School of Biomedical Sciences

The Australian Community Methicillin Resistant *Staphylococcus aureus* Endemic: Clonal Spread or Multiple Evolutionary Events

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DECLARATION

"To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university."

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ABSTRACT

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote indigenous communities living in the sparsely populated Kimberley region of Western Australia (WA). Between 1989 and 1995 five Panton Valentine leucocidin (PVL) negative clones were isolated from these communities: ST1-MRSA-IVa [2B] (WA-MRSA-1), ST78-MRSA-IVa [2B] (WA-MRSA-2), ST5-MRSA-IVa [2B] (WA-MRSA-3), ST45-MRSA-V [5C2] (WA-MRSA-4), and ST8-MRSA-IVa [2B] (WA-MRSA-5).

Between 1995 and 2003, *S. aureus* screening of the indigenous populations living in 11 of these remote communities showed the *S. aureus* population consisted of 13 multilocus sequence type clonal complexes (CCs) and two Singleton lineages. Although five lineages contained MRSA, the MRSA lineages were not the predominant methicillin-susceptible *S. aureus* (MSSA) lineages. There was greater diversity amongst the MSSA, while the MRSA appeared to have emerged clonally following acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) element. The emergence of CA-MRSA clones in different CCs indicates horizontal transmission of the SCC*mec* element into *S. aureus* had occurred on at least six occasions: SCC*mec* IVa [2B] into CC1 (ST1), CC5 (ST5), CC8 (ST8), CC45 (ST45), CC88 (ST78) and SCC*mec* V [5C2] into CC45 (ST45). Based upon the *spa* type and the DNA microarray profile six evolutionary events have subsequently occurred on at least three occasions from these clones (i.e. vertical transmission of the SCC*mec* element): twice from WA-MRSA-1, WA-MRSA-3, and WA-MRSA-5. Vertical transmission of the SCC*mec* element has not been identified for WA-MRSA-4 or WA-MRSA-2. The most prevalent MSSA lineage in the communities was the PVL-positive Singleton ST93 clone. As ST93-MRSA-IVa [2B], colloquially known as Queensland CA-MRSA, has become the most prevalent CA-MRSA in Australia, it was surprising in an environment of high β-lactam use and frequent horizontal transmission of SCC*mec* IVa a methicillin-resistant variant of ST93-MSSA was not found.

Within these indigenous communities people colonised with MSSA tended to harbour clones of a different genetic lineage at each anatomical site while people

colonised with MRSA tended to harbour clones of the same lineage at each site. Although the anterior nares is the preferred screening site for population studies, in this study many isolates of *S. aureus* would have been missed if throat and skin lesions had not also been swabbed. Three MRSA clones (WA-MRSA-1, WA-MRSA-2, and WA-MRSA-3) considered to be endemic in these communities have subsequently become predominant clones in the wider Australian community.

Although WA-MRSA-1, WA-MRSA-2, WA-MRSA-3 and Queensland CA-MRSA predominate, the CA-MRSA population in Australia is genetically diverse. In WA, between 2003 and 2010, 83 unique pulsed-field gel electrophoresis (PFGE) strains were described from which 46 multilocus sequence types have been characterised. Forty five of these sequence types (STs) were from 18 CCs and two Singletons. While SCC*mec* IV and V were the predominant SCC*mec* elements, SCC*mec* VIII and several novel and composite SCC*mec* elements were present. The emergence of MRSA in diverse *S. aureus* CCs suggest horizontal transmission of the SCC*mec* elements has occurred on multiple occasions. Furthermore, DNA microarray and *spa* typing suggest horizontal transfer of SCC*mec* elements has also occurred within the same CC. For many single and double locus variant CA-MRSA clones only a few isolates were detected. This suggests the successful evolution of a CA-MRSA clone may not only depend on the mobility of the SCC*mec* element but also on other genetic determinants.

As WA CA-MRSA, colloquially known as "WA-MRSA" are typically PVL negative many of the MRSA infections in WA have been superficial skin infections. However with the recent introduction of PVL-positive CA-MRSA more severe skin and soft tissues infections accompanied with a significant decrease in the age of patients have been observed.

In 2010, 22% of CA-MRSA isolated in WA were PVL positive, with Queensland CA-MRSA being the predominant PVL-positive clone. The emergence of Queensland CA-MRSA (ST93-MRSA-IVa [2B]) has been due to independent acquisitions of different *dru*-defined type IV and type V SCC*mec* elements in several *spa*-defined ST93-MSSA backgrounds. Rearrangement of the *spa* sequence in ST93-MRSA has subsequently occurred in some of these strains. Although multiple

ST93-MRSA strains were identified, PVL-positive ST93-MRSA-IVa [2B]-t202-dt10 was the predominant strain. Whether this strain arose from one PVL-positive ST93-MSSA-t202 or by independent acquisition of SCC*mec*-IVa [2B]-dt10 into multiple PVL-positive ST93-MSSA-t202 strains is yet to be determined.

Several international PVL-positive clones have been introduced into WA, including the CC59 strain ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA clone), and the CC8 strain ST8-MRSA-IV [2B] (USA300). Genetic analysis of these strains indicated they are distinct from WA CA-MRSA clones.

Although ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA clone) was found to be the most prevalent CC59 clone isolated in WA, independent evolution of PVL-negative CC59 CA-MRSA has occurred. Using a variety of molecular techniques, six distinct groups of CC59 were differentiated. Within these groups at least seven different variants of SCC*mec* elements were distinguished; (IVa [2B], IVb [2B], IVd [2B], IVa [2B]&5, IVv [2B], Vv [5C2], and V [5C2&5]. This suggests rapid evolution and/or multiple transfer events of SCC*mec* have occurred within this CC. Although some CC59 isolates in WA have overseas origins (eg Taiwan CA-MRSA clone and possibly USA1000), PVL-negative CC59 lineages unique to WA have acquired various SCC*mec* types on multiple occasions.

The PVL-positive ST8-MRSA-IV [2B] strain isolated in WA was found to be closely related to USA300, with most isolates unable to be distinguished from USA300-TC1516. Some isolates however varied in their carriage of resistance and virulence determinants and therefore USA300 in Australia cannot be regarded as being genetically homogeneous. Altogether 16 variants were identified. Notably some isolates did not harbour the ACME locus, which is intriguing because this locus is assumed to be involved in facilitating the spread of USA300 by skin contact.

In conclusion, this thesis has shown “WA-MRSA” arose in remote indigeneous communities located in WA, and three of these clones have subsequently become the most prevalent MRSA clones in Australia. However “WA-MRSA” did not arise from the predominant MSSA clones isolated from these remote communities. Although the vertical and horizontal transmission of SCC*mec* elements into *S. aureus*

has occurred on multiple occasions in the WA community only three “WA-MRSA” clones have found an ecological niche. These three PVL negative clones harbour few additional resistance and virulence genes which paradoxically may contribute to their success. PVL-positive CA-MRSA infections have become more prevalent in young Australians. Although primarily due to Queensland CA-MRSA, international PVL-positive CA-MRSA clones are present in Australia.

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LIST OF PEER REVIEWED PUBLICATIONS INCLUDED AS PART OF THE THESIS

PAPER ONE

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PAPER FIVE

The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia.

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STATEMENT OF CONTRIBUTION OF OTHERS

The nature and extent of the intellectual input Geoffrey Coombs (GWC) and the co-authors for the five publications included as part of this thesis are summarised.

Population dynamics of methicillin-susceptible and –resistant *Staphylococcus aureus* in remote communities

GWC designed the study, analysed and interpreted the data, and drafted the manuscipt
FGO designed the study, analysed and interpreted the data, and drafted the manuscipt
JWP critically revised the manuscript for important intellectual content
MG critically revised the manuscript for important intellectual content
FM critically revised the manuscript for important intellectual content
KJC critically revised the manuscript for important intellectual content
WBG designed the study and critically revised the manuscript for important intellectual content

All authors read and approved the final manuscript.

Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region

GWC designed the study, analysed and interpreted the data, and drafted the manuscript
SM assisted in the analysis and interpretation of data and critically revised the manuscript for important intellectual content
JCP, H-LT, Y-KC and LW carried out the laboratory procedures
RE critically revised the manuscript for important intellectual content
FGO assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content

KJC assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content

All authors read and approved the final manuscript.

The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* Strain

GWC designed the study, analysed and interpreted the data, and drafted the manuscript
RVG produced the minimum spanning trees and critically revised the manuscript for important intellectual content
KYC performed the quantification of LukF-PV expression experiments
SM critically revised the manuscript for important intellectual content
BPH critically revised the manuscript for important intellectual content
TPS critically revised the manuscript for important intellectual content
RE critically revised the manuscript for important intellectual content
FGO assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content
KJC assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content

All authors read and approved the final manuscript.

Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia

GWC designed the study, analysed and interpreted the data, and drafted the manuscript
SM assisted in the analysis and interpretation of data and critically revised the manuscript for important intellectual content
RE critically revised the manuscript for important intellectual content
PS critically revised the manuscript for important intellectual content
JCP and H-LT carried out the laboratory procedures

KJC assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content
FGO assisted in the design of the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content

All authors read and approved the final manuscript.

The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia

GWC designed the study, analysed and interpreted the data, and drafted the manuscript
SM assisted in the study design, analysis and interpretation of data and drafting the manuscript
RE critically revised the manuscript for important intellectual content
PS critically revised the manuscript for important intellectual content
H-LT carried out the laboratory procedures

All authors read and approved the final manuscript.

A signed statement of the nature and extent of the intellectual input by Geoffrey Coombs (GWC) has been signed by the co-authors. These statements form part of the appendix

Geoffrey W Coombs	Frances G O'Brien	Keryn J Christiansen
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1. INTRODUCTION and OVERVIEW

Staphylococcus aureus is one of the major bacterial pathogens of man, causing a variety of infections from mild skin and soft-tissue infections (SSTIs) to severe invasive infections with high mortality. The 1990s emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains causing community-onset infections represented a major change in the epidemiology of *S. aureus* with a variety of community-associated MRSA (CA-MRSA) with divergent genetic backgrounds having emerged globally.

Since 1982 colonisation or infection with MRSA has been a notifiable condition in Western Australia (WA). For infection control purposes all MRSA isolated in the state since 1997 have been referred to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (***ACCESS***) Typing and Research where based on molecular markers they are characterised as either healthcare-associated MRSA (HA-MRSA) or CA-MRSA.

Colloquially known as "WA-MRSA", CA-MRSA was first reported in WA in the early 1990s from indigenous people living in different communities in the Kimberley health region, a remote, sparsely populated country health region in the northern-most part of the state [1]. "WA-MRSA" has subsequently been identified throughout the state and now accounts for up to 14% of the state's community *S. aureus* infections (http://www.agargroup.org/files/FED%20REPORT%20SAP210%20MRSA%20FINAL%20shrink.pdf).

In 1983 the overall rate of MRSA notifications was 10/100,000 in the country health regions and 7/100,000 in the metropolitan health regions [2]. By 2010, the state's MRSA notification rate increased to 216/100,000 of which 181/100,000 were CA-MRSA. In the metropolitan health regions, the CA-MRSA notification rate was 154/100,000, whilst in the Kimberley health region the CA-MRSA notification rate has increased 80-fold to 840/100,000 (http://www.asainc.net.au/aasp/wamer)

CA-MRSA is thought to emerge when a locally prevalent strain of methicillin susceptible *S. aureus* (MSSA) acquires a staphylococcal cassette chromosome *mec* (SCC*mec*) element and utilizes mobile genetic elements and single nucleotide polymorphisms to establish local and geographic niches [3]. As WA is a remote region in which all MRSA isolates are referred to a central typing laboratory it is an ideal environment to determine if the Australian Community MRSA endemic is due to the spread of a small number of clones or has involved multiple evolutionary events.

The major objectives of this thesis were:

- To determine the colonisation dynamics and genetics of *S. aureus* in remote indigenous communities and thereby gain an insight into the emergence of "WA-MRSA".

- To determine the genetic relatedness of "WA-MRSA" clones within different multilocus sequence type clonal clusters providing an insight into the frequency of *S. aureus* SCC*mec* acquisition within a region.

- To determine if the increased prevalence of Panton Valentine leucocidin (PVL) positive CA-MRSA in Australia is due to the widespread transmission of a single clone or to multiple independent acquisitions of the SCC*mec* element by PVL positive local MSSA; or by the dissemination of international PVL positive MRSA.

2. LITERATURE REVIEW

2.1. History

Staphylococcus aureus is a ubiquitous bacterium, colonising 20-30% of humans [4], that has the genetic versatility to acquire multiple virulence and resistance genes. Beyond asymptomatic carriage, *S. aureus* causes a wide range of infections, such as skin and soft tissue infections (SSTIs), bone, joint and implant infections, pneumonia, septicaemia and various toxicoses such as toxic shock syndrome. It also occurs in many different species of animals, where it may cause comparable disease such as bovine mastitis.

Shortly after the introduction of penicillin in the 1940s, the first penicillinase-producing *S. aureus* strains were detected [5], leading to the development of the penicillinase-resistant semi-synthetic penicillins such as methicillin, oxacillin, and the first/second generation cephalosporins. Methicillin-resistant *S. aureus* (MRSA) was detected soon after the introduction of methicillin in the UK in 1960 [6] and in Australia in 1968 [7]; and isolation rates increased until the early 1970s [8]. These earlier nonmultiresistant MRSA strains (termed "classic MRSA") were genetically similar to each other and were thought to have evolved from a single clone [9].

Gentamicin-resistant MRSA was first noted in Australia in 1976 [10] and hospital outbreaks occurred in Victoria (Vic) in the late 1970s and early 1980s [10,11]. In 1985 it became evident that these "modern" strains of MRSA carried epidemic potential not possessed by MRSA isolated in the 1960s and early 1970s and that they were genetically different from the earlier "classic MRSA" [12]. Since 1990, international and intercontinental spread of MRSA (known as epidemic MRSA or EMRSA) has increased. In 2002, Enright *et al* using multilocus sequence typing (MLST) combined with staphylococcal cassette chromosome *mec* [also known as staphylococcal chromosome cassette] (SCC*mec*) typing, established that relatively few major EMRSA clones existed [13]. These clones emerged as either descendants of pre-existing EMRSA clones or by horizontal transfer of the SCC*mec* into methicillin-susceptible *S. aureus* (MSSA).

EMRSA became endemic in hospitals in eastern Australian states (New South Wales [NSW], Vic, and Queensland [Qld]) in the late 1980s and 1990s, with some spread to hospitals in South Australia (SA), the Northern Territory (NT) and Tasmania (Tas) [14,15,16]. A state-wide MRSA policy, introduced in 1982 following a hospital outbreak of EMRSA, prevented these strains from becoming established in hospitals located in Western Australia (WA).

In the early 1990s, non multidrug-resistant MRSA (nmMRSA) were observed in WA, initially from indigenous people living in remote communities [1] but subsequently in Perth, the state capital. These strains became known as "WA-MRSA". By 2006 nmMRSA from indigenous people living in remote areas outside of WA were reported in the NT [17], Qld [18] and central Australia [19]. In Qld, NSW, Vic and the Australian Capital Territory (ACT) an association between Polynesian ethnicity and the occurrence of community-acquired MRSA SSTIs was described in 1997 [20]. Isolates causing these infections were indistinguishable by phage typing and pulsed-field gel electrophoresis (PFGE) from those previously reported in New Zealand [21,22,23]. In 2003 community acquired nmMRSA infections were reported in Caucasians living in Qld [24].

Many different strains of community-acquired nmMRSA, also known as "community-acquired" or 'community-associated" MRSA (CA-MRSA) have been reported worldwide. This occurrence of concurrent epidemics of CA-MRSA in many countries due to multiple different clones has been striking [25] and represents a major change in the epidemiology of *S. aureus*. Some of these CA-MRSA strains harbour genes encoding the bi-component PVL toxin [26]. Although this toxin was identified in *S. aureus* as early as 1932, its presence in MRSA is a very recent phenomenon [27]. These strains are frequently associated with chronic/recurrent SSTIs as well as with life-threatening necrotising pneumonia [28], often in previously healthy young people. PVL-positive CA-MRSA have become a serious public health concern because of their virulence, their ability to cause outbreaks in households and close contact social groups, and their rapid spread in many countries.

To fully understand the epidemiology of CA-MRSA requires an understanding of the genetics of MRSA and the molecular epidemiological tools that are available to characterise MRSA strains.

2.2. MRSA Genotyping

Methicillin resistance in staphylococci is due to a modified penicillin binding protein (PBP2' or PBP2a) encoded by the *mecA* gene [29,30]. Apart from ceftobiprole [30] and ceftaroline, the presence of PBP2a confers resistance to all β-lactam antibiotics including the semi-synthetic β-lactamase resistant penicillins, such as methicillin and oxacillin. As methicillin and oxacillin can be used as indicators of resistance, PBP2a- or *mecA*-positive *S. aureus* are referred to as either MRSA or oxacillin-resistant *S. aureus* (ORSA).

2.2.1. SCC*mec* Element

The *mecA* gene is located on a complex mobile genetic element known as SCC*mec* [31,32]. In addition to *mecA*, SCC*mec* elements are comprised of unique site-specific recombinases designated as cassette chromosome recombinase (*ccr*) genes, regulatory elements, and variably additional genes encoding resistance to other antimicrobials, such as aminoglycosides or macrolides, and to heavy metals, and virulence determinants such as the *pls* gene and phenol sobulin modulins [33,34].

The emergence of methicillin-resistant staphylococcal lineages is due to the acquisition and insertion of the SCC*mec* element into the chromosome of susceptible strains. SCC*mec* elements are highly diverse in their structural organization and genetic content and have been classified into types and subtypes [35].

SCC*mec* elements share a number of characteristics:

- Carriage of *mecA* in a *mec* gene complex
- Carriage of *ccr* gene(s) (*ccrAB* or *ccrC*) in a *ccr* gene complex
- Integration at a specific site in the staphylococcal chromosome, referred to as the integration site sequence (ISS) for SCC, which serves as a target for *ccr*-mediated recombination

- The presence of flanking direct repeat sequences containing the ISS
- J, or joining regions

SCC*mec* types are defined by the combination of the class of the *mec* gene complex and the type of *ccr* gene complex, which is represented by *ccr* gene allotype, and subtyped by the content of the J regions (http://www.scc*mec*.org).

2.2.2. *mec* Gene Complex

The *mec* gene complex is composed of *mecA*, its regulatory genes, *mecR1* and *mecI*, and associated insertion sequences.

The class A *mec* gene complex (class A *mec*) is the prototype complex, which contains *mecA*, the complete *mecR1* (encoding the signal transducer protein MecR1) and *mecI* (encoding the repressor protein MecI) regulatory genes upstream of *mecA*, and the hypervariable region (HVR) and insertion sequence IS*431* downstream of *mecA*.

The class B *mec* gene complex (class B *mec*) is composed of *mecA*, a deleted *mecI*, and truncated *mecR1* resulting from the insertion of IS*1272* upstream of *mecA* and HVR and IS*431* downstream of *mecA*.

The class C *mec* gene complex (class C *mec*) contains *mecA*, a deleted *mecI*, and truncated *mecR1* by the insertion of IS*431* upstream of *mecA*, and HVR and IS*431* downstream of *mecA*. There are two distinct class C *mec* gene complexes; in the class C1 *mec* gene complex, the IS*431* upstream of *mecA* has the same orientation as the IS*431* downstream of *mecA* (next to HVR), while in the class C2 *mec* gene complex, the orientation of IS*431* upstream of *mecA* is reversed. C1 and C2 are regarded as different *mec* gene complexes since they have likely evolved independently.

The class E *mec* gene complex (class E *mec*) contains a highly divergent *blaZ-mecA-mecR1-mecI*. The $mecA_{LGA251}$ gene shares only 70% nucleotide homology with *mecA* [36].

2.2.3. *ccr* Gene Complex

The *ccr* gene complex is composed of the *ccr* gene(s) and surrounding open reading frames (ORFs). Currently three phylogenetically distinct *ccr* genes, *ccrA*, *ccrB* and *ccrC*, have been identified in *S. aureus* with DNA sequence similarities below 50%. In general *ccr* genes with nucleotide identities of more than 85% are assigned to the same allotype, whereas, *ccr* genes that belong to different allotypes have lower nucleotide identities of between 60% and 82% of each other. *ccrA* and *ccrB* have been found to be encoded together. Six *ccrA/ccrB* gene allotypes have so far been classified in *S. aureus* (Table 1).

Table 1: Currently identified SCC*mec* types in *S. aureus*

SCC*mec* Type	*ccr* gene complex	*mec* gene complex
I [1B]	1 (A1B1)	B (IS*431*-*mecA*-Δ*mecR1*-IS*1272*)
II [2A]	2 (A2B2)	A (IS*431*-*mecA*-*mecR1*-*mecI*)
III [3A]	3 (A3B3)	A (IS*431*-*mecA*-*mecR1*-*mecI*)
IV [2B]	2 (A2B2)	B (IS*431*-*mecA*-Δ*mecR1*-IS*1272*)
V [5C2]	5 (C1)	C2 (IS*431*-*mecA*-Δ*mecR1*-IS*431*)
		two IS*431*s arranged opposite orientations
VI [4B]	4 (A4B4)	B (IS*431*-*mecA*-Δ*mecR1*-IS*1272*)
VII [5C1]	5 (C1)	C1 (IS*431*-*mecA*-Δ*mecR1*-IS*431*)
		two IS*431*s arranged same orientation
VIII [4A]	4 (A4B4)	A (IS*431*-*mecA*-*mecR1*-*mecI*)
IX [1C2]	1 (A1B1)	C2 (IS*431*-*mecA*-Δ*mecR1*-IS*431*)
		two IS*431*s arranged opposite orientations
X [7C1]	7 (A1B6)	C1 (IS*431*-*mecA*-Δ*mecR1*-IS*431*)
		two IS*431*s arranged in the same direction
XI [8E]	8 (A1B3)	E (*blaZ*-$mecA_{LGA251}$-$mecR1_{LGA251}$-$mecI_{LGA251}$)

All *ccrC* variants identified to date in staphylococci have >86% similarity, and therefore there is only one *ccrC* allotype called *ccrC1*. *ccrC1* genes with sequence

differences of 87% or greater have been given allele numbers. For example, *ccrC1* allele 2 and *ccrC1* allele 8 for the two *ccrC1* genes found in the Tawian clone composite SCC*mec* Type V (5C2&5).

2.2.4. J Regions

Besides the *mec* and *ccr* gene complexes the SCC*mec* element contains three J or joining regions, which constitute nonessential components of the cassette. J1 is the region between the right chromosomal junction and the *ccr* gene complex; J2 is between the *ccr* gene complex and the *mec* gene complex; and J3 is between the *mec* gene complex and the left chromosomal junction. Variations in the J regions within the same *mec*-*ccr* gene complex are used for defining SCC*mec* subtypes.

2.2.5. SCC*mec* Nomenclature

To date eleven SCC*mec* types have been described for *S. aureus* (Table 1 and Figures 1a, 1b and 1c).

The first three SCC*mec* elements were designated as types I, II, and III [32,33]. These were followed by reports of SCC*mec* types IV to VIII [37,38,39,40,41]. Since 2011 three additional SCC*mec* element types have been described in *S. aureus* including IX and X [42] and XI [36,43].

In 2009 the International Working Group on the Classification of SCC*mec* recommended a standardised and more informative system for naming SCC*me*c and SCC elements [35]. The structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an uppercase letter respectively in parenthesis. For example type IVa [2B] SCC*mec* indicates a SCC*mec* harbouring a type 2 *ccr* and a class B *mec* gene complex with a subtype "a" J region structure. Recently SCC*mec* elements carrying two *ccr* gene complexes have been identified. For example, the SCC*mec* carried by *S. aureus* ZH47 is composed of a *ccrC* and SCC*mec* with a class B2 *mec* gene complex (a subclass of class B *mec* gene complex into which a transposon Tn*4001* has been integrated), a type 2 *ccr* gene complex and a J1 region

with homology to type IVc SCC*mec* [44]. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type. For example, as per Figure 1, *S. aureus* ZH47 SCC*mec* element is type IV (2B&5).

For the purpose of this thesis and with an understanding of the limitations of SCC*mec* typing by PCR only, when there is an extra *ccr* element present and the precise location is unknown, it is indicated by an "&" and *ccr* number outside the parentheses.

Three methods have been used to describe subtypes of SCC*mec* elements:

(i) expressing the J1 region differences as lower case letters, e.g., IVa, IVb, and IVc;

(ii) expressing the differences due to the presence or absence of mobile genetic elements as capital letters, e.g., IA, IIA, and IVA;

(iii) expressing the differences in each J1, J2, and J3 region in Arabic numbers, which are given in the order of discovery, e.g., II.1.1.1, II.1.1.2, and II.2.1.1.

In this thesis SCC*mec* subtypes have been expressed using lower case letters, eg IVa.

SCC*mec* types I [1B], II [2A] and III [3A] are typically restricted to MRSA strains associated with healthcare infections and are not found widely among the healthy population [45]. These strains, which were known in the 1990s as EMRSA, are now known as "hospital-acquired" or "healthcare-associated MRSA" (HA-MRSA). The presence of their SCC*mec* elements correlates with a relatively slower growth rate and it has been assumed that the mobile element may confer a selective disadvantage in the absence of antibiotics [46,47]. Consequently, strains carrying these elements may be less fit to survive in a competitive environment with faster growing wild type strains once antibiotic therapy is discontinued.

Strains carrying SCC*mec* types IV [2B] and V [5C2] predominantly evolved outside of healthcare settings or proved capable of spreading outside of hospitals, infecting not only patients but also colonising healthy contact persons.

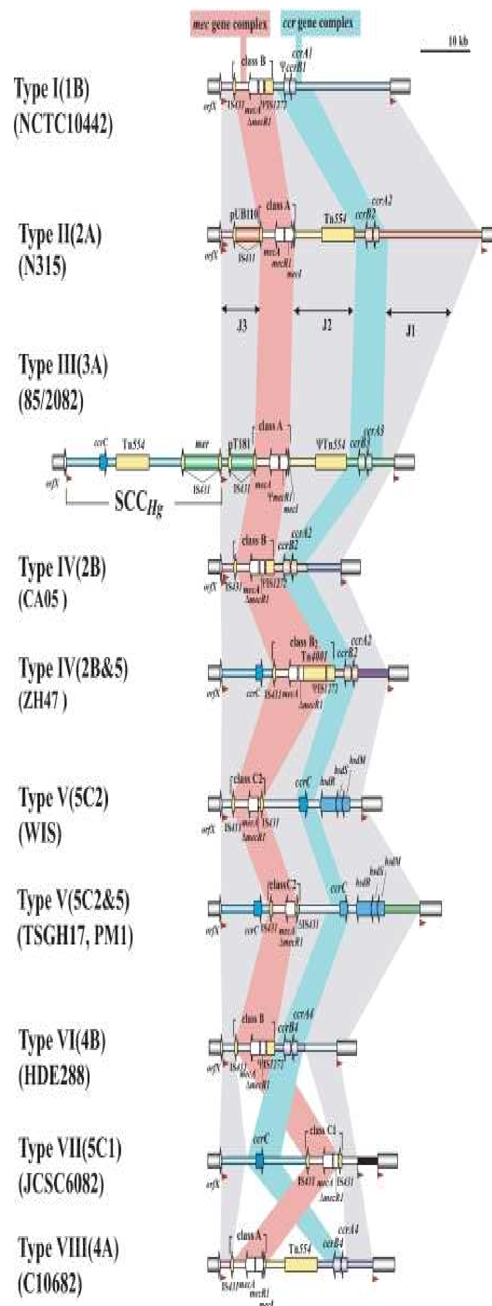

Figure 1a: Basic structures of representative SCC*mec* elements I – VIII.

The structures of SCC*mec* elements of representative strains are illustrated based on the following nucleotide sequences deposited in databases: NCTC10442 (AB033763), N315 (D86934), 85/202 (AB037671), CA05 (AB063172), ZH47 (AM292304), WIS (AB121219), TSGH17 (AB5122767), PM1 (AB462393), HDE288 (AF411935), JCSC6082 (AB373032), and C10684 (FJ390057) [35]. (Reproduced courtesy of American Society for Microbiology)

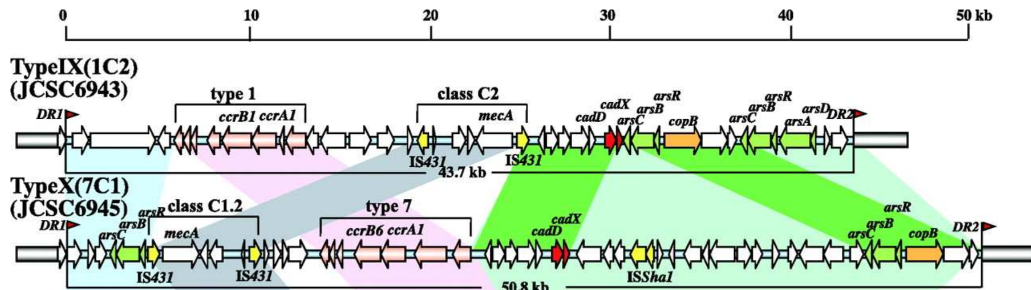

Figure 1b: Basic structures of representative SCC*mec* elements IX and X from MRSA CC398 strains JCSC6943 and JCSC6945 respectively [42].

(Reproduced courtesy of American Society for Microbiology)

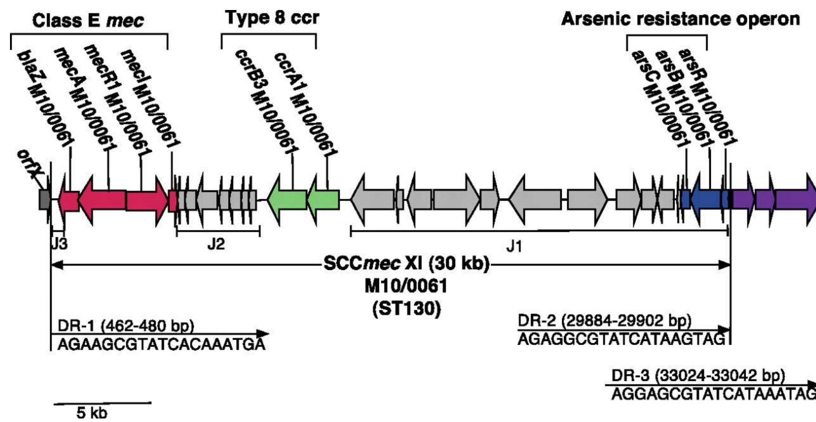

Figure 1c: Schematic diagram showing the genetic organisation of the SCC*mec* element designated SCC*mec* XI in the ST130 MRSA isolate M10/0061 (GenBank accession number FR823292) [43] (Reproduced courtesy of American Society for Microbiology)

Since 2003, some notable MRSA strains carrying SCC*mec* IV, V, IX or X have spread among livestock revealing the zoonotic potential of *S aureus*/MRSA. These strains have been dubbed "livestock-associated MRSA" (LA-MRSA) [42,48].

2.3. MRSA Nomenclature

2.3.1. Multilocus Sequence Typing

Different criteria and different methods have been applied to define and name MRSA strains. As a result the same MRSA strain may be known by several names. For example the Rhine-Hesse Epidemic strain in Germany, EMRSA-3 in the United Kingdom, USA100 in the USA, CMRSA-2 in Canada, AR07.3, AE07.4 or AE11 in Ireland, and New York-Japan MRSA in Australia and Asia are all the same clone [45]. For this reason in Tokyo in 2002, a subcommittee of the International Union of Microbiology Societies accepted the Enright *et al* [13] proposal that MRSA clones be named according to their multilocus sequence type and their SCC*mec* type in the form ST-resistance phenotype (i.e. MRSA and MSSA)-SCC*mec* type (e.g. I, II, III). It was anticipated that this nomenclature would "*replace or at least supplement existing arbitrary designations of MRSA clones, based on geographical location or other less satisfactory typing methods, since multilocus sequence typing (MLST) is systematic and objective, and provides a key for investigators to search for clones in the MLST website databases*" [49]. MLST/SCC*mec* typing is now widely regarded as the reference method for defining MRSA clones.

MLST, which is based on the principles of multilocus enzyme electrophoresis [50], is an unambiguous procedure for characterising isolates of bacterial species using the sequences of approximately 450 – 500 bp internal fragments of seven house-keeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*). Using the MLST website (http://www.mlst.net), different sequences for each house-keeping gene are assigned as distinct alleles, and for each isolate, the alleles at each of the seven loci define the allelic profile or sequence type (ST) [51]. The number of nucleotide differences between alleles is ignored and sequences are given different allele numbers whether they differ at a single nucleotide site or at many sites. The rationale is that a single

genetic event resulting in a new allele can occur by a point mutation (altering only a single nucleotide site), or by a recombinational replacement (that will often change multiple sites) - weighting according to the number of nucleotide differences between alleles would imply that the latter allele was more distantly-related to the original allele than the former, which would be true if all nucleotide changes occurred by mutation, but not if the changes occurred by a recombinational replacement.

The *S. aureus* MLST website currently contains >2,000 isolates from humans and animals from multiple countries.

The "clock speed" or secular rate of change in the coding regions used in MLST is relatively slow, as befits a system designed to analyse the population structure of entire bacterial species. Consequently compared to other molecular methods (e.g. pulsed-field gel electrophoresis), MLST has a lower discriminatory power.

2.3.2 Defining a MRSA Clone

A strain is defined as "*an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic characteristics or genotypic characteristics or both*" [52,53], which is essentially the same as a clone which is defined as "*a group of isolates that are distinguishable from each other by a variety of genetic tests*" [52,53]. Thus the definition of a clone or strain depends on the discriminatory power of the test and/or the number of different tests applied.

Although defining *S. aureus* clones as the ST-resistance phenotype-SCC*mec* type has allowed investigators to unambiguously identify *S. aureus* clones, this nomenclature has shortcomings if clearly different strains have the same ST and SCC*mec* type (such as the ST8-MRSA-IV [2B] strains USA300 and WA-MRSA-5). Additional information such as PVL status or carriage of superantigens may be of relevance in defining a clone or strain. For example the presence or absence of *lukS-PV/lukF-PV*

PVL encoding genes differentiates the PVL-positive ST8-MRSA-IV [2B] (USA300) and PVL-negative ST8-MRSA-IV [2B] (WA-MRSA-5).

Although all previously defined *S. aureus* strains, based upon their variable genes, could be subdivided into a considerable number of variants, it is not practical to regard all variants as different strains and apply different names; for example the variants of ST22-MRSA-IV [2B] which just differ in carriage of *erm*(*C*) or *sec+sel*.

When scrutinised by additional molecular methods, such as genome sequencing or microarray hybridisation, strains have been shown not to be static blocks comprised of identical isolates, but rather consist of isolates with similar sequences. For example ST5-MRSA-II [2A] is thought to have evolved from different ST5-MSSA strains acquiring the same SCC*mec* element independently on several occasions [54]. These sequences might differ in single point mutations (as sometimes obvious in MLST e.g. Taiwan CA-MRSA clone isolates may have different *gmk* alleles); in the composition and sequence of single loci (such as the variable region of the chromosomal *spa* gene or the *dru* region within the SCC*mec* element) or in the presence or absence of complete genes or multi-gene mobile elements. Thus the concept of the "quasispecies" could be applied in which the genome cannot be described as a defined structure, but rather as a weighted average of a large number of individual sequences [45].

In addition to MLST/SCC*mec* typing, methods used in this thesis to discriminate CA-MRSA clones were pulsed-field gel electrophoresis (PFGE), sequencing of the repeat region of the *S. aureus* protein A (*spa*) type and PVL type [55]. Further discrimination of strains was achieved by DNA microarray profiling and sequencing the repeat regions of the *dru* gene.

2.3.3. Clonal Complex

eBURST (Based Upon Related Sequence Types) is a novel clustering algorithm for analysing MLST data (http://eburst.mlst.net/1.asp). In MLST, *S. aureus* isolates with identical sequences for the seven house keeping loci are referred to as clonal. A clonal complex or cluster (CC) comprises genetically related isolates that differ at

only one or two loci (termed single [SLV] and double locus variants [DLV], respectively). The primary founder of a complex is defined as the ST that differs from the largest number of other STs at only a single locus (i.e. the ST that has the greatest number of SLVs). DLVs of the founder are only linked if the intermediate SLV on the path from the founder to the DLV is present.

Some STs may not share alleles at five out of seven loci with any other STs and are termed "Singleton" STs.

S. aureus has a highly clonal population structure, which is dominated by a dozen major CCs and comprises several hundred multilocus sequence types [51]. All major HA-MRSA clones can be grouped into five CCs: CC5, CC8, CC22, CC30 and CC45 (Table 2) [56]. Different SCC*mec* acquisition has on occasion resulted in MRSA from similar MSSA isolates e.g. ST5-MSSA has produced ST5-MRSA-I and ST5-MRSA-II pandemic clones. The "classic MRSA", ST250-MRSA-I, evolved from ST250-MSSA that arose from ST8-MSSA, a common cause of epidemic MSSA disease and itself a genotype containing at least two major MRSA clones (ST8-MRSA-II and ST8-MRSA-IV). ST8-MSSA is therefore the ancestral genotype of the first MRSA [13].

Unlike HA-MRSA, CA-MRSA has evolved from multiple genetic backgrounds [57]. In Australia, by 2008, 33 STs, from 14 CCs and 4 singletons had been identified [2]. In addition to CC5, CC8, CC30 and CC45, CA-MRSA evolved from CC1, CC9, CC59, CC72, CC88, CC121, CC188 and CC361. Although most STs had acquired SCC*mec* IV [2B], SCC*mec* V [5C2] and several novel SCC*mec* types were identified. Thus CA-MRSA represents novel combinations of SCC*mec* type and MSSA clone type.

2.4. Molecular Epidemiological Typing Techniques

In addition to MLST and SCC*mec* typing other molecular techniques were used in this thesis.

2.4.1. Pulsed-field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) is considered the method of choice for studying *S. aureus* epidemiology. It is highly discriminatory, and has been widely used for local outbreak investigation and as part of typing systems for long term surveillance of MRSA infections at regional and national levels [58,59]. The technique enables the resolution of large fragments of DNA that cannot be resolved by conventional gel electrophoresis [60].

Table 2: Details of pandemic HA-MRSA clones and their previous designations

CC	Clone	MST allelic profile	Previous name of MRSA clone
5	ST5-MRSA-I [1B]	1-4-1-4-12-1-10	UK EMRSA-3
5	ST5-MRSA-II [2A]	1-4-1-4-12-1-10	New York-Japan
5	ST228-MRSA-I [1B]	1-4-1-4-12-24-29	Southern German
8	ST8-MRSA-II [2A]	3-3-1-1-4-4-3	Irish-1
8	ST8-MRSA-IV [2B]	3-3-1-1-4-4-3	UK EMRSA-2, -6
8	ST239-MRSA-III [3A]	2-3-1-1-4-4-3	UK EMRSA-1, -4, -11, Portuguese, Brazilian, Viennese
8	ST247-MRSA-I [1B]	3-3-1-12-4-4-16	UK EMRSA-5, -17, Iberian
8	ST250-MRSA-I [1B]	3-3-1-1-4-4-16	Classic MRSA
22	ST22-MRSA-IV [2B]	7-6-1-5-8-8-6	UK EMRSA-15, Barnim
30	ST36-MRSA-II [2A]	2-2-2-2-3-3-2	UK EMRSA-16
45	ST45-MRSA-IV [2B]	10-14-8-6-10-3-2	Berlin

The procedure involves the application of controlled electric fields that change direction at a predetermined angle to samples of DNA that have been embedded in an agarose gel matrix and digested with a restriction endonuclease. By embedding bacterial cultures in an agarose block mechanical shearing of the high molecular mass chromosomal DNA is prevented. The restriction endonuclease selected generates 10 – 20 fragments, the sizes of which range between 10 and 700kb. For *S. aureus*, which has a low genomic GC content, the most frequently used restriction endonuclease is *Sma*1, which cleaves at the high GC recognition site of CCCGGG.

Due to advances in the standardisation of electrophoresis conditions [60,61,62] and the development of normalisation and analysis software [63] multicentre studies have become possible. Interpretive criteria for use in comparing complex PFGE patterns in outbreaks have been applied to non outbreak situations to track the national and international dissemination of *S. aureus* clones [53]. Although the use of PFGE typing with adjusted interpretation criteria for grouping patterns with <7 bands difference has been shown to correspond to MLST CCs [64], attribution of PFGE clusters to genetic lineages can be problematic [65].

2.4.2. *spa* Sequence Typing

Although PFGE is considered a gold standard for strain typing of MRSA, it is time-consuming and the inter-laboratory comparability of results requires extensive effort using harmonization of protocols [61]. DNA sequence-based approaches are becoming more frequently used because of the ease with which sequence data can be transferred between laboratories via the internet.

Epidemiologically useful bacterial strain typing has been achieved by analysing sequence polymorphisms in a single genetic locus. In *S. aureus* HVR consisting of 24bp repeats within the 3' coding region of the *spa* gene (Protein A) is particularly effective for typing MRSA from healthcare institutions [66,67]. The repeat region of the *spa* gene is subject to spontaneous mutations as well as the loss and gain of repeats. By sequencing this polymorphic repeat region, each new repeat variant determined can be assigned a unique repeat code. The repeat succession for a given strain, determines the strain's *spa* type.

spa sequence typing has become one of the primary typing methods for regional and national MRSA surveillance schemes [65]. Its popularity is attributable to its many practical advantages, including absolute reproducibility which allows internet-based type assignment and comparison with a worldwide database [68,69]. However, *spa*-typing has been hampered in the past by the lack of consensus on assignments of new *spa*-repeats and -types. To ensure uniform code terminology usage, repeats and *spa* types are now determined by online software that allows rapid repeat determination,

data management and retrieval. There are two websites to facilitate standardisation of *spa* nomenclature; http://tools.egenomics.com/ and http://ridom.de/staphtype.

In Europe nearly all national reference centres (e.g. Robert Koch Institute, Germany, Health Protection Agency, UK, and the National Institute for Public Health and the Environment, The Netherlands) are Ridom StaphType users. The Ridom SpaServer can be used to collate and harmonize data from various geographic regions. To simplify *spa*-type nomenclature, a numerical repeat code is used, where repeats are assigned a numerical code and the *spa*-type is deduced from the order of specific repeats (Figure 2). This approach was chosen despite the currently existing different alpha-numerical nomenclatures, because numerical codes are now widely used for MLST. An online conversion of the old terminology into the new one is possible.

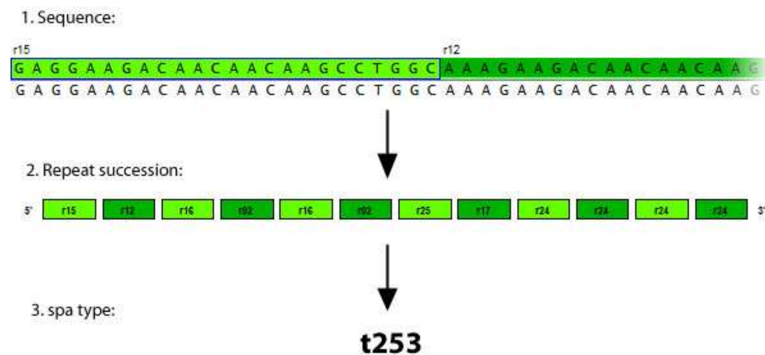

Figure 2: Generation of *spa* type using the numerical code established by Ridom Staphtype (http://ridpm.de/staphtype)

Although PFGE provides better typeability and discriminatory power [70] [71], s*pa* typing has a very good predictive power over the clonal relationships defined by eBURST [70], and is valuable for the tracking of epidemic isolates [72]. The technique has limitations in that some genetic ST lineages (e.g. ST1, ST8, and ST80) are not able to be reliably inferred. In these cases, the use of PCR for the analysis of additional markers, such as toxin and antibiotic resistance genes located on clone-specific mobile genetic elements is needed for correct ST delineation and assignment [69,73]. Repeat-based *spa* locus polymorphism is also subject to misclassification bias, owing to both horizontal DNA transfer and recombinations (e.g. ST239) and homoplasy (e.g. in ST5 subclones) [54].

2.4.3. DNA Microarray-based Typing

DNA microarray-based typing methods allow the detection of clone specific single-nucleotide polymorphisms (SNPs), genes, or genomic islands. Studies using "genome chips" have shown that insertion and deletion of entire genes is a major source of genetic variation among isolates, with as much as 22% of the *S. aureus* genome being "dispensable" [74]. Consequently there are literally hundreds of strain specific genes that may be present in or absent from individual isolates and therefore may provide a uniquely discriminatory typing system.

In this thesis the Alere StaphType DNA microarray was employed using protocols and procedures previously described in detail [75,76]. The DNA microarray covers 334 target sequences (170 distinct genes and their allelic variants) including species markers, SCC*mec*, capsule and *agr* group typing markers, resistance genes, exotoxins, genes encoding microbial surface components recognising adhesive matrix molecules of the host (MSCRAMM) and immune evasion genes carried on beta haemolytic converting phages.

Results are regarded as negative if the normalised intensity for a given probe is below 25% of the median value of species markers (*coa*, *eno*, *fnbA*, *gapA*, *katA*, *nuc*, *rrn*, *sarA*, *sbi*, *spa*, *vraS*) and a biotin staining control. If the normalised intensity of a given probe is higher than 50% of this breakpoint, it is interpreted as positive. If it is between 25% and 50% the result is regarded as ambiguous. For some markers, for which allelic variants are to be discriminated (*bbp*, *clfA*, *clfB* and *fnbB* as well as some *set*/*ssl* genes, *isaB*, *mprF* and *isdA*) a different approach is used because these alleles differ only in single nucleotides. For these markers only the probe with the strongest signal value is regarded as positive, provided that it exceeds the 50% breakpoint. All others are regarded as ambiguous, or if below the 25% breakpoint, as negative.

2.4.4. *dru* Typing

In MRSA the direct-repeat unit (*dru*) HVR region adjacent to IS*431* in SCC*mec* has proved useful in the epidemiological analysis of highly uniform epidemic strains

such as ST22-MRSA-IV [77], ST36-MRSA-II [77,78], ST239-EMRSA-III [79], and in the tracking of the horizontal movement of SCC*mec*. In 2008 Goering *et al* proposed a uniform system of nomenclature [77] (Figure 3). In a manner similar to that applied for staphylococcal protein A gene (*spa*) typing [72], a prefix (dr; *dru* repeat) is used, combined with numbers to identify specific 40-bp repeat sequences, whereas a different prefix (dt; *dru* type), combined with numbers is used to identify repeat combinations. An additional alphabetical designation is used to indicate different locations of change (for example dr2a and dr2b both differ from the consensus by two nucleotides, but at different positions within the sequence. An additional alphabetical designation is also used in the *dru* type to indicate different tandem arrangements of specific repeats; for example dt8a and dt8b both contain the same eight *dru* repeats but in different arrangements

The www.dru-typing.org website allows user generated 40-bp repeat sequences to be searched against the current database of *dru* repeats (dr) and identified, if known. Specific combinations of repeats can be queried against the database and if recognized, the resulting *dru* type (dt) will be identified. New *dru* repeat and/or *dru* type chromatograms can be submitted online for verification and inclusion into the database.

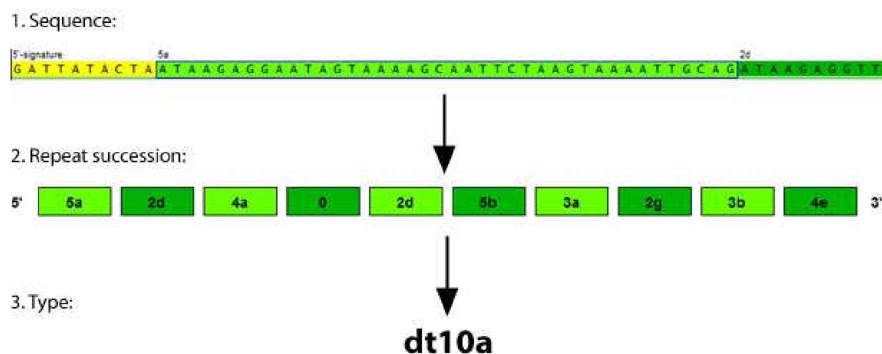

Figure 3: Generation of *dru* type (dt) using the nomenclature system proposed by *Goering et al* [77] and the BioNumerics polymorphic HVR sequencing typing plug in.

When compared to PFGE, MLST and *spa* and SCC*mec* typing, the discriminatory power of *dru* typing in the epidemiological analysis of MRSA has been shown to be similar to PFGE (Simpson's index of diversity values over 89%) [79] and may be

particularly useful in the epidemiological analysis of highly clonal MRSA strains. Although the stability of specific *dru* types over time has not been established, an investigation on EMRSA-16 isolates from the HARMONY collection suggests the *dru* sequences are sufficiently stable to have strain-associated significance [77].

2.4.5. Panton Valentine Leucocidin

The worldwide emergence of CA-MRSA strains has been linked to the carriage of the genes encoding the bi-component Panton-Valentine leucocidin (PVL) toxin.

PVL is a well characterised virulence factor of *S. aureus*, and is composed of two distinct protein components which together form heptameric pores in leucocytes. Although PVL-positive strains have been associated with a variety of diseases including chronic and recurrent SSTIs [25,28,80,81,82], necrotising pneumonia, [83,84,85,86,87] necrotising fasciitis [88], purpura fulminans [89] and Waterhouse-Friderichsen syndrome [90], the contribution PVL has to CA-MRSA pathogenesis remains controversial.

PVL was initially described in 1932. During the 1950s/1960s the pandemic spread of a PVL-positive β-lactamase producing MSSA strain (phage type 80/81) occurred, but receded after the introduction of penicillinase-resistant β-lactam agents such as oxacillin [91]. PVL-positive *S. aureus* strains carrying SCC*mec*-IV [2B] were identified in the 1990s [92], and by 2003 global spread of these strains had been reported [25].

Since PVL is bacteriophage encoded, PVL-positive MRSA clones are found in many CCs and STs including CC1, CC5, CC8, CC22, CC30, CC59, ST72, CC80, CC88, ST45, ST93, ST154, ST398 and ST772 [45]. The PVL genes have been found to have allelic sequence single nucleotide polymorphisms (SNPs) and to be carried on different bacteriophages that appear to have specificities for particular lineages of *S. aureus* [93].

The epidemiology of PVL-positive CA-MRSA can be distinguished by three crude observations. First, in many European countries (e.g. Germany [76,94], United

Kingdom [95], Malta [96,97,98] and Ireland [97,98]) the prevalence of PVL-positive MRSA is low and has remained low for many years. In these countries a variety of different strains have been identified and individual cases can often be traced to travel histories or to foreign patients. Secondly, in Australia PVL-positive MRSA have recently become common and a number of different strains co-exist [2]. As Australia has had a recent immigration of people from all over the world, it can be assumed that these people have introduced epidemic strains from their respective home countries, for example ST30-MRSA-IV [2B] from New Zealand and ST772-MRSA-V [5C2] from India. Thirdly, the extensive spread of a single strain effectively marginalises all other strains. This has occurred in the USA with ST8-MRSA-IV [2B] (USA300) [99], and similarly in Taiwan where most infections are caused by ST59-MRSA-V [5C2&5] [100,101]. A comparable picture may evolve in Australia due to a significant increase in ST93-MRSA-IV [2B] infections [102].

2.5. Community MRSA

Based on phenotypic and genotypic typing methods, community onset MRSA infections are caused by HA-MRSA, which appear to have been transferred from hospitals or healthcare facilities into the community by patients or healthcare workers [103], or by CA-MRSA which have been isolated from people who have had little or no contact with healthcare facilities or healthcare workers [104]. This distinction between community and healthcare facility associated MRSA however has become blurred with the replacement of HA-MRSA with CA-MRSA in hospitals [105,106]. For these reasons differentiating CA-MRSA from HA-MRSA strains by only using epidemiological criteria (onset of infection within 48 hours of hospitalisation) is neither sensitive nor specific.

In contrast to HA-MRSA, CA-MRSA are generally more susceptible to non beta-lactam antibiotics, grow significantly faster, have different clonal backgrounds, carry smaller SCC*mec* elements, have enhanced virulence properties and frequently harbour genes expressing PVL [25,57,107,108]. Rather than a worldwide spread of a single clone, multiple CA-MRSA clones have emerged from diverse genetic backgrounds. Several well characterised CA-MRSA clones predominate in different regions: ST8-MRSA-IV [2B] (USA300) and ST1-MRSA-IV [2B] (USA400) in

North America [109,110]; ST80-MRSA-IV [2B] (European clone) in Europe [25] , North Africa [111], and the middle east [112]; ST59-MRSA-V [5C2&5] (Taiwan CA-MRSA clone) in Taiwan [113]; ST93-MRSA-IV [2B] (Queensland clone) in Australia [58] ; ST30-MRSA-IV [2B] (South West Pacific [SWP] clone) in the Western Pacific [20,21] ; and ST772-MRSA-V [5C2] (Bengal Bay clone) in India and Bangladesh [95]. Transmission of these clones into other regions has occurred [2,114]. Prominent features of these clones have been their ability to cause severe infections in young otherwise healthy people and the carriage of the *lukS-PV/lukF-PV* PVL encoding genes.

It has been suggested two potential markers for defining CA-MRSA are the carriage of the PVL encoding genes and SCC*mec* IV [2B] [25]. However although PVL genes are present in the majority of CA-MRSA reported in the literature, the majority of CA-MRSA strains in Australia lack *lukS-PV/lukF-PV*. Furthermore SCC*mec* V has been well described in CA-MRSA isolates [57,107,115] and SCC*mec* IV [2B] in some HA-MRSA isolates [59,116,117].

Consequently the most sensitive and specific process for differentiating CA-MRSA from HA-MRSA is using molecular typing complemented with patient epidemiology [118].

2.5.1. Community MRSA in Western Australia

2.5.1.1. "Kimberley MRSA"

Although MRSA infections acquired in the community were first reported in the USA in 1982 [119], these patients had predisposing healthcare-associated risk factors for infection such as previous hospital admission or intravenous drug abuse.

The first genuine cases of CA-MRSA infection were reported in 1993 in infected indigenous people from remote communities in the sparsely populated Kimberley region of WA [1]. Approximately one-half of the people living in the Kimberley regions are indigenous, many of whom live in poor socioeconomic conditions. Infected skin lesions and staphylococcal sepsis occur frequently in this population

and antistaphylococcal therapy is often prescribed. Like the “classic MRSA” reported in the 1960s, “Kimberley MRSA” were nonmultiresistant, however while the “Kimberley MRSA” isolates were streptomycin and erythromycin susceptible they harboured a tetracycline resistance plasmid. In addition their PFGE patterns differed from the “classic MRSA” and the epidemic healthcare associated “EA-MRSA” clone found in hospitals on the east coast of Australia. Subsequent MLST/SCC*mec* have characterised “Kimberley MRSA” as ST8-MRSA-IV [2B], and the “classic MRSA” and “EA-MRSA” clones as ST250-MRSA-I [1B] and ST239-MRSA-III [3A] respectively. However using eburst analysis all three clones belong to CC8, suggesting they have evolved from the same *S. aureus* lineage.

During the 1990s, CA-MRSA in WA, subsequently termed “WA-MRSA”, were isolated in most regions of the state [120,121], and a substantial number of cases of infection and colonisation occurred in metropolitan Perth by 1997 [122].

Although CA-MRSA isolated in WA were typically nonmultiresistant, all strains analysed harboured a large β-lactamase plasmid and cadmium resistance plasmid that could vary in size and *Eco*R1 restriction fragment length patterns [123]. In addition some isolates were reported to carry a 41.4 kb plasmid that encoded β-lactamase and resistance to mupirocin, tetracycline, trimethoprim and cadmium, and a smaller plasmid (2 kb) that encoded erythromycin resistance. Chromosomal fusidic acid and tetracycline resistance determinants were also reported [115,123].

2.5.1.2. Notification of MRSA

Following the successful control of an “EA-MRSA” hospital outbreak [124], state legislation was passed in 1982 requiring notification of all MRSA isolates in WA. The referral of these isolates to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (***ACCESS***) Typing and Research Unit allowed unparalleled epidemiological study of CA-MRSA within a defined geographical region. Since 1982 all patients or healthcare workers who are colonised or infected with MRSA are notified to the Western Australia Department of Health and included in an electronic microbiology alert system [125].

2.5.1.3. 1980 - 2002

In the 1980s, the proportion of *S. aureus* that was MRSA varied from 10 to 30% in states other than WA, while WA remained at 0.4% [126]. After a relatively low number of MRSA notifications in the 1980s, the number in WA increased significantly in the 1990s. This was due almost exclusively to community-associated "WA-MRSA" with notifications increasing from 14% in 1989 to 94% of total MRSA notifications in 1998 with the increase occurring mainly in rural areas. In 1983, the overall rate of notifications in the rural regions was 10/100,000 compared with the metropolitan area rate of 7/100,000 [120]. In 2002, notification rates in rural and metropolitan regions were 108 and 104 notifications per 100,000 persons respectively.

2.5.1.4. 2003 - 2004

A further study was conducted on isolates notified between July 2003 and December 2004 [59]. 4,099 MRSA were epidemiologically typed by ***ACCESS*** Typing and Research, of which 77.5% were characterised as CA-MRSA. Twenty two clones were identified by MLST/SCC*mec* typing. Most clones harboured type IV or type V SCC*mec* with three clones harbouring novel SCC*mec* elements. The clones were grouped into ten clonal complexes and two singletons, suggesting horizontal transfer of the SCC*mec* element had occurred on multiple occasions in the WA community. Although polyclonal, 96.5% of CA-MRSA consisted of five PVL negative clones; ST1-MRSA-IVa [2B] (WA-MRSA-1, 55.3%), ST78-MRSA-IVa [2B] (WA-MRSA-2, 29.8%), ST5-MRSA-IVa [2B] (WA-MRSA-3, 8.6%), ST45-MRSA-V [5C2] (WA MRSA-4, 1.9%) and ST8-MRSA-IVa [2B] (WA-MRSA-5, 0.9%). Many clones had acquired plasmids and chromosomal resistance determinants resulting in some isolates to be resistant to five non-β-lactam antimicrobial agents, including erythromycin, tetracycline, trimethoprim, ciprofloxacin, gentamicin, rifampin, fusidic acid, and mupirocin. Unlike CA-MRSA isolated outside WA, "WA-MRSA" did not harbour genes encoding the bi-component toxin PVL. However, five PVL positive CA-MRSA clones were identified in this study, including ST93-MRSA-IV [2B] (Qld CA-MRSA), ST8-MRSA-IV [2B] (USA300), ST59-MRSA-V [5C2&5]

(Taiwan CA-MRSA), ST80slv-MRSA-IV [2B] (European CA-MRSA) and ST30-MRSA-IV [2B] (SWP CA-MRSA).

2.5.1.5. 2009 - 2010

In 2009/2010, 83.8% of the 4,691 non duplicate MRSA isolates referred to ***ACCESS*** Typing and Research were characterised as CA-MRSA (http://www.asainc.net.au/aasp/wamer). Since 2003/2004 PVL-positive and PVL-negative CA-MRSA numbers in WA have increased significantly (P<0001) (Figure 4).

77.7% of CA-MRSA were PVL negative. Using MLST/SCC*mec* typing 34 clones (44 PFGE pulsotypes) consisting of 25 STs (12 CCs and one Singleton) were identified. For the 34 MLST/SCC*mec* clones, 21, 7 and 6 carried SCC*mec* IV [2B], SCC*mec* V [5C2] and novel SCC*mec* element types respectively. The emergence of MRSA in diverse clonal clusters with different SCC*mec* types once again suggested that horizontal transmission of SCC*mec* elements had occurred on multiple occasions. Furthermore, the results suggested that re-arrangement and/or novel generation of the the SCC*mec* was occurring. Although multiple PVL negative CA-MRSA clones were identified, more than 95% of isolates were characterised as ST1-MRSA-IV [2B] (WA-MRSA-1, 52%), ST78-MRSA-IV [2B] (WA-MRSA-2, 31%) and ST5-MRSA-IV [2B] (WA-MRSA-3, 12%). The original CA-MRSA identified in WA, ST8-MRSA-IV [2B] (WA-MRSA-5) accounted for <1% of PVL negative CA-MRSA, suggesting the acquisition of the SCC*mec* by this clone did not necessarily provide an ecological advantage.

22.3% of CA-MRSA harboured the *lukS-PV/lukF-PV* PVL encoding genes. Since 2003/2004 several non WA-MRSA PVL positive clones have increased significantly (P<0.0001); ST93-MRSA-IV [2B] (Qld CA-MRSA), ST8-MRSA-IV [2B] (USA300), ST59-MRSA-V [5C2&5] (Taiwan CA-MRSA), ST772-MRSA-V [5C2] (Bengal Bay CA-MRSA) and ST30-MRSA-IV [2B] (SWP CA-MRSA) (Figure 5). The Qld CA-MRSA clone increased from 0.7% of all MRSA in 2003/2004 to 13.3% in 2009/2010.

The highest prevalence of PVL positive infection and/or colonisation was in the Kimberley and Pilbara regions (287 and 115/100,000 population respectively). As mentioned previously, approximately 50% of people in these regions are indigenous, many of whom live in poor socioeconomic conditions. As many of the original CA-MRSA strains isolated in these regions were PVL negative many of the infections were superficial skin infections such as impetigo. However with the introduction of the PVL-positive Qld clone more severe skin and soft tissues infections have been observed (http://www.asainc.net.au/aasp/wamer), [24].

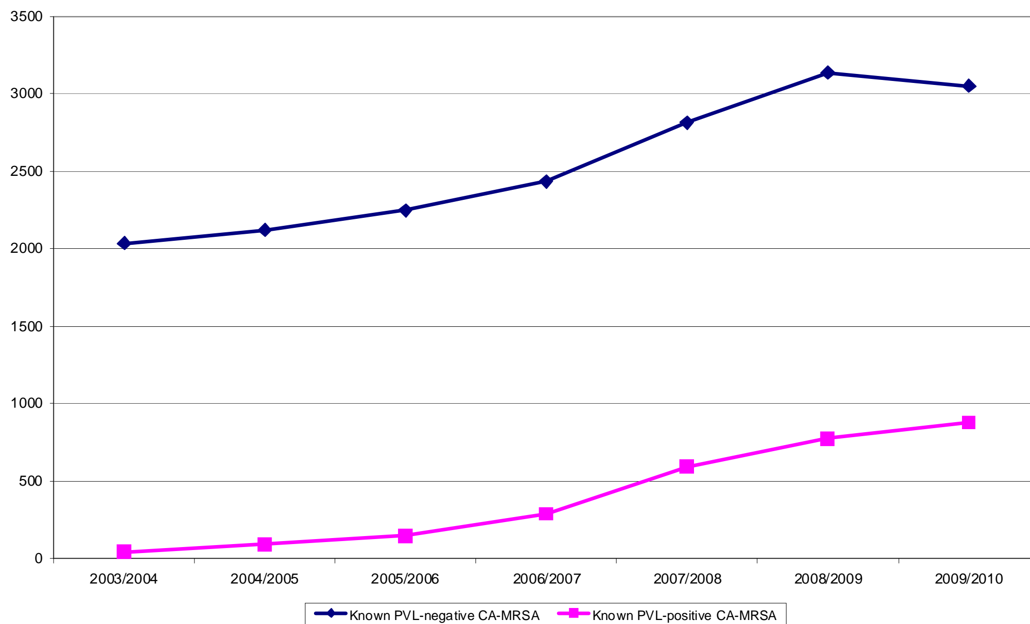

Figure 4: Number of known PVL-positive and PVL-negative CA-MRSA, Western Australia July 2003 to June 2010

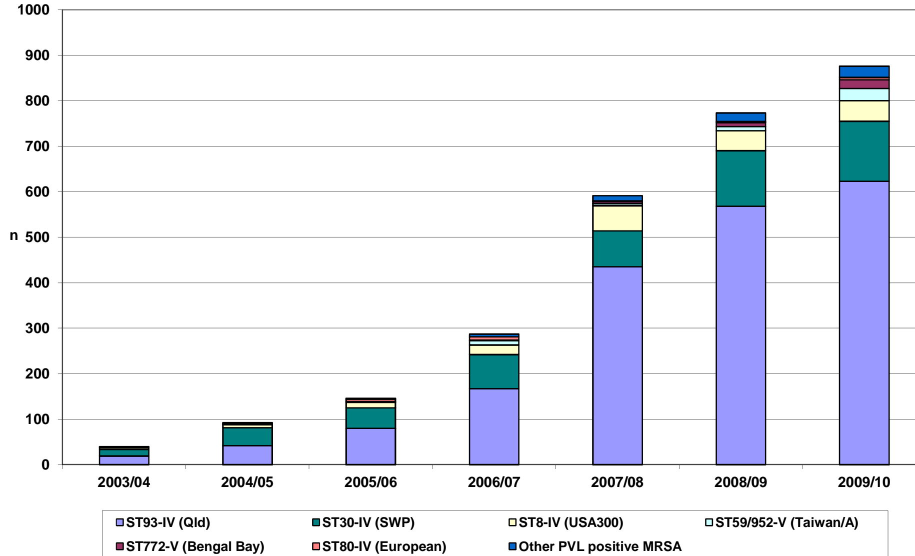

Figure 5: PVL positive clones isolated in WA, 2003/2004 to 2009/2010

This introduction of PVL positive clones has a caused a significant change in the age of patients infected/colonised with CA-MRSA in WA (Figures 6 and 7).

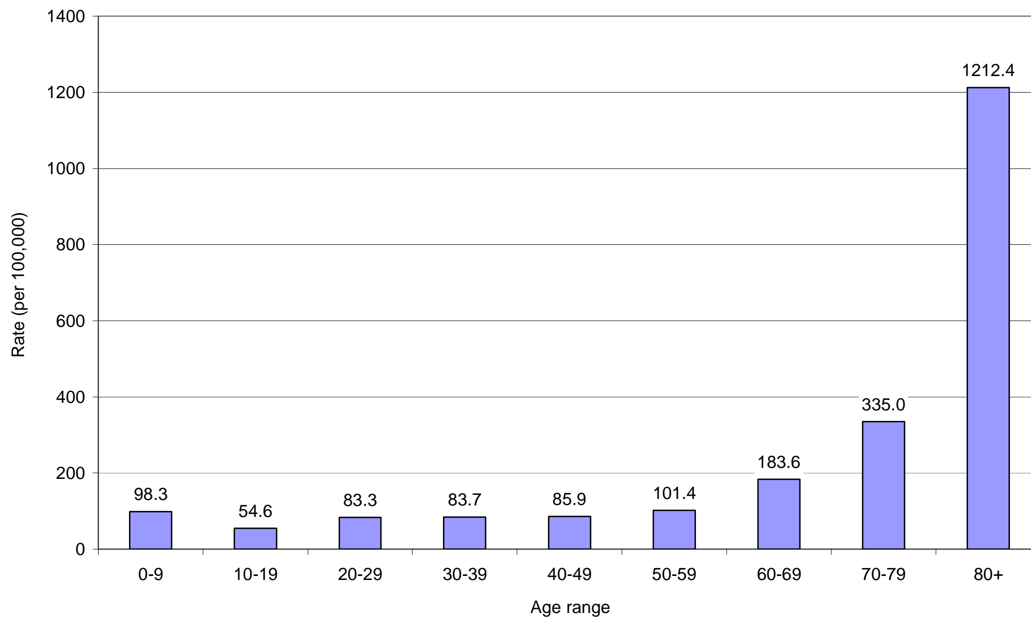

Figure 6: Rate (per 100,000 population) of known PVL-negative CA-MRSA by age, Western Australia July 2009 to June 2010

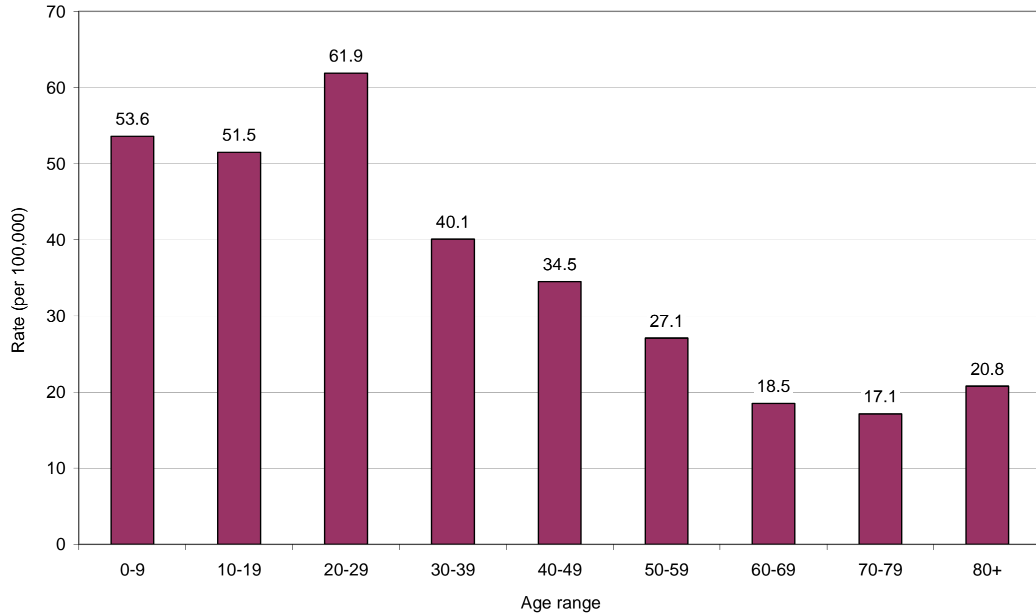

Figure 7: Rate (per 100,000 population) of known PVL-positive CA-MRSA by age, Western Australia July 2009 to June 2010

The average age of patients infected/colonised with PVL-positive CA-MRSA was 29 years (median 26 years) – significantly younger (T=24.9554) than patients with PVL-negative CA-MRSA (mean 55 years, median 60 years) (Figure 8). 93% of PVL positive CA-MRSA strains were isolated from clinical specimens, predominantly SSTIs (as opposed to screening swabs), compared to 72% of PVL-negative CA-MRSA.

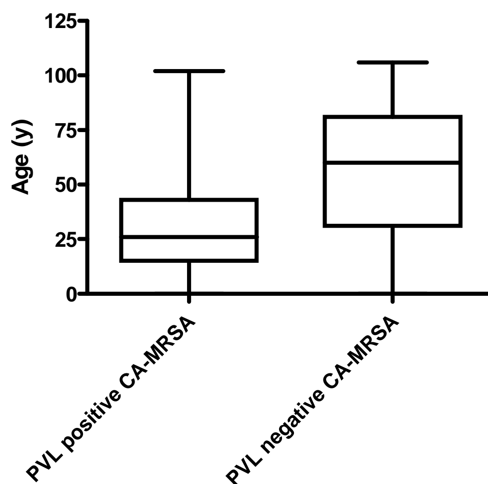

Figure 8: Median age and range of patients infected or colonised with PVL-positive and PVL-negative CA-MRSA

Owing to its geographical isolation and to a state-wide policy of screening all patients and healthcare-associated staff who have lived outside the state for MRSA the epidemiology of MRSA in WA has always differed from that in the rest of Australia [124].

2.5.2. Community MRSA in Central Australia

Emergence of non multiresistant MRSA causing infections outside the healthcare setting was noted in the Northern Territory (NT) not long after the initial observations in WA. Like the Kimberley region, the NT has a high proportion of aborigines and is sparsely populated. A retrospective study in 1995 found CA-MRSA was first isolated at Royal Darwin Hospital (RDH) in 1991 [17]. One half of infections due to CA-MRSA (defined as MRSA susceptible to gentamicin and to tetracycline and/or erythromycin) were community acquired (risk factors for healthcare-associated acquisition were absent), whereas all infections due to HA-MRSA (defined as MRSA resistant to gentamicin and/or to both tetracycline and erythromycin) were nosocomially acquired. Community-acquired infections due to CA-MRSA were far more common in aborigines than non-aborigines; the relative risk (95% confidence interval) was 15.4 (7.9 – 30.3). Overall the rate of CA-MRSA infections was highest in the western region of the NT, which borders the Kimberley region of WA. The aboriginal populations of these two regions are in frequent contact and therefore spread from the Kimberley region to the NT could have occurred. However NT isolates were neither typed nor compared with WA isolates at that time.

A detailed study of community-onset *S. aureus* bacteraemia at RDH between 1998 and 2001 identified 121 episodes, of which 15 (12.4%) were due to CA-MRSA (defined as MRSA resistant to two or fewer non-β-lactam antimicrobials) [127]. The proportion due to CA-MRSA increased steadily from 9% in 1998 to 20% in 2001. Most patients were aboriginal (86%) and most lived in remote areas or rural areas of the northern end of the NT (79%). Two cases were preceded by skin or soft-tissue abscesses and two developed lung abscesses. Endocarditis was diagnosed in three episodes. Ten isolates from nine episodes were available for genetic testing and all

contained the type IV [2B] SCC*mec* element. Three pairs of isolates were indistinguishable by PFGE, but they were not compared with strains from WA.

2.5.3. Community MRSA in Eastern Australia

Infections due to nonmultiresistant MRSA that were not associated with healthcare settings first appeared in eastern Australia in the mid 1990s.

An association between polynesian ethnicity and the occurrence of CA-MRSA SSTIs, particularly furunculosis, was first noted in Brisbane, Sydney, Canberra and Melbourne in 1997 [20]. It seemed likely that the strain causing these infections was the same as that causing similar infections in the polynesian population in Auckland, New Zealand [21]. Subsequent detailed studies in Brisbane and Sydney concluded that the strains in Australia and New Zealand were indistinguishable by PFGE and bacteriophage typing [22,128]. These studies confirmed that people of Polynesian ethnicity were overrepresented in the infected population. They also showed the medium age of patients with CA-MRSA was significantly lower than that of patients with HA-MRSA. SSTIs, most notably furunculosis, predominated. In the Brisbane study, 74% of CA-MRSA infections had no risk factors for healthcare acquisition, while in Sydney the figure was 44%. The clone responsible proved to be PVL-positive ST30-MRSA-IV [2B] (SWP CA-MRSA) [25]. This clone is closely related to the 80/81 MSSA clone that caused major outbreaks of neonatal infection in Australia and became pandemic in the 1950s [91,129].

The number of cases of CA-MRSA infection seen in the Caucasian population increased in the city of Ipswich in southeast Qld in 2000. PFGE showed that some of these cases were due to a unique pulsotype, which was subsequently typed as PVL-positive ST93-MRSA-IV [2B] (Qld CA-MRSA) [24,25]. This has proven to be a very important clone in Australia; it has become the predominant CA-MRSA in Qld and NSW and has also spread widely in the rest of the country [115]. ST93-MRSA-IV [2B] is notably virulent, causing necrotising pneumonia (including fatal cases), deep abscesses, osteomyelitis, septic arthritis and bacteraemia [130,131].

2.5.4. Recent Australian Epidemiology of CA-MRSA

At a national level, the Australian Group for Antimicrobial Resistance (AGAR) has been conducting regular surveys of clinical *S. aureus* in all states and territories since 1986. From the 2000 survey typing of all MRSA isolates has been performed by ***ACCESS*** Typing and Research [59,115,132,133].

2.5.4.1. AGAR 2010 Community-Onset *Staphylococcus aureus* Surveillance Programme

[http://www.agargroup.org/files/FED%20REPORT%20SAP210%20MRSA%20FINAL%20shrink.pdf]

The AGAR biennial community-onset *Staphylococcus aureus* surveillance programme (SAP) commenced in 2000. In the 2010 community programme (SAP2010) up to 100 clinically significant isolates of *S. aureus* from different patients with community onset infections were collected by each of 30 institutions located across Australia. Isolates were collected from GP clinics, hospital outpatients, nursing homes, long-term care facilities and hospice patients. Day surgery and dialysis patients were excluded. MRSA isolates were referred to ***ACCESS*** Typing and Research for clone characterization and detection of the PVL determinants. Of the 539 *S. aureus* classified as MRSA (18.0%) molecular typing was performed on 532 (98.7%) isolates. Overall 66.7% of MRSA were characterised as CA-MRSA. Since the 2000 survey the percentage of *S aureus* characterised nationally as CA-MRSA has almost doubled from 6.6% to 11.6%. Prevalence varied markedly between states and territories ranging from 3.0% in Tas to 29.0% in NT (Figure 9).

As in previous AGAR community-onset *S. aureus* surveys although CA-MRSA was multiclonal (32 clones) the majority of isolates (84.3%) could be characterised into six clones (Figure 10).

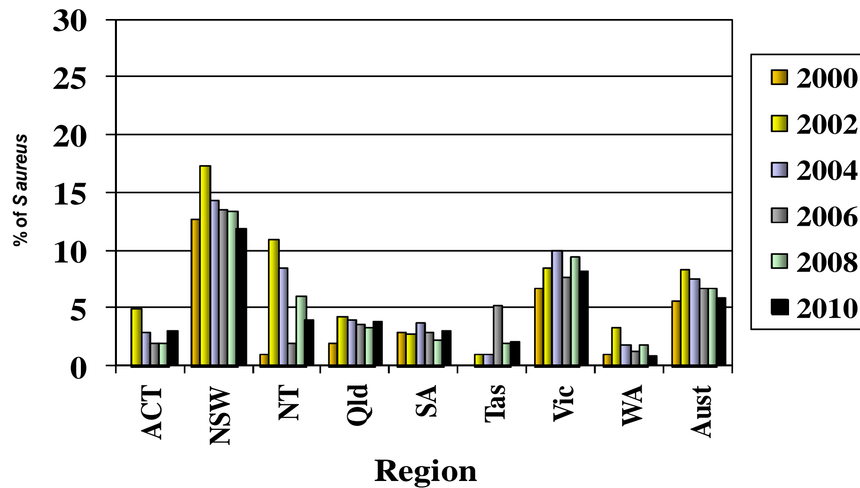

Figure 9: AGAR SAP2010 Community-Onset *Staphylococcus aureus* Surveillance Programme: Percentage of *S. aureus* characterised as CA-MRSA per state and territory. ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victora; WA = Western Australia; Aust = Australia

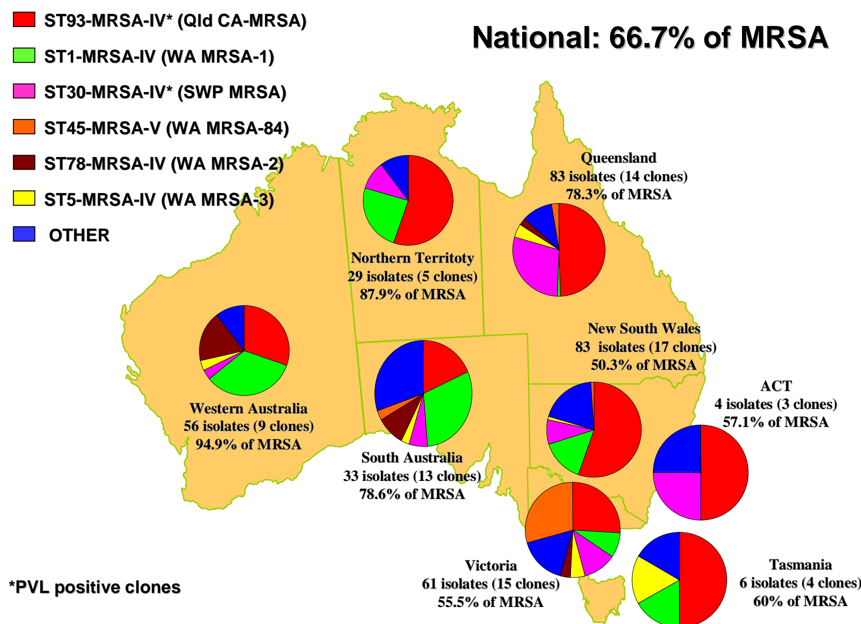

Figure 10: AGAR SAP2010 Community-Onset *Staphylococcus aureus* Surveillance Programme: Percentage of CA-MRSA clones per state and territory

PVL-positive ST93-MRSA-IV [2B] (Qld CA-MRSA) was the most frequently isolated CA-MRSA clone in the Australian community accounting for 41.4% of all CA-MRSA and 27.6% of all MRSA infections. Although the prevalence of Qld CA-MRSA has increased in all states and territories (Figure 11), PVL-negative ST1-MRSA-IV [2B] (WA-MRSA-1) was the most prevalent strain in WA and SA and PVL- negative ST45-MRSA-V [5C2&5] (WA-MRSA-84) the most common in Vic.

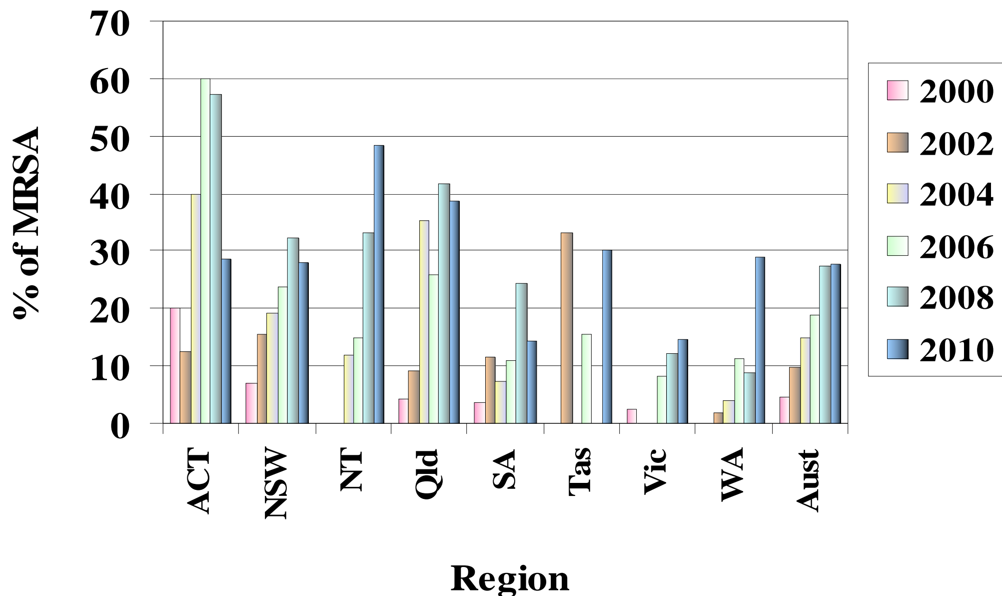

Figure 11: AGAR SAP2000 to SAP2010 Community-Onset *Staphylococcus aureus* Surveillance Programme: Regional Distribution of ST93-MRSA-IV [2B] Queensland CA-MRSA. ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victora; WA = Western Australia; Aust = Australia

Overall 62.5% of CA-MRSA were PVL positive, a 21% increase when compared to the 2006 survey. The mean age of patients with PVL-positive CA-MRSA infections (31 years; median 25 years) was significantly lower (P<0.0001) than the mean age of patients with PVL-negative CA-MRSA (53 years; median 57 years). The increase in PVL-positive MRSA was not only due to ST93-MRSA-IV [2B] but also to the introduction of several international CA-MRSA clones including ST30-MRSA-IV [2B] (SWP CA-MRSA), ST8-MRSA-IV [2B] (USA300), ST59-MRSA-V [5C2&5]

(Taiwan CA-MRSA), ST80-MRSA-IV [2B] (European CA-MRSA) and the hypervirulent ST772-MRSA-V [5C2] (Bengal Bay CA-MRSA).

2.5.4.2. AGAR 2009 Hospital-Onset *S. aureus* Surveillance Programme

http://www.agargroup.org/files/SAP09%20MRSA%20TYPING%20REPORT%20FINAL%20shrink.pdf

The AGAR biennial hospital-onset *Staphylococcus aureus* surveillance programme (SAP) commenced in 2005. In the 2009 hospital-onset programme (SAP2009) up to 100 clinically significant isolates of *S. aureus* from different patients were collected by each of 30 institutions located across Australia. Isolates were collected from hospitalised patients (>48 hours post-admission at the time of specimen collection). MRSA isolates were referred to ***ACCESS*** Typing and Research for clone characterization and detection of the *lukS-PV/lukF-PV* PVL encoding genes. Of the 916 *S. aureus* classified as MRSA (33.6%) molecular typing was performed on 899 (98.1%) isolates. Overall 32.3% of MRSA were characterised as CA-MRSA. Since the 2005 survey the percentage of *S. aureus* characterised nationally as CA-MRSA has significantly increased from 6.5% to 10.6% (P<0.002). Prevalence varied markedly between regions ranging from 8.2% in Tas/Vic to 20.4% in WA (Figure 12).

As in previous AGAR hospital-onset *S. aureus* surveys although CA-MRSA was multiclonal (28 clones) the majority (87.9%) of isolates could be characterised into six clones (Figure 13).

PVL-negative ST1-MRSA-IVa [2B] (WA-MRSA-1) was the most frequently isolated CA-MRSA clone in Australian hospitals accounting for 31.7% of all CA-MRSA and 10.2% of all MRSA infections. Although since 2000 the prevalence of WA-MRSA-1 has increased in most regions (Figure 14), PVL-positive ST93-MRSA-IV [2B] (Qld CA-MRSA) was the most prevalent strain in NT/Qld and NSW/ACT and PVL-negative ST45-MRSA-V [5C2&5] (WA-MRSA-84) in Tas/Vic

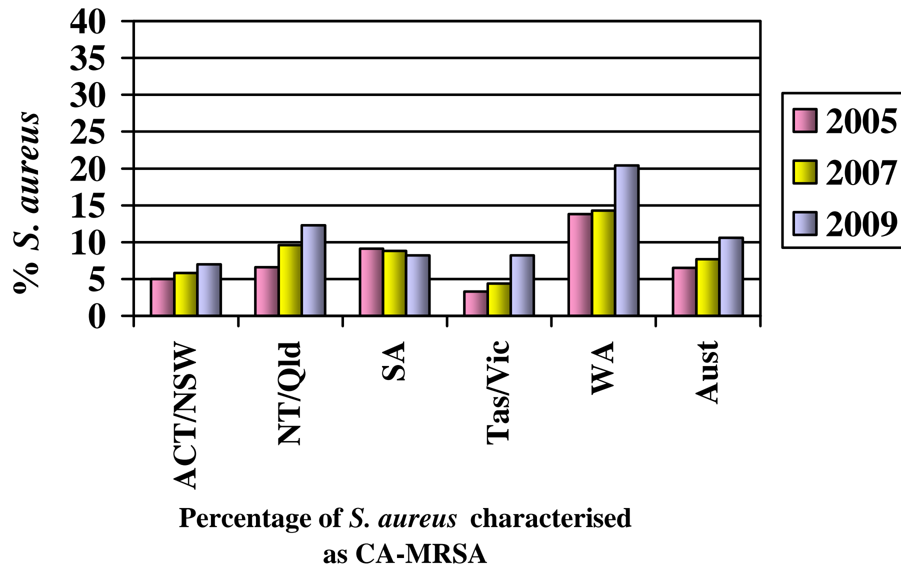

Figure 12: AGAR SAP2009 Hospital-Onset *Staphylococcus aureus* Surveillance Programme: Percentage of *S. aureus* characterised as CA-MRSA per region. ACT/NSW = Australian Capital Territory/New South Wales; NT/Qld = Northern Territory/Queensland; SA = South Australia; Tas/Vic = Tasmania/Victora; WA = Western Australia; Aust = Australia

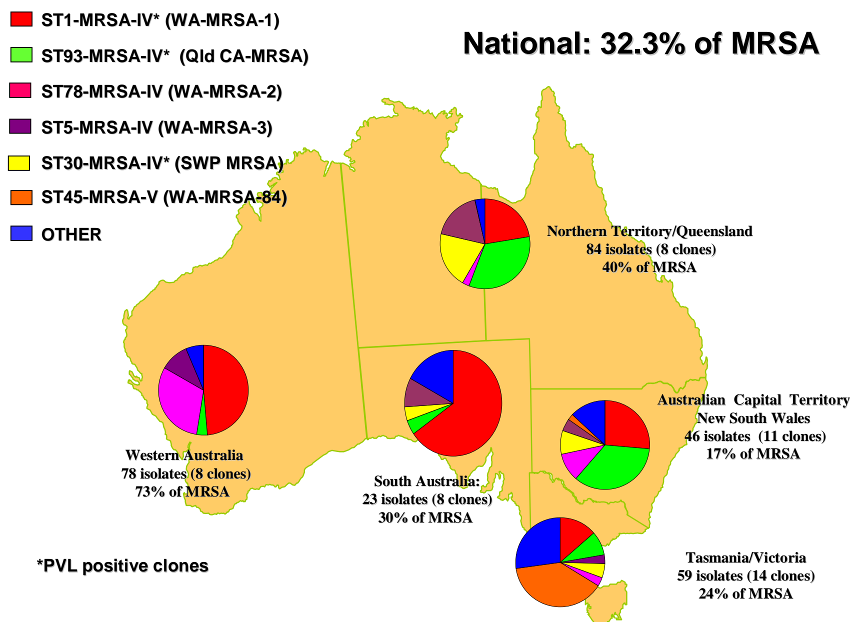

Figure 13: AGAR SAP2009 Hospital-Onset Surveillance *Staphylococcus aureus* Programme: Percentage of MRSA characterised as CA-MRSA per region

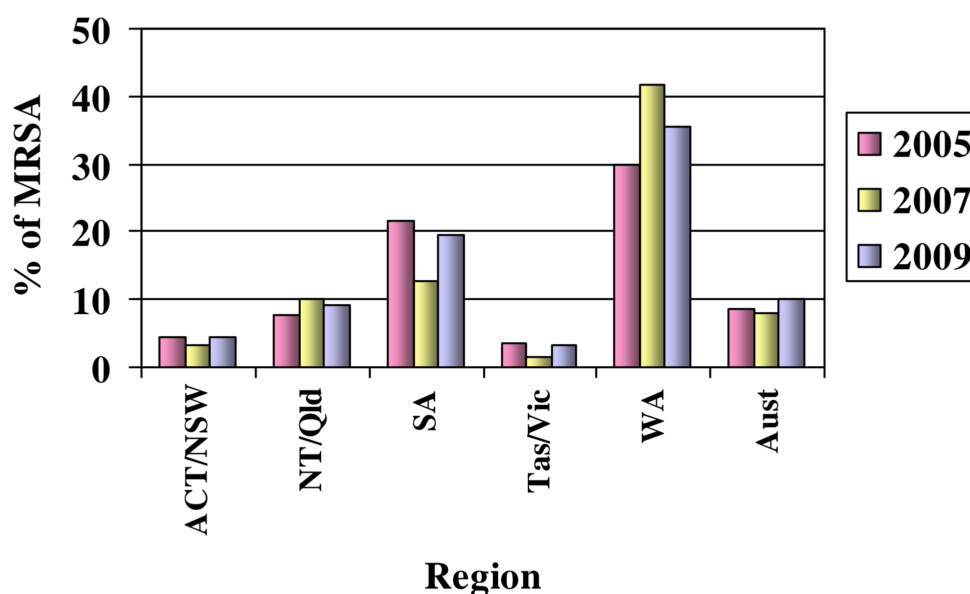

Figure 14: AGAR SAP2005 to SAP2009 Hospital-Onset *Staphylococcus aureus* Surveillance Programme Regional Distribution of ST1-MRSA-IV [2B] WA-MRSA-1. ACT/NSW = Australian Capital Territory/New South Wales; NT/Qld = Northern Territory/Queensland; SA = South Australia; Tas/Vic = Tasmania/Victora; WA = Western Australia; Aust = Australia

Overall 28.3% of CA-MRSA were PVL positive, a much lower proportion than seen in the outpatient community-onset surveys. In this survey an increase in PVL-positive MRSA was observed, whIch was not only due to the ST93-MRSA-IV [2B] clone but also due to two international CA-MRSA clones; ST30-MRSA-IV [2B] (SWP CA-MRSA) and ST59-MRSA-V [5C2&5] (Taiwan CA-MRSA).

The AGAR 2009 hospital-onset *Staphylococcus aureus* surveillance programme has demonstrated how the distinction between community and healthcare facility has become blurred, with 10.6% of *S. aureus* hospital onset infections due to CA-MRSA strains. Similarly in the AGAR 2010 community-onset *Staphylococcus aureus* surveillance programme 5.9% of *S. aureus* community onset infections were due to HA-MRSA.

2.6. Summary

Having started as a series of geographically separate epidemics involving different clones, CA-MRSA has increased in prevalence in all areas of Australia. There has been remarkable diversity in the clones that have acquired SCC*mec* types IV [2B], V [5C2] and [5C2&5], and novel SCC*mec* types. Most CA-MRSA do not harbour genes encoding the bi-component PVL toxin. However the PVL positive clones have been associated with the most severe infections and one of them ST93-MRSA-IV [2B] (Qld CA-MRSA) has now become the most prevalent CA-MRSA clone in Australia.

The notification of MRSA in WA has facilitated the collection of numerous isolates from all parts of the state providing the necessary resource to address the important questions of colonisation dynamics and genetics of *S. aureus*; frequency of SCC*mec* acquisition; and the genetic basis for the increasing prevalence of PVL-positive CA-MRSA in WA.

Furthermore the nationwide AGAR program has allowed comparison of "WA-MRSA" with CA-MRSA isolated elsewhere in Australia.

3. RESEARCH DESIGN

3.1. Objective One

The first true CA-MRSA without known healthcare associated risk factors described in the world were reported in 1993 in infected indigenous people living in remote communities in the sparsely populated Kimberley region of Western Australia (WA) [112]. As a consequence of this remoteness, these communities provided a unique opportunity to study the evolution of CA-MRSA within a confined isolated population which has had limited contact with healthcare institutions and therefore HA-MRSA.

The first objective of this thesis was to "*determine the colonisation dynamics and genetics of* S. aureus *in remote indigenous communities and thereby gain an insight into the emergence of WA-MRSA*".

Within these confined communities we proposed to:

- *Determine the prevalence of different MSSA and MRSA clones in selected remote communities and compare the MRSA prevalence to the rest of the state.*

- *Examine the relationship between MSSA and MRSA STs thereby providing an insight into SCC*mec *acquisition.*

- *Determine if clonal dissemination has occurred.*

- *Investigate the emergence of resistance in* S. aureus.

- *Determine any differences in the colonisation dynamics between MSSA and MRSA.*

3.2. Objective Two

Having examined the MSSA/MRSA dynamics in remote communities we then proposed to expand our analysis to a wider region concentrating on the evolution of CA-MRSA, with particular reference to SCC*mec* acquisition.

The second objective was therefore to "*determine the genetic relatedness of WA-MRSA clones within different multilocus sequence type clonal clusters providing an insight into the frequency of* S. aureus *SCC*mec *acquisition within a region"*.

The genetic profile of these clones may also offer an explanation why only a few "WA-MRSA" clones have successfully adapted to the community environment.

The following minor objective was also to:

- *Determine the antibiotic resistance profile and resistance determinants of "WA-MRSA" isolated in WA.*

3.3. Objective Three

The results from Objective 2 demonstrated the emergence of PVL-positive CA-MRSA in WA. This is of clinical significance given the greater potential of these clones to cause increased SSTIs and life threatening infections particularly in young otherwise healthy people.

The next objective of this thesis was therefore "*to determine if the increased prevalence of Panton Valentine leucocidin (PVL)-positive CA-MRSA in Australia is due to the widespread transmission of a single MRSA clone, or multiple independent acquisitions of the SCCmec element by a local PVL-positive MSSA clone(s); or the dissemination and subsequent evolution of international PVL-positive MRSA*".

PVL is a necrotising toxin that causes leucocyte destruction and tissue necrosis and is associated with abscesses and severe pneumonia. It is present in the majority of CA-MRSA studied in Europe and USA. Initial studies have shown Western Australian CA-MRSA infrequently carried the genes encoding PVL. However, due to the emergence and dissemination of the Qld CA-MRSA clone (ST93-MRSA-IV [2B]) and the introduction of several international CA-MRSA clones the proportion of PVL-positive CA-MRSA in WA increased from 2% of CA-MRSA in 2003/2004 to 22.3% in 2009/2011.

As the increased prevalence of PVL positive CA-MRSA in WA may be due to more than a single evolutionary event the following three PVL-positive CA-MRSA clones were investigated: ST93-MRSA-IV [2B] (Qld CA-MRSA); ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA); ST8-MRSA-IV [2B] (USA300).

3.3.1. ST93-MRSA-IV [2B] (Qld CA-MRSA)

From 2003/2004 to 2009/2010 Qld CA-MRSA, SWP CA-MRSA, Taiwan CA-MRSA, USA300 and Bengal Bay CA-MRSA have increased significantly ($P<0.0001$) in WA. Qld CA-MRSA however has showed the greatest increase from 0.7% of all MRSA in 2003/2004 to 13.3% in 2009/2010. Although Qld CA-MRSA was first detected in WA in 2003, as demonstrated in our first objective, PVL-positive ST93-MSSA was identified as the most prevalent *S. aureus* lineage in WA's remote indigenous communities in the mid 1990s and the early 2000s. By 2009/2010 Qld CA-MRSA were isolated in all public health regions with the highest prevalence in the Kimberley (237/100,000).

The increase in numbers of ST93-MRSA has major clinical implications therefore it was important to determine if the increased prevalence of Qld CA-MRSA in WA was due to the widespread transmission of a single clone or was due to multiple independent acquisitions of the SCC*mec* element. Hence the objective of this study was "*to examine the genetic relatedness of* S. aureus *ST93 isolated throughout Australia over an extended period*."

3.3.2. ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA)

In the Asia Pacific region a distinct genotype, CC59, has become widespread. ST59 CA-MRSA is an important cause of morbidity in Taiwan. The so called "Taiwan CA-MRSA clone" has acquired a composite type V SCC*mec* element (V [5C2&5] also known as V_T). Since 2003 multiple CC59 strains have been characterised in WA (WA MRSA-9, -15, -24, -52, -55, -56 and -73). These strains differ from each other in ST designation, PFGE pattern, SCC*mec* element and PVL carriage. Whether CC59 in WA is the result of the importation and expansion of the ST59-MRSA-V_T [5C2&5] Taiwan CA-MRSA clone with subsequent genetic changes or due to SCC*mec* acquisition by local CC59 MSSA isolates was not known.

The objective of this study was therefore "*to determine if CC59 MRSA isolated in WA are genetically related to Taiwan CA-MRSA*".

3.3.3. ST8-MRSA-IV [2B] (USA300)

PVL-positive USA300 has been the dominant MRSA strain in the North American community for over a decade. It is responsible for increased clinical disease, and has entered the hospital setting. First reported in WA in 2003, by 2009/2010 USA300 was the third most frequently isolated PVL-positive MRSA and the sixth most isolated CA-MRSA in WA. Almost 50% of patients presenting with a USA300 infection reported a recent travel history. The majority of USA300 (80%) were isolated from patients living in the Perth metropolitan region.

Given the importance of this international clone further examination of WA strains was indicated. The objective of this study was therefore "*to determine if USA300-like isolates that have been identified in WA were USA300, and if so had they undergone further diversification*."

The materials and methods used in each study are described in the relevant publication.

4. REVIEW

In Paper One (Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities) we showed that between the years 1995 and 2003 the *S. aureus* population structure in the geographically remote regions of WA was different to other regions. In European and the North American *S. aureus* population studies, five main genotypic clusters have been identified: CC5, CC8, CC22, CC30 and CC45. These clusters form the essential genetic backgrounds of *S. aureus*, with differences occurring principally in the local prevalence of the genotypes and the presence of minor clones [134,135]. In a study performed in Indonesia, which has prehistoric links with remote WA, a similar *S. aureus* population structure to that of Europe and the USA was reported [136]. The differences found in WA are probably a consequence of the geographic and cultural isolation of the remote population in WA; however these differences had an important influence on the epidemiology of MRSA in the entire WA community.

The most prevalent MSSA identified were ST93-MSSA from the Singleton ST93 lineage (21.4% of MSSA), ST15-MSSA from CC15 (14.9%), ST72-MSSA from CC5 (10.1%) and ST45-MSSA from CC45 (5.9%).

When compared with MSSA, we found less diversity in the MRSA lineages. Five MRSA lineages consisting of seven clones were identified in the communities screened in the 1995 surveys. No additional lineages were found in these or other remote communities in subsequent surveys within this study. ST1-MRSA-IVa [2B] (WA-MRSA-1) was the most frequently isolated MRSA clone (42.7%), followed by ST73-MRSA-IVa [2B] (WA-MRSA-65) (17.6%), ST5-MRSA-IVa [2B] (WA-MRSA-3) (13.4%), ST45-MRSA-V [5C2] (WA-MRSA-4) and ST45-MRSA-IVa [2B] (WA-MRSA-75) (12.5%), ST78-MRSA-IVa [2B] (WA-MRSA-2) (12.5%), and ST8-MRSA-IVa [2B] (WA-MRSA-5) (1.3%). Although all lineages containing MRSA also contained MSSA, they were not the most prevalent MSSA lineages. The CC1 MRSA clone, ST1-MRSA-IVa [2B] formed 42.7% of all MRSA while the methicillin susceptible counterpart ST1-MSSA, formed only 1.3% of the MSSA population. Similarly, in CC5, ST5-MRSA-IVa [2B] was 13.4% of the MRSA while ST5-MSSA formed only 0.6% of the MSSA and ST78-MRSA-IVa [2B] was 12.5%

of the MRSA while the corresponding MSSA was only 3.6% of the MSSA population. MRSA were not found in the largest MSSA lineages of Singleton ST93 and CC15.

Two lineages of MSSA harboured PVL determinants; seven of eight ST93-MSSA tested were found to carry *lukS-PV/lukF-PV* and of three ST121-MSSA tested one carried the determinant. No MRSA carried PVL.

Very few of the *S. aureus* isolates were resistant to multiple antimicrobials; however the potential for the emergence of resistance was indicated by the presence of several antimicrobial resistance determinants. In addition to the SCC*mec* element, determinants for resistance to penicillin, fusidic acid, MLS_{Bi} (inducible erythromycin resistance and erythromycin inducible resistance to lincomycin), tetracycline, gentamicin, kanamycin, mupirocin, trimethoprim and chloramphenicol were present. The resistance determinants for β-lactamase production, MLS_{Bi}, mupirocin and trimethoprim in remote WA community MRSA have been shown to be plasmid borne [123,137], and gentamicin and kanamycin resistance have been demonstrated to be on transposons while those for erythromycin, tetracycline and chloramphenicol are on plasmids.

Subsequent data have shown that the four MRSA clones considered endemic in Paper One have become the most prevalent CA-MRSA clones in the wider WA community [59]. State wide surveillance has revealed that in December 2006, ST1-MRSA-IVa [2B], ST78-MRSA-IVa [2B] and the CC5 clones (ST73-MRSA-IVa [2B] and ST5-MRSA-IVa [2B]) comprised 56.7%, 30.5% and 8.9% of CA-MRSA in WA, respectively. The CC45 clones (ST45-MRSA-V [5C2] and ST45-MRSA-IVa [2B]), and ST8-MRSA-IVa [2B], which were not found in the communities after 1998, formed only 1.9% and 0.8% of CA-MRSA respectively in WA in 2006 suggesting that they were not as well adapted to the WA community environment.

With the exception of ST8-MRSA-IVa [2B], the MRSA did not belong to the most prevalent MSSA lineages. However the MRSA strains did form the greater proportion of isolates present in the lineage to which they belonged, suggesting that an advantage was gained by acquisition of the SCC*mec* element. It would appear,

however, that only a limited number of clones acquired and maintained the SCC*mec* element, even though β-lactamase-stable β-lactams were widely used in the communities. The clonal structure of MRSA and the small amount of genetic diversity when compared with MSSA indicate not only the more recent emergence of MRSA, but also the dissemination of MRSA has probably occurred along clonal lines by well-adapted community clones that could support the SCC*mec* element.

The most prevalent MSSA lineage was the PVL-positive Singleton ST93 clone ST93-MSSA, which has been rarely found outside Australia. No Singleton ST93-MRSA was found during the periods of the surveys. PVL-positive ST93-MRSA-IVa [2B], also known as the Qld clone, however, is an important Australian CA-MRSA that was originally found in a Caucasian population in Qld in 2000 and has been reported in indigenous people from Qld [18,24] and the NT [138]. It is interesting that in an environment of high β-lactam use a methicillin-resistant variant of ST93-MSSA was not found in WA during these surveys.

S. aureus isolates from most of the lineages were found at all anatomical sites tested. The highest recovery of MSSA of 42.6% was from the throat while for MRSA the highest recovery of 51.6% was from the anterior nares. Although the anterior nares is the preferred screening site for population studies, in this study many isolates of *S. aureus* would have been missed if the throat and skin lesions had not also been swabbed.

The clonal nature of MRSA and the tendency for people carrying MRSA at multiple anatomical sites to harbour clones of the same genetic lineage as opposed to those with MSSA, who tended to have different lineages at each site, indicates that MRSA in the WA remote communities are well adapted colonisers that could possibly displace MSSA as asymptomatic commensal organisms.

The results of Paper One raised further questions regarding CA-MRSA molecular epidemiology in the wider WA region; in particular is there evidence for either clonal dissemination of these observed clones or ongoing SCC*mec* acquisition in different CCs, or have both occurred?

In Paper Two, which included CA-MRSA from metropolitan and regional WA (Evolution and diversity of CA-MRSA in a geographical region), we identified an additional 41 CA-MRSA to those described in the remote communities.

As the geographical spread of CA-MRSA over long distances and across cultural borders is believed to be a rare event compared to the frequency with which the SCC*mec* element is acquired by *S. aureus* [54], most of these clones are likely to have evolved in WA. Some clones are slvs and dlvs of pre-existing CA-MRSA, and their SCC*mec* type, *spa* type, and DNA microarray profile suggests vertical transmission of the SCC*mec* element has occurred. However the emergence of MRSA in several unrelated *S. aureus* CCs suggests horizontal transmission of the SCC*mec* element has also occurred. SCC*mec* and *spa* type, and DNA microarray results also suggest horizontal transfer of SCC*mec* elements has occurred into the same CC on more than one occasion.

In 2010 approximately 88% of CA-MRSA were identified as WA-MRSA-1 (40% of MRSA), WA-MRSA-2 (24%) and WA-MRSA-3 (8%). For many slv and dlv CA-MRSA only a small number of isolates have been detected suggesting changes in the housekeeping genes may have conferred a fitness cost or did not allow the SCC*mec* element to be maintained. For example WA-MRSA-45 and WA-MRSA-57 are slvs of ST1 and their SCC*mec* and *spa* type and DNA microarray profile suggest they have evolved from WA-MRSA-1. WA-MRSA-45 was first identified in 2006 and WA-MRSA-57 in 2007. Although WA-MRSA-1 has become the most successful CA-MRSA clone in the WA community only one isolate of WA-MRSA-45 and two isolates of WA-MRSA-57 have so far been identified (http://www.asainc.net.au/aasp/wamer).

In Paper Two we identified six PVL-positive globally disseminated MRSA lineages of which three have been shown to have local and national significance. The three clones (ST93-MRSA-IV [Qld CA-MRSA], ST59-MRSA-V_T [Taiwan CA-MRSA], and ST8-MRSA-IV [USA300]) were investigated further to determine if they have emerged due to clonal dissemination following importation, with or without genetic evolution, or due to multiple independent acquisitions of SCC*mec* elements.

In Australia ST93-MRSA-IVa [2B] has recently become the predominant CA-MRSA. [http://www.agargroup.org/files/FED%20REPORT%20SAP210%20MRSA%20FINAL%20shrink.pdf]. This strain is associated with skin infection and severe invasive infection including necrotizing pneumonia, deep-seated abscess, osteomyelitis, septic arthritis and septicaemia [58,130,131]. In the 2010 Australian Group for Antimicrobial Resistance (AGAR) Community-onset *Staphylococcus aureus* Surveillance Programme (SAP10) the mean age of patients with Qld CA-MRSA infections (31 years, median 25 years) was significantly lower (P<0.0001) than the mean age of patients with PVL-negative CA-MRSA infections (53 years; median 57 years).

Although in the 1990s ST93 was the most prevalent MSSA isolated from indigenous people living in WA remote communities, the first ST93-MRSA-IVa [2B] was not identified in WA until 2003 [59]. By 2010 ST93-MRSA-IVa [2B] accounted for 28.8% of the state's CA-MRSA community-onset infections.

Paper Three (The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* strain) showed all 13 ST93-MSSA examined were homogeneous despite being isolated at varying times, 1993 to 2008, and locations (WA remote communities, Perth metropolitan area, NT and Vic).

The 45 MRSA isolates were ≥ 80% related by PFGE with the majority of isolates falling into one pulsotype which was dispersed throughout Australia over eight years.

Although rearrangement of the *spa* sequence has occurred several times, the PFGE patterns and DNA microarray profiles of the 13 ST93-MSSA isolates suggested the ST93 core and accessory genome is very stable. All carried the PVL-encoding phage ΦSa2USA and their *lukS-PV/lukF-PV* genes had the same R variant SNP profile. The isolates produced similar expression levels of LukF-PV with no apparent relationship between PFGE subtypes and PVL expression. The emergence of five different *spa* types, albeit four types assigned to the same cluster, suggested ST93-MSSA emerged some time ago from a common *spa* type. As the *spa* sequences are similar it is not possible to predict the ancestral strain; however one strain, ST93-MSSA-t202, predominated and has successfully disseminated across Australia.

Like ST93-MSSA, ST93-MRSA had multiple *spa* types; including the closely related t202 and t4178, both identified in ST93-MSSA, and t1811 and t6487, all of which could be assigned to the same cluster. t202 had the largest number of isolates; 42 of the 45 ST93-MRSA. SCC*mec* and *dru* typing indicated the SCC*mec* element had been acquired by ST93-MSSA-t202 on at least three occasions; dt10 (SCC*mec* type IVa [2B]), dt3b/dt4d (SCC*mec* type IVa [2B]) and dt11i (SCC*mec* type V [5C2&5]). Unlike ST93-MRSA-IVa [2B]-t202, ST93-MRSA-V [5C2&5]-t202 did not carry the *lukS-PV*/*lukF-PV* genes. The PVL-negative ST93-MRSA-IVa [2B]-t1811 isolate may have arisen by independent acquisition of SCC*mec* IVa [2B] or by the subsequent rearrangement of the *spa* sequence. Forty three of the 45 isolates carried the PVL-encoding phage ΦSa2USA. The *lukS-PV/lukF-PV* genes had the same R variant SNP profile and produced similar expression levels of LukF-PV as reported in ST93-MSSA.

Apart from the *ermC* gene which was identified in several early ST93-MSSA and ST93-MRSA isolates, ST93 *S. aureus* initially carried few antibiotic resistance elements. However since 2008, in addition to *mecA* and *ermC*, some isolates of ST93-MRSA have acquired *msr(A)* and *tetK*. Although the *dfrA* gene was not detected by the microarray, SAPWH53 was phenotypically trimethoprim resistant (presumably due to an alternative trimethoprim resistance gene or a different *dfrA* allele). In addition, the quaternary ammonium compound resistance protein C gene *qacC* was carried by two isolates. The acquisition of additional resistance genes by an epidemic PVL-positive CA-MRSA clone is not unique to ST93-MRSA-IVa [2B]. The USA300 clone (ST8- MRSA-IV [2B]), initially resistant only to semi-synthetic penicillins and macrolides, is now, frequently resistant to other antimicrobial agents including clindamycin, tetracycline, mupirocin, and the fluoroquinolones. In addition, USA300 is occasionally resistant to gentamicin and trimethoprim-sulfamethoxazole, and may have reduced susceptibility to daptomycin [139].

In Paper Three we have shown the increased prevalence of Qld CA-MRSA in Australia is possibly due to independent acquisitions of four different *dru*-defined type IV SCC*mecs* and one type V SCC*mec* in several *spa*-defined ST93-MSSA backgrounds. Although rearrangement of the *spa* sequences in ST93-MRSA had subsequently occurred in some of these strains the PVL-positive ST93-IVa [2B]-

t202-dt10 strain is predominant across Australia. Whether this strain arose from one PVL-positive ST93-MSSA-t202 or by independent acquisitions of SCC*mec*-IVa [2B]-dt10 into multiple ST93-MSSA-t202 strains is not known.

CC59 MRSA is prevalent in WA. Determining the genetic relatedness of various CC59 strains isolated in WA answers the question of whether there has been clonal dissemination following importation of the internationally recognised Taiwan CA-MRSA clone ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA), or whether separate evolutionary events due to SCC*mec* acquisition have occurred.

In Paper Four (Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia) we examined multiple CC59 strains isolated in WA between 2003 and 2008. These strains differed from each other in their ST designation, PFGE pattern, SCC*mec* element, and PVL carriage.

In essence the results of this study indicated the increase in numbers of CC59 in WA arose primarily due to the clonal dissemination of the Taiwan CA-MRSA clone with evidence of further evolutionary diversification within this PVL positive clone.

Although of lower numbers, other CC59 isolates were obtained. These CC59 isolates most likely had different origins. For example WA73 being similar to USA1000 may be either a PVL-negative ancestor or a PVL-negative variant of that clone. Three other groups with varying SCC*mec* types may indicate multiple episodes of SCC*mec* acquisition by PVL negative CC59 lineages unique to WA.

A second important international CA-MRSA identified in WA was USA300 (ST8-MRSA-IVa [2B]/t008). First reported in WA in 2003, by 2010 USA300 had become the third most isolated PVL-positive clone and the sixth most isolated CA-MRSA in WA (http://www.asainc.net.au/aasp.wamer). ST8-MRSA-IV [2B]/t008 has emerged as the dominant MRSA strain in North America, both in the community and hospital setting [84,99,109,140,141,142,143], and has been reported in Australia, Canada, Denmark, Germany, Japan, Switzerland and the UK [141,144,145,146,147]. Because of its rapid spread, it has drawn considerable attention, resulting in the

sequencing of multiple complete genomes including; USA300-FPR3757, GenBank CP000255.1 and USA300-TCH1516, GeneBank CP000730.1 [3,146,148,149].

Locally known as WA MRSA-12, USA300-like isolates have been identified in Australia [2]. To determine whether WA MRSA-12 was USA300 in Paper Five (The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia) we investigated 76 PVL-positive ST8-MRSA-IV [2B] isolated between July 2003 and February 2009 from 72 patients living in the Perth area.

Array hybridisation and PCR demonstrated that all WA-MRSA-12 isolates harboured the *lukS-PV*/*lukF-PV* PVL genes. Carriage of capsule genes (type 5), biofilm (*icaA*, *icaC*, *icaD*) and MSCRAMM genes (*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnB*, *map*, *sdrC*, *sdrD*, and *vwb*) was identical to USA300-FPR3757 and USA300-TCH1516. Genes of the ACME locus (*arcA-SCC*, *arcB-SCC*, *arcC-SCC* and *arcD-SCC*) were detected in 64 (84.2%) of the WA-MRSA-12 isolates. An absence of ACME was confirmed using two different *arcA* PCR primer sets.

Microarray hybridization profiles identified 16 variants (variants A – P) among 76 WA MRSA-12 isolates. Forty seven isolates (61.8%) belonged to variant A. An analysis of the genome sequence of USA300-TCH1516 predicted the same hybridisation pattern as that observed in variant A. Thus USA300 and WA MRSA-12 can be regarded as the same clone. From this clone, most of the other variants may have evolved by a limited number of gene losses or acquisitions. Variations in carriage of virulence and resistance-associated genes allow distinction of variants or sub-clones. The 16 variants differed in the carriage of resistance genes (*blaZ/I/R*, *ermC*, *msrA* + *mpbBM*, *aadD* + *mupR*, *aphA3* + *sat*, *tetK*, *qacC*, *merA*/*B*/*R*/*T*), immune evasion clusters (IEC) and enterotoxins (*sek* + *seq*, were not detected in four isolates). Notably the ACME locus was absent in 12 isolates. The mercury resistance operon (*mer*) was found in several ACME-negative isolates.

In summary in Paper Five we have shown PVL positive WA-MRSA-12 is the USA300 clone. However, in WA USA300 cannot be regarded as genetically homogeneous with variations in the carriage of resistance and virulence determinants

identified. The most common variant in WA was genetically closely related to USA300-TC1516, a geographically widespread strain found in Texas, Germany and in Switzerland.

5. DISCUSSION

5.1. Paper One

"Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities"

5.1.1. Findings

In Paper One we found the *S. aureus* population in the 11 remote indigenous communities consisted of 13 clonal complexes and two Singleton lineages together with 56 sporadic isolates. This population structure was different to that reported in Europe, the USA and in neighbouring Indonesia. Five lineages contained MRSA; however these did not arise from the predominant MSSA lineages. There was greater genetic diversity amongst the MSSA lineages, while the MRSA lineages appear to have emerged clonally following acquisition of SCC*mec* IVa [2B] and V [5C2]. In an environment of high β-lactam use, the horizontal transfer of the SCC*mec* IVa element into the MSSA was found to have occurred on at least five occasions (CC1, CC5, CC8, CC45 and CC88). It was therefore surprising that a methicillin-resistant variant of ST93-MSSA was not found, particularly as ST93-MRSA-IVa [2B] has become the predominant CA-MRSA in Australia and is now frequently isolated in WA. The three MRSA lineages we considered to be endemic in the remote WA communities (CC1, CC5 and CC88) have subsequently become the predominant lineages of CA-MRSA in the wider WA and Australian community. People colonised with MSSA tended to harbour clones of a different genetic lineage at each anatomical site, while people colonised with MRSA tended to harbour clones of the same lineage at each site. The MRSA isolates were resistant to few antimicrobials. However, the potential for the emergence of resistance was demonstrated by the presence of several antimicrobial resistance determinants known to be on mobile elements. PVL determinants were identified in only two MSSA lineages (ST93-MSSA and ST121-MSSA), and no PVL-positive MRSA were isolated.

5.1.2. Limitations and Future Directions

Subsequent to Paper One, PVL-positive ST5-MRSA-IVa (WA-MRSA-3) and ST73-MRSA-IVa (WA-MRSA-65) isolates from the Kimberley region have been identified by ***ACCESS*** Typing and Research (http://www.asainc.net.au/aasp/wamer). To determine if the bacteriophages from the PVL-positive MSSA have subsequently been acquired by "WA-MRSA", the bacteriophages harbouring the PVL determinants in ST121-MSSA and in WA-MRSA-3 and WA-MRSA-65 should be characterised and the *lukS-PV/lukF-PV* genes sequenced. The ability for these bacteriophages to be acquired by other *S. aureus* lineages including PVL negative WA-MRSA-3 and WA-MRSA-65 should also be investigated. PVL-positive ST1-MRSA-IVa [2B] (WA-MRSA-1) and ST78-MRSA-IVa [2B] (WA-MRSA-2) have also been reported in Australia [http://www.agargroup.org/files/FED%20REPORT%20SAP210%20MRSA%20FINAL%20shrink.pdf]. As per WA-MRSA-3 and WA-MRSA-65, the PVL element harboured by these strains should be investigated.

In Paper One, only one representative isolate from each PFGE pulsotype and sub-pulsotype was tested for the presence of the PVL determinant. PCR for the detection of PVL determinants should be performed on all *S. aureus* isolated in this study.

During the period of Paper One, no ST93-MRSA was detected despite the high prevalence of ST93-MSSA. Since the conclusion of the Paper One study ST93-MRSA-IVa [2B] has become the predominant CA-MRSA in Australia. Repeat surveys of the remote communities may shed light on whether ST93-MRSA-IVa [2B] has subsequently emerged in these locations. Genetic investigation would then be required to answer the question of whether SCC*mec* acquisition has occurred locally or if the clone has been imported from other regions.

The potential for the emergence of antibiotic resistance was indicated by the presence of several other antimicrobial resistant determinants among the population. The possible emergence of multiresistant CA-MRSA in these communities should be investigated and linked to the communities' antimicrobial use as a potential resistance selection pressure.

5.1.3. Conclusion

Paper One showed "WA-MRSA" arose in remote indigeneous communities with horizontal transfer of the SCC*mec* element on at least six occasions. This leads on to the next objective where the frequency of SCC*mec* acquisition in the greater WA region was investigated.

5.2. Paper Two

"Evolution and diversity of community-associated methicillin-resistant S*taphylococcus aureus* in a geographical region"

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5.2.1. Findings

In Paper Two we have shown that the CA-MRSA population in WA is genetically diverse consisting of 83 unique PFGE strains from which 46 STs have been characterised. The STs confirm CA-MRSA have not evolved from the major Australian HA-MRSA clones; ST22-MRSA-IV [2B] and ST239-MRSA-III [3A] [133]. Forty five of these STs were from 18 clonal clusters and two Singletons. While SCC*mec* IVa [2B] and V [5C2] were the predominant SCC*mec* elements, SCC*mec* IV subtypes b, c and d, SCC*mec* VIII, and several novel and composite SCC*mec* elements were present. The emergence of MRSA in diverse *S. aureus* clonal clusters suggests horizontal transmission of the SCC*mec* element has occurred on multiple occasions. The DNA microarray and *spa* typing data suggest horizontal and vertical transfer of SCC*mec* elements has occurred within a clonal complex. Despite the movement of different SCC*mec* elements, three clones predominate in WA; WA-MRSA-1, WA-MRSA-2 and WA-MRSA-3. For many single and double locus variant CA-MRSA clones only a few isolates were detected suggesting changes in the housekeeping genes may have conferred a fitness cost or did not allow the SCC*mec* element to be maintained. Multiple PVL positive clones, including ST93-MRSA-IVa [2B] and five international pandemic clones were identified.

5.2.2. Limitations and Future Directions

In Paper Two only the initial isolate of each PFGE pulsotype was investigated. Therefore subsequent genetic changes within each pulsotype would not have been detected. To determine if such evolution is occurring in the successful CA-MRSA clones found in the WA community genetic profiles of subsequent isolates should be investigated. Clonal complexes that should be investigated include CC1, CC5, CC88, CC45 and Singleton ST93.

5.2.3. Conclusion

Although the horizontal and vertical transmission of SCC*mec* elements into *S. aureus* has occurred on multiple occasions in the WA community, only three “WA-MRSA” clones have found an ecological niche. These three PVL negative clones harbour few additional resistance and virulence genes which paradoxically may account for their success. Additional genetic elements may confer a fitness cost. The identification of PVL positive clones signalled the potential for an increased burden of disease. This leads to a more in depth analysis of PVL positive clones to explain their emergence.

5.3. Paper Three

"The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* strain"

5.3.1. Findings

In Paper Three we demonstrated that although multiple rearrangements of the *spa* sequence have occurred, the core genome in ST93 *S. aureus* is very stable. From 2008, PVL-positive ST93-MSSA-t202 has become the predominant ST93-MSSA across Australia. We have shown the emergence of ST93-MRSA has been due to independent acquisitions of different *dru*-defined type IV and type V SCC*mec* elements in several *spa*-defined ST93-MSSA backgrounds. Rearrangement of the *spa* sequence in ST93-MRSA has subsequently occurred in these strains. Although many ST93-MRSA strains were identified in this study, little genetic diversity was identified for most MRSA isolates, with PVL-positive ST93-IVa [2B]-t202-dt10 being predominant across Australia.

5.3.2. Limitations and Future Directions

To determine if ST93-MRSA-IVa [2B]-t202-dt10 has arisen from one PVL-positive ST93-MSSA-t202 or by independent acquisitions of SCC*mec*-IVa [2B]-dt10 into multiple PVL-positive ST93-MSSA-t202 strains will require whole genomic sequencing of the isolates. Furthermore comparative genomic sequencing may further enhance our understanding of the molecular basis for the emergence and increased virulence of ST93 CA-MRSA. At a time when this clone is acquiring additional resistance genes and an increased potential for infections in the healthcare setting, understanding the mechanism of SCC*mec* acquisition, the role of virulence determinants and how MRSA transmission occurs is crucial if we are to prevent this clone from becoming established in hospitals.

5.3.3. Conclusion

Although, multiple ST93-MSSA strains have emerged in Australia, Paper Three has shown, although ST93-MRSA has acquired different SCC*mec*s, ST93-MRSA-IVa [2B]-t202-dt10 is the predominant MRSA strain.

5.4. Paper Four

"Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia"

5.4.1. Findings

Paper Four found in WA there are at least six discernible groups of CC59 CA-MRSA strains, which can be differentiated by PFGE, MLST, presence or absence of PVL, determination of the SCC*mec* type, and microarray analysis. Within the study isolates at least seven different variants of SCC*mec* elements were distinguished. This suggests rapid evolution and/or multiple transfer events of SCC*mec* elements have occurred. Genetically CC59 displays a high degree of variability, affecting not only SCC*mec* markers but also a variety of other mobile genetic elements.

ST59-MRSA-V_T [5C2&5] (Taiwan CA-MRSA) was found to be the most prevalent CC59 clone in WA. We have demonstrated that this clone has undergone further evolutionary events. Some CC59 isolates in WA however have evolved from different origins (e.g. USA1000) while other isolates with varying SCC*mec* types and absence of PVL indicate multiple episodes of SCC*mec* acquisition by WA CC59 clones.

5.4.2. Limitations and Future Directions

Further studies should investigate the variability and evolution of CC59 strains in other geographical locations where this clonal complex has been detected. Apart from data for the Taiwan CA-MRSA clone there are little data available on the distribution of CC59 clones outside WA. It can be assumed that these isolates are usually identified as USA1000 or the Taiwan CA-MRSA clone and their true diversity remains unrecognized. This might also obscure routes of transmission of CC59 CA-MRSA and hinder the understanding of their spread.

As the six CC59 groups showed minimal genetic variation, a marker for the Taiwan CA-MRSA clone should be found among the rather limited number of genes which are variable within CC59. Full genome sequencing of representative strains of CC59 may provide a marker and overcome the limitations of the microarray.

The bacteriophages harbouring the PVL determinants in WA-MRSA-9, WA-MRSA-52, WA-MRSA-55 and WA-MRSA-56 should be characterised and the ability for these bacteriophages to be acquired by PVL-negative WA CC59 strains including WA-MRSA-9, WA-MRSA-15, WA-MRSA-24, WA-MRSA-55, and WA-MRSA-73 should be investigated. To confirm the PVL determinants in CC59 are the same, sequencing of the *lukS-PV/lukF-PV* genes in each strain should be performed.

5.4.3. Conclusion

Paper Four has shown the PVL-positive WA-MRSA-9 and WA-MRSA-52 strains are the Taiwan CA-MRSA clone. Furthermore the closely related PVL positive Group 3 strains (WA-MRSA-55 and WA-MRSA-56) may have evolved from the same branch of the CC59 complex. This suggests PVL positive CC59 isolates have not evolved from WA CC59 MRSA strains but have been introduced, and the PVL negative strains have evolved independently.

5.5. Paper Five

"The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia"

5.5.1. Findings

Paper Five has shown PVL-positive ST8-MRSA-IV [2B] WA-MRSA-12 isolates have not arisen locally but are the USA300 clone. The isolates varied in their carriage of resistance and virulence determinants and therefore cannot be regarded as genetically homogeneous. Several isolates did not harbour the ACME locus. This is intriguing because this locus is assumed to be involved in facilitating the spread of USA300 by skin contact. The most common variant in WA was genetically closely realted to USA300-TC1516, a geographically widespread strain found in Texas, Germany and in Switzerland.

5.5.2. Limitations and Future Directions

Because of the limited presence of USA300 in WA, further studies should focus on possible changes in the ratio of ACME-positive to ACME-negative variants. This may improve our understanding of the clinical significance of this element and its proposed role in the rapid spread of USA300.

In this study subtyping of the SCC*mec* was only performed on two isolates. Both isolates were subtype IVa [2B]. As USA300 has been shown to harbour different SCC*mec* IV [2B] subtypes [3], subtyping of all isolates should be performed.

The explosive expansion of USA300 warrants further study. The first CA-MRSA identified in WA was PVL-negative ST8-MRSA-IVa [2B], known as WA-MRSA-5. Whole genome sequencing of WA-MRSA-5 and ACME negative USA300 isolates may contribute to understanding which genes are responsible for the global spread of the USA300-TC1516 strain.

As the prevalence of USA300 is increasing in the Australian community, the susceptibility of “WA-MRSA” strains to the bacteriophage harbouring the PVL determinant in USA300 should be investigated.

5.5.3. Conclusion

Paper Five confirms PVL-positive USA300 (ST8-MRSA-IV [2B]) has been introduced into WA possibly with subsequent evolutionary changes.

6. CONCLUSIONS

Staphylococcus aureus is one of the most successful bacterial pathogens, responsible for a wide spectrum of infections, from uncomplicated SSTIs through to necrotizing pneumonia, necrotizing fasciitis and bacteraemia. In recent years a MRSA pandemic due to CA-MRSA, which carried highly mobile SCC*mec* elements, has occurred. In addition some CA-MRSA strains harbour the prophage encoded virulence factor PVL, a bicomponent toxin that forms polymeric pores in leucocyte membranes. Multiple PVL-positive CA-MRSA strains from different clonal groups have evolved. Some have been confined to certain regions or localised outbreaks, whereas others have spread worldwide.

In Australia we have shown the initial CA-MRSA arose in remote communities and have subsequently disseminated into the wider community. However these strains did not arise from the predominant MSSA clones in these communities. Although the vertical and horizontal transmission of SCC*mec* elements into *S. aureus* has occurred on multiple occasions in the WA community, only three WA-MRSA clones, ST1-MRSA-IVa [2B] (WA-MRSA-1), ST78-MRSA-IVa [2B] (WA-MRSA-2), and ST5-MRSA-IVa [2B] (WA-MRSA-3), have found an ecological niche and are now isolated Australia wide. These three PVL-negative clones harbor few additional resistance and virulence genes. Low fitness cost paradoxically may account for their success. PVL positive CA-MRSA infections however are becoming more prevalent in Australians, particularly in the young. Although this is primarily due to a single strain of Qld CA-MRSA (ST93-MRSA-IVa [2B]-t202-dt10), international PVL positive CA-MRSA clones are now frequently isolated in Australia.

The studies included in this thesis show the ongoing evolutionary potential of *S. aureus* and in particular CA-MRSA. The movement of SCC*mec* is central to this changing epidemiology. Ultimately defining the external pressures that facilitate SCC*mec* acquisition (antibiotic exposure or other factors), and the ability of certain strains to then successfully maintain the SCC*mec* element may provide insight into control of this epidemic.

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8. PUBLISHED PAPERS

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Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities.

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Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities

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***Objectives*: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote regions of Western Australia (WA) in 1992 and is now the predominant MRSA isolated in the State. To gain insights into the emergence of CA-MRSA, 2146 people living in 11 remote WA communities were screened for colonization with *S. aureus*.**

***Methods*: Antibiogram analysis, contour-clamped homogeneous electric field electrophoresis, multilocus sequence typing, Panton–Valentine leucocidin determinant detection and accessory genetic regulator typing were performed to characterize the isolates. MRSA was further characterized by staphylococcal cassette chromosome *mec* typing.**

***Results*: The *S. aureus* population consisted of 13 clonal complexes and two Singleton lineages together with 56 sporadic isolates. Five lineages contained MRSA; however, these were not the predominant methicillin-susceptible *S. aureus* (MSSA) lineages. There was greater diversity amongst the MSSA while the MRSA appeared to have emerged clonally following acquisition of the staphylococcal cassette chromosome *mec*. Three MRSA lineages were considered to have been endemic in the communities and have subsequently become predominant lineages of CA-MRSA in the wider WA community. People colonized with MSSA tended to harbour clones of a different genetic lineage at each anatomical site while people colonized with MRSA tended to harbour clones of the same lineage at each site. Overall, the isolates were resistant to few antimicrobials.**

***Conclusions*: Although the evidence suggests that in WA CA-MRSA strains arose in remote communities and have now disseminated into the wider community, there is no evidence that they arose from the predominant MSSA clones in these communities.**

Keywords: *S. aureus*, community methicillin-resistant *Staphylococcus aureus*, population structure, colonization

Introduction

Staphylococcus aureus is one of the most successful pandemic bacterial pathogens. It is also a ubiquitous inhabitant of human microbiological flora, with up to 30% of humans persistently colonized asymptomatically, and up to 70% intermittently colonized.[1]

Initially MRSA was found almost exclusively in hospitals where it became known as healthcare-associated MRSA (HA-MRSA). However, it has now emerged in communities around the world where it is known as community-associated MRSA (CA-MRSA). The earliest reports of CA-MRSA involved infections in people from isolated Indigenous[2] or disadvantaged

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communities,[3] suggesting that these were the primary environments from which it emerged. In Australia the first CA-MRSA, colloquially known as 'WA MRSA', was reported in 1993 in infected Indigenous people from remote communities in the sparsely populated Kimberley region of Western Australia (WA).[2] This was followed by reports of CA-MRSA from Indigenous people in the Northern Territory,[4] Queensland[5] and Central Australia.[6]

CA-MRSA is responsible for a wide spectrum of infections, from uncomplicated skin and soft tissue infections through to necrotizing pneumonia, necrotizing fasciitis and bacteraemia, which can be fatal in otherwise healthy people. This virulence has been attributed to the possession of virulence determinants, such as the Panton–Valentine leucocidin (PVL).[7] Apart from isolated instances, CA-MRSA was resistant to few non-β-lactam antibiotics and did not initially spread in hospitals. However, the epidemiology of CA-MRSA is changing and multiply resistant CA-MRSA spreading in hospitals, communities and internationally has been reported.[8,9]

To prevent the transmission of MRSA in WA hospitals, MRSA was made a notifiable organism and a 'search and destroy' policy was introduced in 1982. As part of this policy all isolates are sent to a reference centre for typing and storage.[10] Although this strategy has not prevented the spread of CA-MRSA, which now comprises 77.5% of MRSA isolated in WA,[11] it has enabled its spread to be closely monitored. Surveillance data have shown that between 1983 and 2002 the notification rates for CA-MRSA in WA increased >50- and 70-fold in rural and metropolitan health regions, respectively.[12,13]

CA-MRSA utilizes mobile elements and single nucleotide polymorphisms to establish local and geographic niches[14] and is thought to emerge when a locally prevalent strain of methicillin-susceptible *S. aureus* (MSSA) acquires a staphylococcal cassette chromosome *mec* (SCC*mec*) element. The remote WA Indigenous communities provide an ideal environment in which to study the natural genetics of *S. aureus* and CA-MRSA as the population has limited contact with healthcare institutions and therefore HA-MRSA. Consequently, surveys of populations from remote WA communities were undertaken between 1995 and 2003. The aims of this study were to determine the colonization dynamics and genetics of *S. aureus* in the communities and to gain insights into the emergence of CA-MRSA.

Materials and methods

Ethics approval for the screening of Indigenous communities was obtained from the WA Aboriginal Health Information and Ethics Committee and the Curtin University of Technology Human Research Ethics Committee. Prior to each survey a senior member of the team travelled to each community to obtain permission from the community Elders and Councils. Remote region healthcare professionals and Indigenous aides provided valuable support. Although participation in the survey was voluntary, on most occasions participation was near to 100%. Written informed consent was obtained from each adult individual, parent or guardian.

Communities

The inhabitants of 11 major remote communities from three geographical regions of WA, the Kimberley, the Pilbara and the Goldfields, were screened for *S. aureus* colonization (Figure 1). The community population sizes were between 60 and 400 people. Small fringe or satellite communities with populations of between 9 and 51 were also screened and their results were combined with results for the larger community in their geographical proximity. The communities were 700–2000 km from the capital city Perth and their geographical regions accounted for 6.4% of the total WA population. While for each episode of community screening inhabitants were screened only once, it was not possible to determine the number of times an individual was screened over the 9 year duration of the surveys due to ethical constraints and the nomadic nature of the population. Therefore, each screening episode has been enumerated as a set of screening swabs only.

Screening

Overall, 2146 sets of screening swabs were collected; 924 from three Kimberley communities, 258 from a Pilbara community and 964 from seven Goldfields communities. Community 2 was screened in June (dry season) and December (wet season) of 1995, communities 3 and 4 were screened in 1995, 1999 and 2003, and community 7 was screened in 1999 and 2003. The remaining communities, 1, 5, 6, 8, 9, 10 and 11, were screened once in 1995, 1996, 1998, 1999, 2001, 2001 and 2001, respectively (Figure 1).

The anterior nares, throat and, when applicable, up to two skin lesions were swabbed with moistened cotton wool swabs. Swabs were placed in Amies transport medium (Interpath services, Pty Ltd, West Heidelberg, Australia) and transported in insulated containers by road and air to the laboratory in Perth. All swabs were processed within 48 h of collection.

Laboratory processing

Swabs were plated onto mannitol salt agar (MSA) (Oxoid, Basingstoke, UK) for detection of *S. aureus* and methicillin aztreonam mannitol salt agar (MAMSA)[15] or methicillin MSA (MMSA) (MSA containing 4 μg of methicillin/mL) for detection of MRSA. All plates were incubated at 35°C. The MAMSA plates were read after 20 h incubation and the MSA and MMSA plates were read after 48 h incubation. Mannitol-fermenting colonies were cultured overnight in brain–heart infusion broth (Gibco Diagnostics, Gaithersberg, MD, USA) and identified as *S. aureus* by the tube coagulase test.

Susceptibility testing

Antimicrobial susceptibility testing was performed by disc diffusion on Mueller–Hinton agar (MHA) (BBL, Becton Dickinson, Cockeysville, MD, USA) using Oxoid discs according to the method of the CLSI (formerly the NCCLS),[16] with fusidic acid susceptibility criteria as previously published.[17] All staphylococci were initially tested for methicillin resistance using a 1 μg oxacillin disc (Oxoid, Basingstoke, UK). MRSA was confirmed by detection of the *mecA* and *nuc* genes in a multiplex PCR.[18] Following multilocus sequence typing (MLST) a representative MSSA from each sequence type (ST) was screened by PCR to confirm the absence of the *mecA* gene.[19]

For all MRSA an 18-antimicrobial antibiogram was performed using the following drugs: gentamicin, kanamycin, neomycin, streptomycin, erythromycin, lincomycin, chloramphenicol, minocycline, tetracycline, trimethoprim, sulfamethoxazole, fusidic acid, rifampicin, novobiocin, vancomycin, mupirocin, spectinomycin and ciprofloxacin. Erythromycin-inducible resistance to lincomycin was determined by the D-test.[20] For MSSA isolated after and including 1998 an eight-antimicrobial antibiogram was performed (erythromycin, tetracycline, trimethoprim, mupirocin, gentamicin,

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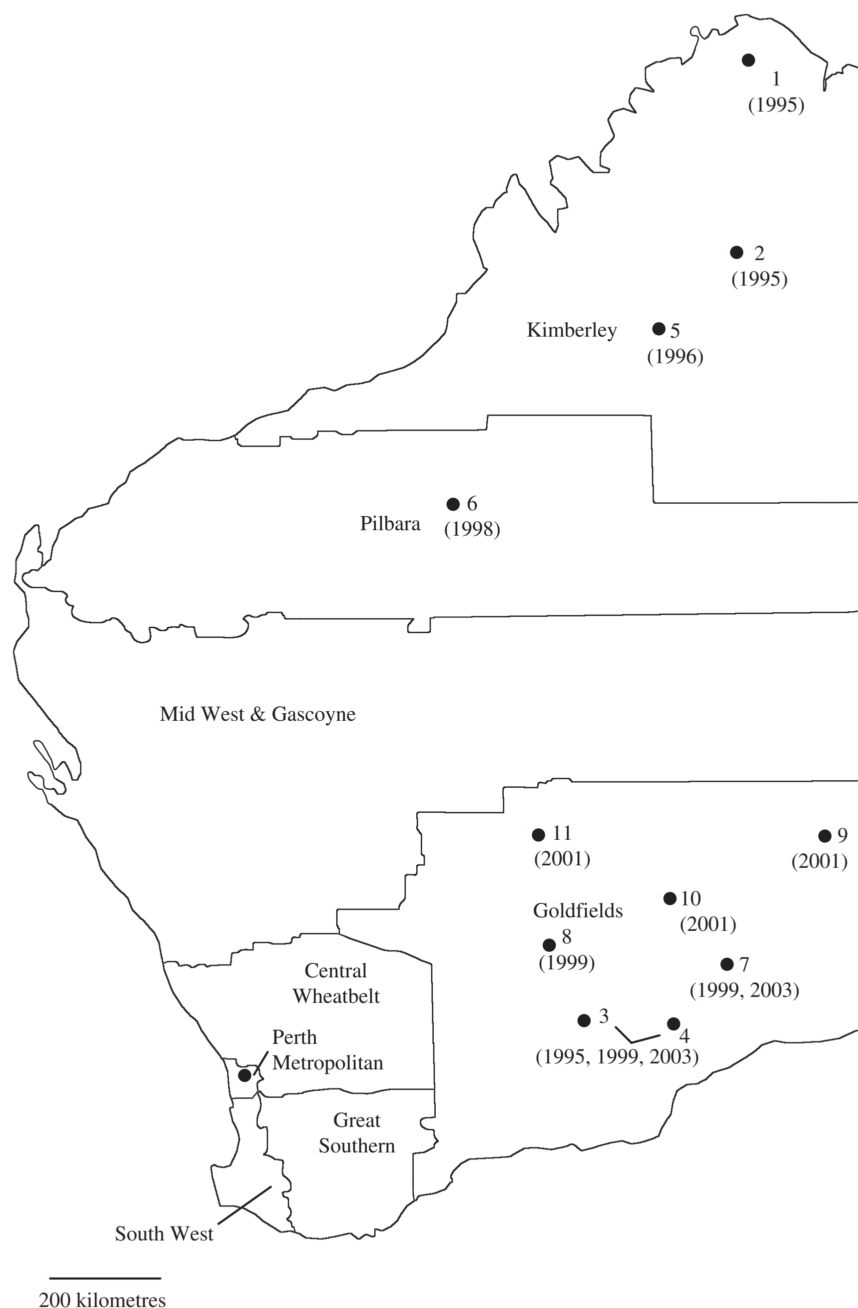

Figure 1. Geographical regions of WA, locations of surveyed communities and years of screening. Geographical regions are named, communities are indicated numerically.

ciprofloxacin, rifampicin and fusidic acid). The 18-antimicrobial antibiogram and penicillin susceptibility testing were performed on representatives of all MSSA STs. All *S. aureus* that had an 18-antimicrobial antibiogram were tested for β-lactamase production using Nitrocefin discs according to the instructions of the manufacturer (BBL, Becton Dickinson, Franklin Lakes, NJ, USA). Resistograms were performed as previously described[21] on all MRSA, and all MSSA isolated after 1998.

Contour-clamped homogeneous electric field electrophoresis

Contour-clamped homogeneous electric field electrophoresis (CHEF) was performed as previously described[22] on all isolates. Chromosomal banding patterns were scanned with a Fluor-S MultiImager and analysed by MultiAnalyst/PC (Bio-Rad Laboratories, Hercules, CA, USA) with a 0.8% band position tolerance. *S. aureus* isolates with ≥80% similarity were considered to belong to the same CHEF pulsotype; sub-pulsotypes were assigned according to the sub-clustering of patterns within the ≥80% similarity threshold. *S. aureus* NCTC8325 was used as the size standard. MRSA CHEF pattern pulsotypes were designated as previously published[23] and MSSA CHEF pattern pulsotypes were designated numerically. Isolates with pulsotypes containing fewer than three isolates were considered to be sporadic and, apart from PVL and antibiogram testing, were not investigated further in this study.

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MLST

MLST was performed as previously described.[24] All MRSA pulsotypes and sub-pulsotypes and all MSSA pulsotypes and sub-pulsotypes that contained three or more isolates were characterized by MLST. The sequences were submitted to http://mlst.net where an allelic profile was generated and an ST assigned. Clonal complex (CC) was determined using the eBURST V3 algorithm at the same website. Clones that diverged at no more than one of the seven MLST loci were considered to belong to the same CC. Double locus variants (dlvs) were included if the linking single locus variant (slv) was present in the MLST database. An *S. aureus* clone was defined by its ST. Isolates that belonged to the same CC were considered to be of the same genetic lineage.

SCC mec typing

SCC*mec* typing was performed using previously published primers that identified the class of *mec* complex and type of cassette chromosome recombinase (*ccr*) complex encoded on the element.[25] Structural architecture was determined with the multiplex PCR primers of Zhang *et al.*[26] and extra primers were utilized to test for SCC*mec* type IV subtypes a, b, c and d.[27] SCC*mec* nomenclature was as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome (IWG-SCC) Elements. Briefly, the structural type is indicated by Roman numerals with a lower case Arabic letter indicating the sub-type and the *ccr* and *mec* complexes are indicated by an Arabic number and letter, respectively, in parentheses.

PVL

The PVL determinant was detected using previously published primers[28] and confirmed by sequencing.

Accessory genetic regulator (agr)

agr was typed using either the ArrayTube System according to the manufacturer's instructions (Clondiag, Jena, Germany) or primers from previous studies.[29,30]

Arginine catabolic mobile element (ACME)

The ACME was detected by PCR as described previously.[31]

Criteria for testing

A colony was selected from each plate from each anatomical site. If there was more than one morphological colony type a representative of each was tested. If these isolates were subsequently found to be different by the typing methods they were included in the study as individual strains.

Results

Of the 2146 sets of screening swabs, 663 sets grew MSSA and 153 grew MRSA. Table 1 presents the sites that were positive for MSSA or MRSA for each set of screening swabs. Of the 1172 *S. aureus* isolated, 933 were MSSA and 239 were MRSA. Overall, 762 isolates of *S. aureus* consisting of 523 MSSA (from 454 sets of screening swabs) and 239 MRSA (from 153 sets of screening swabs) were characterized in this study.

Table 1. Sites of isolation of MSSA and MRSA from people in remote WA communities

Site of isolation	MSSA	MRSA
Anterior nares only	139	49
Anterior nares and throat	56	15
Anterior nares and skin lesions	33	9
Anterior nares, throat and skin lesions	21	6
Throat only	163	30
Throat and skin lesions	43	5
Skin lesions only	208	39
Total positive screening sets	663	153

There was a variation in the ratio of MSSA and MRSA carriage between the three geographical regions. MRSA comprised 4%, 24% and 32% of total *S. aureus* from the Kimberley, Pilbara and Goldfields regions, respectively.

There were differences in the colonization sites of MSSA and MRSA. MSSA was grown from 249 (37.6%), 283 (42.7%) and 305 (46%) anterior nares, throat and skin lesion swabs, respectively, while 79 (51.6%), 56 (36.6%) and 59 (38.6%) anterior nares, throat and skin lesion swabs, respectively, grew MRSA. For MSSA the highest rates of colonization were from skin lesions followed by throat and for MRSA they were anterior nares followed by skin lesions. When considering the overall positive screening sites, the highest recovery of MSSA per screening set was from throat and/or skin lesion swabs (79%) while the highest recovery of MRSA was from anterior nares and/or skin lesion swabs (80.4%). Of 59 sets of screening swabs in which MRSA was cultured from skin lesions, 15 (25%) demonstrated co-colonization with MRSA in the anterior nares.

Genetic lineages

Using CHEF the 523 MSSA were classified into 84 pulsotypes, of which 27 pulsotypes had three or more isolates (Table 2). The 239 MRSA were classified into five pulsotypes (Table 3). These five pulsotypes also contained MSSA, with MRSA pulsotypes WA-1, -2, -3, -4 and -5 corresponding to MSSA pulsotypes MSSA1, 5, 3, 14 and 26, respectively (Tables 2 and 3). Overall 92.7% (467 MSSA and 239 MRSA) of the 762 *S. aureus* clustered into 27 pulsotypes, from which 21 STs belonging to 13 CCs and two Singleton lineages were identified by MLST (Table 4). Eight lineages (CC15, CC121, CC101, CC25, CC20, CC398, CC12 and CC188) and the two Singleton lineages (Singleton 93 and Singleton 760) contained MSSA only. Five lineages (CC1, CC5, CC88, CC45 and CC8) contained MSSA and MRSA. CC5 contained two MRSA clones, ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B) (Figure 2) and CC45 contained two MRSA clones, ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B).

Seven previously undescribed STs were identified: Singleton ST760-MSSA; the CC1 clones ST761-MSSA and ST762-MSSA; the CC5 clone ST73-MSSA; the CC15 clone ST832-MSSA; the CC398 clone ST813-MSSA; and ST833-MSSA from CC188. Together with the previously reported ST73-MRSA-IVa (2B)[32] and ST93-MRSA-IVa (2B), which are rarely found outside

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Table 2. Characteristics of representative methicillin-susceptible *S. aureus* from remote WA communities

Isolate	Resistance/Bla	Pulsotype	CC	ST, allelic profile	PVL	*agr* type
W17S	PCd Bla+	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
N126S	PEL[I]Cd Bla+	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
C229T	Cd Bla+	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
W20S	s Bla−	MSSA2	15	15, 13-13-1-1-12-11-13	−	II
WL90T	PEL[I]Cd Bla+	MSSA25	15	15, 13-13-1-1-12-11-13	−	II
N133T	PCCd Bla+	MSSA29	15	15, 13-13-1-1-12-11-13	−	II
W16S	PCd Bla+	MSSA25	15	15, 13-13-1-1-12-11-13	−	II
P3S	PECd Bla+	MSSA20	15	15, 13-13-1-1-12-11-13	−	II
J27T	PCd Bla+	MSSA30	15	15, 13-13-1-1-12-11-13	−	II
K43T	PCd Bla+	MSSA22	15	832, 13-13-111-1-12-11-13	−	II
WL6N	PCd Bla+	MSSA27	5	5, 1-4-1-4-12-1-10	−	II
K185N	PCd Bla+	MSSA3	5	73, 1-4-27-4-12-1-10	−	II
K153N	PCdEb Bla+	MSSA3	5	73, 1-4-27-4-12-1-10	−	II
WL36N	P Bla+	MSSA3	5	73, 1-4-27-4-12-1-10	−	II
K112L	PCd Bla+	MSSA12	5	6, 12-4-1-4-12-1-3	−	I
Y15S	PFCd Bla+	MSSA1a	1	1, 1-1-1-1-1-1-1	−	III
Y74T	PCd Bla+	MSSA1b	1	761, 1-1-104-1-1-103-1	−	NT
K45S	PECdHg Bla+	MSSA1c	1	762, 1-1-104-1-1-1-1	−	III
K120L	PCd Bla+	MSSA1c	1	762, 1-1-104-1-1-1-1	−	III
C38S	PTCd Bla+	MSSA11	S	760, 10-1-1-1-1-102-1	−	III
C49N	PCd Bla+	MSSA14	45	45, 10-14-8-6-10-3-2	−	I
C54N	s Bla−	MSSA14	45	45, 10-14-8-6-10-3-2	−	I
C30S	P Bla+	MSSA21	45	45, 10-14-8-6-10-3-2	−	IV
M11N	PCd Bla+	MSSA17	45	45, 10-14-8-6-10-3-2	−	I
K102N	PCd Bla+	MSSA28	45	508, 10-40-8-6-10-3-2	−	I
K25S	PCd Bla+	MSSA7	121	121, 6-5-6-2-7-14-5	+	IV
Y1S	P Bla+	MSSA23	121	121, 6-5-6-2-7-14-5	−	IV
WB94E	P Bla+	MSSA19	121	121, 6-5-6-2-7-14-5	−	IV
W67N	PCd Bla+	MSSA5	88	78, 22-1-14-23-12-53-31	−	III
W91T	PCd Bla+	MSSA4	101	101, 3-1-14-15-11-19-3	−	I
W11T	PCd Bla+	MSSA33	25	25, 4-1-4-1-5-5-4	−	I
C57S	PCd Bla+	MSSA13	20	20, 4-9-1-8-1-10-8	−	I
J107N	PCd Bla+	MSSA24	20	20, 4-9-1-8-1-10-8	−	I
N91T	P Bla+	MSSA26	8	8, 3-3-1-1-4-4-3	−	I
W101S	s Bla−	MSSA10	329	813, 3-37-19-2-20-26-32	−	I
C33S	PCd Bla+	MSSA12	12	12, 1-3-1-8-11-5-11	−	II
W36S	PCd Bla+	MSSA8	188	833, 100-1-1-8-12-1-1	−	III

Bla, β-lactamase; C, chloramphenicol; Cd, cadmium acetate; E, erythromycin; Eb, ethidium bromide; F, Fusidic acid; Hg, mercuric chloride; L, lincomycin; P, penicillin; T, tetracycline; superscript I, inducible; superscript +, positive; superscript −, negative; s, susceptible to all antimicrobials tested; S, singleton lineage; NT, non-typeable.

Australia, these isolates appear to represent geographically limited clones that have probably emerged in Australia.

The lineages of *S. aureus* that contained the most isolates were CC1 (18%), CC5 (17.5%), Singleton 93 (14.7%), CC15 (10.6%), CC45 (8.4%), CC88 (6.4%) and CC121 (4.6%). There was no evidence of the emergence of new dominant clones of *S. aureus* during the period of the surveys.

MSSA. The most prevalent MSSA were ST93-MSSA from the Singleton 93 lineage (21.4%), ST15-MSSA from CC15 (14.9%), ST73-MSSA from CC5 (10.1%) and ST45-MSSA from CC45 (5.9%) (Table 4).

Four lineages of MSSA (CC1, CC5, CC15 and CC45) contained slvs, with CC1 also containing a dlv (ST761-MSSA) (Table 2). ST760-MSSA was an ST1-MSSA dlv; however, because the linking allele could not be found in the MLST database it was classified as a new Singleton lineage.

Some of the MSSA lineages showed divergence of CHEF pattern pulsotypes (Table 2). CC15 had diversified into five unrelated pulsotypes, of which one was the slv ST832-MSSA. CC5 contained three pulsotypes representing each of the CC5 clones, ST73-MSSA, ST5-MSSA and ST6-MSSA. There were four unrelated pulsotypes in CC45, one of them being the slv ST508-MSSA clone. CC121 had three pulsotypes, and CC20 had two. The remaining lineages each had one CHEF pulsotype. CC1 isolates also belonged to only one pulsotype; however, there were three sub-pulsotypes that represented the clones ST1-MSSA, ST761-MSSA and ST762-MSSA. ST6-MSSA (CC5) and ST12-MSSA (CC12), although genetically unrelated by MLST, both had the same MSSA12 pulsotype.

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Table 3. Characteristics of representative MRSA from remote WA communities

Isolate	Resistance and Bla	Pulsotype	CC	ST, allelic profile	SCC*mec*	PVL	*agr* type	ACME
WBG8287	$EL^{I}FCd\ Bla^{+}$	WA-1	1	1, 1-1-1-1-1-1-1	IVa (2B)	−	III	−
WBG8375	$EL^{I}Cd\ Bla^{+}$	WA-1a	1	1, 1-1-1-1-1-1-1	IVa (2B)	−	III	−
WBG9409	$EL^{I}FCd\ Bla^{+}$	WA-1c	1	1, 1-1-1-1-1-1-1	IVa (2B)	−	III	−
WBG8361	$EL^{I}Cd\ Bla^{+}$	WA-1d	1	1, 1-1-1-1-1-1-1	IVa (2B)	−	III	−
M28S	$Cd\ Bla^{+}$	WA-1f	1	1, 1-1-1-1-1-1-1	IVa (2B)	−	III	−
WBG8366	$EL^{I}\ Bla^{+}$	WA-2	88	78, 22-1-14-23-12-53-31	IVa (2B)	−	III	−
WL106N	$EL\ Bla^{+}$	WA-2a	88	78, 22-1-14-23-12-53-31	IVa (2B)	−	III	−
C219N	$Cd\ Bla^{+}$	WA-2c	88	78, 22-1-14-23-12-53-31	IVa (2B)	−	III	−
C8N	$EL^{I}Cd\ Bla^{+}$	WA-3	5	5, 1-4-1-4-12-1-10	IVa (2B)	−	II	−
WBG8381	$s\ Bla^{-}$	WA-3a	5	5, 1-4-1-4-12-1-10	IVa (2B)	−	II	−
WB43S	$EL^{I}\ Bla^{+}$	WA-3b	5	73, 1-27-1-4-12-1-10	IVa (2B)	−	NT	−
WL36N	$Cd\ Bla^{-}$	WA-3b	5	73, 1-27-1-4-12-1-10	IVa (2B)	−	II	−
WBG8379	$EL^{I}Cd\ Bla^{+}$	WA-3c	5	5, 1-4-1-4-12-1-10	IVa (2B)	−	II	−
WB101N	$EL^{I}\ Bla^{+}$	WA-3h	5	5, 1-4-1-4-12-1-10	IVa (2B)	−	II	−
WBG8404	$CdAs\ Bla^{+}$	WA-4	45	45, 10-14-8-6-10-3-2 45	V (5C2)	−	Ia	−
WBG8399	$CdAs\ Bla^{+}$	WA-4a	45	45, 10-14-8-6-10-3-2 45	V (5C2)	−	I	−
WBG8355	$CdAs\ Bla^{+}$	WA-4b	45	45, 10-14-8-6-10-3-2 45	IVa (2B)	−	I	−
WBG7583	$EL^{I}TCd\ Bla^{+}$	WA-5	8	8, 3-3-1-1-4-4-3	IVa (2B)	−	I	−

As, sodium arsenate; Bla, β-lactamase; Cd, cadmium acetate; E, erythromycin; F, fusidic acid; L, lincomycin; superscript I, inducible; superscript +, positive; superscript −, negative; s, susceptible to all antimicrobials tested; NT, non-typeable.

Table 4. Genetic lineages of *S. aureus* present in remote WA communities

Genetic lineage, CC and ST	MRSA number (%)	MSSA number (%)
Singleton (93) and 93		112 (21.4)
15 and 15		78 (14.9)
15 and 832		3 (0.6)
5 and 5	32 (13.4)	3 (0.6)
5 and 73	42 (17.6)	53 (10.1)
5 and 6		3 (0.6)
1 and 1	102 (42.7)	7 (1.3)
1 and 761		7 (1.3)
1 and 762		21 (4)
Singleton (760) and 760		19 (3.6)
45 and 45	30 (12.5)	31 (5.9)
45 and 508		3 (0.6)
121 and 121		35 (6.7)
88 and 78	30 (12.5)	19 (3.6)
101 and 101		15 (2.8)
25 and 25		16 (3.1)
20 and 20		13 (2.5)
8 and 8	3 (1.3)	16 (3.1)
398 and 813		5 (1)
12 and 12		4 (0.8)
188 and 833		4 (0.8)
Sporadic		56 (10.7)
Totals	239 (100)	523 (100)

MRSA. When compared with MSSA there was less diversity in the MRSA lineages. Five lineages were identified in the communities screened in the 1995 surveys. No additional lineages were found in these or the other communities in subsequent surveys. ST1-MRSA-IVa (2B) was the most frequently isolated MRSA clone (42.7%), followed by ST73-MRSA-IVa (2B) (17.6%), ST5-MRSA-IVa (2B) (13.4%), ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) (12.5%), ST78-MRSA-IVa (2B) (12.5%) and ST8-MRSA-IVa (2B) (1.3%) (Tables 3 and 4).

The five MRSA lineages corresponded to five CHEF pulsotypes that have previously been identified.[11,33] Four of the pulsotypes had sub-pulsotypes. Although all lineages containing MRSA also contained MSSA, they were not the largest MSSA lineages (Table 4). The CC1 MRSA clone, ST1-MRSA-IVa (2B), formed 42.7% of all MRSA while the methicillin-susceptible counterpart, ST1-MSSA, formed only 1.3% of the MSSA population. Similarly, in CC5, ST5-MRSA-IVa (2B) was 13.4% of the MRSAs while ST5-MSSA formed only 0.6% of the MSSAs and ST78-MRSA-IVa (2B) was 12.5% of the MRSA while the corresponding MSSA was only 3.6% of the MSSA population. MRSAs were not found in the largest MSSA lineages of Singleton 93 and CC15.

Antimicrobial resistance

MSSA. A full 18-antimicrobial antibiogram and penicillin susceptibility testing was performed on 37 MSSA clones representative of the lineages. All were resistant to penicillin and produced β-lactamase except for three, which were fully susceptible (Table 2). Two isolates expressed an MLS_{Bi} resistance phenotype (inducible resistance to erythromycin and erythromycin-inducible resistance to lincomycin), an additional

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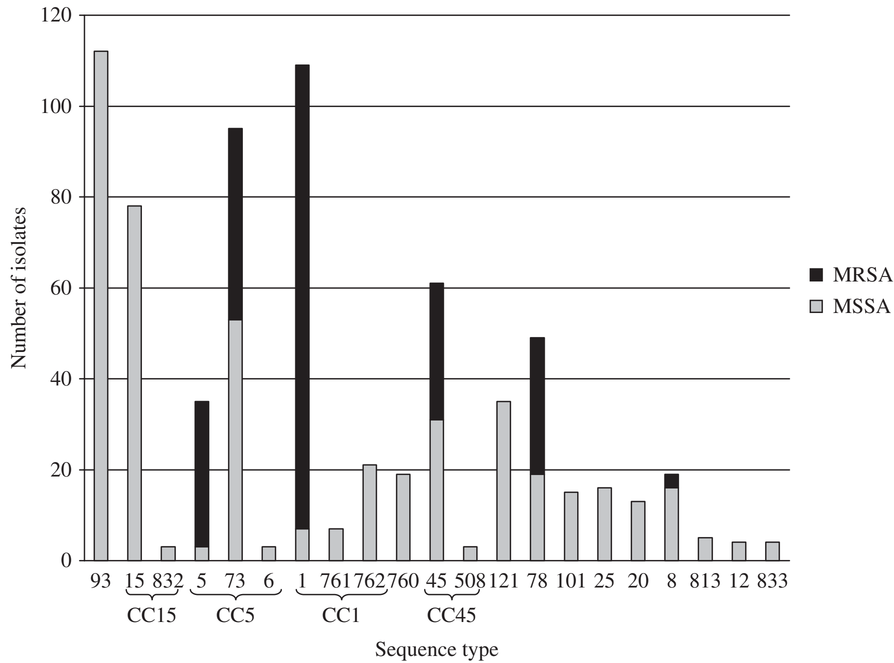

Figure 2. Distribution of MSSA and MRSA amongst the genetic lineages of *S. aureus* present in remote WA communities. Brackets indicate clones that belong to the same CCs.

isolate had constitutive erythromycin resistance and another was chloramphenicol resistant.

An eight-antimicrobial antibiogram was performed on 363 isolates. Eighty-three (22.9%) were fully susceptible to all antimicrobials; 134 (36.9%) were erythromycin resistant, six (1.7%) were fusidic acid resistant, 4 (1.1%) were gentamicin resistant, 6 (1.7%) were trimethoprim resistant and 9 (2.5%) were tetracycline resistant.

A resistogram was performed on 423 MSSA. Of these, 254 (60%) were cadmium resistant, 4 were arsenate resistant, 1 was mercuric chloride resistant, 1 was mercuric chloride and phenyl mercuric acetate resistant, and 4 were ethidium bromide resistant. There were no associations between antimicrobial resistance profile and genetic lineage.

MRSA. All except two of the 239 MRSA isolates were additionally resistant to fewer than two antibiotic classes and therefore non-multi-resistant.[10] Fifty-nine (24.7%) of the isolates were fusidic acid resistant, all of which were ST1-MRSA-IVa (2B). Within CC1, 59 (57.8%) of the 102 MRSA isolates were fusidic acid resistant. One hundred and thirty three isolates (55.6%), including isolates from ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B), ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B), were erythromycin resistant, four (1.7%) isolates, two from ST5-MRSA-IVa (2B) and one each from ST45-MRSA-V (5C2) and ST8-MRSA-IVa (2B), were mupirocin resistant and three (1.3%), two from ST8-MRSA-IVa (2B) and one ST1-MRSA-IVa (2B) were tetracycline resistant. All except two of the representative MRSA (WBG8381 and WL36N) produced β-lactamase (Table 3).

The most prevalent MRSA lineage was also resistant to the most antibiotics. Forty-two of the ST1-MRSA-IVa (2B) isolates that were fusidic acid resistant also expressed the MLS_{Bi} resistance phenotype; one of these was additionally tetracycline resistant and therefore was multi-resistant by definition.[10] The other multi-resistant isolate was an ST45-MRSA-V (5C2) skin lesion isolate that had the MLS_{Bi} resistance phenotype as well as being gentamicin, kanamycin and mupirocin resistant. Interestingly, the individual who harboured this clone also harboured ST45-MRSA-V (5C2) isolates from the anterior nares and throat that were susceptible to all antibiotics except the β-lactams.

All except 10 of the MRSA (95.8%) were cadmium resistant and 26 (10.9%) were arsenate resistant. Arsenate resistance was exclusively linked with ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) clones.

PVL

At least one representative isolate from all MSSA and MRSA CHEF pulsotypes and sub-pulsotypes was tested for the presence of the PVL determinant. Two lineages of MSSA harboured the determinant; seven of eight ST93-MSSA tested were found to carry PVL and of three ST121-MSSA tested one carried the determinant (Table 2). No MRSA carried PVL.

agr and ACME

The *agr* type was determined on representative clones and revealed four major *agr* types in the *S. aureus* isolates (Tables 3 and 4). Five lineages (CC101, CC25, CC20, CC329 and CC8) were *agr* I, two (CC15 and CC12) were *agr* II, five (Singleton 93, CC1, Singleton 760, CC88 and CC188) were *agr* III, and one lineage (CC121) was type IV. CC5 isolates were *agr* II except for a ST6-MSSA clone that was type I. CC45 had members in *agr* types I, Ia and IV. Two isolates (WB43S and Y74T) were non-typeable.

MSSA clones from all *agr* groups were present; however, no *agr* type IV MRSA was found. The PVL-positive clones ST93-MSSA and ST121-MSSA belonged to *agr* groups III and

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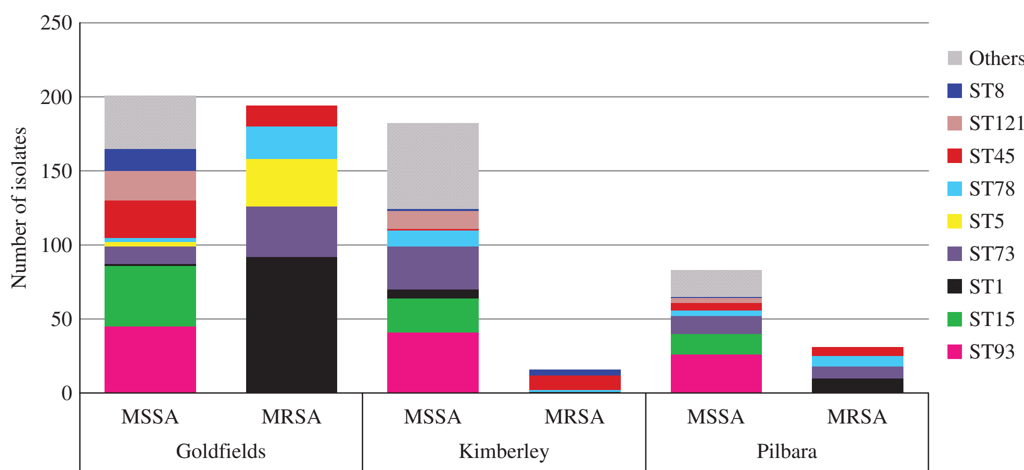

Figure 3. Distribution of the predominant genetic lineages of *S. aureus* throughout remote communities in three geographical regions of WA.

IV, respectively. There was no correlation between site of colonization and *agr* type, and clones with both the same and different *agr* types colonized the same individual and/or sites (not shown).

Representative MRSA were tested for the presence of the ACME and none encoded the element.

Geographical distribution

There were local differences in the *S. aureus* clones present in the geographical regions (Figure 3). ST1 was the predominant clone in the Goldfields region and ST93 and ST73 were predominant in Kimberley and Pilbara. Thirteen of the 21 clones (STs 1, 6, 8, 15, 20, 25, 45, 73, 78, 93, 121, 760 and 762) were found in all geographical regions.

The MRSA clones ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B) and ST45-MRSA-V (5C2) were found in all regions, ST5-MRSA-IVa (2B) and ST45-MRSA-IVa (2B) were found only in the Goldfields, and ST8-MRSA-IVa (2B) was found only in the Kimberley region. The most prevalent MRSA clone in the Goldfields was ST1-MRSA-IVa (2B) (47%) followed by ST73-MRSA-IVa (2B) (18%). Similarly, in the Pilbara region the prevalent MRSA clones were ST1-MRSA-IVa (2B) (32%) and ST73-MRSA-IVa (2B) (26%), while in Kimberley the predominant clone was ST45-MRSA-V (5C2) (62.5%).

Clones ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B), ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B) were found in all years of the surveys and were considered to be endemic in the communities. The ST8-MRSA-IVa (2B), ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) clones were not found subsequent to 1998.

Genetics of colonization

There was no apparent correlation between ST and site of isolation (not shown).

People harboured clones belonging to a diversity of genetic lineages at the same or multiple sites. MRSA and MSSA were found together in 79 sets of screening swabs; all clones from 30 of these were characterized. No person was found to have MRSA and MSSA of the same genetic lineage at either the same or different sites. Two hundred and fifty-seven (12%) sets of screening swabs had MSSA at more than one site. Of 56 with characterized clones at multiple sites, only 12 (21%) had clones of the same genetic lineage at all sites and 44 (79%) had a different lineage at each site. Two sets of screening swabs that yielded MSSA at three sites had isolates of a different genetic lineage at each site.

In contrast to MSSA, of 39 sets of screening swabs where MRSA was found at multiple sites, 36 (92%) had clones of the same genetic lineage at all sites with only three (8%) harbouring MRSA of different lineages.

Discussion

Population studies of *S. aureus* thus far have identified five main genotypic clusters, CC5, CC8, CC22, CC30 and CC45, as forming the essential genetic backgrounds of *S. aureus*, with differences occurring principally in the local prevalence of the genotypes and the presence of minor clones.[34–36] Although these studies have been from Europe and the USA, a study by Melles *et al.*[34] performed in Indonesia, which has prehistoric links with remote WA, reported a similar *S. aureus* population structure to that of Europe and the USA. This study, however, reveals that the population structure of *S. aureus* in the geographically remote regions of WA is different. This difference is probably a consequence of the geographic and cultural isolation of the remote populations of WA; however, it has had an important influence on the epidemiology of MRSA in the entire WA community.

From a genetically diverse background consisting essentially of 21 clones of *S. aureus*, seven clones of MRSA belonging to five CCs were found. Four of the clones were considered to have been endemic in the communities and have subsequently become the most prevalent CA-MRSA clones in the wider WA community.[23] State-wide surveillance has revealed that in December 2006, ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B) and the CC5 clones [ST73-MRSA-IVa (2B) and ST5-MRSA-IVa (2B)] comprised 56.7%, 30.5% and 8.9% of clinical and surveillance CA-MRSA in WA, respectively. The CC45 clones [ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B)], and ST8-MRSA-IVa (2B), which were not found in the remote communities after 1998, formed only 1.9% and 0.8%, respectively, of

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clinical and surveillance CA-MRSA in WA in 2006[11] suggesting that they are not as well adapted to the WA community environment.

The MRSA did not belong to the most prevalent MSSA lineages, yet, with the exception of ST8-MRSA-IVa (2B), they formed the greater proportion of isolates present in the lineage to which they belonged, suggesting that an advantage was gained by acquisition of the SCC*mec* element. It would appear, however, that only a limited number of clones acquired and maintained the SCC*mec* element, even though β-lactamase-stable β-lactams were widely used empirically in the communities. The clonal structure of MRSA and the small amount of genetic diversity when compared with MSSA indicate not only the more recent emergence of MRSA, but also that dissemination of MRSA has probably occurred along clonal lines by well-adapted community clones that could support the SCC*mec* element.

The most prevalent MSSA lineage was the PVL-positive Singleton 93 clone ST93-MSSA, which has been rarely found outside Australia. No Singleton 93 MRSA was found during the period of the surveys. PVL-positive ST93-MRSA-IVa (2B), also known as the Queensland clone, however, is an important Australian CA-MRSA that was originally found in a Caucasian population in Queensland in 2000 and has been reported in Indigenous people from Queensland[5,37] and the Northern Territory.[38] It is interesting that in an environment of high β-lactam use a methicillin-resistant variant of ST93-MSSA was not found in WA during these surveys.

S. aureus isolates from most of the lineages were found at all sites tested. The highest recovery of MSSA of 42.6% was from the throat while for MRSA the highest recovery of 51.6% was from the anterior nares. Although the anterior nares is the preferred screening site for population studies, in this study many isolates of *S. aureus* would have been missed if the throat and skin lesions had not also been swabbed. It has been established previously that there is a high incidence of skin pathology associated with *S. aureus* in remote Australian communities[38] and the recovery figures in this study are clearly influenced by the high numbers of skin lesions found amongst the survey participants.

The clonal nature of MRSA and the tendency for people carrying MRSA at multiple sites to harbour clones of the same genetic lineage as opposed to those with MSSA, who tended to have different lineages at each site, indicates that MRSA in the WA remote communities are well-adapted colonizers that could possibly displace MSSA as asymptomatic commensal organisms. Furthermore, unless the use of β-lactamase-stable antibiotics is curtailed they could become the predominant colonizing organisms in the communities.

Very few remote region *S. aureus* isolates were resistant to multiple antimicrobials; however, the potential for the emergence of resistance was indicated by the presence of several antimicrobial resistance determinants amongst the population. In addition to the SCC*mec* element, determinants for resistance to penicillin, fusidic acid, MLS_{Bi}, erythromycin, tetracycline, gentamicin, kanamycin, mupirocin, trimethoprim and chloramphenicol were present.

The resistance determinants for β-lactamase production, MLS_{Bi}, mupirocin and trimethoprim in remote WA community MRSA are plasmid borne,[33,39] and other studies have shown that those for gentamicin and kanamycin are on a transposon while those for erythromycin, tetracycline and chloramphenicol are on plasmids. In view of the increased isolation rates of CA-MRSA in clinical specimens in WA it would be instructive to assess the current status of CA-MRSA in the remote communities to determine if there is a need to control the local use of antibiotics. Such control could be predicated upon the known resistance determinants in the *S. aureus* populations of the communities. It is imperative that careful antibiotic management guidelines are established and administered in the communities to prevent CA-MRSA acquiring additional resistance determinants and spreading further. The importance of this was indicated from results (not shown) from communities 4 and 5. When these communities were initially screened the prevalence of MRSA was 43% and 22%, respectively. As a consequence, non-β-lactam antibiotics replaced the empirical administration of β-lactam antibiotics for *S. aureus* infections and re-screening of the communities four years later revealed that the prevalence of MRSA had dropped to 11% and 7%, respectively.

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Transparency declarations

None to declare.

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8.2. PAPER TWO

Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region.

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Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region

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Abstract

Background: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote regions of Western Australia and is now the predominant MRSA isolated in the state. The objective of this study is to determine the genetic relatedness of Western Australian CA-MRSA clones within different multilocus sequence type (MLST) clonal clusters providing an insight into the frequency of *S. aureus* SCC*mec* acquisition within a region.

Results: The CA-MRSA population in Western Australia is genetically diverse consisting of 83 unique pulsed-field gel electrophoresis strains from which 46 MLSTs have been characterised. Forty five of these sequence types are from 18 MLST clonal clusters and two singletons. While SCC*mec* IV and V are the predominant SCC*mec* elements, SCC*mec* VIII and several novel and composite SCC*mec* elements are present. The emergence of MRSA in diverse *S. aureus* clonal clusters suggests horizontal transmission of the SCC*mec* element has occurred on multiple occasions. Furthermore DNA microarray and *spa* typing suggests horizontal transfer of SCC*mec* elements has also occurred within the same CC. For many single and double locus variant CA-MRSA clones only a few isolates have been detected.

Conclusions: Although multiple CA-MRSA clones have evolved in the Western Australian community only three clones have successfully adapted to the Western Australian community environment. These data suggest the successful evolution of a CA-MRSA clone may not only depend on the mobility of the SCC*mec* element but also on other genetic determinants.

Background

Based on phenotypic and genotypic typing methods, community onset methicillin-resistant *Staphylococcus aureus* infections are caused by healthcare-associated MRSA (HA-MRSA) strains, which appear to have been transferred from hospitals or healthcare facilities into the community by patients or healthcare workers [1], or by community-associated MRSA (CA-MRSA) strains, which have been isolated from people who have had little or no contact with healthcare facilities or healthcare workers [2]. This distinction between community and healthcare facility however has become blurred with the replacement of HA-MRSA with CA-MRSA in hospitals [3,4].

In contrast to HA-MRSA, CA-MRSA strains are generally more susceptible to non beta-lactam antibiotics, grow significantly faster, have different clonal backgrounds, carry smaller staphylococcal cassette chromosome *mec* (SCC*mec*) elements (most commonly SCC*mec* type IV or type V), have enhanced virulence properties and frequently harbor genes expressing Panton-Valentine leukocidin (PVL) [5-8]. Rather than a worldwide spread of a single clone multiple CA-MRSA clones have emerged from diverse genetic backgrounds. Several well characterized CA-MRSA clones predominate in different regions: Sequence type (ST) 8-IV [2B]

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(USA300) and ST1-IV [2B] (USA400) in North America [9,10]; ST80-IV [2B] (European clone) in Europe [8], North Africa [11] and the Middle East [12]; ST59-V [5C2&5] (Taiwan clone) in Taiwan [13]; ST93-IV [2B] (Queensland clone) in Australia [14], ST30-IV [2B] (South West Pacific [SWP] CA-MRSA) in the Western Pacific [15,16], and ST772-V [5C2] (Bengal Bay clone) in India and Bangladesh [17]. Transmission of these clones into other regions has occurred [18,19]. This occurrence of concurrent epidemics of CA-MRSA in many countries by different clones has been striking. Equally noteworthy are a number of common features of these epidemics, prominent among them the ability to cause severe infections in young otherwise healthy people and the carriage of the *lukF-PV/lukS-PV* PVL encoding genes by the organism.

The earliest report of CA-MRSA infections involved indigenous people living in remote communities in the sparsely populated Kimberley region of Western Australia (WA) [20]. Approximately 50% of the people in this region are indigenous, many of whom live in poor socioeconomic conditions. Infected skin lesions and staphylococcal sepsis occur frequently and empirical antistaphylococcal therapy is often prescribed. Colloquially known as "WA-MRSA", the early isolates have a similar pulsed-field gel electrophoresis (PFGE) pattern and have subsequently been characterized as a single clone; PVL-negative WA5 (ST8-IV/*spa* t008) [21]. By 2006 22 CA-MRSA clones were identified in WA, with PVL-negative WA 1 (ST1-IV [2B]/t127) replacing WA5 as the predominant clone [22]. At this time CA-MRSA from indigenous people living in remote areas outside of WA were reported in the Northern Territory [23], Queensland [24] and Central Australia [25]. As may be expected in a geographically large country with relatively few dense concentrations of population, often separated by large areas of desert, different CA-MRSA clones evolved in these communities.

In 1982 colonization or infection with MRSA became a notifiable condition in WA. For infection control purposes all MRSA isolated in the state since 1997 have been referred to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species* (ACCESS) Typing and Research where based on molecular markers they are characterized as either HA-MRSA or CA-MRSA [26]. Although a state-wide policy of screening all patients and healthcare workers who have lived outside the state for MRSA has prevented HA-MRSA from becoming endemic in Western Australian hospitals, it has not prevented CA-MRSA from becoming established in the community. In WA the public health system is divided into two metropolitan health regions and seven country health regions. The state encompasses an area of 1.02 million square miles and has a population of approximately 2.24 million people. In 1983, the overall rate of MRSA notifications was 10 per 100,000 persons in the rural country health regions and 7/100,000 in the metropolitan regions [27]. By 2006 notifications rates throughout the state had increased to 179/100,000 persons of which 144/100,000 were CA-MRSA. In the metropolitan health regions the CA-MRSA notification rate was 134/100,000 whilst in the Kimberley health region the CA-MRSA notification rate had increased 40-fold to 391/100,000 [18].

CA-MRSA is thought to emerge when a locally prevalent strain of methicillin susceptible *S. aureus* (MSSA) acquires a SCC*mec* element and utilizes mobile genetic elements and single nucleotide polymorphisms to establish local and geographic niches [28]. As WA is a remote region in which all MRSA isolates are referred to a central typing laboratory it is an ideal environment to study the emergence and evolution of CA-MRSA. MLST, SCC*mec*, *spa* typing and microarray DNA is performed on all isolates with a unique PFGE pulsotype. The aim of this study is to determine the genetic relatedness of WA CA-MRSA clones within different MLST clonal clusters (CC) providing an insight into the frequency of *S. aureus* SCC*mec* acquisition within a region. The genetic profile of these clones may also offer an explanation why only a few WA CA-MRSA clones have successfully adapted to the community environment.

Results

The 83 unique PFGE strains isolated in Western Australia from 1989 to 2010 were *nuc* and *mecA* gene positive by PCR. The DNA microarray *S. aureus* species markers *gapA* (glyceraldehyde 3-phosphate dehydrogenase) and *rrn STAU* (*S. aureus* ribosomal marker) were detected in all strains. The array's linear primer elongation method detected the *katA* (catalase A), *coA* (coagulase), *nuc*, *spa* (protein A) and *sbi* (IgG-binding protein) *S. aureus* species markers in 78 strains. These markers were either not detected or detected only by random amplification in five strains (WA8, WA47, WA72, WA76 and WA79).

Forty six STs were identified by MLST. Using the MLST website's eBURST V3 algorithm 45 STs were grouped into 18 CCs and two singletons (Figure 1). The CC for WA76 (ST1303) has not been determined.

Several SCC*mec* types and subtypes, novel SCC*mec*s, and composite SCC*mec*s were identified. Forty five strains harbor SCC*mec* IVa-d [2B] (31 IVa, 2 IVb, 9 IVc, 3 IVd), 12 strains SCC*mec* V [5C2] and two strains SCC*mec* VIII [4A]. Two strains have non typeable SCC*mec* IV subtypes and four strains have a SCC*mec* element with a novel *ccr* gene complex including three with a class B *mec* gene complex and one with a class A *mec* complex. Eighteen strains

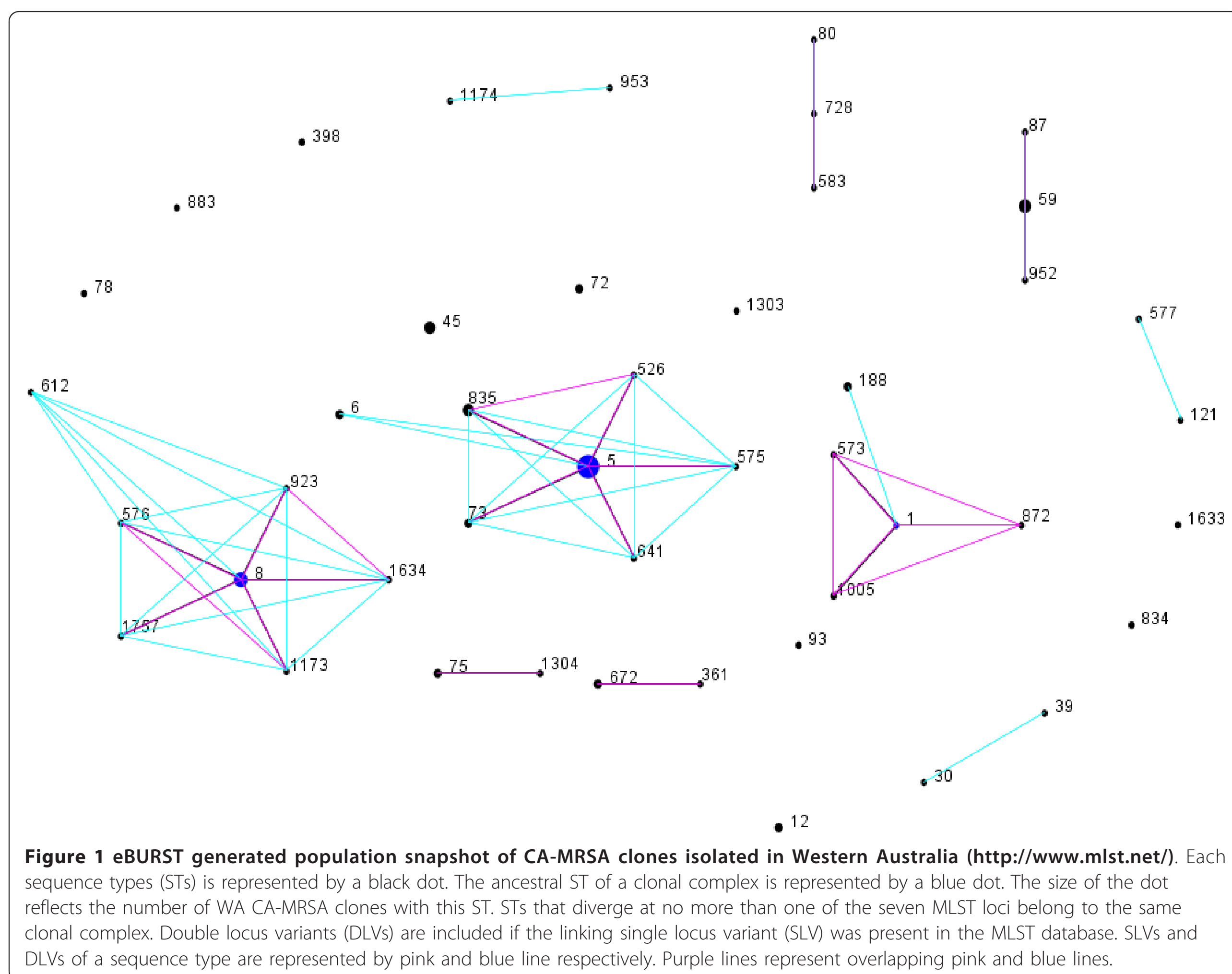

Figure 1 eBURST generated population snapshot of CA-MRSA clones isolated in Western Australia (http://www.mlst.net/). Each sequence types (STs) is represented by a black dot. The ancestral ST of a clonal complex is represented by a blue dot. The size of the dot reflects the number of WA CA-MRSA clones with this ST. STs that diverge at no more than one of the seven MLST loci belong to the same clonal complex. Double locus variants (DLVs) are included if the linking single locus variant (SLV) was present in the MLST database. SLVs and DLVs of a sequence type are represented by pink and blue line respectively. Purple lines represent overlapping pink and blue lines.

harbor SCC*mec* elements with composite *ccr* gene complexes including 12 with SCC*mec* V [5C2&5] (5C2 plus *ccrC1* allele 8), three with SCC*mec* IVa [2B]&5 (2B plus a type 5 *ccr* gene complex), one with V (5C2) &2 (5C2 plus a type 2 *ccr* gene complex) and two with V [5C2&5]&2 (a composite SCC*mec* V element plus a type 2 *ccr* gene complex).

The MLST, *spa* type, *agr* type, capsule type, SCC*mec*, antibiogram, resistance genotype, lukF/S-PVL genes, enterotoxin genes and bacteriophage associated virulence genes of each unique PFGE strain are provided in Additional File 1. Information on target genes, probes, and primers is provided in Additional File 2. Complete hybridization profiles for the individual strains can be provided on request.

Clonal Complex 1

CC1 contains five strains including the PVL positive Bengal Bay clone (ST772 [a single locus variant {slv} of ST1]-V [5C2]/t3387). This strain is epidemiologically linked to a healthcare worker from India and is not considered a WA CA-MRSA.

Based on the *agr*/capsule and SCC*mec* type, the remaining four strains are divided into two groups:

Group 1

agr type III/capsule type 8 SCC*mec* IVa [2B] contains PVL negative WA1 (ST1/t127), WA45 (ST872 [slv of ST1]/t127), and WA57 (ST1005 [ST1 slv]/t127). WA1 and WA45 harbor a *ccrA-1* and *ccB-1* gene complex and Q6GD50 (fusidic acid resistance marker) indicating the presence of the mobile fusidic acid SCC element SCC*fur*. WA1 is known to carry multiple plasmids such as a 2-kb plasmid encoding resistance to erythromycin [29] and this presumably accounts for the differences in the antibiogram and resistance genotype for WA1, WA45 and WA57. In addition to enterotoxin genes the three strains harbor a type D immune evasion cluster [IEC] (*seA+sak+scn*) [30]. Group 2

agr type II/capsule type 5 SCC*mec* V [5C2] contains PVL negative WA10 (ST573 [ST1 slv]/t5073. WA10

carries several enterotoxin genes including the enterotoxin *egc* cluster [*seG+seI+seM+seN+seO+seU/Y*]). Unlike WA1, WA45 and WA57, WA10 does not carry the type D IEC, the pathogenicity island harboring the leukocidin D/E component, the protease *splA* gene and the *hsdS* gene. The *ssl/set* genes and cell surface adhesions encoding genes of WA10 are closely related to the Bengal Bay clone.

Clonal Complex 5

CC5 contains 27 strains. Based on the *agr/*capsule type the isolates are divided into two groups which are further divided into subgroups based on the SCC*mec* type.

Group 1

agr type I/capsule type 8 (2 strains)

i. SCC*mec* IVa [2B] contains WA51 (ST6 [ST5 dlv]). The protein A variable region in WA51 could not be amplified and therefore a *spa* type cannot be allocated.

ii. SCC*mec* IVa [2B]&5 contains WA66 (ST6/t701).

WA51 and WA66 harbor a type D IEC Neither strain harbors the *lukF-PV/lukS-PV* PVL encoding genes.

Group 2

agr type II/capsule type 5 (25 strains)

Unlike Group 1 strains, these 25 strains harbour the enterotoxin *egc* cluster. Ten *spa* types were identified, of which nine are closely related: t002, t045, t071, t442, t688, t1265, t2666, t3378, t4065.

i. SCC*mec* IVa [2B] contains WA3 (ST5/t002), WA64 (ST5/t3778), WA71 (ST5/t002), WA82 (ST5/t002), WA25 (ST575 [ST5slv]/t002), WA50 (ST73 [ST5slv]/t002) and WA65 (ST73/t002). PVL negative WA3, WA71, WA82, WA25, WA50 and WA65 harbor a type F IEC (*seP+sak+chp+scn*). PVL positive WA64 harbors a type A IEC (*seA+sak +chp+scn*). WA64 and WA65 also harbor *edinA* (epidermal cell differentiation inhibitor A gene).

ii. SCC*mec* IVc [2B] contains PVL negative WA74 (ST5/t002) which harbors a type F IEC.

iii. SCC*mec* IV [2B] contains PVL negative WA39 (ST526 [ST5slv]/t4065) which has a non typeable SCC*mec* IV [2B] element and a type B IEC (*sak+chp+scn*).

iv. SCC*mec* V [5C2] contains PVL negative WA14 (ST5/t442), WA35 (ST5/t688), WA81 (ST5/t045) [a non related spa type] and WA90 (ST5/t1265). WA81 harbors a type F IEC; WA14 and WA90 a type G IEC (*seP+sek+scn*) and WA35 a type B IEC.

v. SCC*mec* V [5C2&5] contains PVL negative WA11 (ST5/t045), WA86 (ST5/t002), WA34 (ST5/t458), WA80 (ST5/t071), WA85 (ST5/t2666), and WA87 (ST835 [ST5slv]/t002). WA85 and WA86 harbor a type F IEC; WA34, WA80 and WA87 a type B IEC and WA11 a type E IEC (*sak + scn*). WA80 harbors the ACME (arginine catabolic mobile element) genes.

vi. SCC*mec* V [5C2]&2 contains PVL negative WA61 (ST641 [ST5slv]/t002) which harbors a type E IEC.

vii. SCC*mec* V [5C2&5]&2 contains PVL negative WA40 (ST835 [ST5slv]/t002) and WA46 (ST835/t002). WA40 harbors a type B IEC while WA46 a type E IEC.

viii. SCC*mec* novel [novel B] contains PVL negative WA18 (ST5/t002), WA21 (ST5/t002) and WA48 (ST835/t002) harboring *ccrA-1* and a class B *mec* complex (*mecA* and a truncated *mecR1* genes). WA18 harbors a type F IEC; WA21 a type D IEC; and WA48 a type B IEC.

Clonal Complex 8

The 12 CC8 strains are all *agr* type I/capsule type 5. Seven closely related *spa* types were identified: t008, t024, t064, t334, t711, t1635, t2238.

The CC8 strains include the ST8-MRSA-IVc [2B]/t008 USA300 MRSA clone [31]. Based on the SCC*mec* type the remaining 11 strains are divided into seven subgroups:

i. SCC*mec* IVa [2B] contains WA5 (ST8/t008), WA6 (ST8/t008), WA62 (ST923 [ST8slv]/t1635), and WA83 (ST1634 [ST8slv]/t711). WA5, WA62, and WA83 harbor a type B IEC. An IEC was not detected in WA6. Unlike the other WA CC8 strains, WA62 is PVL positive.

ii. SCC*mec* IVd [2B] contains WA58 (ST1173 [ST8slv]/t064) and WA20 (ST612 [ST8dlv]/t064) which harbor a type D IEC.

iii. SCC*mec* IVa [2B]&5 contains WA92 (ST1757 [ST8slv]/t024) which does not harbor an IEC.

iv. SCC*mec* IV [2B] contains WA31 (ST576 [ST8slv]/t334) which does not harbor an IEC. The SCC*mec* IV element is non typeable.

v. SCC*mec* V [5C2] contains WA77 (ST8/t008) which harbors a type D IEC, the ACME determinant, and SCC*fus*.

vi. SCC*mec* V ([5C2&5]) contains WA53 (ST8/t2238) which harbors a type D IEC.

vii. SCC*mec* VIII (4A) contains WA16 (ST8/t024) which harbors a type D IEC.

Clonal Complex 12

CC12 contains two *agr* group II/capsule type 8 strains which harbor a type G IEC. Neither strain harbor the *lukF-PV/lukS-PV* PVL encoding genes.

Based on the SCC*mec* type the two strains are divided into two subgroups:

i. SCC*mec* IVa [2B] contains WA69 (ST12/t160).
ii. SCC*mec* novelA contains WA59 (ST12/t160) which harbors a class A *mec* complex (*mecA*, complete *mecR1* and *mecI* regulatory genes). The *ccr* genes were not detected by DNA microarray and did not amplify with PCR primers.

Clonal Complex 30

CC30 contains two *agr* group III/capsule type 8 strains: PVL positive ST30-IVc [2B]/t019 and PVL negative WA68 (ST39 [ST30dlv]-IVc [2B]/t2643). Their protease, haemolysin, leukocidin, *ssl*/*set*, *hsdS*, and cell surface adhesion profiles are not homogeneous and their *spa* types are not closely related.

The DNA microarray profile of ST30-IVc [2B]/t019 is homogeneous with the South Western Pacific (SWP) ST30-IV clone as is therefore not considered a WA CA-MRSA.

WA68 harbors a type D IEC and *tst-1*genes.

Clonal Complex 45

CC45 contains four PVL negative strains. Based on the *agr* group/capsule type the four isolates are divided into two groups which are further divided into subgroups based on the SCC*mec* type.

Group 1

agr group I/capsule 8 (two strains)

i. SCC*mec* IVa [2B] contains WA75 (ST45/t1424).
ii. SCC*mec* V [5C2] contains WA4 (ST45/t123) which harbors *tst1* genes.

Both strains harbor a type B IEC. The *spa* types are not closely related.

Group 2

agr group IV/capsule type 8 (two strains)

i. SCC*mec* IVc [2B] contains WA23 (ST45/t1575)
ii. SCC*mec* V [5C2&5] contains WA84 (ST45/t1081).

Both strains harbor a type B IEC and closely related *spa* types.

Clonal Complex 59

CC59 *agr* type I/capsule type 8 contains seven strains.

The DNA microarray profiles of ST59/ST952-V [5C2&5] t437/t1950 are homogeneous with the Taiwan clone and therefore are not considered WA CA-MRSA [32].

Based on the SCC*mec* types the remaining five strains are divided into three subgroups:

i. SCC*mec* IVa [2B] contains PVL positive WA55 and WA56 (ST59/t437). WA55 harbors a type B IEC while WA56 a type A IEC.
ii. SCC*mec* IVb [2B] contains two PVL negative strains with unrelated *spa* types: WA73 (ST59/t528) and WA24 (ST87 [ST59slv]/t216). WA73 harbors a type C IEC (*chp+scn*) and WA24 a type B IEC.
iii. SCC*mec* IVa [2B]&5 contains PVL negative WA15 (ST59/t976) which harbors a type A IEC.

Clonal Complex 72

CC72 contains two *agr* group I/capsule type 5 strains with closely related *spa* types. Based on the SCC*mec* type the two strains are divided into two subgroups:

i. SCC*mec* IVa [2B] contains PVL positive WA44 (ST72/t791) harboring a type B IEC.
ii. SCC*mec* V (5C2) contains PVL negative WA91 (ST72/t3092) harboring a type E IEC and *tst1* genes.

Clonal Complex 75

CC75 contains three PVL negative strains which are *agr* group/capsule nontypeable by DNA microarray: WA8 (ST75-IVa [2B]), WA79 (ST75-IVa [2B]) and WA72 (ST1304 [ST75slv]-IVa [2B]) [33]. The three strains have the same *spa* sequence (259-23-23-17-17-17-23-23-23-17-16) which has not been allocated a *spa* type number by the Ridom website. The three strains harbor a type E IEC.

Clonal Complex 80

CC80 contains three PVL positive *agr* group III/capsule type 8 strains: ST80-IVc [2B]/t044, ST583 [ST80slv]-IVc [2B]/t044, and ST728 [ST80slv]-IVc [2B]/t044. The DNA microarray virulence profiles are identical with the European ST80-IV [2B] clone and therefore the three strains are not considered WA CA-MRSA.

Clonal Complex 97

CC97 contains two PVL negative *agr* group I/capsule type 5 strains with closely related *spa* types: WA54 (ST953 [ST97dlv]-IVa [2B]/t359) and WA63 (ST1174[ST97dlv]-IVa [2B]/t267). The strains harbor a type E IEC.

Clonal Complex 121

CC121 contains two PVL negative *agr* group IV/capsule type 8 strains with closely related *spa* types. The two

strains harbor a type E IEC and based on the SCC*mec* type, are divided into two subgroups:

i. SCC*mec* V [5C2] contains WA22 (ST577 [ST121 dlv]/t3025) which harbors *etA* (exfoliative toxin serotype A) and *edinA* genes.
ii. SCC*mec* V [5C2&5] contains WA93 (ST121/t159).

Clonal Complex 188

CC188 contains two PVL negative *agr* group I/capsule type 8 strains: WA38 and WA78 (ST188-IVa [2B]/t189). The two strains have a type B IEC.

Clonal Complex 361

CC361 contains three PVL negative *agr* group I/capsule type 8 strains. The *spa* types are closely related. Based on the SCC*mec* type the three strains are divided into three subgroups:

i. SCC*mec* IVa [2B] contains WA29 (ST672 (ST361slv)/t1309) which harbors a type E IEC and *tst1* genes.
ii. SCC*mec* V [5C2] contains WA70 (ST672/t1309).
iii. SCC*mec* VIII [4A] contains WA28 (ST361/t315) which harbors a type B IEC.

The following CCs contained a single strain:

Clonal Complex 9

PVL negative WA13 (ST834-IVc [2B]/t3029) is *agr* group I/capsule type 8 and harbors a type B IEC and *tst1* genes.

Clonal Complex 88

PVL negative WA2 (ST78-IVa [2B]/t3205) is *agr* group III/capsule type 8 and harbors a type B IEC.

Clonal Complex 152

PVL positive WA89 (ST1633-V [5C2]/t355) is *agr* group I/capsule type 5 and harbors a type E IEC and *edinB* genes.

Clonal Complex 398

Although PVL negative ST398-V [5C2&5]/t034 is frequently associated with livestock, the strain is increasingly isolated from human patients [34]. Rarely identified in Australia, the DNA microarray profile of this isolate is homogeneous with the European livestock-associated ST398 strain and is therefore not considered a WA CA-MRSA.

WA76 (Clonal Complex not Determined)

PVL negative WA76 (ST1303-IVa [2B]) is *agr* group III with a non typeable capsule by DNA microarray. The *spa* sequence (259-25-17-17-16-16-16-16) has not been allocated a *spa* type number by the Ridom website.

Queensland Clone (Singleton)

PVL positive ST93-IVa [2B]/t202 is *agr* group III/capsule type 8 and harbors a type B IEC. The DNA microarray profile is homogeneous with the Queensland clone. Due to its origin and widespread distribution outside WA the Queensland clone is not considered a WA CA-MRSA.

WA47 (Singleton)

PVL negative WA47 (ST883-IVd [2B]/t7462) has a non typeable *agr* group/capsule type by DNA microarray.

Discussion

As all MRSA isolated in WA are referred to a central typing laboratory it is possible to investigate the emergence and evolution of CA-MRSA in a remote region.

Prior to the global evolution and expansion of CA-MRSA, five CA-MRSA clones were identified in the indigenous population living in the remote communities of the sparsely populated Kimberley, Pilbara and Eastern Goldfield regions of WA [29]. These five PVL negative clones include: WA1 (CC1: ST1-IVa [2B]/t127), WA2 (CC88: ST78-IVa [2B]/t3205), WA3 (CC5: ST5-IVa [2B]/t002), WA4 (CC45 ST45-V (5C2)/t123) and WA5 (CC8: ST8-IVa [2B]/t008). WA5 and WA1 were originally isolated from clinical specimens in 1989 and 1995 respectively, and WA2, WA3 and WA4 from nasal carriage specimens in 1995. The emergence of CA-MRSA clones in different MLST clonal clusters indicates horizontal transmission of the SCC*mec* element into *S. aureus* has occurred on at least five occasions in these remote communities: SCC*mec* IVa [2B] into CC1 (ST1), CC5 (ST5), CC8 (ST8), CC88 (ST78), and SCC*mec* V [5C2] into CC45 (ST45). Based upon the *spa* type and the DNA microarray profile at least six evolutionary events have occurred on at least three occasions from these clones (ie vertical transmission of the SCC*mec* element): twice from WA1, WA3 and WA5 (Figure 2). Vertical transmission of the SCC*mec* element has not been identified for WA4 or WA2.

The emergence of WA1, WA2 and WA3 has been due to the acquisition and insertion of the small and highly mobile type IVa [2B] SCC*mec* element, presumably harbored by methicillin resistant coagulase negative staphylococci (MRCNS). Several hypotheses to explain the transmission of a SCC*mec* element from MRCNS to *S. aureus* have been proposed including the increased use of antimicrobials within a community [35]. Many of the Kimberley indigenous population live in poor socioeconomic conditions. Staphylococcal skin lesions, commonly resulting from scabies infestation, trachoma and venereal

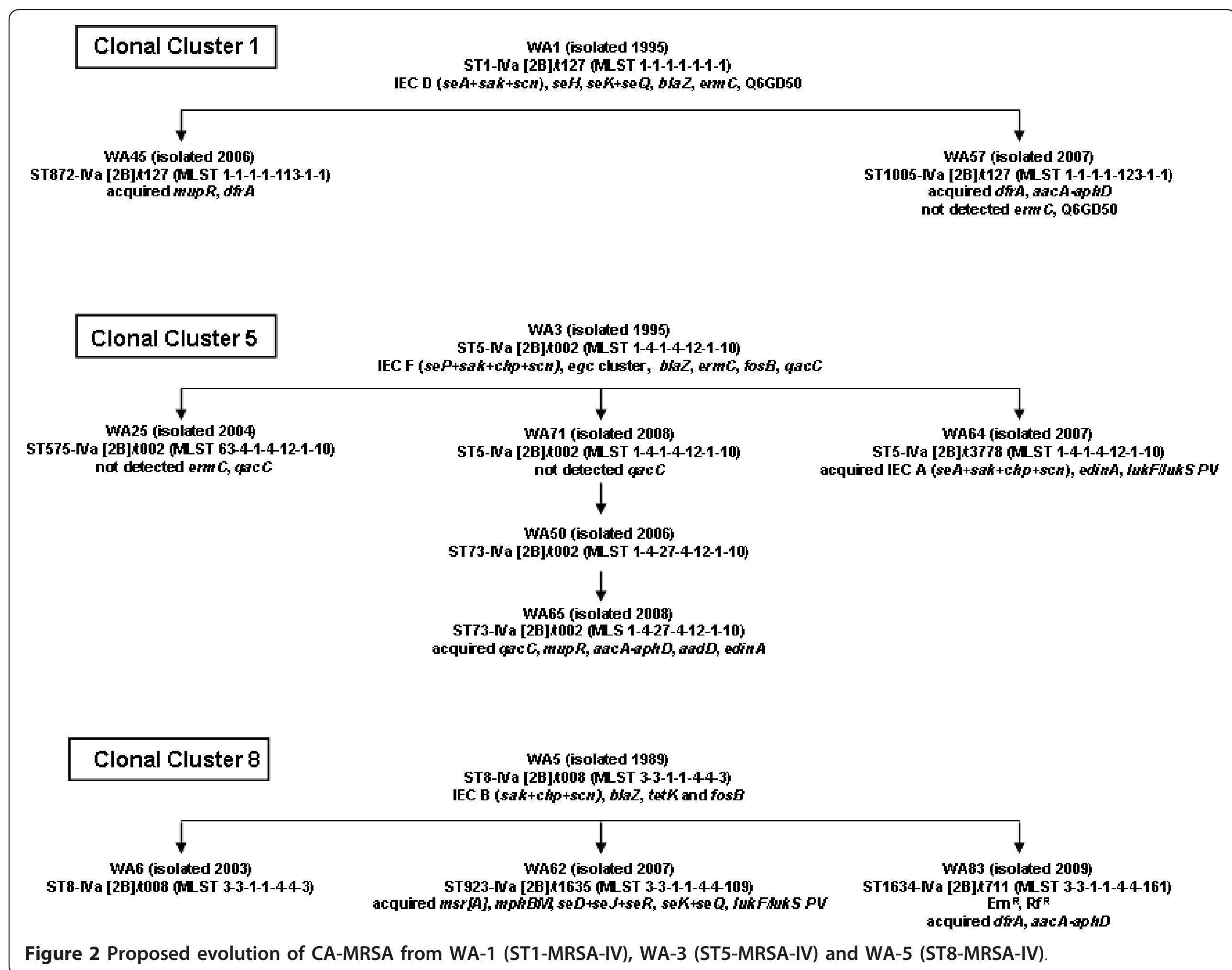

Figure 2 Proposed evolution of CA-MRSA from WA-1 (ST1-MRSA-IV), WA-3 (ST5-MRSA-IV) and WA-5 (ST8-MRSA-IV).

diseases such as chlamydia and gonorrhea occur frequently in this population. Consequently empirical therapy using β-lactamase stable penicillins and azithromycin is often prescribed [36]. The frequent use of these antimicrobials may have assisted in the acquisition of the SCC*mec* element and *erm* genes into *S. aureus*. Genetic studies however have shown these newly emerged CA-MRSA clones did not originate in the predominant methicillin-susceptible *S. aureus* (MSSA) clones found in these communities, suggesting not all clones are able to acquire or retain the SCC*mec* element [37]. The subsequent dissemination of WA1, WA2 and WA3 into the wider community suggests the acquisition of the SCC*mec* element and the *erm* genes has given these clones a selective advantage. WA4 and WA5 however have not been successful in spreading beyond the indigenous communities suggesting the acquisition of the SCC*mec* element does not provide a universal selective advantage.

Many of the remaining 46 CA-MRSA clones, identified between July 2003 and June 2010, were not isolated in remote WA indigenous communities. The geographical spread of CA-MRSA over long distances and across cultural borders is believed to be a rare event compared to the frequency in which the SCC*mec* element is acquired by *S. aureus* [38]. Most of these clones are therefore likely to have evolved in WA. Some clones are slvs and dlvs of pre-existing CA-MRSA, and their SCC*mec* type, *spa* type and DNA microarray profile suggests vertical transmission of the SCC*mec* element has occurred. However the emergence of MRSA in several unrelated *S. aureus* clonal clusters suggests horizontal transmission of the SCC*mec* element has also occurred. SCC*mec* typing and *spa* typing and DNA microarray results also suggests horizontal transfer of SCC*mec* elements has occurred into the same CC on more than one occasion.

Although several SCC*mec* elements have been acquired by multiple *S. aureus* clones from which many CA-MRSA clones have emerged, only a few clones have successfully adapted to the WA community

environment. Between July 2009 to June 2010 4,691 MRSA were referred to ACCESS Typing and Research of which 3,931 were characterized as CA-MRSA. Overall 84% (3,024) of isolates were from clinical infections and the 16% (907) from colonized patients. Approximately 88% of CA-MRSA were identified as WA1 (40%), WA2 (24%) and WA3 (8%). For most clones, including WA4 and WA5 only a few isolates were detected. (http://www.public.health.wa.gov.au/3/896/3/camrsa.pm).

For many slv and dlv CA-MRSA only a small number of isolates have been detected suggesting changes in the housekeeping genes may have conferred a fitness cost or did not allow the SCC*mec* element to be maintained. For example WA45 and WA57 are slvs of ST1 and their SCC*mec* and *spa* type and DNA microarray profile suggest they have evolved from WA1 (Figure 2). WA45 was first identified in 2006 and WA57 in 2007. Although WA1 has become the most successful CA-MRSA clone in the WA community only one isolate of WA45 and two isolates of WA56 have so far been identified (http://www.public.health.wa.gov.au/3/896/3/camrsa.pm).

Six PVL positive pandemic CA-MRSA clones (plus three closely related clones) have been isolated in WA: Bengal Bay CA-MRSA (ST772-V [5C2]/t3387), USA300 MRSA (ST8-IVc [2B]/t008), SWP CA-MRSA (ST30-IVc [2B]/t019), Taiwan CA-MRSA (ST59-V [5C2&5]/t437 and the slv ST952-V [5C2&5]/t1950), European CA-MRSA (ST80-IVc [2B]/t044 and the slvs, ST583-IVc [2B]/t044 and ST728-IVc [2B]/t044), and the Queensland CA-MRSA (ST93-IVa [2B]/t202). The epidemiology of the USA300 and Taiwan CA-MRSA clones in WA and the Queensland and SWP CA-MRSA clones in Australia have previously been reported [18,31,32]. Patients colonized or infected with the Bengal Bay clone have been observed to be epidemiologically linked to Indian healthcare workers (unpublished data). The USA300, European, Taiwanese and Bengal Bay CA-MRSA clones are not frequently isolated in WA. This may be due, in part, to WA Health Department infection control interventions applied to patients who are colonized or infected with international PVL positive pandemic clones. A seventh pandemic clone has recently been identified. The DNA microarray profile and the SCC*mec* element of the PVL negative ST398-V [5C2&5] is indistinguishable from the pandemic ST398 clone initially isolated from pigs and pig farmers in the Netherlands [39]. Only one isolate, from a patient with travel outside of Australia, has been identified in WA.

The Queensland clone (ST93-IVa [2B]) first detected on the east coast of Australia in the Caucasian population in 2000 [40], has become one of the most prevalent CA-MRSA isolated in Australia [18] and in 2010 accounted for 18% of CA-MRSA in WA. This suggests the acquisition of the SCC*mec* element has given this clone a selective advantage. Although the Queensland clone is believed to have been introduced into WA in 2001 [22], PVL positive ST93-MSSA was identified as the most prevalent *S. aureus* clone in WA's remote indigenous communities in surveys performed in the mid 1990s. Although found in an environment of high β-lactam use a methicillin-resistant variant of ST93-MSSA was not found in WA during these surveys.

WA1, WA2 and WA3 are PVL negative and do not harbor multiple virulence genes (Tables 1). Similarly the successful Queensland clone, although PVL positive, carries almost no other exotoxin genes and no additional resistance genes. Although most other WA CA-MRSA clones are also PVL negative, many of these clones have acquired multiple resistance and/or virulence determinants (Tables 1). For example WA78 (ST188-IVa [2B]/t315) in addition to *mecA* and *blaZ*, harbors *aacA-aphD*, *tetK* and *cat* and is phenotypically resistant to erythromycin, trimethoprim and ciprofloxacin; WA64 (ST5-IVa [2B]/t3778) has acquired *seA* enterotoxin genes and *edinA* and *lukF-PV lukS-PV* virulence genes; and WA62 (ST923[ST8slv]-IVa [2B]/t1635) harbors *seD+seJ+seR* and *seK+seQ* enterotoxin genes and *lukF-PV lukS-PV*. The acquisition of multiple resistance and/or virulence genes may have come at a high fitness cost as none of these clones have established a niche in the WA community.

As WA1, WA2 and WA3 CA-MRSA lack PVL as well as other virulence genes that are found in pandemic international CA-MRSA clones, such ACME in USA300, the epidemiology of CA-MRSA disease in WA is different to other regions. Outside of WA the majority of diseases related to CA-MRSA infection are severe skin and soft tissue infections such as soft tissue abscess, carbuncles and furuncles. Many of these infections have occurred in healthy individuals, especially children and adolescents, usually via skin-to-skin contact [41]. In WA the majority of CA-MRSA related diseases were initially associated with the indigenous population and then other groups normally susceptible to *S. aureus* infections such as the elderly. As the original WA CA-MRSA are PVL negative, many of these infections were superficial skin infections such as impetigo. However with the introduction of the PVL-positive Queensland CA-MRSA clone more severe skin and soft tissues infections have been observed.

The limitation of this study is that only the initial isolate of each PFGE pulsotype was included in the study. To determine if the successful CA-MRSA clones found in the WA community are evolving the genetic profiles of subsequent isolates need to be investigated.

Conclusions

In conclusion although the vertical and horizontal transmission of SCC*mec* elements into *S. aureus* has

occurred on multiple occasions in the WA community only three WA CA-MRSA clones have found an ecological niche. These three PVL negative clones harbor few additional resistance and virulence genes which paradoxically may account for their success.

Methods

Isolates

The isolates studied are representative of the 83 CA-MRSA unique PFGE strains identified in WA from 1989 to 2010 (Figure 3). They include five strains isolated

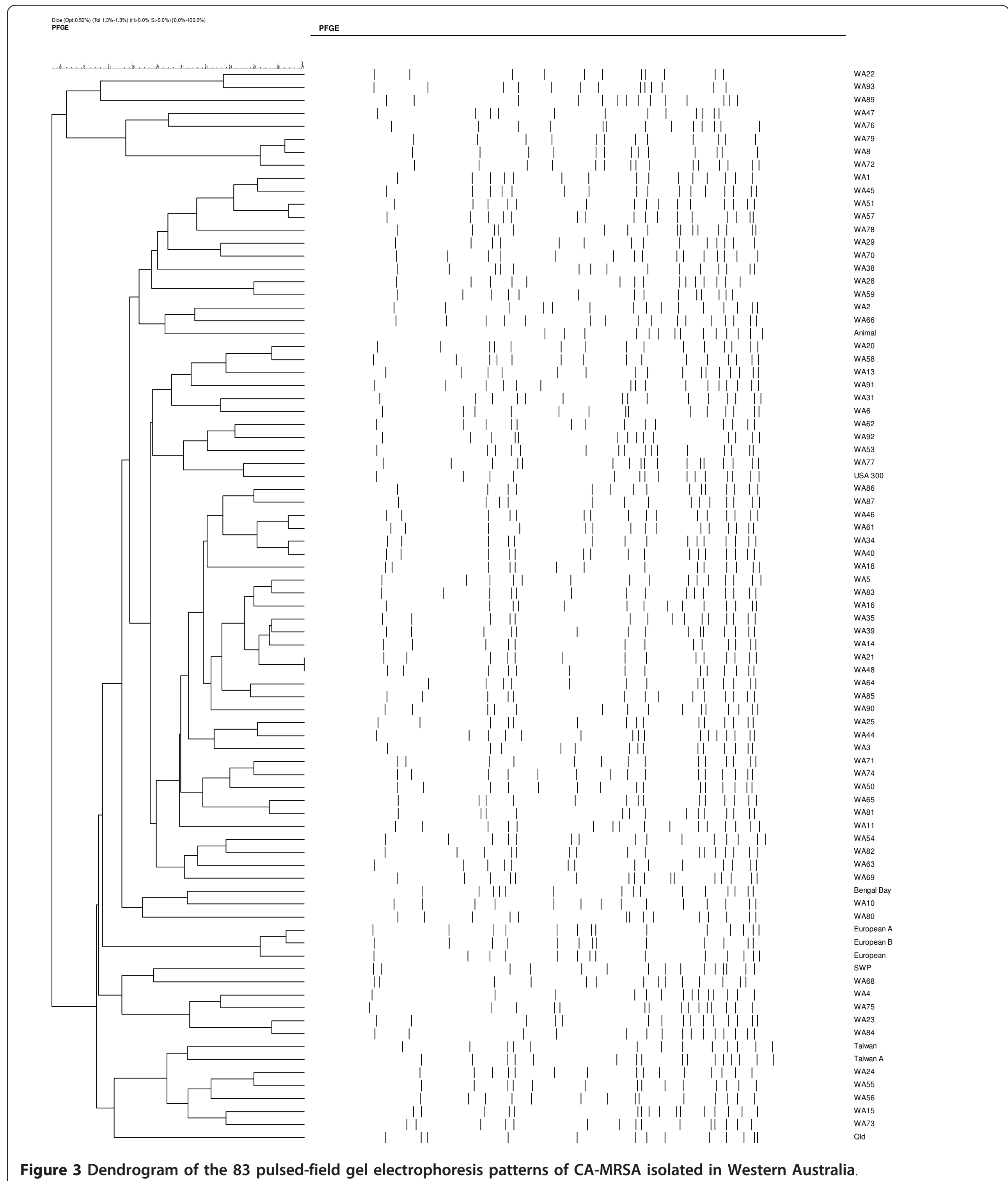

Figure 3 Dendrogram of the 83 pulsed-field gel electrophoresis patterns of CA-MRSA isolated in Western Australia.

from indigenous inhabitants living in remote WA rural communities in 1989 (WA5 {WBG7583} [20]) and 1995 (WA1 {WBG 8287}, WA2 {WB8366}, WA3 {WBG8378}, and WA4 {WBG8404} [42]); and 78 strains identified from 24,368 CA-MRSA referred to ACCESS Typing and Research between July 2003 and June 2010.

nuc and *mecA*

S. aureus species and methicillin resistance was confirmed by the detection of *nuc* (thermostable extracellular nuclease) and *mecA* (methicillin resistance) genes by PCR [43].

Susceptibility testing

An antibiogram was performed by disk diffusion on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [44]. A panel of eight antimicrobial drugs was tested: erythromycin (15 μg), tetracycline (30 μg), trimethoprim (5 μg), ciprofloxacin (5 μg), gentamicin (10 μg), rifampin (5 μg), fusidic acid (10 μg), and mupirocin (5 μg). CLSI interpretive criteria [45] were used for all drugs except fusidic acid [46] and mupirocin [47].

PVL

PCR for the detection of PVL determinants was performed as previously described [48].

PFGE

Electrophoresis of chromosomal DNA was performed as previously described [49], using a contour-clamped homogeneous electric field (CHEF) DR III system (Bio-Rad Laboratories Pty Ltd). Chromosomal patterns were examined visually, scanned with a Quantity One device (Bio-Rad Laboratories Pty Ltd), and digitally analyzed using FPQuest (Bio-Rad Laboratories Pty Ltd). *S. aureus* strain NCTC 8325 was used as a reference strain.

MLST and *spa* typing

Chromosomal DNA for MLST and *spa* typing was prepared using a DNeasy tissue kit (Qiagen Pty Ltd).

MLST was performed as previously described [50]. The sequences were submitted to http://www.mlst.net/ where an allelic profile was generated and an ST assigned. Clonal complex (CC) was determined using the eBURST V3 algorithm at the same website. Clones that diverged at no more than one of the seven MLST loci were considered to belong to the same CC. Double locus variants (dlvs) were included if the linking single locus variant (slv) was present in the MLST database.

spa typing, a DNA sequenced-based analysis of the protein A gene variable region was performed as previously described [51] using the nomenclature as described on the Ridom website (http://spa.ridom.de/).

SCC*mec* typing

The strategy used for SCC*mec* typing was as previously described [32]. SCC*mec* nomenclature is used as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) [52]. Briefly, the structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an uppercase letter respectively in parenthesis. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type. When there is an extra *ccr* element present whose precise location is unknown it is indicated by an "&" and *ccr* number outside the parentheses.

DNA microarray

Arrays and reagents were obtained from Alere Technologies, Jena Germany. The principle of the assay, related procedures, and a list of targets has been described previously [53,54]. An iterated, linear primer elongation was employed for the simultaneous amplification of all targets. An alternative protocol was used for a few isolates in which amplification and labeling was directed by random primers [55]. This method detects target genes for which the binding sites of the primers used in the first protocol were deleted or changed by nucleotide polymorphisms. Target genes included species markers, markers for accessory gene regulator (*agr*) alleles and capsule types, virulence factors, resistance genes, staphylococcal superantigen-like/exotoxin-like genes (*set/ssl* genes) and genes encoding adhesion proteins. Probes for *mecA*, *ugpQ*, *xylR*, and two probes for *mecR* were used for SCC*mec* typing. The last two probes allowed detection and discrimination of untruncated *mecR* and Δ*mecR*, respectively. Probes for the recombinase genes *ccrA1*, *ccrB1*, *ccrA2*, *ccrB2*, *ccrA3*, *ccrB3*, *ccrA4*, *ccrB4*, and *ccrC1;* the fusidc acid resistance marker Q6GD50; and the J region proteins, *dcs*, *pls*-SCC and the *kdp*-operon were also included.

MRSA Strain Definition

MRSA strains are defined according to their unique PFGE pulsotype

MRSA Clone Definition

MRSA clones are defined by the combination of the multilocus sequence type (ST) and the SCC*mec* type [56]. For instance ST1-SCC*mec* IVa [2B] is abbreviated as ST1-IVa [2B].

Additional material

Additional file 1: Characterisation of CA-MRSA isolated in Western Australia.

Additional file 2: DNA Microarray Targets, Primers and Probes.

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Authors' contributions
GC designed the study, analysed and interpreted the data, and drafted the manuscript. SM assisted in the analysis and interpretation of data, and critically revised the manuscript for important intellectual content. JP, HL-T, Y-KC and LE carried out the laboratory procedures. RE critically revised the manuscript for important intellectual content. FGO assisted in the design of the study, analysed and interpreted the data, and critically revised the manuscript for important intellectual content. KJC assisted in the design of the study, analysed and interpreted the data, and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Additional File 1: Characterisation of CA-MRSA isolated in Western Australia

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
Clonal Complex 1												
Group 1												
WA1	1995	WBG8287	1	t127	III	8	IVa [2B]	Ox^R Em^R FA^R	*mecA, blaZ, ermC,* Q6GD50		*seA, seH, seK+seQ*	*sak, scn*
WA45	2006	06-16252	872	t127	III	8	IVa [2B]	Ox^R Em^R FA^R Tm^R Mp^R	*mecA, blaZ, ermC,* Q6GD50, *(dfrA), mupR*		*seA, seH, seK+seQ*	*sak, scn*
WA57	2007	07-16124	1005	t127	III	8	IVa [2B]	Ox^R Gm^R Tm^R	*mecA, blaZ, aacA-aphD, dfrA*		*seA, seH, seK+seQ*	*sak, scn*
Group 2												
WA10	2003	03-16918	573	t5073	II	5	V [5C2]	Ox^R Tm^R	*mecA, blaZ, fosB*		*seC+seL, egc-*cluster, ORF CM14	*scn*
Bengal Bay MRSA												
Bengal Bay	2007	07-17048	772	t3387	II	5	V [5C2]	Ox^R Em^R Gm^R Tm^R Cp^R	*mecA, blaZ, msr(A), mpbBM,aacA-aphD, aphA, sat, fosB*	*lukF/S- PVL*	*seA, seC+seL, egc-*cluster, ORF CM14	*scn*
Clonal Complex 5												
Group 1												
WA51	2007	07-15545	6	DNA	I	8	IVa [2B]	Ox^R	*mecA, blaZ, fosB*		*seA*	*sak, scn*
WA66	2007	07-17366	6	t701	I	8	IVa [2B]&5	Ox^R	*mecA, blaZ, fosB*		*seA, seK+seQ*	*sak, scn*
Group 2												
WA3	1995	WBG8378	5	t002	II	5	IVa [2B]	Ox^R Em^R	*mecA, blaZ, ermC, fosB,qacC*		*seP, egc*-cluster	*sak, chp, scn*
WA64	2007	07-16986	5	t3778	II	5	IVa [2B]	Ox^R	*mecA, blaZ, fosB*	*lukF/S- PVL*	*seA, egc*-cluster	*sak, chp, scn*
WA71	2008	08-17330	5	t002	II	5	IVa [2B]	Ox^R	*mecA, blaZ, fosB*		*seP, egc*-cluster	*sak, chp, scn*
WA82	2009	09-15628	5	t002	II	5	IVa [2B]	Ox^R Tm^R	*mecA, blaZ, dfrA,*		*seP, egc*-cluster	*sak, chp, scn*

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
									fosB, qacA, qacC			
WA25	2004	04-15184	575	t002	II	5	IVa [2B]	Ox^{R}	*mecA, blaZ, fosB*		*seP*, *egc*-cluster	*sak, chp, scn*
WA50	2006	06-18615	73	t002	II	5	IVa [2B]	Ox^{R}	*mecA, blaZ, fosB*		*seP*, *egc*-cluster	*sak, chp, scn*
WA65	2008	08-15231	73	t002	II	5	IVa [2B]	Ox^{R} Gm^{R} Mp^{R}	*mecA, blaZ, aacA-aphD, aadD, mupR, fosB, qacC*		*seP*, *egc*-cluster	*sak, chp, scn*
WA74	2008	08-19202	5	t002	II	5	IVc [2B]	Ox^{R}	*mecA, blaZ, fosB, qacC*		*seP*, *egc*-cluster	*sak, chp, scn*
WA39	2005	05-18015	526	t4065	II	5	IV [2B]	Ox^{R} Em^{R} FA^{R}	*mecA*, Q6GD50, *fosB*		*egc*-cluster	*sak, chp, scn*
WA14	2003	03-17796	5	t442	II	5	V [5C2]	Ox^{R} Te^{R}Tm^{R} FA^{R}	*mecA, tetM,* Q6GD50, *fosB*		*seP*, *egc*-cluster	*sak, scn*
WA35	2005	05-16810	5	t688	II	5	V [5C2]	Ox^{R} Em^{R} Te^{R} FA^{R} Cp^{R}	*mecA, blaZ, ermC, aadD, tetK, tetM, fexA, fosB*		*seD+seJ+seR, egc*-cluster	*sak, chp, scn*
WA81	2009	09-16404	5	t045	II	5	V [5C2]	Ox^{R} Gm^{R}	*mecA, blaZ, fosB, qacC*		*seP*, *egc*-cluster	*sak, chp, scn*
WA90	2009	09-20177	5	t1265	II	5	V [5C2]	Ox^{R} Em^{R} Tm^{R} Cp^{R}	*mecA, blaZ, ermC, fosB*		*seP, seD, seJ, egc-*cluster	*sak, (scn)*
WA11	2003	03-17833	5	t045	II	5	V [5C2&5]	Ox^{R} Gm^{R}	*mecA, blaZ, aacA-aphD, fosB, qacA*		*seD+seJ+seR, egc*-cluster	*sak, scn*
WA86	2009	09-18986	5	t002	II	5	V [5C2&5]	Ox^{R} Gm^{R} Tm^{R}	*mecA, aacA-aphD, fosB, qacC*		*seP*, *egc*-cluster	*sak, chp, scn*
WA34	2005	05-17463	5	t458	II	5	V [5C2&5]	Ox^{R}	*mecA, fosB*		*seD+seJ+seR*, *egc*-cluster	*sak, chp, scn*
WA80	2009	09-15037	5	t071	II	5	V [5C2&5]	Ox^{R}	*mecA, fosB*		*seD, seJ, egc-*cluster	*sak, chp, scn*
WA85	2009	09-17872	5	t2666	II	5	V [5C2&5]	Ox^{R} Tm^{R}	*mecA, blaZ, fosB*		*seP*, *egc*-cluster	*sak, chp, scn*
WA87	2009	09-18264	835	t002	II	5	V [5C2&5]	Ox^{R} Cp^{R}	*mecA, blaZ, fosB*		(seB*), seC+seL, seD+seJ+seR, egc*-cluster	*sak, chp, scn*
WA61	2007	07-18115	641	t002	II	5	V [5C2]&2	Ox^{R} Cp^{R}	*mecA, blaZ, fosB, qacC*		*seD+seJ+seR*, *egc*-cluster	*sak, scn*
WA40	2005	05-18551	835	t002	II	5	V [5C2&5]&2	Ox^{R} Cp^{R}	*mecA, blaZ, fosB,*		*seD+seJ+seR,*	*sak, chp, scn*

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
									qacC		*egc*-cluster	
WA46	2006	06-16677	835	t002	II	5	V [5C2&5]&2	Ox^R Cp^R	*mecA, blaZ, fosB, qacC*		*seD+seJ+seR*, *egc*-cluster	*sak, scn*
WA18	2004	04-16891	5	t002	II	5	novel B	Ox^R	*mecA, blaZ, fosB*		*seP, seD+seJ+seR, seK+seQ, egc*-cluster	*sak, chp, scn*
WA21	2004	04-17091	5	t002	II	5	novel B	Ox^R	*mecA, blaZ, fosB, qacC*		*seA, seD+seJ+seR*, *egc*-cluster	*sak, scn*
WA48	2006	06-17586	835	t002	II	5	novel B	Ox^R Cp^R	*mecA, blaZ, fosB*		*seD+seJ+seR*, *egc*-cluster	*sak, chp, scn*
Clonal Complex 8												
WA5	1989	WBG7583	8	t008	I	5	IVa [2B]	Ox^R Te^R	*mecA, blaZ, tetK, fosB*			*sak, chp, scn*
WA6	2003	03-15521	8	t008	I	5	IVa [2B]	Ox^R Te^R	*mecA, blaZ, tetK, fosB*			
WA62	2007	07-18116	923	t1635	I	5	IVa [2B]	Ox^R Em^R Te^R	*mecA, blaZ, msr(A), mpbBM, tetK, fosB*	*lukF/S- PVL*	*seD+seJ+seR, seK+seQ*	*sak, chp, scn*
WA83	2009	09-17714	1634	t711	I	5	IVa [2B]	Ox^R Em^R Gm^R Tm^R Rf^R	*mecA, blaZ, aacA-aphD, dfrA, fosB*			*sak, chp, scn*
WA58	2007	07-16233	1173	t064	I	5	IVd [2B]	Ox^R Em^R Gm^R Te^R Tm^R Rf^R	*mecA, blaZ, ermA, aacA-aphD, aphA, sat, tetM, dfrA, fosB*		*seA, seB+seK+seQ*	*sak, scn*
WA20	2004	04-17052	612	t064	I	5	IVd [2B]	Ox^R Gm^R Te^R Tm^R Rf^R	*mecA, blaZ, aacA-aphD, tetM, dfrA, cat, fosB*		*seA, seB+seK+seQ*	*sak, scn*
WA92	2010	10-15552	1757	t024	I	5	IVa [2B]&5	Ox^R Tm^R	*mecA, blaZ, dfrA, fosB*			*scn*
WA31	2005	05-15529	576	t334	I	5	IV [2B]	Ox^R	*mecA, blaZ, fosB*			
WA77	2008	08-20001	8	t008	I	5	V [5C2]	Ox^R Te^R FA^R	*mecA, blaZ, tetK,* Q6GD50, *fosB, qacC*		*seA, seK+seQ*	*sak, scn*

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
WA53	2006	06-18088	8	t2238	I	5	V [5C2&5]	Ox^R Em^R Gm^R Cp^R	*mecA, blaZ, ermC, aacA-aphD, fosB*		*seA, seB+seK+seQ*	*sak, scn*
WA16	2003	03-16758	8	t024	I	5	VIII [4A]	Ox^R Em^R Gm^R Cp^R Mp^R	*mecA, blaZ, ermA, aacA-aphD, mupR, fosB, qacC*		*seA*	*sak, scn*
USA300 MRSA												
USA300	2004	04-15086	8	t008	I	5	IVc [2B]	Ox^R Te^R	*mecA, blaZ, tetK, fosB*	*lukF/S- PVL*	*seK+seQ*	*sak, chp, scn*
Clonal Complex 9												
WA13	2003	03-17992	834	t3029	I	8	IVc [2B]	Ox^R Em^R	*mecA, blaZ, msr(A), fosB*		*seC+seL*	*sak, chp, scn*
Clonal Complex 12												
WA69	2007	07-19013	12	t160	II	8	IVa [2B]	Ox^R	*mecA, blaZ, fosB*		*seP, seB*, ORF CM14	*sak, scn*
WA59	2007	07-16590	12	t160	II	8	novel A	Ox^R	*mecA, fosB*		*seP, seB*, ORF CM14	*sak, scn*
Clonal Complex 30												
WA68	2008	08-15775	39	t2643	III	8	IVc [2B]	Ox^R	*mecA, blaZ, fosB*		*seA, seC+seL, seO*	sak, scn
South Western Pacific MRSA												
SWP	2002	02-16663	30	t019	III	8	IVc [2B]	Ox^R	*mecA, blaZ, fosB*	*lukF/S- PVL*	*egc*-cluster	sak, chp, scn
Clonal Complex 45												
Group 1												
WA75	2003	03-17163	45	t1424	I	8	IVa [2B]	Ox^R	*mecA, blaZ*		*(seB), seC+seL, egc*-cluster	*sak, chp, scn*
WA4	1995	WBG8404	45	t123	I	8	V [5C2]	Ox^R	*mecA, blaZ*		*seK+seQ, egc-*cluster	*sak, chp, scn*
Group 2												
WA23	2004	04-16679	45	t1575	IV	8	IVc [2B]	Ox^R	*mecA, blaZ*		*seJ+ seR, egc-*cluster	*sak, chp, scn*

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
WA84	2007	07-16502	45	t1081	IV	8	V [5C2&5]	Cp^R	*mecA, blaZ*		*seJ, egc*-cluster	*sak, chp, scn*
Clonal Complex 59												
WA55	2007	07-15432	59	t437	I	8	IVa [2B]	Em^R Te^R	*mecA, blaZ, aphA, sat, tetK*	*lukF/S- PVL*	*seB+seK+seQ*	*sak, chp, scn*
WA56	2007	07-15443	59	t437	I	8	IVa [2B]	Em^R	*mecA, blaZ, aphA, sat, cat*	*lukF/S- PVL*	*seA, seB+seK+seQ*	*sak, chp, scn*
WA73	2005	05-16512	59	t528	I	8	IVb [2B]	Ox^R	*mecA, blaZ*		*seB+seK+seQ*	*chp, scn*
WA24	2004	04-17626	87	t216	I	8	IVb [2B]	Em^R	*mecA, blaZ, msr(A), mpbBM, aphA, sat*		*seB+seK+seQ*	*sak, chp, scn*
WA15	2003	03-17565	59	t976	I	8	IVa [2B]&5	Ox^R	*mecA, blaZ*		*seA, seB+seK+seQ*	*sak, chp, scn*
Taiwan CA-MRSA												
Taiwan	2003	03-16672	59	t437	I	8	V [5C2&5]	Em^R Te^R	*mecA, blaZ, aphA, sat, tetK, cat*	*lukF/S- PVL*	*seB+seK+seQ*	*chp, scn*
Taiwan[A]	2007	07-15076	952	t1950	I	8	V [5C2&5]	Em^R	*mecA, blaZ, aphA, sat, cat*	*lukF/S- PVL*	*seB+seK+seQ*	*chp, scn*
Clonal Complex 72												
WA44	2006	06-15803	72	t791	I	5	IVa [2B]	Ox^R Tm^R	*mecA, blaZ, dfrA, fosB*	*lukF/S- PVL*	*seC+seL, egc*-cluster	*sak, chp, scn*
WA91	2010	10-15302	72	t3092	I	5	V [5C2]	Ox^R Gm^R Tm^R Cp^R	*mecA, blaZ, aacA-aphD, fosB*		*seC+seL, egc*-cluster	*sak,scn*
Clonal Complex 75												
WA8	2003	03-17848	75	ND	NT	NT	IVa (2B)	Ox^R	*mecA, blaZ, fosB, qacC*		*seB, egc*-cluster	*sak,scn*
WA79	2008	08-18362	75	ND	NT	NT	IVa [2B]	Ox^R	*mecA, blaZ, fosB*		*egc*-cluster	*sak,scn*
WA72	2008	08-16706	1304	ND	NT	NT	IVa [2B]	Ox^R Em^R Mp^R	*mecA, blaZ, ermC, aadD, mupR, fosB*		*seD+seJ+seR, egc*-cluster	*sak,scn*
Clonal Complex 80												
European CA-MRSA												

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
European	2005	05-17006	80	t044	III	8	IVc [2B]	Ox^R Te^R FA^R	*mecA, blaZ, aphA, sat, tetK, far1*	*lukF/S- PVL*		*sak, scn*
European[A]	2004	04-15395	583	t044	III	8	IVc [2B]	Ox^R Te^R FA^R	*mecA, blaZ, aphA, sat, tetK, far1*	*lukF/S- PVL*		*sak, scn*
European[B]	2005	05-15062	728	t044	III	8	IVc [2B]	Ox^R	*mecA, aphA, sat*	*lukF/S- PVL*		*sak, scn*
Clonal Complex 88												
WA2	1995	WBG8366	78	t3205	III	8	IVa [2B]	*Ox*^{R}, *Em*^{R}	*mecA, blaZ, ermA*		*seC+seL*	*sak, chp, scn*
Clonal Complex 97												
WA54	2007	07-15754	953	t359	I	5	IVa [2B]	Ox^R				sak, scn
WA63	2007	07-17920	1174	t267	I	5	IVa [2B]	Ox^R				sak, scn
Clonal Complex 121												
WA22	2004	04-16237	577	t3025	IV	8	V [5C2]	Ox^R Em^R	*mecA, blaZ, ermA, fosB*		*egc*-cluster, ORF CM14	*sak, scn*
WA93	2010	10-15882	121	t159	IV	8	V [5C2&5]		*mecA, blaZ, fosB*		*seB, egc*-cluster, ORF CM14	*sak, scn*
Clonal Complex 152												
WA89	2009	09-20065	1633	t355	I	5	V [5C2]	Ox^R Te^R Tm^R	*mecA, blaZ, teK*	*lukF/S- PVL*		*sak, scn*
Clonal Complex 188												
WA38	2005	05-17762	188	t189	I	8	IVa [2B]	Ox^R Em^R Gm^R Tm^R Cp^R Rf^R	*mecA, blaZ, aacA-aphD*			*sak, chp, scn*
WA78	2008	08-20097	188	t189	I	8	IVa [2B]	Ox^R Em^R Gm^R Te^R Tm^R Cp^R	*mecA, blaZ, aacA-aphD, tetK, cat*			*sak, chp, scn*
Clonal Complex 361												
WA29	2005	05-15441	672	t1309	I	8	IVa [2B]	Ox^R Cp^R	*mecA, blaZ, fosB*		*seB, egc*-cluster	*sak, scn*
WA70	2008	08-18855	672	t1309	I	8	V [5C2]	Ox^R Gm^R Tm^R Cp^R	*mecA, blaZ, aacA-aphD, aphA, sat, fosB*		*egc*-cluster	
WA28	2005	05-16157	361	t315	I	8	VIII [4A]	Ox^R Te^R	*mecA, blaZ, aphA, sat, tetK, fosB*		*egc*-cluster	*sak, chp, scn*

PFGE Pulsotype	Year	Reference Number	MLST	*spa* type	*agr* type	Capsule type	SCC*mec* type	Antibiogram	DNA Microarray Resistance Genotype	*lukF/S- PVL*	Enterotoxins	Bacteriophage Associated Virulence Genes
Clonal Complex 398												
Animal	2009	09-16670	398	t034	I	5	V [5C2&5]	Ox^R Em^R Te^R Tm^R	*mecA, blaZ, ermA,* (*tetK*), *tetM*			
Singletons												
WA47	2006	06-16607	883	t7462	NT	NT	IVd [2B]	Ox^R Em^R	*mecA, ermC, (cat)*		*seB*	
Queensland CA-MRSA												
Qld	2003	03-16790	93	t202	III	8	IVa [2B]	Ox^R	*mecA, blaZ*	*lukF/S- PVL*	ORF CM14	*sak, chp, scn*
Clonal Complex not Determined												
WA76	2006	06-17540	1303	ND	III	NT	IVa [2B]	Ox^R	*mecA, blaZ, fosB*		*seB, egc*-cluster	*scn*

PFGE, pulsed field gel electrophoresis; MLST, multilocus sequence type; *spa* Type: ND, Not Determined; NT, Non typeable; *agr*, accessory gene regulator
SCC*mec*, staphylococcal cassette chromosome *mec*;
Antibiogram: Ox, oxacillin; Cp, ciprofloxacin; Em, erythromycin; FA, fusidic acid; Gm, gentamicin; Mp, mupirocin; Rf, rifampicin; Te, tetracycline
Resistance Genotype: *mecA*, methicilin; *aacA-aphD*, aminoglycoside; *aadD*, tobramycin; *aphA*, neomycin/kanamycin *blaZ*, beta lactamase; *cat*, chloramphenicol; *dfrA*, trimethoprim; *ermA*, erythromycin/clindamycin; *ermC*, erythromycin/clindamycin; *far1*, fusidic acid; *fosB*, fosfomycin; *mpbBM*, lysylphosphatidylglycerol synthetase; *msr[a]*, mercuric; *mupR*, mupirocin; Q6GD50, fusidic acid; *qacA* quaternary ammonium compound; *qacC* quaternary ammonium compound; *sat*, streptomycin; *tetM*, *tetracycline*; tetK, tetracycline;

Additional File 2: DNA Microarray Targets, Primers and Probes							
Gene	**Synonyma**	**Gene product /function**	**Alleles**	**Probe name**	**Probe definition**	**Primer name**	**Primer definition**
aacA-aphD		bifunctional enzyme Aac/Aph, gentamicin resistance		aacA-aphD_10,4	AB096217.1 [28286:28313]	aacA-aphD_PM4	AB096217.1[28367:28386]
aadD		aminoglycoside adenyltransferase,tobramycin resistance		aadD_1,2	BA000017.4 [41203:41230:r]	aadD_PM4	BA000017.4[41144:41164:r]
agrB		accessory gene regulator B	*agrB-I*	agrB-I_11	CP000046.1 [2083620:2083646]	agrB-I_51	CP000046.1[2083674:2083696:r]
			agrB-II	agrB-II_11	BA000017.4 [2156206:2156234]	agrB-II_51	BA000017.4[2156235:2156253:r]
			agrB-III	agrB-III_11	BX571856.1 [2087653:2087682]	agrB-III_51	BX571856.1[2184604:2184626:r]
			agrB-IV	agrB-IV_11	AF288215.1 [1200:1226]	agrB-IV_51	AF288215.1[1255:1275:r]
agrC		accessory gene regulator C	*agrC-I*	agrC-I_12	CP000046.1 [2084385:2084411]	agrC-I_51	CP000046.1[2084470:2084490:r]
			agrC-II	agrC-II_11	BA000017.4 [2156768:2156793]	agrC-II_51	BA000017.4[2156859:2156881:r]
			agrC-III	agrC-III_11	BX571856.1 [2185117:2185143]	agrC-III_51	BX571856.1[2185152:2185173:r]
			agrC-IV	agrC-IV_11	AF288215.1 [1553:1580]	agrC-Ia_51	AF288215.1[2049:2069:r]
						agrC-IV_51	AF288215.1[1609:1631:r]
						agrC-IV_52	AJ617711.1[867:889:r]
agrD		accessory gene regulator D	*agrD-I*	agrD-I_11	CP000046.1 [2083761:2083788]	agrD-I_51	CP000046.1[2083792:2083813:r]
				agrD-I_12	CP000046.1 [2083765:2083788]	agrD-II+I_51	CP000046.1[2083820:2083842:r]
				agrD-I_13	CP000046.1 [2083761:2083783]		
			agrD-II	agrD-II_11	BA000017.4 [2156525:2156554]	agrD-II_51	BA000017.4[2156556:2156577:r]
			agrD-III	agrD-III_11	BX571856.1 [2184647:2184676]	agrD-III_51	BX571856.1[2184683:2184699:r]
aphA3		3'5'-aminoglycoside phosphotransferase, neo-/kanamycin resistance		aphA-3_18,3	AY602209.1 [105:130]	aphA-3_PM4	AY602209.1[206:223]
arcA		ACME-locus	*arcA-SCC*	hp_arcA_611	AE015929.1[102505:102530:r]	lb_arcA_651_rv	AE015929.1[102460:102479]
arcB		ACME-locus: ornithincarbamoyltransferase	*arcB-SCC*	hp_arcB_611	AE015929.1[99281:99307:r]	lb_arcB_651_rv	AE015929.1[99256:99274]
arcC		ACME-locus: carbamatkinase	*arcC-SCC*	hp_arcC_611	AE015929.1[98603:98631:r]	lb_arcC_651_rv	AE015929.1[98571:98590]
arcD		ACME-locus: arginine/ornithine-antiporter	*arcD-SCC*	hp_arcD_611	AE015929.1[101412:101440:r]	lb_arcD_651_rv	AE015929.1[101381:101398]
aur		aureolysin	*aur (cons)*	hp_aur_613	CP000046.1 [2721001:2721030:r]	lb_aur_651_rv	CP000046.1[2721434:2721453]
			aur (Other than MRSA252)	hp_aur_611	CP000046.1 [2721468:2721496:r]	lb_aur_653_rv	CP000046.1[2720967:2720987]
			aur (MRSA252)	hp_aur_612	BX571856.1 [2812419:2812446:r]	lb_aur_652_rv	BX571856.1[2812384:2812405]
bap		surface protein involved in biofilm formation		hp_bap_611	AY220730.1 [7832:7860]	lb_bap_651_rv	AY220730.1[7869:7891:r]
bbp		bone sialoprotein-binding protein	*bbp (cons)*	hp_bbp_614	CP000046.1 [640403:640431]	lb_bbp_654_rv	CP000046.1[640459:640476:r]
			bbp (ST45)	hp_bbp_611	AM076252.1 [3:31]	lb_bbp_656_rv	CP000046.1[642598:642620:r]
			bbp (RF122)	hp_bbp_612	AJ938182.1 [578264:578291]	lb_bbp_651_rv	AM076252.1[37:59:r]
			bbp (MRSA252)	hp_bbp_613	BX571856.1 [621217:621242]	lb_bbp_653_rv	BX571856.1[621266:621285:r]
			bbp (COL+MW2)	hp_bbp_616	CP000046.1 [642547:642573]	lb_bbp_655_rv	BX571856.1[621919:621939:r]
			bbp (Mu50)	hp_bbp_617	BA000017.4 [638429:638457]	lb_bbp_657_rv	BA000017.4[638483:638502:r]
						lb_bbp_652_rv	AJ938182.1[578305:578327:r]
blaI		beta lactamase repressor (inhibitor)		hp_blaI_611	BX571856.1 [1911923:1911949:r]	lb_blaI_651_rv	BX571856.1[1911882:1911903]
blaR		beta-lactamase regulatory protein		hp_blaR_611	BX571856.1 [1912975:1913002:r]	lb_blaR_652_rv	BX571856.1[1912922:1912942]
				hp_blaR_612	BX571856.1 [1912288:1912317:r]	lb_blaR_653_rv	BX571856.1[1912251:1912274]
blaZ		beta-lactamase		blaZ_11	AB074882.1 [417:441]	blaZ_PM4	BX571856.1[1914099:1914121]
				blaZ_4,2	BX571856.1 [1913997:1914021]	lb_blaZ_651_rv	BX571856.1[1914034:1914054:r]
				hp_blaZ_611	BX571856.1 [1914503:1914531]	lb_blaZ_652_rv	BX571856.1[1914553:1914570:r]
						lb_blaZ_653_rv	DQ016047.1[1101:1120:r]
capH		capsular polysaccharide synthesis enzyme CapH of capsule types 1, 5, and 8	*capH1*	hp_capH1_611	U10927.2 [19165:19192]	lb_capH1_651_rv	U10927.2[19210:19230:r]
			capH5	hp_capH5_611	CP000046.1 [161120:161144]	lb_capH5_651_rv	CP000046.1[161160:161180:r]
			capH8	hp_capH8_611	BX571856.1 [176513:176541]	lb_capH8_651_rv	BX571856.1[176544:176565:r]
capI		capsular polysaccharide biosynthesis protein CapI	*capI8*	hp_capI8_612	BX571856.1 [178269:178298]	lb_capI8_651_rv	BX571856.1[178195:178214:r]
						lb_capI8_652_rv	BX571856.1[178312:178332:r]

Additional File 2: DNA Microarray Targets, Primers and Probes							
Gene	**Synonyma**	**Gene product /function**	**Alleles**	**Probe name**	**Probe definition**	**Primer name**	**Primer definition**
capJ		O-antigen polymerase CapJ of capsule types 1, 5, and 8	*capJ1*	hp_capJ1_611	U10927.2 [21322:21350]	lb_capJ1_651_rv	U10927.2[21367:21385:r]
			capJ5	hp_capJ5_611	CP000046.1 [163178:163206]	lb_capJ5_651_rv	CP000046.1[163216:163233:r]
				hp_capJ5_612	CP000046.1 [163344:163373:r]	lb_capJ5_652_rv	CP000046.1[163316:163337]
			capJ8	hp_capJ8_611	BX571856.1 [178770:178800]	lb_capJ8_651_rv	BX571856.1[178809:178826:r]
capK		capsular polysaccharide biosynthesis protein CapK of capsule types 1, 5, and 8	*capK1*	hp_capK1_611	U10927.2 [22439:22466]	lb_capK1_651_rv	U10927.2[22490:22508:r]
			capK5	hp_capK5_611	CP000046.1 [164536:164564]	lb_capK5_651_rv	CP000046.1[164576:164595:r]
			capK8	hp_capK8_611	BX571856.1 [178990:179017]	lb_capK8_651_rv	BX571856.1[179021:179045:r]
				hp_capK8_612	BX571856.1 [179930:179956]	lb_capK8_652_rv	BX571856.1[179958:179980:r]
cat		chloramphenicol acetyltransferase	*cat-pC221*	hp_cat_613	M64281.1 [358:389]	lb_cat_654_rv	AB080798.1[2860:2878:r]
			cat-pC223	hp_cat_611	AF507977.1 [17615:17642]	lb_cat_653_rv	AF507977.1[17658:17682:r]
			cat-pMC524	hp_cat_612	AB080798.1 [2826:2854]	lb_cat_655_rv	AJ312056.2[587:605:r]
			cat-pSBK203R	hp_cat_615	M58515.1 [353:384]	lb_cat_652_rv	M58515.1[407:431:r]
ccrA		cassette chromosome recombinase A	*ccrA-1*	hp_ccrA-1_611	CP000046.1 [48646:48672:r]	lb_ccrA-1_651_rv	CP000046.1[48623:48642]
			ccrA-2	hp_ccrA-2_611	BA000017.4 [66314:66342:r]	lb_ccrA-1_652_rv	CP000046.1[48372:48390]
			ccrA-3	hp_ccrA-3_611	AB014436.1 [254:279]	lb_ccrA-2_651_rv	BA000017.4[66290:66306]
			ccrA-4	hp_ccrA-4_612	AF411935.1 [8756:8782]	lb_ccrA-2_652_rv	BA000017.4[65738:65756]
						lb_ccrA-2_653_rv	AB063173.1[6486:6506:r]
						lb_ccrA-3_651_rv	AB014436.1[283:300:r]
						lb_ccrA-3_652_rv	AB014436.1[826:844:r]
						lb_ccrA-4_651_rv	AF411935.1[8582:8602:r]
						lb_ccrA-4_652_rv	AF411935.1[8803:8823:r]
ccrB		cassette chromosome recombinase B	*ccrB-1*	hp_ccrB-1_612	CP000046.1 [47628:47652:r]	lb_ccrB-1_651_rv	CP000046.1[47771:47788]
				hp_ccrB-1_613	CP000046.1 [47794:47823:r]	lb_ccrB-1_652_rv	CP000046.1[47594:47614]
			ccrB-2	hp_ccrB-2_611	BA000017.4 [63943:63970:r]	lb_ccrB-2_651_rv	BA000017.4[63916:63937]
			ccrB-3	hp_ccrB-3_611	AB014436.1 [2110:2135]	lb_ccrB-2_652_rv	DQ483074.1[378:401:r]
			ccrB-4	hp_ccrB-4_611	AY918294.1 [319:343]	lb_ccrB-2_653_rv	BA000017.4[63678:63697]
						lb_ccrB-3_651_rv	AB014436.1[2160:2179:r]
						lb_ccrB-3_652_rv	AB014436.1[2265:2286:r]
						lb_ccrB-4_651_rv	AE015929.1[59580:59600]
						lb_ccrB-4_652_rv	AE015929.1[59457:59474]
ccrC		cassette chromosome recombinase		hp_ccrC_611	AP008934.1 [57303:57333]	lb_ccrC_651_rv	AB037671.1[60643:60662]
cfr		23S rRNA methyltransferase		hp_cfr_611	AJ249217.1 [1048:1074]	lb_cfr_651_rv	AJ249217.1[1075:1093:r]
chp		chemotaxis-inhibiting protein (CHIPS)		hp_chp_611	BX571856.1 [2126835:2126861]	lb_chp_651_rv	BX571856.1[2126883:2126903:r]
				hp_chp_612	BX571856.1 [2127086:2127114]	lb_chp_652_rv	BX571856.1[2127127:2127150:r]
clfA		clumping factor A	*clfA (cons)*	hp_clfA_611	CP000046.1 [881138:881166]	lb_clfA_651_rv	CP000046.1[881192:881211:r]
			clfA (COL+RF122)	hp_clfA_612	CP000046.1 [882182:882210]	lb_clfA_652_rv	CP000046.1[882220:882241:r]
			clfA (MRSA252)	hp_clfA_613	BX571856.1 [889789:889814]		
			clfA (Mu50+MW2)	hp_clfA_614	BA000017.4 [888713:888737]		
clfB		clumping factor B	*clfB (cons)*	hp_clfB_611	CP000046.1 [2713245:2713275:r]	lb_clfB_651_rv	CP000046.1[2713215:2713233]
			clfB (COL+Mu50)	hp_clfB_612	CP000046.1 [2712297:2712328:r]	lb_clfB_652_rv	CP000046.1[2712249:2712271]
			clfB (MW2)	hp_clfB_613	AM075901.1 [1069:1098]	lb_clfB_654_rv	AM075915.1[1125:1146:r]
			clfB (RF122)	hp_clfB_614	AJ938182.1 [2647736:2647765:r]	lb_clfB_653_rv	BX571856.1[2803198:2803221]
cna		collagen-binding adhesin		hp_cna_611	BX571856.1 [2879879:2879905:r]	lb_cna_651_rv	BX571856.1[2879853:2879871]
coA		coagulase		coa_consens_11	CP000046.1 [246925:246954]	coa_consens_PM4	CP000046.1[246967:246988]
dcs-Q9XB68		hypothetical protein from SCCmec elements		hp_Q9XB68_611	CP000046.1 [34948:34976]	lb_Q9XB68_651_rv	CP000046.1[35004:35027:r]
dfrA		dihydrofolate reductase type 1		dfrA_12	AE017171.1 [2588:2614:r]	dfrA_PM4	AE017171.1[2494:2513:r]
				2,1-dfrA	AB049452.1 [2076:2103]		
ebh		cell wall associated fibronectin-binding protein		hp_ebh-3prime_611	CP000046.1 [1483834:1483860:r]	lb_ebh-3prime_651_rv	CP000046.1[1483793:1483813]

Additional File 2: DNA Microarray Targets, Primers and Probes

Gene	Synonyma	Gene product /function	Alleles	Probe name	Probe definition	Primer name	Primer definition
ebpS		cell surface elastin binding protein	*ebpS (COL)*	hp_ebpS_613	CP000046.1 [1561278:1561303:r]	lb_ebpS_651_rv	CP000046.1[1561493:1561511]
				hp_ebpS_612	CP000046.1 [1561514:1561541:r]	lb_ebpS_652_rv	CP000046.1[1561245:1561268]
				hp_ebpS_614	CP000046.1 [1560815:1560839:r]	lb_ebpS_653_rv	CP000046.1[1560784:1560801]
			ebpS-01-1111 (from CC45)	hp_ebpS_611	AM075954.1 [148:172]		
edinA		epidermal cell differentiation inhibitor precursor		edinA_11	M63917.1 [460:489]	edinA_51	M63917.1[502:520:r]
edinB		epidermal cell differentiation inhibitor B		edinB_11	AB057421.1 [7445:7471]	edinB_51	AB057421.1[7482:7501:r]
edinC		epidermal cell differentiation inhibitor C		edinC_11	AP003088.1 [1810:1839:r]	edinC_51	AP003088.1[1755:1776]
eno		enolase		hp_eno_611	CP000046.1 [870472:870501]	lb_eno_651_rv	CP000046.1[870526:870544:r]
erm(A)	*ermA*	rRNA adenine N-6-methyltransferase, erythromycin/clindamycin resistance		ermA_9,4	BA000017.4 [1762850:1762875]	ermA_PM4	BA000017.4[1762907:1762928]
erm(C)	*ermC*	erythromycin/clindamycin resistance		ermC_8,1	M17990.1 [1775:1799]	ermC_PM4	AF466402.1[130:150]
				ermC_8,2	M17990.1 [1840:1864]		
etA		exfoliative toxin serotype A		etA_8,2	AP001553.1 [42317:42344]	etA_PM4	AP001553.1[42387:42406]
etB		exfoliative toxin serotype B		etB_9,3	AP003088.1 [5389:5416]	etB_PM4	AP003088.1[5438:5460]
etD		exfoliative toxin D		etD_11	AB057421.1 [5648:5677]	etD_51	AB057421.1[5694:5715:r]
far1	*fusB*	fusidic acid resistence		far1_10	AY047358.1 [1787:1814]	far1_11_PM4	AY047358.1[1818:1838:r]
fexA		chloramphenicol/florfenicol exporter		hp_fexA_611	AJ549214.1 [332:357]	lb_fexA_651_rv	AJ549214.1[364:382:r]
fib		fibrinogen binding protein (19 kDa)	*fib*	hp_fib_611	CP000046.1 [1177103:1177127]	lb_fib_651_rv	CP000046.1[1177131:1177148:r]
			fib (MRSA252)	hp_fib_612	BX571856.1 [1178081:1178105]		
fnbA		fibronectin-binding protein A	*fnbA (cons)*	hp_fnbA_615	CP000046.1 [2570812:2570840:r]	lb_fnbA_652_rv	CP000046.1[2571555:2571576]
			fnbA (COL)	hp_fnbA_612	CP000046.1 [2571598:2571624:r]	lb_fnbA_655_rv	CP000046.1[2570764:2570784]
			fnbA (MRSA252)	hp_fnbA_613	BX571856.1 [2662314:2662342:r]	lb_fnbA_653_rv	BX571856.1[2662290:2662308]
			fnbA (Mu50+MW2)	hp_fnbA_611	BA000017.4 [2644424:2644451:r]	lb_fnbA_651_rv	BA000017.4[2644388:2644409]
			fnbA (RF122)	hp_fnbA_614	AJ938182.1 [2510055:2510084:r]	lb_fnbA_654_rv	AJ938182.1[2510030:2510048]
						lb_fnbA_656_rv	AM076033.1[1356:1379:r]
fnbB		fibronectin-binding protein B	*fnbB (COL)*	hp_fnbB_614	CP000046.1 [2567853:2567879:r]	lb_fnbB_657_rv	CP000046.1[2567809:2567829]
			fnbB (COL+Mu50+MW2)	hp_fnbB_616	CP000046.1 [2567182:2567212:r]	lb_fnbB_658_rv	CP000046.1[2567156:2567173]
			fnbB (Mu50)	hp_fnbB_611	BA000017.4 [2640460:2640489:r]	lb_fnbB_653_rv	BA000017.4[2640421:2640441]
			fnbB (MW2)	hp_fnbB_613	BA000033.2 [2578791:2578820:r]	lb_fnbB_654_rv	BA000017.4[2640538:2640560]
			fnbB (ST15)	hp_fnbB_612	AM076087.1 [758:783]	lb_fnbB_656_rv	AM076068.1[893:912:r]
			fnbB (ST45-2)	hp_fnbB_615	AM076078.1 [866:893]	lb_fnbB_651_rv	AM076087.1[905:925:r]
						lb_fnbB_652_rv	AM076079.1[914:933:r]
						lb_fnbB_655_rv	AM076078.1[900:920:r]
fosB		metallothiol transferase	*fosB*	hp_fosB_611	CP000046.1 [2389191:2389221]	lb_fosB_651_rv	CP000046.1[2389252:2389271:r]
			fosB-plasmid	hp_fosB_612	AP006717.1 [448:478]	lb_fosB_652_rv	AP006717.1[508:527:r]
gapA		glyceraldehyde 3-phosphate dehydrogenase, locus 1		gapA_11	CP000046.1 [865778:865806]	gapA_51	CP000046.1[865816:865836:r]
hl		putative membrane protein		hl_11	CP000046.1 [927983:928011]	hl_51	CP000046.1[928034:928052:r]
hla		haemolysin alpha		hla_11	CP000046.1 [1180134:1180163:r]	hla_51	CP000046.1[1180098:1180120]
hlb		haemolysin beta		hp_hlb_611	CP000046.1 [2063898:2063922]	hlb_51	CP000046.1[2063925:2063944:r]
				hp_hlb_612	BA000017.4 [2126171:2126196]		
				hp_hlb_613	S72497.1 [366:390]		
			hlb, un-disrupted	hlb_11	CP000046.1 [2063880:2063906]		
				hlb_12	S72497.1 [347:374]		
hld		haemolysin delta		hld_11	CP000046.1 [2082840:2082864:r]	hld_51	CP000046.1[2082797:2082819]
hlgA		haemolysin gamma, component A		hlgA_11	CP000046.1 [2479145:2479171]	hlgA_51	CP000046.1[2479172:2479193:r]

Additional File 2: DNA Microarray Targets, Primers and Probes							
Gene	**Synonyma**	**Gene product /function**	**Alleles**	**Probe name**	**Probe definition**	**Primer name**	**Primer definition**
hlIII		putative membrane protein	*hlIII-consensus*	hp_hlIII_611	CP000046.1 [2239419:2239444:r]	lb_hlIII_651_rv	CP000046.1[2239376:2239397]
			hlIII- other than RF122	hl-III_11	CP000046.1 [2239828:2239856:r]	hl-III_51	CP000046.1[2239783:2239805]
hsdS1		type I site-specific deoxyribonuclease subunit, 1st locus	*hsdS1 (RF122)*	hp_hsdS-RF122-1_611	AJ938182.1 [317663:317689]	lb_hsdS-RF122-1_651_rv	AJ938182.1[317702:317723:r]
hsdS2		type I site-specific deoxyribonuclease subunit, 2nd locus	*hsdS2 (ST5+ST8)*	hp_hsdS-COL-1_611	CP000046.1 [478949:478977]	lb_hsdS-COL-1_651_rv	CP000046.1[478988:479006:r]
			hsdS2 (MW2+476)	hp_hsdS-MW2-1_611	BX571857.1 [441641:441667]	lb_hsdS-MW2-1_651_rv	BA000033.2[443035:443058:r]
			hsdS2 (RF122)	hp_hsdS-RF122-2_611	AJ938182.1 [422326:422351]	lb_hsdS-RF122-2_651_rv	AJ938182.1[422376:422398:r]
			hsdS2 (MRSA252)	hp_hsdS-MRSA252-1_611	BX571856.1 [463045:463073]	lb_hsdS-MRSA252-1_651_rv	BX571856.1[463099:463120:r]
hsdS3		type I site-specific deoxyribonuclease subunit, 3rd locus	*hsdS3 (Other Than RF122+MRSA252)*	hp_hsdS-CC25_611	CP000046.1 [1913615:1913643:r]	lb_hsdS-COL-2_651_rv	CP000046.1[1913581:1913598]
			hsdS3 (ST8+ST1+RF122)	hp_hsdS-COL-2_611	DQ309452.1 [57:85]		
			hsdS3 (Mu50+N315)	hp_hsdS-Mu50-2_611	BA000017.4 [1935888:1935914:r]	lb_hsdS-Mu50-2_651_rv	BA000017.4[1935844:1935865]
			hsdS3 (CC51+252)	hp_hsdS-CC51_611	BX571856.1 [1983689:1983715:r]	lb_hsdS-CC51_651_rv	BX571856.1[1983667:1983686]
			hsdS3 (MRSA252)	hp_hsdS-MRSA252-2_611	BX571856.1 [1983034:1983063:r]	lb_hsdS-MRSA252-2_651_rv	BX571856.1[1983003:1983023]
hsdSx		type I site-specific deoxyribonuclease subunit, unknown locus	*hsdSx (CC25)*	hp_hsdS-CC25_612	CP000046.1 [1914582:1914609:r]	lb_hsdS-CC25_651_rv	CP000046.1[1914550:1914567]
			hsdSx (CC15)	hp_hsdS-CC15_611	DQ309450.1 [976:1000]	lb_hsdS-CC15_651_rv	DQ309450.1[1009:1031:r]
			hsdSx (etd)	hp_hsdS-etd_611	AB057421.1 [2572:2598:r]	lb_hsdS-etd_651_rv	AB057421.1[2543:2565]
hysA1/2		hyaluronate lyase, first / second locus	*hysA1 (MRSA252)*	hp_hysA_613	BX571856.1 [1975471:1975495]	lb_hysA_652_rv	BX571856.1[1975386:1975408:r]
			hysA1 (MRSA252+RF122) and/or hysA2 (cons)	hp_hysA_614	CP000046.1 [2275950:2275980]	lb_hysA_651_rv	CP000046.1[2275984:2276004:r]
			hysA1 (MRSA252+RF122) / hysA2 (COL+USA300)	hp_hysA_615	BX571856.1 [1975353:1975381]		
hysA2		hyaluronate lyase, second locus	*hysA2 (All Other Than MRSA252)*	hp_hysA_611	CP000046.1 [2274647:2274673]	lb_hysA_653_rv	CP000046.1[2274574:2274597:r]
			hysA2 (COL+USA300+NCTC8325)	hp_hysA_617	CP000046.1 [2274542:2274572]	lb_hysA_654_rv	CP000046.1[2274687:2274705:r]
			hysA2 (All Other Than COL+USA300+NCTC8325)	hp_hysA_616	BX571856.1 [2376035:2376064]		
			hysA2 (All OtherThan COL+USA300+NCTC8325)	hp_hysA_618	BA000017.4 [2343407:2343437]		
			hysA2 (MRSA252)	hp_hysA_612	BX571856.1 [2376142:2376170]		
icaA		intercellular adhesion protein A		hp_icaA_611	CP000046.1 [2764366:2764391]	lb_icaA_651_rv	CP000046.1[2764412:2764432:r]
icaC		intercellular adhesion protein C		hp_icaC_611	CP000046.1 [2766355:2766384]	lb_icaC_651_rv	CP000046.1[2766392:2766410:r]
icaD		biofilm PIA synthesis protein D		hp_icaD_611	CP000046.1 [2764671:2764700]	lb_icaD_651_rv	CP000046.1[2764728:2764750:r]
isaB		immunodominant antigen B	*isaB*	hp_isaB_611	CP000046.1 [2722864:2722888:r]	lb_isaB_651_rv	CP000046.1[2722835:2722853]
			isaB-MRSA252	hp_isaB_612	BX571856.1 [2813801:2813828:r]	lb_isaB_652_rv	BX571856.1[2813775:2813793]
isdA		transferrin-binding protein	*isdA (cons)*	hp_isdA_611	CP000046.1 [1148523:1148547:r]	lb_isdA_651_rv	CP000046.1[1148500:1148518]
			isdA (MRSA252)	hp_isdA_612	BX571856.1 [1149423:1149447:r]	lb_isdA_653_rv	BX571856.1[1149379:1149401]
			isdA (Other Than MRSA252)	hp_isdA_614	CP000046.1 [1148394:1148422:r]	lb_isdA_652_rv	CP000046.1[1148360:1148382]
						lb_isdA_654_rv	AY175448.1[298:320:r]
katA		katalase A		katA_11	CP000046.1 [1374794:1374818]	katA_PM4	CP000046.1[1374825:1374845]
kdpA		potassium-translocating ATPase A, chain 2		hp_kdpA-SCC_611	BA000017.4 [77596:77622]	lb_kdpA-SCC_651_rv	BA000017.4[77655:77675:r]
						lb_kdpA-SCC_652_rv	BA000017.4[77890:77910:r]
kdpB		potassium-transporting ATPase B, chain 1		hp_kdpB-SCC_611	BA000017.4 [79736:79763]	lb_kdpB-SCC_651_rv	BA000017.4[79776:79795:r]
kdpC		potassium-translocating ATPase C, chain 2		hp_kdpC-SCC_612	BA000017.4 [81035:81061:r]	lb_kdpC-SCC_651_rv	BA000017.4[80962:80980:r]
						lb_kdpC-SCC_652_rv	BA000017.4[81011:81031]
kdpD		sensor kinase protein		hp_kdpD-SCC_611	BA000017.4 [76370:76397:r]	lb_kdpD-SCC_651_rv	BA000017.4[76331:76349]
kdpE		KDP operon transcriptional regulatory protein		hp_kdpE-SCC_611	BA000017.4 [73744:73769:r]	lb_kdpE-SCC_651_rv	BA000017.4[73717:73735]
linA		Lincosaminid-Nucleotidyltransferase		linA_19,2	J03947.1 [866:890]	linA_51	J03947.1[1049:1069:r]
				linA_19,3	J03947.1 [938:962]	linA_PM4	J03947.1[1036:1053]
lmrP		hypothetical protein, similar to integral membrane protein LmrP	*lmrP (OtherThanRF122)*	hp_lmrP_611	CP000046.1 [181497:181522]	lb_lmrP_651_rv	CP000046.1[181530:181547:r]
			lmrP (OtherThanRF122)	hp_lmrP_613	CP000046.1 [182184:182210]	lb_lmrP_653_rv	CP000046.1[182214:182234:r]
			lmrP (RF122)	hp_lmrP_612	AJ938182.1 [140620:140646]	lb_lmrP_652_rv	AJ938182.1[140655:140672:r]
			lmrP (RF122)	hp_lmrP_614	AJ938182.1 [141308:141333]	lb_lmrP_654_rv	AJ938182.1[141338:141358:r]
lukD		leukocidin D component		lukD_11	CP000046.1 [1934731:1934760:r]	lukD_51	CP000046.1[1934686:1934706]
lukE		leukocidin E component		lukE_11	CP000046.1 [1935944:1935968:r]	lukE_51	CP000046.1[1935901:1935920]

Additional File 2: DNA Microarray Targets, Primers and Probes

Gene	Synonyma	Gene product /function	Alleles	Probe name	Probe definition	Primer name	Primer definition
lukF-hlg		haemolysin gamma / leukocidin, component B		lukF_10	CP000046.1 [2481634:2481659]	lukF_11b_PM4	CP000046.1[2481663:2481680:r]
lukS-hlg		haemolysin gamma / leukocidin, component C	*lukS*	lukS_10	CP000046.1 [2480644:2480668]	lukS_11_PM4	CP000046.1[2480672:2480692:r]
			lukS-ST45	hp_lukS-ST45_611	EF672356.1[663:686]		EF672356.1[690:710:r]
							EF672356.1[690:711:r]
lukF-PV		Panton Valentine leukocidin F component		lukF_PV_10	AB006796.1 [2256:2284]	lukF-PV_11_PM4	AB006796.1[2295:2316:r]
lukS-PV		Panton Valentine leukocidin S component		lukS_PV_20	AB006796.1 [1628:1656]	lukS-PV_21_PM4	AB006796.1[1679:1699:r]
lukF-PV83		F component from hypothetical leukocidin from ruminants		lukF-PV-P83_11	AB044554.1 [42010:42037]	lukF-PV-P83_51	AB044554.1[42053:42070:r]
lukM		S component from hypothetical leukocidin from ruminants		lukM_11	AB044554.1 [40866:40893]	lukM_51	AB044554.1[40914:40932:r]
"lukX"	*SAV2004*	leukocidin/haemolysin toxin family protein		lukX_11	CP000046.1 [2065056:2065080:r]	lukX_51	CP000046.1[2065011:2065033]
"lukY"	*SAV2005*	leukocidin/haemolysin toxin family protein	*lukY*	lukY-var1_11	CP000046.1 [2066795:2066824:r]	lukY-var2_51	CP000046.1[2066757:2066777]
			lukY-MRSA252	lukY-var2_11	BX571856.1 [2171414:2171443:r]		
map		Major histocompatibility complex class II analog protein	*map (COL)*	hp_map_611	CP000046.1 [2063009:2063037:r]	lb_map_652_rv	CP000046.1[2062965:2062984]
		(=Extracellular adherence protein, eap)	*map (MRSA252)*	hp_map_613	BX571856.1 [2123661:2123688:r]	lb_map_651_rv	BX571856.1[2123618:2123636]
			map (Mu50+MW2)	hp_map_612	BA000017.4 [2082228:2082252:r]	lb_map_653_rv	BA000017.4[2082185:2082206]
mecA		penicillin binding protein 2, betalactam resistance defining MRSA		mecA_1,4	CP000046.1 [39915:39942:r]	mecA_PM4	CP000046.1[39857:39876:r]
				mecA_11	CP000046.1 [40041:40068:r]	mecA_51	CP000046.1[40007:40025]
mecI		meticillin-resistance regulatory protein		hp_mecI_611	BA000017.4 [49133:49162]	lb_mecI_651_rv	BA000017.4[49169:49190:r]
mecR1	*mecR*	signal transducer protein MecR1	*mecR1-truncated only*	hp_mecR_611	CP000046.1 [41853:41882]	lb_mecR_651_rv	CP000046.1[41885:41906:r]
			mecR1-untruncated	hp_mecR_612	BA000017.4 [48685:48711]	lb_mecR_652_rv	BA000017.4[48726:48745:r]
mefA		macrolide efflux protein A		hp_mefA_611	AB011259.1 [536:563]	lb_mefA_651_rv	AB011259.1[570:588:r]
				hp_mefA_612	AB011259.1 [1045:1072]	lb_mefA_652_rv	AB011259.1[1078:1099:r]
merA		mercury-reductase		hp_merA_611	AB179623.1 [2351:2379:r]	lb_merA_651_rv	AB037671.1[39315:39334]
merB		mercuric resistance operon regulatory protein		hp_merB_611	AB179623.1 [1018:1043:r]	lb_merB_651_rv	AB037671.1[38000:38018]
mph(BM)	*mpbBM, mphBM*	probable lysylphosphatidylglycerol synthetase		hp_mpbBM_611	AB013298.1 [2664:2693]	lb_mpbBM_651_rv	AB013298.1[2700:2720:r]
				hp_mpbBM_612	AB013298.1 [2896:2924]	lb_mpbBM_652_rv	AB013298.1[2929:2947:r]
mprF		defensin resistance protein	*mprF (COL+MW2)*	hp_mprF_611	CP000046.1 [1407488:1407518]	lb_mprF_651_rv	CP000046.1[1407519:1407542:r]
			mprF (Mu50+252)	hp_mprF_612	BA000017.4 [1442171:1442201]		
msr(A)	*msrA*	energy-dependent efflux of erythromycin		msrA_15,3	AB013298.1 [1525:1552]	msrA_PM4	AB013298.1[1614:1635]
mupR	*mupA*	mupirocin resistence protein		mupR_13,2	X75439.1 [1504:1531]	mupR_PM4	X75439.1[1623:1642]
nuc1		thermostable extracellular nuclease		hp_nuc1_611	CP000046.1 [888207:888233]	lb_nuc1_651_rv	CP000046.1[888249:888266:r]
ORF CM14		enterotoxin-like protein ORF CM14		hp_entCM14_611	AJ938182.1 [37154:37182]	entCM14_51	U10927.2[32900:32918:r]
				hp_entCM14_612	AJ938182.1 [37532:37557]	lb_entCM14_651_rv	AJ938182.1[37591:37610:r]
pls-SCC		plasmin-sensitive surface protein		hp_plsSCC_611	CP000046.1 [57330:57354]	lb_plsSCC_651_rv	CP000046.1[57378:57398:r]
Q2FXC0		hypothetical protein, located next to serine protease operon		hp_Q2FXC0_611	CP000046.1 [1922667:1922692]	lb_Q2FXC0_651_rv	CP000046.1[1922702:1922721:r]
Q2YUB3		Unspecific efflux/transporter		hp_Q2YUB3_611	AJ938182.1 [2026944:2026969:r]	lb_Q2YUB3_651_rv	AJ938182.1[2026920:2026937]
Q6GD50		hypothetical protein associated with fusidic acid resistance		hp_Q6GD50_611	AF411935.1 [423:452:r]	lb_Q6GD50_651_rv	AF411935.1[372:390]
Q7A4X2		hypothetical protein		hp_Q7A4X2_611	BA000017.4 [1952955:1952984]	lb_Q7A4X2_651_rv	BA000017.4[1953003:1953025:r]
qacA		quaternary ammonium compound resistance protein A		hp_qacA_611	AF053771.1 [976:1004]	qacA_PM4	AF053771.1[2361:2383]
						lb_qacA_651_rv	AB255366.1[19153:19173:r]
						lb_qacA_652_rv	AB255366.1[20475:20498:r]
qacC		quaternary ammonium compound resistance protein C	*qacC (cons)*	hp_qacC_611	AB125342.1 [2382:2411]	lb_qacC_651_rv	AB125342.1[2431:2450:r]
			qacC (equine)	hp_qacC_614	AJ512814.1 [1518:1545]	lb_qacC_653_rv	AJ512814.1[1567:1590:r]
			qacC (SA5)	hp_qacC_613	U81980.1 [2017:2043]	lb_qacC_654_rv	U81980.1[2065:2086:r]
			qacC (Ssap)	hp_qacC_612	Y16945.1 [1951:1981]	lb_qacC_652_rv	AE016833.1[8848:8869:r]
			qacC (ST94)	hp_qacC_615	Y16944.1 [1622:1649]	lb_qacC_655_rv	Y16944.1[1692:1714:r]
rrn STAU		Ribosomal sequence from S. aureus (genusspecific positive control)		s_aur_rrn_1PM4	CP000046.1 [1979941:1979966:r]	saur_rrn_1_6_PM4	CP000046.1[1979903:1979921:r]
						saur_rrn_1_7_PM4	CP000046.1[1979866:1979882:r]
saeS		histidine protein kinase, sae locus		hp_saeS_611	CP000046.1 [788443:788471:r]	lb_saeS_651_rv	CP000046.1[788396:788417]
						lb_saeS_652_rv	CP000046.1[787918:787939]
sak		staphylokinase		hp_sak_611	BA000017.4 [2086572:2086601:r]	lb_sak_651_rv	BA000017.4[2086553:2086571]
				sak_11	BA000017.4 [2086418:2086443:r]	sak_51	BA000017.4[2086376:2086395]

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Gene	Synonyma	Gene product /function	Alleles	Probe name	Probe definition	Primer name	Primer definition
sarA		staphylococcal accessory regulator A		hp_sarA_611	CP000046.1 [700076:700102:r]	lb_sarA_651_rv	CP000046.1[700049:700066]
sasG		Staphylococcus aureus surface protein G	*sasG (COL+Mu50)*	hp_sasG_613	CP000046.1 [2562368:2562396:r]	lb_sasG_651_rv	CP000046.1[2562772:2562794]
			sasG (MW2)	hp_sasG_612	BX571857.1 [2552889:2552918:r]	lb_sasG_652_rv	CP000046.1[2562337:2562358]
			sasG (OtherThan MRSA252+RF122)	hp_sasG_611	CP000046.1 [2562815:2562842:r]	lb_sasG_653_rv	BA000033.2[2573526:2573543]
sat		streptothricine-acetyltransferase		sat_17,2	U51474.1 [393:421]	sat_PM4	U51474.1[488:505]
				sat_17,3	U51474.1 [429:456]		
sbi		IgG-binding protein		sbi-var1_11	CP000046.1 [2476904:2476929]	sbi-var1_51	CP000046.1[2476963:2476982:r]
				sbi-var1_12	CP000046.1 [2477142:2477171]	sbi-var1_52	CP000046.1[2477188:2477210:r]
scn		Staphylococcal complement inhibitor (SCIN)		hp_scn_611	BA000017.4 [2084397:2084425:r]	lb_scn_651_rv	BA000017.4[2084367:2084387]
sdrC		Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein C	*sdrC (cons)*	hp_sdrC_613	CP000046.1 [633257:633283]	lb_sdrC_651_rv	CP000046.1[632900:632919:r]
			sdrC (B1)	hp_sdrC_612	AM076155.1 [1009:1036]	lb_sdrC_652_rv	AM076155.1[1039:1061:r]
			sdrC (COL)	hp_sdrC_615	CP000046.1 [633866:633892]	lb_sdrC_653_rv	CP000046.1[633304:633322:r]
			sdrC (Mu50)	hp_sdrC_614	BA000017.4 [630748:630774]	lb_sdrC_655_rv	CP000046.1[633915:633933:r]
			sdrC (MW2+MRSA252+RF122)	hp_sdrC_616	BX571856.1 [617689:617717]	lb_sdrC_654_rv	BX571856.1[617128:617145:r]
			sdrC (Other Than MRSA252+RF122)	hp_sdrC_611	CP000046.1 [632858:632886]		
sdrD		Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein D	*sdrD (cons)*	hp_sdrD_614	CP000046.1 [637654:637681]	lb_sdrD_652_rv	CP000046.1[637025:637046:r]
			sdrD (COL+MW2)	hp_sdrD_612	CP000046.1 [636995:637020]	lb_sdrD_654_rv	CP000046.1[637691:637712:r]
			sdrD (Mu50)	hp_sdrD_613	BA000017.4 [633896:633924]	lb_sdrD_655_rv	AM076208.1[854:875:r]
			sdrD (other)	hp_sdrD_611	AM076221.1 [157:186]	lb_sdrD_653_rv	BA000017.4[633928:633948:r]
						lb_sdrD_651_rv	AM076221.1[195:212:r]
sea	*entA, entP, sep*	enterotoxin A	*sea*	entA_3,2	BA000017.4 [2088572:2088598:r]	entA-var1_51	BA000017.4[2088473:2088492]
				entA_3,3	BA000017.4 [2088482:2088456]		
				entA-var1_11	BA000017.4 [2088512:2088536:r]		
			sea-320E (entA-320E)	entA-var2_11	AY196686.1 [508:532]		
			sea-N315 (entP, sep)	entA-var3_11	BA000018.3 [2011518:2011545:r]	entA-var3_51	BA000018.3[2011492:2011510]
seb	*entB*	enterotoxin B		entB_11	CP000046.1 [916903:916927]	entB_51	CP000046.1[916957:916976:r]
				entB_4,1	CP000046.1 [916583:916609]	entB-41_PM4	CP000046.1[916642:916663]
sec	*entC*	enterotoxin C		entC_5,2	BA000017.4 [2134733:2134761:r]	entC_PM4	BA000017.4[2134593:2134610:r]
				entC_5,3	BA000017.4 [2134652:2134680:r]		
sed	*entD*	enterotoxin D		entD_11	M94872.1 [675:702]	entD_51	M94872.1[705:723:r]
see	*entE*	enterotoxin E		entE_11	AY518387.1 [305:328]	entE_51	AY518387.1[348:365:r]
seg	*entG*	enterotoxin G		entG_11	BA000017.4 [1954500:1954526:r]	entG_51	BA000017.4[1954468:1954486]
seh	*entH*	enterotoxin H		entH_11	AB060536.1 [139:164]	entH_51	AB060536.1[178:196:r]
sei	*entI*	enterotoxin I		entI_11	BA000017.4 [1957319:1957343:r]	entI_51	BA000017.4[1957273:1957293]
sej	*entJ*	enterotoxin J		entJ_11	AB075606.1 [1849:1876:r]	entJ_51	AB075606.1[1804:1823]
sek	*entK*	enterotoxin K		hp_entK_611	CP000046.1 [905353:905384:r]	entK_PM4	CP000046.1[904957:904979:r]
				hp_entK_612	CP000046.1 [905035:905065:r]	lb_entK_651_rv	CP000046.1[905311:905334]
						lb_entK_652_rv	CP000046.1[904995:905017]
						lb_entK_653_rv	BA000033.2[2087412:2087433:r]
sel	*entL*	enterotoxin L		entL_11	BA000017.4 [2134144:2134171]	entL_51	BA000017.4[2134182:2134202:r]
sem	*entM*	enterotoxin M		entM_11	BA000017.4 [1958262:1958291:r]	entM_51	BA000017.4[1958242:1958260]
						entM_52	BX571856.1[1999451:1999468]
sen	*entN*	enterotoxin N	*sen- other than RF122*	entN_11	BA000017.4 [1955521:1955545:r]	entN_51	BA000017.4[1955492:1955513]
			sen-consensus	hp_entN_611	BA000017.4 [1955741:1955768:r]	lb_entN_651_rv	BA000017.4[1955709:1955731]
seo	*entO*	enterotoxin O		entO_11	BA000017.4 [1958936:1958962:r]	entO_51	BA000017.4[1958904:1958925]
seq	*entQ*	enterotoxin Q		hp_entQ_611	CP000046.1 [906043:906072:r]	entQ_PM4	CP000046.1[905715:905734:r]
				hp_entQ_612	CP000046.1 [905818:905848:r]	lb_entQ_651_rv	CP000046.1[905997:906018]
						lb_entQ_652_rv	CP000046.1[905862:905881]
ser	*entR*	enterotoxin R		entR_11	AB075606.1 [750:775]	entR_51	AB075606.1[783:802:r]

Additional File 2: DNA Microarray Targets, Primers and Probes							
Gene	**Synonyma**	**Gene product /function**	**Alleles**	**Probe name**	**Probe definition**	**Primer name**	**Primer definition**
"setB1"		staphylococcal exotoxin-like protein, second locus	*setB1*	setB-SA1178_11	CP000046.1 [1182940:1182967:r]	setB-SA1178_51	CP000046.1[1182916:1182934]
			setB1-MRSA252	setB-SAR1139_11	BX571856.1 [1185070:1185094:r]	setB-SAR1139_51	BX571856.1[1185043:1185061]
"setB2"		staphylococcal exotoxin-like protein, second locus	*setB2*	setB-SA1179_11	CP000046.1 [1183750:1183777:r]	setB-SA1179_51	CP000046.1[1183717:1183736]
			setB2-MRSA252	setB-SAR1140_11	BX571856.1 [1185785:1185812:r]	setB-SAR1140_51	BX571856.1[1185730:1185749]
"setB3"	*MW0345*	staphylococcal exotoxin-like protein, second locus	*setB3*	setB-SA1180_11	CP000046.1 [1184568:1184596:r]	setB-SA1180	CP000046.1[1184540:1184558]
"setC"		staphylococcal exotoxin-like protein		setC-MW0345_11	CP000046.1 [446394:446420]	setC-MW0345_51	CP000046.1[446441:446462:r]
seu / sey		Enterotoxin U and/or Y		hp_entU_611	BA000017.4 [1956679:1956708:r]	lb_entU_651_rv	BA000017.4[1956633:1956653]
spa		Protein A		proteinA_12	CP000046.1 [107407:107435:r]	proteinA_51	CP000046.1[107900:107917]
						proteinA_52	CP000046.1[107378:107395]
splA		Serinprotease A		splA_11	CP000046.1 [1921021:1921046:r]	splA_51	CP000046.1[1920987:1921005]
splB		Serinprotease B		splB_11	CP000046.1 [1920101:1920126:r]	splB_51	CP000046.1[1920073:1920090]
splE		Serinprotease E		hp_splE_611	CP000046.1 [1917590:1917613:r]	lb_splE_651_rv	CP000046.1[1917552:1917572]
ssl01	*set6, set16*	staphylococcal superantigen-like protein 1	*set6-COL* (SACOL468): probe 1_11+probe 4_11	set6-var1_11	CP000046.1 [470653:470680]	set6-var1_51	CP000046.1[470687:470707:r]
			set6-Mu50 (SAV0422): probe 1_11+probe 1_12/ 4_11	set6-var1_12	BA000017.4 [467359:467382]	set6-var1_52	CP000046.1[470893:470911:r]
			set6-MW2 (MW0382): probe 2_11+probe 2_12	set6-var2_11	BX571856.1 [452856:452881]	set6-var2_51	BX571856.1[452890:452909:r]
			SAR0422 (MRSA 252): probe 2_11+probe 1_12	set6-var2_12	BA000033.2 [429731:429757]		
				set6-var4_11	CP000046.1 [470856:470879]		
			ssl01-RF122	hp_ssl01_611	AJ938182.1 [412017:412045]	lb_ssl01_651_rv	AJ938182.1[412058:412076:r]
ssl02	*set7, set17*	staphylococcal superantigen-like protein 2	*ssl02*	set7-var1_11	CP000046.1 [471582:471608]	set7-var1_51	CP000046.1[471646:471665:r]
			ssl02-MRSA252	set7-var2_11	BX571856.1 [453788:453814]		
ssl03	*set8, set18*	staphylococcal superantigen-like protein 3	*ssl03*	set8_11	CP000046.1 [472456:472481]	set8_51	CP000046.1[472502:472520:r]
				hp_ssl03_611	AJ938182.1 [413800:413827]		
			ssl03-MRSA252	set-SAR0424_11	BX571856.1 [454824:454850]		
ssl04	*set9, set19*	staphylococcal superantigen-like protein 4	*ssl04*	set9-var1_11	CP000046.1 [474254:474283]	set9-var1_51	CP000046.1[474328:474349:r]
				set9-var1_12	CP000046.1 [474511:474537]	set9-var1_52	CP000046.1[474556:474575:r]
			ssl04-MRSA252	set-SAR0425_11	BX571856.1 [456040:456064]	set-SAR0425_51	BX571856.1[454869:454886:r]
				set-SAR0425_12	BX571856.1 [455386:455412]	set-SAR0425_52	BX571856.1[456703:456721:r]
ssl05	*set3, set20*	staphylococcal superantigen-like protein 5	*ssl05*	set3-var1_11	BA000017.4 [470660:470688]	set3-var1_51	BA000017.4[470703:470720:r]
				hp_ssl05_612	BA000017.4 [470300:470327]	lb_ssl05_652_rv	BA000017.4[470338:470359:r]
			ssl05-MRSA252	set3-var2_11	BX571856.1 [457227:457253]	set3-var2_51	BX571856.1[457263:457284:r]
			ssl05-RF122	hp_ssl05_611	AJ938182.1 [415143:415170]	lb_ssl05_651_rv	CP000253.1[393062:393084:r]
ssl06	*set21*	staphylococcal superantigen-like protein 6		set21_11	BA000033.2 [435210:435237]	set21_51	BA000033.2[435244:435264:r]
				hp_ssl06_611	BX571857.1 [433755:433784]	lb_ssl06_651_rv	BA000033.2[435132:435153:r]
						lb_ssl06_652_rv	CP000253.1[394288:394308:r]
ssl07	*set1, set22*	staphylococcal superantigen-like protein 7	*ssl07*	set1-var4_11	BA000017.4 [471560:471589]	set1-var1_51	BA000017.4[471600:471617:r]
			ssl07-MRSA252	set1-var1_11	BX571856.1 [458486:458515]		
			ssl07-FRI326	set1-var2_11	AF188836.1 [165:194]		
ssl08	*set12, set23*	staphylococcal superantigen-like protein 8	*ssl08*	set12_11	BA000017.4 [472594:472621]	set12_51	BA000017.4[472624:472644:r]
				hp_ssl08_611	AJ938182.1 [417435:417464]	lb_ssl08_651_rv	AJ938182.1[417465:417488:r]
ssl09	*set5, set24*	staphylococcal superantigen-like protein 9	*ssl09*	set5-var1_11	CP000046.1 [475126:475152]	set5-var1_51	CP000046.1[475171:475191:r]
				hp_ssl09_611	CP000046.1 [475125:475151]		
			ssl09-MRSA252	set5-var2_11	BX571856.1 [459446:459472]	set5-var2_51	BX571856.1[459498:459519:r]
ssl10	*set4, set25*	staphylococcal superantigen-like protein 10	*ssl10*	set4-var1_11	CP000046.1 [476429:476457]	set4-var1_51	CP000046.1[476474:476491:r]
			ssl10-MRSA252	hp_ssl10_611	AJ938182.1 [419736:419765]		
			ssl10-RF122	set4-var2_11	BX571856.1 [460746:460772]	set4-var2_51	BX571856.1[460783:460800:r]
ssl11	*set2, set26*	staphylococcal superantigene-like protein 11	*ssl11 (COL)*	set2-var4_11	CP000046.1 [480340:480368]	set2-var4_51	CP000046.1[480377:480395:r]
			ssl11 (Mu50+N315)	set2-var3_11	BA000017.4 [478916:478945]	set2-var3_51	BA000017.4[478947:478967:r]
			ssl11 (MW2+MSSA476)	set2-var1_11	BA000033.2 [443788:443816]	set2-var1_51	BA000033.2[443857:443879:r]
			ssl11 (MRSA252)	set2-var2_11	BX571856.1 [464936:464964]	set2-var2_51	BX571856.1[464979:464999:r]

Additional File 2: DNA Microarray Targets, Primers and Probes							
Gene	**Synonyma**	**Gene product /function**	**Alleles**	**Probe name**	**Probe definition**	**Primer name**	**Primer definition**
sspA		glutamylendopeptidase		hp_sspA_611	CP000046.1 [1063762:1063789:r]	lb_sspA_651_rv	CP000046.1[1063730:1063748]
						lb_sspA_653_rv	CP000046.1[1063034:1063053]
						lb_sspA_652_rv	BA000017.4[1097992:1098012]
sspB		staphopain B, Protease		hp_sspB_611	CP000046.1 [1062809:1062834:r]	lb_sspB_651_rv	CP000046.1[1062777:1062797]
				hp_sspB_612	CP000046.1 [1062120:1062149:r]	lb_sspB_652_rv	CP000046.1[1062098:1062116]
sspP		staphopain A (staphylopain A), Protease		hp_sspP_611	CP000046.1 [2034882:2034908]	lb_sspP_651_rv	CP000046.1[2034918:2034936:r]
			sspP (other than ST93)	hp_sspP_612	CP000046.1 [2035388:2035416]	lb_sspP_652_rv	CP000046.1[2035421:2035442:r]
"tetEfflux"		Transport-/Effluxprotein		hp_tetEfflux_611	CP000046.1 [2238561:2238587:r]	lb_tetEfflux_651_rv	CP000046.1[2238525:2238546]
						lb_tetEfflux_652_rv	BX571856.1[2342013:2342032]
tet(K)	*tetK*	tetrazyklin-resistance		tetK_12,3	M16217.1 [1424:1452]	tetK_PM4	M16217.1[1582:1604:r]
				tetK_12,4	M16217.1 [1507:1535]		
tet(M)	*tetM*	tetrazyklin-resistance		tetM_11,3	BA000017.4 [440374:440400:r]	tetM_51	BA000017.4[440340:440358]
tst1		toxic shock syndrome toxin 1	*tst1 (other than RF122)*	tst1_16,2	BA000017.4 [2137855:2137879]	tst1_PM4	BA000017.4[2137910:2137930]
			tst1 (consensus)	hp_tst_611	BA000017.4 [2138016:2138044]	lb_tst_651_rv	BA000017.4[2138051:2138070:r]
ugpQ		glycerophosphoryl diester phosphodiesterase, associated with mecA		hp_ugpQ_611	CP000046.1 [38665:38690]	lb_ugpQ_651_rv	CP000046.1[38701:38719:r]
vanA		vancomycin resistance gene		vanA_18,2	AE017171.1 [35199:35226]	vanA_PM4	AE017171.1[35302:35321]
vanB		vancomycin resistance gene from enterococci and Clostridium		vanB_11	AE016954.1 [78616:78640:r]	vanB_PM4	AE016954.1[78518:78535:r]
				vanB_19,3	AE016954.1 [78613:78638:r]		
vanZ		teicoplanin resistance gene from enterococci		vanZ_20,3	AE017171.1 [37564:37589]	vanZ_PM4	AE017171.1[37613:37632]
vatA		virginiamycin A acetyltransferase		vatA_15,3	AF117258.1 [2296:2323:r]	vatA_PM4	AF117258.1[2229:2247:r]
vatB		acetyltransferase inactivating streptogramin A		vatB_16,3	U19459.1 [543:570]	vatB_PM4	U19459.1[659:680]
vga		ATP binding protein, streptogramin-A-resistance	*vga*	vga_17,3	AF117259.1 [3729:3756]	vga_PM4	AF117259.1[3779:3799]
			vga-BM 3327	vgaA_18,3	AF186237.2 [6470:6497]	vgaA_PM4	AF117259.1[3925:3944]
vgb		virginiamycin B hydrolase		vgb_19,2	M36022.1 [1040:1067]	vgb_PM4	M36022.1[1126:1148]
vraS		sensor protein		hp_vraS_612	CP000046.1 [2005634:2005659:r]	lb_vraS_651_rv	CP000046.1[2005818:2005835]
						lb_vraS_652_rv	CP000046.1[2005605:2005624]
vwb		van Willebrand factor binding protein	*vwb (cons)*	hp_vwb_615	CP000046.1 [884859:884884]	lb_vwb_651_rv	CP000046.1[883877:883899:r]
			vwb (COL+MW2)	hp_vwb_612	CP000046.1 [883836:883864]	lb_vwb_656_rv	CP000046.1[884895:884914:r]
			vwb (MRSA252)	hp_vwb_613	BX571856.1 [891899:891927]	lb_vwb_653_rv	BX571856.1[891952:891972:r]
			vwb (Mu50)	hp_vwb_614	BA000017.4 [890919:890947]	lb_vwb_654_rv	BA000017.4[890960:890980:r]
						lb_vwb_655_rv	BA000017.4[891393:891412:r]
			vwb (RF122)	hp_vwb_611	AJ938182.1 [821019:821046]	lb_vwb_652_rv	AJ938182.1[821064:821083:r]
xylR		homolog of xylose repressor, associated with SCCmec-elements		hp_xylR_611	BA000017.4 [50113:50141]	lb_xylR_651_rv	BA000017.4[50152:50171:r]

8.3. PAPER THREE

The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* strain

Coombs GW, Goering RV, Chua KY, Monecke S, Howden BP, Stinear TP, Ehricht R, O'Brien FG, Christiansen KJ

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The Molecular Epidemiology of the Highly Virulent ST93 Australian Community *Staphylococcus aureus* Strain

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Abstract

In Australia the PVL - positive ST93-IV [2B], colloquially known as ''Queensland CA-MRSA'' has become the dominant CA-MRSA clone. First described in the early 2000s, ST93-IV [2B] is associated with skin and severe invasive infections including necrotizing pneumonia. A singleton by multilocus sequence typing (MLST) eBURST analysis ST93 is distinct from other *S aureus* clones. To determine if the increased prevalence of ST93-IV [2B] is due to the widespread transmission of a single strain of ST93-IV [2B] the genetic relatedness of 58 *S. aureus* ST93 isolated throughout Australia over an extended period were studied in detail using a variety of molecular methods including pulsed-field gel electrophoresis, *spa* typing, MLST, microarray DNA, SCC*mec* typing and *dru* typing. Identification of the phage harbouring the *lukS-PV/lukF-PV* Panton Valentine leucocidin genes, detection of allelic variations in *lukS-PV/lukF-PV*, and quantification of LukF-PV expression was also performed. Although ST93-IV [2B] is known to have an apparent enhanced clinical virulence, the isolates harboured few known virulence determinants. All PVL-positive isolates carried the PVL-encoding phage ΦSa2USA and the *lukS-PV/lukF-PV* genes had the same R variant SNP profile. The isolates produced similar expression levels of LukF-PV. Although multiple rearrangements of the *spa* sequence have occurred, the core genome in ST93 is very stable. The emergence of ST93-MRSA is due to independent acquisitions of different *dru*-defined type IV and type V SCC*mec* elements in several *spa*-defined ST93-MSSA backgrounds. Rearrangement of the *spa* sequence in ST93-MRSA has subsequently occurred in some of these strains. Although multiple ST93-MRSA strains were characterised, little genetic diversity was identified for most isolates, with PVL-positive ST93-IVa [2B]-t202-dt10 predominant across Australia. Whether ST93-IVa [2B] t202-dt10 arose from one PVL-positive ST93-MSSA-t202, or by independent acquisitions of SCCmec-IVa [2B]-dt10 into multiple PVL-positive ST93-MSSA-t202 strains is not known.

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Introduction

The community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) worldwide epidemic is polyclonal, however several well characterized clones predominate in different regions of the world: Sequence type (ST) 8-IV [2B] (USA300) and ST1-IV [2B] (USA400) in North America [1,2]; ST80-IV [2B] (European clone) in Europe [3], North Africa [4] and the Middle East [5]; ST59-V [5C2&5] (Taiwan clone) in Taiwan [6], ST30-IV [2B] (South West Pacific [SWP] CA-MRSA) in the Western Pacific [7,8] and ST772-MRSA-V [5C2] (Bengal Bay clone) in India and Bangladesh [9]. Transmission of these clones into other regions has occurred [10,11]. The occurrence of concurrent epidemics of CA-MRSA in many countries by different clones has been striking. Equally noteworthy are a number of common features of these epidemics, prominent among them the ability to cause severe infections in young otherwise healthy people and the carriage of *lukS-PV/lukF-PV* Panton Valentine Leukocidin (PVL) encoding genes by the organism.

In Australia the PVL - positive ST93-IV [2B], colloquially known as ''Queensland CA-MRSA'', has recently emerged to become the dominant CA-MRSA clone. First described in the early 2000s, ST93 is a singleton by MLST eBURST analysis and is therefore distinct from other *S aureus* clones [12].

In the 2010 Australian Group for Antimicrobial Resistance (AGAR) Community *S aureus* Surveillance Programme (SAP10)

ST93-IV [2B] accounted for 41.4% of all CA-MRSA, 27.6% of all MRSA and 4.9% of all *S aureus* community-onset infections (http://www.agargroup.org/files/FED%20REPORT%20SAP210%20MRSA%20FINAL%20shrink.pdf.). The mean age of patients with ST93-IV [2B] infections (31 years, median 25 years) was significantly lower (P<0.0001) than the mean age of patients with PVL negative CA-MRSA infections (53 years; median 57 years).

ST93-IV [2B] is associated with skin infection and severe invasive infection including necrotizing pneumonia, deep-seated abscess, osteomyelitis, septic arthritis and septicaemia [13,14,15]. Although ST93-IV [2B] has an apparent enhanced clinical virulence, the recently sequenced ST93-IV [2B] strain "JKD6159" has a relative paucity of recognizable virulence determinants [16,17]. This strain however does contain genes encoding three important CA-MRSA virulence factors, Hla, PVL and α-type phenol soluble modulins (PSMs), and when compared to three other well-characterised Australian MRSA strains, ST1-IV [2B], ST30-IV [2B] and ST239-III [3B] and the epidemic North American strain, USA300, was shown to be the most virulent in two *in vivo* models [17].

While predominately an Australian strain, ST93-IV [2B] has been reported in New Zealand, accounting for 5.1% of all MRSA referred to the Institute of Environmental Science and Research in 2010 (http://www.surv.esr.cri.nz/PDF_surveillance/Antimicrobial/MRSA/aMRSA_2010.pdf), and in the United Kingdom, [18], where many cases have epidemiological links to Australia.

In Western Australia (WA) ST93-IV [2B] was first identified in 2003 [19] and in SAP10 accounted for 28.8% of the state's CA-MRSA community-onset infections. In the mid 1990s *S aureus* screening of indigenous people living in WA remote communities demonstrated the most prevalent methicillin susceptible *S aureus* (MSSA) linage isolated was the PVL-positive ST93 MSSA clone [20]. Although seven CA-MRSA clones from genetically diverse backgrounds were identified in these communities, no ST93 MRSA was found during this time.

As Australia is a geographically large country with the majority of the population densely concentrated in a few major cities which are separated in many instances by vast desert areas, it is to be expected that different CA-MRSA clones will have evolved in different areas of Australia. To better understand the molecular epidemiology of ST93-IV [2B], the aim of this study was to analyse the genetic relatedness of *S. aureus* ST93 isolated throughout Australia over an extended period and to determine if the increased prevalence of ST93-IV [2B] has been due to the widespread transmission of a single strain of ST93-IV [2B] or has been due to multiple independent acquisitions of the SCC*mec* element into different strains of ST93 MSSA.

Materials and Methods

Bacterial Strains and Identification

Overall 58 ST93 *S. aureus* were included in the study. The 13 ST93-MSSA included four isolates from remote aboriginal communities in WA, isolated from 1995 to 2003; two isolates from the Northern Territory, isolated in 1992; five isolates from WA, isolated in 2008; and single isolates from Victoria, isolated in 2007, and Queensland, isolated in 2008. The 45 ST93-MRSA included 30 isolates from across Australia from the 2000 to 2008 AGAR Community onset *S. aureus* programs, and 15 isolates from WA from 2003 to 2009. *S. aureus* species and methicillin resistance was confirmed by the detection of *nuc* (thermostable extracellular nuclease) and *mecA* (methicillin resistance) genes by PCR as previously described [21].

Susceptibility Testing

An antibiogram was performed by disk diffusion on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [22]. A panel of eight antimicrobial drugs was tested: erythromycin (15 μg), tetracycline (30 μg), trimethoprim (5 μg), ciprofloxacin (5 μg), gentamicin (10 μg), rifampin (5 μg), fusidic acid (10 μg), and mupirocin (5 μg). CLSI interpretive criteria [23] were used for all drugs except fusidic acid [24] and mupirocin [25].

PFG

Electrophoresis of chromosomal DNA was performed as previously described [26], using a contour-clamped homogeneous electric field (CHEF) DR III system (Bio-Rad Laboratories Pty Ltd). Chromosomal patterns were examined visually, scanned with a Quantity One device (Bio-Rad Laboratories Pty Ltd), and digitally analyzed using FPQuest (Bio-Rad Laboratories Pty Ltd). *S. aureus* strain NCTC 8325 was used as a reference strain.

MLST and *Spa* Typing

Chromosomal DNA for MLST and *spa* typing was prepared using a DNeasy tissue kit (Qiagen Pty Ltd).

MLST was performed as previously described [27]. The sequences were submitted to http://www.mlst.net/where an allelic profile was generated and an ST assigned.

spa typing, a DNA sequenced-based analysis of the protein A gene variable region was performed as previously described [28] using the nomenclature as described on the Ridom website (http://spa.ridom.de/). Cluster analysis of *spa* sequences was performed using the spa typing plug-in tool of the BioNumerics software program (version 6.6; Applied Maths, Ghent, Belgium). The analysis compares and aligns sequences via an algorithm based on potential tandem *spa* repeat duplications, substitutions, and indels (the DSI model) [29]. A minimum spanning tree (MST) was generated from the similarity matrix with the root node assigned to the sequence type with the greatest number of related types. The default software parameters were used for analysis with a bin distance of 1.0%. Thus, the distance between *spa* types of 99% to 100% similarity was 0, 98% to 99% similarity was assigned a distance of 1, etc., on the MST. For cluster analysis, only *spa* types separated by an MST distance of ≤1 (i.e., if they were ≥98% similar) were considered closely related and assigned to the same cluster.

DNA Microarray

Arrays and reagents were obtained from Alere Technologies, Jena Germany. The principle of the assay, related procedures, and a list of targets has been described previously [30,31]. Target genes included species markers, markers for accessory gene regulator (*agr*) alleles and capsule types, virulence factors, resistance genes, staphylococcal superantigen-like/exotoxin-like genes (*set*/*ssl* genes) and genes encoding adhesion proteins and immune evasion factors. Probes for *mecA*, *ugpQ*, *xylR*, *kdp*, *ccr*'s, *mecI* and two probes for *mecR* were used for SCC*mec* typing.

SCC*mec* Typing

The strategy used for SCC*mec* typing was as previously described [32]. SCC*mec* nomenclature is used as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) [33]. Briefly, the structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an

uppercase letter respectively in parenthesis. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type. When there is an extra *ccr* element present whose precise location is unknown it is indicated by an "&" and *ccr* number outside the parentheses.

PVL

PCR for the detection of PVL determinants was performed as previously described [34].

PVL Phage Identification

PCRs were performed to detect the six PVL-encoding phages (ΦSa2MW, ΦSa2958, ΦPVL, Φ108PVL, ΦSLT and ΦSA2USA) as previously described [35,36].

Detection of Allelic Variations in *Luks-PV/lukF-PV* Genes

Detection of single nucleotide polymorphisms (SNPs) in a defined region of the *lukS-PV/lukF-PV* genes were performed as previously described [36,37]. Sequences obtained were compared to the proposed progenitor PVL gene in ΦSLT/ST30.

Quantification of *In vitro* LukF-PV Expression

PVL is a 2-component exotoxin and both LukS-PV and LukF-PV are required for activity. LukF-PV was measured instead of LukS-PV to obtain an anti-LukF-PV antibody with increased specificity of binding as there was more sequence divergence between *lukF-PV* and the orthologous 2-component *S. aureus* exotoxins compared to *lukS-PV*. To produce recombinant LukF-PV *lukF-PV* was PCR amplified using primers, forward 5′-CACCATGGCTCAACATATCACAC and reverse 5′-GCTCA-TAGGATTTTTTTCC. The resulting PCR product was then TOPO cloned into pENTR/SD/D-TOPO (Invitrogen). This plasmid was sequenced using M13 primers to confirm that the insert was present in the correct orientation without mutations. *lukF-PV* was subsequently cloned using an LR recombination reaction into the expression vector pET-DEST42 (Invitrogen) which introduced a C-terminal 6x-Histidine tag. This expression clone was used to transform Rosetta2 *E. coli* (Novagen). Soluble recombinant LukF-PV was produced by the Protein Production Unit, Monash University by growth of the expression strain in Auto Induction media at 28°C. The resulting recombinant LukF-PV was purified by Nickel purification followed by gel filtration and eluted in 100 mM NaP04, pH 7.4, 150 mM NaCl buffer. Aliquots were frozen and stored at −80°C. The concentration of recombinant LukF-PV was determined using the 2100 Bioanalyser P230 kit (Agilent).

Quantification of LukF-PV Expression

Bacteria were grown in CCY media (3% yeast extract (Oxoid), 2% Bacto Casamino Acids (Difco), 2.3% sodium pyruvate (Sigma-Aldrich), 0.63% Na_2HPO_4, 0.041% KH_2PO_4, pH 6.7). Overnight cultures were diluted 1:100 into fresh media and then incubated at 37°C with shaking (180 rpm) until stationary phase (OD600 ~ 1.8). Culture supernatants were harvested by centrifugation and filter sterilized. The LukF-PV expression experiments were performed in at least duplicate for each *S. aureus* strain. Trichloroacetic acid was added to culture supernatants and incubated at 4°C overnight. Precipitates were then harvested by centrifugation, washed with acetone, air-dried and solubilized in a sample buffer containing 1.7% SDS and 1% 2-mercaptoethanol. The proteins were separated on 12% SDS-PAGE.

A peptide sequence specific to LukF-PV, HWIGNNYKDEN-RATHT was synthesized and HRP conjugated polyclonal chicken IgY raised against this peptide (Genscript). This antibody was used to detect LukF-PV with enhanced chemiluminescence. Images generated from the western blots were quantitated using GS800 Calibrated Densitometer and Quantity One (BioRad). 50 µg of recombinant LukF-PV was used as an internal standard on each gel, and was the positive control. Results observed with this standard were set to 1.0. All other results were shown as a ratio relative to this standard. RN4220 was used as a negative control.

Dru Typing

Sequence analysis of the *mec*-associated *dru* region was performed as previously described [38]. A cluster analysis of *dru* sequences was performed using the Polymorphic VNTR plug-in tool of the BioNumerics software program (version 6.6; Applied Maths, Ghent, Belgium). The analysis compares and aligns sequences via an algorithm based on potential tandem *dru* repeat duplications, substitutions, and indels (the DSI model) [29]. A MST was generated from the similarity matrix with the root node assigned to the sequence type with the greatest number of related types. The default software parameters were used for analysis with a bin distance of 1.0%. Thus, the distance between *dru* types of 99% to 100% similarity was 0, 98% to 99% similarity was assigned a distance of 1, etc., on the MST. For cluster analysis, only *dru* types separated by an MST distance of ≤1 (i.e., if they were ≥98% similar) were considered closely related and assigned to the same cluster.

Control Strain

The sequenced ST93-IVa [2B] strain JKD6159 (NCBI GenBank Accession No. CP002114 and CP002115) was included in this study for comparison [17].

Results

Susceptibility results, SCC*mec* typing together with a summary of the resistance genes, *spa* types (using the Ridom Nomenclature) and *dru* type are shown in Table 1. Further characterisations are as follows:

Molecular Typing

By PFGE the 58 isolates (13 MSSA and 45 MRSA) had ≥80% similarity with the sequenced JKD6159 strain (Figure 1). Eleven pulsotypes were identified. The MSSA isolates consisted of three pulsotypes, "A" – "C" with 12 of the 13 isolates grouped into two closely related pulsotypes; "A" (9 isolates) and "C" (3 isolates). The MRSA isolates consisted of eight pulsotypes ("C" – "K") with 39 of the 45 isolates grouped into two closely related pulsotypes; "D" (36 isolates) and "J" (3 isolates). The MSSA pulsotypes "A" and "C" and the MRSA pulsotypes "D" and "J" were 92% related; the difference presumably due to the insertion of the SCC*mec* type IVa [2B] element into an existing restriction fragment in the two MRSA pulsotypes. Single isolates of closely related MRSA pulsotypes "I" (SAPWH71) and "K" (SAPWH53) lacked a PVL-encoding phage. MRSA pulsotype "H" (20198) also lacked a PVL-encoding phage, however unlike the other MRSA, carried the SCC*mec* type V element with an additional *ccr* element [5C2&5]. The remaining MSSA and three MRSA isolates were classified into four unique pulsotypes (pulsotypes B, E, F, G).

Isolates representing each pulsotype were identified as ST93 by MLST.

Seven *spa* types were identified with the majority of isolates characterised as t202 (8/13 MSSA and 42/45 MRSA). The MST algorithm clustered the *spa* types into two significantly different

Table 1. Characterisation of ST93 isolates.

Region	Reference Number	Year	Specimen	Antibiogram	DNA Microarray Resistance Genotype	PFGE	MLST	*spa* Sequence	*Spa* Type	SCC*mec* type	dru Sequence	*Dru* Type	*lukSI lukF PV*
MSSA													
NT	WBG 7735	1992	Unknown	Er^R	*blaZ, blaI, blaR, ermC*	B	93	11-17-23-17-17-16-16-16-25	t4178	NA	NA		POSITIVE
NT	WBG 7762	1992	Unknown	Er^R	*blaZ, blaI, blaR, ermC*	A		11-17-16-16-25	t4699	NA	NA		POSITIVE
Qld	UQ40	2008	Unknown		*blaZ, blaI, blaR*	A		11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
Vic	DP 2039	2007	Unknown		*blaZ, blaI, blaR*	A	93	11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	C229T	2003	Throat		*blaZ, blaI, blaR*	A		11-17-23-17-17-16-16-25-25	t5767	NA	NA		POSITIVE
WA	N126W	2003	Hands	Er^R	*blaZ, blaI, blaR, ermC*	C	93	11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	W17S	1995	Skin		*blaZ, blaI, blaR*	C	93	11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	Y113S	1996	Skin	Er^R	*blaZ, blaI, blaR, ermC*	C		11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	9506160A	2008	Blood		*blaZ, blaI, blaR*	A		11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	9509712N	2008	Pleural Fl		*blaZ, blaI, blaR*	A		04-23-17-17-16-16-25	t6485	NA	NA		POSITIVE
WA	9524093R	2008	Buttock		*blaZ, blaI, blaR*	A		11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	9525206A	2008	Blood		*blaZ, blaI, blaR*	A		11-17-23-17-17-16-16-25	t202	NA	NA		POSITIVE
WA	9529120L	2008	Heel	Er^R	*blaZ, blaI, blaR, ermC*	A		11-17-23-17-17-16-16-25-25	t5767	NA	NA		POSITIVE
MRSA													
ACT	SAPTCH92	2000	Blood	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
ACT	SAPTCH53	2008	Boil	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NSW	SAPRPAH96	2000	Eye	Ox^R Er^R	*mecA, blaZ, blaI, blaR, ermC*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b	dt3b	POSITIVE
NSW	SAPWH23	2000	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b-4e	dt4d	POSITIVE
NSW	SAPWH39	2000	Wound	Ox^R Er^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b-4e	dt4d	POSITIVE
NSW	SAPWH61	2000	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NSW	SAPWH64	2000	Wound	Ox^R	*mecA, blaZ, blaI, blaR, ermC*	E	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NSW	SAPWH94	2000	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NSW	SAPWH71	2004	Blood	Ox^R	*mecA, blaZ, blaI, blaR*	I	93	11-17-23-17-17-16-16	t6487	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	NEGATIVE
NSW	SAPCRGH95	2007	Blood	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-2a-2g-3b-4e	dt10g	POSITIVE
NSW	SAPRPAH21	2007	Sputum	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE

Table 1. Cont.

Region	Reference Number	Year	Specimen	Antibiogram	DNA Microarray Resistance Genotype	PFGE	MLST	*spa* Sequence	*Spa* Type	SCC*mec* type	dru Sequence	*Dru* Type	*lukS/lukF PV*
NSW	SAPRPAH7	2008	Ulcer	Ox^R	*mecA, blaZ, blaI, blaR, ermC*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
													POSITIVE
NSW	SAPWH10	2008	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
													POSITIVE
NSW	SAPWH53	2008	Wound	Ox^R Er^R Tmp^R	*mecA, blaZ, blaI, blaR, msr(A)*	K	93	11-17-17-16-16-25	t1811	IVa [2B]	5a-2d-3b	dt3b	NEGATIVE
NT	SAPRDH61	2006	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NT	SAPRDH27	2007	Thigh	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
NT	SAPRDH2	2008	Leg	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPRBH98	2000	Leg	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPRBH12	2005	Blood	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPGCH3	2006	Abscess	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPRBH14	2006	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPCBH10	2008	Aspirate	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPGCH28	2008	Foot	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Qld	SAPRBH1	2008	Forearm	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
SA	SAPGPSA73	2000	Unknown	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
SA	SAPIMVS24	2006	Abscess	Ox^R	*mecA, blaZ, blaI, blaR*	J		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
SA	SAPIMVS31	2008	Boil	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Vic	SAPRCH74	2000	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b-4e	dt4d	POSITIVE
Vic	SAPAH21	2008	Wound	Ox^R Te^R	*mecA, blaZ, blaI, blaR, tetK*	D		11-17-23-17-17-16-16-16-25	t4178	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	16790	2003	Axilla	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	16815	2005	Abscess	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE

Table 1. Cont.

Region	Reference Number	Year	Specimen	Antibiogram	DNA Microarray Resistance Genotype	PFGE	MLST	*spa* Sequence	*Spa* Type	SCC*mec* type	dru Sequence	*Dru* Type	*lukSl lukF PV*
WA	15586	2008	Lip	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b	dt3b	POSITIVE
WA	15587	2008	Nose	Ox^R Er^R	*mecA, blaZ, blaI, blaR, ermC*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	16414	2008	Thigh	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b	dt3b	POSITIVE
WA	16475	2008	ETT	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-4f-3a-2g-3b-4e	dt10i	POSITIVE
WA	17164	2008	Abscess	Ox^R	*mecA, blaZ, blaI, blaR*	J		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	18158	2008	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b	dt3b	POSITIVE
WA	18385	2008	Shin	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	18418	2008	Nose	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-3b	dt3b	POSITIVE
WA	20198	2008	Nose	Ox^R	*mecA, blaZ, blaI, blaR*	H	93	11-17-23-17-17-16-16-25	t202	V [5C2&5]	5a-2d-4a-0-2d-5b-3a-2g-1c-4e-3e	dt11i	NEGATIVE
WA	SAPRPH48	2008	Wound	Ox^R	*mecA, blaZ, blaI, blaR*	J	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	16908	2009	Leg	Ox^R	*mecA, blaZ, blaI, blaR, (dfrA)*	F	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	17090	2009	Wound	Ox^R	*mecA, blaZ, blaI, blaR, qacC*	G	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	17195	2009	Nose	Ox^R Er^R	*mecA, blaZ, blaI, blaR, ermC, qacC*	D	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
WA	20548	2009	Abscess	Ox^R	*mecA, blaZ, blaI, blaR*	D		11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE
Control Strain													
Vic	JKD6159	2004	Blood	Ox^R	*mecA, blaZ, blaI, blaR*	D	93	11-17-23-17-17-16-16-25	t202	IVa [2B]	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	dt10a	POSITIVE

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia.
Antibiogram: Ox, oxacillin; Em, erythromycin; Te, tetracycline.
Resistance Genotype: *mecA*, alternate penicillin binding protein 2 gene; *blaZ*, beta lactamase gene; *blaI*, beta-lactamase repressor (inhibitor) gene; *blaR* beta-lactamase regulatory protein gene, *ermC*, erythromycin/clindamycin resistance gene; *msr(A)*, macrolide resistance gene; *tetK*, tetracycline resistance gene; *dfrA*, trimethoprim resistance gene; qacC quaternary ammonium compound resistance gene protein C.
PFGE, pulsed field gel electrophoresis; MLST, multilocus sequence type; SCCmec, staphylococcal cassette chromosome mec; *spa, Staphylococcus aureus* protein gene A; *dru*, the direct-repeat unit (*dru*) variable-number tandem repeat region adjacent to IS431 in SCC*mec*.
lukF/*lukS* PV, Panton Valentine leucocidin F and S component genes.
doi:10.1371/journal.pone.0043037.t001

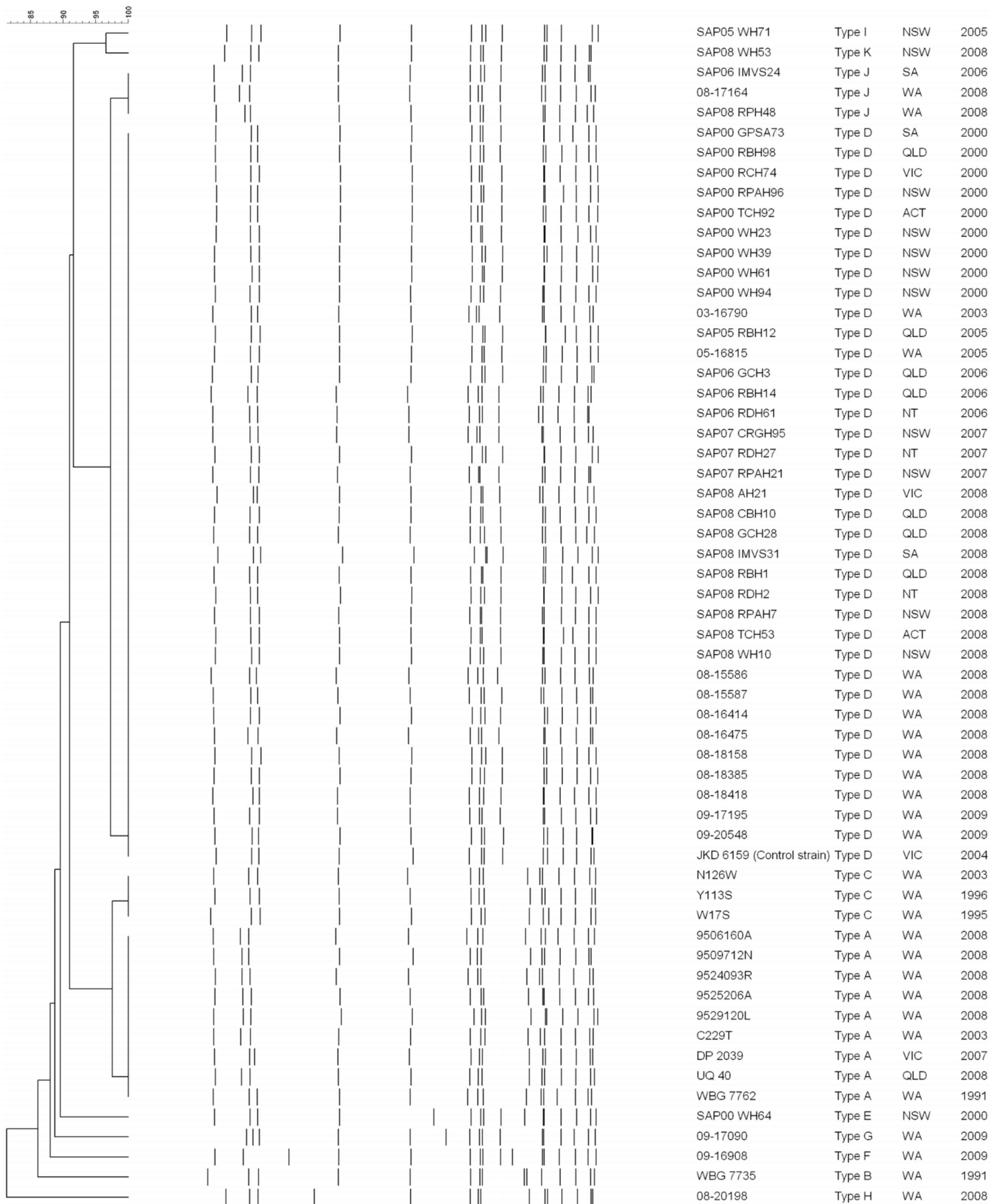

Figure 1. Dendrogram of the 58 pulsed-field gel electrophoresis patterns (PFGE) of ST93 (13 MSSA and 45 MRSA). Sequenced JKD6159 strain was used as the ST93 control. *S. aureus* strain NCTC 8325 was used as the reference strain.
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groups; t202, t4178, t5767; t1811, t4699; plus t6487; and t6485 (Figure 2).

DNA Microarray

The β-lactamase operon (*blaZ*, *blaI*, *blaR*) was detected in all isolates (Table 1). Apart from isolate 20198, the MRSA carried *mecA* as a part of the SCC*mec* type IVa [2B] element. Carriage of other resistance genes was infrequent and variable. Five of the MSSA were phenotypically erythromycin resistant and carried *ermC*. Of the five erythromycin resistant MRSA isolates, three carried *ermC* and one the staphylococcal *msr(A)* macrolide efflux protein gene. A macrolide resistance gene was not detected in one isolate that demonstrated phenotypic resistance (SAPWH39). Two *ermC* harbouring MRSA isolates were not phenotypically erythromycin resistant. A single MRSA isolate harboured the *tetK* tetracycline resistant gene (isolated in Victoria in 2008), and two MRSA isolates carried the quaternary ammonium compound resistance protein C (*qacC*) gene (isolated in WA in 2009).

The 58 isolates were *agr* group III and capsule type 8. Although the enterotoxin and *tst1* genes were absent from all isolates, the enterotoxin homologue ORF CM14 was present in 34 isolates (4 MSSA and 30 MRSA) (Table S1). All isolates carried the *hlb*, *hld* and *hlIII* hemolysin genes; the staphylokinase (*sak*), chemotaxis inhibitory protein (*chp*) and staphylococcal complement inhibitor (*scn*) genes; and the *aur*, *splA*, *sspA*, *sspB*, *sspP* protease genes. Although the gene for a biofilm-associated protein, *bap*, was absent, the biofilm operon icaACD was present in all isolates. Most isolates carried the leukocidin *lukX* and *lukY* genes, the *hl*, *hla* hemolysin genes and the *splE* protease genes.

The staphylococcal superantigen like or exotoxin-like genes (*set* or *ssl* genes) and genes encoding MSCRAMMS (microbial surface components recognizing adhesive matrix molecules) and the immune evasion factors were homogeneous and characteristic for ST93 (Tables S2, S3, S4 and S5).

Panton Valentine Leukocidin (PVL)

Apart from three MRSA isolates (SAPWH71, SAPWH53 and 20198) the *lukS-PV/lukF-PV* genes were detected in all isolates by array hybridisation and PCR. All *lukS-PV/lukF-PV* positive isolates carried the PVL-encoding phage ΦSa2USA. Using the proposed progenitor PVL gene in ΦSLT/ST30 as a reference sequence, all isolates were similar, having the same R variant SNP profile with three substitutions compared to ΦSLT. This SNP profile is associated with the ΦSa2USA phage.

lukF-PV expression is shown in Figure 3 and was measured to determine if there was a consistent expression profile across different ST93 strains. As expected, ST93 isolates which did not contain *lukS-PV/lukF-PV* did not express LukF-PV. However, there

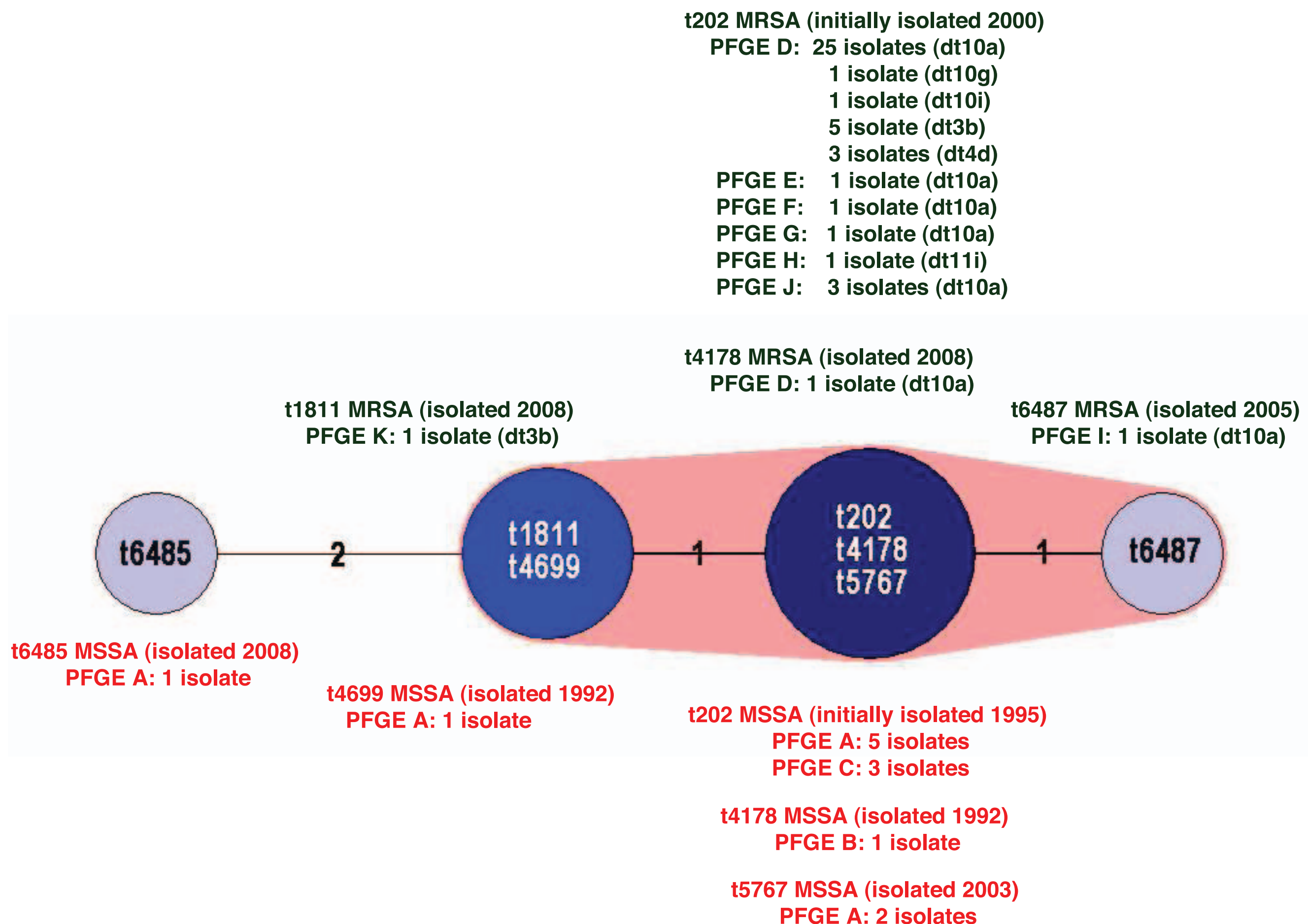

Figure 2. Minimum spanning tree (MST) of the seven ST93 *spa* types. Cluster analysis was performed using the spa typing plug-in tool of the BioNumerics program. *spa* types separated by an MST distance of ≤1 (i.e., if they were ≥98% similar) were considered closely related and assigned to the same cluster. MSSA and MRSA *spa* types are designated in red and green print respectively. Pulsed-field gel electrophoresis (PFGE) pulsotypes and *dru* types (dt) are recorded for each *spa* type.
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were three isolates (SAPCRGH95 isolated in NSW, SAPAH21 isolated in Vic, and 15587 isolated in WA) which were PVL positive by array hybridization and PCR but did not express LukF-PV indicating that there may be regulatory differences such as an *agr* defect in these isolates to account for the absence of LukF-PV. All other isolates produced LukF-PV, with expression levels similar between most strains.

dru Typing

Six *dru* types were identified (Table 1). The majority of isolates (35/45) were dt10 (33 dt10a, 1 dt10 g and 1 dt10i). The remaining nine SCC*mec* type IVa [2B] isolates were *dru* type dt4d (three isolates) and dt3b (6 isolates). The SCC*mec* type V [5C2&5] isolate (20198) was dt11i. The MST algorithm clustered the six *dru* types into three significantly different groups; dt10, dt4d plus dt3b, and dt11 (Figure 4). This suggests the SCC*mec* element may have been acquired on at least three occasions by ST93.

Discussion

CA-MRSA is thought to emerge when a locally prevalent strain of methicillin susceptible *S. aureus* (MSSA) acquires a SCC*mec* element and utilizes mobile genetic elements and single nucleotide polymorphisms to establish local and geographic niches [39]. Although the vertical and horizontal transmission of SCC*mec* elements into *S. aureus* has occurred on multiple occasions in the Australian community only a small number of clones have successfully found an ecological niche to predominate over other CA-MRSA clones [40]. PVL-positive ST93-IV [2B] is one such clone, and since 2000 has been reported across Australia and is responsible for the increasing prevalence of CA-MRSA infections nationwide [13].

Conflicting hypotheses have been proposed to explain the molecular evolution of ST93-MRSA. In 2008 Munckhof and colleagues found little genetic diversity within ST93-IV [2B] suggesting it arose from one PVL-positive binary subtype of ST93 MSSA after the acquisition of SCC*mec* [41]. However in 2009 Tong and colleagues identified multiple *spa* types in ST93-MRSA and ST93-MSSA and proposed their data supported an early acquisition of SCC*mec* with subsequent rearrangement of the *spa* sequence or multiple independent acquisitions of SCC*mec* and coexistence of MSSA and MRSA versions of the same lineage [42]. Although seven *spa* types were described in this study, cluster analysis of the seven *spa* sequences using the Spa typing plug-in tool of the BioNumerics software program shows six of the *spa* types are closely related and can be assigned to a single cluster (data not shown).

Unlike the Tong study, which examined the *spa* types of geographically localized ST93 *S aureus* collected over a short period, the current study examined ST93 *S aureus* isolated across Australia over sixteen years using a variety of molecular tools, providing greater power to detect unique evolutionary events in geographically diverse regions.

Prior to the isolation of ST93-IV [2B], *S aureus* surveillance screening of aboriginal people living in 11 remote Western Australian communities identified ST93 as the most prevalent MSSA lineage [20]. Although located in three geographically distant regions of WA, the ST93-MSSA examined from these communities, (W17S isolated in 1995, Y113S in 1996 and C229T and N126W in 2003, and) exhibit limited diversity within their PFGE patterns, *spa* types and microarray DNA profiles. Their two *spa* types, t202 [3 isolates ("PFGE C")] and t5767 ("PFGE A") are closely related and are assigned to the same cluster. The microarray DNA profiles for the two ST93-MSSA isolated in the Northern Territory in 1992 (WBG7735 and WBG7762) are homogeneous with the four WA remote community ST-93 MSSA. In addition their PFGE patterns are either identical ("PFGE A") or 90% related ("PFGE B"), and their *spa* types, t4699 and t4178,

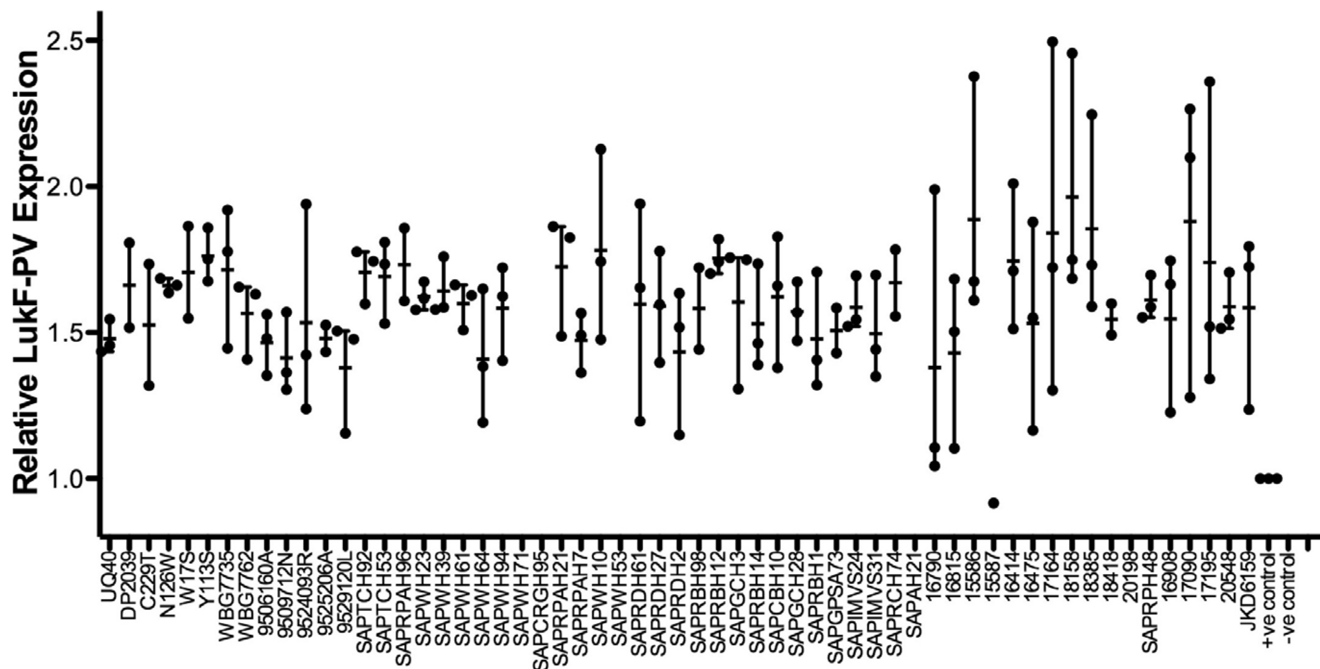

Figure 3. Relative LukF-PV expression in ST93 isolates. All isolates were tested for LukF-PV expression using western blot and LukF-PV specific antibody. Results are expressed as optical density of test strain relative to a 50 µg control of rLukF-PV that was run on every gel. All experiments were performed with multiple replicates and mean and range is shown. Positive control, rLUKF-PV; negative control, RN4220.
doi:10.1371/journal.pone.0043037.g003

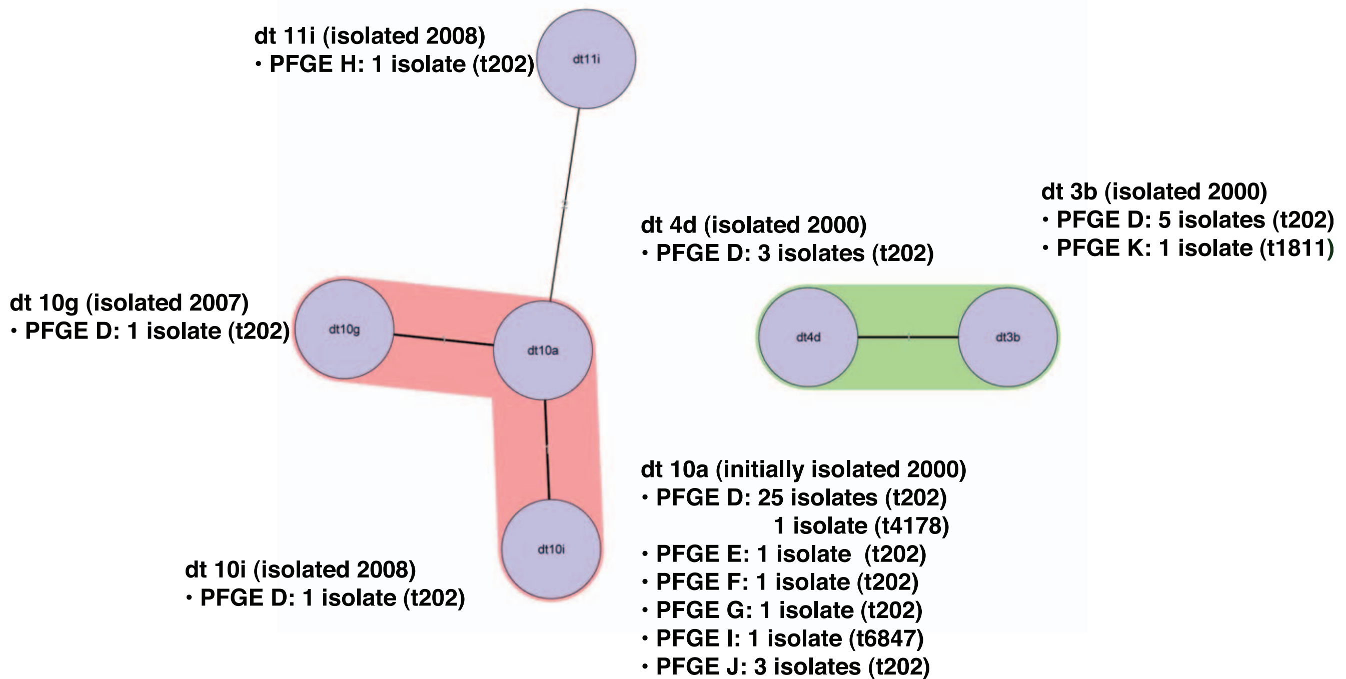

Figure 4. Minimum spanning tree (MST) of the six ST93 *dru* types. Cluster analysis was performed using the Polymorphic VNTR plug-in tool of the BioNumerics program. *dru* types separated by an MST distance of ≤1 (i.e., if they were ≥98% similar) were considered closely related and assigned to the same cluster. Pulsed-field gel electrophoresis (PFGE) pulsotypes and *spa* types are recorded for each *dru* type.
doi:10.1371/journal.pone.0043037.g004

are assigned to the same cluster. The DNA microarray profiles for the five ST93-MSSA, (9506160A, 9509712N, 9524093R, 9525206A and 9529120L) isolated in the state's capital, Perth in 2008 (located 700–2000 km from the remote communities and over 3,000 km from the Northern Territory border) are also homogeneous with the Western Australian remote community strains. The PFGE pattern for these isolates is "PFGE A". The *spa* types for four of these strains are t202 (3 isolates) and t5767. The *spa* type for 9509712N (t6485) cannot be assigned to the same cluster. The PFGE patterns, *spa* types and microarray DNA profiles for the MSSA isolated on the Australian eastern seaboard (UQ40– Queensland in 2008 and DP2039– Victoria in 2007) are identical to three Perth ST93-MSSA-t202 isolates.

As shown in Figure 1 the MRSA isolates are ≥80% related by PFGE with the majority of isolates falling into pulsotype D. Similar to the MSSA pulsotypes, pulsotype D was dispersed throughout Australia over the eight years. Although rearrangement of the *spa* sequence has occurred several times, the PFGE patterns and microarray DNA profiles of the 13 ST-93 MSSA isolates suggests the ST93 core and accessory genome is very stable. All carry the PVL-encoding phage ΦSa2USA and their *lukS-PV*/*lukF-PV* genes have the same R variant SNP profile. The isolates produce similar expression levels of LukF-PV with no apparent relationship between subtype and PVL expression. The emergence of five different *spa* types, albeit four types assigned to the same cluster, suggests ST93-MSSA emerged some time ago from a common *spa* type. As the *spa* sequences are similar it is not possible to predict the ancestral strain; however one strain, ST93-MSSA-t202, predominates and has successfully disseminated across Australia.

Like ST93-MSSA, ST93-MRSA has multiple *spa* types; including the closely related t202 and t4178, identified in ST93-MSSA, t1811 and t6487, all of which are assigned to the same cluster. t202 has the largest number of isolates; 42 of the 45 ST93-MRSA. SCC*mec* and *dru* typing indicates the SCC*mec* element has been acquired by ST93-MRSA-t202 on at least three occasions; dt10 (SCC*mec* type IVa [2B]), dt3b/dt4d (SCC*mec* type IVa [2B]) and dt11i (SCC*mec* type V [5C&5]). Unlike ST93-IVa [2B]-t202, ST93-V [5C&5]-t202 does not carry the *lukS-PV*/*lukF-PV* genes. The PVL-negative ST93-IVa [2B]-t1811 isolate may have arisen by independent acquisition of SCC*mec* IVa [2B] or by the subsequent rearrangement of the *spa* sequence.

As for ST93-MSSA, the PFGE patterns and microarray DNA profiles of the 45 ST-93 MRSA isolates suggests the ST93 core and accessory genome is stable. Forty three of the 45 isolates carry the PVL-encoding phage ΦSa2USA. The *lukS-PV*/*lukF-PV* genes have the same R variant SNP profile and produce similar expression levels of LukF-PV as reported in ST93-MSSA.

Apart from the *ermC* gene which was identified in several early ST93-MSSA and ST93-MRSA isolates, ST93 *S. aureus* initially carried few antibiotic resistance elements. However since 2008, in addition to *mecA* and *ermC*, some isolates of ST93-MRSA have acquired the *msr(A)* and *tetK* resistance genes. Although the *dfrA* gene was not detected by the microarray DNA, SAPWH53 is phenotypically trimethoprim resistant (presumably due to an alternative trimethoprim resistance gene or a different *dfrA* allele). In addition, the quaternary ammonium compound resistance protein C gene *qacC* is carried by two isolates. The acquisition of several resistance genes by an epidemic PVL-positive CA-MRSA clone is not unique to ST93-IV [2B]. The USA300 clone (ST8-IV [2B]), initially resistant only to semi-synthetic penicillins and macrolides, is now, frequently resistant to other antimicrobial agents including clindamycin, tetracycline, mupirocin, and the fluoroquinolones; occasionally resistant to gentamicin and trimethoprim-sulfamethoxazole, and may have reduced susceptibility to daptomycin [43].

Single strain outbreaks of ST93-IV [2B] have not been reported in Australian hospitals, however as has been reported in United States hospitals with USA300 [44], ST93-IV [2B] has become a

major cause of healthcare-associated/onset infection. In 2008 Munckhof and colleagues reported nearly three quarters of nmMRSA infections in their hospital-based study were healthcare associated, of which ST93-IV [2B] predominated [41].

Conclusion

This study has demonstrated that although multiple rearrangements of the *spa* sequence have occurred, the core genome in ST93 *S. aureus* is very stable. Since 2008 PVL-positive ST93-MSSA-t202 has become the predominant ST93-MSSA across Australia. We have shown the emergence of ST93-MRSA has been due to independent acquisitions of different *dru*-defined type IV and type V SCC*mec* elements in several *spa*-defined ST93-MSSA backgrounds. Rearrangement of the *spa* sequence in ST93-MRSA has subsequently occurred in some of these strains. Although several ST93-MRSA strains have been identified in this study, little genetic diversity was identified for most MRSA isolates, with PVL-positive ST93-IVa [2B]-t202-dt10 predominant across Australia. However to determine if ST93-IVa [2B] t202-dt10 has arisen from one PVL-positive ST93-MSSA-t202, or by independent acquisitions of SCCmec-IVa [2B]-dt10 into multiple PVL-positive ST93-MSSA-t202 strains will require whole genomic sequencing of the isolates. Furthermore, comparative genomic sequencing may further enhance our understanding of the molecular basis for the emergence and increased virulence of ST93 CA-MRSA. At a time when this clone is acquiring additional resistance genes and an increased potential for infections in the healthcare setting, understanding the means for SCC*mec* acquisition, virulence determinants and transmission dynamics is crucial if we are to prevent this clone from becoming established in hospitals.

Supporting Information

Table S1 Microarray DNA ST93 virulence profile
(DOCX)

Table S2 Microarray DNA ST93 ST93 staphylococcal superantigen/enterotoxin-like genes (set/ssl) profile.
(DOCX)

Table S3 and S4 Microarray DNA ST93 ST93 MSCRAMMs and adhesion profile.
(DOCX)

Table S5 Microarray DNA ST93 immunevasion and miscellaneous profile.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: GWC FGO KJC. Performed the experiments: GWC KYC. Analyzed the data: GWC KYC SM RVG BPH FGO KJC. Contributed reagents/materials/analysis tools: GWC KYC SM RVG BPH TPS RE FGO KJC. Wrote the paper: GWC KYC BPH.

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Supplementary Table 1: ST93 virulence profile

		lukF/lukS PV	Superantigenic Toxins		Leucocidins and Haemolysins	sak/chp/scn	et	edin	Proteases	Biofilms	ACME
Region	Reference Number		Enterotoxins	tst							
ST93 MSSA											
NT	WBG7735	*lukF/lukS PV*			*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NT	WBG7762	*lukF/lukS PV*			*lukX/(Y), hl,(hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA,(splE), sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	UQ40	*lukF/lukS PV*			*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Vic	DP2039	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	C229T	*lukF/lukS PV*			*lukX, hl, (hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	N126W	*lukF/lukS PV*			*lukX/Y,(hl),(hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	W17S	*lukF/lukS PV*			*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	113S	*lukF/lukS PV*			*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	9506160A	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	9509712N	*lukF/lukS PV*			*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	9524093R	*lukF/lukS PV*	(entCM14)		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	9525206A	*lukF/lukS PV*			*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	9529120L	*lukF/lukS PV*	(entCM14)		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
ST93 MRSA											
ACT	SAPTCH92	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
ACT	SAPTCH53	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPRPAH96	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH23	*lukF/lukS PV*			*lukD, lukX, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH39	*lukF/lukS PV*	entCM14		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH61	*lukF/lukS PV*	entCM14		*lukD, lukX, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH64	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH94	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH71				*lukX, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, (splE), sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPCRGH95	*lukF/lukS PV*			*hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPRPAH21	*lukF/lukS PV*	(entCM14)		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPRPAH7	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH10	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NSW	SAPWH53				*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NT	SAPRDH61	*lukF/lukS PV*			*lukX/(Y), hl, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, (splE), sspA, sspB, sspP*	*icaA, icaC, icaD*	
NT	SAPRDH27	*lukF/lukS PV*			*lukX/Y, hl,(hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
NT	SAPRDH2	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPRBH98	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPRBH12	*lukF/lukS PV*			*lukX, hl, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPGCH3	*lukF/lukS PV*			*lukX/(Y),(hl), hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA,(splE), sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPRBH14	*lukF/lukS PV*			*lukX, hl, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPCBH10	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPGCH28	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Qld	SAPRBH1	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
SA	SAPGPSA73	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	

Supplementary Table 1: ST93 virulence profile

		lukF/lukS PV	Superantigenic Toxins		Leucocidins and Haemolysins	sak/chp/scn	et	edin	Proteases	Biofilms	ACME
Region	**Reference Number**		**Enterotoxins**	**tst**							
SA	SAPIMVS24	*lukF/lukS PV*	(entCM14)		*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
SA	SAPIMVS31	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Vic	SAPRCH74	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Vic	SAPAH21	*lukF/lukS PV*			*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	16790	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	16815	*lukF/lukS PV*	entCM14		*lukD/(E), lukX/Y, hl, hla, hlb, hld, hlIII untruncated hlb*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	15586	*lukF/lukS PV*			*lukX/(Y), hl,(hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	15587	*lukF/lukS PV*			*(lukD), lukX/(Y), hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	16414	*lukF/lukS PV*			*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	16475	*lukF/lukS PV*			*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	17164	*lukF/lukS PV*	entCM14		*lukD/(E), lukX/Y, hl, hla, hlb, hld, hlII, untuncated hlbI*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	18158	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	18385	*lukF/lukS PV*			*lukX/(Y), hl,(hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA,(splE), sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	18418	*lukF/lukS PV*			*lukD, lukX/Y, hl, (hla), hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	20198		entCM14		*lukD, lukX/Y, hl, hla, hlb, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	SAPRPH48	*lukF/lukS PV*	(entCM14)		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	16908	*lukF/lukS PV*	entCM14		*(hlgA)lukD/E, lukX/Y, hl, hla, hlb, hld, hlIII, untruncated hlb*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	17090	*lukF/lukS PV*	entCM14		*lukD, lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	17195	*lukF/lukS PV*	entCM14		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
WA	20548	*lukF/lukS PV*	(entCM14)		*lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	
Control Strain											
Vic	JKD6159	*lukF/lukS PV*	entCM14		*(lukD), lukX/Y, hl, hla, hlb, hld, hlIII*	*sak, chp, scn*			*aur, splA, splE, sspA, sspB, sspP*	*icaA, icaC, icaD*	

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia

lukF/*lukS* PV, Panton Valentine leucocidin F and S component genes; entCM14, enterotoxin-like protein ORF CM14; *lukD/E*, leucocidin D and E component genes; *lukX/ Y*, leucocidin haemolysin toxin family protein genes; *hl*, putative membrane protein gene; *hla*, haemolysin alpha gene, *hlb*, haemolysin beta gene; *hld*, haemolysin delta gene; *hlIII*, putative membrane protein; sak, staphylokinase gene; chp, chemotaxis-inhibiting protein (CHIPS) gene; *scn*, staphylococcal complement inhibitor gene; *aur* aureolysin gene; *splA*, serinprotease A gene; *splE*, serinprotease E gene, *sspA*, glutamylendopeptidase gene, *sspB*, staphopain B protease gene, *sspP*, staphopain A protease gene, *icaA*, intracellular adhesion protein A gene; *icaC*, intracellular adhesion protein C gene; *icaD*, biofilm PIA synthesis protein D gene

( ), gene detected but yielding weak or ambiguous signals

Supplementary Table 2: ST93 staphylococcal superantigen/enterotoxin-like genes (set/ssl) profile

Region	Reference Number	setC (MW0345)	ssl01/set6 (COL/SACOL468)	ssl01/set6 (Mu50/SAV0422)	ssl01/set6 (MW2-MW0382)	ssl01/set6 (SAR0422/MRSA252)	ssl01/set6 (all others)	ssl01/set6 (RF122)	ssl02/set7	ssl03/set8	ssl03/set8 (MRSA252/SAR0424)	ssl04/set9	ssl04/set9 (MRSA252/SAR0425)	ssl05/set3	sl05/set3 (RF122)	ssl05/set3 (MRSA252)	ssl06/set21	ssl06/set21 (NCTC/MW2)	ssl07/set1	ssl07/set1 (MRSA252)	ssl07/set1 (AF188836)	ssl08/set12	ssl09/set5	ssl09/set5 (MRSA252)	ssl10/set4	ssl10/set4 (RF122)	Ssl10/set4 (MRSA252)	ssl11/set2 (COL)	ssl11/set2 (Mu50/N315)	ssl11/set2 (MW2/MSSA476)	ssl11/set2 (MRSA252)	setB3	setB3 (MRSA252)	setB2	setB2 (MRSA252)	setB1
ST93 MSSA																																				
NT	WBG7735	+					w	+					w				+																+			
NT	WBG7762	+						w									+																w			
Qld	UQ40	+			+		+	+					w		w		+					w											+			
Vic	DP32039	+			+		+	+					+		+		+	+				+			+		+						+			w
WA	C229T	+					w	+									+								+		+						+			
WA	N126W	+					w	+									+																+			
WA	W17S	+					+	+					+		w		+	w							+								+			
WA	113S	+			+		+	+					w		w		+								+								+			
WA	9506160A	+			+		+	+					+		w		+	+				+					+						+			
WA	9509712N	+			+		+	+					+				+	w				w			+								+			
WA	9524093R	+			+		+	+					+				+	+				w			+								+			
WA	9525206A	+			+		+	+					w				+																+			
WA	9529120L	+			+		+	+					+				+	w				w			+		+						+			
ST 93 MRSA																																				
ACT	SAPTCH92	+			+		+	+					+		w		+	+				+			+		+						+			
ACT	SAPTCH53	+			+		+	+					+		w		+	+				+					+						+			
NSW	SAPRPAH96	+			+		+	+					w				+	w															+			
NSW	SAPWH23	+						+									+																			
NSW	SAPWH39	+			+		+	+					+				+	+				+			+		+						+			
NSW	SAPWH61	+						+									+								+								+			
NSW	SAPWH64	+			+		+	+					+				+	w				+											+			
NSW	SAPWH94	+			+		+	+					+		w		+	+				+			+		+						+			
NSW	SAPWH71	+						+									+																			
NSW	SAPCRGH95	+															+																			
NSW	SAPRPAH21	+			+		+	+					+				+	w				w			+								+			
NSW	SAPRPAH7	+			+		+	+					+		w		+	+				+			+		+						+			
NSW	SAPWH10	+			+		+	+					+		w		+	+				+			+		+						+			
NSW	SAPWH53	+					w	+					w				+																+			
NT	SAPRDH61	+															+																			
NT	SAPRDH27	+					w	w									+																w			
NT	SAPRDH2	+			+		+	+					+				+	w				+			+		+						+			
Qld	SAPRBH98	+			+		+	+					+		w		+	+				+			+		+						+			
Qld	SAPRBH12	+					+										+																			

Supplementary Table 2: ST93 staphylococcal superantigen/enterotoxin-like genes (set/ssl) profile

Region	Reference Number	setC (MW0345)	ssl01/set6 (COL/SACOL468)	ssl01/set6 (Mu50/SAV0422)	ssl01/set6 (MW2-MW0382)	ssl01/set6 (SAR0422/MRSA252)	ssl01/set6 (all others)	ssl01/set6 (RF122)	ssl02/set7	ssl03/set8	ssl03/set8 (MRSA252/SAR0424)	ssl04/set9	ssl04/set9 (MRSA252/SAR0425)	ssl05/set3	sl05/set3 (RF122)	ssl05/set3 (MRSA252)	ssl06/set21	ssl06/set21 (NCTC/MW2)	ssl07/set1	ssl07/set1 (MRSA252)	ssl07/set1 (AF188836)	ssl08/set12	ssl09/set5	ssl09/set5 (MRSA252)	ssl10/set4	ssl10/set4 (RF122)	Ssl10/set4 (MRSA252)	ssl11/set2 (COL)	ssl11/set2 (Mu50/N315)	ssl11/set2 (MW2/MSSA476)	ssl11/set2 (MRSA252)	setB3	setB3 (MRSA252)	setB2	setB2 (MRSA252)	setB1
Qld	SAPGCH3	+						+									+																			
Qld	SAPRBH14	+						w									+																			
Qld	SAPCBH10	+			+		+	+					+		w		+	+				+					+						+			
Qld	SAPGCH28	+			+		+	+					+				+	w				+			+		+						+			
Qld	SAPRBH1	+			+		+	+					+				+	w				+			+								+			
SA	GPSA73	+			+		+	+					+				+	+				w			+								+			
SA	SAPIMVS24	+					w	+					+				+	w															+			
SA	SAPIMVS31	+			+		+	+					+				+	w				+			+		+						+			
Vic	SAPRCH74	+			+		+	+					+		w		+	+				+			+		+						+			
Vic	SAPAH21	+			+		+	+					+				+	w				+											+			
WA	16790	+			+		+	+					+		w		+	+				+			+		+						+			
WA	16815	+			+		+	+					+	w	+		+	+				+	w		+		+						+		w	+
WA	15586	+						w									+																w			
WA	15587	+					w	+							w		+								+								w			
WA	16414	+						+					w				+																+			
WA	16475	+			+		+	+					+		+		+	w				w			+		+						+			w
WA	17164	+			+		+	+				w	+	w	+		+	+				+	+		+		+						+		+	+
WA	18158	+			+		+	+					+		w		+	+				+			+								+			
WA	18385	+						w									+																			
WA	18418	+			+		+	+					+		+		+	+				+			+		+						+			
WA	20198	+			+		+	+					+				+	+				+					+						+			
WA	SAPRPH48	+			+		+	+					+		+		+	+				+			+		+						+		w	w
WA	16908	+			+		+	+	w			+	+	w	+		+	+				+	+		+		+						+		+	+
WA	17090	+			+		+	+					+		w		+	+				+			+		+						+			
WA	17195	+			+		+	+					+		w		+	+				+			+		+						+			
WA	20548	+			+		+	+					+				+	w				w			+								+			
Control Strain																																				
Vic	JKD6159	+			+		+	+					+				+	+				+											+			

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia

setC, staphylococcal exotoxin-like protein gene; *ssl01/set6*, staphylococcal superantigen-like protein 1 gene (alleles); *ssl021/set7*, staphylococcal superantigen-like protein 2 gene; *ssl03/set8*, staphylococcal superantigen-like protein 3 gene; *ssl04/set9*, staphylococcal superantigen-like protein 4 gene (alleles); *ssl51/set3*, staphylococcal superantigen-like protein 3 gene (alleles); *ssl06/set21*, staphylococcal superantigen-like protein 6 gene (alleles);

ssl07/set1, staphylococcal superantigen-like protein 7 gene (alleles); *ssl08/set6*, staphylococcal superantigen-like protein 8 gene; *ssl09/set9*, staphylococcal superantigen-like protein 9 gene (alleles); *ssl10/set4*, staphylococcal superantigen-like protein 10 gene (alleles); *ssl11/set2*, staphylococcal superantigen-like protein 1 gene (alleles); *setB3,* staphylococcal exotoxin-like protein gene, second locus (alleles); *setB2,* staphylococcal exotoxin-like protein gene, second locus (alleles); *setB1,* staphylococcal exotoxin-like protein gene

+, gene detected; w, gene detected but yielding weak or ambiguous signals

Supplementary Table 3: Microarray DNA ST93 MSCRAMMs and adhesion profile

Region	Reference Number	bbp (all)	bbp (COL /MW2)	bbp (MRSA252)	bbp (Mu50)	bbp (RF122)	bbp (ST45)	clfA (all)	clfA (COL/ RF122)	clfA (MRSA252)	clfaA (Mu50/MW2)	clfB- all	clfB (COL + Mu50)	clfB (MW2)	clfB (RF122)	cna	ebh (all)	eno	fib	fib (MRSA252)	ebpS	epbS (01-1111)	ebpS (COL)	fnbA (all)	fnbA (COL)	fnbA (MRSA252)	fnbA (Mu50/MW2)	fnbA (RF122)	fnbB (COL)	fnbB (COL/Mu50/MW2)	fnbB (Mu50)	fnbB (MW2)	fnbB (ST15)	fnbB (ST45-2)
ST93 MSSA																																		
NT	WBG7735	+			+			+			+	+			+			+		+	+			+						+				
NT	WBG7762	+			+			+			+	+			+			+		+	+			+						+				
Qld	UQ40	+			+			+			+	+			+			+		+	+			+						+	w			
Vic	DP2039	+			+		w	+			+	+			+			+		+	+		+	+						+	+			
WA	C229T	+			+			+			+	+			+			+		+	+			+						+				
WA	N126W	+			+			+			+	+			+			+		+	+			+						+				
WA	W17S	+			+			+			+	+			+			+		+	+			+						+				
WA	Y113S	+			+			+			+	+			+			+		+	+			+						+				
WA	9506160A	+			+			+			+	+			+			+		+	+		+	+						+	+			
WA	9509712N	+			+			+			+	+			+			+		+	+			+						+	w			
WA	9524093R	+			+			+			+	+			+			+		+	+			+						+	w			
WA	9525206A	+			+			+			+	+			+			+		+	+			+						+				
WA	9529120L	+			+			+			+	+			+			+		+	+			+						+	w			
ST93 MRSA																																		
ACT	SAPTCH92	+			+			+			+	+			+			+		+	+		+	+						+	+			
ACT	SAPTCH53	+			+			+			+	+			+			+		+	+		w	+						+	+			
NSW	SAPRPAH96	+			+			+			+	+			+			+		+	+			+						+				
NSW	SAPWH23	+			+			+			+	+			+			+		+	+			+						+				
NSW	SAPWH39	+			+			+			+	+			+			+		+	+		+	+						+	+			
NSW	SAPWH61	+			+			+			+	+			+			+		+	+			+						+				
NSW	SAPWH64	+			+			+			+	+			+			+		+	+			+						+	w			
NSW	SAPWH94	+			+			+			+	+			+			+		+	+		+	+						+	+			
NSW	SAPWH71							+			+	+			+			+		+	+			+						+				
NSW	SAPCRGH95	+			+			+			+	+			+			+		+	+			+						+				
NSW	SAPRPAH21	+			+			+			+	+			+			+		+	+			+						+	w			
NSW	SAPRPAH7	+			+			+			+	+			+			+		+	+		+	+						+	+			
NSW	SAPWH10	+			+			+			+	+			+			+		+	+		+	+						+	+			
NSW	SAPWH53	+			+			+			+	+			+			+		+	+			+						+				
NT	SAPRDH61	+			+			+			+	+			+			+		+	+			+						+				
NT	SAPRDH27	+			+			+			+	+			+			+		+	+			+						+				
NT	SAPRDH2	+			+			+			+	+			+			+		+	+			+						+	+			
Qld	SAPRBH98	+			+			+			+	+			+			+		+	+		w	+						+	+			

Supplementary Table 3: Microarray DNA ST93 MSCRAMMs and adhesion profile

Region	Reference Number	bbp (all)	bbp (COL /MW2)	bbp (MRSA252)	bbp (Mu50)	bbp (RF122)	bbp (ST45)	clfA (all)	clfA (COL/ RF122)	clfA (MRSA252)	clfaA (Mu50/MW2)	clfB- all	clfB (COL + Mu50)	clfB (MW2)	clfB (RF122)	cna	ebh (all)	eno	fib	fib (MRSA252)	ebpS	epbS (01-1111)	ebpS (COL)	fnbA (all)	fnbA (COL)	fnbA (MRSA252)	fnbA (Mu50/MW2)	fnbA (RF122)	fnbB (COL)	fnbB (COL/Mu50/MW2)	fnbB (Mu50)	fnbB (MW2)	fnbB (ST15)	fnbB (ST45-2)
Qld	SAPRBH12	+			+			+			+	+			+			+		+	+			+						+				
Qld	SAPGCH3	+			+			+			+	+			+			+		+	+			+						+				
Qld	SAPRBH14	+			+			+			+	+			+			+		+	+			+						+				
Qld	SAPCBH10	+			+			+			+	+			+			+		+	+		w	+						+	+			
Qld	SAPGCH28	+			+			+			+	+			+			+		+	+			+						+	+			
Qld	SAPRBH1	+			+			+			+	+			+			+		+	+			+						+	+			
SA	SAPGPSA73	+			+			+			+	+			+			+		+	+		w	+						+	w			
SA	SAPIMVS24	+			+			+			+	+			+			+		+	+			+						+	w			
SA	SAPIMVS31	+			+			+			+	+			+			+		+	+		w	+						+	+			
Vic	RCH74	+			+			+			+	+			+			+		+	+		+	+						+	+			
Vic	SAPAH21	+			+			+			+	+			+			+		+	+			+						+	w			
WA	16790	+			+			+			+	+			+			+		+	+		+	+						+	+			
WA	16815	+			+		w	+			+	+			+			+		+	+		+	+						+	+			
WA	15586	+			+			+			+	+			+			+		+	+			+						+				
WA	15587	+			+			+			+	+			+			+		+	+			+						+				
WA	16414	+			+			+			+	+			+			+		+	+			+						+				
WA	16475	+			+			+			+	+			+			+		+	+			+						+	w			
WA	17164	+			+		w	+			+	+			+			+		+	+		+	+						+	+			
WA	18158	+			+		w	+			+	+			+			+		+	+			+						+	+			
WA	18385	+			+			+			+	+			+			+		+	+			+						+				
WA	18418	+			+			+			+	+			+			+		+	+		w	+						+	+			
WA	20198	+			+			+			+	+			+			+		+	+		w	+						+	+			
WA	SAPRPH48	+			+			+			+	+			+			+		+	+		+	+						+	+			
WA	16908	+	+		+		w	+			+	+	+	w	+		+	+		+	+		+	+						+	+			
WA	17090	+			+			+			+	+			+			+		+	+		+	+						+	+			
WA	17195	+			+		w	+			+	+			+			+		+	+		w	+						+	+			
WA	20548	+			+			+			+	+			+			+		+	+			+						+	w			
Control Strain																																		
Vic	JKD6159	+			+			+			+	+			+			+		+	+			+						+	+			

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia

bbp, bone sialoprotein-binding protein gene (alleles); *clfA*, clumping factor A gene (alleles); *clfB*, clumping factor B gene (alleles); *cna*, collagen-binding adhesion gene; *ebh*, cell wall associated fibronectin-binding protein gene; *eno*, enolase gene; *fib*, fibrinogen binding protein gene (alleles); *ebpS*, cell surface elastin binding protein gene (alleles); *fnbA*, fibronectin-binding protein A gene (alleles); *fnbB*, fibronectin-binding protein B gene (alleles)

+, gene detected; w, gene detected but yielding weak or ambiguous signals

Supplementary Table 4: Microarray DNA ST93 MSCRAMMs and adhesion profile cont

Region	Reference Number	map	map (RF122)	map (MRSA252)	map (Mu50/MW2)	sdrC (all)	sdrC (B1)	sdrC (COL)	sdrC (Mu50)	sdrC (MW2/MRSA252/RF122)	sdrc (other than MRSA252/RF122)	sdrD (COL/MW2)	sdrD (Mu50)	sdrD (other)	sdrD (other than MRSA252/RF122)	vwb (all)	vwb (COL/MW2)	vwb (MRSA252)	vwb (Mu50)	vwb (RF122)	sasG	sasG (COL/Mu50)	sasG (MW2)	sasG (Other than MRSA252/RF122)
ST93 MSSA																								
NT	WBG7735					+		+						+	+	+								
NT	WBG7762					+		+						+	+	+								
Qld	UQ40					+		+						+	+	+								
Vic	DP2039					+		+						+	+	+								
WA	C229T					+		+						+	+	+								
WA	N126W					+		+						+	+	+								
WA	W17S					+		+						+	+	+								
WA	Y113S					+		+						+	+	+								
WA	9506160A					+		+						+	+	+								
WA	9509712N					+		+						+	+	+								
WA	9524093R					+		+						+	+	+								
WA	9525206A					+		+						+	+	+								
WA	9529120L					+		+						+	+	+								
ST93 MSSA																								
ACT	SAPTCH92					+		+						+	+	+								
ACT	SAPTCH53					+		+						+	+	+								
NSW	SAPRPAH96					+		+						+	+	+								
NSW	SAPWH23					+		+						+		+								
NSW	SAPWH39					+		+						+	+	+								
NSW	SAPWH61					+		+						+	+	+								
NSW	SAPWH64					+		+						+	+	+								
NSW	SAPWH94					+		+						+	+	+								
NSW	SAPWH71					+		+						+		+								
NSW	SAPCRGH95					+		+						+		+								
NSW	SAPRPAH21					+		+						+	+	+								
NSW	SAPRPAH7					+		+						+	+	+								
NSW	SAPWH10					+		+						+	+	+								
NSW	SAPWH53					+		+						+	+	+								

Supplementary Table 4: Microarray DNA ST93 MSCRAMMs and adhesion profile cont

Region	Reference Number	map	map (RF122)	map (MRSA252)	map (Mu50/MW2)	sdrC (all)	sdrC (B1)	sdrC (COL)	sdrC (Mu50)	sdrC (MW2/MRSA252/RF122)	sdrc (other than MRSA252/RF122)	sdrD (COL/MW2)	sdrD (Mu50)	sdrD (other)	sdrD (other than MRSA252/RF122)	vwb (all)	vwb (COL/MW2)	vwb (MRSA252)	vwb (Mu50)	vwb (RF122)	sasG	sasG (COL/Mu50)	sasG (MW2)	sasG (Other than MRSA252/RF122)
NT	SAPRDH61					+		+						+	+	+								
NT	SAPRDH27					+		+						+	+	+								
NT	SAPRDH2					+		+						+	+	+								
Qld	SAPRBH98					+		+						+	+	+								
Qld	SAPRBH12					+		+						+		+								
Qld	SAPGCH3					+		+						+	+	+								
Qld	SAPRBH14					+		+						+	+	+								
Qld	SAPCBH10					+		+						+	+	+								
Qld	SAPGCH28					+		+						+	+	+								
Qld	SAPRBH1					+		+						+	+	+								
SA	SAPGPSA73					+		+						+	+	+								
SA	SAPIMVS24					+		+						+	+	+								
SA	SAPIMVS31					+		+						+	+	+								
Vic	SAPRCH74					+		+						+	+	+								
Vic	SAPAH21					+		+						+	+	+								
WA	16790					+		+						+	+	+								
WA	16815					+		+						+	+	+								
WA	15586					+		+						+	+	+								
WA	15587					+		+						+	+	+								
WA	16414					+		+						+	+	+								
WA	16475					+		+						+	+	+								
WA	17164					+		+						+	+	+								
WA	18158					+		+						+	+	+								
WA	18385					+		+						+	+	+								
WA	18418					+		+						+	+	+								
WA	20198					+		+						+	+	+								
WA	SAPRPH48					+		+						+	+	+								
WA	16908					+		+	w	w				+	+	+								
WA	17090					+		+						+	+	+								
WA	17195					+		+						+	+	+								
WA	20548					+		+						+	+	+								
Vic	JK06159					+		+						+	+	+								

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia

map, major histocompatability complex class II analogue protein gene (alleles); *sdrC*, ser-asp rich fibrinogen-/bone sialoprotein-binding protein C gene (alleles); *sdrD*, ser-asp rich fibrinogen-/bone sialoprotein-binding protein D gene (alleles);*vwb*, van Willebrand factor binding protein gene (allele); *sasG*, *Staphylococcus aureus* surface protein G gene (alleles)

+, gene detected; w, gene detected but yielding weak or ambiguous signals

Supplementary Table 5: Microarray DNA ST93 immunevasion and miscellaneous profile

Region	Reference Number	isaB	isaB (MRSA252)	mprF	mprF (COL/MW2)	mprF (Mu50/MRSA252)	isdA	isdA (MRSA252)	isdA (Other than MRSA252)	lmrP	lmrP (RF122)	Q2YUB3	hsdS1 (RF122)	hsdS2 (ST5/ST8)	hsdS2 (MW2/476)	hsdS2 (RF122)	hsdS2 (MRSA252)	hsdS3 (other than RF122/MRSA252)	hsdS3 (ST8/ST1/RF122)	hsdS3 (Mu50/N315)	hsdS3 (CC51/MRSA252)	hsdS3 (MRSA252)	hsdSx (CC25)	hsdSx (CC15)	hsdSx (etd)	Q2FXCO	Q7A4X2	hysA1 (MRSA252)	hysA1 (MRSA252/RF122)/hysA2	hysA1 (MRSA252/RF122)/hysA2 (COL/USA300)	hysA2 (all except MRSA252)	hysA2 (COL/USA300/NCTC)	hysA2 (all except COL/USA300/NCTC)	hysA2 (MRSA252)
ST93 MSSA																																		
NT	WBG7735	+	+				+		+	+												+					+		+	+		+		
NT	WBG7762	+	+				+		+	+												+					+		+	+		+		
Qld	UQ40	+	+				+		+	+												+					+		+	+		+		w
Vic	DP2039	+	+	w		w	+	w	+	+		w			w							+	w	w			+		+	+		+		+
WA	C229T	+	+				+		+	+					+							+					+		+	+		+		
WA	N126W	+	+				+		+	+												+					+		+	+		+		
WA	W17S	+	+	w		w	+		+	+		w										+	w				+		+	+		+		w
WA	Y113S	+	+				+		+	+					+							+					+		+	+		+		
WA	9506160A	+	+				+		+	+					+							+					+		+	+		+		+
WA	9509712N	+	+				+		+	+					+							+					+		+	+		+		
WA	9524093R	+	+				+		+	+					+							+					+		+	+		+		
WA	9525206A	+	+				+		+	+												+					+		+	+		+		
WA	9529120L	+	+				+		+	+					+							+					+		+	+		+		
ST93 MRSA																																		
ACT	SAPTCH92	+	+				+		+	+					+							+					+		+	+		+		+
ACT	SAPTCH53	+	+				+		+	+					+							+					+		+	+		+		w
NSW	SAPRPAH96	+	+				+		+	+												+					+		+	+		+		
NSW	SAPWH23	+	+				+		+	+												+					+		w	+		+		
NSW	SAPWH39	+	+				+		+	+												+					+		+	+		+		w
NSW	SAPWH61	+	+				+		+	+												+					+		w	+		+		
NSW	SAPWH64	+	+				+		+	+												+					+		+	+		+		
NSW	SAPWH94	+	+				+		+	+												+					+		+	+		+		w
NSW	SAPWH71	+	+				+		+	+												+					+		+	+		+		
NSW	SAPCRGH95	+	+				+		+	+												+					+			+		+		
NSW	SAPRPAH21	+	+				+		+	+												+					+		+	+		+		
NSW	SAPRPAH7	+	+				+		+	+					+							+					+		+	+		+		+
NSW	SAPWH10	+	+				+		+	+					w							+					+		+	+		+		+
NSW	SAPWH53	+	+				+		+	+												+					+		+	+		+		
NT	SAPRDH61	+	+				+		+	+												+					+		+	+		+		
NT	SAPRDH27	+	+				+		+	+												+					+		+	+		+		
NT	SAPRDH2	+	+				+		+	+												+					+		+	+		+		
Qld	SAPRBH98	+	+				+		+	+					+							+					+		+	+		+		w

Supplementary Table 5: Microarray DNA ST93 immunevasion and miscellaneous profile

Region	Reference Number	isaB	isaB (MRSA252)	mprF	mprF (COL/MW2)	mprF (Mu50/MRSA252)	isdA	isdA (MRSA252)	isdA (Other than MRSA252)	lmrP	lmrP (RF122)	Q2YUB3	hsdS1 (RF122)	hsdS2 (ST5/ST8)	hsdS2 (MW2/476)	hsdS2 (RF122)	hsdS2 (MRSA252)	hsdS3 (other than RF122/MRSA252)	hsdS3 (ST8/ST1/RF122)	hsdS3 (Mu50/N315)	hsdS3 (CC51/MRSA252)	hsdS3 (MRSA252)	hsdSx (CC25)	hsdSx (CC15)	hsdSx (etd)	Q2FXCO	Q7A4X2	hysA1 (MRSA252)	hysA1 (MRSA252/RF122)/hysA2	hysA1 (MRSA252/RF122)/hysA2 (COL/USA300)	hysA2 (all except MRSA252)	hysA2 (COL/USA300/NCTC)	hysA2 (all except COL/USA300/NCTC)	hysA2 (MRSA252)
Qld	SAPRBH12	+	+				+		+	+												+					+			+		+		
Qld	SAPGCH3	+	+				+		+	+												+					+		+	+		+		
Qld	SAPRBH14	+	+				+		+	+												+					+		w	+		+		
Qld	SAPCBH10	+	+				+		+	+					+							+					+		+	+		+		w
Qld	SAPGCH28	+	+				+		+	+					+							+					+		+	+		+		
Qld	SAPRBH1	+	+				+		+	+					w							+					+		+	+		+		
SA	SAPGPSA73	+	+				+		+	+					w							+					+		+	+		+		
SA	SAPIMVS24	+	+				+		+	+												+					+		+	+		+		
SA	SAPIMVS31	+	+				+		+	+					w							+					+		+	+		+		
Vic	SAPRCH74	+	+				+		+	+					+							+					+		+	+		+		w
Vic	SAPAH21	+	+				+		+	+					w							+					+		+	+		+		
WA	16790	+	+				+		+	+					+							+					+		+	+		+		w
WA	16815	+	+	+		+	+	w	+	+		+			w							+	+	w	+		+		+	+		+		+
WA	15586	+	+				+		+	+												+					+		+	+		+		
WA	15587	+	+				+		+	+					+							+					+		+	+		+		w
WA	16414	+	+				+		+	+												+					+		+	+		+		
WA	16475	+	+	w		w	+		+	+					w							+	w				+		+	+		+		w
WA	17164	+	+	+		+	+	w	+	+		+			w							+	+	w			+		+	+		+		+
WA	18158	+	+				+		+	+		w			+							+	w	w			+		+	+		+		w
WA	18385	+	+				+		+	+												+					+		+	+		+		
WA	18418	+	+	w		w	+		+	+												+					+		+	+		+		w
WA	20198	+	+				+		+	+												+					+		+	+		+		
WA	SAPRPH48	+	+	w		w	+		+	+												+	w				+		+	+		+		+
WA	16908	+	+	+	w	+	+	+	+	+		+			w							+	+	w			+		+	+		+		+
WA	17090	+	+				+		+	+		w										+					+		+	+		+		w
WA	17195	+	+	w		w	+		+	+		w										+	w	w			+		+	+		+		w
WA	20548	+	+				+		+	+												+					+		+	+		+		
Control Strain																																		
Vic	JK06159	+	+				+		+	+					+							+					+		+	+		+		

Regions: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory, Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia

isaB, immunodominant antigen B gene (alleles); *mprF*, defensin resistance gene protein gene (alleles); *isdA*, transferrin-binding protein gene (alleles); *lmrP*, hypothetical protein, similar to integral membrane protein LmrP gene (alleles); *Q2YUB3*, Unspecific efflux/transporter gene; hsdS1, type 1 site-specific deoxyribonuclease subunit, 1st locus gene; *hsdS2*, type 1 site-specific deoxyribonuclease subunit, 2nd locus gene (alleles); hsdS3, type 1 site-specific deoxyribonuclease subunit, 3rd locus gene (alleles); hsdSx, type 1 site-specific deoxyribonuclease subunit, unknown locus gene (alleles); *Q2FXCO*, hypothetical protein gene, located next to serine protease operon; *Q7A4X2*, hypothetical protein gene; *hysA1*, hyaluronate lyase first locus gene (alleles); *hysA2*, hyaluronate lyase second locus gene (alleles)

+, gene detected; w, gene detected but yielding weak or ambiguous signals

8.4. PAPER FOUR

Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia.

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Differentiation of Clonal Complex 59 Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Western Australia▽‡

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Clonal complex 59 (CC59) community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains were characterized using pulsed-field gel electrophoresis, *spa* typing, multilocus sequence typing, diagnostic DNA microarrays, and PCRs targeting staphylococcal cassette chromosome *mec* (SCC*mec*) elements and Panton-Valentine leukocidin (PVL). Six distinct groups within CC59 were characterized. At least seven different variants of SCC*mec* elements were identified (IVa [2B], IVb [2B], IVd [2B], IV variant [2B], IVa [2B&5], V variant [5C2], and V [5C2&5]). (The structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an uppercase letter, respectively. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type.) The first group is similar to the American sequence type 59 (ST59) MRSA-IV CA-MRSA strain USA1000. The second group includes a PVL-negative ST87 strain with an SCC*mec* element of subtype IVb (2B). The third group comprises PVL-variable ST59 MRSA-IV strains harboring multiple SCC*mec* IV subtypes. PVL-negative ST59 MRSA strains with multiple or composite SCC*mec* elements (IVa [2B&5]) form the fourth group. Group 5 corresponds to the internationally known "Taiwan clone," a PVL-positive strain with a variant SCC*mec* element (V [5C2&5]). This strain proved to be the most common CC59 MRSA strain isolated in Western Australia. Finally, group 6 encompasses the ST59 MRSA-V variant (5C2). The differentiation of CC59 into groups and strains indicates a rapid evolution and spread of SCC*mec* elements. Observed differences between groups of strains as well as intrastrain variability within a group facilitate the tracing of their spread.

Several well-characterized community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains predominate in different regions of the world. Many of these strains harbor the bicomponent Panton-Valentine leukocidin (PVL). Sequence type 8 (ST8) MRSA-IV (USA300), ST80 MRSA-IV, and ST93 MRSA-IV are the major CA-MRSA strains reported to occur in the United States, Europe, and Australia, respectively. In the Asia Pacific region, a distinct genotype, clonal complex 59 (CC59)/ST59, has become widespread. ST59 CA-MRSA strains are an important cause of morbidity in Taiwan (1, 13, 17, 25, 29) This so-called "Taiwan clone" has acquired a novel type V staphylococcal cassette chromosome *mec* (SCC*mec*) element (V [5C2&5], also known as V_T) and *ermB*, a macrolide-lincosamide-streptogramin B resistance gene (28) frequently reported to be present in streptococci and other bacteria. Its properties have recently been described in detail (24). ST59 MRSA-V and other CC59 strains have now been reported to occur in several countries, including the United States (USA1000) (6), Sweden (11), Germany (20), the United Kingdom (19), Vietnam (26), and Australia (22).

In Western Australia (WA), all MRSA strains are referred to the state's central typing reference laboratory (the Gram-Positive Bacteria Typing and Research Unit) for molecular characterization (5). Multiple CC59 strains, colloquially characterized as WA MRSA-9, -15, -24, -52, -55, -56, and -73, have been identified. They differ from each other in ST designation, pulsed-field gel electrophoresis (PFGE) pattern, SCC*mec* element, and PVL carriage.

To better understand the molecular epidemiology of this clonal complex, all CC59 MRSA strains isolated in Western Australia were examined using PFGE, *spa* typing, multilocus sequence typing (MLST), diagnostic DNA microarrays, and PCRs targeting SCC*mec* elements and PVL.

MATERIALS AND METHODS

Isolates and patients. From July 2003 to June 2008, 43 MRSA strains from 40 individuals living in WA were characterized as CC59 MRSA by the Gram-Positive Bacteria Typing and Research Unit. One person yielded three isolates (WA MRSA-15 04-16657, 05-17619, and 06-17484) over a 3-year period, and a

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second yielded two isolates (WA MRSA-9 06-17363 and 07-17830) over a 2-year period. Three isolates (WA MRSA-52 07-15076, 07-16295, and 07-16320) were obtained from three family members over a 12-month period. Isolates were recovered from 33 skin and soft tissue infections and seven nasal screening swabs.

Control strains. Two reference strains of USA1000 ST59 MRSA-IV (NARSA483 and NARSA676) were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) and have been included in this study for comparison.

Susceptibility testing. An antibiogram was performed by disk diffusion on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (3). A panel of eight antimicrobial drugs, i.e., erythromycin (15 μg), tetracycline (30 μg), trimethoprim (5 μg), ciprofloxacin (5 μg), gentamicin (10 μg), rifampin (5 μg), fusidic acid (10 μg), and mupirocin (5 μg), was tested. CLSI interpretive criteria (4) were used for all drugs except fusidic acid (2) and mupirocin (9).

PFGE. Electrophoresis of chromosomal DNA was performed as previously described (23), using a contour-clamped homogeneous electric field (CHEF) DR III system (Bio-Rad Laboratories Pty Ltd). Chromosomal patterns were examined visually, scanned with a Quantity One device (Bio-Rad Laboratories Pty Ltd), and digitally analyzed using FPQuest (Bio-Rad Laboratories). CHEF patterns were grouped according to the criteria of Tenover et al. (27), and a dendrogram similarity of 80% or greater was used to assign strain relatedness. *S. aureus* strain NCTC 8325 was used as a reference strain.

MLST and *spa* typing. Chromosomal DNA for MLST and *spa* typing was prepared using a DNeasy tissue kit (Qiagen Pty Ltd).

MLST was performed as specified by Enright et al. (7). The method involves bidirectional sequencing of 450- to 500-bp internal fragments of seven housekeeping genes obtained by PCR using highly conserved primer pairs. Each allele sequence is assigned a number by the curator of the MLST database (http://saureus.mlst.net), and the allelic profile determines the sequence type (ST). Allelic profiles can be compared using the BURST (based upon related sequence types) program (http://linux.mlst.net/burst.htm). Clusters of single-locus variants (SLVs) and double-locus variants are referred to as clonal clusters.

To assign an ST, sequences were compared with the sequences on the MLST website. By use of the MLST database, clones were subsequently grouped into CC59.

spa typing, a DNA sequence-based analysis of the protein A gene variable region, was performed as previously described (10) using the nomenclature as described on the Ridom website (http://spa.ridom.de/).

PVL. PCR for the detection of PVL determinants was performed as previously described (8).

SCC*mec* typing. SCC*mec* is a mobile genetic element that carries the *mecA* gene, which encodes broad-spectrum beta-lactam resistance, and unique site-specific recombinases designated cassette chromosome recombinases (*ccr*). SCC*mec* elements are classified into types and subtypes by a hierarchical system (15). Types are defined by the combination of the *ccr* gene complex (types 1 to 5, represented by the *ccr* gene allotypes [*ccrA1*, *ccrA2*, *ccrA3*, and *ccrA4*; *ccrB1*, *ccrB2*, *ccrB3*, and *ccrB4*; and *ccrC1*]) and the *mec* gene complex (classes A, B, C1, and C2). The SCC*mec* element also contains three nonessential components known as the "J regions." Variations in the J regions within the same *mec-ccr* complex are used for defining SCC*mec* subtypes.

SCC*mec* was typed by PCR using the following strategy. The structural architecture and the *mec* complex were determined using primers described by Zhang et al. (30). SCC*mec* type IV was further subtyped using published primers (18). Cassette chromosome recombinase (*ccr*) typing and *ccrC1* allele 2 and 8 allotyping were performed as published previously (12, 16). Nontypeable strains and the type V SCC*mec* were characterized using previously published primers (15). An IS*Sau4*-like transposase (GenBank accession no. DQ680163) insertion in open reading frame (ORF) V011 (GenBank accession no. AB12129) of the type V SCC*mec* was detected by the production of a ca. 1,600-bp PCR product rather than the characteristic 325-bp product in the multiplex reaction performed by Zhang et al. (30) and confirmed by sequencing. SCC*mec* nomenclature is used as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (15). Briefly, the structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an uppercase letter, respectively. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type.

DNA microarray. The DNA microarray used for this study covered 334 target sequences corresponding to 185 distinct genes and their allelic variants. A complete list of targets has been provided previously (20, 21). Target genes included species markers, virulence factors, resistance genes, staphylococcal superantigen-like or exotoxin-like genes (*set* or *ssl* genes), and genes encoding adhesion proteins, as well as markers for accessory gene regulator (*agr*) alleles and capsule types. With regard to SCC*mec* typing, the array included probes for *mecA*, *ugpQ* (GenBank accession no. BA000018.3, locus tag SA0036), *mecI* (BA000018.3, SA0040), *xylR* (BA000018.3, SA0041), the dcs region (BA000018.3, SA0024 [EMBL accession no. Q9XB68]), and two probes for *mecR* (BA000018.3, SA0039). The last two probes allowed detection and discrimination of untruncated *mecR* and Δ*mecR*, respectively. Recombinase genes *ccrA1*, *ccrB1*, *ccrA2*, *ccrB2*, *ccrA3*, *ccrB3*, *ccrA4*, *ccrB4*, and *ccrC1* were also covered. A gene for a "hypothetical protein" accompanying *ccrC1* was also included, and alleles from strain 85-2082 (GenBank accession no. AB037671.1, nucleotides 61250 to 62893) and strain MRSAZH47 (GenBank accession no. AM292304.1, nucleotides 5654 to 7273) were distinguished. The mercury resistance and *kdp* operons were also included, but they are not relevant for the CC59 strains discussed in this study.

Arrays and reagents were obtained from CLONDIAG. The principle of the assay and related procedures have previously been described in detail (20, 21). Briefly, DNA was obtained by enzymatic lysis of overnight cultures. All targets were simultaneously amplified by linear PCR, and the products were labeled by the incorporation of biotin–16-dUTP during the reaction. The labeled sample was then hybridized to the array, followed by washing steps and the addition of a blocking reagent. Horseradish peroxidase-streptavidin conjugate was added to the array, followed again by incubation and washing. Finally, Seramun Green precipitating dye (Seramun, Heidesee, Germany) was added. An image of the array was recorded and analyzed using a dedicated reader and software.

SplitsTree analysis. To analyze similarities between DNA microarray profiles, SplitsTree software (14) was used. DNA microarray results for relevant genes (see the legend for Fig. 2) were converted into strings of information which were handled as "sequences," using "A" for "positive" and "T" for "negative." These "sequences" were used for tree construction using SplitsTree 4.10 (character transformation, uncorrected *P*; distance transformation, Neighbor-Net; and variance, ordinary least squares).

RESULTS

Antibiogram analysis, PVL PCR, PFGE, *spa* typing, MLST, SCC*mec* typing, and DNA microarray analysis (data not shown) were performed on all isolates (Tables 1 and 2).

PFGE identified seven WA CC59 CA-MRSA strains: WA MRSA-9, -15, -24, -52, -55, -56, and -73 (Fig. 1).

Shared properties of CC59 MRSA isolates. All isolates were *agr* group I and capsule type 8 and carried the gamma-hemolysin genes *lukF*, *lukS*, and *hlgA*, as well as the hl (GenBank accession no. CP000046.1, locus tag SACOL0921), *hla*, *hld*, and hlIII hemolysin genes. Three out of five probes for beta-hemolysin yielded signals, which could be interpreted as an indication of an as yet unsequenced allelic variant. Leukocidin genes *lukD* and *lukE* were not detected. All isolates lacked protease genes *splA*, *splB*, and *splE* but harbored *aur*, *sspA*, *sspB*, and *sspP*. The carriage of *set* and *ssl* genes was uniform among all isolates, including the presence of *setC*, *ssl1* (in a Mu50/N315-like allele), *ssl3*, *ssl4*, *ssl5* (an unsequenced allele giving signals with a probe derived from CC1/5/8 sequences as well as with another one based on RF122), *ssl7*, *ssl8*, *ssl9*, *ssl10*, and *setB1* to *setB3*. The probe for *ssl2* gave weaker or variable results, indicating another as yet unsequenced allelic variant.

The biofilm operon *icaACD* was present. The gene for a biofilm-associated protein, *bap*, was absent.

Genes encoding MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), *bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sasG*, *sdrC*, *sdrD*, and *vwb* were detected in all isolates. The gene for collagen-binding adhesin (*cna*) was absent.

Carriage of resistance genes, PVL, beta-hemolysin integrating phages, and superantigens was variable.

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TABLE 1. Antibiogram results, PVL PCR results, PFGE patterns, *spa* types, MLST results, and SCC*mec* types for the CC59 MRSA isolates

Group and isolate[a]	Source	Antibiogram result[c]	PVL PCR result[d]	PFGE pattern	*spa* sequence	*spa* type	MLST sequence	ST	SCC*mec* type
Group 1, "WA MRSA-73"									
05-16512	Colonization		N	WA MRSA-73	04	t528	19-23-15-2-19-20-15	59	IVb (2B)
Group 2, "WA MRSA-24"									
04-17626	SSTI[b]	Ery^r	N	WA MRSA-24	04-20-17-20-17-31-16-34	t216	19-23-15-2-41-20-15	87	IVb (2B)
06-15325	SSTI	Ery^r	N	WA MRSA-24	04-20-17-20-17-31-16-34	t216	19-23-15-2-41-20-15	87	IVb (2B)
06-16824	SSTI	Ery^r	N	WA MRSA-24	04-20-17-20-17-31-16-34	t216	19-23-15-2-41-20-15	87	IVb (2B)
Group 3, "WA MRSA-55/56"									
06-17947	SSTI	Ery^r Tet^r	P	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVa (2B)
06-16367	SSTI	Ery^r Tet^r	P	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVa (2B)
07-15432	SSTI	Ery^r Tet^r	P	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVa (2B)
07-15760	SSTI	Ery^r Tet^r	P	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVa (2B)
08-16180	SSTI	Ery^r Tet^r	P	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVd (2B)
08-17668	Colonization	Ery^r	N	WA MRSA-55	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVv (2B)
07-15443	SSTI	Ery^r	P	WA MRSA-56	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	IVa (2B)
Group 4, "WA MRSA-15"									
03-17565	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
04-16557	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
04-17489	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
05-17037	SSTI	Ery^r Tet^r	N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
05-17619	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
06-15513	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
06-17484	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
07-19251	SSTI	Ery^r	N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
08-15202	SSTI		N	WA MRSA-15	04-20-17-20-31-16-34	t976	19-23-15-2-19-20-15	59	IVa (2B&5)
Group 5, "WA MRSA-9/52," or "Taiwan clone"									
06-15672	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-25-34	t441	19-23-15-2-19-20-15	59	V (5C2&5)
06-17363	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-25-34	t441	19-23-15-2-19-20-15	59	V (5C2&5)
07-16447	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-25-34	t441	19-23-15-2-19-20-15	59	V (5C2&5)
07-17830	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-25-34	t441	19-23-15-2-19-20-15	59	V (5C2&5)
03-16672	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
04-16811	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
04-17021	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
05-15724	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
07-15919	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
07-16753	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
07-16861	SSTI	Ery^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
07-18714	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
08-15039	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
08-18104	SSTI	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
08-18190	Colonization	Ery^r Tet^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-2-19-20-15	59	V (5C2&5)
07-17677	Colonization	Ery^r	P	WA MRSA-9	04-20-17-20-17-25-34	t437	19-23-15-48-19-20-15	338	V (5C2&5)
07-15076	Colonization	Ery^r	P	WA MRSA-52	04-20-17-20-17-34	t1950	113-23-15-2-19-20-15	952	V (5C2&5)
07-16295	Colonization	Ery^r	P	WA MRSA-52	04-20-17-20-17-34	t1950	113-23-15-2-19-20-15	952	V (5C2&5)
07-16320	Colonization	Ery^r	P	WA MRSA-52	04-20-17-20-17-34	t1950	113-23-15-2-19-20-15	952	V (5C2&5)
07-16355	SSTI	Ery^r	P	WA MRSA-9	04-20-16-34	t2365	19-23-15-2-19-20-15	59	V (5C2&5)
Group 6, "WA MRSA-9"									
05-17759	SSTI		N	WA MRSA-9	04-20-17-31-16-34	t316	19-23-15-2-19-20-15	59	Vv (5C2)
08-15683	SSTI	Ery^r	N	WA MRSA-9	04-20-17-31-16-34	t316	19-23-15-2-19-20-15	59	Vv (5C2)
06-18653	SSTI	Cip^r	N	WA MRSA-9	04-20-17-25-34	t441	19-23-15-2-19-20-15	59	Vv (5C2)
NARSA control strains, "USA1000"									
NARSA483		Ery^r	P	USA1000	04-20-17-31-16-34	t316	19-23-15-2-19-20-15	59	IVa (2B)
NARSA676			P	USA1000	04-20-17-20-17-31-16-34	t216	19-23-15-2-19-20-15	59	IVa (2B)

[a] Group 1, PVL-negative ST59 MRSA-IVb (2B) (*ccrA2B2* and *mec* complex class B); group 2, PVL-negative ST87 (59SLV) MRSA-IVb (2B) (*ccrA2B2* and *mec* complex class B); group 3, PVL-variable ST59 MRSA-IVa, -IVd, and -IVv (2B) (*ccrA2B2* and *mec* complex class B); group 4, PVL-negative ST59 MRSA-IVa (2B&5) (*ccrA2B2*, *mec* complex class B, and *ccrC1*); group 5, PVL-positive ST59/338 (59SLV)/952 (59SLV) MRSA-V (5C2&5) (*mec* complex class C2 and *ccrC1*); group 6, PVL-negative ST59 MRSA-Vv (5C2) (*mec* complex class C2 and *ccrC1*); NARSA control strains, PVL-positive ST59 MRSA-IVa (2B) (*ccrA2B2* and *mec* complex class B).

[b] SSTI, skin and soft tissue infection.

[c] Ery^r, erythromycin resistant; Tet^r, tetracycline resistant; Cip^r, ciprofloxacin resistant.

[d] N, negative; P, positive.

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TABLE 2. Grouping of CC59 isolates based on DNA microarray hybridization results

Group and no. of isolates[a]	Presence (+) or absence (−) of:																												
	mecA	Δ*mecR*	*ugpQ*	dcs region	*ccrA2*	*ccrB2*	85-2082 *ccrA*	MRSAZH47 *ccrA*	85-2082 *ccrC*	*blaZ*	*blaI*	*blaR*	*ermA*	*ermB*	*ermC*	*msrA*	*mpbBM*	*aphA3*	*sat*	*tetK*	pC221 *cat*	pC223 *cat*	*entA*	*entB*	*entK*	*entQ*	*lukF-PV*	*lukS-PV*	*sak*
Group 1, "WA MRSA-73"																													
1	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	+
Group 2, "WA MRSA-24"																													
3	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	+	+	−	−	+
Group 3, "WA MRSA-55/56"																													
4	+	+	+	+	+	+	−	−	−	+	+	+	−	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+
1	+	+	+	+	+	+	−	−	−	+	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+	+	+	+	−
1	+	+	+	+	+	+	−	−	−	+	+	+	−	+	−	−	−	+	+	−	−	+	−	+	+	+	−	−	+
1	+	+	+	+	+	+	−	−	−	+	+	+	−	+	−	−	−	+	+	−	−	+	+	+	+	+	+	+	+
Group 4, "WA MRSA-15"																													
7	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	+
1	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	+	+	+	−	−	+
1	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	−	−	−	−	−	+	−	−	−	−	−	+
Group 5, "WA MRSA-9/52," or "Taiwan clone"																													
4	+	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	+	−	−	−	−	+	+	−
2	+	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	−
8	+	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+	+	+	+	−
5	+	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	−	−	+	−	+	+	+	+	+	−
1	+	−	+	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−
Group 6, "WA MRSA-9"																													
1	+	−	+	−	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
1	+	−	+	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	+	+	−	−	−
1	+	−	+	−	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	+
NARSA control strains, "USA1000"																													
1 (NARSA483)	+	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−
1 (NARSA676)	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−

[a] Group 1, PVL-negative ST59 MRSA-IVb (2B); group 2, PVL-negative ST87 (59SLV) MRSA-IVb (2B); group 3, PVL-variable ST59 MRSA-IVa, -IVd, and -IVv (2B); group 4, PVL-negative ST59 MRSA-IVa (2B&5); group 5, PVL-positive ST59/338 (59SLV)/952 (59SLV) MRSA-V (5C2&5); group 6, PVL-negative ST59 MRSA-Vv (5C2); NARSA control strains, PVL-positive ST59 MRSA-IVa (2B).

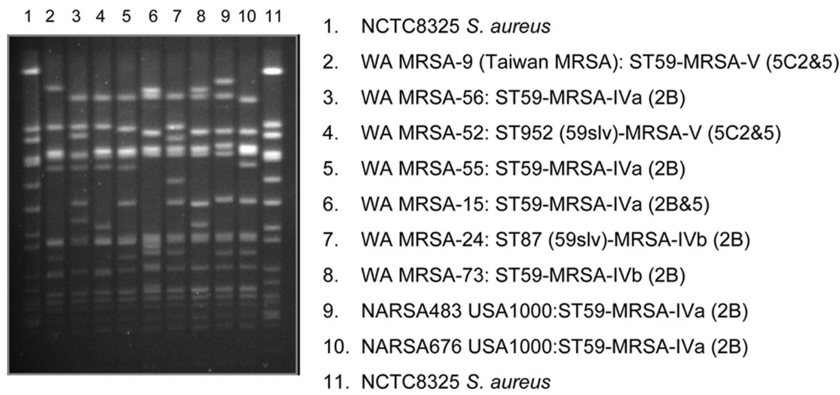

FIG. 1. Representative pulsed-field gel electrophoresis patterns of the seven Western Australian CC59 MRSA strains.

DNA microarray-based analysis of the CC59 isolates clustered them into six groups (Fig. 2).

Group 1, "WA MRSA-73" (PVL-negative ST59 MRSA-IVb [2B]). "WA MRSA-73" (isolate 05-16512) is an ST59/*spa* type t528 strain that has acquired an SCC*mec* IVb (2B) (*ccrA2B2* and *mec* complex class B) element. Although lacking PVL, this strain has a DNA microarray profile similar to that of the NARSA ST59 MRSA-IVa "USA1000" control strain (NARSA483). Both strains have acquired s*eb*, *sek*, and *seq* enterotoxin genes and lack antimicrobial resistance genes apart from *mecA* and *blaZ*.

Group 2, "WA MRSA-24" (PVL-negative ST87 MRSA-IVb [2B]). Three "WA MRSA-24" isolates (04-17626, 06-15325, and 06-16824), collected from unrelated patients in 2004 and 2006, were identified as ST87 (an SLV of ST59 [59SLV])/*spa* type t216 with a type IVb (2B) SCC*mec*. The beta-lactamase operon (*blaZ*, *blaI*, and *blaR*), the *msrA*, *mpbBM* (macrolide), *aphA3* (neomycin), and *sat* (streptothricin) resistance genes,

FIG. 2. SplitsTree graph visualizing similarities of CC59 isolates and strains based on hybridization results for *mecA*, Δ*mecR*, *ugpQ*, the dcs region, *ccrA2*, *ccrB2*, 85-2082 *ccrC1*, hypothetical ORF accompanying *ccrC1*, *blaZ*, *blaI*, *blaR*, *ermB*, *ermC*, *msrA*, *mpbBM*, *aphA3*, *sat*, *tetK*, pC221 *cat*, pC223 *cat*, *sea*, *seb*, *sek*, *seq*, *lukF-PV*, *lukS-PV*, and *sak* (Table 2). Isolate designations in boxes indicate PVL-positive isolates.

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and the *seb*, *sek*, and *seq* enterotoxin genes were present in all three isolates. The isolates were also positive for staphylokinase (*sak*), chemotaxis-inhibiting protein (*chp*), and staphylococcal complement inhibitor (*scn*) genes, which are known to be located on beta-hemolysin integrating phages. However, the enterotoxin A (*sea*) gene, which is also located on beta-hemolysin integrating phages, was not detected. PVL was not detected.

Group 3, "WA MRSA-55/56" (PVL-variable ST59 MRSA-IV [2B], structural subtypes IVa, IVd, and IVv). Group 3, a group of seven ST59/*spa* type t437 isolates, is comprised of four very similar strains that have acquired *ermB* (macrolide-lincosamide-streptogramin B), *aphA3*, *sat*, and *tetK* resistance genes. In this regard, they resemble the "Taiwan clone" (see below). The other three strains in this group differ by variable carriage of PVL and enterotoxin A (*sea*) and SCC*mec* type IV subtype.

The first strain, PVL-positive "WA MRSA-55," included four isolates (06-16367, 06-17947, 07-15432, and 07-15760) collected in 2006 and 2007. All four isolates harbored an SCC*mec* IVa (2B) element, and their DNA microarray-based profiles were identical. The beta-lactamase operon (*blaZ*, *blaI*, and *blaR*), *ermB*, *aphA3*, *sat*, and *tetK* resistance genes as well as the *seb*, *sek*, and *seq* enterotoxin genes, PVL genes (*lukF-PV* and *lukS-PV*), and *chp*, *scn*, and *sak* genes were all detected in this strain.

The second strain (08-16180) was also PVL positive and, except for the absence of the *sak* gene and the presence of the chloramphenicol resistance gene *cat*, yielded the same DNA microarray hybridization pattern as the first strain in this group. However, SCC*mec* analysis by PCR revealed that this isolate harbored a type IVd (2B) SCC*mec* element.

The third strain, a PVL-negative isolate (08-17668), closely resembled the PVL-positive strain "WA MRSA-55" in terms of microarray hybridization and PFGE pattern. However, it has acquired a novel type IV (2B) SCC*mec* which could not be subtyped with primers specifying subtypes a to h.

The fourth strain, "WA MRSA-56," consisted of one isolate (07-15443) collected in 2007. It was also identified as PVL-positive ST59 MRSA-IVa (2B). However, unlike the other group 3 strains, "WA MRSA-56" carried the enterotoxin gene *sea*.

Group 4, "WA MRSA-15" (PVL-negative ST59 MRSA-IVa [2B&5]). Group 4 consisted of nine isolates (03-17565, 04-16557, 04-17489, 05-17037, 05-17619, 06-15513, 06-17484, 07-19251, and 08-15202) collected from seven individual patients between 2003 and 2008. One patient with various wound infections yielded three identical isolates in three subsequent years (2004 to 2006).

These isolates were identified as PVL-negative ST59 MRSA-IVa (2B&5) (*ccrA2B2 mec* complex class B and *ccrC1*)/*spa* type t976.

All nine isolates carried the beta-lactamase operon (*blaZ*, *blaI*, and *blaR*). Variable resistance genes, including *msrA* and *mpbBM* (in two isolates), *aphA3* and *sat* (in one isolate), and *tetK* (in one isolate), were detected. All isolates were negative for PVL genes but positive for the enterotoxin A gene (*sea*). Seven out of nine isolates harbored *seb*, *sek*, and *seq* enterotoxin genes.

Isolate 05-17037 differed in its carriage of resistance genes (*msrA*, *mpbBM*, *aphA3*, *sat*, and *tetK*). DNA microarray-based markers indicated that all isolates in this group encoded an unusual SCC*mec* element with *mec* complex class B and *ccr* complex type 2, characteristic of SCC*mec* type IV, and *ccrC1*, which has thus far been found in SCC*mec* type V or SCC elements. Positive hybridization signals for SCC*mec* markers *mecA*, Δ*mecR*, *ugpQ*, the dcs region, *ccrA2*, *ccrB2*, 85-2082 *ccrA*, and 85-2082 *ccrC1* were obtained. SCC*mec* PCR confirmed this observation. All isolates in the group were positive for SCC*mec* type IVa (2B) structural elements, and all encoded a class B *mec* complex, a type 2 *ccr* complex, and *ccrC1* allele 2. This suggests either the presence of a composite IVa (2B) and V (5C2) SCC*mec* element or the additional presence of an SCC element encoding *ccrC1* allele 2.

Group 5, "WA MRSA-9/52," or "Taiwan clone" (PVL-positive ST59/338/952 MRSA-V [5C2&5]). Group 5 consisted of 17 "WA MRSA-9" isolates identified as ST59/*spa* type t437, t441, or t2365 and three "WA MRSA-52" isolates identified as ST952 (a single-locus variant of ST59)/*spa* type t1950 (Table 1). The DNA microarray-based profiles for these 20 isolates were similar, and consequently the two strains were classified into one group. "WA MRSA-9" was the first SCC*mec* V (5C2&5) (*mec* complex class C2 and *ccrC1*) CC59 strain found in Western Australia (in 2003), and it appears to be the most common and clinically relevant strain in this clonal complex.

For SCC*mec* markers on the array, all isolates yielded hybridization signals with probes for *mecA*, *ugpQ*, 85-2082 *ccrA*, and MRSAZH47 *ccrA*, as well as for *ccrC1*. When tested by PCR, all 20 strains had *mec* complex C2 and *ccrC1*. *ccrC1* allotyping revealed the presence of two *ccrC1* complexes, *ccrC1* allele 2 and *ccrC1* allele 8, which is characteristic of the SCC*mec* encoded by the Taiwan clone. An ISSau4-like transposase was found inserted into the structural gene V011 of all isolates in this group.

All 20 "Taiwan clone" isolates carried the beta-lactamase operon (*blaZ*, *blaI*, and *blaR*) as well as *ermB*, *aphA3*, and *sat*. Variable resistance genes were *tetK* (in 14 isolates) and *cat* (in 17 isolates). All isolates were positive for *lukF-PV* and *lukS-PV*. Enterotoxin genes *seb*, *sek*, and *seq* were present in 15 of the 20 isolates.

Group 6, "WA MRSA-9" (PVL-negative ST59 MRSA-Vv [5C2]). Although characterized as "WA MRSA-9" by PFGE, three isolates (05-17759, 08-15683, and 06-18653) were classified into group 6. These isolates carried an SCC*mec* type V variant (5C2) (*ccrC1* and *mec* complex class C2) element. However, they yielded a DNA hybridization pattern different from that of the "Taiwan clone" (group 5). They were positive for *mecA*, *ugpQ*, 85-2082 *ccrA*, and 85-2082 *ccrC1* but negative with the MRSAZH47 *ccrA* probe.

PCR SCC*mec* analysis revealed that isolates from this group did not amplify the SCC*mec* type V (5C2) specific structural ORF V011 (GenBank accession no. AB12129) with or without the ISSau4-like transposase insertion. However, they were positive for the type V (5C2) core genes for *mec* complex C2 and *ccrC1*. Allotyping of the *ccrC1* gene revealed that it was neither allele 2 nor allele 8. On the basis of a lack of amplification of the structural gene, the SCC*mec* of these strains has been classified as a type V variant (Vv [5C2]). It is evident that this group of isolates harbors an SCC*mec* element that is significantly different from that of the Taiwan clone.

Two isolates carried the beta-lactamase operon, and one was

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positive for *ermC* (macrolide-lincosamide resistance) and *cat*. All three isolates harbored *seb*, *sek*, and *seq* enterotoxin genes as well as *chp* and *scn*. One isolate also yielded hybridization signals for the staphylokinase gene *sak*. All isolates were PVL negative.

DISCUSSION

In Western Australia there are at least six discernible groups of CC59 CA-MRSA strains, which can be differentiated by PFGE, MLST, determination of the presence or absence of PVL, determination of the SCC*mec* type, or microarray analysis. Within the study strains, at least seven different variants of SCC*mec* elements (IVa [2B], IVb [2B], IVd [2B], IVa [2B&5], IVv [2B], Vv [5C2], and V [5C2&5]) were distinguished. This suggests rapid evolution and/or multiple transfer events of SCC*mec* elements. In a recent study by Takano et al. (24), at least six SCC*mec* elements were described to occur in a collection of ST59 MRSA strains isolated in Taiwan, including V (5C), V (5C2&5), IVc (2B), IV (2B), and two novel elements. This diversity of SCC*mec* types and subtypes can be expected to cause ambiguities in nomenclature, which underscores the need for sequence information. Consequently, the novel SCC*mec* elements described in this study warrant further characterization, although sequencing is beyond the scope of this study. Another observation suggesting a rapid evolution within CC59 is that groups 3 and 5 ("Taiwan clone") appear to be closely related to each other with regard to all markers but SCC*mec*. Both groups share *aphA3*, *sat*, *ermB*, and usually also *cat* as well as PVL. This indicates that groups 3 and 5 might represent one branch of the CC59 complex that evolved into separate groups by acquiring different SCC*mec* elements. Generally, CC59 displayed a high degree of variability, affecting not only SCC*mec* markers but also a variety of other mobile genetic elements. For instance, "USA1000" and "WA MRSA-73" differ only in the presence of PVL. It cannot yet be determined whether "USA1000" evolved from a "WA MRSA-73"-like ancestor by acquiring PVL, whether "WA MRSA-73" was a deletion variant of USA1000, or whether both represent independently evolved branches of one lineage. Similarly, isolate 08-17668 might represent either a PVL-negative ancestor or a mere deletion mutant of group 3 strains. A high degree of variability can also be detected within CC59 groups. For example, the Taiwan clone (group 5) can be subdivided based on resistance and toxin genes and *spa* and MLST sequences. Such variation within a supposed "clone" might be used to trace individual chains of infection, as in the case of the patients with the ST952 variant of the "Taiwan clone" ("WA MRSA-52") who belonged to the same family.

Further studies should investigate the variability and evolution of CC59 strains in other locations where this clonal complex has been detected. Apart from data for the Taiwan clone (group 5), there are little data available on the distribution of CC59 clones outside Western Australia. It can be assumed that these strains are usually identified as "USA1000" or the "Taiwan clone" and that their true diversity remains unrecognized. This might also obscure routes of transmission of CC59 CA-MRSA strains and hinder the understanding of their international spread.

If a variety of closely related strains exist simultaneously, it can be assumed that they are in competition with each other for "ecological" resources, i.e., for susceptible, as yet uncolonized hosts. The ecological success of strains should result in wide distribution and/or relatively high prevalence. The Taiwan clone (group 5) could be regarded as the most successful strain among the CC59 CA-MRSA strains isolated in Western Australia. In our study, nearly as many isolates belonged to group 5 as to all other CC59 groups combined. Since the groups are nearly isogenic, a marker determining such success should be found among the rather limited number of genes which are variable within CC59, and it should be present in the Taiwan clone. Full genome sequencing of representative strains of CC59 may provide the answer and overcome the limitations of this study. Among the genes which were examined in this study, PVL genes could be related to the success of the Taiwan clone.

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8.5. PAPER FIVE

The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia.

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The molecular epidemiology and evolution of the Panton–Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia

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Abstract

Between 2003 and 2008, 76 clinical isolates of the Panton–Valentine leukocidin-positive *Staphylococcus aureus* strain 'West Australian methicillin-resistant *Staphylococcus aureus* (MRSA)-12' (WA MRSA-12) were recovered from 72 patients living in the Perth area in Western Australia. These isolates were found to belong to multilocus sequence type 8, and had a USA300-like pulsed-field gel electrophoresis pulsotype. All isolates were genotyped using diagnostic DNA arrays covering species markers, resistance factors, virulence-associated, as well as MSCRAMM (microbial surface components recognizing adhesive matrix molecules) genes to prove the identity between WA MRSA-12 and the pandemic strain USA300, as well as to detect possible genetic variability. In general, WA MRSA-12 isolates were similar to USA300, and the most common variant was identical to USA300-TC1516. From this clone, most of the other variants may have evolved by a limited number of gene losses or acquisitions. Variations in carriage of virulence and resistance-associated genes allow distinction of variants or sub-clones. Altogether, 16 variants could be distinguished. They differed in the carriage of resistance genes (*blaZ/I/R*, *ermC*, *msrA* + *mpbBM*, *aadD* + *mupR*, *aphA3* + *sat*, *tetK*, *qacC*, *merA/B/R/T*) of β-haemolysin-converting phages and of enterotoxins (*sek* + *seq*, which were deleted in four isolates). Notably, the arginine catabolic mobile element (ACME) was absent in 12 isolates (15.8%). The mercury resistance (*mer*) operon, which is usually associated with SCC*mec* type III elements, was found in several ACME-negative isolates. The present study emphasises the importance of genotyping in detecting the introduction and evolution of significant MRSA strains within a community.

Keywords: Arginine catabolic mobile element (ACME), diagnostic DNA microarray, molecular typing, MRSA, Panton–Valentine leukocidin (PVL), *Staphylococcus aureus*, USA300

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Introduction

In recent years, a pandemic unfolded of methicillin-resistant *Staphylococcus aureus* (MRSA) strains that carry novel and apparently highly mobile chromosomal staphylococcal chromosomal cassette elements harbouring *mecA* (SCC*mec* elements). Such strains have emerged outside the hospital setting and have been designated as 'community-associated MRSA' (CA-MRSA). Some of them harbour the prophage-encoded [1,2] virulence factor Panton–Valentine leukocidin (PVL). PVL is a bicomponent toxin that forms polymeric pores in leukocyte membranes [3], although its role in pathogenesis is still subject to discussion [4–6]. Several PVL-positive CA-MRSA strains from different clonal groups have evolved. Some have been confined to certain regions or localised outbreaks, whereas others have spread worldwide.

One of these strains, a clonal complex 8, *spa* type t008 MRSA carrying a SCC*mec* type IV element, has recently emerged as the dominant MRSA strain in North America, both in community and hospital settings [7–14]. Colloquially known as USA300, it has also been reported from Australia, Canada, Denmark, Germany, Japan, Switzerland and the UK [10,15–18]. Because of its rapid spread, it has drawn considerable attention, resulting in the sequencing of two complete

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genomes (USA300-FPR3757, GenBank CP000255.1 [19] and USA300-TCH1516, CP000730.1 [20]). One remarkable finding was the detection of an arginine catabolic mobile element (ACME), which previously only has been found in *Staphylococcus epidermidis*. It was hypothesised that ACME contributed to the ability to metabolically alter the local pH on the skin. This could increase the ability of USA300 to persist on intact skin and, consequently, facilitate spread by skin contact [19,21].

Locally known as WA MRSA-12, several USA300-like isolates have been identified in Australia [22]. To determine whether WA MRSA-12 was identical to USA300, we applied previously developed DNA microarrays on a collection of Australian PVL-positive ST8-MRSA IV isolates. Variations detected within this strain may be relevant for typing or therapeutic interventions.

Material and methods

Isolates and patients

Between July 2003 and February 2008, 76 MRSA isolates from 72 patients living in the Perth area, Western Australia (WA), were characterised as PVL-positive WA MRSA-12 using pulsed-field gel electrophoresis (PFGE) [23] and PCR for the detection of PVL genes using previously published primers [24]. Representative PFGE patterns are shown in Fig. 1. MLST and SCC*mec* typing were performed on 16 isolates (see Supporting Information, Table S1) using previously published methods [25–28]. Some 62.5% of the patients were male, and approximately 55% were older than 30 years (Table S1).

Skin and soft tissue infections were reported in 94.4% of cases. The remaining cases were asymptomatic, and subjected to contact screening. Necrotising pneumonia was not observed.

To determine the stability of variants, follow-up isolates from individual patients were also characterised.

The sequenced strain USA300-FPR3757 was included in the study. Although the second sequenced strain, USA300-TCH1516, was not tested, a prediction of its hybridisation profile based on the published genome sequence allowed a comparison to be made with the WA isolates.

Array procedures

The DNA array used in the present study covers 334 target sequences. Depending on the nomenclature used, this corresponds to approximately 185 distinct genes and their allelic variants, and includes mainly clinically relevant genes on mobile elements that are not covered by whole-genome arrays derived from sequenced genomes [29]. The targets, related protocols, data interpretation and evaluation procedures used have been described previously [30,31].

Briefly, cultures were grown overnight on Columbia blood agar. Culture material was harvested, lysed using lysostaphin, lysozyme and ribonuclease A and treated with proteinase K. DNA was purified using the Qiagen device EZ1 (Qiagen, Valencia, CA, USA) according to the manufacturer's tissue lysis protocol. An iterated, linear primer elongation was employed for the simultaneous amplification of all targets. Within this step, amplicons were labelled by incorporation of biotin-16-dUTP.

The labelled sample was denatured and hybridised to the array. This was followed by washing steps and by the addition of a blocking reagent. Horseradish–peroxidase–streptavidin conjugate was then added to the array, followed by incubation and washing. The array tube was placed into the ATR01 reading device (Clondiag, Jena, Germany), and Seramun Green precipitating dye (Seramun, Heidesee, Germany)

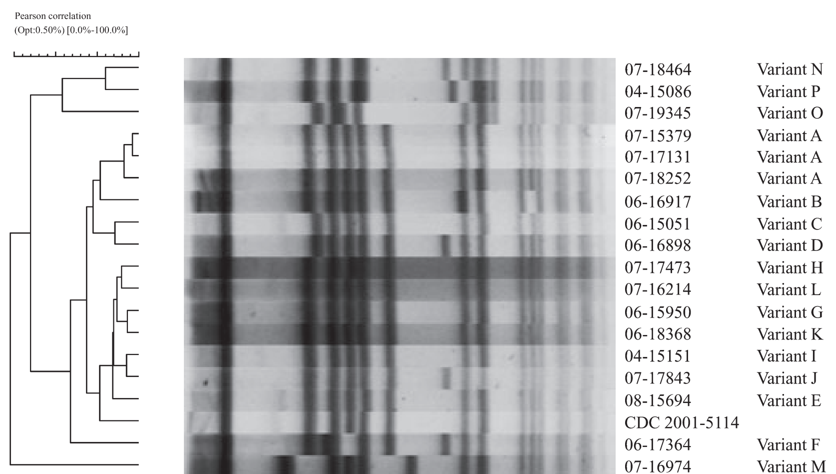

FIG. 1. Pulsed-field gel electrophoresis patterns of representative USA300 isolates from the Perth area and their affiliations to variants based on array hybridisations.

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was added. After 5 min, an image of the array was recorded and analysed.

Results

Virulence-associated genes and the ACME locus

Array hybridisation and PCR [24] demonstrated that all WA MRSA-12 isolates harboured the PVL genes, *lukF-PV* and *lukS-PV*.

All but four isolates (94.7%) yielded hybridisation signals for enterotoxin genes *sek* and *seq*. Negative results were confirmed by PCR (primers *sek*_forward, ACAGAGAATTT TCATTTGGATGT and *sek*_reverse, CACATTTTGCTTAT CCCTCCT, with a melting temperature during PCR of 55°C, as well as primers *seq*_forward_2 GCTTCAAGGAGT TAGTTCTGG and *seq*_reverse_2 CTTGACCAGTTCCGG TGT, with a melting temperature of 54°C; see Supporting Information, Table S1).

One isolate was negative for genes encoding staphylokinase (*sak*), chemotaxis-inhibiting protein (*chp*) and staphylococcal complement inhibitor (*scn*). Two isolates were positive for *sak* and *scn*, but lacked *chp*. Carriage of *set*/*ssl* genes was identical to USA300-FPR3757 and USA300-TCH1516 genome sequences and to previously described USA300 isolates from Germany [30].

Genes of the ACME locus (*arcA-SCC*, *arcB-SCC*, *arcC-SCC* and *arcD-SCC*) were detected in 64 (84.2%) of the WA MRSA-12 isolates. An absence of ACME was confirmed using two different *arcA* PCRs [19,32].

Capsule, biofilm and microbial surface components recognizing adhesive matrix molecules (MSCRAMM) genes

Carriage of capsule genes (type 5), biofilm (*icaA*, *icaC*, *icaD*) and MSCRAMM genes (*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD* and *vwb*) was identical to USA300-FPR3757 and USA300-TCH1516.

Antibiotic resistance determinants

Apart from one isolate, all WA MRSA-12 isolates carried *mecA* as a part of the SCC*mec* type IV element. This isolate initially did not yield hybridisation signals for any of the SCC*mec*-associated genes.

However, growth on a broth containing cefoxitin was observed. DNA from this culture yielded signals for *mecA* and all other SCC*mec* type IV probes (*ugpQ*, *crrA-2*, *crrB-2*, truncated *mecR*). Except for these markers, the hybridisation pattern remained unchanged and allowed assignment to variant L (Figure 2).

The β-lactamase operon (*blaZ*, *blaI*, *blaR*), a gene encoding a putative transport protein (SAUSA300_2128, USA300-HOU_2160), and the fosfomycin resistance gene *fosB* (SAUSA300_2280, USA300HOU_2313) were detected in all isolates.

The neo-/kanamycin resistance gene *aphA3* and the streptothricin resistance gene *sat* were jointly detected in 64 (84.2%) isolates. Two genes for macrolide efflux proteins, *msrA* and *mpbBM* were also always found together in 61 (80.3%) isolates.

Comparatively rare resistance determinants included the rRNA adenine *N*-6-methyltransferase gene *ermC* (in seven isolates, 9.2%), a gene for a tetracycline efflux protein, *tetK* (eight isolates, 10.5%), a gene encoding an unspecific efflux pump (*qacC*, one isolate, 1.3%), as well as the aminoglycoside adenyltransferase gene *aadD* and a gene conferring high level mupirocin resistance, *mupR* (two isolates, 2.6%). In WA MRSA-12 isolates, *aadD* and *mupR* genes occurred together, whereas, in USA300-FPR3757, only *mupR* was present.

MsrA/*mpbBM* or *ermC* were mutually exclusive in all isolates but one.

The *mer* operon

The genes *merA* (encoding mercuric reductase), *merB* (organomercurial lyase), *merR* (regulatory protein) and *merT* (transport protein) were detected in eight (10.5%) isolates, with all of them being ACME-negative.

Variants of the WA MRSA-12 strain

Hybridization profiles identified 16 variants (variants A–P, Fig. 2) among 76 WA MRSA-12 isolates. Forty-seven isolates (61.8%) belonged to variant A. An analysis of the genome sequence of USA300-TCH1516 predicted the same hybridisation pattern as that observed in variant A. Strain CDC 2001-5114, which was used as reference for PFGE, represents a seventeenth variant. It differed from variant A only in being *tetK*-positive. Thus, 'USA300' and 'WA MRSA-12' can be regarded as synonyms for one strain. An eighteenth variant is represented by USA300-FPR3757. Its hybridisation pattern was in full accordance with a prediction based on its genome sequence. No clinical isolates of this variant were found in WA.

To assess the stability of variants, attention focused on follow-up isolates from individual patients and on isolates from family members (Table S1). Identical follow-up isolates from individual cases were recovered after 3, 76 and 580 days. In one case, a patient initially infected with variant A cultured a follow-up isolate after 205 days, which differed in the presence of *aadD* and *mupR* (variant G).

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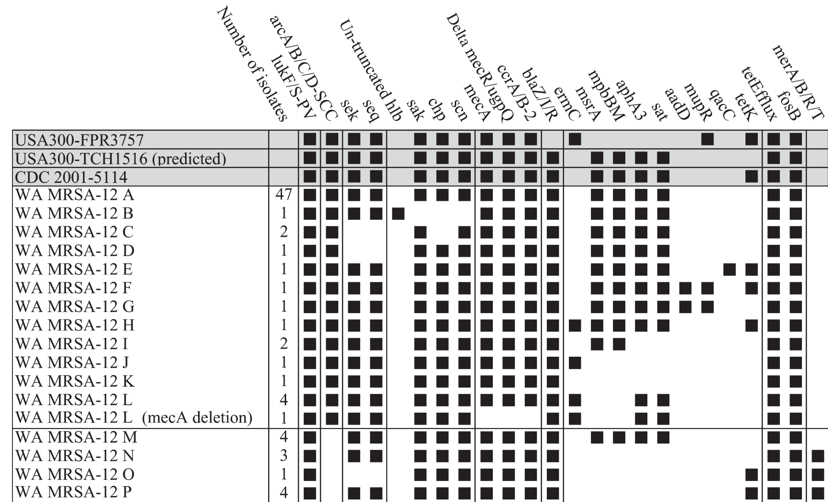

FIG. 2. Variants of clinical West Australian MRSA-12 isolates. Experimental data for the sequenced strain USA300-FPR3757 and for strain CDC 2001-5114 (which was used as reference strain for pulsed-field gel electrophoresis experiments), as well as the predicted hybridisation profile for the sequenced strain USA300-TCH1516, are shown for comparison.

In three separate episodes, USA300 was isolated from family members. In one case, a girl was diagnosed with a variant I infection, 295 days after the same variant was recovered from her mother. In another case, two isolates of variant A were sampled within 1 month from two siblings.

In a third family, both parents and their two children were, within a period of 7 months, infected with variant A.

Discussion

USA300 is a PVL-positive, CA-MRSA strain that has spread rapidly in North America. Sporadic cases and outbreaks have been reported from various European countries and in Australia. In WA, the first infection with USA300/WA MRSA-12 was reported in 2003. Subsequently, an increase in the number of cases has occurred, particularly within 2007 and in 2008 (Fig. 3).

USA300 isolates were not identical to each other. Thus, USA300 cannot be regarded as a genetically homogenous unit. Whether, for example, ACME-positive and -negative isolates should be regarded as variants of one strain, or as two different strains, is a matter of definition, raising the question of how to define a strain in general and USA300 in particular. Variations within USA300 affected virulence factors and antibiotic resistance determinants. MSCRAMM, capsule or *set/ssl* gene carriage was uniform, and closely resembled the other clonal complex 8 strains [30], including NCTC8325 [33]. It can be assumed that the lack of variability of these genes was due to the relatively short time-span of USA300 proliferation, whereas virulence- and resistance-associated genes varied on a faster time-scale because they are situated on mobile elements.

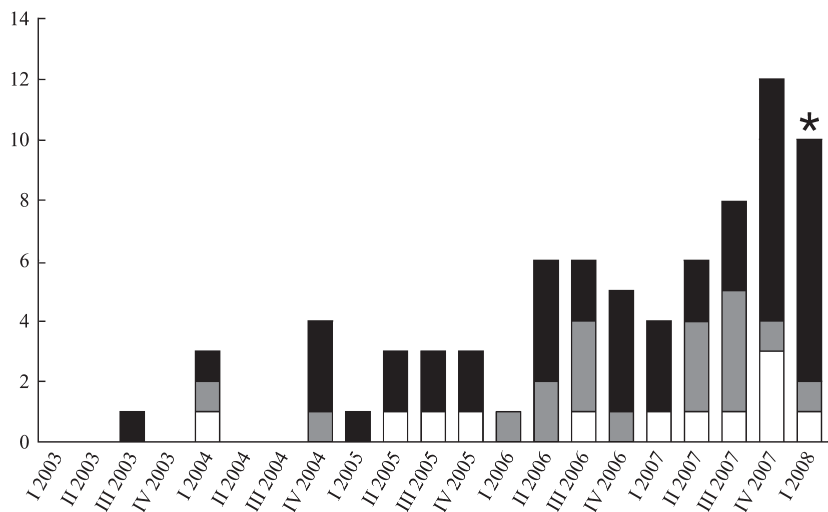

FIG. 3. Number of isolates per 3 months interval. Black, West Australian MRSA-12 variant A; Grey, isolates of other ACME-positive variants; White, ACME-negative isolates; asterisk, January and February only.

One isolate lacked the phage-born innate immune evasion cluster (which truncates the *hlb* gene by introducing *sak*, *chp* and *scn* [34,35]), and some isolates were negative for *sek* and *seq*. Because ST80-MRSA IV also lacks enterotoxin genes [36], it could be speculated that, for PVL-positive strains, some virulence factors were expendable without selective disadvantage.

Carriage of ACME was also variable. This is especially intriguing because this locus is assumed to be involved in facilitating the spread of USA300 by skin contact [19,21]. ACME-negative variants of USA300 appear to be common in WA. They also exist in the USA but appear to be extremely rare (one study found none [37] and another identified a single isolate [38]), and they have been reported in Germany [39]. Thus, they are not restricted to WA, and a strategy to identify USA300 by multiplex PCR detection of PVL, *mecA* and ACME can confirm, but not rule out, the presence of USA300. Because of the limited time of the presence of USA300 in WA, further studies should focus on possible changes of the ratio of ACME-positive to -negative variants. This may improve our understanding of the clinical significance of this element and its proposed role in the rapid spread of USA300.

The variability of resistance genes was not unexpected because these genes are subjected to a high, but variable selective pressure.

In one isolate, negative signals for SCC*mec* probes were observed but, after passage on a cefoxitin-containing medium, the resulting culture was positive for these genes. We assume that a majority of cells lost the SCC*mec* element, but that a small *mecA*-positive subpopulation below the detection limit of linear amplification was still present. In the presence of cefoxitin, this subpopulation had a selective advantage resulting in displacement of the deletion variant. A loss of SCC*mec* elements from MRSA has occasionally been observed [40–42], emphasising the mobility of the SCC*mec* gene cluster.

Other common, but variable resistance genes included the β-lactamase operon and a fixed combination of *sat* and *aphA3*. The latter genes are frequently detected in diverse MRSA strains, including ST8-MRSA IV, ST45-MRSA IV, ST80-MRSA IV and ST228-MRSA I [30]. Because neomycin is commonly used as topical preparation, *aphA3* might confer an advantage to a strain usually associated with skin infections.

Another apparently fixed combination of resistance genes comprised *aadD* and *mupR*, encoding resistance to neomycin, tobramycin and mupirocin. Although *mupR* was rare, it deserves further attention because mupirocin is crucial for the eradication of MRSA. Macrolide resistance genes were common. The genes *msrA/mpbBM* or *ermC*, apart from a single exception, proved to be mutually exclusive. This might indicate that the maintenance of multiple genes conferring similar resistance properties could result in an unnecessary fitness cost. Because *ermC* encodes not only macrolide, but also clindamycin resistance, it might confer a more significant advantage. In other MRSA strains, *erm* genes are more abundant than *msrA* [43] but, in USA300, *msrA/mpbBM* positive isolates predominate. Thus, clindamycin can be considered as therapeutic option, although a widespread application might favour *ermC* positive variants of USA300.

Surprisingly, the mercury resistance (*mer*) operon was found in ACME-negative USA300 isolates. It can be plasmid-borne (GenBank L29436) but, similar to ACME, it can also be associated with recombinases (GenBank AB037671, [44]) forming some kind of SCC element. Thus, its genetic background and its position in the USA300 genome remains to be clarified.

Further isolates from diverse regions and over a longer time-span need to be studied to determine whether the described variability represents random variations, or an early stage of a rift into separate strains. It will also be interesting to observe the competition of variants which are—except for a small number of genes—essentially isogenic. An especially successful variant can be expected to combine genetic traits that also are responsible for the success of USA300, regardless of whether this might be the carriage of PVL, ACME [19,21] or another factor yet to be identified. In the present study, variant A appeared to be most successful. Because it was the first USA300 variant detected in WA, it can be regarded as the founder variant, from which variants B–L, and possibly M, may have been derived by a limited number of gene losses or acquisitions. Variant A appears to be geographically widespread because it has been found in Texas (USA300-TC1516), in the German states Saxony [16,30] and Brandenburg, and in Switzerland (isolates courtesy of T. Juratzek and B. Berger-Baechi).

The explosive expansion of USA300 still warrants further study. DNA microarray technology might contribute to the understanding of this phenomenon by resolving variants below strain level. This might be helpful for tracing chains of transmissions and elucidating the sources of importation of that strain into a given region.

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Transparency Declaration

R. Ehricht and P. Slickers are employees of Clondiag. The authors declare no other conflicting interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Patient data and isolate characteristics.

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Isolate	Date of sample collection	Gender	Date of birth	Postal code	Clinical Presentation	Comments	PFGE TYPE	MLST	SCCmec Typing	PVL-PCR as described by Fey 2003	sek-PCR as described in this paper	seq-PCR as described in this paper	arcA-PCR as described by Diep 2006	arcA-PCR as described by Zhang 2008	GENT	ERYT	CLIN	TETR	CIPR	TMP	FUSI	RIFA	MUPR	Varint according to array analysis
03-16593	10-Jul-03	F	30-Sep-79	6029	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
04-15400	10-Feb-04	F	10-Feb-60	6211	Surgical wound		WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-18066	22-Nov-04	M	04-Mar-98	6014	Abscess, leg	Sibling of 04-18340	WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-18334	26-Dec-04	F	03-Oct-62	6111	SSTI		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-18340	23-Dec-04	F	13-Mar-00	6014	Wound swab	Sibling of 04-18066	WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
05-15242	29-Jan-05	M	27-Aug-30	6010	Sputum	Same patient as 05-15965	WA 12	ST8	IV						S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-15965	15-Apr-05	M	27-Aug-30	6010	SSTI, neck	Same patient as 05-15242	WA 12								S	R	S (non inducible)	S	R	S	S	S	R	Variant A
05-16235	14-May-05	F	03-Mar-94	6025	SSTI, shoulder		WA 12	ST8	IV	POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-16940	21-Jul-05	M	19-Apr-79	6155	SSTI, Wrist		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
05-17243	19-Aug-05	M	09-Aug-34	6010	Scalp wound		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-18090	10-Nov-05	M	08-Sep-67	6164	SSTI, elbow	Same patient as 05-18104	WA 12	ST8	IV				POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-18104	07-Nov-05	M	08-Sep-67	6164	SSTI, elbow	Same patient as 05-18090	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-16044	12-Apr-06	M	14-Jun-37	6008	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	R	Variant A
06-16229	03-May-06	M	15-Jun-61	6025	Wound, knee		WA 12	ST8	IVa	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-16691	14-Jun-06	F	03-Jul-41	6019	Nasal Swab (Patient)	Same patient as 08-15180	WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-16786	21-Jun-06	M	16-Aug-85	6230	SSTI, leg and forearms		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-17275	16-Aug-06	F	25-Oct-82	6062	Bartholin. Abscess		WA 12	ST8	IVa	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-17519	10-Sep-06	F	20-Jul-95	6168	Abscess, thigh	Same family as 06-18270, 07-15379, 07-15590	WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-17899	18-Oct-06	F	25-Jul-65	6057	Abscess		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-18011	24-Oct-06	M	03-Jun-65	6062	Nasal Swab (Patient)	Same patient as 06-15950	WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-18195	16-Nov-06	F	28-Feb-79	6230	Drainage of an abscess		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-18270	21-Nov-06	M	02-Mar-77	6169	unknown	Same family as 06-17519, 07-15379, 07-15590	WA 12			POS			NEG		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-15220	15-Jan-07	M	19-Oct-55	6019	Wound swab		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-15379	02-Feb-07	F	21-Sep-63	6169	SSTI, finger	Same family as 06-17519, 06-18270, 07-15590	WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-15590	20-Feb-07	M	02-Mar-99	6169	Abscess, knee	Same family as 06-17519, 06-18270, 07-15379	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-16431	02-May-07	F	16-Nov-78	6155	SSTI, back		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-17131	28-Jun-07	F	20-Sep-33	6122	Swab, eye		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-17307	18-Jul-07	F	31-Jul-75	6164	Wound swab		WA 12			POS			POS		S	R	S	S	S	S	S	S	S	Variant A
07-17723	16-Aug-07	M	04-Nov-38	9999	Sputum		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-17974	11-Sep-07	F	23-Aug-30	6105	SSTI		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18252	05-Oct-07	M	05-May-87	6102	Abscess		WA 12			POS			POS		S	R	S	S	S	S	S	S	S	Variant A
07-18298	10-Oct-07	M	19-Sep-95	6169	SSTI		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18413	21-Sep-07	M	23-Nov-35	6027	Abscess, neck		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18756	12-Nov-07	M	14-Mar-66	8888	Abscess, neck		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18831	17-Nov-07	M	14-Aug-79	6008	SSTI, leg		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18848	13-Nov-07	M	03-May-53	6010	Boil, axilla		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18878	20-Nov-07	M	27-Aug-65	6017	SSTI		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18956	17-Nov-07	F	27-Aug-71	6025	SSTI		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-19150	10-Dec-07	M	15-Mar-59	6050	Furuncle		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15158	14-Jan-08	Sydney	20-Oct-34	6021	Abscess, axilla		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15180	15-Jan-08	F	03-Jul-41	6019	SSTI, leg	Same patient as 06-16691	WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15332	21-Jan-08	M	21-Jan-88	6018	SSTI, elbow		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15361	28-Jan-08	M	18-Jan-70	6169	SSTI, elbow		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
08-15503	08-Feb-08	M	21-Oct-47	6284	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
08-15635	15-Feb-08	F	12-Jan-66	6030	Infected Boil		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15653	18-Feb-08	F	27-May-83	6025	Pus		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15674	20-Feb-08	F	01-Mar-68	6059	SSTI, buttock		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-16917	03-Jul-06	M	14-May-44	6110	Nasal Swab (Patient)		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant B
06-15051	06-Jan-06	M	03-Nov-88	6027	Elbow aspirate		WA 12			POS	NEG	NEG		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant C
06-16748	14-Jun-06	M	26-Mar-71	6152	SSTI, cheek		WA 12			POS	NEG	NEG		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant C
06-16898	06-Jul-06	F	21-Jan-85	6009	SSTI		WA 12			POS	NEG	NEG	POS	POS	S	R	S (non inducible)	S	R	S	S	S	S	Variant D
08-15694	19-Feb-08	M	09-Nov-84	6014	SSTI, leg		WA 12			POS					S	R	S (non inducible)	R	R	S	S	S	S	Variant E
06-17364	23-Aug-06	M	30-Oct-66	6281	SSTI, finger		WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	R	R	S	S	S	R	Variant F
06-15950	02-Apr-06	M	03-Jun-65	6062	Perianal abscess	Same patient as 06-18011	WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	R	S	S	S	R	Variant G
07-17473	28-Jul-07	M	01-Jul-85	6162	SSTI, buttock		WA 12			POS			POS		S	R	constitutive R	R	S	S	S	S	S	Variant H
04-15151	19-Jan-04	F	25-Feb-74	6152	SSTI, Right hip	Mother of 04-17898	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant I
04-17898	09-Nov-04	F	12-Apr-92	6152	Facial swab	Daughter of 04-15151	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant I
07-17843	31-Aug-07	M	04-Mar-92	6011	SSTI, labial tissue		WA 12			POS					S	R	constitutive R	S	S	S	S	S	S	Variant J
06-18368	25-Nov-06	M	12-Oct-87	6030	SSTI, leg		WA 12			POS			POS		S	S		S	S	S	S	S	S	Variant K
07-16214	03-Apr-07	F	06-Feb-66	6060	SSTI, groin		WA 12			POS			POS		S	R	constitutive R	S	S	S	S	S	S	Variant L
07-16814	06-Jun-07	F	30-Jan-93	6011	Putrid lesion		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant L
07-17877	04-Sep-07	F	31-Jul-91	6009	Perianal abscess		WA 12			POS			POS		S	R	constitutive R	S	S	S	S	S	S	Variant L
07-19108	07-Dec-07	M	14-Nov-88	6010	SSTI, groin		WA 12			POS					S	R	constitutive R	S	S	S	S	S	S	Variant L
07-16859	09-Jun-07	F	03-Feb-91	6003	Pus, thigh		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant L (with/without SCC deletion)
06-16974	05-Jun-07	M	22-Sep-76	6152	Abscess		WA 12	ST8	IV	POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	S	Variant M
07-17882	06-Sep-07	M	20-Apr-86	6014	SSTI		WA 12			POS			NEG	NEG	S	R	S	S	R	S	S	S	S	Variant M
05-18180	14-Nov-05	M	09-Dec-51	6162	SSTI		WA 12	ST8	IV	POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	R	Variant M
07-19147	12-Dec-07	M	30-Jun-84	6160	Abscess, forearm		WA 12			POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	S	Variant M
05-15946	12-Apr-05	M	12-Jun-59	6005	Abscess		WA 12	ST8	IV	POS			NEG	NEG	S	S		S	S	S	S	S	S	Variant N
05-17686	27-Sep-05	M	08-Jan-63	6025	SSTI, thigh		WA 12	ST8	IV	POS			NEG	NEG	S	S		S	S	S	S	S	S	Variant N
07-18464	15-Oct-07	F	21-Jan-67	6450	SSTI, leg		WA 12			POS				NEG	S	S	S	S	S	S	S	S	S	Variant N
07-19345	28-Dec-07	M	27-Sep-85	6230	Facial abscess		WA 12			POS	NEG	NEG		NEG	S	S		R	S	S	S	S	S	Variant O
04-15086	08-Jan-04	M	12-Dec-78	6020	SSTI, swab		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P
08-15094	02-Jan-08	M	20-Mar-80		Putrid boils		WA 12			POS				NEG	S	S	S	R	S	S	S	S	S	Variant P
07-15566	13-Feb-07	F	14-Aug-41	6210	SSTI, buttock		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P
06-17244	31-Jul-06	M	31-Jan-69	6160	Nasal swab (screening)		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P

Isolate	Date of sample collection	Gender	Date of birth	Postal code	Clinical Presentation	Comments	PFGE TYPE	MLST	SCCmec Typing	PVL-PCR as described by Fey 2003	sek-PCR as described in this paper	seq-PCR as described in this paper	arcA-PCR as described by Diep 2006	arcA-PCR as described by Zhang 2008	GENT	ERYT	CLIN	TETR	CIPR	TMP	FUSI	RIFA	MUPR	Varint according to array analysis
03-16593	10-Jul-03	F	30-Sep-79	6029	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
04-15086	08-Jan-04	M	12-Dec-78	6020	SSTI, swab		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P
04-15151	19-Jan-04	F	25-Feb-74	6152	SSTI, Right hip	Mother of 04-17898	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant I
04-15400	10-Feb-04	F	10-Feb-60	6211	Surgical wound		WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-17898	09-Nov-04	F	12-Apr-92	6152	Facial swab	Daughter of 04-15151	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant I
04-18066	22-Nov-04	M	04-Mar-98	6014	Abscess, leg	Sibling of 04-18340	WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-18340	23-Dec-04	F	13-Mar-00	6014	Wound swab	Sibling of 04-18066	WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
04-18334	26-Dec-04	F	03-Oct-62	6111	SSTI		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
05-15242	29-Jan-05	M	27-Aug-30	6010	Sputum	Same patient as 05-15965	WA 12	ST8	IV						S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-15946	12-Apr-05	M	12-Jun-59	6005	Abscess		WA 12	ST8	IV	POS			NEG	NEG	S	S		S	S	S	S	S	S	Variant N
05-15965	15-Apr-05	M	27-Aug-30	6010	SSTI, neck	Same patient as 05-15242	WA 12								S	R	S (non inducible)	S	R	S	S	S	R	Variant A
05-16235	14-May-05	F	03-Mar-94	6025	SSTI, shoulder		WA 12	ST8	IV	POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-16940	21-Jul-05	M	19-Apr-79	6155	SSTI, Wrist		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
05-17243	19-Aug-05	M	09-Aug-34	6010	Scalp wound		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-17686	27-Sep-05	M	08-Jan-63	6025	SSTI, thigh		WA 12	ST8	IV	POS			NEG	NEG	S	S		S	S	S	S	S	S	Variant N
05-18104	07-Nov-05	M	08-Sep-67	6164	SSTI, elbow	Same patient as 05-18090	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-18090	10-Nov-05	M	08-Sep-67	6164	SSTI, elbow	Same patient as 05-18104	WA 12	ST8	IV				POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
05-18180	14-Nov-05	M	09-Dec-51	6162	SSTI		WA 12	ST8	IV	POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	R	Variant M
06-15051	06-Jan-06	M	03-Nov-88	6027	Elbow aspirate		WA 12			POS	NEG	NEG		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant C
06-15950	02-Apr-06	M	03-Jun-65	6062	Perianal abscess	Same patient as 06-18011	WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	R	S	S	S	R	Variant G
06-16044	12-Apr-06	M	14-Jun-37	6008	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	R	Variant A
06-16229	03-May-06	M	15-Jun-61	6025	Wound, knee		WA 12	ST8	IVa	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-16691	14-Jun-06	F	03-Jul-41	6019	Nasal Swab (Patient)	Same patient as 08-15180	WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-16748	14-Jun-06	M	26-Mar-71	6152	SSTI, cheek		WA 12			POS	NEG	NEG		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant C
06-16786	21-Jun-06	M	16-Aug-85	6230	SSTI, leg and forearms		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-16917	03-Jul-06	M	14-May-44	6110	Nasal Swab (Patient)		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant B
06-16898	06-Jul-06	F	21-Jan-85	6009	SSTI		WA 12			POS	NEG	NEG	POS	POS	S	R	S (non inducible)	S	R	S	S	S	S	Variant D
06-17244	31-Jul-06	M	31-Jan-69	6160	Nasal swab (screening)		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P
06-17275	16-Aug-06	F	25-Oct-82	6062	Bartholin. Abscess		WA 12	ST8	IVa	POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-17364	23-Aug-06	M	30-Oct-66	6281	SSTI, finger		WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	R	R	S	S	S	R	Variant F
06-17519	10-Sep-06	F	20-Jul-95	6168	Abscess, thigh	Same family as 06-18270, 07-15379, 07-15590	WA 12	ST8	IV	POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-17899	18-Oct-06	F	25-Jul-65	6057	Abscess		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
06-18011	24-Oct-06	M	03-Jun-65	6062	Nasal Swab (Patient)	Same patient as 06-15950	WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-18195	16-Nov-06	F	28-Feb-79	6230	Drainage of an abscess		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-18270	21-Nov-06	M	02-Mar-77	6169	unknown	Same family as 06-17519, 07-15379, 07-15590	WA 12			POS			NEG		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
06-18368	25-Nov-06	M	12-Oct-87	6030	SSTI, leg		WA 12			POS			POS		S	S		S	S	S	S	S	S	Variant K
07-15220	15-Jan-07	M	19-Oct-55	6019	Wound swab		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-15379	02-Feb-07	F	21-Sep-63	6169	SSTI, finger	Same family as 06-17519, 06-18270, 07-15590	WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-15566	13-Feb-07	F	14-Aug-41	6210	SSTI, buttock		WA 12	ST8	IV	POS			NEG	NEG	S	S		R	S	S	S	S	S	Variant P
07-15590	20-Feb-07	M	02-Mar-99	6169	Abscess, knee	Same family as 06-17519, 06-18270, 07-15379	WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-16214	03-Apr-07	F	06-Feb-66	6060	SSTI, groin		WA 12			POS			POS		S	R	constitutive R	S	S	S	S	S	S	Variant L
07-16431	02-May-07	F	16-Nov-78	6155	SSTI, back		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-16974	05-Jun-07	M	22-Sep-76	6152	Abscess		WA 12	ST8	IV	POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	S	Variant M
07-16814	06-Jun-07	F	30-Jan-93	6011	Putrid lesion		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant L
07-16859	09-Jun-07	F	03-Feb-91	6003	Pus, thigh		WA 12			POS			POS		S	R	S (non inducible)	S	S	S	S	S	S	Variant L (with/without SCC deletion)
07-17131	28-Jun-07	F	20-Sep-33	6122	Swab, eye		WA 12			POS			POS		S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-17307	18-Jul-07	F	31-Jul-75	6164	Wound swab		WA 12			POS			POS		S	R	S	S	S	S	S	S	S	Variant A
07-17473	28-Jul-07	M	01-Jul-85	6162	SSTI, buttock		WA 12			POS			POS		S	R	constitutive R	R	S	S	S	S	S	Variant H
07-17723	16-Aug-07	M	04-Nov-38	9999	Sputum		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-17843	31-Aug-07	M	04-Mar-92	6011	SSTI, labial tissue		WA 12			POS					S	R	constitutive R	S	S	S	S	S	S	Variant J
07-17877	04-Sep-07	F	31-Jul-91	6009	Perianal abscess		WA 12			POS			POS		S	R	constitutive R	S	S	S	S	S	S	Variant L
07-17882	06-Sep-07	M	20-Apr-86	6014	SSTI		WA 12			POS			NEG	NEG	S	R	S	S	R	S	S	S	S	Variant M
07-17974	11-Sep-07	F	23-Aug-30	6105	SSTI		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18413	21-Sep-07	M	23-Nov-35	6027	Abscess, neck		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18252	05-Oct-07	M	05-May-87	6102	Abscess		WA 12			POS			POS		S	R	S	S	S	S	S	S	S	Variant A
07-18298	10-Oct-07	M	19-Sep-95	6169	SSTI		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18464	15-Oct-07	F	21-Jan-67	6450	SSTI, leg		WA 12			POS				NEG	S	S	S	S	S	S	S	S	S	Variant N
07-18756	12-Nov-07	M	14-Mar-66	8888	Abscess, neck		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18848	13-Nov-07	M	03-May-53	6010	Boil, axilla		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18831	17-Nov-07	M	14-Aug-79	6008	SSTI, leg		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
07-18956	17-Nov-07	F	27-Aug-71	6025	SSTI		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-18878	20-Nov-07	M	27-Aug-65	6017	SSTI		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-19108	07-Dec-07	M	14-Nov-88	6010	SSTI, groin		WA 12			POS					S	R	constitutive R	S	S	S	S	S	S	Variant L
07-19150	10-Dec-07	M	15-Mar-59	6050	Furuncle		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A
07-19147	12-Dec-07	M	30-Jun-84	6160	Abscess, forearm		WA 12			POS			NEG	NEG	S	R	S (non inducible)	S	R	S	S	S	S	Variant M
07-19345	28-Dec-07	M	27-Sep-85	6230	Facial abscess		WA 12			POS	NEG	NEG		NEG	S	S		R	S	S	S	S	S	Variant O
08-15094	02-Jan-08	M	20-Mar-80		Putrid boils		WA 12			POS				NEG	S	S	S	R	S	S	S	S	S	Variant P
08-15158	14-Jan-08	Sydney	20-Oct-34	6021	Abscess, axilla		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15180	15-Jan-08	F	03-Jul-41	6019	SSTI, leg	Same patient as 06-16691	WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15332	21-Jan-08	M	21-Jan-88	6018	SSTI, elbow		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15361	28-Jan-08	M	18-Jan-70	6169	SSTI, elbow		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
08-15503	08-Feb-08	M	21-Oct-47	6284	Wound swab		WA 12			POS					S	R	S (non inducible)	S	R	S	S	S	S	Variant A
08-15635	15-Feb-08	F	12-Jan-66	6030	Infected Boil		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15653	18-Feb-08	F	27-May-83	6025	Pus		WA 12			POS					S	R	S (non inducible)	S	S	S	S	S	S	Variant A
08-15694	19-Feb-08	M	09-Nov-84	6014	SSTI, leg		WA 12			POS					S	R	S (non inducible)	R	R	S	S	S	S	Variant E
08-15674	20-Feb-08	F	01-Mar-68	6059	SSTI, buttock		WA 12			POS	POS	POS		POS	S	R	S (non inducible)	S	S	S	S	S	S	Variant A

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Clin Microbiol Infect. 2009 Aug;15(8):770-6. Epub 2009 Jun 24.
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- Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia.
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- The molecular epidemiology and evolution of the Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia.
  Monecke S, Ehricht R, Slickers P, Tan HL, Coombs G.
  Clin Microbiol Infect. 2009 Aug;15(8):770-6. Epub 2009 Jun 24.
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- Figure 1a: Basic structures of representative SCC*mec* elements I – VIII. The structures of SCC*mec* elements of representative strains are illustrated based on the following nucleotide sequences deposited in databases: NCTC10442 (AB033763), N315 (D86934), 85/202 (AB037671), CA05 (AB063172), ZH47 (AM292304), WIS (AB121219), TSGH17 (AB5122767), PM1 (AB462393), HDE288 (AF411935), JCSC6082 (AB373032), and C10684 (FJ390057) [150] (Reproduced courtesy of American Society for Microbiology)

- Figure 1b: Basic structures of representative SCC*mec* elements IX and X from MRSA CC398 strains JCSC6943 and JCSC6943 respectively [42]. (Reproduced courtesy of American Society for Microbiology)

- Figure 1c: Schematic diagram showing the genetic organisation of the SCC*mec* element designated SCC*mec* XI and the adjacent 3-kb downstream region in the ST130 MRSA isolate M10/0061 (GenBank accession number FR823292) [43] (Reproduced courtesy of American Society for Microbiology)

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Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities

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Objectives: **Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote regions of Western Australia (WA) in 1992 and is now the predominant MRSA isolated in the State. To gain insights into the emergence of CA-MRSA, 2146 people living in 11 remote WA communities were screened for colonization with *S. aureus*.**

Methods: **Antibiogram analysis, contour-clamped homogeneous electric field electrophoresis, multilocus sequence typing, Panton–Valentine leucocidin determinant detection and accessory genetic regulator typing were performed to characterize the isolates. MRSA was further characterized by staphylococcal cassette chromosome *mec* typing.**

Results: **The *S. aureus* population consisted of 13 clonal complexes and two Singleton lineages together with 56 sporadic isolates. Five lineages contained MRSA; however, these were not the predominant methicillin-susceptible *S. aureus* (MSSA) lineages. There was greater diversity amongst the MSSA while the MRSA appeared to have emerged clonally following acquisition of the staphylococcal cassette chromosome *mec*. Three MRSA lineages were considered to have been endemic in the communities and have subsequently become predominant lineages of CA-MRSA in the wider WA community. People colonized with MSSA tended to harbour clones of a different genetic lineage at each anatomical site while people colonized with MRSA tended to harbour clones of the same lineage at each site. Overall, the isolates were resistant to few antimicrobials.**

Conclusions: **Although the evidence suggests that in WA CA-MRSA strains arose in remote communities and have now disseminated into the wider community, there is no evidence that they arose from the predominant MSSA clones in these communities.**

Keywords: *S. aureus*, community methicillin-resistant *Staphylococcus aureus*, population structure, colonization

Introduction

Staphylococcus aureus is one of the most successful pandemic bacterial pathogens. It is also a ubiquitous inhabitant of human microbiological flora, with up to 30% of humans persistently colonized asymptomatically, and up to 70% intermittently colonized.[1]

Initially MRSA was found almost exclusively in hospitals where it became known as healthcare-associated MRSA (HA-MRSA). However, it has now emerged in communities around the world where it is known as community-associated MRSA (CA-MRSA). The earliest reports of CA-MRSA involved infections in people from isolated Indigenous[2] or disadvantaged

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Coombs et al. BMC Microbiology 2011, 11:215
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BMC Microbiology

RESEARCH ARTICLE Open Access

Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region

Geoffrey W Coombs[1,2*], Stefan Monecke[3], Julie C Pearson[1], Hui-leen Tan[1], Yi-Kong Chew[1], Lynne Wilson[1], Ralf Ehricht[3], Frances G O'Brien[2] and Keryn J Christiansen[1,2]

Abstract

Background: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote regions of Western Australia and is now the predominant MRSA isolated in the state. The objective of this study is to determine the genetic relatedness of Western Australian CA-MRSA clones within different multilocus sequence type (MLST) clonal clusters providing an insight into the frequency of *S. aureus* SCC*mec* acquisition within a region.

Results: The CA-MRSA population in Western Australia is genetically diverse consisting of 83 unique pulsed-field gel electrophoresis strains from which 46 MLSTs have been characterised. Forty five of these sequence types are from 18 MLST clonal clusters and two singletons. While SCC*mec* IV and V are the predominant SCC*mec* elements, SCC*mec* VIII and several novel and composite SCC*mec* elements are present. The emergence of MRSA in diverse *S. aureus* clonal clusters suggests horizontal transmission of the SCC*mec* element has occurred on multiple occasions. Furthermore DNA microarray and *spa* typing suggests horizontal transfer of SCC*mec* elements has also occurred within the same CC. For many single and double locus variant CA-MRSA clones only a few isolates have been detected.

Conclusions: Although multiple CA-MRSA clones have evolved in the Western Australian community only three clones have successfully adapted to the Western Australian community environment. These data suggest the successful evolution of a CA-MRSA clone may not only depend on the mobility of the SCC*mec* element but also on other genetic determinants.

Background

Based on phenotypic and genotypic typing methods, community onset methicillin-resistant *Staphylococcus aureus* infections are caused by healthcare-associated MRSA (HA-MRSA) strains, which appear to have been transferred from hospitals or healthcare facilities into the community by patients or healthcare workers [1], or by community-associated MRSA (CA-MRSA) strains, which have been isolated from people who have had little or no contact with healthcare facilities or healthcare workers [2]. This distinction between community and healthcare facility however has become blurred with the replacement of HA-MRSA with CA-MRSA in hospitals [3,4].

In contrast to HA-MRSA, CA-MRSA strains are generally more susceptible to non beta-lactam antibiotics, grow significantly faster, have different clonal backgrounds, carry smaller staphylococcal cassette chromosome *mec* (SCC*mec*) elements (most commonly SCC*mec* type IV or type V), have enhanced virulence properties and frequently harbor genes expressing Panton-Valentine leukocidin (PVL) [5-8]. Rather than a worldwide spread of a single clone multiple CA-MRSA clones have emerged from diverse genetic backgrounds. Several well characterized CA-MRSA clones predominate in different regions: Sequence type (ST) 8-IV [2B]

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The Molecular Epidemiology of the Highly Virulent ST93 Australian Community *Staphylococcus aureus* Strain

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Abstract

In Australia the PVL - positive ST93-IV [2B], colloquially known as "Queensland CA-MRSA" has become the dominant CA-MRSA clone. First described in the early 2000s, ST93-IV [2B] is associated with skin and severe invasive infections including necrotizing pneumonia. A singleton by multilocus sequence typing (MLST) eBURST analysis ST93 is distinct from other *S aureus* clones. To determine if the increased prevalence of ST93-IV [2B] is due to the widespread transmission of a single strain of ST93-IV [2B] the genetic relatedness of 58 *S. aureus* ST93 isolated throughout Australia over an extended period were studied in detail using a variety of molecular methods including pulsed-field gel electrophoresis, *spa* typing, MLST, microarray DNA, SCC*mec* typing and *dru* typing. Identification of the phage harbouring the *lukS-PV/lukF-PV* Panton Valentine leucocidin genes, detection of allelic variations in *lukS-PV/lukF-PV*, and quantification of LukF-PV expression was also performed. Although ST93-IV [2B] is known to have an apparent enhanced clinical virulence, the isolates harboured few known virulence determinants. All PVL-positive isolates carried the PVL-encoding phage ΦSa2USA and the *lukS-PV/lukF-PV* genes had the same R variant SNP profile. The isolates produced similar expression levels of LukF-PV. Although multiple rearrangements of the *spa* sequence have occurred, the core genome in ST93 is very stable. The emergence of ST93-MRSA is due to independent acquisitions of different *dru*-defined type IV and type V SCC*mec* elements in several *spa*-defined ST93-MSSA backgrounds. Rearrangement of the *spa* sequence in ST93-MRSA has subsequently occurred in some of these strains. Although multiple ST93-MRSA strains were characterised, little genetic diversity was identified for most isolates, with PVL-positive ST93-IVa [2B]-t202-dt10 predominant across Australia. Whether ST93-IVa [2B] t202-dt10 arose from one PVL-positive ST93-MSSA-t202, or by independent acquisitions of SCCmec-IVa [2B]-dt10 into multiple PVL-positive ST93-MSSA-t202 strains is not known.

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Introduction

The community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) worldwide epidemic is polyclonal, however several well characterized clones predominate in different regions of the world: Sequence type (ST) 8-IV [2B] (USA300) and ST1-IV [2B] (USA400) in North America [1,2]; ST80-IV [2B] (European clone) in Europe [3], North Africa [4] and the Middle East [5]; ST59-V [5C2&5] (Taiwan clone) in Taiwan [6], ST30-IV [2B] (South West Pacific [SWP] CA-MRSA) in the Western Pacific [7,8] and ST772-MRSA-V [5C2] (Bengal Bay clone) in India and Bangladesh [9]. Transmission of these clones into other regions has occurred [10,11]. The occurrence of concurrent epidemics of CA-MRSA in many countries by different clones has been striking. Equally noteworthy are a number of common features of these epidemics, prominent among them the ability to cause severe infections in young otherwise healthy people and the carriage of *lukS-PV/lukF-PV* Panton Valentine Leukocidin (PVL) encoding genes by the organism.

In Australia the PVL - positive ST93-IV [2B], colloquially known as "Queensland CA-MRSA", has recently emerged to become the dominant CA-MRSA clone. First described in the early 2000s, ST93 is a singleton by MLST eBURST analysis and is therefore distinct from other *S aureus* clones [12].

In the 2010 Australian Group for Antimicrobial Resistance (AGAR) Community *S aureus* Surveillance Programme (SAP10)

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Differentiation of Clonal Complex 59 Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Western Australia[▽][‡]

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Clonal complex 59 (CC59) community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains were characterized using pulsed-field gel electrophoresis, *spa* typing, multilocus sequence typing, diagnostic DNA microarrays, and PCRs targeting staphylococcal cassette chromosome *mec* (SCC*mec*) elements and Panton-Valentine leukocidin (PVL). Six distinct groups within CC59 were characterized. At least seven different variants of SCC*mec* elements were identified (IVa [2B], IVb [2B], IVd [2B], IV variant [2B], IVa [2B&5], V variant [5C2], and V [5C2&5]). (The structural type is indicated by a Roman numeral, with a lowercase letter indicating the subtype, and the *ccr* complex and the *mec* complex are indicated by an Arabic numeral and an uppercase letter, respectively. Where there is an extra *ccr* element, this is indicated by "&" and an Arabic numeral designating the *ccr* type.) The first group is similar to the American sequence type 59 (ST59) MRSA-IV CA-MRSA strain USA1000. The second group includes a PVL-negative ST87 strain with an SCC*mec* element of subtype IVb (2B). The third group comprises PVL-variable ST59 MRSA-IV strains harboring multiple SCC*mec* IV subtypes. PVL-negative ST59 MRSA strains with multiple or composite SCC*mec* elements (IVa [2B&5]) form the fourth group. Group 5 corresponds to the internationally known "Taiwan clone," a PVL-positive strain with a variant SCC*mec* element (V [5C2&5]). This strain proved to be the most common CC59 MRSA strain isolated in Western Australia. Finally, group 6 encompasses the ST59 MRSA-V variant (5C2). The differentiation of CC59 into groups and strains indicates a rapid evolution and spread of SCC*mec* elements. Observed differences between groups of strains as well as intrastrain variability within a group facilitate the tracing of their spread.

Several well-characterized community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains predominate in different regions of the world. Many of these strains harbor the bicomponent Panton-Valentine leukocidin (PVL). Sequence type 8 (ST8) MRSA-IV (USA300), ST80 MRSA-IV, and ST93 MRSA-IV are the major CA-MRSA strains reported to occur in the United States, Europe, and Australia, respectively. In the Asia Pacific region, a distinct genotype, clonal complex 59 (CC59)/ST59, has become widespread. ST59 CA-MRSA strains are an important cause of morbidity in Taiwan (1, 13, 17, 25, 29) This so-called "Taiwan clone" has acquired a novel type V staphylococcal cassette chromosome *mec* (SCC*mec*) element (V [5C2&5], also known as V_T) and *ermB*, a macrolide-lincosamide-streptogramin B resistance gene (28) frequently reported to be present in streptococci and other bacteria. Its properties have recently been described in detail (24). ST59 MRSA-V and other CC59 strains have now been reported to occur in several countries, including the United States (USA1000) (6), Sweden (11), Germany (20), the United Kingdom (19), Vietnam (26), and Australia (22).

In Western Australia (WA), all MRSA strains are referred to the state's central typing reference laboratory (the Gram-Positive Bacteria Typing and Research Unit) for molecular characterization (5). Multiple CC59 strains, colloquially characterized as WA MRSA-9, -15, -24, -52, -55, -56, and -73, have been identified. They differ from each other in ST designation, pulsed-field gel electrophoresis (PFGE) pattern, SCC*mec* element, and PVL carriage.

To better understand the molecular epidemiology of this clonal complex, all CC59 MRSA strains isolated in Western Australia were examined using PFGE, *spa* typing, multilocus sequence typing (MLST), diagnostic DNA microarrays, and PCRs targeting SCC*mec* elements and PVL.

MATERIALS AND METHODS

Isolates and patients. From July 2003 to June 2008, 43 MRSA strains from 40 individuals living in WA were characterized as CC59 MRSA by the Gram-Positive Bacteria Typing and Research Unit. One person yielded three isolates (WA MRSA-15 04-16657, 05-17619, and 06-17484) over a 3-year period, and a

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The molecular epidemiology and evolution of the Panton–Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain USA300 in Western Australia

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Abstract

Between 2003 and 2008, 76 clinical isolates of the Panton–Valentine leukocidin-positive *Staphylococcus aureus* strain 'West Australian methicillin-resistant *Staphylococcus aureus* (MRSA)-12' (WA MRSA-12) were recovered from 72 patients living in the Perth area in Western Australia. These isolates were found to belong to multilocus sequence type 8, and had a USA300-like pulsed-field gel electrophoresis pulsotype. All isolates were genotyped using diagnostic DNA arrays covering species markers, resistance factors, virulence-associated, as well as MSCRAMM (microbial surface components recognizing adhesive matrix molecules) genes to prove the identity between WA MRSA-12 and the pandemic strain USA300, as well as to detect possible genetic variability. In general, WA MRSA-12 isolates were similar to USA300, and the most common variant was identical to USA300-TC1516. From this clone, most of the other variants may have evolved by a limited number of gene losses or acquisitions. Variations in carriage of virulence and resistance-associated genes allow distinction of variants or sub-clones. Altogether, 16 variants could be distinguished. They differed in the carriage of resistance genes (*blaZ/I/R, ermC, msrA + mpbBM, aadD + mupR, aphA3 + sat, tetK, qacC, merA/B/R/T*) of *β*-haemolysin-converting phages and of enterotoxins (*sek + seq*, which were deleted in four isolates). Notably, the arginine catabolic mobile element (ACME) was absent in 12 isolates (15.8%). The mercury resistance (*mer*) operon, which is usually associated with SCC*mec* type III elements, was found in several ACME-negative isolates. The present study emphasises the importance of genotyping in detecting the introduction and evolution of significant MRSA strains within a community.

Keywords: Arginine catabolic mobile element (ACME), diagnostic DNA microarray, molecular typing, MRSA, Panton–Valentine leukocidin (PVL), *Staphylococcus aureus*, USA300

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Introduction

In recent years, a pandemic unfolded of methicillin-resistant *Staphylococcus aureus* (MRSA) strains that carry novel and apparently highly mobile chromosomal staphylococcal chromosomal cassette elements harbouring *mecA* (SCC*mec* elements). Such strains have emerged outside the hospital setting and have been designated as 'community-associated MRSA' (CA-MRSA). Some of them harbour the prophage-encoded [1,2] virulence factor Panton–Valentine leukocidin (PVL). PVL is a bicomponent toxin that forms polymeric pores in leukocyte membranes [3], although its role in pathogenesis is still subject to discussion [4–6]. Several PVL-positive CA-MRSA strains from different clonal groups have evolved. Some have been confined to certain regions or localised outbreaks, whereas others have spread worldwide.

One of these strains, a clonal complex 8, *spa* type t008 MRSA carrying a SCC*mec* type IV element, has recently emerged as the dominant MRSA strain in North America, both in community and hospital settings [7–14]. Colloquially known as USA300, it has also been reported from Australia, Canada, Denmark, Germany, Japan, Switzerland and the UK [10,15–18]. Because of its rapid spread, it has drawn considerable attention, resulting in the sequencing of two complete

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Title: Classification of Staphylococcal Cassette Chromosome mec (SCCmec): Guidelines for Reporting Novel SCCmec Elements

Author: International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC)*

Publication: Antimicrobial Agents and Chemotherapy

Publisher: American Society for Microbiology

Date: Dec 1, 2009

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AMERICAN SOCIETY FOR MICROBIOLOGY

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

Author: Anna C. Shore, Emily C. Deasy, Peter Slickers, et al.

Publication: Antimicrobial Agents and Chemotherapy

Publisher: American Society for Microbiology

Date: Aug 1, 2011

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Title: Novel Types of Staphylococcal Cassette Chromosome mec Elements Identified in Clonal Complex 398 Methicillin-Resistant Staphylococcus aureus Strains

Author: Shanshuang Li, Robert Leo Skov, Xiao Han, et al.

Publication: Antimicrobial Agents and Chemotherapy

Publisher: American Society for Microbiology

Date: Jun 1, 2011

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11. LIST OF ADDITONAL PUBLICATIONS RELEVANT TO THE THESIS BUT NOT FORMING PART OF IT

11.1. 2012 PEER REVIEWED PUBLICATIONS

1. Illness severity in community-onset invasive *Staphylococcus aureus* infection and the presence of virulence genes.

   Wehrhahn MC, Robinson JO, Pascoe EM, **Coombs GW**, Pearson JC, O'Brien FG, Tan HL, New D, Salvaris P, Salvaris R, Murray RJ.

   J Infect Dis. **2012** Apr 5. PMID: 22492857

2. A multidrug-resistant *Staphylococcus epidermidis* clone (ST2) is an ongoing cause of hospital acquired infection in a Western Australian hospital.

   Widerström M, McCullough CA, **Coombs GW**, Monsen T, Christiansen KJ.

   J Clin Microbiol. **2012** Mar 21. PMID: 22442320

3. Formal infectious diseases consultation is associated with decreased mortality in *Staphylococcus aureus* bacteraemia.

   Robinson JO, Pozzi-Langhi S, Phillips M, Pearson JC, Christiansen KJ, **Coombs GW**, Murray RJ.

   Eur J Clin Microbiol Infect Dis. **2012** Mar 3. PMID: 22382823

4. Distribution of SCC*mec*-associated phenol-soluble modulin in staphylococci.

   Monecke S, Engelmann I, Archambault M, Coleman DC, **Coombs GW**, Cortez de Jäckel S, Pelletier-Jacques G, Schwarz S, Shore AC, Slickers P, Ehricht R.

   Mol Cell Probes. **2012** Jan 11. PMID: 22251619

11.2. 2011 PEER REVIEWED PUBLICATIONS

1. Comparison of a multiplexed MassARRAY system with real-time allele-specific PCR technology for genotyping of methicillin-resistant *Staphylococcus aureus*.

   Syrmis MW, Moser RJ, Whiley DM, Vaska V, **Coombs GW**, Nissen MD, Sloots TP, Nimmo GR.

   Clin Microbiol Infect. **2011** Dec;17(12):1804-10. Epub 2011 May 20. PMID: 21595795

2. Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR.

   Howden BP, McEvoy CR, Allen DL, Chua K, Gao W, Harrison PF, Bell J, **Coombs G**, Bennett-Wood V, Porter JL, Robins-Browne R, Davies JK, Seemann T, Stinear TP.

   PLoS Pathog. **2011** Nov;7(11):e1002359. Epub 2011 Nov 10. PMID: 22102812

3. The dominant Australian community-acquired methicillin-resistant *Staphylococcus aureus* clone ST93-IV [2B] is highly virulent and genetically distinct.

   Chua KY, Seemann T, Harrison PF, Monagle S, Korman TM, Johnson PD, **Coombs GW**, Howden BO, Davies JK, Howden BP, Stinear TP.

   PLoS One. **2011**;6(10):e25887. Epub 2011 Oct 3. PMID: 21991381

4. Prevalence of nasal methicillin-resistant *Staphylococcus aureus* colonisation in healthcare workers in a Western Australian acute care hospital.

Verwer PE, Robinson JO, **Coombs GW**, Wijesuriya T, Murray RJ, Verbrugh HA, Riley T, Nouwen JL, Christiansen KJ.

Eur J Clin Microbiol Infect Dis. **2011** Sep 10. PMID: 21909648

5. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from hospital inpatients, 2009: report from the Australian Group on Antimicrobial Resistance.

   Nimmo GR, Pearson JC, Collignon PJ, Christiansen KJ, **Coombs GW**, Bell JM, McLaws ML; Australian Group on Antimicrobial Resistance.

   Commun Dis Intell. **2011** Sep;35(3):237-43. PMID: 22624484

6. Community-acquired methicillin-resistant *Staphylococcus aureus* pneumonia: a clinical audit.

   Thomas R, Ferguson J, **Coombs G**, Gibson PG.

   Respirology. **2011** Aug;16(6):926-31. PMID: 21382130

7. Methicillin-resistant *Staphylococcus aureus* in a population of horses in Australia.

   Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, Kiehne J, **Coombs G**

   Aust Vet J. **2011** Jun;89(6):221-5. PMID: 21595643

8. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*.

   Monecke S, **Coombs G**, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S, Ehricht R.

PLoS One. **2011** Apr 6;6(4):e17936. PMID: 21494333.

9. Antimicrobial resistance: Not community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA)! A clinician's guide to community MRSA - its evolving antimicrobial resistance and implications for therapy.

   Chua K, Laurent F, **Coombs G**, Grayson ML, Howden BP.

   Clin Infect Dis. **2011** Jan 1;52(1):99-114. Review. PMID: 21148528

11.3. 2010 PEER REVIEWED PUBLICATIONS

1. Characterisation of Australian MRSA strains ST75- and ST883-MRSA-IV and analysis of their accessory gene regulator locus.

   Monecke S, Kanig H, Rudolph W, Müller E, **Coombs G**, Hotzel H, Slickers P, Ehricht R.

   PLoS One. **2010** Nov 17;5(11):e14025. PMID: 21103340

2. Incidence, risk factors, and outcomes of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* infections in Auckland, New Zealand.

   Muttaiyah S, **Coombs G**, Pandey S, Reed P, Ritchie S, Lennon D, Roberts S.

   J Clin Microbiol. **2010** Oct;48(10):3470-4. Epub 2010 Aug 4. PMID: 20686081

3. Clinical and laboratory features of invasive community-onset methicillin-resistant *Staphylococcus aureus* infection: a prospective case-control study.

   Wehrhahn MC, Robinson JO, Pearson JC, O'Brien FG, Tan HL, **Coombs GW**, Pascoe EM, Lee R, Salvaris P, Salvaris R, New D, Murray RJ.

   Eur J Clin Microbiol Infect Dis. **2010** Aug;29(8):1025-33. Epub 2010 Jun 12.

   PMID: 20549534

4. Rapid detection of H and R Panton-Valentine leukocidin isoforms in *Staphylococcus aureus* by high-resolution melting analysis.

   Tong SY, Lilliebridge RA, Holt DC, **Coombs GW**, Currie BJ, Giffard PM.

   Diagn Microbiol Infect Dis. **2010** Aug;67(4):399-401. PMID: 20638613

5. Community-acquired pneumonia due to pandemic A(H1N1)2009 influenzavirus and methicillin resistant *Staphylococcus aureus* co-infection.

   Murray RJ, Robinson JO, White JN, Hughes F, **Coombs GW**, Pearson JC, Tan HL, Chidlow G, Williams S, Christiansen KJ, Smith DW.

   PLoS One. **2010** Jan 14;5(1):e8705. PMID: 20090931

11.4. 2009 PEER REVIEWED PUBLICATIONS

1. Intrafamilial transmission of methicillin-resistant *Staphylococcus aureus*

   Pozzi Langhi SA, Robinson JO, Pearson JC, Christiansen KJ, **Coombs GW**, Murray RJ.

   Emerg Infect Dis. **2009** Oct;15(10):1687-9. PMID: 19861077

2. *Staphylococcus aureus* bacteraemia: a major cause of mortality in Australia and New Zealand.

   Turnidge JD, Kotsanas D, Munckhof W, Roberts S, Bennett CM, Nimmo GR, **Coombs GW**, Murray RJ, Howden B, Johnson PD, Dowling K; Australia New Zealand Cooperative on Outcomes in Staphylococcal Sepsis.

   Med J Aust. **2009** Oct 5;191(7):368-73. PMID: 19807625

3. Community-associated versus healthcare-associated methicillin-resistant *Staphylococcus aureus* bacteraemia: a 10-year retrospective review.

   Robinson JO, Pearson JC, Christiansen KJ, **Coombs GW**, Murray RJ.

   Eur J Clin Microbiol Infect Dis. **2009** Apr;28(4):353-61. Epub 2008 Oct 11.

   PMID: 18850122

4. Prevalence of MRSA strains among *Staphylococcus aureu*s isolated from outpatients, 2006.

   Coombs GW, Nimmo GR, Pearson JC, Christiansen KJ, Bell JM, Collignon PJ, McLaws ML; Australian Group for Antimicrobial Resistance.

   Commun Dis Intell. **2009** Mar;33(1):10-20. PMID:19618763

11.5. 2008 PEER REVIEWED PUBLICATIONS

1. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*.

   Nübel U, Roumagnac P, Feldkamp M, Song JH, Ko KS, Huang YC, **Coombs G**, Ip M, Westh H, Skov R, Struelens MJ, Goering RV, Strommenger B, Weller A, Witte W, Achtman M.

   Proc Natl Acad Sci U S A. **2008** Sep 16;105(37):14130-5. Epub 2008 Sep 4.

   PMID: 18772392

2. Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) in Australia.

   Nimmo GR, **Coombs GW**.

   Int J Antimicrob Agents. **2008** May;31(5):401-10. Epub 2008 Mar 14. Review.

   PMID: 18342492

11.6. 2007 PEER REVIEWED PUBLICATIONS

1. Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton-Valentine leukocidin.

   Monecke S, Berger-Bächi B, **Coombs G**, Holmes A, Kay I, Kearns A, Linde HJ, O'Brien F, Slickers P, Ehricht R.

   Clin Microbiol Infect. **2007** Mar;13(3):236-49. PMID: 17391377

11.7. 2006 PEER REVIEWED PUBLICATIONS

1. Molecular typing of methicillin-resistant staphylococci isolated from cats and dogs.

   Malik S, **Coombs GW**, O'Brien FG, Peng H, Barton MD.

   J Antimicrob Chemother. **2006** Aug;58(2):428-31. Epub 2006 Jun 16.

   PMID: 16782740

2. Non-multiresistant methicillin-resistant *Staphylococcus aureus* bacteraemia in Sydney, Australia: emergence of EMRSA-15, Oceania, Queensland and Western Australian MRSA strains.

   Gosbell IB, Barbagiannakos T, Neville SA, Mercer JL, Vickery AM, O'Brien FG, **Coombs GW**, Malkowski MJ, Pearson JC.

   Pathology. **2006** Jun;38(3):239-44. PMID: 16753746

3. Methicillin-resistant *Staphylococcus aureus* in the Australian community: an evolving epidemic.

   Nimmo GR, **Coombs GW**, Pearson JC, O'Brien FG, Christiansen KJ, Turnidge JD, Gosbell IB, Collignon P, McLaws ML.

   Med J Aust. **2006** Apr 17;184(8):384-8. PMID: 16618236

4. Methicillin-resistant *Staphylococcus aureus* clones, Western Australia.

   Coombs GW, Pearson JC, O'Brien FG, Murray RJ, Grubb WB, Christiansen KJ.

   Emerg Infect Dis. **2006** Feb;12(2):241-7. PMID: 16494749

11.8. 2005 PEER REVIEWED PUBLICATIONS

1. Type V staphylococcal cassette chromosome *mec* in community staphylococci from Australia.

   O'Brien FG, **Coombs GW**, Pearson JC, Christiansen KJ, Grubb WB.

   Antimicrob Agents Chemother. **2005** Dec;49(12):5129-32. PMID: 16304184

2. Macrolide, lincosamide and streptogramin B resistance in a dominant clone of Australian community methicillin-resistant *Staphylococcus aureus*.

   O'Brien FG, Zaini Z, **Coombs GW**, Pearson JC, Christiansen K, Grubb WB.

   J Antimicrob Chemother. **2005** Nov;56(5):985-6. Epub 2005 Oct 4. PMID: 16204342

3. Methicillin-resistant *Staphylococcus aureus*, Western Australia.

   Dailey L, **Coombs GW**, O'Brien FG, Pearman JW, Christiansen K, Grubb WB, Riley TV.

   Emerg Infect Dis. **2005** Oct;11(10):1584-90. PMID: 16318700

12. RELEVANT CONFERENCE PAPERS

12.1. International Symposium on Staphylococci and Staphylococcal Infections (ISSSI)

2012

15th International Symposium on Staphylococci and Staphylococcal Infections (ISSI2012): Lyon, France

Molecular Epidemiology of the Highly Virulent ST93 Australian Community *S. aureus* Strain

Coombs G, Goering R, Chua K, Monecke S, Howden B, Stinear T, Ehricht R, O'Brien F, Christiansen K

2010

14th International symposium on Staphylococci and Staphylococcal Infections (ISSSI2010): Bath, United Kingdom

Introduction of a Multi-Resistant Panton-Valentine Leucocidin Positive Community Associated MRSA into Western Australia

Pearson J, **Coombs G**, Tan H-L, Cramer S, Wilson L, Chew YK, O'Brien F, Christiansen K

Staphylococcus aureus Infection and Colonisation in the Community in Melbourne, Australia

Bennett C, **Coombs G**, Wood G, Howden B, Quek T, Parrott C, Craven J, Johnson P

2008

13th International Symposium on Staphylococci and Staphylococcal Infections (ISSI 2008): Cairns, Australia

Molecular Diversity of MRSA in Australia

Coombs G

Culture Confirmation of Positive BD GeneOhm MRSA Assay Results

Coombs G, Lee R, Cramer S, Kay I, Christiansen

Intra-strain Variability of USA300 MRSA Isolated in Western Australia

Monecke S, Ehricht R, Slickers P, Tan HL, **Coombs G**

Genotyping of Aberrant Australian CA-MRSA Strains which may contain a Novel *agr* Group

Monecke S, Ehricht R, Slickers P, Tan HL, **Coombs G**

Prevalence of MRSA Carriage in Health Care Workers Working in a Western Australian Acute Care Hospital

Verwer P, Robinson O, **Coombs G**, Wijesuriya T, Murray R, Riley T, Nouwen J, Christiansen K

Microarray Analysis of *Staphylococcus aureus* Colonising People in Remote Western Australia

O'Brien FG, Chew YK, **Coombs G**, Christiansen KC, Grubb WB

Community and Healthcare Associated *Staphylococcus aureus* Bacteraemia: 10 year Review

Robinson JO, Pozzi-Langhi S, Pearson JC, Christiansen KJ, **Coombs G**, Murray RJ

Community Onset *Staphylococcus aureus* Infections in Melbourne

Bennett CM, **Coombs G**, Wood GM, Howden BP, Johnson LEA, Johnson PDR

Evaluation of *spa* and Diversilab rep-PCR Typing in Characterising Western Australian Community MRSA Clones as Defined by MLST/SCC*mec*

Coombs G, Pearson J, Wilson L, Tan H-L, Cramer S, O'Brien F

Laboratory Features of Invasive Community-Onset Methicillin-Resistant *Staphylococcus aureus* – Correlation with Clinical Features in a Prospective Case-Control Study

Wehrhahn MC, Pearson JC, O'Brien HG, Tan H-L, Robinson JO, Lee R, Chan J, **Coombs G**, Murray RJ

International MRSA Clones Identified in Western Australia

Tan H-L, Pearson J, **Coombs G**, Christiansen K, Murray R, Robinson O, O'Brien F

Knowing MRSA Colonisation Status Increases Empiric Use of Glycopeptide in MRSA Bacteraemia and May Reduce Mortality

Robinson JO, Pearson JC, Christiansen KJ, **Coombs G**, Murray RJ

Dynamics of Colonisation with *Staphylococcus aureus* in Remote Western Australian Communities

O'Brien FG, Pearman JW, **Coombs G**, Christiansen KC, Grubb WB

Dissemination of USA300 MRSA in Western Australia

Pearson J, **Coombs G**, Christiansen K, Murray R, Robinson O, O'Brien F

2006

12th International Symposium on Staphylococci and Staphylococcal Infections: Maastricht, The Netherlands

Virulence Determinants in Community-Associated MRSA Isolated in Australia are Clone Specific

Coombs G, Pearson JC, Ngan P, Hui-Leen T, Pryce T, Kay I, Christiansen K, O'Brien F

Population Genetics of *Staphylococcus aureus* and the Staphylococcal Cassette Chromosome *mec* in Remote Western Australian communities

O'Brien FG, **Coombs G**, Christiansen KJ, Grubb WB

Microarray-Based Genotyping of Epidemic Strains of *Staphylococcus aureus*

Monecke S, Berger-Bächi B, **Coombs G**, Holmes A, Kearns A, Kay I, Linde H-J, O´Brien F, Slickers P, Ehricht R

Variations in Epidemic Methicillin Resistant *Staphylococcus aureus*-16 (EMRSA-16) from the UK

Monecke S, Berger-Bächi B, **Coombs G**, Holmes A, Kearns A, Kay I, Linde H-J, O´Brien F, Slickers P, Ehricht R

12.2. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)

2011

51st Interscience Conference on Antimicrobial Agents and Chemotherapy: Chicago, USA

Importation of Bengal Bay MRSA (ST772-V [5C2]) into Australia

Coombs G, Peterson A, Pearson J, Tan H-L, O'Brien F, Christiansen K

Community Onset *Staphylococcus aureus* Household Cohort Study (COSAHC): Household Colonisation Patterns in Melbourne, Australia

Bennett CM, **Coombs GW**, Wood GM, Howden BP, Quek T, Craven J, Parrot C, Johnson PDR

Global Survey of Antibiotic Susceptibility and Molecular Epidemiology of Panton Valentine (PVL) Positive Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Macedo-Vinas M, Conly J, Aschbacher R, Blanc D, **Coombs G**, Daikos G, Dhawan B, Empel J, Etienne J, Figueiredo A, Hoang L, Ishii J, Kim H, Koeck R, Larsen A, Layer F, Li Q, Lo Y, Mulvey M, G Golding & CNISP, Pantosti A, Saga T, Schrenzel J, Simor A, Skov R, Tsiodras S, van Rijen M, Wang H, Zakaria Z, Harbarth S, for the Global PVL+MRSA Survey, Geneva (Switzerland).

2010

50th Interscience Conference on Antimicrobial Agents and Chemotherapy: Boston, USA

Long Term Care Facility (LTCF) Patients and Healthcare Workers (HCW) are a Reservoir of EMRSA-15 in Western Australia (WA)

Coombs G, Pearson J, Peterson A, Christiansen K

Community-Associated MRSA (CA-MRSA) in Western Australia (WA): The Emerging Patterns

Coombs G, Pearson J, O'Brien F, Christiansen K

2009

49th Interscience Conference on Antimicrobial Agents and Chemotherapy: San Francisco, USA

Evaluation of the BD GeneOhm™ StaphSR and Cepheid Xpert™ MRSA/SA Blood Culture Assays to Detect MRSA with Diverse Lineages

Coombs G, Crammer S, Kay ID, Morgan J, Christiansen K

PVL Positive EMRSA-15 in Western Australia: A Changing Epidemiology

Coombs G, Pearson J, Heitz L, O'Brien FG, Christiansen K.

2008

48th Interscience Conference on Antimicrobial Agents and Chemotherapy: Washington, USA

Characterisation of USA300 MRSA Introduced into Western Australia

Coombs G, Monecke S, Tan H-L, Pearson J, Christiansen F

Evaluation of Positive BD GeneOhm MRSA Results with Culture

Coombs G, Lee R, Cramer S, Kay I, Christiansen K

Patient and Infection Characteristics Associated with Panton-Valentine Leukocidin in Community On-Set *Staphylococcus aureus* Infections in Australia

Bennett CM, Wood GM, **Coombs G**, Howden BP, Johnson PDR

2007

47th Interscience Conference on Antimicrobial Agents and Chemotherapy: Chicago, USA

Epidemiology of Community-Acquired *Staphylococcus aureus* Infections in Australia

Bennett CM, Johnson PDR, **Coombs G**, Wood GM, Howden BP, Johnson L

Detection of International Panton-Valentine Leucocidin (PVL) Positive cMRSA Clones in Western Australia

Coombs G, Pearson J, O'Brien F, Murray R, Christiansen K

Australian Nosocomial Infections due to Community MRSA Clones
Coombs G, Pearson J, O'Brien F, Nimmo G, Christiansen

Evaluation of the IDI-MRSA™ PCR Assay to Detect MRSA with Variable SCC*mec* Types and Clonal Backgrounds

Coombs G, Kay IS, Gray K, Pearson JC, O'Brien FG, Christiansen KJ

Community-Onset versus Hospital-Onset Methicillin Resistant *Staphylococcus aureus* (MRSA) Bacteraemia: 10-year Retrospective Review

Robinson JO, Pearson JC, Christiansen, **Coombs G**, Murray RJ

2006

46th Interscience Conference on Antimicrobial Agents and Chemotherapy: San Francisco, USA

New York/Japan Epidemic MRSA Isolated in Australia.

Coombs G, Pearson J, Christiansen K, Tan T, Van Gessel H, Godsell M, O'Brien F

2005

45th Interscience Conference on Antimicrobial Agents and Chemotherapy: Washington, USA

Epidemiology of Epidemic and Community MRSA Clones Isolated in Western Australia

Coombs G, Pearson J, O'Brien F, Murray R, Grubb W, Christiansen K

12.3. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)

2011

21st European Congress of Clinical Microbiology and Infectious Diseases: Milan, Italy

The Emergence of Novel and Composite SCC*mec* Element Types into Western Australian Community *S aureus*

Coombs GW, Pearson JC, Monecke S, O'Brien FG, Christiansen KJ

A Case Control Study Comparing Infections due *Staphylococcus aureus* with Panton Valentine Leucocidin (PVL) to those without PVL

Boan P, Pearson J, **Coombs G**, Tan H-L, Christiansen K, Robinson O

Distribution of the Arginine Catabolic Mobile Element in Staphylococci

Monecke S, Coleman D, **Coombs G**, Deasy E, Ehricht R, Ip M, Shore A

2010

20th European Congress of Clinical Microbiology and Infectious Diseases Vienna, Austria

Widespread dissemination of the Panton-Valentine leucocidin positive ST93-MRSA-IV clone in the Australian community

Coombs G, Pearson J, Nimmo G, Christiansen K on behalf of the Australian Group for Antimicrobial Resistance

Development of an Automated Method for High-throughput Multilocus Sequence Typing of *Staphylococcus aureus*

Pryce T, **Coombs G**, Lim L, O'Brien F, Smith L,

2009

19th European Congress of Clinical Microbiology and Infectious Diseases: Helsinki, Finland

Differentiation of CC59 Community Acquired MRSA Strains

Monecke S, O'Brien F, **Coombs G**, Tan H-L, Pearson J, Ehricht R

2008

18th European Congress on Clinical Microbiology and Infectious Diseases: Barcelona, Spain

Emergence and Dissemination of USA300 MRSA in Australia

Coombs G, Christiansen K, Pearson J, Murray R, Robinson O, O'Brien F

2005

15th European Congress of Chemotherapy and Infectious Diseases: Copenhagen, Denmark

Efficient Genotyping of Methicillin Resistant *Staphylococcus aureus* Using a Combination of Single Nucleotide Polymorphisms and Binary Markers

Huygens F, Stephens AJ, Nimmo GR, Schooneveldt JM, **Coombs G**, Price EP, Giffard PM

Community Acquired MRSA in Europe at Work: The Evolution of Community MRSA in Australia – Lessons for Europe

Riley TV, **Coombs G**, O'Brien F, Pearman J, Christiansen K, Grubb W

12.4. Australian Society for Antimicrobials Annual Scientific Meeting (ASA)

2012

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2012. Brisbane, Australia

AGAR SAP10: Molecular Epidemiology of MRSA in the Australian Community

Coombs G, Pearson J, Nimmo G, Christiansen K

Searching for Organism Factors that may explain the Association between Elevated Vancomycin Minimum Inhibitory Concentration and Mortality in *Staphylococcus aureus* Bacteraemia

Holmes N, Turnidge J, Munckhof W, Robinson JO, Korman T, O'Sullivan M, Anderson T, Roberts S, **Coombs G**, Gao W, Johnson P, Howden B

2011

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2011: Melbourne, Australia

Panton-Valentine Leukocidin (PVL) positive CA-MRSA clones in Western Australia

Tan H-L, Pearson J, **Coombs G**, Christiansen K, Robinson O, O'Brien F

2010

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2010: Sydney, Australia

Community Onset Infections in Australia: A Tale of Two Clones

Coombs G, Nimmo G, Pearson J, Cramer S, Christiansen K

CC59 Community Associated Methicillin-Resistant *Staphylococcus aureus* in Western Australia: Clonal Spread or Multiple Evolutionary Events?

Coombs G, Monecke S, Ehricht R, Slickers P, Pearson J, Tan H-L, Christiansen K, O'Brien F

Simultaneous Detection of Panton-Valentine leucocidin, *mecA* and *nuc* Genes in Clinical isolates of Staphylococci Using a Triplex Real-Time (Light Cycler 2.0) PCR Assay

Kay I, **Coombs G**

2009

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2009: Melbourne, Australia

PVL-positive EMRSA-15 Detected in Western Australia

Pearson J, **Coombs G**, Cramer S, Tan H-L, Chew Y, Wilson L, Kay I, O'Brien F, Christiansen K

Whole Genome Sequence of ST93-MRSA-IV, a Unique Australian Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus*

Chua K, **Coombs G**, Seeman T, Davies JK, Moore R, Haring V, Stinear TP, Howden BP

2008

Australia Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2008: Sydney, Australia

More Common than we Thought? – Methicillin Resistance and PVL in Community-Onset *Staphylococcus aureus* Infections in Melbourne

Bennett CM, **Coombs G,** Wood GM, Johnson L, Howden BP, Johnson PDR

USA300 MRSA Identified in the Australian Community

Pearson J, **Coombs G**, Christiansen K, Murray R, Robinson O, O'Brien F

Evaluation of Positive BD GeneOhm™ IDI-MRSA Assay Results from Nasal and Throat MRSA Screening Swabs

Coombs G, Cramer S, Kay I, Perry P, Christiansen K

Evaluation of the BD GeneOhm™ StaphSR Assay for Direct Detection of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* in Positive Blood Cultures

Coombs G, Lloyd P, Kay I, O'Brien F, Wijesuriya T, Murray R, Christiansen K

2007

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2007: Melbourne, Australia

Molecular Epidemiology of MRSA in Australian Hospitals

Coombs G, Pearson J, O'Brien F, Christiansen K

Detection of Epidemic and Community MRSA Strains in Western Australia Using the IDI-MRSA® PCR Assay

Coombs G, Kay I, Gray K, Pearson J, O'Brien F, Christiansen K

2006

Australian Society for Antimicrobials Annual Scientific Meeting: Antimicrobials 2006: Sydney, Australia

An Outbreak of New York/Japan EMRSA in Rural Western Australia

Pearson J, **Coombs G**, O'Brien F, Tan H-L, Van Gessell H, Godsell M-R, Christiansen K.

CLSI Cefoxitin Disc Diffusion Susceptibility Testing of Coagulase-Negative Staphylococci

McCullough C, **Coombs G**, Pryce T, Price D, Christiansen K

2005

Australian Society for Antimicrobials Annual Scientific Meeting, Antimicrobials 2005: Lorne, Australia

Methicillin-Resistant *Staphylococcus aureus* in Australia – the 2003 AGAR Data

Coombs G, Pearson J, Christiansen K, O'Brien F, Nimmo G, Collignon P

NCCLS Cefoxitin Susceptibility Testing of MRSA Clones Isolated in Western Australia

McCullough C, **Coombs G**, Christiansen K

12.5. Annual Scientific Meeting of the Australian Society for Microbiology (ASM)

2010

Annual Scientific Meeting of the Australian Society for Microbiology: Sydney, Australia

A Shift in the Epidemiology of Community Onset MRSA Infections in Australia

Coombs G, Pearson J, Cramer S, Nimmo G, Christiansen K on behalf of the Australian Group for Antimicrobial Resistance

2009

Annual Scientific Meeting of the Australian Society for Microbiology: Perth, Australia

Characterization of a Novel Staphylococcal Cassette Chromosome *mec* in a Western Australian Community-Associated Methicillin-Resistant *Staphylococcus aureus*

Wilson L, **Coombs G**, Christiansen K, Pearson J, O'Brien F

Automated High-throughput Multilocus Sequence Typing of *Staphylococcus aureus*

Pryce T, **Coombs G**, Lim L, O'Brien FG, Smith LK

Rapid Detection of Histidine (H) and Arginine (R) Panton-Valentine Leukocidin Variants by High-Resolution Melt Analysis

Tong S, Lilliebridge R, Holt D, **Coombs G**, Currie B, Giffard P

2008

Annual Scientific Meeting of the Australian Society for Microbiology: Melbourne, Australia

Dynamics of Colonisation with *Staphylococcus aureus* in Remote Western Australian Communities

O'Brien FG, Pearman J, Gracey M, **Coombs G**, Christiansen K, Grubb W

Comparison of the BD GeneOhm StaphSR with Traditional Culture for Direct Detection of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* in Positive Blood Cultures

Lloyd P, **Coombs G**, Kay I, O'Brien FG, Murray R, Christiansen

2005

Annual Scientific Meeting of the Australian Society for Microbiology: Canberra, Australia

Detection of Panton-Valentine Leucocidin (PVL) Toxin in Australian Community MRSA (cMRSA)

Coombs G, Tan H-L, Pearson J, Morgan D, O'Brien F, Christiansen K

13. BIBLIOGRAPHY

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