *spa* types were clustered into related CCs using the Based Upon Repeat Patterns (BURP) algorithm in the Ridom StaphType software using a calculated cost between members of ≤4 and excluding *spa* types shorter than 5 repeats. The stringent calculated cost was chosen to increase the resolution between *spa* CCs. At least one representative isolate from each *spa* CC was typed by MLST (section 6.10, p.113) and PFGE (section 6.11, p.114). Cluster analysis was performed on PFGE profiles using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and visualised as a dendrogram by the unweighted pair group method applying 1% optimisation and 1% tolerance settings, as described by Strommenger *et al*.[121] A similarity cut-off of 70% was used to define a cluster. In addition, MLST was performed on a representative isolate from *spa* singleton lineages that contained >1 isolate.

6.14.4 Statistical analysis

Descriptive statistics were performed using Microsoft Excel 2003. Statistical comparisons were made using the Mann-Whitney or Kruskal-Wallace tests for continuous variables and the Chi-square test for discrete variables using GraphPad Prism version 5.00. Trends were analysed using regression models in Microsoft Excel 2003.

6.15 Assessment of antimicrobial susceptibility based algorithms for the presumptive identification of CA-MRSA

In order to test the hypothesis that AMS-based algorithms can be used as an accurate screening marker for the presumptive identification of CA-MRSA in a collection of predominantly HA-MRSA isolates, I studied a prospective collection of all MRSA reported at GSTT for a three month period. Fluoroquinolone susceptibility and other AMS-based algorithms have been useful for the presumptive identification of CA-MRSA in large collections of HA-MRSA isolates.[112,113,205,390,443,444] However, these studies probably underestimate the prevalence of CA-MRSA because of the presence of multiresistant CA-MRSA strains.

6.15.1 Selection of isolates

I investigated all MRSA cases reported for a three month period from the 1st March to the 30th June 2008 and defined them as HA-MRSA or CA-MRSA by genotype. One MRSA isolate per patient is routinely stored on a nutrient agar slope and tested for AMS by automated broth microdilution by the clinical laboratory (section 6.3.4, p.92). Three-hundred and eighty six unique patient isolates were reported during the three month study period and 239 (61.9%) were available for analysis.

6.15.2 Molecular characterisation

Subculture of isolates from storage and DNA extraction (section 6.4, p.92) was performed by Dahir Mohamed (GSTT). Confirmed *S. aureus* isolates were tested for SCC*mec* type (section 6.7.3, p.101), PVL (section 6.8.1, p.103) and *spa* type (section 6.9, p.112). *spa* PCR products were sequenced by GATC. *spa* types were grouped into related clonal CCs using the BURP algorithm using a calculated cost between members of ≤6 and excluding *spa* types of <5 repeats.

6.15.3 Definition of MRSA isolates as HA- or CA-MRSA strains

CA-MRSA are classically defined as SCC*mec* types IV or V.[205,444] However, one of the most common causes of HA-MRSA in the UK is EMRSA-15, which is SCC*mec* IV.[99] Therefore, I used a combination of *spa* type and SCC*mec* type to define HA- and CA-MRSA. CA-MRSA strains were defined as isolates that were SCC*mec* IV or V that did not have a *spa* type in the same CC as EMRSA-15. Isolates with non-typeable (NT) SCC*mec* regions were defined as CA-MRSA because they were considered unlikely to represent epidemic hospital lineages. All other isolates were classified as HA-MRSA.

6.15.4 Epidemiological classifications

Clinical and demographic data were obtained from patient electronic medical records. These included patient demographics, the body site from which MRSA was isolated, admission and discharge dates (including outpatient and day surgery visits), underlying medical conditions, previous healthcare contact or evidence of transfer from other healthcare facilities and previous history of MRSA. Each MRSA case (infection or colonisation) was reviewed and classified as either healthcare-acquired or community-acquired based on available epidemiological information. Cases were classified as healthcare-acquired if their first MRSA-positive specimen during the study period was collected >48 hours after hospital admission

or less than 12 months after a previous inpatient stay. Infections in patients with (1) previous MRSA episodes, (2) regular day care, (for example haematology and renal patients), (3) day surgery or (4) evidence of risk factors (for example long term indwelling devices), were also classified as healthcare-acquired. Cases were classified as community-acquired if the patient had had no inpatient stay in the previous 12 months and the first MRSA-positive specimen was collected in the community or within 48 hours of hospital admission. Cases were defined as 'hospital onset' if the first specimen during the study period was cultured either on admission or during an inpatient stay and as 'community onset' if the first specimen during the study period was cultured during an outpatient visit.

6.15.5 Algorithms

AMS patterns were grouped by *spa* type and used to develop algorithms for distinguishing HA- and CA-MRSA isolates. Algorithms that were highly significantly associated ($p < 0.001$) with CA-MRSA were tested for their performance characteristics in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

6.15.6 Statistical analysis

Descriptive statistics were performed using SPSS v17.0. Statistical comparisons were made using the Mann-Whitney test for continuous variables and the Chi-square test for discrete variables using SPSS v17.0. To further define the relative utility of susceptibility to individual antimicrobial agents, a binomial logistic regression model was used to predict isolate classification with susceptibility to each antimicrobial agent as covariates. Binomial logistic regression is a form of multiple regression that is used when the dependent variable is dichotomous, in this case, HA-MRSA or CA-MRSA. The model is used to assess the relative importance of each covariate on the dependent variable.[445]

6.16 The prevalence and molecular epidemiology of CA-MRSA identified on admission screens

To test the hypothesis that CA-MRSA strains account for a considerable proportion of MRSA colonising patients admitted to GSTT, all MRSA identified on admission to GSTT during the first six months of universal screening in 2008 were saved and analysed to determine the prevalence and molecular epidemiology of MRSA carriage.

6.16.1 Screening policy and culture methods

GSTT has approximately 120,000 admissions per annum including day visits. A policy to screen all admissions was implemented across the Trust from 1st April 2008. Elective admissions were screened during outpatient or pre-assessment visits. Inpatients were screened within the first 48 hours of admission. Patients were screened by clinical staff using three cotton tipped swabs to sample the nose, throat and perineum. Screening swabs from each patient were pooled onto one quarter of an MRSA selective chromogenic agar by the clinical microbiology laboratory (Brilliance™ MRSA, Oxoid). Presumptive MRSA isolates were confirmed by standard methods and tested for antimicrobial susceptibility by automated broth microdilution by the clinical laboratory (section 6.3.4, p.92). One MRSA isolate per patient was collected prospectively and stored on a nutrient agar slope by Dahir Mohamed (GSTT) during the study period.

6.16.2 Determination of MRSA prevalence

A database of all screens for MRSA from 1st April 2008 to 30th September 2008 was downloaded from electronic hospital records. Admission screens did not have a specific code so admission screens were defined based on timing relative to other specimens from the same patient and hospital admissions by Dr Trent Herdman (GSTT). Repeated screens from the same patient were excluded. The patients' age, gender, previous visits to

GSTT, previous history of MRSA, specialty of admission and location in which the screen was collected were available for all patients.

6.16.3 Epidemiological classifications, molecular characterisation and definition of MRSA isolates as HA- or CA-MRSA strain types

Each patient's isolate was defined as HA- or CA-MRSA strain types (section 6.15.3, p.127) and each MRSA-positive patient was classified epidemiologically as healthcare-acquired or community-acquired (section 6.15.4, p.127). Subculture of isolates from storage and DNA extraction (section 6.4, p.92) was performed by Dahir Mohamed (GSTT). Slopes were saved for 85% of the 304 positive patients and MRSA was recovered from 97% of saved slopes. MRSA isolates were tested for SCC*mec* type (section 6.7.3, p.101), PVL (section 6.8.1, p.103) and *spa* type (section 6.9, p.112). *spa* PCR products were sequenced by GATC. *spa* types were grouped into related clonal CCs using the BURP algorithm using a calculated cost between members of ≤6 and excluding *spa* types of <5 repeats.

6.16.4 Statistical analysis

Statistical comparisons were made using the Mann-Whitney test for continuous variables and the Chi-square test for discrete variables using SPSS v17.0 (SPSS Inc., Chicago, USA). Four binomial logistic regression models were used to investigate risk factors using variables that were significant by univariate analysis as covariates.[445] The dependent variable in the first model was whether the patient was MRSA-positive or MRSA-negative (Table 7-6, p.153); the dependent variable in the second model was whether the MRSA isolate was HA-MRSA or CA-MRSA (Table 7-7, p.158); the dependent variable in the third model was whether the patient had an MRSA-negative screen followed by an MRSA-positive specimen or an MRSA-negative screen only (Appendix 9-7, p.224); and the dependent variable in the forth model was whether the patient was MRSA-negative or had an HA-MRSA isolate (Appendix 9-8, p.227).

6.17 PVL-encoding bacteriophage and gene sequence variation

To test the hypothesis that polymorphisms in the PVL genes vary with the PVL-encoding phage, I analysed 22 PVL-positive clinical MRSA isolates identified in the retrospective study (section 6.14, p.122) in detail. Isolates were chosen to reflect all MLST clonal complexes reported, namely CC1, 5, 8, 59, 80, 88 and 154. ST80 was the predominant PVL-positive clone, so one representative PVL-positive ST80 isolate was chosen for each year of the study in which ST80 isolates were identified.

6.17.1 Microbiological and molecular characterisation

Subculture of isolates from storage was performed by Dahir Mohamed (GSTT). All confirmed *S. aureus* isolates were tested for AMS by automated broth microdilution (section 6.3.4, p.92) and the BSAC disc diffusion method (section 6.3.1, p.88). The oxacillin MIC was determined using E-test strips (section 6.3.2.2, p.90). A D-test for inducible clindamycin resistance was performed on strains that were erythromycin-resistant but clindamycin-susceptible (section 6.3.3, p.91).

DNA was extracted (section 6.4, p.92) and the SCC*mec* type was determined using the Milheirico *et al.* method (section 6.7.2, p.98); SCC*mec* IV isolates were sub-typed (section 6.7.3, p.101). PVL carriage (section 6.8.1, p.103) was determined. All isolates were tested for a range of toxin genes and antimicrobial resistance determinants using the Clondiag oligonucleotide array (section 6.12, p.116).

The PVL genes were amplified and sequenced (section 6.8.2, p.104) and the PVL-encoding bacteriophage was identified using the PCR assay described by Ma *et al*. (section 6.8.3, p.106).

6.17.2 *spa,* PFGE and cluster analysis

All isolates were typed by *spa* (section 6.9, p.112) and PFGE (section 6.11, p.114), and representative isolates from each *spa* CC were typed by MLST (section 6.10, p.113). *spa* and MLST PCR products were sequenced by GATC. MLST was performed on representative isolates because PFGE and *spa* have been shown to have more resolution than MLST.[121,297,446]

spa types were clustered into related CCs using the BURP algorithm using a calculated cost between members of ≤4, excluding *spa* types with <5 repeats. Cluster analysis was performed on PFGE profiles using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and visualised as a dendrogram by the unweighted pair group method applying 1% optimisation and 1% tolerance settings, as described by Strommenger *et al*.[121] A similarity cut-off of 80% was used to define a cluster. Differing pulsotypes within a cluster were assigned unique cluster numbers; indistinguishable isolates were assigned the same number.

Discriminatory indices (DIs) for *spa*/BURP and PFGE profiles and clusters were calculated using the method described by Hunter and Gaston.[447] This DI provides an estimate of the probability that two randomly selected strains would differ and was calculated as:

$$DI = 1 - [(1/N(N-1))\Sigma n_i(n_i - 1)]$$

where n is the number of strains belonging to the *i*th type, and N is the total number of strains.

Ninety-five percent confidence intervals (CIs) for DIs were calculated using the method described by Grundmann *et al*.[448] If repeated samples of a fixed size N are drawn from the sample population, the values for DI will be distributed around the true discriminatory index with the variance (σ^2):

$\sigma^2 = (4/N)[\Sigma n_i^3 – (\Sigma n_i^2)^2]$

An estimate of the 95% CI is given by:

$CI = [DI – 2(\sqrt{\sigma^2}), DI – 2(\sqrt{\sigma^2})]$.

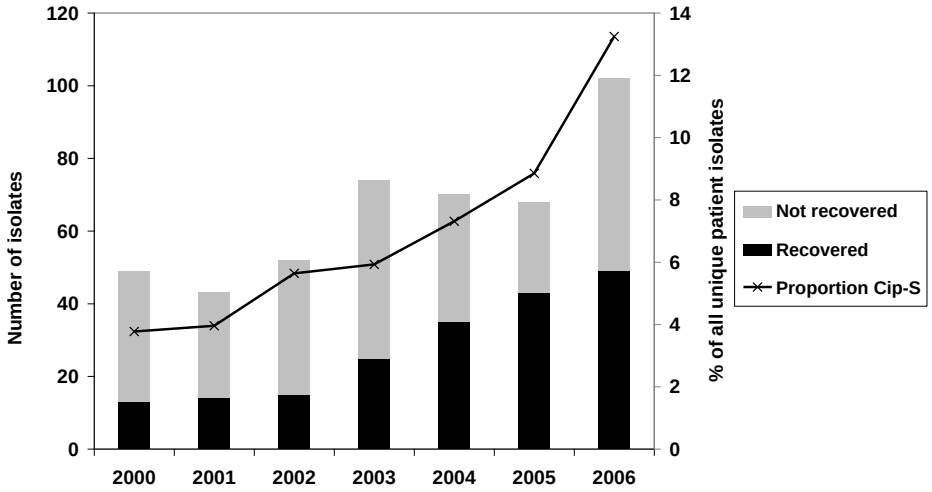
7 RESULTS

7.1 Retrospective study: the molecular epidemiology of ciprofloxacin-susceptible (Cip-S) MRSA at GSTT, 2000-2006

7.1.1 Prevalence of Cip-S MRSA

Four hundred and fifty-eight (6.4%) of the 7146 unique patient MRSA isolates reported during 2000-2006 were Cip-S; 194 (42.2%) of Cip-S the MRSA isolates were recovered from storage. The trends in the number of Cip-S isolates reported each year and in the proportion of Cip-S isolates among all MRSA were analysed using regression models in Microsoft Excel 2003. The exponential regression model was the best fit for both trends. The number of Cip-S isolates reported increased year by year from 49 in 2000 to 102 in 2006 which correlated strongly with an exponential regression model (r^2 = 0.80). Although there was a decrease in the total number of MRSA reported during 2003-2005, the proportion of Cip-S MRSA rose from 3.7% in 2000 to 13.2% in 2006 which correlated strongly with an exponential regression model (r^2 = 0.96) (Figure 7-1).

Figure 7-1. Total number of ciprofloxacin-susceptible (Cip-S) MRSA reported by year (bars) and the proportion of 458 Cip-S MRSA among all 7146 unique patient MRSA isolates reported by year (line), 2000-2006.



7.1.2 Epidemiological comparison of Cip-S and Cip-R MRSA

The features of the reported Cip-S and ciprofloxacin-resistant (Cip-R) MRSA are compared in Table 7-1.

Table 7-1. Features of ciprofloxacin-resistant MRSA (Cip-R) compared with ciprofloxacin-susceptible MRSA (Cip-S) reported at GSTT.
(Percentage of total in parenthesis unless otherwise stated.)

	Cip-R MRSA		Cip-S MRSA		p value[a]
Total number reported	6688		458		-
Patient demographics					
Male[b]	1002	(56.7)	289	(63.1)	**0.0162**
Median age (range)	68.5 (0.0-104.0)		44.8 (0.0-95.8)		**<0.0001[c]**
Children <1 yrs	106	(2.0)	31	(6.8)	**<0.0001**
Children <15 yrs	181	(3.3)	66	(14.4)	**<0.0001**
Antimicrobial resistance[d]					
Erythromycin	5566	(89.4)	230	(51.7)	**<0.0001**
Gentamicin	1718	(25.8)	28	(6.1)	**<0.0001**
Fusidic acid	465	(7.5)	192	(43.1)	**<0.0001**
Tetracycline	366	(11.5)	51	(16.6)	**0.0118**
Neomycin	2610	(40.2)	82	(19.2)	**<0.0001**
Mupirocin	717	(10.9)	11	(2.5)	**<0.0001**
Rifampicin	68	(1.0)	11	(2.4)	**0.0126**
Trimethoprim	1790	(27.1)	78	(17.1)	**0.0126**
Collection location					
Adult inpatient	5024	(75.1)	205	(44.8)	**<0.0001**
Paediatric inpatient	113	(1.7)	33	(7.2)	**<0.0001**
Accident and Emergency	290	(4.3)	69	(15.1)	**<0.0001**
General practitioner	1105	(16.5)	116	(25.3)	**<0.0001**
Outpatient clinic	124	(1.9)	16	(3.5)	**0.0248**
GUM[e]	1	(0.0)	9	(2.0)	**<0.0001**
Obs & gynae[f] / maternity	31	(0.5)	10	(2.2)	**<0.0001**
Epidemiological classification[g]					
Hospital acquisition	1259	(50.8)	56	(26.3)	**<0.0001**
Present on admission	1012	(40.8)	144	(67.6)	**<0.0001**
Previous positive	208	(8.4)	13	(6.1)	**0.0007**

	Cip-R MRSA		Cip-S MRSA		p value[a]
Specimen[h]					
SSTI[i]	8799	(45.6)	449	(64.1)	**<0.0001**
Abscess	212	(1.1)	41	(5.9)	**<0.0001**
Ulcer	1160	(6.0)	50	(7.1)	0.2596
Surgical site infection	1294	(6.7)	33	(4.7)	**0.043**
Respiratory	2971	(15.5)	77	(11.0)	**0.0016**
Tracheotomy	460	(2.4)	12	(1.7)	0.3019
Bronchoalveolal lavage	763	(4.0)	20	(2.9)	0.1654
Sputum	1670	(8.7)	35	(5.0)	**0.0008**
Urine	815	(4.2)	13	(1.9)	**0.0027**
Mucosal	572	(3.0)	52	(7.4)	**<0.0001**
Eye	291	(1.5)	12	(1.7)	0.7876
Ear	103	(0.5)	12	(1.7)	**0.0002**
Nose	57	(0.3)	12	(1.7)	**<0.0001**
Vaginal	71	(0.4)	16	(2.3)	**<0.0001**
Invasive / line / tip	2536	(13.2)	53	(7.6)	**<0.0001**
Blood	779	(4.1)	27	(3.9)	0.874
Line / tip	1066	(5.5)	20	(2.9)	**0.0028**
Other	3535	(18.4)	56	(8.0)	**<0.0001**

[a] p values calculated using Chi-square tests of 2 x 2 contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] Gender information was not available for all patients; percentages represent the number as a proportion of the total for which information was available.

[c] p value calculated using a two-tailed Mann-Whitney test.

[d] Not all antimicrobial agents were tested for all reported isolates; percentages represent the number positive as a proportion of the total tested.

[e] Genitourinary medicine.

[f] Obstetrics and gynaecology..

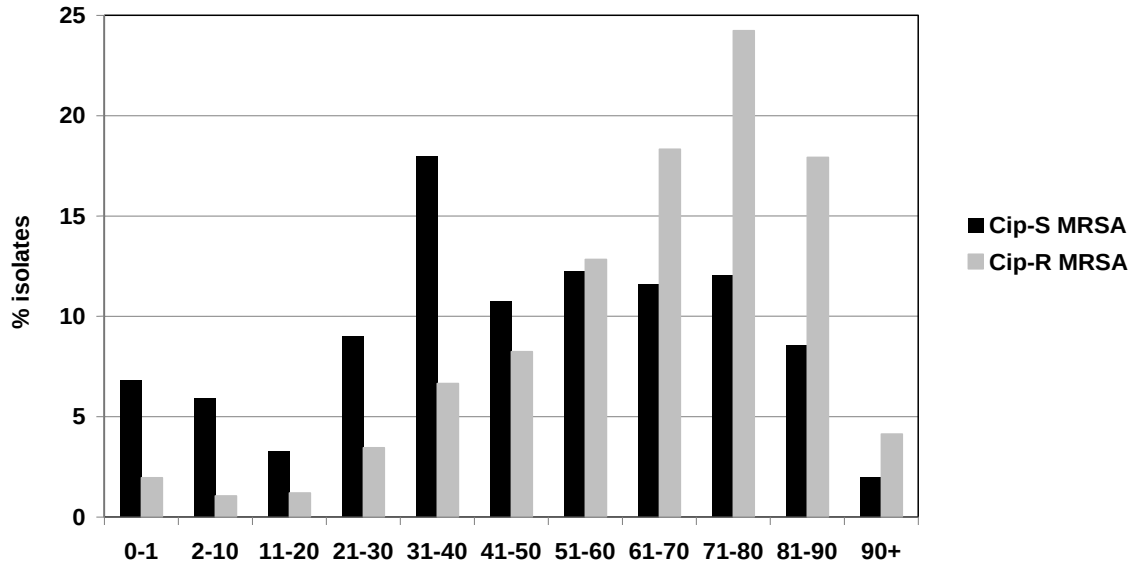
[g] Since 2003, infections were classified as ‘hospital-acquired’, ‘present on admission’ or ‘previously positive’ if the patient had a previous MRSA episode. 2479 Cip-R and 144 Cip-S infections were classified; classification data were not available for many of the outpatients.

[h] Multiple specimens from different body sites were included. Multiple specimens collected from the same body site from the same patient were excluded.

[i] Skin and soft tissue infection.

The age distribution of the Cip-R and Cip-S MRSA is compared in Figure 7-2.

Figure 7-2. Age distribution of all 5423 ciprofloxacin-resistant (Cip-R) MRSA compared with 458 ciprofloxacin-susceptible (Cip-S) MRSA reported 2000-2006 for which age data were available.



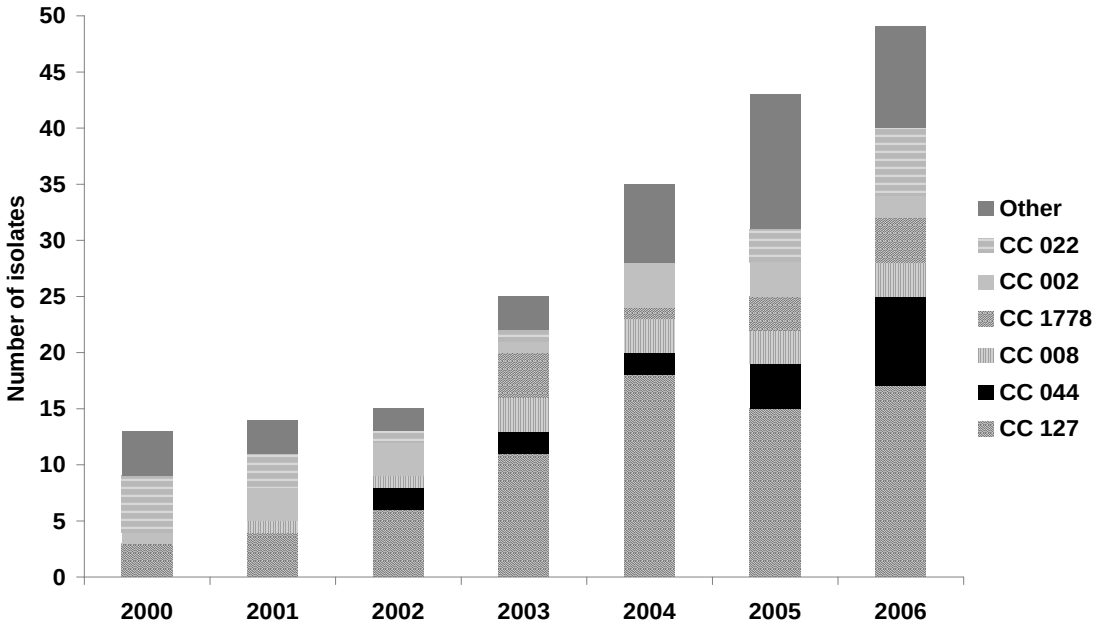
Compared with Cip-R MRSA, Cip-S MRSA isolates affected younger patients (median age 44.8 vs. 68.5 years), were significantly less likely to be resistant to erythromycin, gentamicin and mupirocin but significantly more likely to be resistant to fusidic acid and tetracycline (Table 7-1, p.135). Cip-R MRSA were significantly more likely to be obtained from adult inpatients whereas Cip-S MRSA were more likely to be isolated from patients attending Accident and Emergency, General Practice and hospital specialties in which MRSA is uncommon, such as paediatrics, obstetrics and gynaecology and genito-urinary medicine. Furthermore, 50.8% of the Cip-R MRSA were classified as hospital-acquired compared with only 26.3% of the Cip-S MRSA. Cip-S MRSA were significantly more likely to be cultured from SSTIs and mucosal sites than Cip-R MRSA. MRSA isolates from sites associated with HA-MRSA (such as respiratory, urine and intravascular catheter insertion sites and tips), were significantly more likely to be Cip-R, but there was no significant difference between the proportion of Cip-R and Cip-S isolates from blood.

7.1.3 Characteristics of recovered Cip-S MRSA

One hundred and ninety-four (42.2%) of the 458 reported Cip-S MRSA isolates were recovered from storage. Although all 194 isolates recovered from storage were *mec*A positive, 23 (11.9%) were phenotypically susceptible to methicillin by either disc diffusion or agar dilution. The median methicillin MIC was ≥16 μg/mL. One hundred and fifty-nine (82.0%) of the isolates were SCC*mec* IV and a further 28 (14.4%) were non-typeable by the multiplex PCR method used; only 7 (3.6%) of the isolates were SCC*mec* types I, II or III.

spa typing identified 62 unique *spa* types among the 194 Cip-S MRSA isolates, which were grouped into six *spa* CCs and 30 singleton lineages using the BURP algorithm (Appendix 9-2, p.214). The proportion of isolates from each *spa* CC remained relatively constant throughout the study (Figure 7-3).

Figure 7-3. *spa* clonal complexes by year, retrospective study.



The features of the most common *spa* types known to relate to CA-MRSA lineages are summarised in Table 7-2, 139.

Table 7-2. Features of 194 ciprofloxacin-susceptible MRSA reported at GSTT, 2000-2006.

(Percentage of total in each *spa* type in parenthesis unless otherwise stated.)

	t127	%	*t044*	%	*t022*	%	*t002*	%	*t008*	%	*Other*[a]	%	Total	%	p value[b]
Total	72	(37.1)	12	(6.2)	12	(6.2)	11	(5.7)	7	(3.6)	80	(41.2)	194	(100.0)	-
spa CC	127		044		022		002		008		-		-		-
MLST[c]	1		80		22		5		8		-		-		-
SCC*mec*[d] type															
I, II or III	2	(2.8)	0	-	0	-	0	-	0	-	5	(6.3)	7	(3.6)	0.6508
Non-typeable	9	(12.5)	0	-	0	-	2	(18.2)	1	(14.3)	16	(20.0)	28	(14.4)	0.2693
IV	61	(84.7)	12	(100.0)	12	(100.0)	9	(81.8)	6	(85.7)	59	(73.8)	159	(82.0)	0.0953
IV, no subtype	4	(5.6)	0	-	9	(75.0)	3	(27.3)	0	-	9	(11.3)	25	(12.9)	**<0.0001**
IVa	48	(66.7)	1	(8.3)	1	(8.3)	2	(18.2)	5	(71.4)	25	(31.3)	82	(42.3)	**<0.0001**
IVc	9	(12.5)	11	(91.7)	2	(16.7)	4	(36.4)	1	(14.3)	24	(30.0)	51	(26.3)	**<0.0001**
IVd	0	-	0	-	0	-	0	-	0	-	1	(1.3)	1	(0.5)	0.9275
PVL[e]	6	(8.3)	12	(100.0)	0	-	0	-	4	(57.1)	27	(33.8)	49	(25.3)	**<0.0001**
Antimicrobial resistance															
Erythromycin	37	(51.4)	6	(50.0)	1	(8.3)	1	(9.1)	0	-	22	(27.5)	67	(34.5)	**0.0004**
Fusidic acid	55	(76.4)	6	(50.0)	0	-	1	(9.1)	2	(28.6)	18	(22.5)	82	(42.3)	**<0.0001**
Tetracycline	3	(4.2)	5	(41.7)	0	-	1	(9.1)	1	(14.3)	16	(20.0)	26	(13.4)	**0.0023**
Neomycin	1	(1.4)	11	(91.7)	0	-	1	(9.1)	1	(14.3)	16	(20.0)	30	(15.5)	**<0.0001**
Antibiogram[f]															
None	7	(9.7)	0	-	11	(91.7)	9	(81.8)	2	(28.6)	37	(46.3)	66	(34.0)	**<0.0001**
1 class	36	(50.0)	2	(16.7)	1	(8.3)	1	(9.1)	4	(57.1)	14	(17.5)	58	(29.9)	**<0.0001**
2 classes	26	(36.1)	5	(41.7)	0	-	0	-	1	(14.3)	19	(23.8)	51	(26.3)	0.0537
≥3 classes	3	(4.2)	5	(41.7)	0	-	1	(9.1)	0	-	10	(12.5)	19	(9.8)	**0.0030**
Patient demographics															
Male	50.0	(69.4)	6.0	(50.0)	8.0	(66.7)	6.0	(54.5)	3	(42.9)	53	(66.3)	126	(64.9)	0.5616
Median age	41.6		27.7		51.9		29.1		27.2		39.6		39.0		0.3684[g]
Age range	0.1-95.8		4.1-78.1		0.0-94.4		0.0-85.8		0.8-57.3		0.0-94.5		0.0-95.8		-
Children <15 years	7.0	(9.7)	3.0	(25.0)	4.0	(33.3)	3.0	(27.3)	1	(14.3)	19	(23.8)	37	(19.1)	0.1766
Risk factors															

	t127	%	*t044*	%	*t022*	%	*t002*	%	*t008*	%	*Other*[a]	%	Total	%	p value[b]
Drug or alcohol abuse	28	(39.4)	0	-	1	(8.3)	2	(18.2)	1	(14.3)	5	(6.6)	37	(22.2)	**<0.0001**
Neoplasm	1	(1.4)	0	-	5	(41.7)	1	(9.1)	0	-	8	(10.5)	15	(9.0)	**0.0001**
Collection location															
Inpatient	31	(43.1)	4	(33.3)	12	(100.0)	4	(36.4)	1	(14.3)	39	(48.7)	91	(46.9)	**0.0024**
Outpatient or A&E[h]	41	(56.9)	8	(66.7)	0	-	7	(63.6)	6	(85.7)	41	(51.3)	103	(53.1)	**0.0024**
Specimen															
SSTI[i]	70	(60.3)	12	(75.0)	11	(45.8)	11	(78.6)	6	(66.7)	66	(62.9)	176	(62.0)	0.3375
SSTI – abscess	7	(6.0)	4	(25.0)	0	-	2	(14.3)	3	(33.3)	6	(5.7)	22	(7.7)	**0.0021**
Respiratory	12	(10.3)	0	-	7	(29.2)	0	-	0	-	10	(9.5)	29	(10.2)	**0.0163**
Urine	2	(1.7)	0	-	0	-	0	-	0	-	3	(2.9)	5	(1.8)	0.8682
Mucosal	6	(5.2)	2	(12.5)	2	(8.3)	1	(7.1)	2	(22.2)	6	(5.7)	19	(6.7)	0.4110
Invasive & line or tip	15	(12.9)	1	(6.3)	2	(8.3)	2	(14.3)	1	(11.1)	10	(9.5)	31	(10.9)	0.9265
Other	11	(9.5)	1	(6.3)	2	(8.3)	0	-	0	-	10	(9.5)	24	(8.5)	0.7705
Epidemiological classification[j]															
Healthcare-acquired	30	(41.7)	4	(33.3)	11	(91.7)	5	(45.5)	0	-	37	(46.3)	90	(46.4)	**0.0062**
Community-acquired	41	(56.9)	8	(66.6)	1	(8.3)	6	(54.5)	7	(100.0)	39	(48.8)	101	(52.1)	**0.0037**
No data	1	(1.4)	0	-	0	-	0	-	0	-	4	(5.0)	27	(13.9)	-

[a] 'Other is a heterogeneous group comprising 11 *t1778* isolates, 4 *t012* isolates, 2 *t096, t076, t0690, t437, t216, t186, t015, t005* isolates and single representatives of 48 other *spa* types.

[b] P values calculated using Chi-square tests of 2 x 6 contingency tables unless otherwise stated. P values <0.05 highlighted in bold.

[c] Multi-locus sequence type (MLST); the MLST type of one representative isolate from the most common *spa* type within each *spa* clonal complex was determined.

[d] Staphylococcal cassette chromosome *mec.*

[e] Panton-Valentine leukocidin.

[f] The number of antimicrobial resistance classes in addition to the β-lactams.

[g] P value calculated using a Kruskal-Wallace test.

[h] Includes isolates from genitourinary medicine and obstetrics and gynaecology.

[i] Skin and soft tissue infection.

[j] See methods (section 6.14.1, p.40) for a detailed explanation of epidemiological classification criteria.

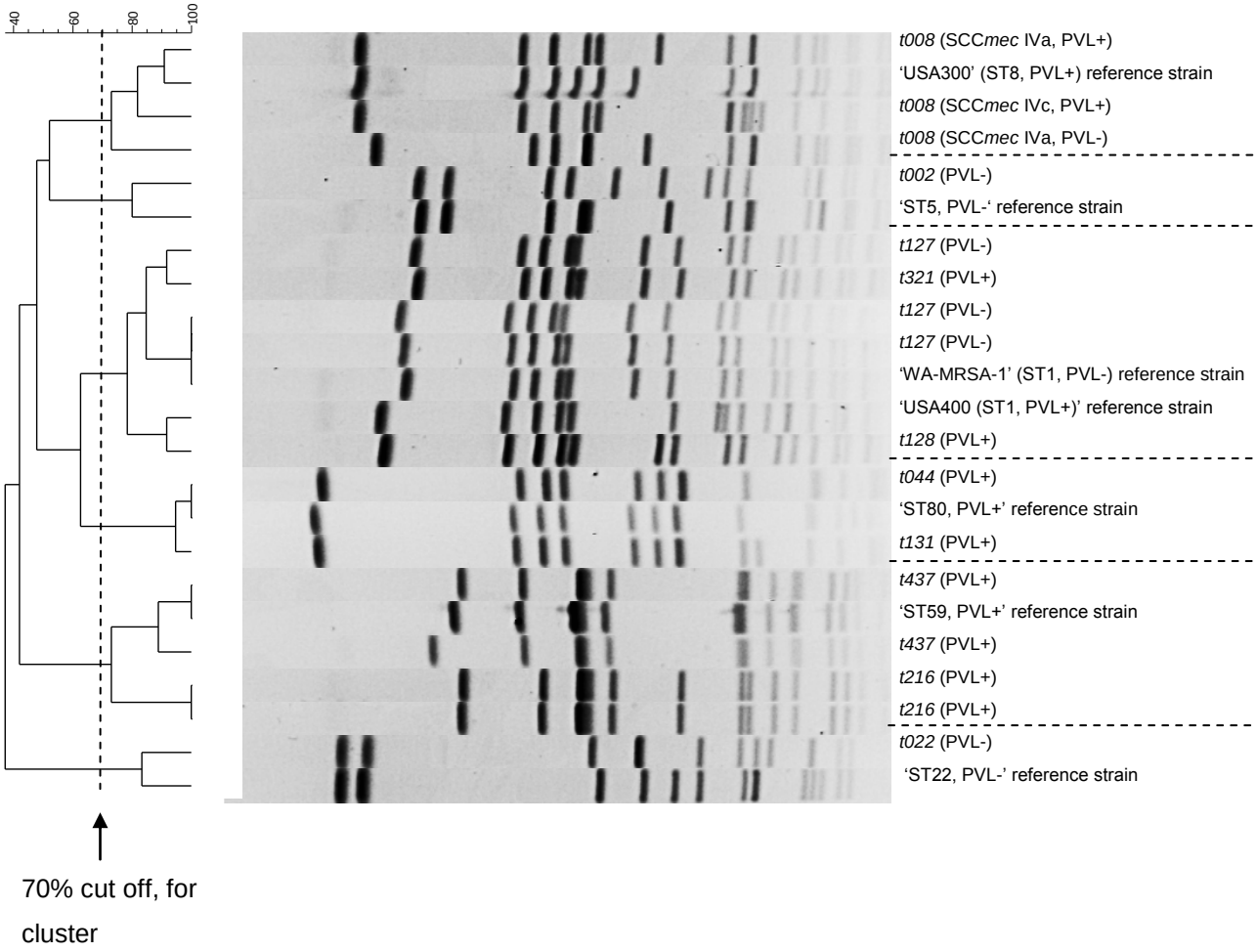
MLST was performed on at least one representative isolate from each *spa* CC and on a representative isolate from *spa* singleton lineages that contained >1 isolate (Table 7-3).

Table 7-3. MLST data.

spa type	*spa* CC	MLST
t127	127	1
t008	008	8
t986	008	8
t022	022	22
t005	022	22
t002	022	5
t756	022	217
t044	044	80
t1778	1778	1
t012	Singleton	30
t015	Singleton	45
t216	Singleton	59
t437	Singleton	59
t186	Singleton	88
t690	Singleton	88

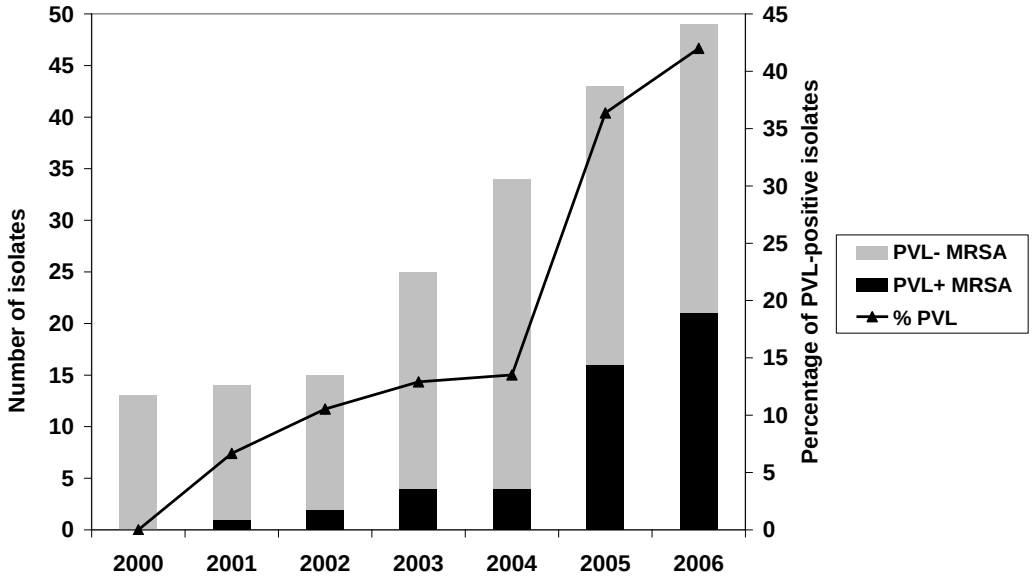
PFGE was performed on at least one isolate from each *spa* CC and on several isolates from *spa* singleton lineages (Figure 7-4, p.142).

Figure 7-4. PFGE data from selected Cip-S MRSA.



Forty-nine (25.3%) of the Cip-S isolates were PVL-positive: the frequency increased from 12 (11.8%) of the 102 isolates from 2000-2004 to 37 (40.2%) of 92 from 2005-2006 (P < 0.0001) (Figure 7-5).

Figure 7-5. Number and percentage of PVL-positive isolates among recovered isolates.



The characteristics of the 49 PVL-positive Cip-S MRSA are compared with the PVL-negative Cip-S MRSA in Appendix 9-3 (p.216). Compared with PVL-negative Cip-S MRSA, PVL-positive Cip-S MRSA affected significantly younger patients (median age 33.6 vs. 41.2 years, p=0.02), were more frequently associated with abscesses (26.5% vs. 9.0%, p=0.003), less likely to be associated with drug or alcohol abuse (2.0% vs. 22.8%, p=0.0004), more likely to be classified epidemiologically as community-acquired (51.0 vs. 31.0, p=0.024), more likely to be resistant to tetracycline (44.9% vs. 4.1%, p<0.0001) and neomycin (32.7% vs. 6.2%, p<0.0001) and more likely to be resistant to ≥3 non-β-lactam classes of antimicrobial agents (28.6% vs. 9.0%).

Two patients who had different surnames but shared the same postcode had PVL-positive MRSA isolates. They turned out to be a 22 year old mother and her newborn daughter. A *t216* (ST59) isolate was cultured from a vaginal swab of the mother on the day of delivery and seven days

later, a *t216* isolate indistinguishable by PFGE (Figure 7-4, p.142) was cultured from a skin wound on the newborn, which suggests vertical transmission.

The PVL-positive isolates were diverse with 24 distinct *spa* types clustering into five *spa* CCs and eight singleton lineages. The ST80 *t044* European clone was the most common PVL-positive isolate, accounted for 24.9%. Recognised CA-MRSA clones that occurred infrequently in the collection included four PVL-positive ST59 isolates (two *t437* and two *t216* types), three of which occurred in 2006, and individual occurrences of PVL-positive USA400/ST1 *t128* and *t311* types (Figure 7-4, p.142). No single clone was responsible for the increase in PVL-positive MRSA in the latter years of the study (Figure 7-5, p.143); among the 37 PVL-positive Cip-S MRSA from 2005 to 2006, there were 21 different *spa* types grouped into three *spa* CCs and 12 singleton lineages by BURP analysis.

The most common *spa* type among all 194 recovered isolates was *t127*, which accounted for 72 isolates (37.1% of the total); it was ST1 and usually PVL-negative, consistent with WA-MRSA-1, which has a PFGE profile that is closely related to USA-400 (Figure 7-4, p.142).[449] Six (8.3%) *t127* isolates were PVL-positive and showed considerable phenotypic and genotypic heterogeneity, exhibiting five distinct antibiograms and five distinct SCC*mec* types related to type IV. Drug or alcohol abuse was noted in the medical records of 39.4% of the *t127* patients (Table 7-2, p.139) and 47.2% were homeless or living in temporary sheltered accommodation; 12.5% of the patients shared the same homeless shelter. Although 30 (41.7%) of the *t127* infections were classified epidemiologically as healthcare-acquired, 21 of these had unusual features: six were in IDUs with inpatient healthcare contact, six were from paediatrics and two were in maternity.

Twelve (6.2%) of the isolates were *t044* and they were all PVL positive; a representative isolate was ST80 and had a PFGE profile consistent with the European clone (Figure 7-4, p.142). Nine (75.0%) of the *t044* isolates

were classified epidemiologically as community-acquired and the other three were atypical healthcare-acquired infections (Table 7-2, p.139): one was an infected caesarean-section, another was a bone culture from a paediatric patient and the third was an abscess in an out-patient with previous multiple sclerosis-related inpatient stays.

In contrast, the 12 *t022* isolates (6.2%) were consistent with EMRSA-15; a representative isolate was ST22 and had a common UK EMRSA-15 PFGE profile (Figure 7-4, p.142). All 12 isolates were PVL-negative, SCC*mec* IV and classified epidemiologically as healthcare-acquired (Table 7-2, p.139). BURP clustering included 7 further Cip-S MRSA in a *spa* CC with the *t022* isolates (Appendix 9-2, p.214), which were all PVL-negative and had similar healthcare-associated characteristics.

Seven (3.6%) of the 194 isolates were *t008.* A representative isolate was ST8 and these isolates were associated with abscess formation, PVL production and community-acquired infections (Table 7-2, p.139). They showed considerable heterogeneity: 4 were PVL-positive, and there were five different antibiograms and three different SCC*mec* IV-related types (Figure 7-4, p.142).

t002 is a common *spa* type that includes a range of HA-MRSA clones. Eleven diverse isolates of *t002* were identified that were associated with both hospital and community infections (Table 7-2, p.139).

A high proportion (33.8%) of the 80 remaining isolates, were PVL-positive.

7.2 Assessment of antimicrobial susceptibility-based algorithms for the presumptive identification of CA-MRSA

I decided to assess whether ciprofloxacin susceptibility or some other antimicrobial-susceptibility based algorithm is a suitable screening marker for CA-MRSA at GSTT. This was done with a three month study of available MRSA isolates. Three-hundred and eighty six unique patient

isolates were reported during the three month study period and 239 (61.9%) were available for analysis.

7.2.1 Comparison of isolates defined as HA- or CA-MRSA

The clinical, epidemiological and molecular characteristics of the isolates defined as HA- or CA-MRSA are summarised in Table 7-4, p.147.

EMRSA-15, primarily *t032* which accounted for almost half of HA-MRSA isolates. A further 24.2% were *spa* CC 012, which contained the *spa* types associated with ST32-II EMRSA-16. The remaining 6% of the HA-MRSA was made up by sporadic isolates. SCC*mec* types among the HA-MRSA isolates reflected the predominance of EMRSA-15 and -16, with 68.2% SCC*mec* IV and 21.7% SCC*mec* II.

The 41 CA-MRSA isolates were significantly more diverse than the HA-MRSA isolates (48.8 vs. 16.1% unique *spa* types and 36.6 vs. 0.5% singleton lineages) (Table 7-4, p.147). The majority of isolates were SCC*mec* IV (73.2%) or V (17.1%). Almost 15% of the CA-MRSA isolates were clustered in *spa* CC012, but were different *spa* and SCC*mec* types to the EMRSA-16 isolates. Similarly, 19.5% were *spa* CC008, but these were different *spa* and SCC*mec* types to the multiresistant *t190* / ST8-VI HA-MRSA clone. The CA-MRSA group contained several recognised CA-MRSA clones including PVL-negative *t127* / ST1-IV or NT (31.7%), PVL-positive *t008* or *t024* / ST8-IV (presumptive USA300, 9.7%), PVL-positive *t019* or *t021* / ST30-IV (7.3%) and PVL-positive *t044* / ST80-IV (2.4%) (European clone).

Table 7-4. Clinical, microbiological, epidemiological and molecular characteristics of MRSA isolates defined genetically as HA-MRSA or CA-MRSA.

	HA-MRSA (n=198)		CA-MRSA (n=41)		p value[a]	Multivariate analysis[b] p value
	n[c]	%	n	%		
Age / years						
Mean	60.2		48.2		**0.002**[d]	-
Median (range)	65.0 (0.1-94.0)		49.0 (0.1-90.0)		-	-
Gender						
Male	123	62.1	25	61.0	>0.999	-
Body site of isolation[e]						
Screen	163	55.6	31	59.6	0.353	-
SSTI[f]	80	27.3	17	32.7	0.261	-
Abscess	*0*	*0.0*	*2*	*11.8*	***0.003***	-
Invasive	34	11.6	3	5.8	0.156	-
Respiratory	10	3.4	1	1.9	0.487	-
Mucosal	4	1.4	0	0.0	0.519	-
Other	2	0.7	0	0.0	0.721	-
Total	293	-	54	-	0.645	-
Epidemiological classifications[g]						
Community-acquired	20	10.1	12	29.3	**0.002**	-
Community-onset	46	23.2	18	43.9	**0.007**	-
Antimicrobial resistance, by agent						
Ciprofloxacin	195	98.5	15	36.6	**<0.001**	**<0.001**
Erythromycin	147	74.2	9	22.0	**<0.001**	0.141
Fusidic acid	23	11.6	14	34.1	**0.001**	**0.044**
Gentamicin	41	20.7	2	4.9	**0.009**	0.328
Tetracycline	15	7.6	7	17.1	0.060	0.102
Trimethoprim	39	19.7	4	9.8	0.095	0.759
Mupirocin	14	7.1	2	4.9	0.460	0.736
Rifampicin	2	1.0	0	0.0	0.686	0.999
Resistance to non-β lactam classes						
None	3	1.5	13	31.7	**<0.001**	-
<2 classes	41	20.7	24	58.5	**<0.001**	-
<3 classes	129	65.2	35	85.4	**0.007**	-
3 or more classes	69	62.5	6	12.9	**0.007**	-
Specific resistance patterns[h]						
CIP	38	19.2	1	2.4	**0.003**	-
CIP/ERY	78	39.4	5	12.2	**<0.001**	-
CIP or CIP/ERY	116	58.6	6	14.6	**<0.001**	-
CIP susceptible or CIP/FA	10	5.1	29	75.6	**<0.001**	-
PVL						
PVL positive	0	0.0	9	22.0	**<0.001**	-
SCC*mec*[i]						
I	1	0.5	-	-	-	-
II	43	21.7	-	-	-	-
III	7	3.5	-	-	-	-
IV	135	68.2	30	73.2	-	-
V	1	0.5	7	17.1	-	-

	HA-MRSA (n=198)		CA-MRSA (n=41)		p value[a]	Multivariate analysis[b] p value
	n[c]	%	n	%		
VI	8	4.0	-	-	-	-
NT	3	1.5	9	9.8	-	-
spa diversity						
Unique *spa* types	32	16.1	20	48.8	**<0.001**	-
Common *spa* types (inferred MLST CC)[j]						
spa CC032 (CC22)	139	70.2	-	-	-	-
t032	*97*	*49.0*	*-*	*-*	-	-
t022	*9*	*4.5*	*-*	*-*	-	-
t020	*2*	*1.5*	*-*	*-*	-	-
spa CC012 (CC30)	48	24.2	6	14.6	-	-
t018	*29*	*14.6*	*0*	*0.0*	-	-
t012	*11*	*5.6*	*3*	*7.3*	-	-
t019	*0*	*0.0*	*2*	*4.9*	-	-
spa CC008 (CC8)	9	4.5	8	19.5	-	-
t190	*9*	*4.5*	*0*	*0.0*	-	-
t008	*0*	*0.0*	*4*	*9.8*	-	-
Singleton	1	0.5	15	36.6	-	-
t127 (CC1)	*0*	*0.0*	*13*	*31.7*	-	-
Other	1	0.5	12	29.2	-	-

[a] p values determined using Chi-square tests of 2x2 contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] A binomial logistic regression model using resistance to individual antimicrobial agents was used for the multivariate analysis. Resistance to non-β-lactam classes and specific algorithms could not be included because the model requires independent covariates. p values <0.05 highlighted in bold.

[c] n = number.

[d] p value determined using the Mann-Whitney U test.

[e] Multiple specimens from different body sites were included. Multiple specimens collected from the same body site from the same patient were excluded.

[f] SSTI = skin and soft tissue infection. Abscesses were included as a sub-classification of SSTIs.

[g] See methods (section 6.15.4, p.40) for a detailed explanation of classification criteria.

[h] CIP= ciprofloxacin, ERY = erythromycin, FA = fusidic acid.

[i] SCC*mec* = Staphylococcal cassette chromosome *mec.* p values were not calculated for SCC*mec* types because they were used as part of the definition of healthcare and community associated isolates.

[j] MLST CC = multilocus sequence typing clonal complex. Inferred MLST CC obtained from the *spa* server, www.spaserver.ridom.de (accessed 29/07/09). Statistical comparisons were not performed based on *spa* types (except *spa* type diversity) because they were used as part of the definition of healthcare and community associated isolates.

BURP analysis clustered *spa* types into seven CCs and two singleton lineages (Appendix 9-4, p.218). The majority (70.2%) of the 198 HA-MRSA were *spa* CC 032, which contained the *spa* types associated with ST22-IV The age distribution of the HA-MRSA and CA-MRSA patients is compared in Figure 7-6.

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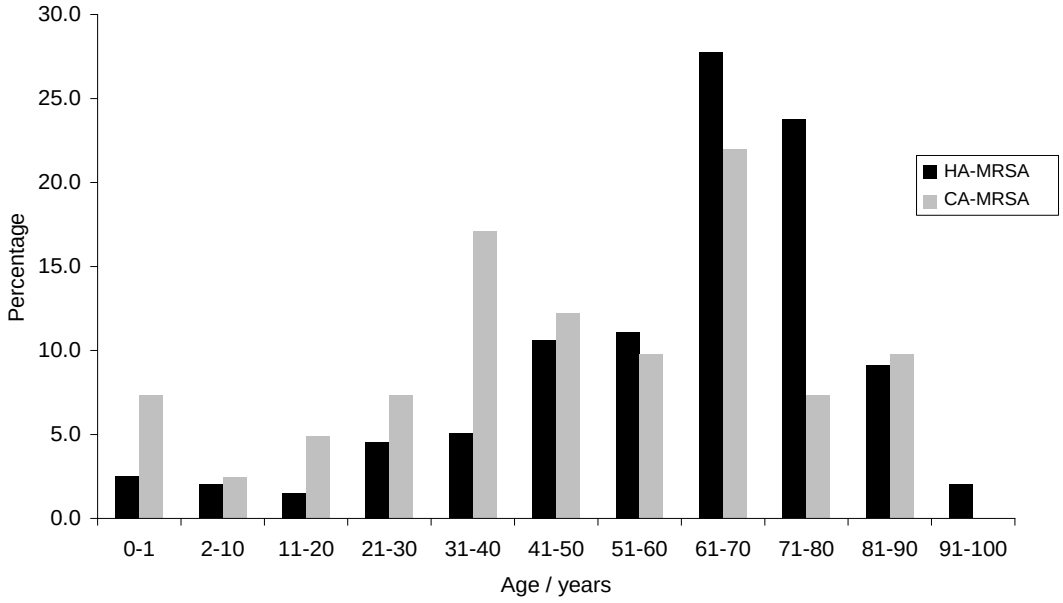
Figure 7-6. Age distributions for isolates defined as HA-MRSA or CA-MRSA.



HA-MRSA were associated with significantly younger patients (Figure 7-6), were less likely to be classified epidemiologically as CA and community-onset and were less likely to be cultured from abscesses or encode the PVL genes (Table 7-4, p.147). Seventy-one percent of the CA-MRSA isolates were classified epidemiologically as hospital-acquired.

AMS-based algorithms

HA-MRSA isolates were significantly more likely to be ciprofloxacin, erythromycin and gentamicin resistant but significantly less likely to be resistant to fusidic acid (Table 7-4, p.147). Ciprofloxacin susceptibility and fusidic acid resistance remained independent predictors of CA-MRSA isolates in a multivariate binomial regression analysis including all antimicrobial agents tested (Table 7-4, p.147). CA-MRSA isolates were

generally resistant to fewer non-β-lactam classes, with significant association with resistance to no or <2 classes (Table 7-4, p.147). AMS profiles were grouped by *spa* type and analysed to develop algorithms for the differentiation of HA- and CA-MRSA strain types (Appendix 9-5, p.220; Table 7-4, p.147).

The performance characteristics of algorithms highly significantly associated (p <0.001) with CA-MRSA are summarised in Table 7-5.

Table 7-5. Performance characteristics of AMS-based algorithms as predictors of isolates defined as CA-MRSA, sorted by increasing sensitivity.

Algorithm[a]	Sensitivity	Specificity	PPV[b]	NPV[c]
Resistant to no non-β lactam classes	31.7	98.5	81.2	87.4
Resistant to <2 non-β lactam classes	58.5	79.3	36.9	90.2
Ciprofloxacin-susceptible	63.4	98.5	89.6	92.9
Ciprofloxacin-susceptible or specific antibiogram of CIP/FA	70.7	94.9	74.3	94.0
Erythromycin susceptible	78.0	74.2	38.6	94.2
Does not have a specific antibiogram of CIP or CIP/ERY	85.3	58.6	29.9	95.1
Does not have a specific antibiogram of CIP/ERY	87.8	39.3	23.0	94.0

[a] CIP= ciprofloxacin resistant, ERY = erythromycin resistant, FA = fusidic acid resistant.

[b] PPV = positive predictive value.

[c] NPV = negative predictive value.

The sensitivity of the algorithms (the proportion of CA-MRSA isolates classified correctly) ranged from 31.7% to 87.9% but the specificity (the proportion of HA-MRSA isolates classified correctly) ranged from 98.5% to 39.3% (Table 7-5, p.150). The PPVs ranged from 23.0% to 89.6% and the NPVs ranged from 87.4% to 95.1% (Table 7-5, p.150). Combinations of ciprofloxacin susceptibility and fusidic acid resistance provided the most useful phenotypic markers. Ciprofloxacin susceptibility alone had

sensitivity, 63.4%; specificity, 98.5%; PPV, 89.6% and NPV, 92.6%. An algorithm of ciprofloxacin-susceptible or a specific antibiogram of resistance to ciprofloxacin and fusidic acid only had sensitivity, 70.7%; specificity, 94.9%; PPV, 74.3% and NPV, 94.0%. The limited sensitivity of these algorithms means that 30-35% of isolates would be misclassified as HA-MRSA. Importantly, four of the nine PVL-positive CA-MRSA isolates were resistant to ciprofloxacin and three of these were susceptible to fusidic acid.

Due to the limited sensitivity of the best AMS-based algorithms, the hypothesis that AMS-based algorithms can be used as an accurate screening marker for the presumptive identification of CA-MRSA in a collection of predominantly HA-MRSA isolates is rejected. Therefore, in order to conduct an accurate assessment of the prevalence of CA-MRSA on admission to GSTT, all isolates were typed.

7.3 The prevalence and molecular epidemiology of CA-MRSA identified on admission screens

7.3.1 Prevalence of positive screens on admission

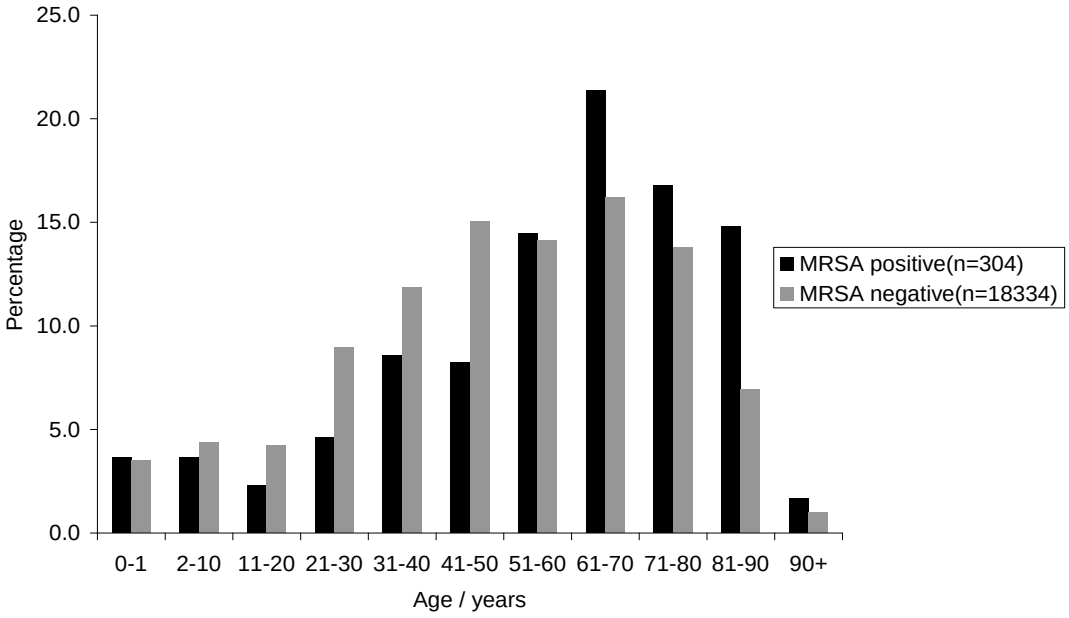
Over the first six months of universal admission screening from 1st April 2009, 304 (1.6%) of 18636 screens were positive for MRSA. Approximately half (54.0%) of the screens were admission screens from inpatients, 45% were pre-admission screens from outpatients and the other 1% were collected from patients admitted through the accident and emergency department (A&E); most (82.3%) of the pre-admission screens were taken in surgical specialties. Five percent of screens were collected from non-standard sites; 2% were from incomplete standard sets, 2% were rectal screens (standard in the intensive care unit) and 1% were from non-infected clinical sites. The frequency of MRSA in non-standard sites was significantly greater than the frequency of MRSA from standard screening sites (7.2% vs. 1.6%, $p<0.001$) (Appendix 9-6, p.223). Overall, approximately 40% of all admissions were screened for MRSA; however,

the admissions denominator included day visits which are not included for screening in the GSTT universal screening policy, excepting day surgery.

The prevalence of MRSA by specialty and location is summarised in Table 7-6. p.153.

The age distribution of MRSA-positive patients is compared with MRSA-negative patients in Figure 7-7.

Figure 7-7. Age distribution of MRSA-positive and MRSA-negative patients screened on admission to GSTT.



The highest prevalence of MRSA colonisation occurred in patients admitted to critical care units (7.7% of 84 admissions) and respiratory medicine (7.3% of 267 admissions) and the lowest prevalence occurred in pre-admission screens collected by general practitioners (none of 68 admissions) and patients admitted to paediatric A&E (none of 43 admissions) (Table 7-6, p. 153). The prevalence of MRSA colonisation was low in obstetrics and gynaecology (0.7% of 897 admissions), neonatology (0.8% of 252 admissions) and paediatrics (1.3% of 1679 admissions) (Table 7-6, p. 153).

Table 7-6. The prevalence of MRSA colonisation on admission by specialty and location.

	MRSA-negative (n=18334)		MRSA-positive (n=304)			Univariate[a]		Multivariate[b]	
	n[c]	% of all negatives	n	% positive	% of all positives	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
DEMOGRAPHICS									
Mean age / years	50.0	-	57.6	-	-	-	**<0.001**[d]	-	-
Median age / years	52.0	-	63.0	-	-	-	-	-	-
Age >60 years	6951	37.9%	166	-	54.6%	2.0 (1.6-2.5)	**<0.001**	1.7 (1.3-2.3)	**<0.001**
Male	9014	54.0%	176	-	61.3%	1.3 (1.1-1.7)	**0.014**	-	0.155
Hospital day visit in the past 12 months	1728	9.4%	29	-	9.5%	-	0.469	-	-
Overnight hospital stay in past 12 months	1825	10.0%	63	-	20.7%	2.4 (1.8-3.1)	**<0.001**	-	0.418
Previous positive for MRSA	350	1.9%	102	-	33.6%	25.9 (20.0-33.7)	**<0.001**	19.9 (15.0-26.5)	**<0.001**
SPECIALTY									
Surgery									
Orthopaedics	2286	12.5%	22	1.0%	7.2%	0.6 (0.4-0.8)	**0.006**	-	0.626
Urology	1458	8.0%	19	1.3%	6.3%	-	0.334	-	-
General	1445	7.9%	21	1.4%	6.9%	-	0.584	-	-
Cardiothoracic	1346	7.3%	14	1.0%	4.6%	-	0.081	-	-
ENT/Oral	1171	6.4%	23	1.9%	7.6%	-	0.412	-	-
Plastic	795	4.3%	6	.7%	2.0%	0.4 (0.2-1.0)	**0.044**	-	0.421
Paediatric	657	3.6%	6	.9%	2.0%	-	0.159	-	-
Vascular	304	1.7%	6	1.9%	2.0%	-	0.647	-	-
Breast	234	1.3%	2	.8%	.7%	-	0.598	-	-
Ophthalmology	60	.3%	2	3.2%	.7%	-	0.268	-	-

	MRSA-negative (n=18334)		MRSA-positive (n=304)			Univariate[a]		Multivariate[b]	
	n[c]	% of all negatives	n	% positive	% of all positives	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Total surgery	9756	53.2%	121	1.2%	39.8%	0.6 (0.5-0.7)	**<0.001**	-	-
Medicine									
General	2468	13.5%	77	3.0%	25.3%	2.2 (1.7-2.8)	**<0.001**	-	0.120
Cardiology	1350	7.4%	9	.7%	3.0%	0.4 (0.2-0.7)	**0.003**	0.4 (0.2-0.8)	**0.014**
Paediatric	733	4.0%	14	1.9%	4.6%	-	0.555	-	-
Renal	539	2.9%	4	.7%	1.3%	-	0.118	-	-
Oncology	308	1.7%	7	2.2%	2.3%	-	0.366	-	-
Respiratory	267	1.5%	21	7.3%	6.9%	5.0 (3.2-7.9)	**<0.001**	3.0 (1.6-5.5)	**<0.001**
Elderly Care	123	.7%	4	3.1%	1.3%	-	0.651	-	-
Haematology	120	.7%	2	1.6%	.7%	-	1.000	-	-
Gastroenterology	98	.5%	2	2.0%	.7%	-	0.679	-	-
Critical Care	84	.5%	7	7.7%	2.3%	5.1 (2.3-11.2)	**0.001**	3.9 (1.5-10.4)	**0.010**
Rheumatology	84	.5%	2	2.3%	.7%	-	0.653	-	-
Dermatology	65	.4%	3	4.4%	1.0%	-	0.100	-	-
Endocrinology	58	.3%	1	1.7%	.3%	-	0.622	-	-
Neurology	47	.3%	1	2.1%	.3%	-	0.546	-	-
Total medicine	6344	34.6%	154	2.4%	50.7%	1.9 (1.5-2.4)	**<0.001**	-	-
A&E/GP/Other									
Accident & Emergency	662	3.6%	18	2.6%	5.9%	1.7 (1.0-2.7)	**0.043**	-	0.926
Other	312	1.7%	3	1.0%	1.0%	-	0.497	-	-
General practitioner	68	.4%	0	.0%	.0%	-	0.631	-	-

	MRSA-negative (n=18334)		MRSA-positive (n=304)			Univariate[a]		Multivariate[b]	
	n[c]	% of all negatives	n	% positive	% of all positives	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Paed A&E/GP/Other	43	.2%	0	.0%	.0%	-	1.000	-	-
Total A&E/GP/Other	1085	5.9%	21	1.9%	6.9%	-	0.469	-	-
Obstetrics/Gynaecology/Neonatology									
Obstetrics/Gynaecology	897	4.9%	6	.7%	2.0%	0.4 (0.2-0.9)	**0.015**	-	0.705
Neonatology	252	1.4%	2	.8%	.7%	-	0.449	-	-
Total obs/gynae/neonatology	1149	6.3%	8	.7%	2.6%	0.4 (0.2-0.8)	**0.009**	-	-
GRAND TOTAL	18334	100.0%	304	1.6%	100.0%				
LOCATION OF SCREEN									
Adult inpatient	8126	44.3%	168	2.1%	55.3%	1.6 (1.2-1.9)	**<0.001**	-	0.974
Adult pre-assessment	4570	24.9%	42	0.9%	13.8%	0.5 (0.3-0.7)	**<0.001**	0.5 (0.3-0.9)	**0.032**
Adult outpatients	3064	16.7%	27	0.9%	8.9%	0.5 (0.3-0.7)	**<0.001**	-	0.137
Adult ITU/HDU[e]	670	3.7%	34	5.1%	11.2%	3.3 (2.3-4.8)	**<0.001**	-	0.405
Adult A&E	225	1.2%	11	4.9%	3.6%	3.0 (1.6-5.6)	**0.002**	-	0.318
Paediatrics	1679	9.2%	22	1.3%	7.2%	-	0.271	-	-

[a] p values determined using Chi-square tests of 2x2 contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] A binomial logistic regression model was used for the multivariate analysis using variables that were significant (p<0.05) by univariate analysis as covariates.

[c] n = number.

[d] p value determined using the Mann-Whitney U test.

[e] ITU = intensive care unit; HDU = high dependency unity.

Compared with MRSA-negative screens, MRSA-positive screens were significantly associated with patients with a history of MRSA (odds ratio, OR, 25.9), overnight stay at GSTT in the 12 months prior to admission (OR 2.4) but not day visits (p=0.469), age over 60 years (OR 2.0), and male gender (OR 1.3); positive screens were significantly more likely in medical specialties (OR 1.9), specifically critical care (OR 5.1), respiratory medicine (OR 5.0), general medicine (OR 2.2), but less likely in cardiology (OR 0.4); less likely in surgical specialties (OR 0.6), specifically orthopaedics (OR 0.6) and plastic surgery (OR 0.4); more likely to be taken from adult patients admitted to ITU (OR 3.3) or through A&E (OR 3.0) and adult inpatients (OR 1.6) but less likely to be taken from adult pre-assessment clinic (OR 0.5) or outpatient (OR 0.5) pre-admission screens (Table 7-6, p. 153). History of MRSA (adjusted odds ratio, AOR, 19.9), critical care medicine (AOR 3.9), respiratory medicine (AOR 3.0) and age >60 years (AOR 1.7) were significant predictors of MRSA-positive screens whereas cardiology (AOR 0.4) and screens taken in adult pre-assessment clinics (AOR 0.5) were significant predictors of MRSA-negative screens in the multivariate binomial logistic regression model (Table 7-6, p.153).

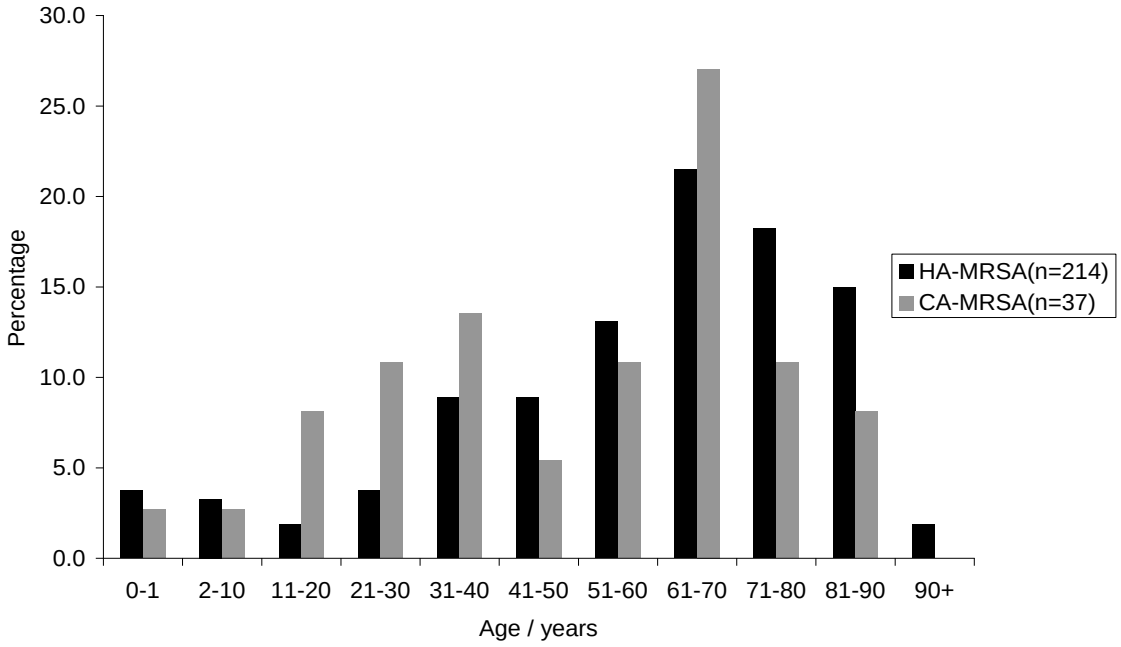
Over the six month period, 148 patients screened negative on admission and had a subsequent inpatient screen or clinical culture positive for MRSA, which was considered likely to represent a hospital acquisition. The characteristics of patients with a negative admission screen and a subsequent positive screen or culture are compared with patients with a negative admission screen and no subsequent positive screen or culture in Appendix 9-7, p.224. In multivariate analysis, history of MRSA (AOR 10.7), age >60 years (AOR 2.5), male gender (AOR 1.5), admission to dermatology (AOR 18.6), critical care medicine (AOR 5.1), vascular surgery (AOR 3.3), general medicine (AOR 1.8), and screens collected in the adult ITU or HDU (AOR 2.7) were risk factors for a negative admission screen followed by a subsequent positive screen or culture (Appendix 9-7, p.224). Since patients with a history of MRSA were more than ten times more likely to have a negative admission screen and a subsequent positive screen or culture, I repeated the analysis excluding patients

previously positive for MRSA because these I judged these patients more likely to be false negatives on the admission screen. However, the results of multivariate analysis were similar: age >60 years (AOR 3.0), male gender (AOR 1.6), admission to dermatology (AOR 24.5), critical care medicine (AOR 3.8), vascular surgery (AOR 3.7), general medicine (AOR 2.0), and screens collected in the adult ITU or HDU (AOR 4.0) were risk factors for a negative admission screen followed by a subsequent positive screen or culture (Appendix 9-7, p.224).

7.3.2 Molecular epidemiology of recovered MRSA isolates from admission screens

Slopes were saved for 85% of the 304 positive patients and MRSA was recovered from 97% of saved slopes. The age distribution of the isolates defined as HA-MRSA or CA-MRSA are compared in Figure 7-8.

Figure 7-8. Age distributions for isolates defined as HA-MRSA or CA-MRSA.



The molecular epidemiology of MRSA carried on admission is summarised in Table 7-7, p.158.

Table 7-7. The molecular epidemiology of MRSA carried on admission defined as HA-MRSA or CA-MRSA.

	HA-MRSA (n=214)		CA-MRSA (n=37)		Univariate[a]		Multivariate[b]		%CA-MRSA among recovered MRSA	%CA-MRSA among MRSA from specialty
	n[c]	% HA-MRSA	n	%CA-MRSA	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value		
DEMOGRAPHICS										
Mean age	58.5		50.1		-	**0.041**[d]	-	0.836		
Median age (range)	63.5 (0-93)		55.0 (1-85)							
Age >60 years	121	56.5%	17	45.9%	-	0.283	-	-	6.8%	-
Gender (male)	125	62.2%	23	63.9%	-	0.590	-	-	9.2%	-
Previous positive for MRSA	75	35.0%	10	27.0%	-	0.452	-	-	4.0%	-
SPECIALTY										
Surgery	74	34.6%	22	59.5%	2.8 (1.4-5.8)	**0.006**	-	0.525	8.8%	22.9%
Medicine	105	49.1%	10	27.0%	0.4 (0.2-0.8)	**0.019**	-	0.292	4.0%	8.7%
Obstetrics / gynaecology	6	2.8%	0	0.0%	-	0.596	-	-	0.0%	0.0%
A&E/GP/Other[e]	14	6.5%	3	8.1%	-	0.723	-	-	1.2%	17.6%
Paediatrics	15	7.0%	2	5.4%	-	1.000	-	-	0.8%	11.8%
LOCATION OF SCREEN										
Adult A&E	6	2.8%	2	5.4%	-	0.335	-	-	0.8%	25.0%
Adult ITU/HDU[f]	32	15.0%	0	0.0%	0.8 (0.8-0.8)	**0.006**	-	0.998	0.0%	0.0%
Adult outpatient	22	10.3%	4	10.8%	-	1.000	-	-	1.6%	15.4%
Adult re-assessment	27	12.6%	7	18.9%	-	0.302	-	-	2.8%	20.6%
Adult inpatient	112	52.3%	22	59.5%	-	0.478	-	-	8.8%	16.4%
Paediatrics	15	7.0%	2	5.4%	-	1.000	-	-	0.8%	11.8%
EPIDEMIOLOGICAL CLASSIFICATION[g]										
Healthcare-acquired	169	79.0%	24	69.4%	-	0.089	-	-	-	-
Hospital-onset	161	75.2%	24	69.4%	-	0.224	-	-	-	-
Previous hospital visit in the past 12 months	72	33.6%	8	21.6%	-	0.182	-	-	-	-

	HA-MRSA (n=214)		CA-MRSA (n=37)		Univariate[a]		Multivariate[b]		%CA-MRSA among recovered MRSA	%CA-MRSA among MRSA from specialty
	n[c]	% HA-MRSA	n	%CA-MRSA	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value		
ANTIMICROBIAL RESISTANCE										
Ciprofloxacin	206	96.3%	12	32.4%	0.02 (0.07-0.05)	**<0.001**	0.02 (0.004-0.1)	**<0.001**	-	-
Erythromycin	148	69.2%	12	32.4%	0.2 (0.1-0.4)	**<0.001**	-	0.871	-	-
Fusidic acid	21	9.8%	15	40.5%	6.2 (2.8-13.9)	**<0.001**	8.6 (1.7-44.0)	**0.009**	-	-
Gentamicin	37	17.3%	2	5.4%	-	0.084	-	-	-	-
Tetracycline	13	6.1%	6	16.2%	3.0 (1.1-8.4)	**0.043**	-	0.635	-	-
Trimethoprim	37	17.3%	7	18.9%	-	0.816	-	-	-	-
Mupirocin	7	3.3%	1	2.7%	-	1.000	-	-	-	-
Rifampicin	2	0.9%	0	0.0%	-	1.000	-	-	-	-
NUMBER OF NON-β-LACTAM RESISTANCE CLASSES										
None	3	1.4%	10	27.0%	26.0 (6.7-100.6)	**<0.001**	-	0.612	-	-
<2 classes	53	24.8%	18	48.6%	2.8 (1.4-5.7)	**0.006**	-	0.774	-	-
PVL[h]	0	0.0%	8	21.6%	1.3 (1.1-1.5)	**<0.001**	-	0.998	3.2%	-
SCC*mec* TYPE[i]										
I	1	.5%	0	.0%	-	-	-	-	-	-
II	33	15.4%	0	.0%	-	-	-	-	-	-
III	4	1.9%	0	.0%	-	-	-	-	-	-
IV	161	75.2%	23	62.2%	-	-	-	-	-	-
V	2	.9%	6	16.2%	-	-	-	-	-	-
VI	8	3.7%	0	.0%	-	-	-	-	-	-
Non-typeable	5	2.3%	8	21.6%	-	-	-	-	-	-
SPA DIVERSITY										
Unique *spa* types	43	20.1%	23	62.2%	-	**<0.001**	-	-	-	-
COMMON *SPA* TYPES (INFERRED MLST CC)[j]										
spa CC032 (CC22)	172	80.4%	-	-	-		-	-	-	-
t032	*102*	*47.7%*	-	-	-		-	-	-	-
t022	*15*	*7.0%*	-	-	-		-	-	-	-
spa CC012 (CC30)	32	15.0%	5	13.5%	-		-	-	-	-

		HA-MRSA (n=214)		CA-MRSA (n=37)		Univariate[a]		Multivariate[b]		%CA-MRSA among recovered MRSA	%CA-MRSA among MRSA from specialty
		n[c]	% HA-MRSA	n	%CA-MRSA	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value		
	t018	*20*	*9.3%*	*0*	*.0%*	-		-	-	-	-
	t012	*8*	*3.7%*	*2*	*5.4%*	-		-	-	-	-
spa CC008 (CC8)		8	3.7%	5	13.5%	-		-	-	-	-
	t190	*7*	*3.3%*	*0*	*.0%*	-		-	-	-	-
	t008	*0*	*.0%*	*2*	*5.4%*	-		-	-	-	-
Singleton		1	.5%	17	45.9%	-		-	-	-	-
	t127	*0*	*.0%*	*12*	*32.4%*	-		-	-	-	-
Other		1	0.5%	10	4.7%	-		-	-	-	-

[a] p values determined using Chi-squared tests of contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] A binomial logistic regression model was used for the multivariate analysis using variables that were significant by univariate analysis as covariates.

[c] n = number.

[d] p value determined using the Mann-Whitney U test.

[e] A&E = Accident and Emergency; GP = General Practitioner.

[f] ITU = intensive care unit; HDU = high dependency unity.

[g] See methods (section 6.15.4, p.40) for a detailed explanation of classification criteria.

[h] PVL = Panton-Valentine leukocidin.

[i] SCC*mec* = Staphylococcal cassette chromosome *mec.* p values were not calculated for SCC*mec* types because they were used as part of the definition of healthcare and community associated isolates.

[j] MLST CC = multilocus sequence typing clonal complex. Inferred MLST CC obtained from the *spa* server, www.spaserver.ridom.de (accessed 30/08/09). Statistical comparisons were not performed based on *spa* types (except *spa* type diversity) because they were used as part of the definition of healthcare and community associated isolates.

Isolates defined as molecular CA-MRSA types accounted for 37 (14.7%) of the recovered isolates. Therefore, CA-MRSA types accounted for approximately 0.25% of all screens.

Compared with isolates defined as HA-MRSA types, CA-MRSA isolates were associated with younger patients (median age 55.0 vs. 63.5 years, p<0.041) (Figure 7-8, p.157) and were more likely to be identified in surgical specialties (OR 2.8) but less likely to be identified in medical specialties (0.4) and patients admitted to ITU (0.8) (Table 7-7, p.158). CA-MRSA strain types represented 23% of the MRSA identified in surgery, 25% in A&E and 20% in pre-assessment clinics (Table 7-7, p.158).

There were no significant differences between patients with CA-MRSA and patients with negative screens apart from a history of MRSA (OR 19.0) (Appendix 9-8, p.227). In contrast, compared with patients with negative screens, patients with HA-MRSA strains were older (mean age 58.5 vs. 50.0, p <0.001), more likely to be male (OR 1.4), have a history of MRSA (OR 27.7), have had a previous hospital visit in the 12 months prior to the admission screen (OR 2.1), be admitted to medical specialties (OR 2.2), and were more likely to have the admission screen collected in ICU/HDU (OR 4.6) or from inpatients (OR 1.4); patients with HA-MRSA were less likely to be admitted to surgical specialties (OR 0.5) and were less likely to have admission screen collected in adult outpatients (OR 0.6) or pre-assessment clinics (OR 0.4) (Appendix 9-8, p.227). History of MRSA (AOR 22.5), screens collected in ITU/HDU (AOR 3.3) and age >60 years (AOR 1.8) remained significantly associated with HA-MRSA strains in multivariate analysis.

There was no significant difference in the proportion of HA or CA-MRSA isolates with previous hospital contact or those classified epidemiologically as hospital-acquired or hospital-onset, supporting the breakdown of epidemiological definitions of CA-MRSA (Table 7-7, p.158). CA-MRSA were significantly less likely to be resistant to ciprofloxacin (OR 0.02) and erythromycin (OR 0.2) but more likely to be resistant to fusidic acid (OR

6.2) and tetracycline (OR 3.0); resistance to no non β-lactam classes (OR 26.0) or less than two classes (OR 2.8) was more common in CA-MRSA strain types (Table 7-7, p.158). Twelve (32.6%) of the CA-MRSA isolates were ciprofloxacin-resistant. Ciprofloxacin (AOR 0.02) and fusidic acid (AOR 8.6) resistance were significant predictors of CA-MRSA strain types in multivariate binomial logistic regression model.

BURP analysis clustered *spa* types into seven CCs and two singleton lineages (Appendix 9-9, p.229). The majority (80.4%) of the HA-MRSA isolates were *spa* CC1 related to ST22 EMRSA-15, with *t032* (47.7% of isolates) predominating (Table 7-7, p.158). A further 15.0% were *spa* CC2 related to ST36 EMRSA-16. The remaining 4.6% were made up of sporadic hospital types. SCC*mec* types among the HA-MRSA isolates reflected the predominance of EMRSA-15 and -16 type isolates, with 75.2% SCC*mec* IV and 15.4% SCC*mec* II. None of the HA-MRSA isolates were PVL positive.

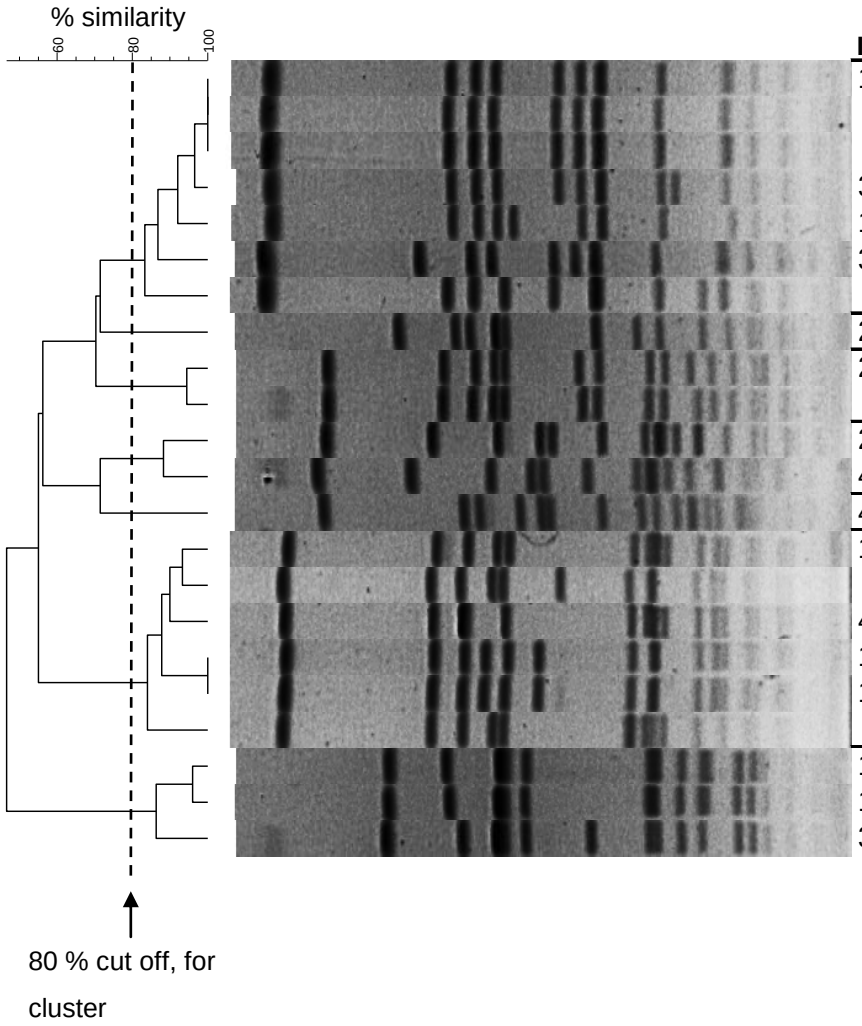
CA-MRSA were significantly more diverse than the HA-MRSA isolates. Eight (21.6%) of the CA-MRSA isolates were PVL positive (compared with none of the HA-MRSA isolates), and thus 3.2% of all recovered MRSA were PVL positive (Table 7-7, p.158). The eight PVL-positive isolates were represented by eight *spa* types, three of which were CC30 and three of which were CC8. Almost half of the CA-MRSA isolates were from singleton lineages, with *t127* representing 32.4% of the isolates.

7.4 PVL-encoding bacteriophage and gene sequence variation

7.4.1 *spa* types and PFGE profiles

The clustering of *spa* types and PFGE profiles for the 22 PVL-positive isolates and MLST data for selected isolates identified from the retrospective study and investigated in detail are compared in Figure 7-9, p.163.

Figure 7-9. PFGE, *spa* and MLST data for 22 PVL-positive MRSA clinical isolates.



Isolate ID	*spa* repeats	*spa*	*spa* CC	MLST	MLST CC	PFGE
16	07-23-12-34-34-33-34	t131	1	-	80	01.1
2	07-23-12-34-34-33-34	t044	1	80	80	01.1
9	07-23-12-34-34-33-34	t044	1	-	80	01.1
30	07-23-12-34-34-33-34	t044	1	-	80	01.2
18	07-23-12-34-34-33-34	t044	1	-	80	01.3
37	07-23-12-34-34-33-34	t1247	1	-	80	01.4
5	07-23-12-34-34-33-34	t044	1	-	80	01.5
21	26-23-17-34-20-17-12-17-16	t311	Singleton	5	5	02.1
20	07-23-23-21-16-34-33-13	t127	5	1	1	03.1
6	07-23-23-21-16-34-33-13	t128	5	1	1	03.2
25	07-12-21-17-13-13-34-34-34-33-34	t690	4	88	88	04.1
41	07-12-21-17-34-13-34-34-34-33-34	t1816	4	88	88	04.2
40	07-13-34-33-34	t667	1	154	154	05.1
19	11-19-12-21-17-34-24-34-22-25	t024	3	8	8	06.1
4	11-19-12-21-17-34-24-34-22-25	t008	3	-	8	06.2
44	11-19-12-21-17-34-24-34-22-25	t008	3	-	8	06.3
10	11-19-12-21-17-34-24-34-22-25	t622	3	-	8	06.4
13	11-19-12-21-17-34-24-34-22-25	t008	3	8	8	06.4
7	11-19-12-21-17-34-24-34-22-25	t008	3	-	8	06.5
12	04-20-17-20-17-31-17-25-34	t437	2	-	59	07.1
17	04-20-17-20-17-31-17-25-34	t1894	2	59	59	07.2
31	04-20-17-20-17-31-16-12-34	t216	2	59	59	07.3

The discriminatory power of *spa*/BURP and PFGE clustering is compared in Table 7-8.

Table 7-8. Discriminatory indices for *spa* and PFGE.

Method	n[a] types / groups	DI[b]	95% CI[c]
PFGE profiles	19	0.98	0.96-1.01
spa types	15	0.93	0.86-1.00
PFGE cluster analysis	7	0.82	0.73-0.91
spa / BURP	6	0.79	0.69-0.89

[a] n = number.

[b] DI = discriminatory index.

[c] CI = confidence interval.

The 22 isolates comprised 15 *spa* types that were grouped into five *spa* CCs and one singleton lineage using the BURP algorithm and 19 distinguishable PFGE profiles that were clustered into seven groups (Figure 7-9, p.163). *spa*/BURP and PFGE clustering were concordant for 21 (95%) of the 22 isolates; one isolate (ID 40, *t667*, ST154) was incorrectly clustered with the CC80 isolates by *spa*/BURP (Figure 7-9, p.163). The Discriminatory Index (DI) of PFGE was marginally better than *spa*, but the DIs of both were high and confidence intervals overlapped for most groups due to the small sample size (Table 7-8, p.164). However, the concordance between different *spa* types and distinguishable PFGE profiles within clusters was poor. For example, among the CC80 isolates, five were *t044* but these five isolates had four distinguishable PFGE profiles (Figure 7-9, p.163). Similarly, two of the three CC80 isolates indistinguishable PFGE profiles had different *spa* types (Figure 7-9, p.163).

7.4.2 Characteristics of study isolates

The detailed characteristics of the 22 isolates are summarised in Table 7-9, p.166. Most of the isolates were cultured from SSTIs and the mean age of affected patients was 32 years (Table 7-9, p.166). All isolates were

*mec*A positive by PCR but two were negative for *mec*A on the Clondiag array (isolate IDs 21 and 40) and five isolates were oxacillin-susceptible by Etest (range 0.25 - 1 mg/L) (Table 7-9, p.166 and Table 7-10, p.169). Two of these isolates (isolate IDs 13 and 17) were oxacillin-resistant (MIC >=4 mg/L) by Vitek. The isolates were selected by ciprofloxacin susceptibility and were generally not multiresistant; 16 (73%) were resistant to less than two non-β-lactam classes and the median oxacillin MIC was 20 mg/L although two isolates were from a multiresistant ST59 lineage.[94,236]

7.4.3 Sequence variation in the PVL genes

Single nucleotide polymorphisms (SNPs) were noted at four locations in the *luk*S-PV gene and at two locations in the *luk*F-PV gene; one was non-synonymous (Table 7-9, p.166). Phylogenetic analyses by both Wolter *et al*. and Takano *et al*.[94,243] propose the ΦSLT / ST30 gene sequence as the progenitor *lukSF-PV* sequence. Therefore, I compared the SNP profiles in the isolates with ΦSLT (Table 7-9, p.166). With the exception of the two ST1 isolates (isolate IDs 6 and 20), SNPs varied with lineage. Although ΦSa2mw was the only definitively identified PVL-encoding bacteriophage, in CC80 and CC1 isolates, it appeared that the PVL-encoding bacteriophage also varied with lineage. In contrast, there was considerable variation between members of the same lineage in terms of SCC*mec* type.

Table 7-9. Characteristics of 22 PVL-positive CA-MRSA isolates.

Isolate ID	Clincial details[a]			Phenotype[b]		Genotype[c]						PVL gene sequence variation[d]																Phage type[f]
												luk S-PV							*luk* F-PV	PVL phage determination[e]								
	Source	Age	Year	Antibiogram	Ox MIC	*spa*	*spa* CC	MLST	MLST CC	SCC*mec*	PFGE	33	105	345	527	663	1396	1729	PCR1	PCR2	PCR3	PCR4	PCR5	PCR6	PCR7	PCR8	
ΦSLT	NC 002661					-	-	-	-	-	-	G	T	C	A	G	A	A										
MW2	Sequenced genome					*t128*	5	-	1	IVa	-	G	T	C	G	T	A	A	+	+	-	+	-	-	Sa2MW	-	Sa2MW
USA300	Sequenced genome					*t008*	3	-	8	IVc	-	G	T	C	G	T	A	G	-	+	-	+	(PVL)	-	-	-	EH-type
16	SSTI	43	2005	Neo	**8**	*t131*	1	-	80	IVc	01.1	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
2	SSTI	34	2002	Ery,Clin*,Neo	**16**	*t044*	1	80	80	IVc	01.1	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
9	SSTI	26	2004	Ery,Clin*,Neo	**24**	*t044*	1	-	80	IVc	01.1	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
30	SSTI	5	2006	Ery,Clin*,Neo	**32**	*t044*	1	-	80	IVc	01.2	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
18	SSTI	42	2005	Neo	**>256**	*t044*	1	-	80	IVc	01.3	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
37	SSTI	62	2006	None	**48**	*t1247*	1	-	80	IVc	01.4	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
5	SSTI	12	2003	None	**16**	*t044*	1	-	80	IVa	01.5	A	T	T	A	G	A	A	+	+	-	+	(PVL)	-	Sa2MW	-	Sa2MW
21	SSTI	50	2005	Tet,Trim	0.5	*t311*	Single	5	5	IV	02.1	G	T	C	A	G	A	A	-	-	-	-	-	-	-	-	Unknown
20	SSTI	29	2005	Tet	1	*t127*	5	1	1	NT	03.1	G	T	C	G	T	A	G	+	+	-	-	(PVL)	-	-	-	Unknown
6	SSTI	35	2003	Chl	**4**	*t128*	5	1	1	IVa	03.2	G	T	C	G	T	A	A	+	+	-	+	-	-	Sa2MW	-	Sa2MW
25	SSTI	7	2005	Tet	**>256**	*t690*	4	88	88	IVa	04.1	G	C	C	A	G	G	A	-	+	(-)	+	(PVL)	-	-	-	EH-type
41	SSTI	27	2006	Tet	**12**	*t1816*	4	88	88	IVa	04.2	G	C	C	A	G	G	A	-	+	(-)	+	(PVL)	-	-	-	EH-type
40	SSTI	28	2006	Tet	0.5	*t667*	1	154	154	IV	05.1	A	T	C	A	G	G	A	-	+	(-)	+	(PVL)	-	-	-	EH-type
19	SSTI	44	2005	Tet	**64**	*t024*	3	8	8	IVc	06.1	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
4	SSTI	34	2003	Trim	**24**	*t008*	3	-	8	IVa	06.2	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
44	SSTI	38	2006	None	**32**	*t008*	3	-	8	IVc	06.3	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
10	Trach.	65	2004	Neo	**96**	*t622*	3	-	8	IVa	06.4	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
13	SSTI	19	2004	Rif	0.75	*t008*	3	8	8	IVa	06.4	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
7	SSTI	27	2003	None	**96**	*t008*	3	-	8	IVc	06.5	G	T	C	G	T	A	G	-	+	(-)	+	(PVL)	-	-	-	EH-type
12	SSTI	40	2004	Ery,Clin,Chl,Neo	**12**	*t437*	2	-	59	V	07.1	G	T	C	A	G	G	A	-	+	(-)	-	(PVL)	Sa2958	-	-	Sa2958 variant
17	SSTI	22	2005	Ery,Clin,Chl,Neo	0.25	*t1894*	2	59	59	IVa	07.2	G	T	C	A	G	G	A	-	+	(-)	-	(PVL)	Sa2958	-	-	Sa2958 variant
31	HVS	22	2006	None	**48**	*t216*	2	59	59	IVa	07.3	G	T	C	A	G	G	A	-	-	-	-	PVL	-	-	-	Unknown

[a] SSTI = skin and soft tissue infection, Trach. = tracheotomy, HVS = high vaginal swab.

[b] Tet = Tetracycline, Trim = Trimethoprim, Rif = Rifampicin, Chl = Chloramphenicol, Ery = Erythromycin, Clin* = inducible Clindamycin resistance, Clin = constitutive Clindamycin resistance; Neo = neomycin; None = resistant to β-lactams only. Ox MIC = oxacillin minimum inhibitory concentration (mg/L); values in bold type are above the breakpoint for oxacillin-resistance.

[c] NT = non-typeable.

[d] SNP at location 527 results in an amino acid change from Histidine (H) to Arginine (R); all other SNPs are silent. Black shaded cell indicate that the base is different from the reference sequence.

[e] PCR reactions specific for each type of phage is described by Ma *et al.*[43] (+) or (-) indicates PCR results that would not be included if the assay described by Ma *et al*. was followed strictly.

[f] EH-type = Elongated head-type phage.

The regulation, resistance and toxin gene profiles for the 22 isolates are summarised in Table 7-10, p.169 and included in full in Appendix 9-10, p.231. The profiles tended to vary with lineage, although there were strain differences that did not generally vary with *spa* type or PFGE profile. Analysis of the oligonucleotide array yielded several ambiguous results, most likely due to suboptimal hybridisation. These were most evident among the various alleles of the *ssl / set* genes (Table 7-10, p.169). This persisted despite repeats, including analysis from a pure culture including a repeated extraction stage, by Dr Stefan Monecke (Dresden University) who has pioneered the method.[141,142]

7.4.3.1 MLST CC80

Seven *agr* III CC80 "European" clone isolates were included for study. Six were SCC*mec* IVc, one was SCC*mec* IVa and all were within a single *spa* repeat change of *t044* with related PFGE profiles (Table 7-9, p.166) and Figure 7-9, p.163). The PVL genes in all CC80 isolates had two silent SNPs compared with ΦSLT, and all isolates carried ΦSa2mw, the PVL-encoding phage present in USA400 (Table 7-9, p.166).[322] All CC80 isolates were positive for the 1411bp ΦPVL band in PCR5. However, the PCR for ΦPVL lacked specificity, with 20/22 isolates positive for the ΦPVL band; BLAST analysis of these primers showed poor specificity, sharing 100% sequence homology with the PVL genes from ΦSa2usa and Φ2958.

All seven CC80 isolates contained *etD* and *edin*C, which were unique to CC80 isolates in this small set of isolates (Table 7-10, p.169). Six of seven contained the *aph*A3 and *sat* genes and five of these expressed resistance to neomycin; five of the seven contained an allotype of *ssl11 / set6*. The isolate lacking *aph*A3 and *sat* (isolate ID 5) was also the only isolate in the group with SCC*mec* IVa although it was *t044*. The CC80 European clone is usually characterised by resistance to fusidic acid and tetracycline with variable resistance to ciprofloxacin.[84,299] However, none of the seven CC80 isolates had fusidic acid or tetracycline resistance genes.

Table 7-10. Clondiag data.

Black cells = positive; white cells = negative; grey cells = ambiguous result. MLST CC is inferred from *spa* type (table continued overleaf).

			agr			Antimicrobial resistance									Enterotoxins												Two-component toxins								
Isolate ID	spa type	MLST CC	agrI	agrII	agrIII	mecA	blaZ	ermC	linA	msrA	aphA_3	sat	tetK	tetM	entA	entA-N315 (aka entP)	entB	entC	entG	entH	entI	entK	entL	entM	entN_other than RF122	entO	entQ	entX	lukF	lukS	hlgA	lukF_PV	lukS_PV	lukD	lukE	lukX	lukY_var1
16	*t131*	80																																			
2	*t044*	80																																			
9	*t044*	80																																			
30	*t044*	80																																			
18	*t044*	80																																			
37	*t1247*	80																																			
5	*t044*	80																																			
21	*t311*	5																																			
20	*t127*	1																																			
6	*t128*	1																																			
25	*t690*	88																																			
41	*t1816*	88																																			
40	*t667*	154																																			
19	*t024*	8																																			
4	*t008*	8																																			
44	*t008*	8																																			
10	*t622*	8																																			
13	*t008*	8																																			
7	*t008*	8																																			
12	*t437*	59																																			
17	*t1894*	59																																			
31	*t216*	59																																			

			Other toxins																																											
Isolate ID	spa type	MLST CC	hl	hla	hld	hl_III_Other than RF122	un-truncated hlb	sak	etA	etB	etD	edinA	edinB	edinC	splA	splB	setC_MW0345	ssl1 / set6 (COL)	ssl1 / set6(Mu50/N315)	ssl1 / set6(MW2/MSSA476)	ssl1 / set6 (MRSA252)	ssl1 / set6_other variants	ssl2 / set7	ssl2 / set7 (MRSA252)	ssl3 / set8	ssl4 / set9	ssl4 / set9 (MRSA252, SAR0	ssl5 / set3	ssl5 / set3 (MRSA252)	ssl6 / set21	ssl7 / set1	ssl7 / set1 (MRSA252)	ssl7 / set1 (AF188836)	ssl8 / set12	ssl9 / set5	ssl9 / set5 (MRSA252)	ssl10 / set4	ssl10 / set4 (MRSA252)	ssl11 / set2 (COL)	ssl11 / set2(Mu50/N315)	ssl11 / set2(MW2/MSSA476	ssl11 / set2 (MRSA252)	setB3	setB2	setB1	setB1 (MRSA252)
16	*t131*	80																																											
2	*t044*	80																																											
9	*t044*	80																																											
30	*t044*	80																																											
18	*t044*	80																																											
37	*t1247*	80																																											
5	*t044*	80																																											
21	*t311*	5																																											
20	*t127*	1																																											
6	*t128*	1																																											
25	*t690*	88																																											
41	*t1816*	88																																											
40	*t667*	154																																											
19	*t024*	8																																											
4	*t008*	8																																											
44	*t008*	8																																											
10	*t622*	8																																											
13	*t008*	8																																											
7	*t008*	8																																											
12	*t437*	59																																											
17	*t1894*	59																																											
31	*t216*	59																																											

7.4.3.2 MLST 5

The one ST5 isolate was *agr* II, *t311*, SCC*mec* IVa, tetracycline (*tet*K) and trimethoprim resistant (despite being negative for *dfr*A, which encodes trimethoprim resistance). It was identified as a singleton by *spa* typing, MLST and PFGE analysis (Figure 7-9, p.163). The ST5 isolate had the same PVL gene SNP profile as ΦSLT and all PVL phage PCR reactions were negative suggesting the isolate harboured a variant or novel PVL-encoding phage (Table 7-9, p.166). The isolate contained *seA, seG, seH, seM, seN* and *seO* (Table 7-10, p.169).

7.4.3.3 MLST CC1

Two isolates were CC1 and *agr* III with different *spa* types (*t127, t128*), distinguishable but related PFGE profiles and distinct SCC*mec* types (non-typeable and IVa) (Table 7-9, p.166). Both carried *setA, H, K* and *Q* whereas isolate ID 6 (*t128*) additionally carried *seC* and L (Table 7-10, p.169). Both isolates carried the *ssl11 / set2* gene (MW2/MSSA476 allotype) and isolate ID 20 (*t127)* was tetracycline resistant (*tetK*).

The *t128* (isolate ID 6) isolate carried ΦSa2mw and had PVL genes homologous to USA400 (Table 7-9, p.166). The *t127* isolate (isolate ID 20) had PVL genes homologous with USA300, and the PVL-encoding phage could not be identified because PCRs 3 and 4 to identify the linkage between the phage and the PVL genes were negative (Table 7-9, p.166). Two randomly selected *t127* isolates obtained from the national Staphylococcus Reference Unit both had PVL genes homologous with USA300. The PVL-encoding phage in one of these isolates could not be identified and the other appeared to carry ΦPVL. However, the identification of ΦPVL remains uncertain because of the poor specificity of the primers for ΦPVL in PCR5.

7.4.3.4 MLST CC88

The two *agr* III CC88 isolates were both *SCCmec* IVa but had different *spa* types and distinguishable PFGE patterns (Table 7-9, p.166). Both were tetracycline resistant but only one (isolate ID 41) had *tet*K (Table 7-10, p.169). Both carried a variant of *se*A and one (isolate ID 25) also carried *seK* and *Q.* Isolate ID 41 also carried *ssl1 / set6* and *ssl11 / set 2.* The two CC88 isolates had two silent SNPs in the PVL genes compared with ΦSLT and carried an elongated head-type PVL-encoding phage, which was not identified definitively (Table 7-9, p.166).

7.4.3.5 MLST154

The one ST154 isolate was *agr* III, *t667* and SCC*mec* IV, which could not be sub-typed (Table 7-9, p.166). It was identified as a singleton lineage by MLST and PFGE analysis but incorrectly clustered with the CC80 isolates by BURP analysis (Figure 7-9, p.163). The isolate was tetracycline resistant (*tetM*), and carried *seC* and *seL.* The PVL gene sequence in this isolate had two silent SNPs compared with ΦSLT and contained an elongated head-type PVL-encoding phage, as did the MLST CC8 and CC88 isolates.

7.4.3.6 MLST CC8

Six isolates were CC8 and *agr* I, with USA300-like PFGE profiles. Three were SCC*mec* IVc and three were SCC*mec* IVa; these two groups of three isolates did not vary with *spa* type or PFGE profile (Figure 7-9, 163). All six CC8 isolates had PVL genes homologous with USA300 and contained elongated-head type PVL-encoding phages (Table 7-9, p.166). Primers to detect ΦSa2usa, the PVL-encoding phage in USA300[240] were not included in the assay described by Ma *et al*.[43]

All CC8 isolates contained *seQ* and *seK* and all but one contained *ssl11 / set6* and *ssl2 / set7* (Table 7-10, p.169). One unusual CC8 isolate (isolate

ID 10, *t622*) contained the *aph*A3 and *sat* genes, which encode neomycin and streptothricin resistance, respectively and lacked all four *ssl1 / set6* and both *ssl2 / set7* allotypes.[450]

7.4.3.7 MLST CC59

Three of the isolates were CC59 and *agr* I, with three *spa* types and related PFGE profiles (Figure 7-9, p.163). Two of the isolates were multiresistant with closely related PFGE profiles (>95%) but one was SCC*mec* IVa and the other was SCC*mec* V (Table 7-9, p.166). The other CC59 isolate was non-multiresistant and SCC*mec* IVa (Table 7-9, p.166).

The CC59 isolates had a similar profile of resistance and toxin gene carriage (Table 7-10, p.169). All three isolates carried *seB*, *seK, seQ*, the untruncated version of *hlb* and lacked *lukD, lukE, sak* and *splA/B.* The non-multiresistant isolate (ID 31) lacked *lukX* and one allelic variant of *ssl4* / *set 9* and *ssl7* / *set1.*

All three isolates had a silent SNP at position 1396 compared with ΦSLT. Both multi-resistant isolates possibly carried a variant of ΦSa2958 because PCR6 (specific for ΦSa2958) was positive; however, PCR4 to detect the linkage between the phage and the PVL genes was negative (Table 7-9, p.166). The PVL-encoding phage in the non multi-resistant CC59 isolate could not be determined.

8 DISCUSSION

8.1 The molecular epidemiology of ciprofloxacin-susceptible MRSA at GSTT, 2000-2006

The results of this study are published in *Clinical Microbiology and Infection* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**).

HA-MRSA is common in the UK[187] but reports of CA-MRSA in the UK were rare at the time of my retrospective study. At the start of the project in 2005, reports of CA-MRSA in the UK were restricted to a small number of isolates referred to the HPA Staphylococcus reference laboratory.[359] Since then there have been a few outbreak reports[354,357] and case series.[101,252,360,451,452] This prompted me to hypothesise that CA-MRSA were present in UK hospitals but that the volume of HA-MRSA had masked their appearance. In order to test this hypothesis, I evaluated ciprofloxacin susceptibility as a screening marker for CA-MRSA.

The epidemiological and microbiological characteristics of Cip-S MRSA reported at GSTT from 2000-2006 were generally consistent with published characteristics of CA-MRSA. Compared with Cip-R MRSA, Cip-S MRSA were associated with younger patients, resistance to fewer antimicrobial agents, low-level expression of methicillin resistance, a greater frequency of PVL production, presentation in outpatient settings or hospital specialties in which MRSA is uncommon and SSTIs, especially abscesses (Table 7-1, p.135).[198,204,207,220]

I observed marked increases during 2000-2006 in both the number (from 49 in 2000 to 102 in 2006) and proportion of Cip-S MRSA isolates (from 3.7% in 2000 to 13.2% in 2006), which is consistent with other reports from the UK and other countries.[84,198,230,360,363,453] For example, a retrospective analysis of the UK General Practice Research Database by Schneider-Lindner *et al.* reported that the prevalence of epidemiologically-

defined presumptive CA-MRSA increased 45.8% from 332 cases in 2000 to 484 cases in 2004.[363] I also noted a marked increase in the frequency of PVL-positive MRSA isolates among the recovered Cip-S MRSA in the latter years of the study. The proportionate increase in PVL-producing isolates in the latter years of the study could be explained by loss of bacteriophage-mediated PVL genes from older isolates stored at room temperature on slopes; however, this is unlikely because the proportion of a given *spa* type that was PVL-positive tended to remain constant.

The increase in the proportion of isolates that were PVL-positive was due to a variety of clones, and not the establishment of a predominant CA-MRSA, as has been seen in the USA (Figure 7-3, p.138). Except in the US, clonal heterogeneity is a feature of CA-MRSA, with many clones circulating concurrently in the same country, presumably resulting from repeated *de novo* insertion of SCC*mec* into multiple *S. aureus* lineages.[112,220,229,449] I noted clonal heterogeneity throughout the study period with representatives of ST80, ST59, ST8 and both PVL-positive and PVL-negative ST1 CA-MRSA lineages but rarely USA-300, USA-400 or the successful ST30 CA-MRSA clone.[84,299]

The PVL-positive Cip-S MRSA were more likely to be associated with abscesses that PVL-negative Cip-S MRSA (Appendix 9-3, p.216), supporting the clinical association of PVL with abscess formation identified by others.[238,454] The PVL-positive Cip-S MRSA isolates were also associated with younger patients and were more likely to be classified epidemiologically as community-acquired, supporting the association of PVL with CA-MRSA.[84,112,207,238,248] PVL-positive Cip-S MRSA were less likely to be associated with drug or alcohol use that PVL-negative Cip-S MRSA; this is because, in this study, the Cip-S clone associated with drug or alcohol abuse was PVL-negative. An interesting finding was that the PVL-positive Cip-S MRSA isolates were more likely to be resistant to ≥3 classes of non-β-lactam antimicrobial agents than the PVL-negative Cip-S MRSA; resistance to erythromycin, tetracycline and neomycin were common in a selection of referred PVL-positive MRSA isolates reported by

the UK MRSA reference laboratory.[112] We also identified a case of likely vertical transmission of a PVL-positive ST59 isolate; MRSA vertical transmission has been reported previously.[455,456]

The most common clone was PVL-negative ST1 *t127*, which was closely associated with IDUs and homeless people living in sheltered accommodation. Nine (12.5%) of the patients with *t127* MRSA shared the same homeless shelter in the same two year period, which could be evidence of a focus of community transmission.[227,277] Some discussion on this clone based on data from this study are published as a letter in the *Journal of Hospital Infection* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**). The ST1 *t127* CA-MRSA clone has been reported as a cause of CA-MRSA infection in IDUs in the UK.[228,358]

The types of CA-MRSA identified in my study are similar to those reported by other investigators in the UK (Table 7-2, p.139). The most recent report of PVL-positive isolates referred to the Health Protection Agency in 2005-2006 found that the European clone accounted for 32% of the isolates, 25% were ST8 (USA300-like) and 18% were the SWP clone.[112] Another UK study using ciprofloxacin susceptibility as a phenotypic marker found that 24% of isolates were PVL-positive (very similar to the 25% in my study); 24% of these were PVL-positive ST8 and 12% were the European clone.[362] A very low prevalence of ST1 isolates were identified in this study, suggesting differences in the local epidemiology of CA-MRSA in the UK, as is evident in other countries.[231,231]

A subset of isolates from my retrospective study was compared with isolates collected at a US teaching hospital in New Haven, Connecticut in a recent study.[390] Patients presenting with MRSA in the first 72 hours of hospital admission or in outpatient settings at both hospitals from January 2004 to March 2006 were studied. Fluoroquinolone susceptibility was used as a screening marker to select presumptive CA-MRSA. One hundred and eighteen and 49 such strains were identified, representing an incidence of 0.1 and 0.2 isolates per 1,000 patient days in the UK and US, respectively.

PVL-positive ST8-IVa (USA300)-type strains predominated among 43 surviving US isolates, whereas PVL-negative ST1-IV predominated among 71 surviving UK isolates. There were also differences in the demographics of patients affected by CA-MRSA in the UK and USA: US isolates tended to affect younger patients, who were more frequently black or Hispanic. However, the interpretation of these differences is uncertain without detailed demographic data for the populations served by the hospitals. Nonetheless, this study provides further evidence of differences in the molecular epidemiology of CA-MRSA in different parts of the world.

The reasons for the differences in molecular epidemiology of CA-MRSA in the UK and in the USA are unknown. Environmental factors and patient demographics (in particular ethnicity and associated host factors) and socioeconomic factors, are likely involved but have not yet been properly investigated. Other factors are also likely to be important: for example, the differences in the structure of the UK and US healthcare systems and differences in the choice and amount of antimicrobial agents used in both hospitals in the in community are also likely to play a role. Given the localisation of established HA-MRSA clones in the UK (EMRSA-15 and -16) and USA (USA100),[181,319] it is currently uncertain whether USA300, which has been so successful in the USA, will become established in the UK or elsewhere in Europe.

My retrospective study has several limitations. First, I collected isolates from a single centre, although the hospital receives isolates from GPs and primary care facilities through the London Borough of Lambeth. Second, my data collection was retrospective, which resulted in incomplete clinical information. Third, although ciprofloxacin susceptibility appeared to be a useful marker for CA-MRSA at GSTT, 90 (46.4%) of the Cip-S isolates were epidemiologically defined as healthcare-acquired; ciprofloxacin-susceptible EMRSA-15 has been reported at low frequency in the UK, and was responsible for a small outbreak of MRSA on a GSTT neonatal unit in 2006.[99] Furthermore, ciprofloxacin-resistant CA-MRSA have been reported in the UK and elsewhere.[359,457] Fourth, I was only able to recover

42% of the reported Cip-S MRSA from storage so clones with poor survival characteristics may be under-represented. Fifth, with the exception of *t127,* the number of isolates with the same *spa* type was small so it is difficult to draw reliable *spa* type-related associations.

Despite these limitations, the results of the retrospective study confirm my hypothesis that CA-MRSA have emerged at GSTT but that their appearance has been masked by the volume of HA-MRSA cultured by the clinical laboratory. Furthermore, I found that the prevalence of CA-MRSA increased during 2000-2006 and isolates displayed considerable clonal heterogeneity.

In order to better understand the limitations of ciprofloxacin susceptibility as a screening marker for CA-MRSA, I conducted a prospective collection of all MRSA reported for a three month period hypothesising that antimicrobial susceptibility (AMS) based algorithms can be used as an accurate screening marker for the presumptive identification of CA-MRSA among predominantly HA-MRSA isolates.

8.2 Assessment of antimicrobial susceptibility based algorithms for the presumptive identification of CA-MRSA

The results of this study are published in the *European Journal of Clinical Microbiology and Infectious Diseases* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**).

Broad susceptibility to non-β-lactam antimicrobials was a striking feature of the early reports of CA-MRSA.[84,204] The insertion of small types of SCC*mec* without the additional antimicrobial resistance genes associated with most SCC*mec* cassettes in HA-MRSA into diverse lineages community *S. aureus* strains explains the non-multiresistant phenotype of early CA-MRSA.[29] Therefore, antimicrobial-susceptibility has proven to be a useful phenotypic marker of CA-MRSA since their emergence.[112,113,362,390,443,444]

In order to test whether AMS algorithms can be used as a reliable phenotypic marker of CA-MRSA at GSTT, I chose a molecular definition of CA-MRSA based on a combination of SCC*mec* and *spa* type to determine the genetic background. SCC*mec* type alone or in combination with PVL status has been used as a useful marker of CA-MRSA in other countries.[100,429,458-460] However, the inclusion of *spa* data was important in my set of isolates to allow differentiation of EMRSA-15, which is SCC*mec* IV, from CA-MRSA strains.[99] EMRSA-15 and -16 together accounted for 94% of the isolates defined as HA-MRSA, confirming the dominance of these two clones in the UK (Table 7-4, p.147).[179,181]

CA-MRSA accounted for 17% of the isolates tested (Table 7-4, p.147). They were characterised by clonal heterogeneity including several recognised CA-MRSA lineages previously reported in the UK, such as the ST1 PVL-negative clone that has been associated with the homeless and IDUs, the ST80 European clone and the ST30 Southwest Pacific clone.[205,227,362,444] Significantly, PVL-positive ST8-IV isolates, which are presumptively USA300, accounted for 7% of the CA-MRSA isolates, suggesting that USA300 may be on the rise in the UK as it is in other parts of Europe.[391,392]

Compared with isolates defined as HA-MRSA, CA-MRSA were associated with younger patients and were more likely to encode PVL (Table 7-4, p.147).[112,444] A striking finding was that more than 70% were of CA-MRSA strains were defined epidemiologically as healthcare-acquired, supporting the view that CA-MRSA strains are becoming an increasingly common cause of healthcare-acquired infection.[82] However, my impression is that many of the affected patients, such as intravenous drug users, probably have community acquisition followed by repeated hospital contacts and are misclassified as HA-MRSA.[227]

Many investigators have used AMS as a phenotypic marker of CA-MRSA,[112,362,390,444] but there are few systematic studies to validate the

performance of AMS-based markers. In my study, ciprofloxacin susceptibility and fusidic acid resistance were the strongest predictors of CA-MRSA in a multivariate analysis (Table 7-4, p.147). Fluoroquinolone-resistance develops readily in *S. aureus* under antimicrobial pressure and most HA-MRSA are fluoroquinolone-resistant.[204,390,430,461] Fusidic acid is commonly used as a topical agent to treat SSTI in the community, providing a selective pressure for the development of fusidic acid resistance in community methicillin-susceptible *S. aureus* and in CA-MRSA.[162]

Ciprofloxacin-susceptible isolates were very likely to be CA-MRSA in my collection (PPV = 89.6%) (Table 7-5, p.150). However, ciprofloxacin alone was not a sensitive marker to differentiate HA- and CA-MRSA isolates (sensitivity = 63.4%) due to the presence of ciprofloxacin-resistant CA-MRSA, including 44% of the PVL-positive isolates. While the addition of fusidic acid resistance into the algorithm improved the sensitivity to 70.7%, my data suggest that these algorithms would miss 30-37% of CA-MRSA at GSTT. This finding has clear implications for other studies that have used ciprofloxacin susceptibility as a screening marker for CA-MRSA.[112,362,390]

Two published studies have validated AMS antibiograms for the presumptive identification of CA-MRSA. Popovich *et al.* developed phenotypic prediction rules to predict the genotype of 137 MRSA blood isolates.[113] Popovich *et al.* defined CA-MRSA based on pulsed-field gel electrophoresis (PFGE) profiles and found that fluorquinolone susceptibility (sensitivity, 73%; specificity, 86%) or clindamycin susceptibility (sensitivity, 95%; specificity, 80%) were the best predictors of genotype. Modelling showed that the resolution of the prediction rules decreased as the prevalence of resistance increased and resistance to multiple classes was strongly predictive of HA-MRSA. Gbaguidi-Haore *et al.* used a case-control design to validate various AMS algorithms to identify PVL and toxic shock syndrome toxin (TSST) producing MRSA in their hospital collection.[443] Their algorithms had a sensitivity and specificity of 77.8% and 100%, respectively, for their nine PVL-positive isolates and

100% and 72.4%, respectively, for their 21 TSST-positive isolates. However, this study is limited by using fluoroquinolone susceptibility to select the isolates that were tested for PVL and TSST production. Nevertheless, these studies, like mine, suggest that AMS-based algorithms can be a guide for the presumptive identification of CA-MRSA.

The limitations of the AMS algorithms tested in my study and in others' are not surprising because phenotype is never an absolute marker of genotype and bacterial populations tend to develop resistance when selective pressure is exerted.[462] For this reason, the resolution of antimicrobial susceptibility algorithms is likely to decrease further over time as CA-MRSA develop broader antimicrobial resistance by continuing antimicrobial selective pressure in hospitals.[82,234,236] Furthermore, given the global variation in the molecular epidemiology and antimicrobial resistance profiles of CA-MRSA worldwide,[84,299] the results of my study cannot be generalised beyond the GSTT population. Also, my study was conducted over a relatively short period in a single centre, not all of the isolates were saved, my molecular definition of CA-MRSA may have included sporadic HA-MRSA isolates and my findings are based on AMS data from only 41 CA-MRSA isolates.

Despite these limitations, ciprofloxacin-susceptible isolates are likely to be CA-MRSA strains but need to be confirmed by molecular methods. However, ciprofloxacin susceptibility and other AMS-based algorithms are unreliable screening markers to identify CA-MRSA in collections including HA-MRSA because of the prevalence of resistant CA-MRSA isolates. Therefore, in order to investigate the prevalence and molecular epidemiology of CA-MRSA on admission to GSTT, I decided not to use an AMS-based screening marker but to characterise each isolate in order to make a molecular definition of CA-MRSA.

8.3 The prevalence and molecular epidemiology of CA-MRSA identified on admission screens

Prospective studies of colonisation with MRSA in the community usually identify low rates of carriage, typically 1-2%, in contrast to higher rates of carriage by patients admitted to hospital, typically 3-10%.[155,396-400] However, the prevalence of CA-MRSA can be substantially higher in areas of high prevalence or in high risk community groups.[290,401,402] The prevalence of colonisation with MRSA was 7% in patients admitted to medical and surgical specialties at GSTT in 2006-2007.[399] The same prevalence of MRSA colonisation was reported for patients admitted to A&E at a hospital in Lewisham, London in 2004-2005.[463] Lower rates of colonisation (3%) were reported on admission to a hospital in Birmingham in 2005-2007.[464] Broadly similar rates of MRSA colonisation at hospital admission have been reported from US hospitals.[196,465] Large community-based surveys of MRSA colonisation are rare in the UK. A large 2002 survey of elderly residents in Nottingham found a colonisation rate of <1%[397] and an older survey of healthy adults in Birmingham in 1998 found a prevalence of 1.5%.[396]

In my study at GSTT in 2008, only 1.6% of all admissions were MRSA-positive, which is surprisingly low and strikingly similar to the prevalence of MRSA in large community screens (Table 7-6, p.153).[155,397] This prevalence is substantially lower than the 7% colonisation rate among patients admitted to medical and surgical specialties at GSTT in 2007.[399]

This surprisingly low rate may be the result of several factors. Firstly, this was the first six months of universal admission screening. Previously, it was national policy to generally screen only patients who were at high risk of carrying HA-MRSA. The introduction of universal screening, which includes screening of low risk patients such as those in paediatrics, obstetrics and gynaecology, and short-stay admissions, means that the

overall admission carriage rate in a setting of low prevalence of CA-MRSA was inevitably lower than in previous studies. In support of this, many screens were taken from patients in pre-assessment clinics for elective surgery; these patients lack the traditional risk factors for MRSA and screening in pre-assessment clinics was an independent predictor of a negative result (Table 7-6, p.153). Secondly, the rate of MRSA infection and transmission in GSTT and other London hospitals has greatly reduced in the last few years. This has probably resulted in a smaller number of new patient carriers being discharged with consequently a lower rate of 'revolving door' carriers being admitted. In support of this, it is noteworthy that in my study, previous hospital admission was not independently associated with a positive result, which is inconsistent with previous studies on risk factors for MRSA (Table 7-6, p.153).[155,196,466] However, there could have been inaccuracies in the admission history, in particular relating to transfers from other hospitals and healthcare facilities, which is more difficult to trace than previous treatment at GSTT.

The low prevalence of MRSA identified in my study questions the value of widespread implementation of universal screening and suggests that a focused screening policy based on known risk factors would be a more cost-effective approach.[18,467,468]

The risk factors for HA-MRSA colonisation and infection are well established and include previous MRSA episodes, older age, prior antimicrobial use, exposure to healthcare facilities and underlying medical conditions.[155,466] The risk factors for CA-MRSA differ, and include socio-economic factors, contact sport participation and injecting drug use, with less bias towards older individuals.[155,469] In my study, factors independently associated with MRSA-positive screens were previous MRSA episodes, older age and admission to critical care and respiratory medicine, which is consistent with other studies (Table 7-6, p.153).[155,196,466] Similar healthcare-associated risk factors were predictive of patients who had a negative admission screen followed by a positive screen or culture during their stay, including older age, male gender and

admission to critical care medicine (Appendix 9-7, p.214). However, I lacked the detailed epidemiological and demographic data for the MRSA-negative patients, such as ethnicity, socioeconomic factors and health status, which are required to make a thorough investigation of risk factors.

The emergence of CA-MRSA has made an appreciable impact on admission MRSA carriage rates in areas of high prevalence. For example, 22% of drug addicts admitted to an Egyptian hospital were colonised and over half were colonised or infected.[289] The rate of colonisation among patients admitted to a hospital in Austin, Texas, an area of high CA-MRSA prevalence, was more than 10%.[465] Paediatric patients are traditionally less affected by HA-MRSA than adults, but paediatric patients seem to be at particularly high risk of CA-MRSA colonisation in areas of high prevalence. For example, 22% of paediatric admissions to a US hospital colonised with MRSA in a study from Corpus Christi, Texas.[470] However, rates of MRSA in paediatric patients elsewhere remain low, for example 0.2% paediatric patients admitted to several Swiss hospitals were colonised with MRSA.[471] In my study, paediatric patients were rarely colonised (1.3%) and no paediatric specialties were associated with positive screens (Table 7-6, p.153).

Notwithstanding the low prevalence of MRSA in paediatric patients in my study, CA-MRSA strain types accounted for 15% of my recovered MRSA and 0.25% of all screens (Table 7-7, p.158). CA-MRSA strains accounted for approximately 15% of MRSA and up to 25% in certain specialties, for example in A&E and pre-admission clinics where patients are primarily from the community.

Several other studies have examined the prevalence of CA-MRSA strains types on admission to hospitals. Harbarth *et al*.[469] reported that CA-MRSA were present on 0.1% of all admissions in Geneva, Switzerland whereas Hidron *et al.*[472] reported that USA300 was present on 2.2% of admissions to a hospital in Atlanta, Georgia, reflecting the apparent difference in the epidemiology of CA-MRSA between the USA and Europe.

Compared with HA-MRSA strains, CA-MRSA strains identified on admission affected younger patients, were more frequent in surgical admissions but less frequency in medical and ITU admissions, which is consistent with the findings of others (Table 7-7, p.158).[155,469] HA-MRSA strains were dominated by EMRSA-15 and -16 whereas CA-MRSA strains were characterised by clonal heterogeneity; the most common single CA-MRSA strain was the ST1 PVL-negative clone previously reported in IDUs[227] and several different PVL-positive CA-MRSA clones were identified.

It is noteworthy that patients with CA-MRSA were not significantly different from patients with negative screens in terms of age, frequency in surgery, medicine and ITU admission, supporting the idea that patients affected by CA-MRSA are more closely related to the community than the hospital population (Appendix 9-8, p.227). Indeed, community strains were disproportionately represented among certain specialties, accounting for almost 25% of the recovered MRSA identified in surgery (mainly pre-admissions), A&E and pre-assessment screens which are likely to reflect a community rather than hospital population (Table 7-7, p.158). However, there was no significant difference between the proportion of CA- and HA-MRSA isolates classified epidemiologically as community-acquired, suggesting that a purely epidemiological definition of CA-MRSA is no longer useful.[82,226]

The finding that 33% of the CA-MRSA isolates were ciprofloxacin-resistant justifies the decision not to use fluoroquinolone-susceptibility as a phenotypic marker of CA-MRSA.[112,205,444]

The prevalence of PVL-positive MRSA (exclusively CA-MRSA types) was 3.2% of recovered isolates, higher than previously reported in the UK,[101] which represented approximately 0.05% of all admissions.

My study is limited by the fact that the coverage of screening was not 100%, 17% of the reported positive isolates were not recovered from storage and the body sites that were sampled may not identify all sites of MRSA colonisation, especially for CA-MRSA.[155,212] Notwithstanding these limitations, my study shows that the overall prevalence of MRSA on admission to a London teaching hospital is currently low, and much lower than previously reported. CA-MRSA strains account for approximately 15% of the MRSA identified on admission, and the usefulness of universal screening at GSTT may be limited by the low prevalence of MRSA.

8.4 PVL-encoding bacteriophage and gene sequence variation

The results of this study are published in *Clinical Microbiology and Infection* (**Error! Reference source not found.**, p.**Error! Bookmark not defined.**).

Analysis of the 22 PVL-positive clinical isolates showed that the PVL-encoding phage and the DNA sequence of PVL genes varied with lineage.

The young age of the affected patients, often low level expression of oxacillin-resistance and non-multiresistant phenotype are consistent with CA-MRSA (Table 7-9, p.166).[204,222] Antimicrobial resistance and SCC*mec* type were variable within lineages, most likely due to the loss or acquisition of mobile genetic elements.[34,450]

There was good concordance between *spa* typing and PFGE for the clustering of 22 PVL-positive MRSA isolates (Figure 7-9, p.163). PFGE profiles were marginally more discriminatory than *spa* types (DI 0.98 vs. 0.93) and PFGE cluster analysis was marginally more discriminatory than BURP analysis of *spa* types (DI 0.82 vs. 0.79), although 95% confidence intervals overlapped for both of these comparisons (Table 7-8, p.164). Other studies have demonstrated the similar discriminatory power of these two methods.[121,297,446] For example, a study of 98 MRSA isolates from the European HARMONY collection also found that indistinguishable PFGE

profiles were marginally more discriminatory than *spa* types (DI 0.99 vs. 0.91); BURP clustering of *spa* types was not analysed.[297] One study of 217 *S. aureus* isolates in Belgium found that *spa* types were marginally more discriminatory than indistinguishable PFGE profiles (DI 0.98 vs. 0.96) but PFGE cluster analysis was marginally more discriminatory than BURP analysis of *spa* types (DI 0.93 vs. 0.89).[446] A study of 99 *S. aureus* isolates from Europe found PFGE profiles were marginally more discriminatory than *spa* types (DI 0.99 vs. 0.97) and PFGE cluster analysis was marginally more discriminatory than BURP analysis of *spa* types (DI 0.89 vs. 0.83).[121] However, in that study, PFGE profiles were considered to represent the same strain if bands differed by ≤3 bands whereas I considered indistinguishable profiles to represent the same strain.[121] These studies have demonstrated consistently that the discriminatory power of MLST is lower than PFGE or *spa*, so I restricted my analysis to PFGE and *spa.*[121,297,446]

All but one of the 22 PVL-positive isolates were clustered into the same PFGE and BURP group; one ST5 isolate was incorrectly clustered with the CC80 isolates by *spa*/BURP. The occasional misclassification by BURP analysis of *spa* types has been reported previously.[121,446] Although *spa* typing is a sequence-based method, it is restricted to a single locus whereas PFGE covers the whole genome approach and MLST includes sequence data from seven loci. Therefore, it is to be expected that *spa* typing occasionally misclassifies an isolate. My comparison of PFGE and *spa* was limited to a small number of isolates but my data and those of others support the use of *spa* typing for the rapid assignment of MRSA to lineages, as defined by MLST clonal complexes.

Initial investigations of sequence variation in the PVL genes suggested that a non-synonymous SNP resulting in an amino acid change from histidine (H) (“H” type) to arginine (R) (“R” type) may have had functional implications.[241,473] The R and H PVL types have comparable biological activity on human PMNs and pore forming ability *in vitro*[242,474] but, while the H type is present in a wide variety of successful CA-MRSA lineages,

the R haplotype was initially found in only USA300 and USA400 isolates.[94,242,243] This striking finding is reflected in my study where it was strictly associated with MLST CC1 and CC8 isolates. However, the R haplotype has been identified in an ST93 “Queensland” CA-MRSA clone, so is not exclusive to CC1 and CC8.[475] This suggests that the R and H haplotypes are incidental and the key variation is the sequence of the genes.

In order to monitor the distribution and spread of the PVL-encoding phages, Ma *et al.* developed a PCR-based identification scheme.[43] It is important to note that this algorithmic approach to detect various phage structures in a series of PCR reactions could result in misleading findings because *S. aureus* can carry multiple lysogenised phages.[40] In order to address this problem, the scheme includes reactions to detect linkage regions between the phage and the PVL genes, but the linkage region, if detected, is not necessarily associated with the same phage as other positive reactions. In my study, the CC8 and CC80 isolates were positive for both PCR1 and PCR2, indicating the presence of both icosahedral and elongated-head type phages. PCR4 (specific for the linkage region between elongated head-type phages and the PVL genes) was also positive, suggesting the presence of a PVL-encoding elongated head-type phage. However, the linkage reactions were negative for 5 of the 22 clinical isolates tested, limiting the utility of the assay and suggesting the presence of variant or even novel PVL-encoding phages, which is consistent with the findings of Ma *et al.*[43] Also, the observed poor specificity of one of the primers reported by Ma *et al.*[43] means that the identification of a 1411 bp fragment in the multiplex PCR5 reaction should not be used, *a priori*, to infer the presence of ΦPVL, but rather, it can be used as part of the prescribed algorithmic approach.

In my study, seven SNPs were identified in *lukSF-PV*, which tended to vary according to lineage and which correlated with the PVL-encoding phage in the clinical isolates (Table 7-9, p.166). These seven SNPs in *lukSF-PF* have been reported previously.[94,241-243] Despite intra-lineage

variations in SCC*mec* type and toxin gene profile, particularly in my MLST CC8, 80 and 88 isolates, the PVL gene sequence and phage were identical. However, several different phage types were noted in CC1 and CC59 isolates. It was striking that the two multi-resistant CC59 isolates apparently carried the same phage, whereas the non multi-resistant CC59 isolate had a marginally different toxin gene carriage profile and appeared to carry a different phage, despite having the same SNP profile (Table 7-9, p.166 and Table 7-10, p.169). Similarly, the SNP profile and phage in CC1 isolates seemed to vary with clone: *t128* isolates are usually PVL-positive whereas *t127* isolates are usually PVL-negative,[444] so *t127* CC1 isolates and *t128* CC1 isolates seem to represent different clones within the CC1 PVL-MRSA lineage. This is borne out by differences in toxin gene profile (Table 7-10, p.169). Therefore, it seems that the PVL sequence and phage vary with the clone, even within the same lineage. However, given the small number of isolates from each lineage in my study, and the discovery of variation in the PVL SNP profile within lineages in other studies,[241,475] further work is required to confirm this finding.

Variations in PVL phage could influence phage induction efficiencies, which may in turn influence the level of PVL expressed with resulting clinical implications.[39,264,265] However, it seems from a recent study that the PVL phage in USA300 is defective and cannot be induced, so it seems unlikely that phage induction is important in the pathogenesis of CA-MRSA given the success of USA300.[261,264]

From an evolutionary perspective, these data support the notion that PVL-encoding phages firstly infected MSSA, and some subsequently acquired SCC*mec* to give rise to PVL-positive CA-MRSA.[34,241,243] The finding of MSSA and MRSA from the same lineage with identical SNP profiles also supports this model.[241,242]

PVL gene sequence and the PVL-encoding phage vary with lineage in PVL-positive CA-MRSA, confirming my hypothesis.

8.5 The molecular epidemiology of CA-MRSA at GSTT

8.5.1 Defining characteristics of CA-MRSA

While the classical definition of CA-MRSA is beginning to break down because MRSA originating in the community have begun to cause transmission in hospitals[82,226,341] and acquire multiresistance,[233-236] based on the results of my studies, CA-MRSA can be distinguished from HA-MRSA in terms of their microbiology and molecular epidemiology at GSTT. In all sections of this study I found that MRSA classified as CA-MRSA by AMS profiles and/or genotype were associated with younger patients, often in outpatient settings, were generally less resistant to non-β lactam antimicrobials and were more likely to be PVL-positive and to have diverse genetic types, which are common with CA-MRSA from other studies.[72,83,84,204,368] Singleton PVL-positive and PVL-negative *spa* lineages were common among the CA-MRSA isolates but rare among the HA-MRSA isolates; these likely represent recent acquisitions of *mecA.*

PVL has been proposed as a marker of CA-MRSA.[112,206,230] I found that PVL-positive Cip-S isolates affected younger patients and were more frequently classified epidemiologically as community-acquired compared with PVL-negative Cip-S isolates (Appendix 9-3, p.216). However, the IDU/homeless patient group identified during the project were most commonly affected by PVL-negative strains. Therefore, although PVL-positive MRSA are very likely to be CA-MRSA, PVL is not a useful marker of CA-MRSA in our population. The association between PVL and CA-MRSA frequently noted in the literature is partly due to the many North American reports of the PVL-positive USA300.

Presentation of MRSA in patients without healthcare contact, which was one of the hallmarks of early reports of CA-MRSA, was not always evident

in the present investigations. In the retrospective study, the Cip-S MRSA were significantly more likely to be classified epidemiologically as community-acquired (Table 7-1, p.135) whereas in the admission screening study, isolates defined as having a CA-MRSA genotype were not significantly less likely to be defined epidemiologically as healthcare-acquired as those isolates with an HA-MRSA genotype (Table 7-7, p.158). Despite important differences in the methodology of these studies, it seems that epidemiological definitions for CA-MRSA are becoming less useful. As more patients in the community are affected by CA-MRSA, repeat episodes are likely to be classified as HA-MRSA and epidemiological definitions are confused further by the continued emergence of MRSA of community origin as a cause of healthcare-acquired infection.[82,225,226] A particular challenge to epidemiological definitions of CA-MRSA at GSTT is the transmission of CA-MRSA among the IDU/homeless group.[227,228] Patients in this group often have previous hospital admissions for other reasons and their infections may originally have been acquired in the community. Cooke *et al.* also discussed the difficulties associated with epidemiological classification of infections in the homeless / IDU population which is characterised by frequent healthcare contact.[228,476]

Due to the difficulties in developing meaningful epidemiological definitions of CA-MRSA, a genotypic definition is more useful. A genotypic definition is easier to derive in the USA, where predominant HA-MRSA lineages are not SCC*mec* IV and USA300 is so dominant.[319,429] Developing a genotypic definition is more difficult in Europe where CA-MRSA are currently characterised by genotypic heterogeneity.[299,326] My genotypic definition of CA-MRSA at GSTT included a combination of *spa* and SCC*mec* to define the lineage as a 'HA-MRSA' or 'CA-MRSA' clone. I had to consider how to differentiate ST22-IV EMRSA-15, which is SCC*mec* IV and relatively susceptible to non-β-lactam antimicrobials. Any genotypic definition needs to be combined with clinical and epidemiological data to be a useful tool.

The widespread emergence of CA-MRSA as a cause of nosocomial and healthcare-acquired infection would result in a convergence with HA-MRSA and a need to reconsider the labels “CA-MRSA” and “HA-MRSA” – however defined.[225] At the current time, however, CA-MRSA have not emerged as a widespread cause of hospital infection at GSTT so “CA-MRSA” remains a useful label.

8.5.2 Typing methods

I used various different *S. aureus* typing methods during the project. All typing methods have advantages and disadvantages and my choices about which methods to use were influenced by discriminatory power, reproducibility, portability of results, cost and time considerations (Table 5-5, p.40). Due to the excellent inter-laboratory repeatability and good concordance of *spa* typing with PFGE and MLST, I used *spa* typing and BURP analysis of *spa* types in combination with SCC*mec* type and PVL carriage to assign isolates to lineages.[121,130,297,446] In a subset of the PVL-positive clinical isolates, I confirmed the findings of others in that there was >95% concordance between *spa*/BURP and PFGE cluster analysis.[121,297,446]

BURP analysis of *spa* types is analogous to BURST analysis of MLST allelic profiles in that it provides an objective method for grouping related profiles based on inferred genetic relatedness. However, the BURST algorithm is freely accessible in the public domain whereas the BURP algorithm is written into proprietary software and is not available in the public domain. Furthermore, the BURST algorithm can be used to compare the sequence types of *S. aureus* in a local collection with sequence types in a large, publicly available online database. In contrast, the BURP algorithm can only be applied to a local collection of *spa* types. Another limitation of *spa* typing is the existence of two different nomenclature systems, one commonly used in Europe and the other in the USA, which makes international comparison of *spa* data difficult.[121,130,133,134]

spa/BURP, MLST/BURST and the clustering of PFGE profiles all have user defined parameters. In the case of comparing PFGE profiles, clustering parameters, such as optimisation and tolerance settings and the similarity cut off to define a cluster are not standardised and are selected based on the dataset. In the case of MLST/BURST, the minimum number of identical alleles in common to define a cluster is user-defined. Similarly, in the case of *spa*/BURP, the calculated cost between members is chosen to best reflect the dataset.

I used two different versions of the BURP algorithm, either using a calculated cost between members of ≤4 or ≤6. Reducing the calculated cost increases the discrimination of the algorithm. A range of calculated costs have been used to cluster *spa* types using BURP. For example, Strommenger *et al.* (2006) used ≤8,[121] Strommenger *et al.* (2008) used ≤6,[477] Mellmann *et al.* used ≤4[135] and Deurenberg *et al.* used ≤8, ≤6 and ≤4.[478] Similarly, I used two different similarity cut offs to define a cluster for my PFGE analyses: 70% and 80%. Increasing the similarity cut off increases the discrimination of the cluster analysis. A variety of PFGE similarity cut offs for *S. aureus* have been used in the literature. For example, Strommenger *et al.* (2006 used 70%,[121] Malachowa *et al.* used 75% and 92%[479] and Bosch *et al.* used 80%.[480]

Several different methods are currently available for the characterisation of SCC*mec* in *S. aureus*. I used three different methods for the characterisation of SCC*mec*: two were multiplex reactions for the determination of SCC*mec* types I-IV[45] and in an updated version, types I-VI,[46] and I also used a composite method including primers from two previously published assays for the sub-typing SCC*mec* IV.[220,442] The Oliveira and de Lencastre[45] assay does not have primers to detect SCC*mec* V and it was striking that 14% of the recovered Cip-S isolates in the retrospective study where this method was used were non-typeable whereas only 5% of isolates identified in the admission screening and assessment of algorithms were non-typeable when the Milheirico *et al.*[46]

assay, which has primers for SCC*mec* V, was used. Several other multiplex PCR methods are available for the characterisation of SCC*mec* in *S. aureus.*[98,232,441,481] As new SCC*mec* types and sub-types emerge, new assays will need to be developed. New methods should follow recently published guidelines from the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) that attempt to reconcile several existing nomenclature systems[46,232] and standardise future nomenclature using a web-based portal.[96] PCR-based methods are currently the most commonly used to characterise SCC*mec* but other methods are available. For example, the latest generation of the Clondiag oligonucleotide array includes probes for SCC*mec* I-VI[482] and rapid sequencing of the SCC*mec* cassette may be a possibility in the near future.

The assay described by Lina *et al.*[238] for the determining whether the PVL genes were present was objective and robust.

As an alternative to a selection of multiplex PCR reactions, I used the Clondiag oligonucleotide array for the simultaneous detection of a range of toxin and antimicrobial resistance genes and species specific markers. Although the method allowed the rapid detection of most of the genes included in the array, ambiguous results were obtained from several of the target sequences and this persisted despite repeats. Also, the analysis of the pictures requires proprietary software and the database for comparison with other strains is not in the public domain. I did not have access to the necessary software so had to send digital images to Dr Stefan Monecke (Dresden University, Germany) for analysis, which resulted in delays in the analysis time.

8.5.3 Prevalence of CA-MRSA

This project provides several measures of the prevalence of CA-MRSA in the patient population served by GSTT. The retrospective study identified a year-on-year increase in the number of Cip-S MRSA reported, the

proportion of all MRSA that were Cip-S MRSA and in the proportion of recovered Cip-S MRSA isolates that were PVL-positive. However, the lack of a reliable denominator and the fact that not all Cip-S MRSA isolates were saved, means that, although the frequency of CA-MRSA appeared to increase during the study period, the actual prevalence of CA-MRSA cannot be determined.

The study of the prevalence and molecular epidemiology of MRSA on admission provides an accurate measure of the prevalence of CA-MRSA on patients admitted to GSTT. The overall prevalence of MRSA carriage on admission was 1.6% and of CA-MRSA strains was 0.25%. Thus the carriage of CA-MRSA is low at the present time. However, CA-MRSA strains accounted for up to a quarter of the MRSA identified in certain specialties, for example in A&E and pre-admission clinics where patients are primarily from the community.

8.5.4 Control of CA-MRSA

My work has provided some information to help formulate strategies for the control of CA-MRSA. It appears that CA-MRSA are present in specific community-based patient groups in the population served by GSTT; for example, the PVL-negative ST1-IV clone was associated with community-based homeless / IDU patients. There is a need to implement community-based policies to control the spread of MRSA in this group. Patients in this group are often described as having the 'tri-morbidity' of concurrent physical health, mental health, and addiction problems.[483,484] This results in a patient group that is difficult to track and manage, and the implementation of a community-based control policy will be challenging. Furthermore, since these patients present at both hospital and community medical centres, collaboration is required between the NHS Trust (GSTT) and the Primary Care Trust (Lambeth Primary Care Trust) to ensure a coordinated response.

The community-hospital interface is another area where current control measures will need to be modified in the era of CA-MRSA. Historically, MRSA screening policies are targeted towards patient admissions with a high risk of MRSA colonisation based on well-established risk factors.[18] However, individuals with CA-MRSA have different risk factors[287-292] and lack traditional ones (they often have no prior healthcare contact, for example) and they may have different sites of colonisation.[211,212] Therefore, CA-MRSA may not be identified by screening policies formulated for HA-MRSA. Given the apparent propensity of CA-MRSA strains to spread in hospitals and the supplanting of HA-MRSA clones by USA300 in the USA,[82,407] the identification of CA-MRSA strain types colonising patients at admission is important for the elucidating the changing epidemiology of MRSA in UK hospitals. GSTT introduced universal screening during this project so I was able to conduct a formal analysis of the prevalence of CA-MRSA identified on hospital admission during this study. The prevalence of CA-MRSA strains at GSTT is low at the current time. However, patient groups in whom HA-MRSA has historically been uncommon, for example young, previously healthy adults without previous healthcare contact, were found to be affected by CA-MRSA strains. This raises important questions regarding the control of MRSA in these patient groups. The successful identification of CA-MRSA strains on hospital admission will help to prevent these strains from becoming established as hospital pathogens. This is one argument for continuing the present controversial UK policy of universal admission screening.

Hospital outbreaks of CA-MRSA strain types in other parts of the world have been controlled successfully by the implementation of methods used to control the spread of HA-MRSA.[216-217,355,408-413] However, these methods have not prevented the supplanting of HA-MRSA clones by successful CA-MRSA clones in some parts of the world.[82,407] I did not identify outbreaks of CA-MRSA strain types during the study but the effectiveness of strategies for preventing the spread of CA-MRSA once introduced into hospitals needs to be addressed.

The successful control of MRSA in any setting depends on accurate and rapid diagnostics to detect and on occasion rapidly type isolates, for example during outbreaks. The molecular methods that I used to differentiate CA-MRSA and HA-MRSA strain types took several days to perform from a pure culture, are expensive, have relatively low throughput and require specialist expertise in molecular biology; they are therefore not suitable for adoption in routine clinical laboratory. However, technological developments may allow these tests to be performed routinely in the future. My attempt to use antimicrobial susceptibility algorithms for the presumptive identification of CA-MRSA strain types was unsuccessful due to low sensitivity of even the best algorithm. PVL genes can be rapidly identified by a simple PCR and the incorporation of this in emerging PCR MRSA screening tests may be useful for identifying at least some CA-MRSA strain types.

GSTT has made substantial reductions in the incidence of MRSA bacteraemias and other infections in recent years.[192,485] If the prevalence of CA-MRSA continues to increase at GSTT, the present control systems may no longer be successful. The focus for control of MRSA may need to be shifted from the hospital wards to the hospital doors and beyond into the wider community.

8.5.5 Molecular epidemiology

The molecular epidemiology of CA-MRSA in most parts of the world is characterised by clonal heterogeneity[72,299] and there is evidence of local variation in the prevalence of various clones.[112,362] The molecular types of CA-MRSA identified at GSTT were heterogeneous and similar to those reported elsewhere in the UK and Europe.[84,112,299,390]

The most common CA-MRSA clone identified at GSTT was the *t127,* ST1-IV PVL-negative clone, which was often associated with the IDU/homeless patient group, so likely represents transmission in a semi-closed

community based group rather than transmission among healthy members in the open community. This clone appears to be closely related to WA-MRSA-1 in Australia[231,368,449] and has been reported by others as a cause of infections in IDUs in the UK.[228,358,476] In contrast to my findings in London, PVL-negative ST1 isolates were rare among a small collection of Cip-S MRSA isolates in Yorkshire.[362]

Other clones reported at low frequency in my project include PVL-positive CA-MRSA clones such as the ST80-IV European clone, ST8-IV (USA300), ST1-IV (USA400), ST59-IV or V and ST30-IV (SWP).

The frequency of PVL production in CA-MRSA identified in the project was 25% of Cip-S MRSA in the retrospective study, and 22% of CA-MRSA isolates identified on admission and CA-MRSA isolates identified during the assessment of AMS-based algorithms. The relatively low frequency of PVL production in the CA-MRSA isolates identified in my study (20-25%) compared with other studies of CA-MRSA reflects the dominance of the PVL-negative ST1 clone in the GSTT patient population.[341,351,486-488]

EMRSA-15 and 16 accounted for approximately 95% of HA-MRSA isolates at GSTT, which is similar to the national reports on bacteraemia isolates from reference laboratories.[179,181] In a study of UK bacteraemia isolates referred between 1998-2000, EMRSA-15 and EMRSA-16 accounted for 60% and 25% of isolates, respectively.[181] A more recent study of MRSA bacteraemia isolates analysed as part of the British Society for Antimicrobial Chemotherapy's bacteraemia surveillance programme in 2001, 2003, 2005 and 2007 found that EMRSA-15 and -16 together accounted for approximately 95% of the isolates, but that the prevalence of EMRSA-15 increased from 76% in 2001 to 85% in 2007 whereas EMRSA-16 declined from 21% to 9%.[179] In my project, *spa* types related to EMRSA-15 accounted for 70-80% of HA-MRSA isolates while those related to EMRSA-16 accounted for 10-15%, reflecting the findings of the HPA reference laboratory.[179,181]

8.5.6 PVL

The role of PVL in CA-MRSA disease remains controversial. Successful CA-MRSA lineages worldwide are usually PVL-positive, notably USA300 and USA400, ST80 (European), SWP, ST59 (Taiwan) and ST93 (Queensland).[72,299] Furthermore, there is a strong epidemiological association between CA-MRSA disease and PVL-positive isolates.[341,351,486-488] However, this association is biased somewhat because many studies use the presence of PVL to define CA-MRSA.[84,112,299] Furthermore, much of the literature regarding CA-MRSA comes from the USA, where the most successful CA-MRSA lineage is PVL-positive USA300.[323] This has encouraged the view that CA-MRSA is nearly always positive.

I did find evidence that PVL-positive Cip-S MRSA isolates were associated with abscess formation and younger patients and were more likely to be classified epidemiologically as community-acquired than PVL-negative Cip-S MRSA (Appendix 9-3, p.216).[84,112,238,454] The propensity for PVL-positive CA-MRSA to cause primary skin infection may explain why some studies have found that nasal colonisation with CA-MRSA is uncommon and CA-MRSA and community spread is independent of nasal colonisation.[212,214,289,296] However, as my study shows, not all successful CA-MRSA clones are PVL-positive, which suggests that PVL is not the primary virulence factor in CA-MRSA disease, which is supported by several studies.[251,252] Successful PVL-negative CA-MRSA clones include several in Australia, such as the ST1-IV (WA-MRSA-1) clone, which is the most common CA-MRSA clone in Western Australia, and ST129-IV (WA-MRSA-2);[231,368] the ST398-V pig-associated clone in Europe[124,337] and the ST72-IV clone in Korea.[489] One study reports the co-existence of sibling clones of PVL-positive and PVL-negative USA400 in Canada.[229] In the study by Zhang *et al*.,[229] PCR and sequence analysis indicated the presence of ΦSa2mw in the PVL-positive clone but not in the PVL-negative clone, suggesting that the phage had either excised from the

PVL-positive clone to generate the PVL-negative clone or that the phage was never introduced into the PVL-negative clone.

I identified a link between the sequence of the PVL-encoding genes and the PVL phage type. Although this does not appear to have a direct bearing on the pathogenesis of CA-MRSA disease, it is an important epidemiological finding, supporting the model that PVL-encoding phages firstly infected MSSA, and some subsequently acquired SCC*mec* to give rise to PVL-positive CA-MRSA.[34,241,243]

8.6 Future work and recommendations

My study has identified important areas for future work. Future studies should base definitions of CA-MRSA around genotype rather than phenotype or epidemiological classifications. Individual hospital laboratories should type MRSA strains involved in outbreaks to determine whether they are CA-MRSA or HA-MRSA strain types because a wider group of patients and staff may be at risk and novel control strategies may be required for CA-MRSA. Periodic investigation of antimicrobial resistance profiles among community-acquired *S. aureus* infections, perhaps combined with periodic typing of local sets of isolates, would also be useful to ensure that empiric therapy is appropriate, mindful that the emergence of CA-MRSA in some parts of the world has forced a change of empiric therapy of staphylococcal skin infections to cover MRSA.[490,491] Reference laboratories should continue to periodically type representative sets of isolates to ensure that MRSA trends and emerging strain types are monitored adequately.

There is a suggestion that the ST1-IV clone may be a particular problem in the IDU/homeless community served by GSTT. Community-based screening focussed on the IDU/homeless community served by GSTT would be useful to define the scale of the problem. Investigations into the molecular epidemiology of CA-MRSA in other parts of the UK would be

useful to allow a more detailed comparison of the molecular types of CA-MRSA around the UK.

In other countries, a high burden of CA-MRSA has been reported among patients presenting to emergency departments, particularly in the USA.[313,492] Therefore, an investigation into the prevalence and molecular of *S. aureus* and MRSA causing SSTIs in the A&E department at GSTT and elsewhere in the UK would be useful.

Ciprofloxacin susceptibility provided poor sensitivity as a phenotypic marker for CA-MRSA at GSTT. Therefore, studies of CA-MRSA in the UK should either avoid using ciprofloxacin susceptibility as a phenotypic marker or conduct a systematic study of the performance of AMS-algorithms in their population.

My study into the prevalence of MRSA on admission was limited to known colonisation sites for HA-MRSA. Future studies should investigate other sites of colonisation on hospital admission in light of the finding that CA-MRSA may be more commonly associated with non-nasal and other colonisation sites.[211,212]

Further work is needed to understand the reasons for the partnership between polymorphism in the PVL genes and the PVL-encoding phage, and whether these polymorphisms have clinical significance. Investigation of PVL gene sequence and phage type in other CA-MRSA of the same lineage, or in older versions of the same lineage, and in related MSSA, would shed further light on the population biology of PVL-positive *S. aureus*.

There is evidence that exposure to environmental stress such as UV light, and certain antimicrobial agents can result in phage induction mediated by the bacterial SOS response.[28,39,59,264,493] This can result in hyper-expression of the genes encoded by the phage and could influence bacteriophage transmission to other strains.[494,495] The host strain

background is also an important factor in the expression of the PVL genes.[244,264] Further research needs to be done to investigate the factors influencing the differential induction of the PVL-encoding bacteriophages and expression of the PVL genes.

The combination of *spa* typing, the assignment of the SCC*mec* allotype and a PCR to detected PVL carriage performed well as a system for the assignment of MRSA to lineages. However, *spa* typing would benefit from a publicly available clustering algorithm and database to facilitate clustering of a local set of isolates with a larger number of well-characterised MRSA isolates. Reconciliation of the two *spa* nomenclature systems would be useful. The Clondiag oligonucleotide array would also benefit from a publicly available analysis platform and database to facilitate comparative analysis of gene profiles. Future SCC*mec* typing schemes should adhere to nomenclature guidelines from the IWG-SCC.[96]

8.7 Conclusions

The definition of CA-MRSA has become more problematic as the global epidemic has evolved. It is becoming apparent that a microbiological and molecular definition is more useful than an epidemiological one. At the current time therefore, a combination of a genotypic method such as MLST, *spa* or PFGE, together with SCC*mec* analysis to infer the likely origin of the MRSA, remains the most useful definition of CA-MRSA.

In common with the CA-MRSA reported elsewhere in the world, CA-MRSA identified at GSTT are generally associated with younger patients, presentation in community settings or hospital specialties in which MRSA have historically been uncommon, SSTIs (specifically abscesses), certain community-based groups (specifically IDUs), resistance to fewer classes of antimicrobial agents, SCC*mec* types IV and V, genetic diversity compared with HA-MRSA and carriage of PVL. Unlike areas of high prevalence, there were no outbreaks of CA-MRSA strain types at GSTT

during the study period and cases of CA-MRSA did not appear to be linked in space and time.

The molecular epidemiology of CA-MRSA at GSTT is dominated by clonal heterogeneity. The most common CA-MRSA clone is PVL-negative ST1-IV, which is associated with the IDU/homeless patient group. Other previously reported clones of CA-MRSA occur at low frequency.

Although HA-MRSA is common at GSTT and in other UK hospitals, true CA-MRSA have been identified only rarely in the UK. Using ciprofloxacin susceptibility as a phenotypic marker of CA-MRSA in a collection of MRSA isolates from inpatients, outpatients and primary care clinics, there was a significant increase in the prevalence of CA-MRSA in the GSTT patient population during 2000-2006 both in terms of the number of cases (rising from 49 in 2000 to 102 in 2006) and proportion of recovered isolates that were PVL-positive (rising from 3.7% in 2000 to 13.2% in 2006).

Ciprofloxacin susceptibility and other AMS-based phenotypic markers are useful for presumptive identification of CA-MRSA but need to be confirmed by molecular methods. However, these markers have poor sensitivity for the identification of CA-MRSA in collections including HA-MRSA because of the presence of resistant CA-MRSA isolates and relatively susceptible HA-MRSA isolates. Among 41 CA-MRSA isolates at GSTT defined by molecular methods, 37% were ciprofloxacin resistant.

The prevalence of MRSA carriage in patients admitted to GSTT in 2008 was 1.6%, a substantially lower rate than previously reported form this hospital and from other UK centres. This lower rate appears to have resulted from the general reduction of HA-MRSA infection in the UK and from the national introduction of universal rather than selected admission screening. The prevalence of MRSA colonisation varied significantly by specialty, with the highest prevalence of colonisation in patients admitted to medical specialties (2.4%), in particular critical care (5.1%), and the lowest rates of colonisation in patients admitted to surgical specialties

(1.2%), many of which were admitted for elective surgery. This suggests that the cost-effectiveness of universal screening needs to be reviewed.

CA-MRSA strains accounted for approximately 15% of all MRSA identified on admission, giving an overall carriage rate of 0.25% of admissions. In contrast to the overall rates of carriage, CA-MRSA strains were significantly less likely to occur in medical specialties, specifically critical care, but were significantly more likely to occur in surgical specialties.

Detailed investigation of a selection of PVL-positive CA-MRSA clinical isolates found that the PVL gene sequence and the PVL-encoding phage vary with lineage.

In summary, CA-MRSA are present at GSTT, but their prevalence is relatively low compared with other parts of the world. CA-MRSA strain types were characterised by genetic heterogeneity and the presence of both PVL-positive and PVL-negative types. I identified evidence of increasing prevalence of CA-MRSA from 2000-2006. Based on the experience of other countries, this is likely to continue as the community reservoir of CA-MRSA expands due to the spread of existing clones, the emergence of new clones and the importation of successful clones from elsewhere. It also seems likely that community strains of MRSA will begin to cause hospital outbreaks at GSTT. Widespread emergence of CA-MRSA at GSTT could force a change in antimicrobial therapy to agents active against MRSA for the treatment of community-acquired *S. aureus* infection, which increases the likelihood of further development of resistance. Also, as CA-MRSA strains are exposed to the healthcare environment, they are likely to develop resistance to more classes of antimicrobial agents. Further emergence of CA-MRSA will result in disease in a wider group of individuals in the community and in hospitals, including high risk groups such as IDUs and but also previously healthy adults, children and healthcare workers. Therefore, there is an urgent need to define the epidemiology of CA-MRSA and to develop effective

systems for the identification and control of these organisms in the community, in hospitals and at the community-hospital interface.

9 APPENDICES

9.1 Rates of colonisation with *S. aureus* and MRSA in hospital and community-based screens

Appendix 9-1. Rates of colonisation with *S. aureus* and MRSA in non-hospitalised persons or at hospital admission.

Two PUBMED searches were performed: “MRSA colonisation community” and “MRSA colonisation admission”. Relevant articles from the bibliographies of articles identified by PUBMED searches were also included. Only papers written in English were included.

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
Community-based surveys								
290	2008	Columbus/ Cleveland, OH, USA	Homeless adults	Nasal swabs	215	34.9	25.6	Recent antibiotic use and alcoholism were risk factors for MRSA; living with a friend was protective
401	2006	Vancouver, Canada	Injecting drug users	Nasal swabs	301	39.5	18.5	USA300 accounted for 75% of all MRSA
402	2007	Pokhara, Nepal	Schoolchildren, <15 yrs	Nasal swabs	184	31.0	17.4	56% of *S. aureus* were MRSA

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
403	2006	Baltimore, MD, USA	Newly arrested men	Nasal swabs	602	40.4	15.8	1.3% of the men had MRSA wound infections
404	2004	Indigenous community, Queensland, Australia	Healthy schoolchildren	Nose, throat and wounds	92	29.3	15.2	MRSA were isolated from 29% of wounds, 8% of nasal swabs and 1% of throat swabs
496	2007	Taipei, Taiwan	Healthy children, <7 yrs	Nasal swabs	68	25.0	13.2	All MRSA were PVL-negative and 8/9 were ST59
406	2004	Nashville, TN, USA	Healthy children, age 2 weeks-21 yrs	Nasal swabs	500	36.4	9.2	22% of MRSA were PVL positive, representing 2% of the total
286	2004-6	Taipei, Taiwan	Healthy children, <15 yrs	Nasal swabs	1615	-	8.1	19% of MRSA were PVL positive, representing 1.5% of the total
497	2005-6	Three centres, Taiwan	Healthy children, 2 months-5 yrs	Nasal swabs	3046	23.4	7.3	MRSA colonisation significantly more frequent in Northern Taiwan
211	1995-2001	11 remove communities, Western Australia	Healthy adults and children	Nose, throat, wounds	2146	30.9	7.1	3.7% of nasal swabs positive
498	2008	Galveston, TX, USA	Healthy children, hospital day centre	Nasal swabs	104	-	6.7	35% of 17 family members were colonised with MRSA
499	2005	San Francisco, CA, USA	Homeless / runaway youths	Nasal swabs	308	26.7	6.2	USA300 and USA1000 (ST59) accounted for 84% of CA-MRSA
500	2005-2006	Suwon, Korea	Paediatric outpatients	Nasal swabs	296	32.1	6.1	ST72-IV accounted for the majority of the MRSA isolates

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
501	2002	Delhi, India	Healthy parents at a well-baby clinic	Nasal swabs	319	29.4	5.3	76% of the MRSA were gentamicin-resistant and likely HA-MRSA
502	2001-2002	Tokyo, Japan	Healthy children, day care centres	Nasal swabs	818	28.2	4.3	32% of tested MRSA carried SCC*mec* IV
503	2005/6	San Antonio, TX, USA	Healthy soldiers	Nasal swabs	3447	-	3.9	1.3% of the men developed abscesses
288	2007	Taipei, Taiwan	Healthy adults	Nasal swabs	3098	-	3.8	Antibiotics use and having children at home were risk factors for MRSA whereas smoking was protective
504	2001	Taipei, Taiwan	Community residents	Nasal swabs	1838	25.2	3.5	7.6% of residents in healthcare facilities were colonised with MRSA
505	2007	Patras, Greece	Healthy children, <15 yrs	Nasal swabs	123	59.3	3.2	Children <5 yrs had a higher risk of MRSA
295	2004	Fort Sam Houston, TX, USA	Healthily soldiers	Nasal swabs	812	31.1	3.0	Soldiers with CA-MRSA were more likely to develop infections than those with MSSA
506	1999-2000	San Francisco, CA, USA	Homeless	Nasal swabs	833	22.8	2.8	Injecting drug use, endocarditis and hospitalisation were MRSA risk factors
507	2005-6	St Louis, MO, USA	Healthy or outpatient children, 0-18 yrs	Nasal swabs	1300	-	2.5	66% of MRSA were community types
508	2006	Ottawa, Canada	Homeless shelter staff and residents	Nasal swabs	84	-	2.4	Colonisation 4.5% of residents vs. 0% of staff

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
291	1999-2000	New York, USA	Community-based drug users	Nasal swabs	500	24.0	2.0	HIV was a risk factor for MRSA
509	2007	Toronto, Canada	Men who have sex with men	Nasal and rectal swabs	500	-	1.6	60% of the men were HIV-positive. 1.2% of nasal swabs positive
155	2003-4	USA	Community based sample	Nasal swabs	9004	28.7	1.5	Gender specific risk factors for MRSA colonisation identified
396	1998	Birmingham, UK	Healthy adults	Nasal swabs	274	23.0	1.5	2/4 MRSA carriers had had recent contact with healthcare facilities
219	2006-2007	Niigata, Japan	Paediatric outpatients / healthy children	Nasal swabs	562	32.0	1.4	3.7% of healthy children were colonised compared with 0.7% of outpatients
510	2004	Hong Kong, China	Students and family members	Nasal swabs	663	28.0	1.4	Working in healthcare significantly associated with MRSA colonisation
365	2005	New Orleans, LA, USA	Community based, 2-65 yrs	Nasal swabs	259	-	1.2	Persons with HA-MRSA risk factors were excluded
292	2004	Atlanta, GA, USA	American Indian community	Nasal swabs	469	27.3	1.1	Crowded housing and antimicrobial use were risk factor for MRSA
511	2004-5	Newark, DE, USA	Healthy volunteers in the community	Nasal swabs	295	26.8	1.0	All three MRSA isolates were community types
512	2006/7	Boston, MA, USA	Healthy or outpatient children <7 yrs	Nasal swabs	974	14.1	0.9	MRSA colonisation increased from 0.2% in the same population in 2003/4

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
513	2001-2	USA	Community based sample	Nasal swabs	9622	32.4	0.8	Prevalence of MRSA colonisation increased to 1.5% in 2003-4
405	2001	Nashville, TN, USA	Healthy children, age 2 weeks-21 yrs	Nasal swabs	500	29.0	0.8	Household members working in a hospital associated with MRSA
397	2002	Nottingham, UK	Senior citizens	Nasal swabs	962	26.7	0.8	All MRSA isolates were HA-MRSA types
393	2002	Tel-Aviv, Israel	Children and parents, primary care	Nasal swabs	3373	17.2	0.8	Only two of the five MRSA identified were CA-MRSA (ST45-IV)
398	2005-2006	Brisbane, Queensland, Australia	Healthy adults	Nasal swabs	699	28.0	0.7	3/5 MRSA were healthcare-associated strains
514	1999	Orange, CA, USA	Senior citizens	Nasal swabs	165	26.1	0.6	9% of nursing home residents were colonised with MRSA
515	1999	Chicago, IL, USA	Healthy children, <16 yrs	Nasal and perineum swabs	500	24.4	0.6	Several isolates had borderline resistance to methicillin
516	2003	Jerusalem, Israel	Healthy children	Nasal swabs	831	23.5	0.6	8% of chronically hospitalised children were colonised with MRSA
517	2004	New York, USA	Healthy adults	Nasal swabs	739	23.4	0.5	Two of the four MRSAs were classified as CA-MRSA
296	2008	New York, USA	Healthy adults	Nasal swabs	823	24.6	0.4	Nasal colonisation with *S. aureus* was not associated with infection

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
518	2004/5	Afyonkarahisar, Turkey	Healthy children, age 4-6 yrs	Nasal swabs	1143	28.3	0.3	All MRSA isolates were in healthcare workers' children
519	2006	Selangor, Malaysia	Healthy adults	Nasal swabs	346	23.4	0.3	The one MRSA isolate was different from local hospital clones
520	2002/3	Lahore, India	Healthy adults	Nasal swabs	1660	14.8	0.3	*S. aureus* and MRSA significantly more likely from urban areas
512	2003/4	Boston, MA, USA	Healthy or outpatient children <7 yrs	Nasal swabs	588	14.6	0.2	MRSA colonisation increased to 0.9% in the same population in 2006/7
521	2000	New York, USA	Children and their guardians	Nasal swabs	500	-	0.2	Colonisation with *S. aureus* was more common in children
522	2001	Oeiras, Portugal	Soldiers and students	Nasal and throat swabs	1414	31.6	0.1	Both MRSA isolates were HA-MRSA clones
523	2007	Ankara, Turkey	School children	Nasal swabs	4050	24.7	0.1	Two of the three MRSA were PVL-negative ST30
Hospital admission / pre-admission								
289	2007	Cairo, Egypt	Adult drug addicts vs. non-addicts, hospital admission	Nasal, throat and clinical sites	120	-	14.2	22% of drug addicts colonised; 52% colonised or infected
465	2005	Austin, TX, USA	General medicine admissions	Nasal swabs	401	-	10.2	Nursing home stay, history of MRSA and various community-based activities were risk factors for MRSA

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
524	2005-6	Plymouth, UK	Admissions to critical care units	Nasal, throat, skin, wounds	612	-	7.0	Monthly rates ranged from 4-11%
525	1997	Paris, France	Admissions to intensive care units	Nasal and skin swabs	2347	-	6.9	3.7% of nasal swabs positive
399	2006-7	London, UK	Admissions, surgical wards	Nasal swabs	8971	-	6.7	Prevalence of MRSA colonisation on admission ranged from 3-20% by ward
463	2004-5	London, UK	Adult emergency admissions	Nasal swabs	6469	-	6.7	Previous MRSA or admission and care home residence were risk factors for MRSA colonisation
196	2005-7	Evanston, IL, USA	Hospital admissions	Nasal swabs	62035	-	6.3	8.3% of ICU admissions were MRSA-positive
526	2000-1	Abergavenny, Wales	Hospital admission	Nasal and wound swabs	430	-	5.4	3.5% of nasal swabs positive
472	2005	Atlanta, GA, USA	Hospital admission	Nasal swabs	726	23.7	5.3	Hospitalisation, antibiotic use, skin infection and HIV were MRSA risk factors
400	2004-6	Geneva, Switzerland	Admissions, surgical wards	Nasal swabs	10193	-	5.1	65% of patients MRSA-positive on admission had previous episodes
527	2002	Houston, TX, USA	Five hospital units	Nasal swabs	758	21.5	3.4	MRSA colonised patients were more likely to develop an MRSA infection

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
466	2003	Geneva, Switzerland	Hospital admissions	Nasal, perineum, skin, wounds	12072	-	3.3	MRSA colonisation prevalence ranged from 0.2-12.8% by specialty
528	2002	Auckland, New Zealand	Adults, hospital admission	Nose, perineum, wounds	201	-	3.0	Strains were healthcare-associated
529	2004	Tours, France	Admissions to vascular surgery	Nasal swabs	308	-	2.9	1.3% of patients acquired MRSA
530	2005	New York, USA	Pregnant women, prenatal GBS screens	Vaginal and rectal swabs	2963	17.1	2.8	13/14 MRSA isolates were community types
464	2005-7	Birmingham, UK	Admissions, surgical wards	Nasal swabs	6671	-	2.8	Study investigated 'patient ward episodes' rather than admissions, so may include duplicate patient visits
531	1998	Atlanta, GA, USA	Hospital admission, adults	Nasal swabs	974	21.1	2.7	Previous healthcare contact was a risk factor for MRSA
304	1999	Chicago, IL, USA	Emergency admissions, children	Nasal swabs	500	26.4	2.2	4/11 children colonised with MRSA had no HA-MRSA risk factors
532	2005-6	Pittsburgh, PA, USA	Pregnant women on a labour unit	Nasal swabs	96	20.8	2.1	Both MRSA-positive women also had vaginal colonisation
533	2008	Birzeit, Palastine	Hospital admissions	Nasal swabs	843	25.9	2.0	14% of healthcare workers were colonised with MRSA

Ref	Year	Location	Patients	Method	Number	%SA	%MRSA	Notes
517	2004	New York, USA	Emergency admissions, adults	Nasal swabs	156	-	1.9	One of the three MRSAs were classified as CA-MRSA
534	2003-5	Denver, CO, USA	Preoperative surgical outpatients	Nasal swabs	284	30.3	1.8	MRSA colonisation increased from 0% in 2003 to 4% in 2005
535	1997-8	Pavia, Italy	Hospital admission	Nasal and clinical swabs	7640	-	1.1	85/86 MRSA carriers had positive nasal cultures
536	2004	Al Hasa, Saudi Arabia	Hospital admissions	Nasal swabs	600	20.2	1.1	*S. aureus* colonisation rates higher in old, very young and female patients
339	2005	Bamako, Mali	Emergency admissions	Nasal swabs	448	19.4	0.2	A PVL-positive ST152 clone predominated among MSSA
537	1999	Riyadh, Saudi Arabia	Hospital admission, eye hospital	Nasal swabs	306	33.3	0	No significant risk factors for *S. aureus* colonisation were identified

9.2 *spa* types and BURP clustering, retrospective study

Appendix 9-2. *spa* types and BURP clustering, retrospective study.

BURP clustering of *spa* types from 192 isolates using a calculated cost between members of ≤4. The stringent calculated cost was chosen to increase the resolution between *spa* CCs. No *spa* type was obtained from two of the isolates.

CC 127	n	CC 022	n	CC 044	n	CC 002	n	CC 008	n	CC 1778	n	Singleton	n
Total	74	Total	19	Total	18	Total	18	Total	14	Total	12	Total	39
%	38.1	%	9.8	%	9.1	%	9.3	%	7.2	%	6.2	%	20.1
t127	72	*t022*	12	*t044*	12	*t002*	12	*t008*	7	*t1778*	11	*t012*	4
t128	1	*t005*	2	*t131*	1	*t001*	1	*t986*	2	*t2478*	1	*t015*	2
t321	1	*t756*	2	*t267*	1	*t088*	1	*t024*	1			*t186*	2
		t032	1	*t359*	1	*t105*	1	*t190*	1			*t216*	2
		t1214	1	*t667*	1	*t214*	1	*t334*	1			*t437*	2
		t2436	1	*t1247*	1	*t311*	1	*t451*	1			*t037*	1
				t2297	1	*t952*	1	*t622*	1			*t084*	1
												t1081	1
												t116	1
												t148	1
												t1777	1
												t1816	1

CC 127	n	CC 022	n	CC 044	n	CC 002	n	CC 008	n	CC 1778	n	Singleton	n
												t1894	1
												t1895	1
												t1894	1
												t1895	1
												t2298	1
												t230	1
												t2479	1
												t2480	1
												t275	1
												t315	1
												t318	1
												t380	1
												t559	1
												t688	1
												t690	1
												t742	1
												t782	1
												t883	1

9.3 Comparison of PVL-positive and PVL-negative Cip-S MRSA

Appendix 9-3. Comparison of PVL-positive and PVL-negative CA-MRSA

	PVL-[a]	%	PVL+[b]	%	p[c]
Patient demographics					
Male	94	64.8	32	65.3	1.0000
Median age (range)	41.2 (0-95.8)		33.6 (0-84.5)		**0.0200[d]**
Children <15 years	26	17.9	11	22.4	0.5297
Risk factors		0.0		0.0	
Drug or alcohol abuse	33	22.8	1	2.0	**0.0004**
Neoplasm	13	9.0	3	6.1	0.7651
Collection location					
Outpatient or A&E[e]	63	43.4	27	55.1	0.1859
Specimen					
SSTI[f]	99	68.3	37	75.5	0.3724
SSTI – abscess	13	9.0	13	26.5	**0.0033**
Respiratory	12	8.3	4	8.2	1.0000
Urine	6	4.1	0	0.0	0.3402
Mucosal	10	6.9	4	8.2	0.7544
Invasive & line or tip	15	10.3	1	2.0	0.0767
Other	3	2.1	3	6.1	0.1704
Epidemiological classification[g]					
Healthcare-acquired	87	60.0	19	38.8	**0.0126**
Community-acquired	45	31.0	25	51.0	**0.0236**
No data	13	9.0	5	10.2	0.7799
Antimicrobial resistance					
Erythromycin	63	43.4	22	44.9	0.8693
Fusidic acid	76	52.4	22	44.9	0.4104
Tetracycline	6	4.1	22	44.9	**<0.0001**
Neomycin	9	6.2	16	32.7	**<0.0001**
Antibiogram[h]					
None	41	28.3	9	18.4	0.1909
1 class	50	34.5	9	18.4	**0.0473**
2 classes	41	28.3	17	34.7	0.4706
≥3 classes	13	9.0	14	28.6	**0.0014**

[a] Panton-valentine leukocidin (PVL)-negative.

[b] PVL-positive.

[c] p values calculated using Chi-square tests of 2 x 6 contingency tables unless otherwise stated. P values <0.05 highlighted in bold.

[d] p value calculated using a Mann-Whitney U test.

[e] Includes isolates from General Practitioners, outpatient clinics, genitourinary medicine and obstetrics and gynaecology.

[f] SSTI = skin and soft tissue infection.

[g] See methods (section 6.14.1, p.40) for a detailed explanation of epidemiological classification criteria.

[h] The number of antimicrobial resistance classes in addition to the β-lactams.

9.4 *spa* types and BURP clustering, AMS algorithm

Appendix 9-4. *spa* types and BURP clustering, AMS algorithm.

BURP clustering of *spa* types from 237 isolates using a calculated cost between members of ≤6 and excluding *spa* types of <5 repeats. No *spa* type was obtained from one isolate and one *spa* type was too short for clustering.

CC 032	n	CC 012	n	CC 008	n	CC 010	n	CC 044	n	CC6 (no founder)	n	CC7 (no founder)	n	Singletons	n
Total	139	Total	54	Total	17	Total	5	Total	3	Total	2	Total	2	Total	15
%	58.6	%	22.8	%	7.2	%	2.1	%	1.3	%	0.8	%	0.8	%	6.3
t032	97	*t018*	29	*t190*	9	*t002*	3	*t044*	1	*t004*	1	*t5068*	1	*t127*	13
t022	9	*t012*	14	*t008*	4	*t010*	1	*t359*	1	*t370*	1	*t5121*	1	*t316*	2
t020	2	*t037*	7	*t024*	2	*t149*	1	*t5064*	1						
t025	2	*t019*	2	*t064*	1										
t294	2	*t253*	1	*t622*	1										
t432	2	*t021*	1												
t515	2														
t749	2														
t879	2														
t1148	2														
t1214	2														
t223	1														

CC 032	n	CC 012	n	CC 008	n	CC 010	n	CC 044	n	CC6 (no founder)	n	CC7 (no founder)	n	Singletons	n
t608	1														
t651	1														
t1021	1														
t1378	1														
t1771	1														
t1864	1														
t2236	1														
t5065	1														
t5066	1														
t5067	1														
t5069	1														
t5176	1														
t5177	1														
t5178	1														

9.5 Antimicrobial resistance patterns grouped by *spa* type

Appendix 9-5. Antimicrobial resistance patterns grouped by *spa* type.

Resistance patterns[a]	Total	% total	*spa* type	n	% profile	% total
CIP/E	81	33.9	*t032*	36	44.4	15.1
			t018	12	14.8	5.0
			t012	6	7.4	2.5
			t022	6	7.4	2.5
			t294	2	2.5	0.8
			t515	2	2.5	0.8
			t879	2	2.5	0.8
			t002	1	1.2	0.4
			t008	1	1.2	0.4
			t020	1	1.2	0.4
			t025	1	1.2	0.4
			t037	1	1.2	0.4
			t127	1	1.2	0.4
			t253	1	1.2	0.4
			t432	1	1.2	0.4
			t458	1	1.2	0.4
			t608	1	1.2	0.4
			t651	1	1.2	0.4
			t1021	1	1.2	0.4
			t1148	1	1.2	0.4
			t1864	1	1.2	0.4
			t5066	1	1.2	0.4
CIP	39	16.3	*t032*	22	57.9	9.2
			t022	2	5.3	0.8
			t5178	1	2.6	0.4
			t5069	1	2.6	0.4
			t5068	1	2.6	0.4
			t5067	1	2.6	0.4
			t2236	1	2.6	0.4
			t1214	1	2.6	0.4
			t1148	1	2.6	0.4
			t749	1	2.6	0.4
			t432	1	2.6	0.4
			t025	1	2.6	0.4
			t020	1	2.6	0.4
			t018	1	2.6	0.4

Resistance patterns[a]	Total	% total	*spa* type	n	% profile	% total
			t012	1	2.6	0.4
			t002	1	2.6	0.4
NONE	18	7.5	*t127*	3	16.7	1.3
			t012	2	11.1	0.8
			t019	2	11.1	0.8
			t024	2	11.1	0.8
			t002	1	5.6	0.4
			t010	1	5.6	0.4
			t022	1	5.6	0.4
			t032	1	5.6	0.4
			t223	1	5.6	0.4
			t359	1	5.6	0.4
			t370	1	5.6	0.4
			t5065	1	5.6	0.4
			t749	1	5.6	0.4
CIP/E/G	16	6.7	*t032*	14	82.4	5.9
			t1378	1	5.9	0.4
			t149	1	5.9	0.4
			t5177	1	5.9	0.4
CIP/E/TM	14	5.9	*t018*	8	57.1	3.3
			t032	6	42.9	2.5
CIP/FA	9	3.8	*t032*	6	66.7	2.5
			t004	1	11.1	0.4
			t044	1	11.1	0.4
			t5176	1	11.1	0.4
CIP/E/G/TM/MUP	8	3.3	*t018*	6	75.0	2.5
			t012	2	25.0	0.8
FA	7	2.9	*t127*	6	85.7	2.5
			t622	1	14.3	0.4
CIP/E/FA/TE/TM/MUP	4	1.5	*t190*	4	100.0	1.7
CIP/E/G/TM	4	1.7	*t018*	2	50.0	0.8
			t012	1	25.0	0.4
			t032	1	25.0	0.4
CIP/E/FA	3	1.3	*t032*	1	33.3	0.4
			t316	1	33.3	0.4
			t1771	1	33.3	0.4
CIP/E/FA/G	3	1.3	*t032*	2	66.6	0.8
			t190	1	33.3	0.4
TE	3	1.3	*t008*	1	33.3	0.4
			t127	1	33.3	0.4

Resistance patterns[a]	Total	% total	*spa* type	n	% profile	% total
			t5065	1	33.3	0.4
CIP/E/FA/TM	3	1.3	*t190*	2	66.6	0.8
			t032	1	33.3	0.4
CIP/E/G/TE	3	1.3	*t037*	3	100.0	1.3
CIP/E/G/TE/TM	3	1.3	*t037*	3	100.0	1.3
CIP/E/TE	3	1.3	*t032*	2	66.7	0.8
			t008	1	33.3	0.4
CIP/G	3	1.3	*t032*	2	66.6	0.8
			t5121	1	33.3	0.4
CIP/E/FA/TE/TM	2	0.8	*t190*	1	50.0	0.4
			NST[b]	1	50.0	0.4
CIP/TM	2	0.8	*t032*	1	50.0	0.4
			t316	1	50.0	0.4
E/FA	2	0.8	*t012*	1	50.0	0.4
			t127	1	50.0	0.4
CIP/E/FA/G/TE	1	0.4	*t032*	1	100.0	0.4
CIP/E/FA/TE	1	0.4	*t190*	1	100.0	0.4
CIP/E/G/TE/RIF	1	0.4	*t032*	1	100.0	0.4
CIP/E/MUP	1	0.4	*t012*	1	100.0	0.4
CIP/E/TM/MUP	1	0.4	*t008*	1	100.0	0.4
CIP/FA/RIF	1	0.4	*t020*	1	100.0	0.4
CIP/TE/TM	1	0.4	*t021*	1	100.0	0.4
FA/G/MUP	1	0.4	*t127*	1	100.0	0.4
TE/TM	1	0.4	*t064*	1	100.0	0.4

[a] CIP = Ciprofloxacin, ERY = Erythromycin, FA = Fusidic acid, G = Gentamicin, TE = Tetracycline, TM = Trimethoprim, MUP = Mupirocin, RIF = Rifampicin.

[b] NST = no *spa* type obtained from this isolate.

9.6 Screening cultures from non-standard sites

Appendix 9-6. Frequency of positive screening cultures from non-standard sites.

	n	% total	n positive	% positive	p value[a]
STANDARD SETS	17694	94.9	281	1.6	-
RECTAL	381	2.0	5	1.3	0.826
INCOMPLETE SETS	354	1.9	3	0.8	0.372
Nose throat	65	0.3	0	0.0	-
Groin	63	0.3	1	1.6	-
Nose	61	0.3	0	0.0	-
Throat	45	0.2	1	2.2	-
Nose perineum	35	0.2	1	2.9	-
Throat perineum	24	0.1	0	0.0	-
Axilla	19	0.1	0	0.0	-
Nose groin	15	0.1	0	0.0	-
Perineum	13	0.1	0	0.0	-
Nose axilla groin	7	0.0	0	0.0	-
Nose axilla	3	0.0	0	0.0	-
Axilla groin	1	0.0	0	0.0	-
Nose throat axilla	1	0.0	0	0.0	-
Skin	1	0.0	0	0.0	-
Throat groin	1	0.0	0	0.0	-
CLINICAL SITES	209	1.1	15	7.2	**<0.001**
Wound (non-operative)	53	0.3	4	7.5	-
Wound	33	0.2	1	3.0	-
Drain	31	0.2	1	3.2	-
Tracheostomy	28	0.2	5	17.9	-
Gastrostomy/Jejunostomy	22	0.1	2	9.1	-
Urine	11	0.1	0	0.0	-
Intravenous access device	9	0.0	0	0.0	-
Stoma	8	0.0	0	0.0	-
Sputum	5	0.0	1	20.0	-
Ear	4	0.0	0	0.0	-
Eye	4	0.0	1	25.0	-
Nephrostomy	1	0.0	0	0.0	-
GRAND TOTAL	18638	100.0	304	1.6	0.777

[a] The frequency of MRSA-positive screens were compared with the frequency of MRSA-positive screens in standard sets using a Chi-squared test of 2x2 contingency tables. p values <0.05 highlighted in bold.

9.7 Analysis of possible acquisitions

Appendix 9-7. Characteristics of patients with a negative admission screen and a subsequent positive screen or culture compared with patients a negative admission screen and no subsequent positive screen or culture.

	MRSA-negative (n=18186)		MRSA-negative to positive (n=148)		Univariate[a]		Multivariate[b]	
	Count	%	Count	%	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
DEMOGRAPHICS								
Mean age / years	49.9		62.4		-	**<0.001**[c]	-	-
Median age / years	52.0 (0-108)		66.0 (0-97)					
Age >60 years	6854	37.7%	97	65.5%	3.1 (2.2-4.4)	**<0.001**	2.5 (1.7-3.7)	**<0.001**
Male	8920	53.9%	94	66.2%	1.7 (1.2-2.4)	**0.004**	1.5 (1.1-2.2)	**0.022**
Hospital day visit in the past 12 months	1717	9.4%	11	7.4%	-	0.481	-	-
Overnight hospital stay in the past 12 months	1800	9.9%	25	16.9%	1.8 (1.2-2.8)	**0.006**	-	0.714
Previous positive for MRSA	319	1.8%	31	20.9%	14.8 (9.8-22.4)	**<0.001**	10.7 (6.8-17.0)	**<0.001**
SPECIALTY								
Surgery								
Orthopaedics	2277	12.5%	9	6.1%	0.4 (0.2-0.9)	**0.021**	-	0.810
Urology	1447	8.0%	11	7.4%	-	1.000	-	-
General Surgery	1433	7.9%	12	8.1%	-	0.878	-	-
Cardiothoracic Surgery	1337	7.4%	9	6.1%	-	0.750	-	-
ENT/Oral Surgery	1165	6.4%	6	4.1%	-	0.310	-	-
Plastic Surgery	793	4.4%	2	1.4%	-	0.099	.-	-

	MRSA-negative (n=18186)		MRSA-negative to positive (n=148)		Univariate[a]		Multivariate[b]	
	Count	%	Count	%	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Paediatric surgery	657	3.6%	0	.0%	-	**0.007**	-	0.992
Vascular Surgery	294	1.6%	10	6.8%	4.4 (2.3-8.5)	**<0.001**	3.3 (1.6-6.8)	**0.001**
Breast Surgery	234	1.3%	0	.0%	-	0.270	-	-
Ophthalmology	60	.3%	0	.0%	-	1.000	-	-
Total surgery	9697	53.3%	59	39.9%	0.6 (0.4-0.8)	**0.001**	-	-
Medicine								
General medicine	2428	13.4%	40	27.0%	2.4 (1.7-3.5)	**<0.001**	1.8 (1.2-2.9)	**0.007**
Cardiology	1344	7.6%	6	4.1%	-	0.153	-	-
Paediatric Medicine	730	4.0%	3	3.0%	-	0.291	-	-
Renal Medicine	536	2.9%	3	2.0%	-	0.804	-	-
Oncology	305	1.7%	3	2.0%	-	0.741	-	-
Respiratory Medicine	261	1.4%	6	4.1%	2.9 (1.3-6.6)	**0.021**	-	0.166
Elderly Care	123	.7%	0	.0%	-	0.629	-	-
Haematology	119	.7%	1	.7%	-	0.623	-	-
Gastroenterology	98	.5%	0	.0%	-	1.000	-	-
Critical Care Medicine	79	.4%	5	3.4%	8.0 (3.2-20.0)	**0.001**	5.1 (1.7-16.8)	**0.004**
Rheumatology	82	.5%	2	1.4%	-	0.148	-	-
Dermatology	68	.3%	7	4.7%	15.5 (7.0-34.6)	**<0.001**	18.6 (7.3-47.4)	**<0.001**
Endocrinology	58	.3%	0	.0%	-	1.000	-	-
Neurology	47	.3%	0	.0%	-	1.000	-	-
Total medicine	6268	34.5%	76	51.4%	2.0 (1.4-2.8)	**<0.001**	-	-
A&E/GP/Other[d]								
Accident & Emergency	658	3.6%	4	2.7%	-	0.823	-	-
Other	311	1.7%	1	.7%	-	0.525	-	-
GP	64	.4%	4	2.7%	7.9 (2.8-21.9)	**0.002**	-	0.123

	MRSA-negative (n=18186)		MRSA-negative to positive (n=148)		Univariate[a]		Multivariate[b]	
	Count	%	Count	%	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Paed A&E/GP/Other	42	.2%	1	.7%	-	0.295	-	-
Total A&E/GP/Other	1075	5.9%	10	6.8%	-	0.600	-	-
Obstetrics/Gynaecology/Neonatology								
Obstetrics/Gynaecology	895	4.9%	2	1.4%	-	0.052	-	-
Neonatology	251	1.5%	1	.7%	-	0.726	-	-
Total obs/gynae/neonatology	1146	6.3%	3	2.0%	0.3 (0.1-1.0)	**0.026**	-	-
LOCATION OF SCREEN								
Adult inpatients	8043	44.2%	83	56.1%	1.6 (1.2-2.2)	**0.005**	-	0.298
Adult pre-assessment	4553	25.0%	17	11.5%	0.4 (0.2-0.6)	**<0.001**	-	0.698
Adult outpatients	3042	16.7%	22	14.9%	-	0.651	-	-
Adult ITU/HDU[e]	650	3.6%	20	13.5%	4.2 (2.6-6.8)	**<0.001**	2.7 (1.2-5.8)	**0.014**
Adult A&E	224	1.2%	1	0.7%	-	1.000	-	-
Paediatrics	1674	9.2%	5	3.4%	-	0.069	-	0.544

[a] p values determined using Chi-square tests of 2x2 contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] A binomial logistic regression model was used for the multivariate analysis using were significant by univariate analysis as covariates.

[c] p value determined using the Mann-Whitney U test.

[d] A&E = Accident and Emergency; GP = General Practitioner.

[e] ITU = intensive care unit; HDU = high dependency unit.

9.8 CA-MRSA or HA-MRSA strain types versus patients with negative screens

Appendix 9-8. Characteristics of patients with CA-MRSA or HA-MRSA strain types versus patients with negative screens.

	MRSA-negative (n=18334)		CA-MRSA strain types (n=37)				HA-MRSA strain types (n=214)					
	n	%	n	%	Unadjusted OR (95% CI)	Univariate[a] p value	n	%	Unadjusted OR (95% CI)	Univariate[a] p value	Adjusted OR (95% CI)	Multivariate[b] p value
DEMOGRAPHICS												
Mean age	50.0		50.1		-	0.838[c]	58.5		-	**<0.001**[c]	-	-
Median age	52.0		55.0				63.5					
Age >60 years	6951	37.9%	17.0	45.9%	-	0.314	121	56.5%	2.1 (1.6-2.8)	**<0.001**	1.8 (1.3-2.5)	**<0.001**
Gender (male)	9014	54.0%	23	63.9%	-	0.247	125	62.2%	1.4 (1.0-1.9)	**0.023**	-	0.168
Previous positive for MRSA	350	1.9%	10	27.0%	19.0 (9.1-39.6)	**<0.001**	75	35.0%	27.7 (20.5-37.4)	**<0.001**	22.5 (16.2-31.3)	**<0.001**
Previous hospital visit in the past 12 months	3553	19.4%	8	21.6%	-	0.680	72	33.6%	2.1 (1.6-2.8)	**<0.001**	-	0.277
SPECIALTY												
Surgery	9099	49.6%	22	59.5%	-	0.252	74	34.6%	0.5 (0.4-0.7)	**<0.001**	-	0.407
Medicine	5611	30.6%	10	27.0%	-	0.723	105	49.1%	2.2 (1.7-2.9)	**<0.001**	-	0.880
Obstetrics/gynaecology	903	4.9%	0	0.0%	-	0.261	6	2.8%	-	0.200	-	-
A&E/GP/Other[d]	1042	5.7%	3	8.1%	-	0.467	14	6.5%	-	0.552	-	-
Paediatrics	1679	9.2%	2	5.4%	-	0.577	15	7.0%	-	0.339	-	-
LOCATION OF SCREEN												
Adult A&E	225	1.2%	2	5.4%	-	0.076	6	2.8%	-	0.052	-	-
Adult ITU/HDU[e]	670	3.7%	0	0.0%	-	0.646	32	15.0%	4.6 (3.2-6.8)	**<0.001**	3.3 (1.4-7.8)	**0.007**
Adult outpatients	3064	16.7%	4	10.8%	-	0.506	22	10.3%	0.6 (0.4-0.9)	**0.012**	-	0.460

	MRSA-negative (n=18334)		CA-MRSA strain types (n=37)				HA-MRSA strain types (n=214)					
	n	%	n	%	Unadjusted OR (95% CI)	Univariate[a] p value	n	%	Unadjusted OR (95% CI)	Univariate[a] p value	Adjusted OR (95% CI)	Multivariate[b] p value
Adult pre-assessment	4570	24.9%	7	18.9%	-	0.453	27	12.6%	0.4 (0.3-0.6)	**<0.001**	-	0.221
Adult inpatients	8126	44.3%	22	59.5%	-	0.069	112	52.3%	1.4 (1.0-1.8)	**0.022**	-	0.787

[a] p values determined using Chi-square tests of 2x2 contingency tables unless otherwise stated. p values <0.05 highlighted in bold.

[b] A binomial logistic regression model was used for the multivariate analysis using were significant by univariate analysis as covariates.

[c] p value determined using the Mann-Whitney U test.

[d] A&E = Accident and Emergency; GP = General Practitioner.

[e] ITU = intensive care unit; HDU = high dependency unit.

9.9 *spa* types and BURP clustering, MRSA admission screening

Appendix 9-9. spa types and BURP clustering, MRSA admission screening.

BURP clustering of *spa* types from 251 isolates using a calculated cost between members of ≤6.

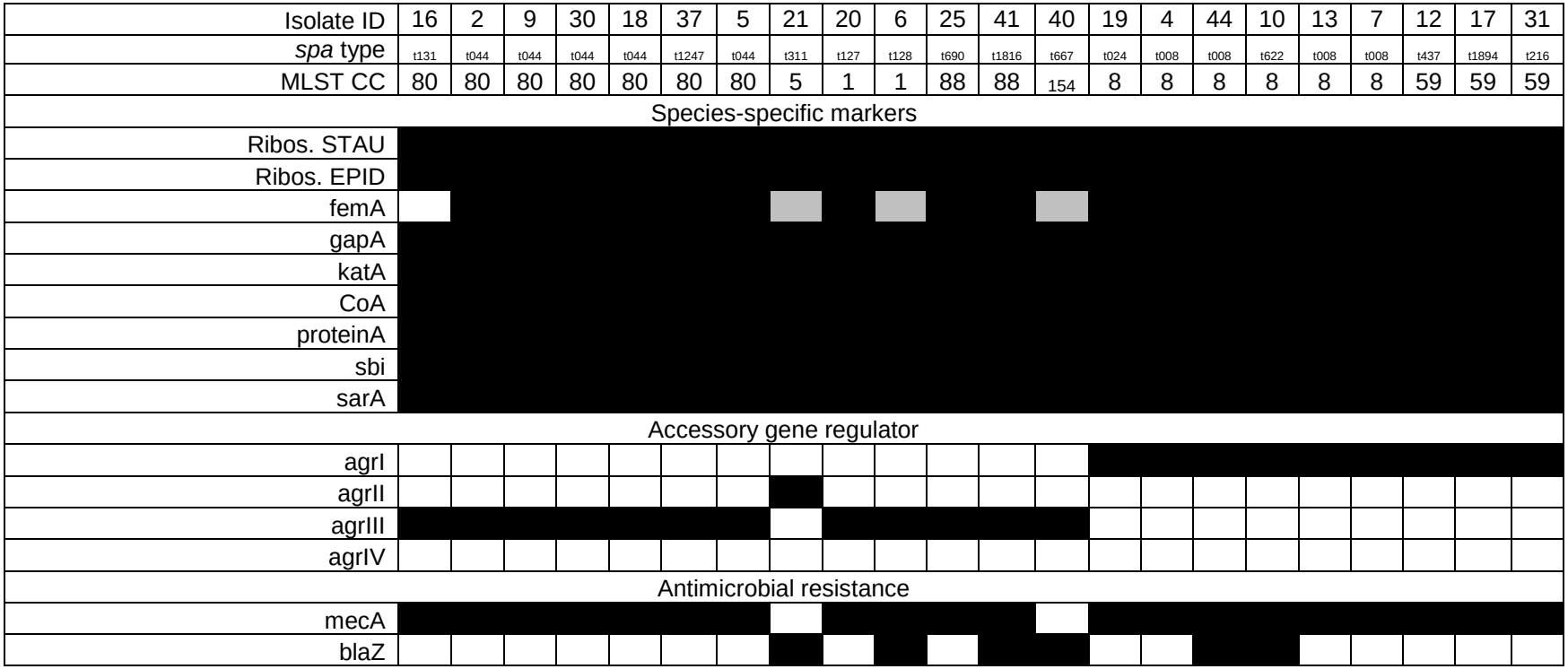
CC 032	n	CC 012	n	CC 008	n	CC 010	n	CC 044	n	CC6 (no founder)	n	CC7 (no founder)	n	Singletons	n
Total	172	Total	37	Total	13	Total	4	Total	3	Total	2	Total	2	Total	18
%	68.5	%	14.7	%	5.2	%	1.6	%	1.2	%	0.8	%	0.8	%	7.2
t032	102	*t018*	20	*t190*	7	*t002*	2	*t044*	1	*t216*	1	*t004*	1	*t127*	12
t022	15	*t012*	10	*t008*	2	*t010*	1	*t359*	1	*t316*	1	*t370*	1	*t084*	1
t037	6	*t019*	1	*t024*	1	*t149*	1	*t376*	1					t1778	1
t1036	6	*t021*	1	*t064*	1									t2393	1
t020	5	*t253*	1	*t068*	1									t5068	1
t1214	3	*t268*	1	t622	1									t5624	1
t294	3	*t318*	1											t5625	1
t515	3	*t5549*	1												
t025	2	*t5550*	1												
t1625	2														
t379	2														
t749	2														
t1021	1														

CC 032	n	CC 012	n	CC 008	n	CC 010	n	CC 044	n	CC6 (no founder)	n	CC7 (no founder)	n	Singletons	n
t1032	1														
t1148	1														
t1370	1														
t1378	1														
t1733	1														
t1771	1														
t1864	1														
t223	1														
t310	1														
t3507	1														
t5066	1														
t5067	1														
t5069	1														
t5176	1														
t5551	1														
t608	1														
t612	1														
t651	1														
t670	1														
t879	1														

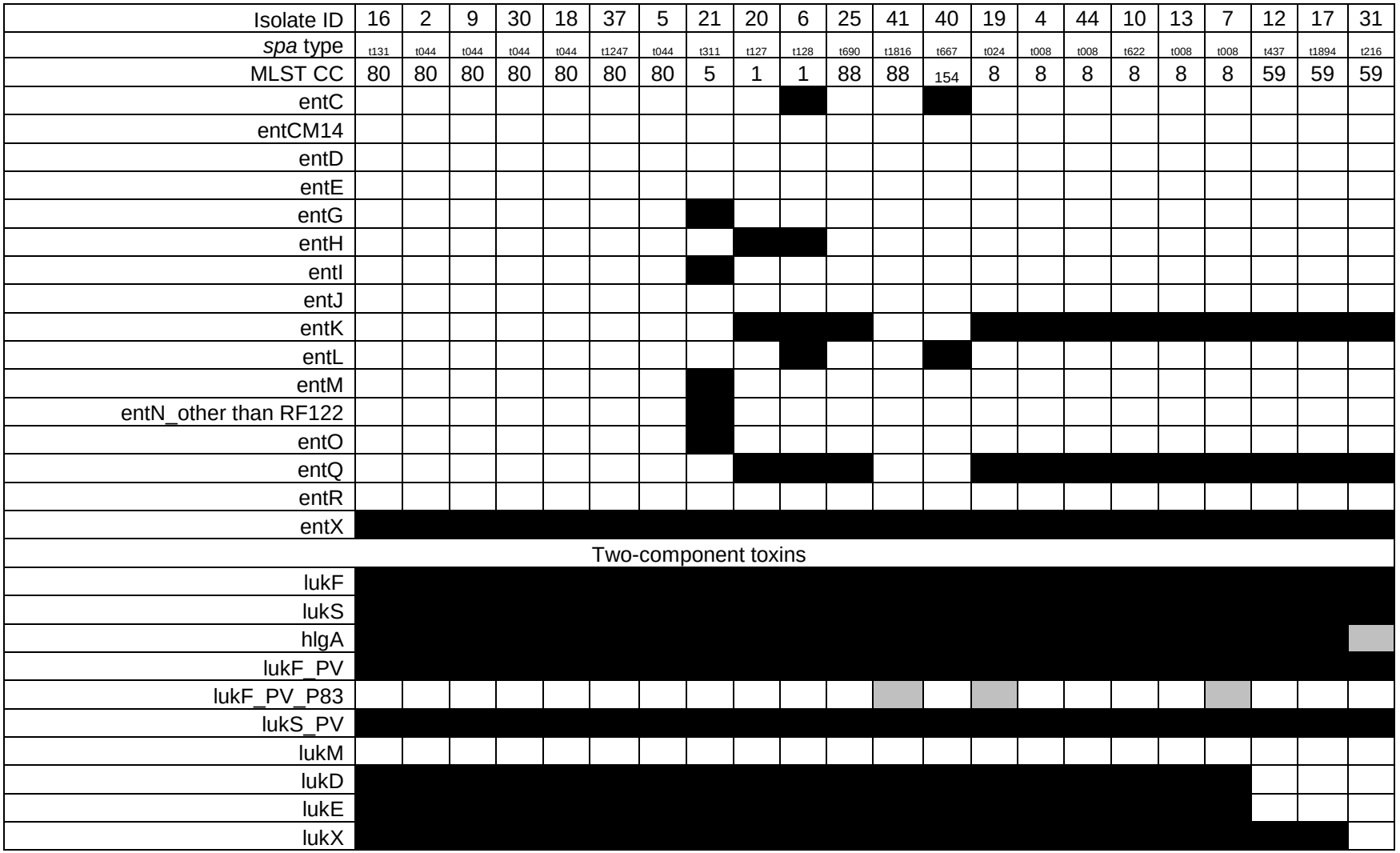
9.10 Clondiag raw data

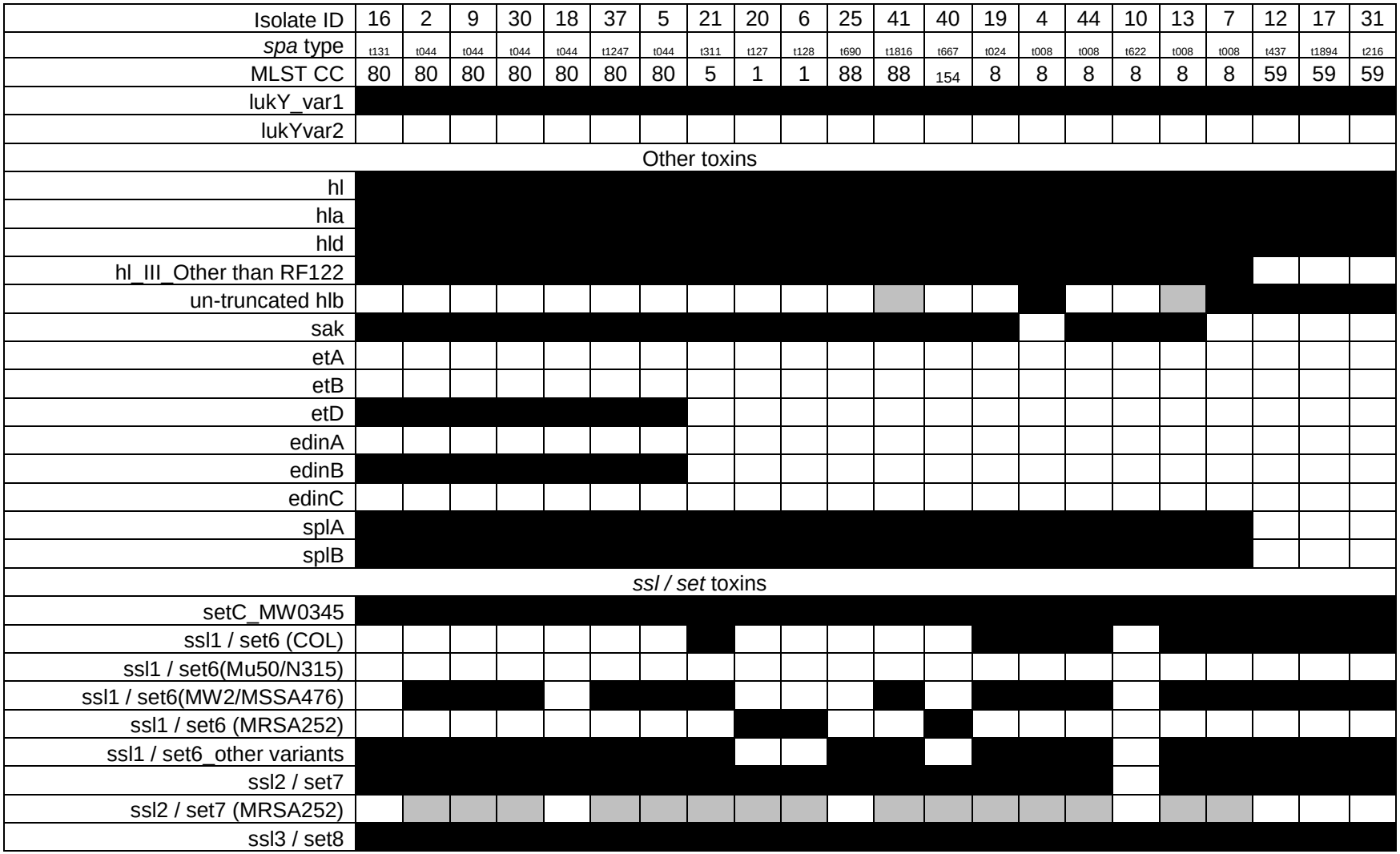
Appendix 9-10. Clondiag raw data.

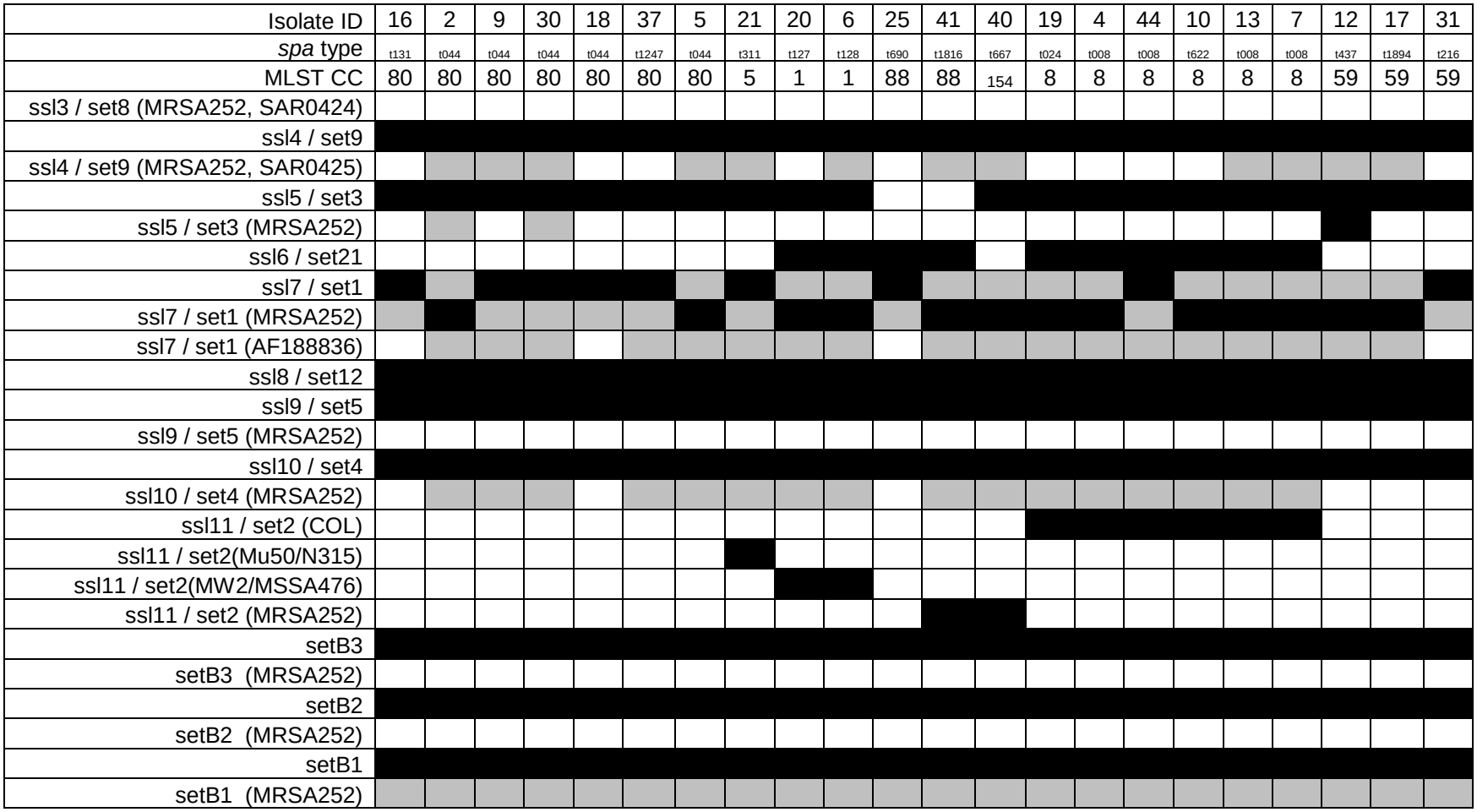
Black shaded cells = positive; white shaded cells = negative; grey shaded cells = ambiguous result.



Isolate ID	16	2	9	30	18	37	5	21	20	6	25	41	40	19	4	44	10	13	7	12	17	31
spa type	t131	t044	t044	t044	t044	t1247	t044	t311	t127	t128	t690	t1816	t667	t024	t008	t008	t622	t008	t008	t437	t1894	t216
MLST CC	80	80	80	80	80	80	80	5	1	1	88	88	154	8	8	8	8	8	8	59	59	59
ermA																						
ermC		■		■																		
linA															■							
msrA																	■					
vatA																						
vatB																						
vga																						
vgaA																						
vgb																						
aacA-aphD																						
aadD																						
aphA_3	■	■	■	■	■	■											■			■	■	
sat	■	■	■	■	■	■											■			■	■	
dfrA																						
far1																						
mupR																						
tetK									■			■		■								
tetM													■									
vanA																						
vanB																						
vanZ																						
Superantigens																						
tst1																						
entA								■	■	■												
entA-320E																						
entA-N315 (aka entP)											■	■										
entB																				■	■	■


Isolate ID
16 2 9 30 18 37 5 21 20 6 25 41 40 19 4 44 10 13 7 12 17 31
spa type
t131 t044 t044 t044 t044 t1247 t044 t311 t127 t128 t690 t1816 t667 t024 t008 t008 t622 t008 t008 t437 t1894 t216
MLST CC
80 80 80 80 80 80 80 5 1 1 88 88 154 8 8 8 8 8 8 59 59 59
entC
entCM14
entD
entE
entG
entH
entI
entJ
entK
entL
entM
entN_other than RF122
entO
entQ
entR
entX
Two-component toxins
lukF
lukS
hlgA
lukF_PV
lukF_PV_P83
lukS_PV
lukM
lukD
lukE
lukX


Isolate ID
16 2 9 30 18 37 5 21 20 6 25 41 40 19 4 44 10 13 7 12 17 31
spa type
t131 t044 t044 t044 t044 t1247 t044 t311 t127 t128 t690 t1816 t667 t024 t008 t008 t622 t008 t008 t437 t1894 t216
MLST CC
80 80 80 80 80 80 80 5 1 1 88 88 154 8 8 8 8 8 8 59 59 59
lukY_var1
lukYvar2
Other toxins
hl
hla
hld
hl_III_Other than RF122
un-truncated hlb
sak
etA
etB
etD
edinA
edinB
edinC
splA
splB
ssl / set toxins
setC_MW0345
ssl1 / set6 (COL)
ssl1 / set6(Mu50/N315)
ssl1 / set6(MW2/MSSA476)
ssl1 / set6 (MRSA252)
ssl1 / set6_other variants
ssl2 / set7
ssl2 / set7 (MRSA252)
ssl3 / set8


Isolate ID 16 2 9 30 18 37 5 21 20 6 25 41 40 19 4 44 10 13 7 12 17 31
spa type t131 t044 t044 t044 t044 t1247 t044 t311 t127 t128 t690 t1816 t667 t024 t008 t008 t622 t008 t008 t437 t1894 t216
MLST CC 80 80 80 80 80 80 80 5 1 1 88 88 154 8 8 8 8 8 8 59 59 59
ssl3 / set8 (MRSA252, SAR0424)
ssl4 / set9
ssl4 / set9 (MRSA252, SAR0425)
ssl5 / set3
ssl5 / set3 (MRSA252)
ssl6 / set21
ssl7 / set1
ssl7 / set1 (MRSA252)
ssl7 / set1 (AF188836)
ssl8 / set12
ssl9 / set5
ssl9 / set5 (MRSA252)
ssl10 / set4
ssl10 / set4 (MRSA252)
ssl11 / set2 (COL)
ssl11 / set2(Mu50/N315)
ssl11 / set2(MW2/MSSA476)
ssl11 / set2 (MRSA252)
setB3
setB3 (MRSA252)
setB2
setB2 (MRSA252)
setB1
setB1 (MRSA252)

9.11 Papers published during the PhD

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