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Studies of community-associated meticillin resistant *Staphylococcus aureus* (caMRSA) carriage in people and animals

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Certificate

This is to certify that the thesis entitled *Studies of community-associated meticillin resistant Staphylococcus aureus (caMRSA) carriage in people and animals* submitted by Danijela Stancic in fulfilment of the requirements for the Doctor of Philosophy is ready for examination.

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Declaration of Originality

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Abstract

Meticillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of bloodstream infections in Australia and is one of the top ten pathogens with an adverse impact on human health worldwide. Infections caused by this organism were originally associated with the healthcare setting, termed healthcare-associated MRSA (haMRSA). New strains of MRSA termed community-associated caMRSA (caMRSA), with different genetic characteristics to haMRSA strains, were first identified in patients residing in remote communities in Western Australia during the early 1990's. Colonisation with caMRSA precedes and increases the risk of subsequent infection with this organism. Research on caMRSA carriage in community settings has mainly come from Europe and the US. Community specific data for caMRSA carriage in Australia is sparse. This study aimed to detail the prevalence of MRSA in community members (n=283), as well as in groups at risk for the acquisition of MRSA, comprising contact sports participants (n=199), dogs (n=108), dog handlers (n=94), horses (n=310), horse handlers (n=38), veterinary nurses (n=48) and veterinarians (n=60). The role of household contacts and the environment as sources of caMRSA was investigated in the veterinary cohort. Genotypic analyses were used to differentiate strains of caMRSA from haMRSA, and unique strain characteristics were detailed. Genotypic tests included multiplex real-time PCR (RT-PCR), macro-restriction pulsed field gel electrophoresis (PFGE), SCC*mec* typing, multilocus sequence typing (MLST) and DNA microarrays. The findings of the present study revealed caMRSA poses a greater risk to veterinarians (20%), veterinary nurses (6.25%) and horse handlers (5.26%) than to all other cohorts investigated in which prevalence of caMRSA was found to be low (<2%) or absent.

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Dedication

Dedicated to my Family.

To my father Nikola Stančić and my Grandfather Svetozar Stančić, even though I lost you both at a very young age, barely a day goes past that I do not think about you both.

I would also like to dedicate this thesis to my Grandmother Vida and Grandfather Ranko Sarić, who I was fortunate enough to have in my life. You have both been such positive influences. Even though you are no longer with me I will always hold you dear to my heart. For my dogs Bela and Bobby you were my little sunshine's and I am glad that you were a part of my life.

Isaiah 54:17

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List of Abbreviations

ACME	Arginine catabolic mobile element
ApaI	*Acetobacter pasteurianus* restriction endonuclease enzyme
caMRSA	Community-associated meticillin resistant *Staphylococcus aureus*
CC	Clonal complex
CDC	Centers for Disease Control and Prevention
CN	Gentamicin
CP	Ciprofloxacin
CSLI	Clinical and Laboratory Standards Institute
DA	Clindamycin
dNTP	Deoxyribonucleotide triphosphate
E	Erythromycin
FOX	Cefoxitin
haMRSA	Healthcare-associated meticillin resistant *Staphylococcus aureus*
laMRSA	Livestock-associated meticillin resistant *Staphylococcus aureus*
MGE	Mobile genetic elements
MLST	Multilocus sequence typing
M-PCR	Multiplex polymerase chain reaction
MRSA	Meticillin resistant *Staphylococcus aureus*
MSCRAMM	Microbial surface components recognising adhesive matrix molecules
orf X	Open reading frame X

PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PSM	Phenol soluble modulins
PVL	Panton-Valentine Leukocidin toxin
RD	Rifampicin
RT-PCR	Real-time polymerase chain reaction
SaPI	Staphylococcal pathogenicity islands
SCC*mec*	Staphylococcal cassette chromosome *mec*
*Sma*I	Type II restriction endonuclease enzyme
SNP	Single nucleotide polymorphisms
ST	Sequence type
TAFE	College of Technical And Further Education
TE	Tetracycline
Tn	Transposons
VA	Vancomycin
VRSA	Vancomycin resistant *Staphylococcus aureus*
W	Trimethoprim
WHO	World Health Organization

Chapter 1 Review of literature

Staphylococcus aureus is a bacterium that can exist as part of the normal flora in humans. Strains of this organism began to exhibit resistance to meticillin during the 1960's (Jevons, 1961, Barber, 1961), and were termed meticillin resistant *S. aureus* (MRSA). These resistant strains were confined to healthcare settings (haMRSA) (Alex and Letizia, 2007, Beam and Buckley, 2006). Infections acquired outside of a healthcare setting were first observed during the early 1990's in healthy individuals in community settings, termed community-associated MRSA (caMRSA) (Wallin et al., 2008, Kobayashi and DeLeo, 2009). More recently, a third type of MRSA has been described in livestock – termed livestock-associated MRSA (laMRSA) (Köck et al., 2013). Certain MRSA strains have developed resistance over time to a wide range of other antibiotics and are termed multi-drug resistant MRSA. Since then MRSA has been isolated from healthcare, community and animal settings. The focus of this literature review is on caMRSA.

Most studies on caMRSA carriage have been conducted in the US and Europe. In Australia there have been studies specifically examining caMRSA prevalence of clinical and carrier isolates in healthcare settings (Brennan et al., 2013, Verwer et al., 2012, Nimmo et al., 2006, Coombs et al., 2004, Coombs et al., 2009b, Coombs et al., 2013a, Coombs et al., 2012a). Apart from one study that investigated nasal caMRSA carriage in healthy adult members of the general community in Australia (Munckhof et al., 2009), very little research has been reported on caMRSA carriage in healthy Australian communities and in risk groups. It is important to investigate the natural reservoirs of this organism in the community as *S. aureus* infection places a great burden on public health resources.

1.1 Classification of *Staphylococcus aureus*

Staphylococcus aureus, also commonly known as "golden staph", is a prokaryote belonging to the bacterial *Staphylococcaceae* family (Ravcheev et al., 2011). *S. aureus* is a Gram positive, coccoid shaped bacterium and is classed as a facultative anaerobe. The *Staphylococcus* genus contains over 40 species, among which *S. aureus* is the most common species to cause skin and soft tissue infections in humans (Sampedro et al., 2014, McNeil et al., 2014). The bacterium often appears golden in colour when grown on an agar plate.

1.1.2 History of Staphylococcus aureus and MRSA

S. aureus was first discovered in the pus of an abscess by Scottish surgeon Sir Alexander Ogston in 1880. In 1928 Alexander Fleming discovered the first antibiotic penicillin. Carrying on from Sir Alexander Fleming's work, Professor Howard Florey and Drs Ernst Chain and Norman Heatley carried out essential experiments in Britain that contributed to the purification process of penicillin in 1939. In 1941, Florey and Heatley travelled to the USA to work on mass production of the drug and by 1944 many laboratories were manufacturing the drug on mass scale, including the Merck, Squibb and Pfizer companies in the USA and the Commonwealth Serum Laboratories in Melbourne, Australia. Australia was the first country that made the drug available for civilian use to treat staphylococcal and other infections.

As a consequence of the increasingly widespread use of penicillin, certain strains of *S. aureus* began to exhibit resistance to the antibiotic by the late 1940s. To combat the emerging penicillin resistant strains meticillin, a derivative of penicillin, was introduced in 1959 (Rolinson et al., 1960). By 1961 strains of this organism began to develop resistance to meticillin (Jevons, 1961, Barber, 1961). These resistant strains were designated meticillin resistant *S. aureus* (MRSA). Today meticillin is no longer manufactured and has been replaced by more stable antibiotics such as oxacillin; however the acronym remains in common usage. Over time MRSA strains have developed resistance to a wide range of other antibiotics, and hence are usually multi-drug resistant. The first confirmed case of MRSA infection in Sydney was reported in 1965 (Rountree and Beard, 1968). In the United States of America (USA) the first MRSA infection was reported 3 years later in 1968 (Barrett et al., 1968).

Traditionally, MRSA infections have been associated with the healthcare setting (haMRSA), typically affecting immunocompromised patients and those undergoing surgery (Beam and Buckley, 2006, Alex and Letizia, 2007). Strains of caMRSA were first reported within a community setting in 1993 in persons without healthcare-associated risk factors living in a remote region of the Kimberley in Western Australia (Udo et al., 1993), and in the late 1990's in North America (Rybak and LaPlante, 2005). By 1999 caMRSA was acknowledged as a potentially virulent pathogen following the death of four healthy infants in America as a consequence of caMRSA infection (Farley, 2008).

Widespread antibiotic use and selective pressure have contributed to the rapid dissemination of caMRSA strains in both the community and healthcare settings (Okuma

et al., 2002, Dukic et al., 2013). The World Health Organisation (WHO) considers MRSA to be one of the leading pathogens with the greatest adverse impact on human health on a global scale (WHO, 2013).

1.2 Staphylococcus aureus genome

The *S. aureus* genome is made up of three major components (Stefani et al., 2012). The first component is a core genome, which is found in all strains and shows little or no variation between strains. Genes that make up the core genome are associated with cell survival, including housekeeping functions and metabolism of the bacteria. They make up approximately 75% of the *S. aureus* genome. Virulence genes *spa* (encodes protein A) and *hla* (encodes alpha-haemolysin) are a part of the core genome. Secondly, disseminated throughout the backbone are 700 core variable genes (CV), which are responsible for encoding surface proteins or structures. The unique distribution pattern of CV genes is used to define *S. aureus* lineages (Lindsay et al., 2006). The third component of the genome consists of large pieces of DNA, called mobile genetic elements (MGE's), which encode mobilisation functions and are frequently transferred between strains (Malachowa and DeLeo, 2010). MGE's are known to carry numerous antimicrobial and virulence factors (Stefani et al., 2012, Lindsay, 2010). MGE's include pathogenicity islands, chromosome cassettes, plasmids and transposons. Pathogenicity islands (SaPIs) generally carry one or more superantigen genes (SAg) that encode numerous enterotoxins (*sea-sed, sef-ser, seu)* and the toxic shock syndrome toxin (*tst1*) (Wu et al., 2011). Chromosome cassettes are genetic elements that encode meticillin resistance (*mecA)* and carry cassette chromosome recombinase genes (*crr)* (Elements, 2009). Plasmids carry resistance determinants and transfer resistance genes by a process of transduction or conjugation (Chambers, 1997). Transposons (Tn) are small transferable DNA fragments involved in

inactivation or modification of cellular functions and encode resistance genes (Jensen and Lyon, 2009). Important MGE's found in MRSA include *mecA* (meticillin resistance) and the PVL toxin, as well as other resistance determinants.

1.2.1 Genetic and phenotypic characteristics of caMRSA & haMRSA

Strains of caMRSA possess different phenotypic and genotypic characteristics compared to traditional haMRSA strains (Dukic et al., 2013, Coffman, 2007) and vary in their antibiotic sensitivity profiles (Farley, 2008). There is also compelling data indicating that the pathogenic mechanisms of caMRSA are unique and distinct from haMRSA (Otto, 2013, Charlebois et al., 2004, Miller and Diep, 2008, Wallin et al., 2008, Vandenesch et al., 2003).

The *mecA* gene is a defining feature of both caMRSA and haMRSA. It codes for an altered penicillin binding protein (PBP2A), responsible for coding resistance to meticillin, due to its low binding affinity for β-lactams (Hartman and Tomasz, 1984, Utsui and Yokota, 1985, Ito et al., 2001). The *mecA* gene is located on a mobile genetic island known as the staphylococcal cassette chromosome *mec* (SCC*mec*). The SCC*mec* is a segment of DNA that contains genes for antibiotic resistance and which can be transferred between bacteria through horizontal spread. SCC*mec* is inserted at the 3' end of open reading frame X (*orfX)* and is located near the replication origin in *S. aureus* (Elements, 2009). The SCC*mec* is made up of the *mec* gene complex and *ccr* gene complex. The *ccr* gene complex encodes recombinase genes *ccrA* and *ccrB,* which are responsible for encoding mobility. There are five currently identified *mec* gene complexes in MRSA (classes A, B, C1, C2 and E) and three distinct *ccr* genes (*ccrA, ccrB* and *ccrC*) (Elements, 2009).

Different combinations of *mec* gene complex classes and *ccr* gene complex types define the SCC*mec* typing (Malachowa and DeLeo, 2010).

Five major SCC*mec* types predominate (I-V) in MRSA, although additional SCC*mec* types have been described (VI-XI) (Elements, 2009, Li et al., 2011, Shore et al., 2011, Vestergaard et al., 2012). Differences between the SCC*mec* types can be used to distinguish caMRSA from haMRSA. Strains of caMRSA typically possess a type IV or V SCC*mec*, while haMRSA strains possess a type I, II or III SCC*mec*. An exception to this is the epidemic EMRSA-15 hospital strain which carries an SCC*mec* type IV. In caMRSA, the smaller SCC*mec* types IV and V carry fewer resistance genes, whereas haMRSA strains possess larger SCC*mec* types, which are reported to hold a greater number of resistance genes (Chua et al., 2011, Gorwitz, 2008). Table 1.1 shows the currently established SCC*mec* types found in *S. aureus* and their combination of *mec* and *crr* genes.

Table 1.1 Currently established SCC*mec* types in *Staphylococcus aureus*

SCC*mec* type	*mec* gene complex	*ccr* gene complex	MRSA type*
Type I	class B	type-1 (*ccrA1* and *ccrB1)*	haMRSA
Type II	class A	type-2 (*ccrA2* and *ccrB2)*	haMRSA
Type III	class A	type-3 (*ccrA3* and *ccrB3)*	haMRSA
Type IV	class B	type-2 (*ccrA2* and *ccrB2)*	caMRSA
Type V	class C2	type-5 (*ccrC1)*	caMRSA
Type VI	class B	type-4 (*ccrA4* and *ccrB4)*	haMRSA
Type VII	class C1	type-5 (*ccrC1)*	caMRSA
Type VIII	class A	type-4 (*ccrA4* and *ccrB4)*	caMRSA
Type IX	class C2	type-1 (*ccrA1* and *ccrB1)*	caMRSA
Type X	class C1	type-7 (*ccrA1* and *ccrB6)*	caMRSA
Type XI	class E	type-8 (*ccrA1* and *ccrB3)*	caMRSA

Adapted from the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (Elements, 2009)

*MRSA type – SCC*mec* type commonly identified in and associated with caMRSA or haMRSA (Coombs et al., 2013a)

SCC*mec* - *staphylococcal* cassette chromosome *mec* *mec* – meticillin resistance

ccr – cassette chromosome recombinase genes

1.3 Origins of caMRSA

Initial speculation on the emergence of caMRSA suggested that caMRSA isolates were haMRSA strains that had escaped and spread into the community (Eady and Cove, 2003). However, the currently held hypothesis is that caMRSA isolates emerged *de novo* in the community setting, where SCC*mec* was inserted into diverse *S. aureus* lineages (Kreiswirth et al., 1993, Musser and Kapur, 1992, Charlebois et al., 2004, Chini et al., 2006). This view is supported by a fifteen-year study examining the changing epidemiology of MRSA that showed caMRSA and haMRSA isolates from a single geographic region were epidemiologically and genetically diverse from each other. Although, in some instances community strains were traceable to a healthcare setting (Crum et al., 2006).

1.4 Defining caMRSA

Inconsistencies exist in the literature in the definition of caMRSA. This has been illustrated in two meta-analyses (Salgado et al., 2003, Dukic et al., 2013) and in a systematic review (Beam and Buckley, 2006). In the latter review it was noted that thirteen different definitions of caMRSA have been described in 31 studies. The lack of a standard definition is reflected in the variety of names given to the non-healthcare acquired strains, including community-acquired *S. aureus*, community-associated *S. aureus*, community-onset *S. aureus* and non-multiresistant meticillin-resistant *S. aureus.* As a consequence of these differing definitions, estimating the overall burden of caMRSA is difficult (Köck et al., 2010b, Chua et al., 2011). For consistency, in this review, for studies in which MRSA is referred to without any prefix, the caMRSA or haMRSA status was either unclear or unspecified.

1.4.1 Epidemiological definition of caMRSA

In 2000 the Centers for Disease Control and Prevention (CDC) advocated a standardised definition of caMRSA based on clinical data, timing of MRSA isolation relative to time of patient admission to hospital and a lack of healthcare risk factors for the acquisition of MRSA (CDC, 2009a). Accordingly, MRSA isolates that were identified in a community setting or within 48 to 72 hours of hospital admission were classified as caMRSA (Salgado et al., 2003, Carleton et al., 2004). However, epidemiological approaches to defining caMRSA, based on place of acquisition (i.e. hospital or community) do not take into account the differing molecular profiles of MRSA strains circulating in the community and the health care settings. This suggests some MRSA isolates circulating in the community setting are in fact identical to strains identified as haMRSA strains according to epidemiological definitions (Chua et al., 2011, David et al., 2008).

A recent paper has suggested that a multivariable model to classify MRSA as community-associated or healthcare-acquired be implemented to simplify and more accurately distinguish between MRSA types in healthcare settings (Sievert et al., 2012). The authors suggest that age, type of infection, susceptibility pattern and hospitalisation of patient during infection are a more reliable method for classifying MRSA types.

1.4.2 Molecular definition of caMRSA

To address the concerns in defining caMRSA epidemiologically, Kluytmans-VandenBergh & Kluytmans (2006) proposed the most reliable way to distinguish caMRSA from

haMRSA is through a combination of molecular typing and collection of epidemiologic data from the patient. Such an approach is more comprehensive, as both time-based criteria and genetic analysis of the strain are included. Molecular definitions of caMRSA rely on SCC*mec* typing and polymerase chain reactions (multiplex PCR/real-time PCR). PCR is used to identify the presence of *mecA* (resistance gene) and *femA* (housekeeping gene unique to *S. aureus*), and is a reliable, time efficient and reproducible technique. However PCR only confirms MRSA status, and fails to differentiate between strains of haMRSA and caMRSA. Similarly, relying on SCC*mec* typing gives the researcher information on the SCC*mec* carried by a strain and provides information on the origins and spread of MRSA, but is unreliable in distinguishing caMRSA from haMRSA, as a pandemic strain of haMRSA characteristically carries SCC*mec* IV, a SCC*mec* usually associated with caMRSA. As a result, more discriminatory genotypic techniques have been utilised to produce a definitive distinction between the different types of MRSA.

1.4.3 Genotypic typing of caMRSA and classification of strain type

Typing of MRSA varies from laboratory to laboratory depending on the depth of analysis required. For research purposes highly discriminatory techniques are used in the analyses of MRSA strain types. Typing of MRSA strains to achieve intralaboratory and interlaboratory comparisons rely on unambiguous and highly reproducible data, as well as international standardised nomenclature (Tong et al., 2012). Three different genetic techniques are commonly used in the classification and differentiation of strains for research purposes; these are macro-restriction pulsed field gel electrophoresis (PFGE), multi locus sequence typing (MLST) and *S. aureus* specific staphylococcal protein A (*spa* typing) (Stefani et al., 2012).

PFGE is considered a gold standard in the investigation of outbreaks (Deurenberg and Stobberingh, 2008). This technique separates *Sma*I-digested DNA fragments of the genome by size. The PFGE pattern of an isolate can be compared to patterns already encountered using the FPQuest database. PFGE typing assigns isolates into clusters based on strain similarity and is particularly useful during epidemiological investigations due to the highly discriminatory power of the technique (Reed et al., 2007). However, PFGE has limited portability and uses numerous nomenclatures depending on the currently utilised classification system in each country (Tian et al., 2013).

MLST is another technique used in the classification of MRSA for research purposes. The basis of this technique involves sequencing seven housekeeping genes (*arc, aro, glp, gmk, pta, tpi, yqi*), which assigns a sequence type (ST) number to isolates that are identical based on the combination of different alleles (Enright et al., 2000b). The isolates' ST can be compared with other strains using the eBURST V3 program on the *Stapylococcus aureus* – Database (saureus.mlst.net) [Accessed 17 August 2015]. MLST is also used to group MRSA into clonal complexes (e.g. CC22), which comprise genetically related ST types and share at least five loci in common with at least one other member of the group. Together, MLST and SCC*mec* typing are used to describe an MRSA genotype (e.g. ST93-IV). As MLST is both time-consuming and expensive to perform, this technique is not considered to be suitable for routine use (Strommenger et al., 2006b). However, as this typing method is sequence based it is more portable and allows for strain comparisons geographically.

For clinical use, *spa* typing is a preferred alternative as it is time efficient, highly reproducible and uses standardised nomenclature. The standard nomenclature for *spa* typing is given as *t* followed by a number (e.g. *t008)*. *Spa* typing sequences the polymorphic X region of the protein A gene (present in all strains of *S. aureus*). The *spa* database is the leading global database for the typing of *S. aureus* and can be freely accessed using the Ridom SpaServer (www.spa.ridom.de) (Stefani et al., 2012). However this technique is not without flaws as distinct lineages can contain the same *spa* type due to the fact this technique only sequences a small section of the *S. aureus* genome (Strommenger et al., 2006b). For example ST22 can contain several different *spa* types (e.g. *t005, t022, t032, t310)*. Conversely a *spa* type can be assigned as several distinct ST types by MLST, for example *t001* can be classed as an ST5, ST222 or ST228. A combination of genotypic testing can resolve this issue.

For more in-depth molecular investigations into transmission, persistence, virulence and identification of different strains at a gene expression level DNA microarrays and single nucleotide polymorphisms (SNP's) can be utilised. DNA microarrays provide a large amount of information about an isolate's genome and are able to detect genes associated with antibiotic resistance, adherence, virulence and pathogenicity, as well as identify SCC*mec* type. This technique allows for concurrent hybridisation of an isolate's genome against the complete available gene content of multiple *S. aureus* genomes (Miller and Tang, 2009, Shore et al., 2012). Use of this technique is however, restricted to specialised research laboratories, as sophisticated equipment is required for bioinformatic analyses of the data generated by the assay. Another disadvantage of this technique relates to the limited discriminatory power between irregular single locus variant ST types with pandemic ST types. SNP investigations are useful in determining evolutionary similarities

and in epidemiological tracking, and are able to differentiate between related isolates that are specific to certain healthcare settings (Prosperi et al., 2013, Nübel et al., 2012, Harris et al., 2010). As with DNA microarrays, use of this technique is considered time consuming and expensive and therefore not suitable for routine typing (Nübel et al., 2012, Shore et al., 2012).

1.5 Antibiotic resistance

Antibiotic resistance and infection with caMRSA and haMRSA is of growing concern to the health of the general public (WHO, 2013). At present Australia is one of the leading prescribers of antibiotics in primary care in the developed world, with over 22 million prescriptions written annually. The most commonly prescribed antibiotic classes include penicillins and macrolides. It is also reported that of all medicinal classes dispensed in general practice, antibiotics are the most frequently prescribed as a treatment option (McKenzie et al., 2013).

1.5.1 Antibiotic resistance and treatment of infections

Over 90% of all *S. aureus* strains are resistant to penicillin (Kennedy and DeLeo, 2009). Strains of caMRSA are typically resistant to beta-lactam (β-lactam) antimicrobials, and are usually susceptible to non-β-lactam antimicrobials, although there is some variability between strains (Alex and Letizia, 2007). This pattern of resistance is different to haMRSA, which are typically resistant to β-lactams and are also commonly resistant to three of more classes of non-β-lactam antibiotics (erythromycin, clindamycin, tetracycline, co-trimoxazole, ciprofloxacin, gentamicin) (Coombs et al., 2013b). Resistance in haMRSA

is usually observed against three or more classes of non-β-lactam antibiotics (Chavez and Decker, 2008).

A systematic review and meta-analysis of 76 clinical studies investigating the relationship between the use of antibiotics and MRSA isolation among 4365 MRSA positive patients and 19865 control participants reported that those who used quinolones had the highest risk of acquiring MRSA, followed by patients who had received treatment with glycopeptides and then cephalosporins (Tacconelli et al., 2008). This study further found that those who had received antibiotic therapy as opposed to those patients who did not receive antibiotics had an almost two-fold risk of acquiring MRSA. Additionally, a study by Lo and colleagues (2007) investigated the association of caMRSA resistance with antibiotic use in the 12 months prior to testing of participants. The authors reported a strong association between use of antibiotics and nasal caMRSA carriage. A previous study has also reported a strong association exists between antibiotic use in the last six months and MRSA infection in participants (Baggett et al, 2002).

Of further concern has been the emergence of vancomycin resistant *S. aureus* (VRSA), as vancomycin was once considered a last line treatment option for MRSA infections (Appelbaum, 2006). Resistance to vancomycin in *S. aureus* is conferred by the *vanA* gene (Holmes et al., 2012). VRSA strains have been reported in the US, India, Pakistan, Africa and Iran (Zarifian et al., 2013, Holmes et al., 2012, Gould, 2013, Saravolatz et al., 2012, Moravvej et al., 2013, Goud et al., 2011, Tenover et al., 2004, Sievert et al., 2008, Onanuga and Temedie, 2011, Azimian et al., 2012). However, confirmed cases of VRSA remain infrequent globally (Gould, 2013).

1.5.2 Empirical treatment of caMRSA infections and drug development

Commonly used antibiotic treatment regimes vary from country to country depending on each country's clinical guidelines for the treatment of MRSA infections, and are also guided by the antibiotic susceptibility profile of the isolate causing the infection. Recommended antibiotics to treat caMRSA infections in Australia include clindamycin, doxycycline and trimethoprim-sulfamethoxazole (Queensland Government, 2012, Eliopoulous et al., 2011). In the US the Infectious Diseases Society of America provides clinicians with guidelines for the treatment of MRSA infections (Bhargava et al., 2013, Liu et al., 2011). Commonly used antibiotics to treat caMRSA infections include clindamycin, doxycycline, trimethoprim-sulfamethoxazole, rifampicin, vancomycin and linezolid (Eliopoulous et al., 2011, Skov et al., 2012, Moellering, 2008, Cosgrove and Fowler, 2008). In Europe, clindamycin is the predominantly prescribed antibiotic used to treat caMRSA infections (Eliopoulous et al., 2011). Inducible clindamycin resistance has been reported globally, including in Australia, in isolates initially observed to be sensitive to clindamycin but resistant to erythromycin (Vlack et al., 2006, Ellington et al., 2009, Lewis and Jorgensen, 2005, Chavez-Bueno et al., 2005, Delialioglu et al., 2005, Das et al., 2013).

Due to the increasing resistance of MRSA to currently available antibiotics, newer therapeutic antibiotics have been developed as potential alternatives to treat caMRSA infections (Wilcox, 2011, Welte and Pletz, 2010). These include ceftobiprole (available in the UK to registered users), ceftaroline fosamil (US FDA approved and approved by the European Commission) (Laudano, 2011), telavancin (approved in Canada and US FDA approved) (Plotkin et al., 2011, Rubinstein et al., 2011) and tigecycline (US FDA

approved) (Stein and Craig, 2006). Newer antibiotics for the treatment of MRSA include dalbavancin (awaiting US FDA approval) (Lin et al., 2006, Paknikar and Narayana, 2012), and oritavancin, (under standard review by the US FDA) (Paknikar and Narayana, 2012). More recent antibiotic developments include epimerox, which has been tested in preclinical studies (Schuch et al., 2013), tedizolid, which is expected to be submitted to the US FDA as a new drug for approval during the later months of 2013, and a naturally derived antibiotic anthracimycin, which is awaiting human trials (Jang et al., 2013).

In Australia a potential new antibiotic that targets a critical enzyme involved in metabolic processes has been developed by mutating a derivative of *S. aureus* Biotin Protein ligase (SaBPL) known as 'leaky mutant' *Sa*BPL-R122G. However, this drug is in the very early stages of development and is awaiting testing in animal models (Tieu et al., 2013). (Burrowes et al., 2011)

Historically, an alternative to antibiotic therapy in the treatment of infections has included bacteriophage (phage) therapy (Burrowes et al., 2011, Miedzybrodzki et al., 2007, Housby and Mann, 2009); a therapy first used in the 1920's. With the increased incidence of antibiotic resistance, renewed interest in old therapies such as bacteriophage therapy has increased since the late 1990's (Vandamme, 2014, Mediavilla et al., 2012).

1.6 Carriage, colonisation and pathogenic mechanisms of caMRSA

1.6.1 Mechanisms of adherence and immune evasion

Although often used interchangeably, the term 'carriage' of MRSA simply refers to the presence of the bacterium at the time an individual was sampled whereas 'colonisation' refers to the persistence of the bacterium in an individual, on multiple sampling occasions, without signs of infection or disease. Researchers have proposed that colonisation by *S. aureus* is in part due to envelope-associated proteins produced by the organism, which promote adherence to cell surfaces through biofilm and capsule formation, which are deemed essential for attachment to occur (Patti et al., 1994, Pohlmann-Dietze et al., 2000, Otto, 2012, Otto, 2008). Successful adherence of *S. aureus* is also reliant on host factors. The role host factors play in colonisation is important as persistence may be due to secretion of antimicrobial peptides, variability in host adhesions or differences in the regulation of the immune response (Peacock, 2010, Zanger et al., 2011). Another factor that may influence colonisation is the composition of the microflora present at the body site. However, further research is needed to understand factors involved in the establishment of colonisation and the role bacterial and host factors play in persistence (Wertheim et al., 2005, Johannessen et al., 2012).

Biofilms and other proteins involved in microbial adhesion

Biofilms enable *S. aureus* to attach to a surface through production of extracellular matrix, and afford the bacteria protection from host defences as well as antibiotics (Otto, 2012, Otto, 2008). An important class of adhesins expressed by *S. aureus* are microbial surface components recognising adhesive matrix molecules (MSCRAMMs). These proteins enable attachment of *S. aureus* to host tissues and are highly associated with the ability to cause

infection (Atshan et al., 2012, Mazmanian et al., 2000). MSCRAMMs typically comprise clumping factors A and B (*clfA, clfB*), *Staphylococcal* protein A (*spa*), fibronectin-binding proteins A and B (*fnbA, fnbB*), bone sialoprotein-adhesin gene (*bbp)*, collagen binding protein (*cna*) and *Staphylococcus aureus* surface protein G (*sasG)* (Gordon and Lowy, 2008, Bien et al., 2011, Lowy, 1998).

In addition to proteins that encode specifically for adherence functions, several other bacterial factors are known to play a role in *S. aureus* adhesion during nasal colonisation. These comprise wall teichoic acid (WTA), autolysin SceD (*sceD*) and the iron-regulated transferrin-binding protein (*isdA*) (Weidenmaier et al., 2004, Wertheim et al., 2008, Kiser et al., 1999, Corrigan et al., 2009).

Polysaccharide capsule and other S. aureus-specific proteins involved in immune evasion

In *S. aureus,* the polysaccharide capsule plays an important role in immune system evasion and adhesion during colonisation, and has been reported to increase resistance (Watts et al., 2005). To date, 11 capsule serotypes have been identified in *S. aureus*, with serotypes 5 and 8 being predominantly involved in infection (Havaei et al., 2013, Watts et al., 2005, Sompolinsky et al., 1985).

Other *S. aureus*-specific proteins that have been shown to have a major role in immune evasion include staphylococcal complement inhibitor (*scn*), chemotaxis inhibitory protein

of *S. aureus* (*chp*), staphylokinase (*sak*), extracellular fibrinogen binding protein (*fib*) and extracellular adherence protein (*map*) (Bien et al., 2011).

1.6.2 Gene expression patterns during colonisation

An important study by Burian and colleagues (2010) has shed light on the gene expression patterns of *S. aureus* during nasal colonisation in humans and has contributed significantly to the understanding of the interaction between host and pathogen. Their study directly characterised global regulators, toxins, adhesion and cell-wall modification enzymes, stress response and metabolic regulators, and immune evasion and immune modulation factors to profile the expression pattern of nasal colonisation in persistent carriers. They reported that adhesive molecules, some genes involved in cell surface dynamics and immune-modulation factors are prominently expressed during colonisation of the nose, whereas major toxins are not transcribed.

1.6.3 Regulation of virulence and adherence factors

In vitro, virulence and adherence factors are influenced by several regulators, including accessory gene regulator (*agr*), *Staphylococcal* accessory regulator (*sarA*), the sae locus and alternative sigma factor B (*SigB*) (Bien et al., 2011). Of these regulators, many critical virulence mechanisms and toxins in caMRSA are controlled by *agr* in particular (Novick, 2003, Cheung et al., 2011).

The accessory gene regulator (*agr)* controls pathways involved in exotoxin production and adhesion expression. It is responsible for binding proteins at the beginning of infection

when cell density is low, thereby facilitating adherence to host tissue. Once infection is established, production of toxins and exoenzymes occurs. Timely activation of agr *in vivo* and its importance for *S. aureus* virulence has been demonstrated in laboratory strains (Wright et al., 2005). Another study has reported that *agr* regulation in the USA300 strain resulted in strong expression of toxins and coenzymes and upregulation of fibrinogen binding proteins, which resulted in an increased capacity to bind fibrinogen and increased the expression of meticillin resistance genes (Cheung et al., 2011). The finding that *agr* influences *mecA* expression is in agreement with a previously reported study in which regulatory interdependence of *mec* and *agr* systems was observed (Queck et al., 2008).

1.6.4 Virulence determinants

Virulence factors in *S. aureus* play a crucial role in the damage of the immune system or immunoevasion and in the production of toxic effects (Bien et al., 2011). This section details the major virulence determinants in caMRSA.

Toxins

Toxins are a key group of virulence factors secreted by *S. aureus*. The pore forming alpha-haemolysin or α-toxin (*hla*) gene is a major virulence factor for both caMRSA and haMRSA (Gordon and Lowy, 2008, Bubeck Wardenburg et al., 2007, Kobayashi et al., 2011). Many cell types are susceptible to the action of *hla,* which has been implicated as a cause of cytolytic, hemolytic, dermonecrotic, and lethal activity. Its role in pathogenesis has been demonstrated in several studies of infection with *S. aureus* in animal models (Kernodle et al., 1997, McElroy et al., 1999, Montgomery et al., 2008, Wardenburg and Schneewind, 2008). Other cytolytic toxins produced by caMRSA and haMRSA are the

gamma-haemolysin group (*hlgA, hlgB, hlgc), which* also play a role in the destruction of leucocytes (Yoong and Torres, 2013, Foster, 2005).

Both caMRSA and haMRSA produce an additional group of superantigen toxins, which include staphylococcal enterotoxins (*egc-cluster*, *sea*, *seb*, *sec*, *sed*, *sef*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *seq* and *ser*), exfoliative toxins A and B (*etA* and *etB*) and toxic shock syndrome toxin-1 (*tst1*) (Hu et al., 2008, Robert et al., 2011). Of the enterotoxins, *sea* is the predominant type in clinical and food related strains (food poisoning) (Lim et al., 2012, Yan et al., 2012). The *egc-cluster* includes superantigen genes *seg*, *sei*, *selm*, *seln* and *selo* (Chini et al, 2006), and is usually found in 46-66% of *S. aureus* strains known to cause invasive infection (Grumann et al, 2013). Exfoliative toxins A or B are involved in scalded skin syndrome, although only a small subset of strains can produce this toxin, and *tst1* is responsible for toxic shock syndrome (Berk and Bayliss, 2010). An additional virulence factor unique to caMRSA and predominantly found in the USA300 strain is the arginine catabolic mobile element (ACME). ACME is responsible for the evasion of host defensive responses and assists in the colonisation of various tissues (Miller and Diep, 2008, Diep et al., 2006). However, the direct link between ACME and the ability to cause colonisation needs to be further explored (Liu, 2009).

Panton-Valentine Leukocidin toxin (PVL)

Panton-Valentine Leukocidin toxin (PVL) is a virulence factor found in many caMRSA strains, but absent in haMRSA (David and Daum, 2010). PVL is a two-component membrane toxin (*lukF-PV, lukS-PV)* that targets leukocytes and causes tissue necrosis (Chi et al., 2013, Lina et al., 1999). Isolates positive for PVL production have been

epidemiologically linked to recurrent skin infections (Ritz and Curtis, 2012, Chambers, 2005). Monecke and colleagues (2006) have reported PVL positive isolates are capable of causing deep and chronic skin infections, and necrotising pneumonia, associated with a high mortality rate, and Tseng and colleagues (2009) have reported PVL contributes to inflammation and muscle tissue injury. However, the role of PVL as a major virulence determinant in caMRSA has been questioned after it was observed that strains lacking the PVL toxin were as virulent as PVL positive strains in a mouse model of sepsis and abscess (Voyich et al., 2006, Otto, 2010).

The majority of epidemiological and clinical findings have reported that PVL has little effect on caMRSA virulence in studies that have used murine or simian models of infection (Lo & Wang, 2011; Bubeck et al, 2008). However, it has been suggested that the contribution of PVL to caMRSA disease could be limited to a specific host genetic background or that an effect may be too subtle to detect in these models of pathogenesis, or that animal models are unable to accurately reflect human disease (Kobayashi and DeLeo, 2009). PVL was demonstrated to bring about rapid cell death in human and rabbit neutrophils, whereas this effect was not observed in simian cells or murine cells (Löffler et al., 2010, Crémieux et al., 2009). Simian neutrophils were found to have reduced susceptibility to PVL compared to human neutrophils (Loffler et al., 2010, Szmigielski et al., 1999), whereas rabbit neutrophils were observed to have an increased susceptibility to cytolysis mediated by PVL (Diep et al., 2010).

Phenol-soluble modulins (PSMs)

A protein family of phenol-soluble modulins (PSMs) is suspected to play a greater role than PVL in the virulence of this organism (Kobayashi et al., 2011, Otto, 2012, Wang et al., 2007, Li et al., 2009). Strains that produce PSMs are reported to have enhanced *in vitro* and *in vivo* leukocidal, pro-inflammatory and chemotactic activities (Wang et al., 2007, Queck et al., 2009). Previous research has reported that the production of this protein was typically higher among caMRSA strains known to be more virulent (Wang et al., 2007, Li et al., 2009). It has also been reported that haMRSA strains often lack production of this protein or produce PSMs at a reduced level (Wang et al., 2007). Wang and colleagues have reported that over-expression of PSMs in a haMRSA strain had the ability to cause leukocidal activity equal to that observed in caMRSA strains (Wang et al., 2007). Their study indicated expression of these peptides is the main cause for the observed difference in lytic activity between caMRSA and haMRSA and that PSM's may be a major contributor to increased pathogenic potential of caMRSA strains. The key genetic differences between caMRSA and haMRSA, as outlined in the previous sections, are summarised in Table 1.2.

Table 1.2 Genotypic and phenotypic differences between caMRSA and haMRSA types

Characteristic	caMRSA	haMRSA
SCC*mec*[a, b, h]	Type IV, V, VII or VIII	Type I, II, III or VI
Antibiotic susceptibility[a, c, j]	Typically resistant to β-lactam antibiotics	Typically resistant to β-lactam antibiotics and commonly resistant to three or more classes of non-β-lactam antibiotics (erythromycin, clindamycin, tetracycline, co-trimoxazole, ciprofloxacin, gentamicin)
PVL[a, d, f, g, h]	More common*[h]	Less common^[h]
PSMs[e, f, g, i]	Found in highly virulent strains	Protein absent or expressed at a lower level

SCC*mec* - staphylococcal cassette chromosome *mec* PVL – Panton-Valentine leukocidin toxin PSMs – phenol soluble modulins

*[h] 31.6% (87/275) of total caMRSA in Australia from 31 different laboratories nationwide in 2011 were found to possess this toxin

^[h] 0.23% (1/428) of total caMRSA in Australia from 31 different laboratories nationwide in 2011 was found to possess this toxin

[a](David and Daum, 2010) [b](Elements, 2009) [c](Chua et al., 2011) [d](DeLeo et al., 2010) [e](Gonzalez et al., 2012)

[f](Otto, 2012) [g](Otto, 2013) [h](Coombs et al., 2012a) [i](Li et al., 2009) [j](Coombs et al., 2013b)

1.7 Carriage of caMRSA in humans

Carriage refers to the presence of an organism on or in the body without clinical signs and symptoms of infection. Carriage status can be classified into three categories (persistent, intermittent and non-carrier) depending on the organism's ability to colonise the host (Muthukrishnan et al., 2013, Kajita et al., 2007, Van Belkum et al., 2009). Persistent carriers are reported to carry a single strain, whereas intermittent carriers may be carriers of different strains over time (Chen et al., 2013, Peacock et al., 2001). Colonised persons are at greater risk of developing a subsequent infection with the same strain (Wertheim et al., 2005, Von Eiff et al., 2001, Murphy et al., 2011).

The most important site for MRSA carriage on the human body is the anterior nares (Mermel et al., 2011), followed by the throat, although some researchers have reported a greater number of participants carrying MRSA in their throats compared to the nares (Nilsson and Ripa, 2006, Hamdan-Partida et al., 2010, Nakamura et al., 2010). It is estimated that around one third of the general healthy population asymptomatically carries the *S. aureus* bacterium in their nares (Frank et al., 2010, Muthukrishnan et al., 2013). A recent study has observed an increased risk of invasive disease in patients with a high nasal burden of MRSA after accounting for host factors for infection (Datta et al., 2014). Other body sites known to harbour caMRSA and haMRSA include the axilla, groin, perineum, gastrointestinal tract and vagina (Bourgeois-Nicolaos et al., 2010, Wallin et al., 2008, McKinnell et al., 2013). Carriage sites for MRSA can act as a reservoir in the dissemination of this bacterium to other body sites, or other people, or animals.

1.7.1 Assessment of multiple anatomical swab sites for detecting MRSA carriage

Although nasal carriage of caMRSA and haMRSA has been examined extensively in the majority of prevalence studies, some authors have suggested that collecting samples from the nares only is insufficient for accurate MRSA detection (Mertz et al., 2009, Widmer et al., 2008, Ringberg et al., 2006, McKinnell et al., 2013). A study examining persistent nasal and throat colonisation of 1243 healthy community members reported that had they only sampled the nares 38% of exclusive throat carriers would have been missed (Hamdan-Partida et al., 2010). They also reported that MRSA can colonise the throat persistently over a period of many years and may contribute to the spread of MRSA. The consensus among these and other researchers is that sampling of nares and an additional site increases the isolation rate of MRSA (Bitterman et al., 2010, Widmer et al., 2008, Marshall and Spelman, 2007, Batra et al., 2008, Currie et al., 2008, Eveillard et al., 2006, Senn et al., 2012, Hamdan-Partida et al., 2010).

1.7.2 Reservoirs of caMRSA in the community

Reservoirs of caMRSA in the community include humans and companion animals colonised or infected with MRSA, as well as contaminated household fomites (Silbergeld et al., 2008, Issmat I, 2011, Knox et al., 2012). Several common modes of spread of caMRSA have been identified. Significant routes for the transfer of caMRSA include skin-to-skin, and skin-to-fomite contact. One study suggested that skin-to-skin contact with persons colonised or infected with caMRSA was primarily responsible for the transfer of organisms (Kazakova et al., 2005). Furthermore, individuals colonised with caMRSA often spread the organism via hand contamination (Calfee et al., 2003, Grundmann et al., 2006). This finding, coupled with findings reported by Ojima and colleagues (2002),

suggest that inadequate personal hygiene is a major factor that assists in the spread of MRSA (Ojima et al., 2002). Recent studies have also shown that MRSA may be spread between close household contacts as well as companion animals (Rafee et al., 2012, Morris et al., 2012).

1.8 Prevalence of caMRSA

Studies on the prevalence of caMRSA infection and carriage have been difficult to compare due to a lack of consistency in the criteria used to distinguish caMRSA from haMRSA. Reported prevalence of caMRSA carriage in the general community tends to vary considerably from study to study and across different geographic regions. Numerous studies in community and in persons in non-institutionalised settings have reported a low caMRSA prevalence of less than 3% in the general population (Munckhof et al., 2009). In these studies, where genotypic analyses of MRSA were performed, caMRSA strains ST1649 (Bartoloni et al., 2013), ST95 and CC5 (Munckhof et al., 2009) were isolated. Reported prevalence of caMRSA carriage in general community groups ranges from 0.26% to 9.2% (den Heijer et al., 2013, Bratu et al., 2006, Nakamura et al., 2002, Farley, 2008). The prevalence at least partly depends on the definition of caMRSA used and the type of setting investigated. Therefore, comparisons of prevalence of this organism are difficult due to the vast differences in criteria used. This section explores and highlights the prevalence of caMRSA in different geographical regions and the predominant MRSA ST and clonal types by region. Strain or clone characterisation is dependent on the genotypic test/s used to type the isolate and the test/s power of discrimination. Tenover and colleagues (1995) define a strain 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" and define a clone as "*a group of isolates that are indistinguishable from each other by a variety of genetic tests... or that are so similar that they are presumed to be derived from a common parent*". Hence the definitions of strain and clone are similar.

1.8.1 Prevalence of caMRSA in the US and predominant strain sequence types (STs)

In the USA the first prototypical caMRSA strain was isolated in 2000, termed the USA300 strain (ST8). The USA300 strain has since become epidemiologically important as a cause of infection and carriage in several distinct populations across various geographic regions, not only in the US, but in other parts of the world (Miller and Diep, 2008). The international spread of this strain has led to an increasing incidence of infection in both Canada and parts of Europe (Adam et al., 2007, Wannet et al., 2005). The predominant caMRSA and haMRSA strains isolated in the US from the community and healthcare establishments include ST1 (caMRSA), ST5 (caMRSA and haMRSA), ST8/USA300 (caMRSA), ST22 (haMRSA), ST36 (haMRSA), ST45 (caMRSA and haMRSA), ST105 (haMRSA), ST225 (haMRSA) and ST239 (haMRSA) (Stefani et al., 2012, Hudson et al., 2013, Mediavilla et al., 2012).

The majority of studies investigating caMRSA carriage have come from the US, where several population-based studies examining the prevalence of MRSA in the community as well as healthcare settings have been undertaken (Albrich and Harbath, 2008, Bratu et al., 2006, Dimitrov et al., 2003, Gorwitz et al., 2008, Tenover et al., 2008, Klein and Laxminarayan, 2013, Jarvis et al., 2012). A US study by Tenover et al (2008) of nasal MRSA carriage in 19,412 non-institutionalised patients reported 1.08% (209/19412) of all

participants carried MRSA. Another significant US population-based study has also reported the prevalence of nasal MRSA carriage in healthy adults in the general community to be low, with a 0.84% (75/9622) carriage prevalence (Graham et al., 2006).

Community-associated MRSA carriage studies in university and college students have also been conducted (Bearman et al., 2010, Ammons et al., 2010, Morita et al., 2007, Shen et al., 2013). In a study of healthy university students the prevalence of caMRSA was reported to be 1.6% (16/1000) (Bearman et al., 2010). Similarly, a prevalence of 1.6% (375) caMRSA nasal carriage has been reported in another study of university students (Ammons et al., 2010). A smaller US study of college students has, however, reported a higher caMRSA carriage prevalence of 3% (3/100) (Morita et al., 2007).

The carriage of caMRSA has also been investigated in countries adjoining the US, where prevalence has been reported to be higher in certain cohorts. For example, a 5.63% (33/586) caMRSA carriage prevalence has been reported in Canadian non-healthcare and healthcare workers (Kottler et al., 2010). Whilst, in a Mexican study of nasal and throat carriage from 1243 healthy volunteers attending schools or working in factories showed 107 participants carried MRSA, a prevalence of 8.6% (Hamdan-Partida et al., 2010). This study also reported throat carriage was most common and observed the majority of MRSA carriers to be of a young age, between 1 to 10 years old.

1.8.2 Prevalence of caMRSA in Europe and predominant strain sequence types (STs)

In Europe, the ST80 strain is generally considered to be the predominant caMRSA type (David and Daum, 2010, Mediavilla et al., 2012). An investigation of caMRSA in certain parts of Europe has reported an increase in the prevalence of the ST80 strain (Grisold et al., 2009). Other frequently isolated caMRSA and haMRSA strains across Europe in both community and healthcare epidemiological investigations, include ST5 (caMRSA and haMRSA), ST8 (caMRSA), ST22 (haMRSA), ST30 (caMRSA), ST36 (haMRSA), ST45 (caMRSA and haMRSA), ST105 (haMRSA), ST228 (haMRSA), ST239 (haMRSA) and ST250 (haMRSA) (Espadinha et al., 2013, Fankhauser et al., 2013, Stefani et al., 2012, Johnson, 2011, Grundmann et al., 2010, Aschbacher et al., 2012, Bartels et al., 2007, Mediavilla et al., 2012).

Prevalence of caMRSA in healthy community members in Europe tends to vary from country to country. Countries such as Sweden and the Netherlands experience rates of less than 1% of MRSA carriage. A low carriage rate of MRSA (0.12%) has also been reported in an Italian study of 812 volunteers (Zanelli et al., 2002). On the other hand, carriage rates of around 3% have been reported in Belgium (Stam-Bolink et al., 2007, Tiemersma et al., 2004, Elston and Barlow, 2009). In contrast, a more recent study of nasal MRSA carriage in healthy volunteers attending healthcare institutions in Malta reported a high caMRSA prevalence of 6.99% (23/329) (Scerri et al., 2013). In clinical settings in Austria and other parts of Europe the prevalence of caMRSA was reported to have increased from 4% in 2005 to 8% in 2006 (Diederen and Kluytmans, 2006, Krziwanek et al., 2007).

In the UK a low prevalence of caMRSA has been observed in a community-based prevalence study (Abudu et al., 2001). Findings from this study reported a nasal MRSA carriage rate of 1.5% (4/274) in the general adult population. An Irish study of caMRSA nasal carriage of 1000 healthy university students reported a low prevalence with a total of seven strains isolated. Interestingly, when the demographic data was analysed to exclude those with healthcare-associated risk factors only five of 879 participants were considered to have true community MRSA carriage, a prevalence of 0.7%. Of these positive carriers, it was noted that all participated in sports. In a recent study of MRSA carriage in adults (n=362) and children (n=168) attending general practices in the UK a prevalence of 1.1% in adults and 3.6% in children was reported (Gamblin et al., 2013). Notably, it has been 12 years since MRSA carriage was investigated in a community population in the UK since the previous study undertaken by Abudu and colleagues (2001).

Possible explanations for the variance in MRSA carriage rates between European countries could be due to current infection control policies within each country, such as Netherland's active 'search and destroy' policy associated with one of the lowest rates of colonisation with MRSA in the world (van Rijen and Kluytmans, 2009). However, no common European strategy has been developed or implemented to control the spread of caMRSA and only a limited number of European countries have developed national strategies for the prevention and surveillance of caMRSA (Köck et al., 2010b).

1.8.3 Prevalence of caMRSA in Australia and predominant strain sequence types (STs)

Novel MRSA strains were first isolated in persons without prior risk factors for the acquisition of MRSA in 1989 from an indigenous community in Western Australia. The

first isolated caMRSA strains were designated as the WA strain (WA-MRSA-1) (Udo et al., 1993). A second strain of caMRSA termed the ST93 strain was identified in Queensland in 2000 (Munckhof et al., 2003). A third caMRSA strain, the Southwest Pacific clone (ST30) has also been isolated from various regions across Australia (Gosbell et al., 2001). Since the initial documentation of these three strains they have been identified Australia wide (Coombs et al., 2012a) At present the most frequently isolated MRSA strains recovered from healthcare settings in Australia, comprising community and healthcare-associated strains, include ST1 (caMRSA), ST5 (caMRSA and haMRSA), ST22 (haMRSA), ST30 (caMRSA), ST45 (caMRSA), ST78 (caMRSA), ST93 (caMRSA) and ST239 (haMRSA) (Stefani et al., 2012, Coombs et al., 2012a, Verwer et al., 2012, Mediavilla et al., 2012).

The majority of studies detailing caMRSA prevalence in Australia have been conducted in a clinical setting (Vlack et al., 2006, Coombs et al., 2012b). The epidemiology of MRSA from clinical and surveillance isolates recovered from healthcare settings has been well documented nationwide by the Australian Group on Antimicrobial Resistance (AGAR). In a recent publication the AGAR group reported the total proportion of all *S. aureus* isolates identified as caMRSA rose from 6.5% in 2005 to 12.5% in 2012, with ST1, ST30 and ST93 being the top three predominant caMRSA clones isolated during 2012 (Coombs et al., 2013a).

In an earlier study detailing the prevalence of MRSA in patients across healthcare settings and residing in long-term care facilities in Australia, Nimmo and colleagues reported an increase in caMRSA prevalence over a period of four years, with the total number of

caMRSA strains recovered rising from 4.7% (118/2498) in 2000 to 7.3% (194/2652) in 2004 (Nimmo et al., 2006). In this study conducted in the year 2000, the three most prominent caMRSA strains were the ST1, ST30 and ST93. These strains were also reported to be the predominant types during 2004. In an Australian study of nasal carriage in rural inpatients an MRSA prevalence of 7% (13/185) was reported, and all strains isolated in their study were identified to be haMRSA (Mitchell et al., 2009). A more recent Australian study investigating nasal MRSA carriage in healthcare workers reported 3.4% (52/1542) of participants to be carriers (Verwer et al., 2012). In another recent study of MRSA in Australia examining nasal, throat and skin lesion carriage in two groups attending the Royal Darwin Hospital, caMRSA carriage was found to be 10.4% (21/201) in inpatients and 0.9% (2/225) in an admission group (Brennan et al., 2013).

There are very few studies detailing caMRSA carriage rates in healthy members of the general community in Australia. Munckhof and colleagues formally assessed the nasal carriage of caMRSA in the healthy adult population (n=303) and in participants attending general practices (n=396) (Munckhof et al., 2009). In their study they defined caMRSA strains as per the criteria recommended by the Centers for Disease Control (CDC). The overall findings in their study indicated that nasal carriage rates in the community were low, with only 0.3% (2/699) of participants harbouring caMRSA. Genetic analysis of caMRSA isolates in their study identified one caMRSA ST93 strain and one CC5. Another Australian study examined nasal, throat and wound carriage of MRSA in indigenous children, and a prevalence of 15% (14/157) was reported (Vlack et al., 2006), with 8% (7/92) of children carrying MRSA in their noses and 1% (1/91) of children carrying MRSA in their throats, while 29% (7/24) of children with wound lesions were also

carriers. In their study, eight isolates from carriers were identified to be caMRSA strains, comprising ST93 (n=6) and ST30 (n=2), and six isolates were haMRSA ST5.

1.8.4 Predominant MRSA strains and clones by region

Certain clones of MRSA are noted to predominate in certain geographical regions (Mediavilla et al., 2012, Chua et al., 2011, Cheng et al., 2013, Tenover et al., 2006). Globally the most frequently isolated MRSA strains spanning 1961 to 2008 belong to clonal complexes CC5, CC8, CC22, CC30 and CC45 (Campanile et al., 2010, Stefani et al., 2012). Table 1.3 illustrates the predominant caMRSA and haMRSA clones and sequence types (ST) reported in different geographic regions (isolates arranged according to ST type in ascending order).

Table 1.3 Most commonly isolated strains of caMRSA and haMRSA - by region

Region	Strain/clone*	Sequence type (ST)	Clonal complex (CC)	MRSA type
Australia[a,b,i,m,n]	WA-MRSA-1	ST1	CC1	caMRSA
	WA-MRSA-3, New York/Japan clone	ST5	CC5	caMRSA/haMRSA
	UK-EMRSA-15	ST22	CC22	haMRSA
	South West Pacific clone	ST30	CC30	caMRSA
	WA-MRSA-23	ST45	CC45	caMRSA
	WA-MRSA-2	ST78	CC88	caMRSA
	Queensland MRSA	ST93	Singleton^	caMRSA
	Aus2 and Aus3 EMRSA	ST239	CC8	haMRSA
Europe[a,c,f,g,h,k,l,n]	Paediatric clone, UK-EMRSA-3	ST5	CC5	caMRSA/haMRSA
	E-MRSA-2/6, USA500	ST8	CC8	haMRSA
	UK-EMRSA-15	ST22	CC22	haMRSA
	South West Pacific clone	ST30	CC30	caMRSA
	UK-EMRSA-16	ST36	CC30	haMRSA
	Berlin clone	ST45	CC45	caMRSA
	European clone	ST80	CC80	caMRSA
	New York/Japan clone~	ST105	CC5	haMRSA
	Italian/Southern German epidemic clone	ST228	CC5	haMRSA
	Brazilian strain/Vienna clone	ST239	CC8	haMRSA
	Archaic haMRSA type	ST250	CC8	haMRSA
Asia[a,d,j,n]	Paediatric clone, New York/Japan clone	ST5	CC5	caMRSA/haMRSA
	USA300	ST8	CC8	caMRSA
	UK-EMRSA-15	ST22	CC22	haMRSA
	South West Pacific clone	ST30	CC30	caMRSA
	Taiwan clone	ST59	CC59	caMRSA
	USA700	ST72	CC72	caMRSA
	Brazilian clone	ST239	CC8	haMRSA
America & Canada[a,e,n]	USA400	ST1	CC1	caMRSA
	USA100, USA800	ST5	CC5	caMRSA/haMRSA
	USA300, USA500	ST8	CC8	caMRSA, haMRSA
	UK-EMRSA-15	ST22	CC22	haMRSA
	USA200	ST36	CC30	haMRSA
	Berlin clone	ST45	CC45	caMRSA
	New York/Japan clone~	ST105	CC5	haMRSA
	Brazilian clone	ST239	CC8	haMRSA

*Strain/clone– defined according to genotypic characterisation and MRSA classification system used in country of isolation

^Distinct clone of *S. aureus* designated by MLST analysis (Chua et al., 2011) ~single -locus variant of ST5 MRSA

[a](Stefani et al., 2012) [b](Coombs et al., 2012a) [c](Johnson, 2011) [d](Rolo et al., 2012) [e](Hudson et al., 2013)
[f](Grundmann et al., 2010) [g](Aschbacher et al., 2012) [h](Bartels et al., 2007) [i](Verwer et al., 2012) [j](Cheng et al., 2013)
[k](Fankhauser et al., 2013) [l](Espadinha et al., 2013) [m](Coombs et al., 2013a) [n](Mediavilla et al., 2012)

1.9 Risk factors for caMRSA carriage

A review of literature investigating risk factors for caMRSA colonisation (Beam and Buckley, 2006) and a meta-analysis by Salgado and colleagues (Salgado et al., 2003), has reported risk factors for caMRSA carriage to include recent hospitalisation (within 24 months), recent outpatient visit (within 12 months), recent nursing home admission (within 12 months), recent antimicrobial use (1-12 months), chronic illness (end stage renal disease), injection drug use, close contact with person/s with risk factors for MRSA acquisition, underlying skin disease, and a high level of physical contact.

1.10 Carriage of caMRSA in risk groups

Globally, high risk groups for caMRSA carriage and or infection have been reported among competitive sport participants (Collins and O'Connell, 2012, Garcia et al., 2012, Kazakova et al., 2005), veterinary personnel (Jordan et al., 2011, Burstiner et al., 2010), homeless people (Borgundvaag et al., 2008), homosexual males (Diep et al., 2008), military recruits (Ellis et al., 2004), prison inmates (Mukherjee et al., 2013, Main et al., 2005, Tattevin et al., 2008), indigenous populations (Baggett et al., 2004, Groom et al., 2001), and children less than five years of age (Huang and Chen, 2011, Sdougkos et al., 2008).

The following risk groups are examined in this literature review: sports participants, household contacts of MRSA positive carriers, dogs and horses and their respective handlers (Section 1.11), and small animal and equine veterinary personnel (Section 1.12). All studies examining carriage of caMRSA in these risk groups have been included in Tables 1.4 to 1.8.

1.10.1 caMRSA carriage in contact sports – American football, soccer and rugby union players

Increased prevalence of caMRSA has been noted in participants of sports teams, in particular amongst American football players (Malachowa et al., 2012). An increased incidence has also been reported among soccer, baseball, basketball, and volleyball players, as well as among martial arts participants and rowers (Huijsdens et al., 2006b, Gantz et al., 2003). Risk factors for caMRSA transfer in athletes include compromised skin integrity, inadequate hygiene, sharing equipment, personal items and sports facilities, crowding and position of athlete on team (Garcia et al., 2012, Nguyen et al., 2005, CDC, 2009b, Kirkland and Adams, 2008, Drews et al., 2006, Cohen, 2005). For the purposes of this literature review MRSA carriage in football, soccer and rugby union players will be examined in further detail due to the high level of physical contact these athletes experience during game play.

Outbreaks of MRSA in sports teams in the US have been documented, although most of the evidence has come from case reports (Camargo et al., 2013, Saben, 2004, Begier et al., 2004, Hall et al., 2009, Romano et al., 2006, Bowers et al., 2008). A frequently referenced paper in which the prevalence of caMRSA was reported involved an investigation into an outbreak of infection among American football players of the St. Louis Rams in 2003 (Kazakova et al., 2005). Results from this study showed that no nasal carriage was recorded in any of the 58 players, suggesting that infection with caMRSA amongst this group preceded colonisation. The authors proposed that caMRSA was spread directly between the linemen through turf burns, draining infections, towel sharing or through the

unwashed hands of the trainers. This trend is evident in a MRSA carriage study of 108 professional American football players and staff where again no carriage was observed but where five caMRSA infections were reported during the competitive season (Garza et al., 2009). Similarly, in a surveillance study of MRSA in 190 American junior varsity football players no carriage was reported (Lear et al., 2011). This was the largest study of caMRSA carriage in football players undertaken without a prior outbreak of MRSA and the inclusion of follow-up testing strengthened this study. However, this study is not without limitations. Firstly, no statistical analysis could be performed for caMRSA carriage risk factors due to the non-existent colonisation rate. Secondly, the authors reported a cofounding variable, being the provision of education to staff, students and adults for ways in which MRSA could be reduced prior to the swabs being taken. Finally, the authors report that their results may have been different had the swabbed participants over a period greater than one season.

In contrast to these findings, an MRSA prevalence of 4% (4/100) has been reported in American male football players during the off-season and 19% (19/100) carriage has been reported during playing season, with the authors suggesting that colonisation alone was inadequate in causing an outbreak (Creech et al., 2010). A limitation of this study pertained to only a single nasal swab being taken to assess carriage, and had the throat or an additional skin site been sampled there may have been potential to increase the yield of MRSA. While another study examining nasal MRSA carriage in American college football and soccer players reported these groups to have a carriage prevalence of 1.87% (2/107) and 4.35% (2/46) respectively (Rackham et al., 2010). The authors noted the carriage rate of 1.87% in football players was similar to the carriage rate reported in the general

community. In addition, no significant correlations between MRSA carriage and risk factors were reported in their study.

Based on the review of literature, only four community studies (as previously discussed) have reported on the prevalence of MRSA carriage in healthy American football players (Lear et al., 2011, Garza et al., 2009, Creech et al., 2010, Rackham et al., 2010) and one US study has examined MRSA carriage in healthy soccer players (n=46) (Rackham et al., 2010). A common feature of these studies investigating MRSA carriage in healthy soccer and football players was the use of convenience sampling to recruit participants, which in turn limits generalisations. To the knowledge of the author, there have been no studies published which have examined MRSA prevalence in soccer and rugby union players in Australia. These groups are of importance as healthy football players can acquire MRSA and in some rare cases infection with this organism has been implicated as a cause of death in contact sports players (Andrews et al., 2007, Mihoces and McLean, 2006). Table 1.4a shows the prevalence of MRSA in American football and soccer participants in community carriage studies, MRSA strain type as defined by the classification system and typing technique in each study, and environmental contamination where applicable. Table 1.4b shows the prevalence of MRSA in American football, soccer and rugby union participants in outbreak studies, MRSA strain type as defined by the classification system and typing technique used in each study, and environmental contamination where applicable.

Table 1.4a MRSA prevalence in American football and soccer participants in community carriage studies

Author/s	Country	Sports participants	Swab site	MRSA carriage	Carriage prevalence	Strain type
(Garza et al., 2009)	USA	American football team and staff^^	Nares	0/108	0%	n/a
(Creech et al., 2010)	USA	American football	Nares	4/100	4%	Carriage^ – USA200, USA300, USA400, USA600, USA800, USA900
		Follow –up at end of season	Nares	19/100	19%	
(Rackham et al., 2010)	USA	American football	Nares	2/107	1.87%	n/t
		Soccer	Nares	2/46	4.35%	n/t
(Lear et al., 2011)	USA	American football	Nares	0/190	0%	n/a
		Follow–up at end of season	Nares	0/190	0%	n/a

*cultures occurred during course of playing season ^exact number of isolates for each strain not specified in football players

^^Five players developed infection with MRSA during the course of the playing season. Isolates did not have further genetic tests performed on them

Note: None of the surveillance studies of MRSA colonisation in soccer and American football players have reported on environmental contamination with this organism

Table 1.4b Community MRSA prevalence in American football, soccer and rugby union participants in studies involving prior outbreak/s

Author/s	Country	Sports participants	Swab site	MRSA carriage	Carriage prevalence	MRSA infection	Infection prevalence	Strain type
Retrospective outbreak studies in which infections were reported in team members prior to undertaking further investigation								
(Stacey et al., 1998)	UK	Rugby union	Nares, SSTI	1/20	5%	5/20	25%	n/t
(CDC, 2003)	USA	American football year 2000	SSTI	n/a	n/a	10/10	100%	n/s
		American football year 2002	SSTI	n/a	n/a	2/2	100%	n/s
(Begier et al., 2004)	USA	American football	Nares, SSTI	0/100	0%	10/100 (6 confirmed)	10%	6 infection isolates typed - USA300 (6)
(Cohen, 2005)	USA	American football	SSTI	n/a	n/a	1/7 (all sports - 1 football player)	14.29%	n/t
(Kazakova et al., 2005)	USA	American football	Nares, SSTI	0/58	0%	5/58	8.62%	5 infection isolates typed - USA300 (5)
		Staff	Nares, SSTI	0/26	0%	n/a	n/a	
		Environment	Artificial turf	n/s	0%	n/a	n/a	
(Muller-Premru et al., 2005)	Slovenia	Soccer	SSTI	n/a	n/a	12/26	46.16%	12 infection isolates typed - ST5 (11), ST152 (1)

Table 1.4b Community MRSA prevalence in American football, soccer and rugby union **participants in studies involving prior outbreak/s continued…**

Author/s	Country	Sports participants	Swab site	MRSA carriage	Carriage prevalence	MRSA infection	Infection prevalence	Strain type
(Nguyen et al., 2005)	USA	American football	Nares, SSTI	8/99	8.08%	11/107`	10.28%	4 infection isolates typed - USA300 (4)
		Staff	Nares	0/28	0%	n/a	n/a	6 nasal carrier isolates typed – USA300 (4), n/s (2)
(Rihn et al., 2005)	USA	American football	Nares, STTI	3/90	3.33%	13/90	14.44%	6 typed – n/s
		Staff members	Nares	0/12	0%	0/12	0%	
(Huijsdens et al., 2006b)	Denmark	Soccer	Nares, GIT, SSTI	6/42	14.29%	9/42	21.43%	Colonising and infecting isolates - ST80 (15)
		Soccer participant roommates	Nares, GIT, SSTI	1/14	7.14%	2/14	18.18%	Colonising and infecting isolates - ST80 (3)
(Romano et al., 2006)^^	USA	American football year 2002	SSTI	n/a	n/a	2/107	1.87%	n/t
		American football year 2003	Nares, SSTI	7/106	6.00%	11/107	10.28%	Infecting isolates - USA300 (11)
		American football year 2004	Nares, SSTI	3/104	2.88%	1/104	0.96%	n/t
(CDC, 2009b)	USA	American football	SSTI	n/a	n/a	6 /51~	11.76%	3 typed - USA300 (3)

Table 1.4b Community MRSA prevalence in American football, soccer and rugby union participants in studies involving prior out**break/s continued…**

Author/s	Country	Sports participants	Swab site	MRSA carriage	Carriage prevalence	MRSA infection	Infection prevalence	Strain type
(Hall et al., 2009)	USA	American football	SSTI	n/a	n/a	25/109`	22.94%	3 typed - USA300 (2), USA800 (1)
(Oller et al., 2010)	USA	American football	Nares, fingertips, knuckles, forearms, SSTI	7/70	10%	9/70	12.86%	n/t
		Control group	Nares, fingertips, knuckles, forearms	0/50	0%	n/a	n/a	n/a
		Environment	Locker room, weight room	33/108	30.56%	n/a	n/a	n/a
(Sutton et al., 2014)	USA	American Football	Nares	9/20	45%	5º	n/t	n/t
			Helmets	5/20	25%			
			Shoulder pads	7/20	35%			

*cultures occurred during course of playing season ^exact number of isolates for each strain not specified in football players ~4 confirmed `6 confirmed

ST – sequence type SSTI – skin and soft tissue infections

ºstudy specified only 5 players were initially infected, the authors did not comment on the total number of players at the institution

^^ authors mentioned examining environmental contamination in training room and athletic locker, however did not specify further or provide details of results

1.10.2 Household contacts of MRSA carriers

Household contacts of carriers are considered to be at an increased risk of carriage or infection with MRSA, and are potential reservoirs for the spread of this organism in the community. Several studies examining the prevalence of caMRSA carriage in household contacts of MRSA carriers have reported high prevalence rates of 20% or higher (Rafee et al., 2012, Mollema et al., 2010, Zafar et al., 2007). In a study investigating nasal caMRSA carriage in patients initially identified to be infected with caMRSA, it was revealed 20% (10/49) of household contacts were carriers of this organism (Zafar et al., 2007). The authors reported the parents of the patient had the highest level of risk (60% risk ratio; p=0.05) for caMRSA carriage when compared to other household members based on relative risk assessment. However, this study was limited due to the low number of subjects sampled and did not include all of the household contacts of the initial patients.

In another recent study of carriage in household contacts of patients with *S. aureus* infections, in which a multiple swab site approach (nares, throat, inguinal region) and an enrichment step was incorporated, the authors reported 22% (177/812) of household contacts were carriers of MRSA, and identified the USA300 strain as the most frequently isolated strain in this cohort (Miller et al., 2012). They also reported that had they only performed nasal swabs, 51% of the total MRSA isolates would have been missed. This study is however not without limitations. Participants in this study were of a low socioeconomic status and may not be representative of the US population. In addition, the directionality of strain transfer could not be determined due to the study being cross sectional.

In contrast to the high carriage rates reported in household contacts of MRSA colonised or infected carriers, a study examining nasal carriage in 914 participants sampled from 321 randomly selected households reported a lower carriage rate of 0.4%, and identified antibiotic use as the only significant risk factor for carriage in this group (Miller et al., 2009). The authors note that their study is limited due to sampling a single site and that this particular population may not represent the general population due to the majority of participants being Hispanic.

Several studies have examined the transfer of MRSA in household settings between close household contacts and family members. In one study examining the transfer of MRSA in 10 families known to be carriers of this organism it was reported isolates obtained from family members in each household shared the same strain characteristics (Huijsdens et al., 2006c). The significance of these results is not known as the authors made no attempt at statistical analysis and a limitation of this study is the small number of families sampled. In another study, Nerby and colleagues (2011) investigated household transfer of MRSA in 712 household contacts of 236 caMRSA infected children and reported 13% (29/236) of index patients and 12% (82/712) of household contacts were nasal carriers (Nerby et al., 2011). This study was hampered by a low participation rate (30%), which may have biased the results. In addition the sampling method in the study may not have been sensitive enough as a single site had been sampled and no pre-enrichment step was included (Brown et al., 2005, Hamdan-Partida et al., 2010). Similarly, an epidemiological study of household transfer of caMRSA in household contacts of carriers in Hong Kong reported a carriage rate of 13% (6/46) (Ho et al., 2007). In another study investigating transfer of MRSA from 62 initially infected index cases to their household contacts (n=160), it was found that transfer of MRSA occurred from 47% of index cases (Mollema et al., 2010).

The investigators identified prolonged exposure to MRSA in the household environment, increased number of household contacts and being the partner of an MRSA index case as significant risk factors for the acquisition of MRSA in household contacts of MRSA infected participants. Factors associated with transfer of MRSA in the index cases showed that carrying MRSA in the throat, being of a younger age and having eczema all significantly increased transmission.

Together, these studies highlight the importance of household contacts as a viable source in the spread of both caMRSA and haMRSA strains in the community. To date, no Australian surveillance studies have been published investigating the prevalence of caMRSA in household contacts of community members' found to be carriers of MRSA.

1.11 Origins, prevalence and strain sequence types of caMRSA in animals and handlers

MRSA was first isolated from a cow with mastitis in 1972 (Devriese et al., 1972). The first documented case of animal to human MRSA transmission was reported in 1994, in which a husband and wife were reinfected after initial clearance. The source of infection was identified as being the family dog (Cefai et al., 1994).

In 2003 a novel type of MRSA was detected in humans from a reservoir belonging to the CC398 lineage, initially identified in pigs and cattle, termed livestock-associated MRSA (laMRSA) (Huijsdens et al., 2006a, van Cleef et al., 2011). Livestock-associated MRSA were originally identified as non-typeable by *Sma1* restriction analysis (Voss et al., 2005).

The enzyme *Apa1* is used instead to characterise these isolates (Kadlec et al., 2009). Compared to haMRSA and caMRSA, laMRSA have low virulence and reduced resistance to antibiotics. Resistance to trimethoprim, tetracyclines, macrolides, aminoglycosides and/or lincosamides have been observed in isolates of laMRSA (Kadlec et al., 2009, Monecke et al., 2011a). Pigs are the main reservoir of this strain (Voss et al., 2005, Weese, 2010), although strains of ST398 have been identified in horses and dogs, without contact to livestock or contact with at risk persons for the acquisition of MRSA (Witte et al., 2007, Sieber et al., 2011). In addition infection with livestock-associated MRSA is not limited to animals, ST398 is also able to cause infections in human hosts (Köck et al., 2013, Fitzgerald, 2012). To determine the origin of laMRSA, whole genome sequencing (WGS) of human and animal MSSA and MRSA isolates has been performed (Price et al., 2012). Isolates of MSSA and MRSA were collected from 19 different countries in their study. The researcher's data showed laMRSA originated in humans, before spreading to livestock.

MRSA carriage has since been observed in a diverse range of animals, particularly among horses, dogs and pigs (Kottler et al., 2010, Schwaber et al., 2013, Cuny et al., 2010, Huijsdens et al., 2006a, Broens et al., 2011, Cui et al., 2009, Weese, 2010, Voss et al., 2005, Leonard and Markey, 2008). MRSA has also been isolated from other animal species such as cats, cattle, sheep and poultry (Eriksson et al., 2013, Köck et al., 2013, Loeffler and Lloyd, 2010).

Recent findings have indicated companion animals may serve as a potential reservoir of MRSA transfer to their owners (Ferreira et al., 2011, Pantosti, 2012). Zoonotic transfer is

possible and various studies have reported transfer of human strains to animals, but also vice versa (Nienhoff et al., 2009, Morgan, 2008, Faires et al., 2009, Weese et al., 2005b, Manian, 2003). Studies examining the relationship between human and animal MRSA carriage have reported individuals who have regular contact with animals outside of a household setting have an increased risk of becoming infected or being a carrier of this organism (Graveland et al., 2011, Köck et al., 2009, van Cleef et al., 2010). Horses and dogs are reported to be at increased risk for MRSA carriage and infection as a result of their close contact with humans (van Duijkeren et al., 2010, Loeffler et al., 2005, Faires et al., 2009, Loeffler et al., 2010b, Schwaber et al., 2013, Van den Eede et al., 2009, Abbott et al., 2010). The next section details what is currently known about MRSA carriage, risk factors and predominant strain types identified in horses, dogs and their handlers in both clinical settings and within community settings.

1.11.1 Community-associated MRSA in horses and their handlers

MRSA was first isolated in horses in 1996 in Japan as a cause of metritis (Anzai et al., 1996). The primary site for MRSA carriage in horses is the nostrils (Axon et al., 2011), although the skin can also be colonised (Sieber et al., 2011). Other sites evaluated for carriage include the pastern, perineum, corner of the mouth, neck and previous infection site (Busscher et al., 2006b, Bergström et al., 2013). Risk factors for caMRSA carriage in horses include horses previously colonised with MRSA, horses that had been administered antimicrobial therapy, those residing in an MRSA positive farm and those receiving clinical interventions (Weese et al., 2006c, Weese and Lefebvre, 2007). Regular contact with more than 20 horses has been reported as a risk factor for caMRSA carriage in horse handlers (Weese et al., 2006c, Weese and Lefebvre, 2007).

Carriage of MRSA in horses and their handlers attending veterinary clinics and during outbreak investigations

Currently, most of the studies examining MRSA carriage in horses have taken place in a clinical setting or during periods of MRSA outbreak (Schwaber et al., 2013, van Duijkeren et al., 2010), and MRSA has been found to be a growing problem in these situations (Weese and van Duijkeren, 2010b). Carriage studies on MRSA in horses attending veterinary clinics typically report the prevalence to range from 0% to 16% (Baptiste et al., 2005, Bergström et al., 2013, Weese, 2010), however some farms have experienced rates higher than 40% (Weese et al., 2005b). Such a high level of carriage is of concern as colonisation precedes infection and, although horses are unlikely to become sick from MRSA when in good health, immunocompromised horses and those receiving multiple doses of antibiotics may develop an infection (Weese et al., 2006c). As a consequence, infected horses may then spread this organism to other horses as well as their handlers. One Dutch study examining MRSA carriage in horses during 2006-2008 reported 9.3% of horses carried MRSA upon admission to hospital (van Duijkeren et al., 2010). This study also reported that of the 36 horse-related environmental sites sampled, 53% were MRSA positive. A similar MRSA carriage prevalence of 10.9% (12/110) has been observed in horses originating from four different countries (Belgium, Netherlands, Luxembourg and France) attending a European equine clinic (Van den Eede et al., 2009). The authors suggested this high carriage rate observed in these horses could be due to the screening protocol implemented, which included a pre-enrichment step. Typing of strains in their study confirmed all 12 MRSA isolates to be community-associated ST398 strains.

An Australian study has reported on the prevalence of MRSA in 216 thoroughbred horses admitted to an intensive care unit of a veterinary practice (Axon et al., 2011). There were two parts to their study; part A assessed nasal carriage upon admission to veterinary clinic and part B assessed clinical and uterine/clitoral swabs of horses submitted to the clinic. The authors reported a caMRSA nasal carriage prevalence of 3.7% and subsequent typing of isolates identified all strains to be ST612. It is important to note that, as the horses were either post surgical cases, admissions from breeding farms or accompanying horses they were not representative of the general healthy population. Also, the authors commented that theirs and other studies might underestimate the carriage rate in horses due to not including a pre-enrichment step.

Similarly, Loeffler et al (2011) reported a lower MRSA carriage prevalence in horses attending a veterinary clinic in which 1.97% (3/152) of clinically treated horses carried MRSA whereas a prevalence of 0% (0/296) was found in healthy horses. The low isolation rate of MRSA in the horses may have been due to a sampling effect as a result of animals from only two stables participating in the study.

Relatively high carriage rates have been observed in horse handlers in both professional veterinary and non-professional (owners or farm personnel) settings. MRSA carriage rates of 11% (16/139) (Schwaber et al., 2013), 12% (8/66) (Weese et al., 2005a) 14% (17/125) (Weese et al., 2005a) and 18% (12/66) (Weese et al., 2005b) have been reported, however these high prevalence rates should be interpreted with caution as samples were collected from MRSA identifiable farms or following MRSA outbreaks and therefore do not necessarily reflect the carriage rate of horse handlers in contact with healthy horses.

Carriage of caMRSA in healthy horses and their handlers

In contrast to the high prevalence of caMRSA and haMRSA in clinically treated horses or horses attending clinical settings, the prevalence of caMRSA in healthy horses is reported to be low or absent. Globally, only a few studies have reported on the prevalence of caMRSA carriage in the healthy horse population in a community setting (Van den Eede et al., 2012, Burton et al., 2008, Vengust et al., 2006, Peterson et al., 2012, Mallardo et al., 2013). Of these, (Vengust et al., 2006) and (Burton et al., 2008) did not find any caMRSA present in healthy horse populations (0/300 and 0/497 horses respectively), despite implementing pre-enrichment steps. However, interpretation of the Slovenian study (Vengust et al., 2006) is difficult as it relied on convenience sampling. In a more recent community study of nasal MRSA carriage in healthy Italian horses it was reported 1.05% (2/191) of horses were carriers of MRSA, with harness racing horses experiencing significantly greater carriage than riding and breeding horses (Mallardo et al., 2013). As with previous studies, difficulty with recruitment resulted in convenience sampling of horses from three different locations.

At present, data on caMRSA carriage in the healthy horse population and their handlers in a community setting in Australia is lacking. There are few published studies investigating caMRSA carriage in both horses and their handlers (Busscher et al., 2006b, Van den Eede et al., 2013, Weese et al., 2005b). Busscher and colleagues (2006) examined nasal and pastern carriage of MRSA in 200 healthy horses housed at 23 different farms and one clinic in the Netherlands, and examined MRSA nasal and throat carriage of 42 persons in close contact with horses (comprising horse handlers, veterinarians and veterinary

students). One MRSA isolate was recovered from a veterinarian, a carriage prevalence of 2.38%, while caMRSA was absent in the healthy horses. This study was cross-sectional in nature and included sampling of veterinary professionals handling these horses; hence this carriage rate cannot therefore be considered to be representative of the general horse handlers. The authors further noted the absence of MRSA in healthy horses might be reflective of the low carriage of MRSA in the Netherlands in general.

Similarly, in the Canadian and US study caMRSA was found to be absent in healthy horses (0/581) from farms without MRSA, although caMRSA was recovered from two of the horse handlers, a prevalence of 5% (Weese et al., 2005b). It should be noted this study by Weese and colleagues (2005b) consisted of two parts, one with a focus on investigating carriage in MRSA notifiable farms, the other in horses and their handlers in non-targeted farms. Hence it can be argued the study is not a true community study. A recent Belgium study has reported caMRSA nasal carriage in two of 166 horses (1.2%) and in four handlers (2.4%) (Van den Eede et al., 2013). This study used convenience sampling to recruit horses and their respective handlers from five different locations. Statistical analysis examining risk factors associated with MRSA carriage could not be performed due to the low isolation rate of MRSA. Table 1.5a summarises the prevalence of caMRSA carriage in horses in a community setting and Table 1.5b summarises the prevalence of caMRSA carriage in horses in attending or treated in clinical setting, including genetic typing of strains and environmental contamination where reported. Table 1.6a shows caMRSA prevalence in horse handlers and horses in a community setting and Table 1.6b shows community and healthcare-associated MRSA prevalence in horses and in personnel in contact with those horses in a clinical setting, including genetic typing of strains and environmental contamination where applicable.

Table 1.5a MRSA carriage prevalence in horses in a community setting

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	ST/*spa* type (n)
(Vengust et al., 2006)†	Slovenia	Community	Healthy horses	Nostrils	0/300	0%	n/a
(Burton et al., 2008)	Canada	Farms	Healthy horses	Nostrils	0/497	0%	n/a
(Peterson et al., 2012)†	US	Farms (F)	Healthy horses (F)	Nostrils	0/26	0%	n/a
		Racetracks (R)	Healthy horses (R)	Nostrils	8/13	61.54%	USA500 (8)
			Environment	Human and animal touch surfaces	5/7	71.43%	USA500 (5)
(Van den Eede et al., 2012)	Belgium	Farms	Healthy horses	Nostrils	1/189	0.53%	ST398 (1)
(Mallardo et al., 2013)	Italy	Racetrack, reproduction centre, riding centre	Healthy horses	Nostrils	2/191	1.05%	n/t

n – number ST – sequence type *t* – *spa* type

†Study examined other animal species; MRSA prevalence in horses was reported in this table only

Table 1.5b MRSA carriage prevalence in horses attending or treated in a clinical setting

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	ST/*spa* type (n)
(Bagcigil et al., 2007)†	Denmark	Equine clinic and farms	Horses *	Nostrils	0/100	0%	n/a
(Tokateloff et al., 2009)	Canada	Veterinary hospital	Horses*	Nostrils	6/458	1.31%	n/t
(Van den Eede et al., 2009)	Belgium	Equine clinic	Horses*	Nostrils	12/110	10.91%	ST398 (12)
(Abbott et al., 2010)†	Ireland	Private veterinary practices	Study 1: Retrospective - Horses*	Clinical specimens	20/383	5.22%	n/t
	Ireland	Veterinary practices	Study 2: Prospective - Healthy horses	Nostrils	2/129	1.55%	n/t
		University teaching hospital	Study 3: Prospective - Healthy horses	Nostrils	2/107	1.87%	n/t
(Lin et al., 2011) †	US	Veterinary clinic	Horses*	Clinical specimens SSTIs	5/12	41.67%	ST8 (4), ST830 (1)
(Loeffler et al., 2011)†	UK	Veterinary clinic	Healthy horses	Nostrils, mouth, axilla, perineum	0/296	0%	n/a
			Unhealthy horses	Nostrils, mouth, axilla, perineum	3/152	1.97%	n/t
(Axon et al., 2011)	Australia	Veterinary clinic	Healthy horses	Nostrils	8/216	3.70%	ST612 (8)
(Couto et al., 2011b)	Portugal	Veterinary clinic	Healthy horses	Nostrils	1/20	5%	ST398 (1)
			Unhealthy horses	Nostrils	1/51	1.96%	ST5 (1)
(Maddox et al., 2012)	UK	Veterinary visited horses mainly sampled on their premises	Horses*	Nostrils	4/678	0.59%	*t064* (2), *t451* (1), *t032* (1)

n – number ST – sequence type *t* – *spa* type †Study examined other animal species; MRSA prevalence in horses was reported in this table only *health status unspecified

Note: none of the above studies examined environmental contamination with MRSA

Table 1.6a MRSA prevalence in horse handlers and horses in a community setting

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	ST/*spa* type (n)
(Weese et al., 2005b)	Canada and USA	Farms without MRSA (non-targeted)	Healthy horses	Nostrils	0/581	0%	n/a
			Horse handlers	Nares	2/41	4.88%	ST8 (2)
(Busscher et al., 2006b)	Netherlands	23 farms, 1 clinic	Healthy horses	Nostrils, pastern	0/200	0%	n/a
			Horse handlers, veterinarians, veterinary students	Nares, throat	1/42	2.38%	n/t
(Van den Eede et al., 2013)	Belgium	Equine meetings (competitive events etc.)	Healthy horses	Nostrils	2/166	1.20%	ST398 (2)^
			Horse handlers	Nares	4/166	2.41%	ST398 (3), CC5 (1)

n – number ST – sequence type *t* – *spa* type ^ Both handler and animal pairs carried ST398

Note: none of the above studies examined environmental contamination with MRSA

Table 1.6b MRSA prevalence in horses and in personnel in contact with those horses in a clinical setting

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	ST/*spa* type (n)
(Baptiste et al., 2005)	UK	Equine hospital	Unhealthy horses (clinic)	Nostrils	11/67	16.42%	CMRSA-5 (1), further typing required (10)
			Healthy horses (community)	Nostrils	0/40	0%	n/a
			Veterinary personnel	Nares	0/12	0%	n/a
(Weese et al., 2005a)	Canada	Veterinary hospital and farms prior MRSA notification	Horses sampled in year 2000 (veterinary clinic)	Nostrils	2/57	3.51%	CMRSA-5 (2)
			Horses sampled in year 2002 (veterinary clinic)	Nostrils	25/320	7.81%	CMRSA-5 (25)
			Horses sampled in year 2002 (farm A)	Nostrils	41/321	13.60%	37– CMRSA-2 like (3), CMRSA-5 (34)
			Horses year 2002 (farm 1 notified with MRSA)	Nostrils	3/64	4.69%	CMRSA-5 (3)
			Horses year 2002 (other 8 farms notified with MRSA)	Nostrils	0/277	0%	n/a
			Veterinary personnel	Nares	17/125	13.60%	CMRSA-5 (16), CMRSA-2 like (1)
			Farm personnel (farm A)	Nares	8/68	11.76%	CMRSA-5 (8)
			Household contact	Nares	1/1	100%	CMRSA-5 (1)
			Owner of infected horse	Nares	1/1	100%	CMRSA-5 (1)

Table 1.6b MRSA prevalence in horses and in personnel in contact with those horses in a clinical setting continued...

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	ST/*spa* type (n)
(Weese et al., 2005b)	Canada/USA	Farms with prior MRSA notification (targeted)	Targeted horses	Nostrils	46/391	11.76%	ST8 (46)
			Targeted horse personnel	Nares	12/66	18.18%	ST8 (12)
(van Duijkeren et al., 2010)	Netherlands	Veterinary teaching hospital^	1st outbreak~ Veterinary personnel in contact with infected horses	Nares	4/61	6.56%	*t2123* (4)
			2nd outbreak` Veterinary and personnel and general staff	Nares	16/170	9.41%	*t011* (11), *t2123* (5)
			Unhealthy horses upon admission	Nostrils	24/259	9.27%	*t011* (21), *t064* (1), *t2123* (1), lost isolate (1)
			Unhealthy horses during hospitalisation	Nostrils	62/149	41.61%	*t2123* (1), *t011* (61)
			Environment	Human touch surfaces	19/36	52.78%	*t011* (19)
(Schwaber et al., 2013)	Israel	Veterinary teaching hospital^	Horses*	Nostrils	12/84	14.29%	*t535* (12)
			Veterinary personnel	Nares	16/139	11.51%	*t535* (14), t002 (1), *t1816* (1)

*health status unspecified

n – number

ST – sequence type

t – *spa* type

^ cohort was sampled as a result of MRSA outbreak

~seven infected horses identified in initial outbreak

`12 infected horses identified in second outbreak

Sequence types of MRSA identified in horses

Certain strains of caMRSA have been observed to predominate in horses in Europe, Canada and America. The majority of MRSA strains isolated from horses have been genotyped and confirmed to be strain types of human origin (Cuny et al., 2008, Cuny et al., 2010, Lin et al., 2011, Walther et al., 2009). In addition as the majority of studies on MRSA carriage in horses have come from clinical settings it is expected that veterinary personnel treating these horses would frequently come into close contact, hence allowing the human strains of MRSA to transfer to their animal patients. However the direct role of initial transfer and transmission of MRSA from persons to horses needs to be investigated further (Cuny et al., 2010). In horses the most commonly isolated MRSA strains (as shown in Table 1.7) are the ST1 (Cuny et al., 2008), ST8 (Cuny et al., 2010, Lin et al., 2011), ST22 (Walther et al., 2009), ST254 (Walther et al., 2009, Cuny et al., 2010), ST398 (Tokateloff et al., 2009, Sieber et al., 2011) and ST1173 (Kinnevey et al., 2010, Weese et al., 2005a). Of these commonly isolated strains in horses the ST398 (laMRSA) is usually identified in pigs and livestock (Weese, 2010, Köck et al., 2009, van Duijkeren et al., 2014). Furthermore, strains such as the ST254, ST398 and ST1173 circulating in equids do not necessarily reflect those predominant in human populations (see Table 1.3).

Table 1.7 Commonly isolated MRSA sequence types in horses

Horse strain sequence types (ST)	MRSA type	Country of isolation
ST1[a]	caMRSA	Germany
ST8[b,c,j]	caMRSA	USA, Germany
ST22[d]	haMRSA	Germany
ST254[b,d,j]	haMRSA	Germany
ST398[e,f,i]	laMRSA	Switzerland, Canada, Austria
ST1173[g,h]	caMRSA	Canada, Austria

[a] *Cuny, Strommenger, Witte, & Stanek, 2008* [b] *Cuny et al., 2010* [c] *Lin et al., 2011*

[d] *Walther et al., 2009* [e] *Sieber et al., 2011* [f] *Tokateloff et al., 2009*

[g] *(Weese et al., 2005a)* [h] *(Kinnevey et al., 2010)* [i] *(Loncaric et al., 2014)*

[j] *(Moodley et al., 2006)*

1.11.2 Community-associated MRSA in dogs and their handlers

MRSA was first reported in a pet dog in 1994 (Walther et al., 2008, Vanderhaeghen et al., 2012, Zhang et al., 2011, Griffeth et al., 2008, Loeffler et al., 2005, Weese et al., 2006b). Recommended sampling sites to assess MRSA carriage in dogs are the anterior nares and perineum (Weese and van Duijkeren, 2010b). It has however, been reported that dogs are not preferentially colonised by *S. aureus* and MRSA carriage in dogs is usually the result of transfer from humans (Walther et al., 2008, Vanderhaeghen et al., 2012, Zhang et al., 2011, Griffeth et al., 2008, Loeffler et al., 2005, Weese et al., 2006b). A qualitative risk assessment of the acquisition of MRSA by dogs reported humans to be the most significant reservoir of MRSA (Heller et al., 2010). On the other hand, a coagulase positive staphylococci frequently isolated in dogs, and rarely isolated from humans, is *S. pseudointermedius* (Ruscher et al., 2010, Ishihara et al., 2010, Walther et al., 2012). Both *S. aureus* and *S. pseudointermedius* carry the *mecA* gene (Wedley et al.), and appear to be

similar phenotypically. Genetic typing targeting the *femA* gene (specific to *S. aureus*) has been used to differentiate *S. aureus* from *S. pseudointermedius* (Ishihara et al., 2010). Although other tests to distinguish between *S. aureus* from *S. pseudointermedius* are available.

Studies investigating risk factors for caMRSA carriage in dogs are scarce. Recent studies reported that carriage was significantly more frequent in female dogs, in adult dogs versus puppies (<12 months), in dogs who had undergone previous surgery (<90 days), in dogs who stayed in a veterinary hospital for over three days, sleeping in the bedroom with a colonised owner and in dogs with owners employed in the healthcare and veterinary fields (Hamilton et al., 2013, Boost et al., 2008, Hoet et al., 2013). An incidental observation by Hamilton and colleagues investigating acquisition and persistence of antimicrobial-resistant bacteria found that dogs housed only indoors had a significantly reduced risk of MRSA carriage (Hamilton et al., 2013). However, as all these studies were conducted in a veterinary setting, the results should be interpreted with caution, as they may not apply to the broader healthy dog population. More studies are required to assess caMRSA in healthy dogs. To date, no studies have reported on significant risk factors for carriage with caMRSA specifically in dog owners in a community setting. One study by Boost and colleagues attempted to identify risk factors for MRSA carriage in 736 dog owners, but were unable to perform statistical analyses due to the low number of MRSA carriers (n=4) observed (Boost et al., 2008).

Prevalence of MRSA carriage in dogs and their handlers attending veterinary clinics and during outbreak investigations

The majority of studies examining carriage in companion animals and their handlers have taken place in a veterinary setting and research is sparse on caMRSA carriage in healthy companion animals and their owners (Umaru et al., 2011, Loeffler et al., 2011). Prevalence of caMRSA carriage in dogs has been reported to range from 0% to 4% (Weese, 2010, Baptiste et al., 2005, Gosbell, 2011, Kottler et al., 2010, Wedley et al., 2014). Higher prevalence rates of MRSA have been observed in dogs admitted to veterinary clinics and dog shelters. In one particular study a relatively high MRSA carriage prevalence of 9% (4/45) in dogs in a single clinic was reported (Loeffler et al., 2005). However, in this study a small number of dogs were enrolled and risk factors for carriage were not assessed. In another study, a relatively high prevalence of MRSA in dogs residing in a rescue shelter during an outbreak was reported, with 7.8% (10/129) of dogs found to be carriers of MRSA (Loeffler et al., 2010a). Interestingly in this study all dogs were found to be carriers of the same strain, ST22 (E-MRSA-15), as the originally infected dog.

In contrast to these high prevalence rates of MRSA in dogs attending veterinary settings or rescue shelters during outbreaks, a much lower prevalence rate has been reported in a study of 200 shelter dogs in the US with MRSA present in only 0.5% of dogs (Gingrich et al., 2011). A similar prevalence of 0.5% (1/193) has been reported in another study of dogs presented to a veterinary hospital, with the single strain typed as community-associated ST8 (Hanselman et al., 2008). Both of these studies assessed meticillin resistant staphylococci, not just MRSA (Gingrich et al., 2011, Hanselman et al., 2008). Couto et al (2011) have also reported a low prevalence of MRSA carriage in dogs attending a veterinary teaching hospital where nasal prevalence was found to be 0.7% (1/146), with

the common human ST22 strain being isolated from this dog (Couto et al., 2011a). Their study also assessed meticillin resistant *Staphylococci*. A study by Loeffler et al (2011) investigating MRSA prevalence and risk factors in dogs reported that 0.66% (2/302) of healthy dogs carried MRSA and 3.23% (13/402) of dogs attending a veterinary clinic were carriers (Loeffler et al., 2011). Unfortunately, the significance of the data presented in the studies discussed above is difficult to assess as they were limited by the use of convenience sampling and the small sample numbers recruited (Gingrich et al., 2011, Hanselman et al., 2008, Loeffler et al., 2011). In addition, one study of 102 dogs presented to a veterinary clinic in Canada and another study of 36 healthy dogs residing at a Hong Kong dog rescue shelter failed to identify any caMRSA (Lefebvre et al., 2006, Epstein et al., 2009).

In Ireland a retrospective study by Abbott et al (2010) examining MRSA carriage from clinical samples reported that MRSA accounted for 1.1% (32/2864) prevalence in dogs (Abbott et al., 2010), however as this study is retrospective and assessed clinical samples from dogs this prevalence cannot be deemed to be representative of dogs in the general community. A similar prevalence of 1.1% (2/177) was observed in dogs upon admission to a veterinary clinic in Belgium (Vanderhaeghen et al., 2012). Sequence types ST45 and ST5 were recovered from these patients. As this study indicated a low overall carriage rate of MRSA in this particular dog population, the authors stated that this figure could be similar to the general community prevalence of caMRSA as all swabs were collected prior to hospitalisation. However their population is not representative of the community as the majority of dogs were suffering from chronic or serious disease and some of these dogs had received antibiotics. The authors also suggested that the environment in which a dog resides might play an important role in the acquisition of this organism.

Only one Australian study has reported on MRSA carriage in healthy and unhealthy dogs attending a clinic in Adelaide (Malik et al., 2006a, Malik et al., 2006b). It was reported that no MRSA was present on the skin of 51 healthy dogs, while MRSA was isolated from skin lesions of two diseased dogs (n=141), both isolates were typed to be the ST239 haMRSA strain. Isolates in this study displayed multiple resistance against antibiotics tested. Both isolates were resistant to oxacillin, tetracycline, gentamicin, amoxicillin-clavulanic acid, clindamycin, cephalothin, erythromycin, and chloremphenicol. Susceptibility to vancomycin and rifampicin was recorded. As with many of the other studies investigating carriage of MRSA in dogs attending clinical settings, this study also used convenience sampling and assessed other meticillin resistant staphylococci.

In studies which have examined caMRSA carriage in dogs and their veterinary handlers in veterinary healthcare settings carriage of MRSA is reported to range from 2% to 27% in veterinary handlers (Aklilu et al., 2012, Loeffler et al., 2005, Baptiste et al., 2005, Zhang et al., 2011), whereas carriage prevalence in dog handlers presenting their dogs to a veterinary clinic has been reported to range from 0% to 17.9% (Abdel-moein et al., 2012, Faires et al., 2009, Ferreira et al., 2011, Boost et al., 2008). A comprehensive study by Boost et al (2008) has examined carriage of MRSA in 736 dog owners and 830 dogs attending a veterinary practice in which MRSA was isolated from four humans and six dogs, a prevalence of 0.72% and 0.54% respectively (Boost et al., 2008). A carriage rate of 0.72% in dog handlers is within the typically observed prevalence reported for general community members (Farley et al., 2008, Munckhof et al., 2009, Graham et al., 2006), however as this study was conducted in a veterinary setting it is not truly representative of

the general population. In their study, Boost and colleagues reported relatively few MRSA carrier owner and dog pairs, which could in part be due to transient colonisation. Due to the cross-sectional nature of the study colonisation status could not be determined. A slightly higher nasal MRSA carriage prevalence of 1.96% (1/51) has been reported in another study of veterinary personnel attending to dogs and cats (Zhang et al., 2011). This study included a pre-enrichment step. However the low number of participants enrolled and having sampled only one carriage site for MRSA limited the study design. Whilst a particularly high MRSA prevalence of 27% (3/11) has been reported in veterinary personnel, albeit being absent in dogs sampled in the same study (Baptiste et al., 2005). This high prevalence should be interpreted with caution as only 11 veterinary personnel were swabbed and cannot therefore be representative of the general veterinary population.

Prevalence of caMRSA carriage in healthy dogs and their handlers

Globally, only five community studies have examined caMRSA carriage in the healthy dog population (Kottler et al., 2010, Walther et al., 2012, Vengust et al., 2006, Hanselman et al., 2009, Schmidt et al., 2014), three of which also examined caMRSA carriage in their handlers. Vengust and colleagues (2010) reported there was no carriage of caMRSA, even with the inclusion of a pre-enrichment step, in a convenience sample of 200 healthy dogs attending agility training, enrolled in rescue/working dog training camps and household pets in Slovenia (Vengust et al., 2006). This trend was confirmed in another recent study assessing carriage in a convenience sample of 108 dogs and their handlers attending a dog show in Germany where nasal caMRSA carriage was absent (Walther et al., 2012). The researchers did not include a pre-enrichment step; although it is highly unlikely MRSA would have been undetected as the swabs were directly inoculated onto a general-purpose culture agar (Columbia agar containing 5% sheep's blood). In addition, it should be noted

that both the studies by Vengust et al (2006) and Walther et al (2012) assessed carriage of staphylococci, and that the researchers did not specifically focus on MRSA.

In contrast, Kottler and colleagues (2010) reported a prevalence of 3.29% (7/213) in healthy dogs and cats who resided in non-healthcare households in the US, and a prevalence of 4.69% (10/213) was observed in their handlers (Kottler et al., 2010). The authors did not report a significant difference in the occurrence of person or pet carriage with MRSA between non-health care and healthcare households. These results are in contrast to previous studies, which have reported a higher carriage of *S. aureus* in healthcare settings (Lu, 2005, Hanselman et al., 2006, Loeffler et al., 2005). Reasons for the higher prevalence in this population could be due to including a pre-enrichment step which resulted in the effective screening of MRSA or that in this particular region MRSA may be higher in prevalence, although more studies are required if this is the case. Additionally, the authors may have underestimated the prevalence of MRSA as only one human and one pet from each household was sampled.

A slightly lower MRSA prevalence of 1.52% (2/132) in dogs and 3.31% (8/242) in dog handlers has been reported in Canada (Hanselman et al., 2009). As with previous studies assessing carriage in dogs and their handlers the main focus of this study was not specific for MRSA. The cross-sectional nature of this study prevented the authors in determining whether transmission occurred between dogs and their owners, although a strength of this study lies in the recruitment approach of participants who were selected at random. At present, data on carriage prevalence with caMRSA in healthy Australian dogs and their handlers is lacking. Table 1.8a details the caMRSA carriage prevalence in healthy dogs

and their handlers in a community setting and Table 1.8b details the community and healthcare-associated MRSA carriage prevalence in dogs in veterinary settings, rescue shelters or outpatient households. Table 1.8c details the community and healthcare-associated MRSA carriage prevalence in both dogs and their handlers attending veterinary settings.

Table 1.8a Carriage of MRSA in healthy dogs and their handlers in a community setting

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type ST/*spa* type (n)
(Vengust et al., 2006)†	Slovenia	Community	Healthy dogs	Nares, perineum	0/200	0%	n/a
(Hanselman et al., 2009)	Canada	Community	Healthy dogs	Nares, rectum	2/132	1.52%	n/s●
			Dog handlers	Nares	8/242	3.31%	n/s●
(Kottler et al., 2010)	US	Community, University and household settings based on household type (households with veterinary or healthcare or non-healthcare personnel)	Non- healthcare household (NH)	Nares	10/213	4.69%	Human isolates – USA100 (17), USA200 (2), USA400 (1), USA500 (6), USA700 (1)
			Veterinary healthcare (VH)	Nares	13/211	6.16%	
			Human healthcare (HH)	Nares	10/162	6.17%	
			Healthy dogs and cats (NH)	Nares, rectum	7/213	3.29%	Dog isolates – USA100 (10), USA200 (1), USA500 (7), USA700 (2)
			Healthy dogs and cats (VH)	Nares, rectum	5/211	2.37%	
			Healthy dogs and cats (HH)	Nares, rectum	8/162	4.94%	
(Walther et al., 2012)	Germany	Dog show	Healthy dogs	Nares	0/108	0%	n/a
			Dog handlers	Nares	0/108	0%	n/a
(Schmidt et al., 2014)	UK	Dog shows	Healthy dogs	Nares	0/73	0%	n/a
				Perineum	0/73	0%	n/a

n – number ST – sequence type *t* – *spa* type n/a – not applicable to study †Study examined other animal species; MRSA prevalence in dogs was reported in this table only

●PFGE typing performed to test for animal-handler strain relatedness, however no ST or MRSA type were stated in this study

Table 1.8b Carriage of MRSA in dogs in clinical veterinary settings, rescue shelters or outpatient households

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Malik et al., 2006a, Malik et al., 2006b)[†]	Australia	Veterinary clinic	Healthy dogs	Skin	0/51	0%	n/a
			Unhealthy dogs	Skin, skin lesions	2/141	1.42%	ST239 (2)
(Bagcigil et al., 2007)[†]	Denmark	Veterinary clinic	Dogs*	Nares	0/100	0%	n/a
(Griffeth et al., 2008)	US	Veterinary clinic	Healthy dogs	Dorsal skull, buccal and gingival mucosa, nares, ventromedial inguinal fold, external anus	0/50	0%	n/a
		Veterinary clinic or owners home	Dermatopathy unhealthy dogs		5/59	8.47%	n/t
(Hanselman et al., 2008)	Canada	Veterinary teaching hospital	Dogs*	Nares, axilla, perineum	1/193	0.52%	n/t
(Walther et al., 2008)	Germany	University teaching hospital	Unhealthy dogs	Clinical specimens	18/589	3.06%	n/t
(Epstein et al., 2009)	Hong Kong	Dog shelter	Healthy dogs	Nares	0/36	0%	n/a
(Murphy et al., 2009)	Canada	Private veterinary hospital	Healthy dogs	Perineum, rectum	0/188	0%	n/a

Table 1.8b Carriage of MRSA in dogs in clinical veterinary settings, rescue shelters or outpatient households **continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Abbott et al., 2010)†	Ireland	Private veterinary practices	Study 1: Retrospective – Dogs*	Clinical specimens	32/2864	1.12%	n/t
		Veterinary practices in Ireland	Study 2: Prospective - Healthy dogs	Nares	1/133	0.75%	n/t
		University teaching hospital Dublin	Study 3: Prospective - Healthy dogs	Nares	0/153	0%	n/a
(Lin et al., 2011)†	US	Veterinary clinic	Unhealthy dogs	Clinical specimens SSTIs	12/478	2.46%	USA100 (10), USA800 (1), USA300 (1)
(Loeffler et al., 2010a)	UK	Rescue kennel	Apparently healthy dogs●	Nares, buccal mucosa, axilla, perineum	10/129	7.75%	E-MRSA15 (10)^
(Bender et al., 2012)†	US	Households of 28 MRSA infected child	Healthy dogs	Nares, perineum	1/18	5.56%	USA300 (1)
(Couto et al., 2011a)†	Portugal	Veterinary teaching hospital	Dogs*	Nares	1/146	0.68%	ST22 (1)
(Gingrich et al., 2011)	US	Dog shelter	Dogs*	Nares, perineum	1/200	0.50%	n/t

Table 1.8b Carriage of MRSA in dogs in clinical veterinary settings, rescue shelters or outpatient households **continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Loeffler et al., 2011)[†]	UK	Veterinary clinic, rescue kennels	Healthy dogs	Nares, mouth, axilla, perineum	2/302	0.66%	ST36 (2)
			Unhealthy dogs	Nares, mouth, axilla, perineum	13/402	3.23%	CC22 (13)
(Morris et al., 2012)[†]	USA	Outpatient households`	Healthy dogs	Nares, perineum, groin, oral mucosa	7/47	14.89%	*t091 (1), t084(1), t008 (2), t002 (2), t3230 (1)*
(Vanderhaeghen et al., 2012)	Belgium	Veterinary teaching hospital	Dogs*	Nares	2/177	1.13%	ST45 (1), ST5 (1)
(Hamilton et al., 2013)[†]	USA	Small animal veterinary teaching hospital	Dogs*	Nares, rectum, oropharynx	7/506	1.38%	ST5 (5), ST72 (1), non-typeable (1)
(Hoet et al., 2013), (van Balen et al., 2013)	USA	University -Veterinary medical centre	Dogs*	Nares, perineum, skin lesions	25/435	5.79%	USA100 (20), USA500 (1), USA800 (2), non typeable (1)
			Environment	Human and animal touch surfaces	77/569	13.53%	USA100 (71), USA300 (2), USA500 (1), USA800 (1), Iberian (1), novel type (2), no type (1)
(Wedley et al., 2014)	UK	Veterinary practices	Unhealthy dogs	Nares	7/724	0.97%	ST22 (7)

Table 1.8b Carriage of MRSA in dogs in clinical veterinary settings, rescue shelters or **outpatient households continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Davis et al., 2014)[†]	US	Veterinary clinics	Healthy dogs	Nares, rectum, oral cavity, hindquarters, stomach	11/155	7.10%	ST5

●sampled after dog with index case of MRSA infection was reported ^ all carried same strain as originally infected dog ‘outpatient households of veterinary dermatology staff

n/a – not applicable to study n/t – not typed, isolates did not have further genotypic tests performed on positive MRSA isolates SSTIs – skin and soft tissue infections

*health status unspecified [†]Study examined other animal species; MRSA prevalence in dogs was reported in this table only

Table 1.8c Carriage of MRSA in dogs and their handlers in clinical veterinary settings

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Baptiste et al., 2005)	UK	Small animal hospital	Healthy and unhealthy dogs	Nares, perineum	0/55	0%	n/a
			Veterinary personnel	Nares	3/11	27.27%	E-MRSA15 (3)
(Loeffler et al., 2005)†	UK	Small animal hospital	Dogs*	Nares, buccal mucosa	4/45	8.89%	E-MRSA15 (4)
			Veterinary personnel	Nares	14/78	17.95%	E-MRSA15 (14)
			Environment	Human and animal touch surfaces	3/30	10%	E-MRSA15 (3)
(Weese et al., 2006b)	Canada and USA	Household contacts of four index case infected dogs^	Household contacts case 1«	Nares	4/37	10.81%	CMRSA-2 (4)
			Household contacts case 2«	Nares	3/23	13.04%	CMRSA-2 (3)
			Household contacts case 3«	Nares	1/1	100%	CMRSA-2 (1)
			Household contacts case 4«	Nares	1/1	100%	CMRSA-2 (1)
(Boost et al., 2008)	Hong Kong	Veterinary practice	Healthy dogs	Nares	6/830	0.72%	n/s●
			Dog handlers	Nares	4/736	0.54%	
(Faires et al., 2009)	Canada	Outpatients in households with prior MRSA infection in pets	Dogs«*	Nares, perineum	2/24	8.33%	USA100 (1), USA300 (1)
			Dog handlers«	Nares	10/56	17.86%	USA100 (5), USA300 (3), n/s (2)
		Outpatients in households with prior MRSA infection in humans	Dogs«*	Nares, perineum	2/21	9.52%	USA100 (1), USA300 (1)
			Dog handlers«	Nares	1/16	6.25%	USA300 (1)

Table 1.8c Carriage of MRSA in dogs **and their handlers in clinical veterinary settings continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Loeffler et al., 2010b)	UK	Small animal veterinary clinic	MRSA infected dogs and cats	Nares	106/106	100%	CC8 (1), CC22 (103), CC30 (2)
			MSSA control group - dogs and cats	Nares	0/91	0%	n/a
			Owners of MRSA pet	Nares	9/120	7.50%	44 isolates recovered from humans in this study - CC22 (43), CC30 (1)○
			Owners of MSSA pet	Nares	0/100	0%	n/a
			Veterinarians handling dogs and cats known to have MRSA	Nares	27/220	12.3%	
			Veterinarians handling dogs and cats known to have MSSA	Nares	8/168	4.76%	
(Ferreira et al., 2011)†	US	Outpatient household setting from 49 infected MRSA outpatients	Healthy dogs	Nares	2/76	2.63%	*t002 (1), t176 (1)*
			Household contacts	Nares	0/13	0%	n/a
		Veterinary wellness clinic	Healthy dogs (control group)	Nares	0/45	0%	n/a
			Dog owners (control group)	Nares	0/50	0%	n/a
(Zhang et al., 2011)	China	Small animal hospitals	Dogs and cats*	Nares	21/2745	0.77%	ST59 (18), ST239 (2), ST398 (1)
			Veterinary personnel	Nares	1/51	1.96%	ST239 (1)

Table 1.8c Carriage of MRSA **in dogs and their handlers in clinical veterinary settings continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Abdel-moein et al., 2012)	Egypt	Veterinary clinic	Apparently healthy dogs	Nares, oral, ear, wound	1/48	2.08%	n/t
			Unhealthy dogs	Nares, oral, ear, wound	1/22	4.55%	n/t
			Dog handlers	Nares, throat	1/28	3.57%	n/t
(Aklilu et al., 2012)†	Malaysia	Veterinary clinic	Dogs*	Nares, perineum	5/50	10%	2 isolates typed - ST59 (2)
			Veterinary personnel	Nares, throat	2/28	7.14%	ST5 (1), ST1241 (1)
			Environment	Human and animal touch surfaces	9/28	32.14%	2 isolates typed from environment - ST658 (1), ST1156 (1)

«most MRSA isolates obtained from dogs and members of the same household were indistinguishable by PFGE `Four human and dog pairs carried the same strain

^Four index dogs identified as being infected with MRSA and human household contacts of these infected dogs agreed to participate

~Six households had dog and pet pair carriers. Of these six households, four dog handlers and pet pairs carried identical strains as one each other, as typed by PFGE

●PFGE typing performed to test for animal-handler strain relatedness, however no ST or MRSA type were stated in this study

○Authors did not specify from which human group CC30 was recovered, rather they reported on the MRSA isolates recovered from both dog owners and veterinary personnel together

†Study examined other pet animals; MRSA prevalence in dogs was reported in this table only

n/a – not applicable to study n/s – not specified n/t – not typed, isolates did not have further genotypic tests performed on positive MRSA isolates

*health status unspecified

Sequence types of MRSA identified in dogs

In dogs the most commonly isolated MRSA sequence types (as shown in Table 1.9) are the ST5 (Vanderhaeghen et al., 2012, Kwon et al., 2006, Lin et al., 2011), ST22 (Walther et al., 2008, Zhang et al., 2011, Loeffler et al., 2005, Strommenger et al., 2006a, Harrison et al., 2014), ST45 (Vanderhaeghen et al., 2012), ST59 (Zhang et al., 2011), ST105 (Lin et al., 2011) and ST239 (Zhang et al., 2011, Malik et al., 2006a). A common feature applicable to these commonly isolated strains from dogs is that these sequence types reflect the most common strains of human origin. A possible reason for human strains commonly isolated in companion animals is due to their close contact with humans (Walther et al., 2012). Table 1.10 shows the sequence types (ST) of commonly isolated MRSA strains in dogs.

Table 1.9 Commonly isolated MRSA sequence types in dogs

Dog strain sequence types (ST)	MRSA type	Country of isolation
ST5[a,b,c]	caMRSA and haMRSA	USA, Korea, Belgium
ST22[d,e,f,g]	haMRSA	China, UK, Germany
ST45[c]	caMRSA	Belgium
ST59[g]	caMRSA	China
ST105[a]	haMRSA	USA
ST239[g,h]	haMRSA	Australia, China

[a] *Lin et al., 2011* [b] *Kwon et al., 2006* [c] *Vanderhaeghen et al., 2012*

[d] *Loeffler et al., 2005* [e] *Strommenger et al., 2006* [f] *Walther et al., 2008*

[g] *Zhang et al., 2011* [h] *Malik, Coombs, O'Brien, Peng, & Barton, 2006*

From the current literature few reports have shown that dogs and horses may act as vectors in the transfer of MRSA to their human hosts, although human to animal transfer has been reported more commonly (Loeffler et al., 2011, Loeffler and Lloyd, 2010). Due to the scarcity of literature that exists detailing MRSA carriage in healthy companion animals and their owners, this literature review suggests further investigation into the carriage of MRSA in companion community animals, as well as an investigation into the risk factors for colonisation in order to aid infection control measures in the community and veterinary settings is warranted.

1.12 Prevalence of caMRSA in veterinarians and veterinary personnel working with small animals and horses

People in certain occupations, such equine veterinarians (Jordan et al., 2011), small animal veterinarians and other staff at veterinary clinics, have been found to have relatively high carriage rates of caMRSA (Ishihara et al., 2010, Swaber et al., 2011). This group is of importance as they are at a greater risk for the acquisition of MRSA and may serve as potential reservoirs to their close household contacts, colleagues, pets and animal patients.

1.12.1 Risk factors for caMRSA carriage in veterinarians and veterinary personnel

Risk factors for caMRSA carriage in companion animal veterinarians have been reported by Ishihara and colleagues, with two risk factors independently associated with carriage, when compared to veterinary students: i). being an employee of a veterinary hospital or a non-clinical laboratory, and ii). contact with an identified animal MRSA case (Ishihara et al., 2010). The type of clinical practice in which veterinarians work may also be an

occupational health hazard, as has been reported for equine veterinarians (Jordan et al., 2011).

1.12.2 Carriage of MRSA in small animal and equine veterinarians and veterinary personnel

MRSA carriage in veterinarians and veterinary personnel is typically reported to range from 0.7% to 23% globally (Wulf et al., 2006, Zemlickova et al., 2009, Gosbell, 2011, Jordan et al., 2011). A recent Swiss study spanning 2005-2010 reported MRSA carriage in veterinary personnel rose from non-existent (0/26) to 18.2% (6/33) by the end of 2010 (Sieber et al., 2011). In the same study, horses were also tested. In 2005 none of the horses (0/349) were found to be carriers of MRSA, whereas 15.9% (13/318) of horses were found to be carriers in 2010. Findings from the study revealed that veterinary personnel were found to be carriers of the same strain as the infected horses, indicating transmission had occurred. However, the small number of veterinary personnel sampled limits this study. Similar to the prevalence of MRSA carriage in equine veterinarians, small animal veterinarians working in clinics in the UK are reported to have a carriage rate ranging from 3.1% to 17.9% (Loeffler et al., 2010b, Loeffler et al., 2005, Heller et al., 2009).

In Canada, a study of veterinarians and veterinary personnel attending a conference found 12% (4/34) of technicians, 16% (15/96) of large animal veterinarians and 4% (12/271) of small animal veterinarians carried MRSA, whereas those who did not have any animal contact did not carry the bacterium (0/50) (Hanselman et al., 2006). Their study included a pre-enrichment step, which could perhaps account for the high isolation of MRSA isolates. A limitation of this study was the potential for bias due to convenience sampling and due

to a greater proportion of participants working in specialty practice, which may not adequately represent general veterinarians. In addition, participants collected their own swabs, which may have led to an underestimation of the prevalence in this group. Other studies investigating the carriage of MRSA in general veterinarians attending conferences have reported a 10% (26/257) and 17% (59/341) carriage rate in the US (Burstiner et al., 2010, Anderson et al., 2008) and a 0.7% (2/280) prevalence in the Czech Republic (Zemlickova et al., 2009). In the two US studies the two most prevalent strains were the USA100 and USA500, whereas in the European study ST30 and ST45 were identified. A possible explanation for the difference between carriage rates experienced in US veterinary personnel compared to veterinary personnel in Europe could be a result of higher MRSA prevalence in the US and stricter guidelines on the stringent use of antimicrobials in Europe.

In America and Canada, Weese and colleagues (2005b) have investigated the carriage of MRSA among veterinarians and horse owners using targeted and non-targeted surveillance and found an overall carriage prevalence of 13% (14/107) in this group (Weese et al., 2005b). The high prevalence of MRSA in this cohort compared to the general population is not unexpected as participants who were included in the study had prior contact with MRSA infected horses. In another study by Weese and colleagues published the same year, it was reported that veterinary clinic staff had an MRSA carriage rate of 13.6% (17/125) and equine farm personnel had a prevalence of 12% (8/68) (Weese et al., 2005a). In another American study, Morris et al (2010) examined nasal, groin, oral and anal mucosa carriage with MRSA in a convenience sample of 171 veterinary dermatology personnel, 258 dogs and 160 cats attending a veterinary clinic (Morris et al., 2010). Carriage prevalence rates were 3.5% (6/171), 0.8% (2/258) and 3.75% (6/160)

respectively. PFGE analysis of the 14 recovered isolates showed the majority of these strains belonged to the USA 100 lineage, three belonged to the USA300 lineage and two could not be assigned a lineage. However, carriage of MRSA may have been under estimated in this study due to self-collection of swabs by the participants and due to sampling only one carriage site for MRSA. Additionally, the authors could not determine colonisation status due to the observational nature of their study.

Similarly, typing of MRSA isolates recovered from veterinary personnel and animal patients in Ireland by O'Mahony and colleagues (2005) revealed all non-equine animal isolates were indistinguishable from one another and were genetically indistinguishable from the isolates recovered from veterinary personnel in close contact with those animals (O'Mahony et al., 2005). The MRSA isolates recovered from horses were also indistinguishable from one another. However, they were unlike the patterns obtained from the other non-equine isolates and unlike any isolates reported from the human lineages in the area in which the study took place. The finding different PFGE types are present in horses is expected when compared to companion animals, as has been highlighted in this literature review. However an unexpected finding in their study was that none of the horse isolates shared the same PFGE pattern as humans. A possible explanation of why these equine isolates were unlike human isolates previously encountered in Ireland may be due to this pattern being novel or it may be due to the authors comparing the PFGE pattern in only their country of investigation. Furthermore the authors failed to perform MLST, which limited global comparison. This study was further limited as the researchers made no attempt to collect samples from the owners of the animal patients, who may have been carriers of the same strain as their horses, nor was an attempt made to collect samples from uninfected animals in the study, leading to a bias in their results.

There has been only one study on MRSA carriage in veterinarians and veterinary personnel in Australia (Jordan et al., 2011). In their study, a convenience sample of 771 individuals attending veterinary four conferences in 2009 was sampled. The cohort included veterinarians working in small and large animal practices (clinical roles group) and industry and government veterinarians (control group). Participants had nasal swabs collected during the veterinary conferences. It was reported only one of 107 participants in the control group was a carrier of MRSA, while veterinarians whose major work emphasis related to horses (but not exclusively) had a prevalence of 11.88% (24/202), a 13-fold greater prevalence. Furthermore, they reported that veterinarians who only treated horses had a prevalence of 21.35% (19/89) versus small animal veterinarians treating mainly dogs and cats had a prevalence of 4.80% (12/250). It was proposed that equine veterinarians had a higher prevalence of MRSA due to their frequent contact with horses' nostrils, as the nostrils are the primary site for MRSA carriage in horses. Other veterinarians assessed in their study with a 'single major emphasis' who were working in clinical roles included pig (n=12), cattle (n=3) and exotic and wild animals (n=6) veterinarians, of which only one pig veterinarian was found to be a carrier of MRSA. As with many of the other studies assessing carriage, prevalence may have been under represented due to sampling only one site known to harbour MRSA and due to self-collection of swabs by the participants. In addition, the cross-sectional nature of this study prevented the researchers from determining whether colonisation was transient or persistent in participants found to be carriers of MRSA.

Table 1.10 Carriage of MRSA in small and large animal veterinarians and veterinary personnel

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Baptiste et al., 2005)	UK	Veterinary hospital (small animal)	Veterinary personnel	Nares	3/11	27%	E-MRSA15 (3)
		Veterinary hospital (equine specific)	Veterinary personnel	Nares	0/12	0%	n/a
(Loeffler et al., 2005)	UK	Veterinary hospital (small animal)	Veterinary personnel	Nares	14/78	17.9%	E-MRSA15 (14)
(Weese et al., 2005a)	Canada	Veterinary hospital and farms (equine specific)*	Veterinary personnel	Nares	17/125	14%	CMRSA-5 (16), CMRSA-2 like (1)
(Hanselman et al., 2006)	USA	Veterinary conference (small and large animal)	Small animal veterinarians	Nares	12/271	4.4%	CMRSA-2 (11), non-typeable (1)
			Large animal veterinarians	Nares	15/96	15.6%	CMRSA-2 (2), CMRSA-5 (13)
			Veterinary technicians	Nares	4/34	12%	n/t
			Others	Nares	0/38	0%	n/a
(Anderson et al., 2008)	US	Veterinary practitioners conference (equine)	Veterinary personnel	Nares	26/257	10.1%	USA100 (9), USA500 (15), USA300 (1), non-typeable (1)

Table 1.10 Carriage **of MRSA in small animal and large animal veterinarians and veterinary personnel continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Moodley et al., 2008b)	Denmark	Veterinary conference (small and large animal)	Veterinary practitioners	Nares	9/231	3.9%	CC1 (1), CC8 (1), CC22 (2), CC59 (1), CC88 (1), CC398 (3)
			Veterinary personnel	Nares	0/72	0%	n/a
			Farmers	Nares	0/98	0%	n/a
			Persons without professional exposure to animals	Nares	2/301	0.7%	CC5 (1), CC398 (1)
(Heller et al., 2009)	UK	Veterinary hospital (small animal)	Veterinary staff	Nares	2/64	3.1%	ST22 (2)
			Environment (day 1)	Surface samples	2/140	1.4%	ST22 (2)
(Zemlickova et al., 2009)	Czech Republic	Veterinary conference (small and large animal)	Veterinary personnel	Nares	2/280	0.7%	ST30 (1), ST45 (1)
(Burstiner et al., 2010)	US	Veterinary conference (small and large animal)	Veterinarians	Nares	53/308	17%	CMRSA-2/USA 100 (32), CMRSA-4/USA200 (8), CMRSA-5/USA500 (16), CMRSA-8 (2), CMRSA-10/USA300 (1)
			Veterinary technicians	Nares	6/33	18%	

Table 1.10 Carriage **of MRSA in small animal and large animal veterinarians and veterinary personnel continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Ishihara et al., 2010)	Japan	Veterinary clinics (small animal)	Veterinarians 2007	Nares	5/20	25%	Total of 13 isolates typed in 2007 *- t002 (9), t008 (2), t062 (1), t1265 (1)*
			Veterinarians 2008	Nares	8/34	23.5%	Total of 10 isolates typed in 2008 *- t002 (7), t008 (1), t1767 (2)*
			Veterinary staff 2007	Nares	3/21	14.3%	
			Veterinary staff 2008	Nares	0/19	0%	n/a
			Students 2007	Nares	3/51	5.9%	
			Students 2008	Nares	2/74	2.7%	
			Staff and student in nonclinical laboratories 2007	Nares	0/36	0%	n/a
			MRSA carriers after treatment	Nares	2/10	20%	
			Environment in veterinary hospital 2007	Surface samples	5/75	6.7%	*t002 (2), t008 (3)*
			Environment in veterinary hospital 2008	Surface samples	3/81	3.7%	*t002 (2), t1767 (1)*

Table 1.10 Carriage **of MRSA in small animal and large animal veterinarians and veterinary personnel continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Loeffler et al., 2010b)	UK	Veterinary clinic (small animal)	Veterinarians handling dogs and cats known to have MRSA	Nares	27/220	12.3%	35 isolates – CC22, CC30○
			Veterinarians handling dogs and cats known to have MSSA	Nares	8/168	4.8%	
(Boost et al., 2011)	Hong Kong	Veterinary clinics (small and large animal)	Veterinary personnel	Nares	1/150	0.67%	CC5/*t002 (1)*
*(Jordan et al., 2011)^^*	Australia	Veterinary conference (small and large animal)	`A. Government/industry	Nares	1/107	0.93%	n/t^ (CC22, CC8)
			`A. Other◦		2/88	2.27%	n/t
			`B. Dogs, cats	Nares	21/430	4.88%	n/t
			`B. Horses		24/202	11.88%	n/t
			`C. Dogs, cats	Nares	12/250	4.80%	n/t
			`C. Horses		19/89	21.35%	n/t
(Zhang et al., 2011)	China	Veterinary hospitals (small animal)	Veterinary personnel	Nares	1/51	1.96%	ST239 (1)

Table 1.10 Carriage of MRSA in small animal and large animal veterinarians and veterinary personnel **continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Aklilu et al., 2012)	Malaysia	Veterinary hospital (small animal)	Veterinary personnel	Nares	2/28	7.14%	ST5 (1), ST1241 (1)
			Veterinary students	Nares	24/103	23.3%	3 isolates typed – ST15 (1), ST149 (1), ST508 (1)
(Schwaber et al., 2013)	Israel	Veterinary teaching hospital (large animal)*~	Equine veterinary personnel	Nares	14/69	20.29%	*t535* (13), t002 (1)
			Non-equine veterinary personnel	Nares	2/70	2.86%	*t535* (1), *t1816* (1)
(Wettstein et al., 2014)	Switzerland	Veterinary conference (small and large animal)	Small animal veterinarians	Nares	4/146	2.73%	ST225 (1), ST5 (2), ST88 (1)
			Large animal veterinarians	Nares	2/31	6.45%	ST398 (2)
			General veterinarians	Nares	5/111	4.50%	ST398 (5)
			Equine	Nares	0/7	0%	n/a
			Zoo	Nares	0/2	0%	n/a
			Veterinary assistants	Nares	1/29	3.44%	ST225 (1)

Table 1.10 Carriage **of MRSA in small animal and large animal veterinarians and veterinary personnel continued…**

Author/s	Country	Setting	Participants	Swab site	MRSA carriage	Prevalence	Strain type
(Wettstein et al., 2014) continued…	Switzerland	Veterinary conference	Non-animal related (laboratory, administration, industry, other)	Nares	1/44	2.27%	ST225 (1)
		(small and large animal)	Unknown	Nares	1/1	n/a	ST5 (1)

Unique – novel *spa* type of MRSA identified *t* – *spa* type ST – sequence type

*cohort sampled as a result of MRSA notification ^not typed in their study, although a follow-up study typed 46 isolates ◦non-clinical veterinarians and non-veterinarians

~performed follow-up testing, in which none of the initially colonised veterinary personnel were found to be carriers of MRSA

○Study examined dog owners also and the authors did not specify from which human group CC30 was recovered, rather they reported on the MRSA isolates recovered from both dog owners and veterinary personnel together

`A – Group A: Nonclinical veterinarians (categories are mutually exclusive)

`B – Group B: All clinical veterinarians with any number of areas of 'major emphasis' (categories are not mutually exclusive)

`C – Group C: Subset of clinical veterinarians in group B with 'single major emphasis'

^^NOTE: only data collected from small animal (dog/cat) and/or large animal (equine) veterinarians and veterinary personnel have been included in this table.

1.13 Environmental sources of caMRSA

Fomites represent an important reservoir for the transfer of MRSA not only in a healthcare setting but also within a community setting, and may be an occupational issue among veterinarians and persons in close contact with animals. Strains of caMRSA are able to colonise household objects, including doorknobs, toilet handles and kitchen sinks (Miller and Diep, 2008, Ojima et al., 2002). Sharing belongings (e.g. towels, body suits) or equipment (e.g. razors, whirlpools, sauna benches) may facilitate the spread of this organism (Redziniak et al., 2009, Begier et al., 2004, Cohen, 2005, Nguyen et al., 2005).

1.13.1 MRSA contamination in the environment

The environment represents an important reservoir of caMRSA transfer. Studies that have investigated the duration of MRSA survival on different surfaces report that MRSA is able to colonise fomites for extended periods of time. In one study it was shown that MRSA was able to survive for 21 days on cotton and up to 40 days on polyester (Neely and Maley, 2000). However, this study did not perform genotypic analyses and as such could not designate MRSA type. In another study of haMRSA survival on nine different surfaces (bed sheets, ceramic, plastic toys, razors, shoulder pads, soap, towels, vinyl, wood) it was found transfer of the bacteria was successful on all fomites, excluding soap, and that length of survival was greater on non-porous fomites than porous and metal fomites (63 days on vinyl versus five minutes on razor) (Desai et al., 2011). This finding is in contrast to a previous study, which has been successful in isolating caMRSA from soap (Nguyen et al., 2005). A possible explanation for this discrepancy could be due to the type of soap tested in each study and the difference of antibacterial agents present in those soaps.

MRSA has been reported to colonise numerous household objects and frequently touched surfaces in both household and public settings (Desai et al., 2011, Dancer, 2008, Garcia et al., 2012, Uhlemann et al., 2011, Brady et al., 2007, Chang et al., 2010). In a study of environmental MRSA contamination from public surfaces at a university, student homes and in the local community in the US the prevalence was reported to be 2.7% (8/294), 11.8% (10/85) and 2.3% (3/130) respectively (Roberts et al., 2011b). Of the total MRSA strains isolated from these surfaces 14 were genotyped to be caMRSA strains, 11 of which were USA300. In another US study examining environmental contamination and nasal carriage with MRSA the authors reported MRSA was isolated from 4.1% (44/1064) surfaces swabbed in fire stations across two districts, five of which were USA300, three of which contained *SCCmec* type IV and 26 of which were non-typeable (Roberts et al., 2011a). In their study a particularly high nasal MRSA carriage rate of 22.5% (9/40) was reported in one fire district. This high prevalence is likely to be the result of clustering within the cohort sampled. Upon genotypic analyses, human strains and strains recovered from the environment were confirmed to be genetically related, which is suggestive of a transfer route. (Tolba et al., 2008) tested caMRSA and haMRSA survival in different types of water (sea, river and pool) and concluded there was no significant difference in survival dynamics between the two, however, a recent study investigating MRSA contamination has reported a contamination rate of 68% (25/40) on public buses, 62.9% of which were typed to be caMRSA (Lutz et al., 2014), suggests that caMRSA isolates may have better survival in the environment.

High MRSA contamination rates of 16% (78/500) (Murphy et al., 2012) and 27% (96/350) (Boyce et al., 1997) have been observed in the environment of MRSA carriers in healthcare settings. The high prevalence of MRSA in the environment of carriers in

healthcare settings is expected due to the MRSA carriers, their visitors and their healthcare professionals frequently having contact with surfaces in their surrounding environment (Boyce et al., 1997).

Environmental contamination with MRSA has also been documented in veterinary settings. Weese et al (2004) examined environmental contamination with MRSA in a veterinary teaching hospital in Canada. In this study 260 samples were taken from the hospital environment (Weese et al., 2004). The authors reported a 9.6% (25/260) prevalence of MRSA in the environment, with the stalls of clinically treated MRSA positive horses yielding the highest isolation rate. Hoet et al (2011) assessed environmental MRSA contamination within a veterinary teaching hospital in America during a non-outbreak period. A total of 157 samples were collected from the small animal, equine and food animal areas. Surfaces to be tested in the study were chosen based on multiple touch sites by humans and animals. MRSA contamination in their study was found to be 16%, 12% and 0% respectively, with the most commonly isolated strain being the caMRSA USA100 (16/19) (Hoet et al., 2011). Twelve percent (19/157) of the veterinary teaching hospital environmental surfaces were found to be contaminated with MRSA. This study concluded that there was no statistically significant difference in contamination between animal and human touch surfaces. Of the total MRSA isolates recovered, eleven were from animal contact surfaces, seven from the human contact surfaces and only one was from the equine section, which was obtained from a doorknob. The authors concluded environmental contamination was greatest on surfaces that are touched by multiple persons, as was the case for doors and carts being frequently contaminated in their study.

1.14 Research justification

Infections caused by community-associated MRSA are of increasing importance throughout the world. The World Health Organization considers MRSA to be one of the top ten pathogens worldwide with an adverse effect on human health (WHO, 2013). Despite substantial research on caMRSA since it was first identified in the early 1990's, there is still much work to be undertaken in order to provide a detailed understanding of the carriage of this organism in healthy members of the general community and in at risk groups for the acquisition of caMRSA.

A standardised definition of caMRSA is vital and should be utilised in all studies in order to report on the true prevalence of caMRSA and address inconsistencies in literature. In the present study MRSA status will be designated as either caMRSA or haMRSA based on established criteria using the following genotypic analyses: macro-restriction pulsed field gel electrophoresis (PFGE) (assigns isolates into clusters and is highly discriminatory) (Reed et al., 2007), DNA microarrays (identifies *SCCmec* type and genes associated with virulence, resistance and adhesion factors) (Miller and Tang, 2009, Shore et al., 2012) and multi locus sequence typing (MLST) (assigns ST and CC) (Enright et al., 2000a).

This review of literature has shown that studies detailing caMRSA carriage in community-based settings and in risk groups in Australia are lacking. Research that better defines the prevalence of caMRSA and risk factors for carriage and infection is needed as colonisation often precedes infection. Together, these combined research efforts have the potential to assist in the prevention and control of this organism in healthy members of the community and in risk groups identified to have higher carriage of this organism.

1.15 Main aim of the study

- To identify caMRSA carriage in the Australian community, in previously identified risk groups and in dogs and horses and their handlers.

- To characterise caMRSA strains isolated from these groups using molecular testing (biochemical, morphological, antibiotic susceptibility and multiplex end-point PCR) and genotypic analyses (multiplex real-time PCR, PFGE, DNA microarrays and MLST).

Research plan

Chapter 3: To assess the carriage and characterise caMRSA strains in the community and in University and Technical and Further Education (TAFE) staff and students.

Chapter 4: To determine the carriage and characterise caMRSA strains in contact sports players (soccer and rugby union players).

Chapter 5: To assess carriage and characterise caMRSA strains in horses and dogs and their respective handlers.

Chapter 6: To determine the carriage and characterise caMRSA strains in small animal and equine veterinarians and veterinary personnel, close household contacts of caMRSA carriers, and to assess the environmental caMRSA contamination (both household and workspace) of carriers.

Chapter 2 General Materials and Methods

2.1 Ethics, participant recruitment and collection of demographic information

Ethics approvals for this study were obtained from both the Human Research Ethics Committee (HREC) and Animal Ethics Committee (AEC) at the University of Sydney.

2.1.1 Participant recruitment

A number of community and risk groups for MRSA carriage were recruited for the study. Community participants comprised members of the general community, sporting teams, University and TAFE staff and students. University staff and students were recruited from two different universities in Sydney. TAFE staff and students were recruited from a single TAFE campus in Sydney. Soccer participants were recruited from four different clubs and rugby union players were recruited from a single sports club. All sporting clubs were located in Sydney. Dogs and their handlers were recruited from two dog training clubs in Sydney. Horses and their handlers were recruited from three riding schools (two located in Sydney and one in Glenworth Valley), three horse stables (two located in Sydney and one in Camden), one horse racing stable (located in Menangle Park) and one equine centre (located in Wagga Wagga). Veterinarians and veterinary personnel were recruited from four small animal clinics (all located in Sydney) and from four equine clinics (two located in Sydney, one located in Camden and one located in Richmond). Sample collection spanned from March 2010 to April 2013.

Prospective participants and clubs for each cohort were identified using an Internet search engine, with a focus on the Sydney region, with the exception being the recruitment of general community members. General community participants were recruited from local parks through poster notification in which the study details were provided. The recruitment approach for all the other groups was to advise prospective participants through general notification (e.g. via the phone and email). Presidents of dog clubs, head coaches and managers of sports teams, managers of veterinary practices, and owners of horse stables and riding schools were first contacted via the telephone. During the telephone conversation brief details regarding the study were provided. An email detailing the study was then sent out to these prospective participants, who then forwarded the email to members in their organisation. Interested participants replied via return email. All participants were provided with a participant information statement, a consent form and a written close-ended questionnaire (see Appendix A). The researcher and supervisors developed the questionnaire in relation to risk factors associated with MRSA carriage. With the exception of demographic information, all other questions in the survey were in a nominal format. In the case of non-responders to the questionnaire follow-up was attempted if the non-respondent provided the researcher with contact information. As a general rule in order to test correlations between caMRSA carriage and the independent variables in the questionnaire a minimum of 50 participants from each cohort were required (VanVoorhis and Morgan, 2007). However, due to the low expected MRSA carriage prevalence of 1-3% in the general population and in healthy animals and an expected prevalence of 2-4% in healthy sports participants (refer to Tables 1.4a to 1.10) a greater number of participants was required for this study. Based on an expected prevalence of 2% for these cohorts, with a precision of 1% and 95% confidence intervals 753 participant were required (Wedley et al., 2014). For the veterinary cohort assuming a

prevalence of 15%, a sample size of 206 was required to enable a power of 80% ($p < 0.05$) (Tirosh-Levy et al., 2015).

2.1.2 Demographic data and data storage

Demographic data were collected by means of a questionnaire provided to participants at the time of specimen collection. Data indicators such as age, number of people in the participant's household and information regarding direct and indirect contact with healthcare facility(s) were collected. Additional data collected sought to gain information concerning reported risk factors for colonisation with MRSA. This included the number of household pets, recent antibiotic use, clinical skin conditions, participation in contact sports and whether they had been in recent contact with a horse farm (see Appendix A).

Demographic data was coded and entered into an Excel spread sheet for later data analysis and interpretation. Personal details gathered from the questionnaire were kept confidential, with only the researchers having access to the data in accordance with human and animal ethics approvals.

2.2 Specimen collection, preparation of media and inoculation of media

2.2.1 Specimen collection

Specimen collection was performed using the Liquid Stuart BD BBL™ CultureSwab™ (Becton Dickinson – Australia). All participants had swabs taken from their anterior nares and throat by the researcher. The human swab sites were chosen based on previous studies that have reported the anterior nares and throat to yield the highest amount of *S. aureus*

when compared to other sites (Bourgeois-Nicolaos et al., 2010, Wallin et al., 2008, McKinnell et al., 2013). Swabs from dogs were taken from the nose and perineum by the researcher, while only a nostril swab was taken from the horses' by a qualified veterinarian, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). All swabs were transported in an insulated container at room temperature and were cultured within 12 hours of being collected as recommended by the manufacturer. Swabs were collected to assess the prevalence of caMRSA carriage.

2.2.2 Preparation of media

Nutrient broth with 4% sodium chloride (for enrichment), nutrient agar (for non-selective subculture) and Mueller Hinton broth (culture for DNA extraction) were all prepared in the laboratory according to manufacturers' instructions (Oxoid – Australia). Commercially prepared *Brilliance*™ MRSA 2 Agar (Oxoid – Australia) was used for MRSA screening.

2.2.3 Inoculation of enrichment media

Collected swab samples were used to inoculate enrichment broth (nutrient broth with 4% NaCl to enhance Staphylococcal detection) for 24 hours at 37^{o}C prior to being subcultured onto Brilliance™ MRSA 2 selective agar (Oxoid – Australia).

2.3 Organism identification - preliminary tests for MRSA

To confirm the identity of presumptive MRSA strains isolated from selective Brilliance™ MRSA 2 agar a sequence of standard tests was used (Brown et al., 2005). This included standard morphological and biochemical tests, including Gram stain, catalase test, rabbit

plasma tube coagulase test, DNase test, antibiotic sensitivity tests and multiplex end-point polymerase chain reaction (M-PCR), as shown in Figure 2.1.

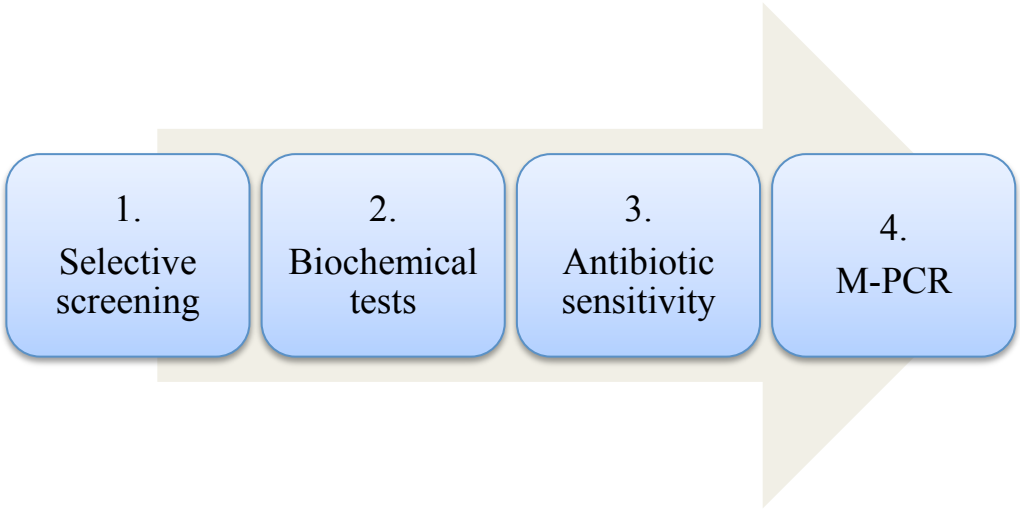

Figure. 2.1 Protocol for caMRSA identification

*2.3.1 Selective screening using **Brilliance™ MRSA 2 agar***

Brilliance™ MRSA 2 agar (Oxoid – Australia), is a selective medium for meticillin resistant strains of *Staphylococcus aureus*. The chromogenic agar plates were incubated at 37^{o}C for 24 hours. Following incubation, colonies showing the typical blue denim colour of MRSA were subcultured onto nutrient agar (Oxoid – Australia). Colony size was typically 0.5mm in diameter (Horstmann et al., 2012). Nutrient agar plates were incubated for 24 hours at 37^{o}C. Figure 2.2 shows the blue denim colonies of presumptive MRSA isolates on Brilliance™ MRSA 2 agar plates.

Figure 2.2 Positive MRSA colonies on ***Brilliance™*** *MRSA 2 agar*

2.3.2 Morphological and biochemical tests

Gram stain

Gram stains were performed on presumptive MRSA isolates. Gram-positive cocci were subsequently tested for catalase production to distinguish *S. aureus* from other Gram-positive cocci.

Catalase test

S. aureus typically produces a catalase enzyme. A catalase test was performed by immersing colonies from a pure nutrient agar culture plate of the suspect organism in a drop of 3% hydrogen peroxide (Sigma-Aldrich – Australia) on a glass slide. Catalase production was confirmed by the release of oxygen, manifested by the appearance of bubbling.

Coagulase test

Coagulase is a type of enzyme produced by *S. aureus* used to differentiate pathogenic *S. aureus* from other staphylococci species. Five other species of staphylococci are known to be coagulase positive; *S. delphini, S. pseudointermedius* (formerly *S. intermedius*), *S. hyicus, S. lutrae* and *S. schleiferi* subspecies *coagulans.* Coagulase tests were performed on isolates that were confirmed as Gram and catalase positive. Strains were tested using the tube test method with citrated rabbit plasma (Becton Dickinson – Australia). An overnight pure culture of the test organism was grown on nutrient agar and several colonies of the bacteria were emulsified with 0.5ml reconstituted rabbit plasma. Test tubes were incubated for 24 hours at 37^{o}C. Tubes were examined for clot formation (positive reaction) at 2 hours, 4 hours and 24 hours. Positive and negative controls were included with each test. A standard strain of *S. aureus* ATCC 29123 was used as a positive control, while water was used as the negative control.

DNase test

A DNase test was used to distinguish *S. aureus* from *S. epidermidis*. The test was performed on all suspected MRSA isolates. Each DNase plate (Oxoid – Australia) was streaked with a fresh culture of the test organism, approximately 2cm x 0.5cm in diameter. Organisms were incubated overnight at 35^{o}C and flooded with hydrochloric acid the next day. DNase positive organisms were confirmed with a clear zone around the test organism.

2.3.3 Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid – Australia), in accordance with the CLSI guidelines

(CLSI, 2012). All isolates were tested for sensitivity to cefoxitin (FOX, 30 μg), erythromycin (E, 15 μg), tetracycline (TE, 30 μg), trimethoprim (W, 5 μg), ciprofloxacin (CIP, 5μg), gentamicin (CN, 10 μg), rifampicin (RD, 5 μg), fusidic acid (FD, 10 μg), and mupirocin (MUP, 5μg). Zone diameters for all antibiotics were interpreted according to CLSI criteria with the exception of fusidic acid (Courvalin and Soussy, 1996) and mupirocin (Finlay et al., 1997b).

The Kirby-Bauer method required three to four well isolated colonies of the suspect organism from a pure overnight culture. These colonies were selected with a sterile loop and inoculated in 4 ml of nutrient broth. Broths were cultured at room temperature to a 0.5 McFarland standard. Within 15 minutes of adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The entire surface of the Mueller Hinton agar plate was streaked with the suspension. Antibiotic discs (Oxoid – Australia) were dispensed aseptically onto the surface of the inoculated plate. Plates were placed in an incubator at 35°C and incubated for 16 to 18 hours. The diameters of the zones of complete inhibition were measured and read. Table 2.3.5 shows the interpretation of antibiotic susceptibility results and concentration of antibiotics used.

Table 2.1 Interpretation of antibiotic resistance for MRSA as per the Kirby Bauer method (CLSI, 2012, Finlay et al., 1997a, Courvalin and Soussy, 1996)

Antibiotic	Resistant* (mm)	Intermediate* (mm)	Sensitive* (mm)	Concentration (μg)
CEF	≤19	-	≥22	30
E	≤13	14-22	≥23	15
CN	≤12	13-14	≥15	10
W	≤10	(11-15)	≥16	5
RD	≤16	17-19	≥20	5
MUP	≤13	-	≥14	5
FD	≤20	19-22	≥23	10
CIP	≤15	16-20	≥21	5
TE	≤14	15-18	≥19	30

* resistance defined by zone diameter measured in millimetres (mm)

cefoxitin – FOX erythromycin – E tetracycline - TE trimethoprim – W

ciprofloxacin - CP gentamicin - CN rifampicin – RD

2.3.4 Multiplex polymerase chain reaction (M-PCR) for femA and mecA

Presumptive MRSA isolates were analysed for the presence of *femA* (universally present in all *S. aureus)* and *mecA* (meticillin resistance) genes. The presence of *femA* was used to confirm *S. aureus* status and differentiate *S. aureus* from other coagulase positive staphylococci (Ishihara et al., 2010). Isolates were grown in a shaking incubator overnight at 37^{o}C in Mueller Hinton broth. DNA was extracted using the extraction buffer (Buffer A)

included in the BacReady Multiplex PCR System reagent kit (GenScript, New Jersey, USA) as per the manufacturer's instructions. Briefly, 2µl of the pure bacterial log phase culture in a Mueller Hinton broth was added to 18µl Buffer A (lysis buffer) and centrifuged to remove cell debris. Two µl of the supernatant containing the DNA template was used in the PCR reaction. Five controls were included in each run; MRSA (NCTC 10443), *S. aureus* (ATCC 8235) (*mecA* negative control), MSSA (clinical isolate), meticillin resistant coagulase negative *Staphylococcus* (*femA* negative control) and MRSA (clinical isolate). Control isolates were obtained from Concord Hospital, Australia.

Multiplex PCR (M-PCR), based on the method of Al-Talib and colleagues (Al-Talib et al., 2009), was performed on all suspected MRSA isolates with the following modification to the PCR reaction mix and running conditions. The final PCR reaction volume of 20µl contained: 10mM Tris-HCl, pH 8.3, 50mM KCl, 2.75mM $MgCl_2$, 0.001 % gelatin, 0.2mM dNTP mix, stabilisers, 0.03 unit/µl of *Taq*DNA Polymerase, 80nM *mecA* primers, 80nM *femA* primers, 3µl H_2O and 2µl of DNA extract. DNA amplification was carried out on a Bio-Rad iCycler PCR Thermal Cycler model 3.021 (Bio-Rad Laboratories, Inc, Australia) with the following PCR cycling profile: one cycle of initial denaturation at 94°C for 5 minutes, 30 cycles of amplification (denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds) with a final extension step at 72°C for 10 minutes. PCR products were analysed by electrophoresis on a 2% agarose gel and visualised with ethidium bromide. Figure 2.3 (see Appendix E) shows a representative gel image of M-PCR performed on MRSA and MSSA isolates recovered from community participants. Details of primers used in M-PCR are available in Appendix B (Table B1). In

the present study the original M-PCR assay described by Al-Talib and colleagues (2009) was altered to optimise the identification of MRSA.

2.3.5 Storage of isolates on bacterial preservers

Strains confirmed as MRSA were stored for later use using the Protect bead system (Oxoid - Australia) as per manufacturer's instructions. The beads containing MRSA positive isolates were placed in a -20^{o}C freezer for storage. All MRSA isolates were also stored in a 1ml solution of Mueller Hinton broth containing 15% glycerol and placed for long-term storage in an -80^{o}C freezer.

2.4 Genetic analyses of caMRSA isolates

Specialised genetic tests were performed on MRSA isolates by the candidate at the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (ACCESS), Royal Perth Hospital, to gather detailed information on their unique genetic characteristics and to discriminate between strain types. These included real-time multiplex polymerase chain reaction (RTM-PCR), macro-restriction pulsed field gel electrophoresis (PFGE), DNA microarrays and multilocus sequence typing (MLST).

2.4.1 Multiplex Real-time polymerase chain reaction (RT-PCR) for nuc, mecA and PVL gene

The MRSA status of isolates was confirmed using a multiplex RT-PCR assay. MRSA isolates were tested for the presence of *nuc* (housekeeping gene of *S. aureus*), *mecA* (a gene indicative of meticillin resistance) and PVL gene (toxin produced by some strains of

S. aureus) based on previously described methods (Costa et al., 2005, Fey et al., 2003). Previously published primer and probe sequences were used identification of *nuc, mecA* and PVL genes (Brakstad et al., 1993, Fey et al., 2003). For all primer and probe sequences used in this assay see Appendix B, Table B2. Five control strains were included in this assay. Control strains were obtained from Royal Perth Hospital, Australia. These consisted of MRSA (NCTC 10443), *S. aureus* (ATCC 25923; PVL positive control), meticillin resistant coagulase negative *Staphylococcus* (clinical isolate), meticillin sensitive coagulase negative *S. epidermidis* (ATCC 14990) and a mutant strain of MRSA (Library isolate). A negative reagent control was included in this assay.

Bacterial DNA was extracted using a previously described method (Costa et al., 2005). Briefly, bacterial colonies from blood agar plates were subcultured and incubated overnight at 37^{o}C. A single colony was resuspended in 50µl of lysostaphin solution (100µg/ml), followed by digestion in Proteinase K (20mg/ml). After centrifugation, 2µl of extracted DNA was used in the PCR reaction mix. The RT-PCR assay was performed on bacterial DNA extracts from 5 control strains and all positive *S. aureus* isolates. The final RT-PCR reaction volume of 20µl contained: 2.0µl FastStart 10 X reaction mix hybridisation probes, 3.2µl $MgCl_2$, 1.0µl UNG 1U/µl, 1.0µl *nuc* primers, 1.0µl *mecA* primers, 1.0µl PVL primers, 0.4µl *nuc* probes, 0.4µl *mecA* probes, 0.4µl PVL probes, 7.6µl H_2O and 2µl DNA extract. The RT-PCR assay was performed using the Roche Light Cycler 2.0 machine (Roche Diagnostics, Australia). The following PCR cycling profile was used: one cycle of UNG at 37°C for 5 minutes, one cycle of activation at 95°C for 10 minutes, 45 cycles of amplification (denaturation at 95°C for 15 seconds, annealing at 50°C for 15 seconds, and extension at 72°C for 15 seconds), one cycle of melting (95°C

for 0 seconds, 45°C for 10 seconds, 95°C for 0 seconds) with a final cooling step at 40°C for 60 seconds.

PCR data obtained from all isolates were analysed using the absolute quantification method. Figures 2.4 a, b and c (see Appendix E) illustrate the amplification curves of these MRSA and MSSA isolates (*nuc, mecA,* PVL gene). Real-time PCR crossing point (Cp) data points for *nuc, mecA* and PVL gene were obtained and analysed using the Roche LightCycler 2.0 Instrument software for all MRSA and MSSA isolates. Table 2.2 shows Cp values for MRSA and MSSA isolates recovered from community participants.

Table 2.2 Representative multiplex Real-time PCR (*nuc, mecA, PVL*) crossing point (Cp) values for MRSA and MSSA isolates recovered in this study

Lane	Isolate	Participant type	*nuc* (LC705)	*mecA* (LC640)	PVL (LC610)	MRSA/MSSA type
1	Control 1	-	19.33	16.26	-	MRSA (NCTC 10443)
2	Control 2	-	26.92	-	19.83	MSSA (ATCC 25923)
3	Control 3	-	-	19.39	-	MRCN (clinical isolate)
4	Control 4	-	-	-	-	MSCN (ATCC 14990)
5	Control 5	-	48.47*	17.64	-	MRSA (library isolate)
6	1n	SP	18.02	16.66	-	MRSA
7	13n	V	19.66	17.19	-	MRSA
8	5t	C	18.27	-	-	MSSA
9	6n	C	18.61	-	-	MSSA
10	7n	C	18.69	-	-	MSSA
11	11n	C	19.82	-	-	MSSA
12	12e	E	21.36	19.00	-	MRSA
13	9n	C	20.66	-	-	MSSA
14	3n	C	14.40	20.42	-	MRSA
15	3n	SP	21.05	17.65	-	MRSA
16	Reagent control	-	-	-	-	Injectable H_2O

n- isolated from nose t - isolated from throat *control strain has a low efficiency for *nuc*

C - community SP – sports participant V – veterinarian

E –contamination with MRSA present on a telephone in the workspace environment of veterinary nurse

MRCN - meticillin resistant coagulase negative *Staphylococcus*

MSCN - meticillin sensitive coagulase negative *Staphylococcus*

MSSA - meticillin sensitive *Staphylococcus aureus*

MRSA - meticillin resistant *Staphylococcus aureus*

Note: A representative image of the absolute quantification for Cp values in MRSA and MSSA isolates recovered from the community, including controls, are shown in Figures 2.4 a, b and c.

2.3.5 Storage of isolates on bacterial preservers

Strains confirmed as MRSA were stored for later use using the Protect bead system (Oxoid - Australia) as per manufacturer's instructions. The beads containing MRSA positive isolates were placed in a -20^{o}C freezer for storage. All MRSA isolates were also stored in a 1ml solution of Mueller Hinton broth containing 15% glycerol and placed for long-term storage in an -80^{o}C freezer.

2.4.2 Macro-restriction pulsed field gel electrophoresis (PFGE) of MRSA

Macro-restriction pulsed field gel electrophoresis (PFGE) was used in the molecular typing of MRSA due to its highly discriminatory characteristics. PFGE was performed on all isolates and compared with a database of fully characterised Australian MRSA and MSSA (MLST, SCC*mec* for MRSA, DNA microarray for MRSA and *spa* typing).

PFGE of bacterial DNA was performed according to a previously published method (O'Brien et al., 2006) using a contour-clamped homogeneous electric field (CHEF) DRIII apparatus (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). A *S. aureus* control strain (NCTC8325) was included in this assay as a size standard. The PFGE assay required a blood agar purity plate of the *S. aureus* isolate to be tested for 24-48 hours. Following incubation pure isolates were inoculated in tripticase soy broth overnight at 37°C. Cells were then washed twice in EDTA and the washed pallet was resuspended in EC buffer to McFarland 6. Briefly, lysostaphin and Proteinase K were used to in the extraction of DNA. PFGE blocks were run on a 1% gel using 150ml 0.5X TBE buffer and 1.5g pulse field certified agarose. Chromosomal patterns were visually examined and scanned with a Quantity One device (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). FPQuest

software was used to digitally analyse the electrophoresis patterns (Bio-Rad Laboratories, Gladesville, Australia). Figure 2.5 shows a representative MRSA and MSSA PFGE gel and Table 2.3 gives the PFGE results for the gel image and the corresponding participants from which the isolates were recovered. CHEF patterns were interpreted according to the criteria of Tenover et al (1996). Patterns 100% related by dendrogram to another subtype were assigned the same name. Isolates that have not been identified before but are 80% related to the existing patterns are considered to be a 'new subtype' of that group. Isolates less than 80% related were considered to be a new clone. Figure 2.6 (see Appendix E) shows a representative dendrogram of the 29 MRSA isolates recovered from veterinarians, veterinary nurses, household contacts and the environment.

2.4.3 DNA Microarray

The DNA microarray array (StaphyType system by Alere Technologies GmbH, Jena, Germany) used in this study included a total of 334 target sequences that correspond to 185 distinct genes and their allelic variants. Target genes consisted of species markers, regulatory markers (*agr*), virulence determinants, resistance genes, recombinase genes (*ccrA1*, *ccrB1*, *ccrA2*, *ccrB2*, *ccrA3*, *ccrB3*, *ccrA4*, *ccrB4*, and *ccrC1),* adhesion determinants and capsule types. A complete list of target genes is available in previously published papers, see Appendix D (Table D1) (Monecke et al., 2008a, Monecke et al., 2008b, Coombs et al., 2011). A blood agar purity plate of the MRSA isolate to be tested was incubated overnight at 37°C. The *S. aureus*-specific DNA microarray assay was performed as previously described (Monecke et al., 2008b) using DNA extracts prepared using the DNeasy Tissue Kit (Qiagen, Australia). The final Master Mix reaction volume of 12.3µl contained: 4.9µl of B1 (2 x labelling buffer), 0.1µl B2 (DNA polymerase) and 7.3µl

of DNA extract. Reagents used in the DNA Microarray reaction were provided in the StaphyType Kit (Alere Technologies GmbH, Jena, Germany).

Linear PCR was used to simultaneously amplify all targets. Linear amplification of PCR was carried out with the following PCR cycling profile: one cycle of initial denaturation at 96°C for 5 minutes, 55 cycles of amplification (denaturation at 96°C for 60 seconds, annealing at 50°C for 20 seconds, and extension at 72°C for 40 seconds) with a final hold step at 4°C for infinity. During the reaction all products were labelled by the incorporation of biotin–16-dUTP (Roche Diagnostics, Australia). Labelled samples were hybridized to the array. Washing steps and addition of a blocking reagent followed this. Subsequently, horseradish peroxidase-streptavidin conjugate (Thermo Fisher Scientific, Australia) was added to the array, followed by incubation and washing. Lastly, peroxidase substrate was added (Seramun green precipitating dye; Seramun, Germany). Upon test completion an image of the grid was captured using the ArrayMate™ reader (CLONDIAG® Chip Technologies GmbH, Jena, Germany). Results were interpreted by the Clondiag software system (CLONDIAG® Chip Technologies GmbH, Jena, Germany). Figure 2.7 (see Appendix E) shows a representative image of a DNA microarray chip captured following test completion on an equine veterinarian MRSA isolate (14n).

2.4.4 Multilocus sequence typing (MLST)

The multilocus sequence typing (MLST) method described in this section was performed by the staff at the Royal Perth Hospital ACCESS laboratory, to characterise isolates of MRSA. MLST was performed according to a previously described method (Enright et al., 2000b).

Two controls were included in the assay, UK-EMRSA-15 (ST22) and reagent control (negative). The MLST assay required a 24-hour blood agar purity plate of the *S. aureus* isolate to be tested. Chromosomal DNA samples were processed using a MagNA Pure Total Nucleic Acid extraction kit (Roche Diagnostics, Australia). Briefly, a single colony of the test organism was inoculated into nutrient broth and grown until equivalent to a 0.5 McFarland standard. Lysostaphin and lysozyme were added to a spun deposit. This solution was incubated for 15 minutes at 37°C in a dri-bath followed by incubation at 95°C for 10 minutes. The solution was then added to MagNA Pure sample cartridge and DNA extracted according to manufacturer's instructions.

This assay involves the amplification and sequencing of the internal fragments of seven housekeeping genes bidirectionally: *arc, aro, glp, gmk, pta, tpi* and *yqi*. The final PCR master mix reaction volume of 50µl for genes *arc, aro, glp, gmk, pta* and *yqi* contained: 5.0µl 10 X Polymerisation buffer, 4.0µl $MgCl_2$, 1.5µl forward primer (20µM), 1.5µl reverse primer (20µM), 1.0µl *d*NTPs (100µM of each), 0.5µl FastStart *Taq* Polymerase (5 U/µl), 31.5µl H_2O and 5.0µl target DNA. The final PCR master mix reaction volume of 50µl for gene *tpi* contained: 5µl 10 X Polymerisation buffer, 4.0µl $MgCl_2$, 2.14µl forward primer (20µM), 2.4µl reverse primer (20µM), 1.0µl *d*NTPs (100µM of each), 0.5µl FastStart *Taq* Polymerase (5 U/µl), 30.21µl H_2O and 5.0µl target DNA. An aliquot of 45µl of each PCR mastermix was added to each well in the 96-well PCR plate (Bio-Rad Laboratories, Inc, Australia), subsequently followed by 5 µl of target DNA. The 96-well PCR was then transferred to a MyCycler (Roche Diagnostics, Australia) for amplification. The following PCR cycling profile was used: one cycle at 95°C for 9 minutes, 30 cycles of

amplification (denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute), and a final extension step of 72°C for 5 minutes. Upon PCR completion, the 96-well PCR tray was delivered to the sequencing laboratory at Royal Perth Hospital, along with forward and reverse sequencing primers. Sample sequences were aligned and edited in BioEdit and uploaded to http://www.mlst.net for comparison with an international dataset. Numbers are allocated to each allele and the allelic profile used to determine the sequence type. Sequences for each housekeeping gene fragment from the control were matched 100% with each housekeeping gene fragment from the reference (UK-EMRSA-15, ST22).

2.5 Follow-up tests of colonised participants (relevant to Chapter 6 only)

Participants found to be carriers of caMRSA were contacted via email or mail, to seek permission to perform follow-up tests. Details of follow-up tests that were performed on consenting household contacts of initially colonised MRSA participants and their household environment are given in Chapter 6.

Chapter 3 Carriage of MRSA in the community, and in University and TAFE staff and students and their genotypes

3.1 Introduction

Staphylococcus aureus is a bacterium commonly found in the nose, throat and on the skin of humans and is the staphylococcal species that most often causes purulent infection (de Kraker et al., 2011). Resistance of *S. aureus* to meticillin was first reported in 1961 (Jevons, 1961). Traditionally meticillin resistant *S. aureus* (MRSA) infections have been associated with the healthcare setting, termed hospital-associated MRSA (haMRSA). The emergence of a distinct type of MRSA in the community setting, termed community-associated MRSA (caMRSA), during the early 1990's has not only placed significant burden on the healthcare system but has also has been implicated in infections in healthy community members (Köck et al., 2010b, Skov et al., 2012).

Strains of caMRSA possess different phenotypic and genotypic characteristics compared to haMRSA, which includes variations in their antibiotic susceptibility profiles (Farley, 2008, Coffman, 2007). In addition, the pathogenic mechanisms of caMRSA are reported to be distinct from haMRSA (Charlebois et al., 2004, Miller and Diep, 2008). The *mecA* (meticillin resistance) gene is a defining feature of caMRSA and haMRSA (Arêde et al., 2012). The *mecA* gene is located on a mobile genetic island, the staphylococcal chromosome cassette *mec* (SCC*mec*). Five SCC*mec* types predominate (types I-V, although 11 have been described to date) and differences between the SCC*mec* types can be used to distinguish caMRSA from haMRSA (Elements, 2009, Li et al., 2011, Shore et

al., 2011). Community-associated MRSA typically possess SCC*mec* Type IV or V, while haMRSA strains typically possess SCC*mec* Type I, II or III, with the exception of the haMRSA clone EMRSA-15 that carries SCC*mec* Type IV. In caMRSA, the smaller SCC*mec* Types IV and V generally harbour fewer resistance genes, whereas haMRSA strains possess larger SCC*mec* types and carry a greater number of resistance genes (Said-Salim et al., 2003, Gorwitz, 2008).

Reservoirs of MRSA in the community include humans and companion animals colonised or infected with MRSA, as well as contaminated household fomites (Uhlemann et al., 2011). Since the 1960's five major MRSA clonal complexes (CC) have been reported to predominate worldwide, namely CC5, CC8, CC22, CC30 and CC45 (Campanile et al., 2010, Stefani et al., 2012). Reported prevalence of caMRSA carriage in the general community across various regions varies, ranging from 0.26% to 9.2% (Farley, 2008), although the majority of studies report a rate of 2% or less (Graham et al., 2006, Lu, 2005, Munckhof et al., 2009). Furthermore, most research studies detailing MRSA prevalence in the general community have been conducted in either the US or Europe, where prevalence of caMRSA carriage has been reported to range from 0.12% to 3.6% (Gamblin et al., 2013, Abudu et al., 2001, Zanelli et al., 2002, Bearman et al., 2010, Morita et al., 2007, Ammons et al., 2010, Tenover et al., 2008, Graham et al., 2006).

At present, studies detailing caMRSA carriage in healthy community members in Australia are lacking. To date, there has been only one published Australian community study, which has formally assessed the carriage rate of caMRSA and haMRSA in the general adult population. In that study nasal carriage was found to be 0.3% (2/699) in healthy

community members and 0.4% (3/699) in people attending general practices (Munckhof et al., 2009). On the other hand, the epidemiology of MRSA clinical isolates recovered from healthcare settings across Australia has been well documented by the Australian Group on Antimicrobial Resistance (AGAR) (Coombs et al., 2009a, Coombs et al., 2013a, Coombs et al., 2012a). However, their data is not representative of MRSA carriage in the healthy adult population. Rather, their data reports on the prevalence of infecting isolates and their genotypes. More community-based studies are needed across Australia in order to shed greater light on the current MRSA situation in this country and to better detail MRSA strain types circulating in the community setting. Additionally, no risk factors for MRSA carriage in healthy community members in Australia have been published. The current study aims to examine the carriage and strain characteristics of MRSA isolates in healthy community volunteers and in a convenience sample of University and TAFE staff and students.

3.2 Methods

3.2.1 Participants

A total of 301 participants were enrolled into the study. Participants comprised members from the community (n=123) and University and Technical And Further Education (TAFE) staff and students (n=178, predominantly University students enrolled in a Bachelor of Health Sciences degree). Two Universities and one TAFE organisation located in Sydney participated. The 178 University and TAFE staff and students were considered to be atypical community participants as the majority had, at some point, been on clinical placement or worked in a healthcare facility (HCF). Participants were required to complete a questionnaire examining demographic factors and previously reported risk factors for

MRSA carriage. The University of Sydney Human Ethics Research Committee (HREC) approved the study.

3.2.2 Sample collection and initial screening tests for MRSA identification

Anterior nares and throat swabs were collected by the researcher and used to inoculate nutrient broth (Oxoid Microbiology Products, Australia) containing 4% sodium chloride as described previously in Section 2.2. Following incubation, the cultured broth was used to inoculate chromogenic Brilliance™ MRSA 2 agar (Oxoid Microbiology Products, Australia) and incubated at 37^{o}C for 24 hours. Presumptive identification of *S. aureus* was confirmed by Gram stain, catalase test, DNase test and rabbit plasma tube coagulase test (Becton, Dickinson and Company, Australia). MRSA isolates were confirmed using both antibiotic susceptibility tests, and M-PCR. All of the above preliminary identification tests were performed on five MRSA and 22 MSSA isolates as described in Section 2.3 of General methods. MRSA were stored for later use using the Protect bead system (Oxoid - Australia), described previously in Section 2.3.

Data analysis

Fisher's exact tests were performed on categorical variables where there were less than five cases present in each cell. Tests were performed using IBM SPSS Statistics version 21.0 software (IBM, Australia) to examine associations between the dependent variable (carrier status) and independent variables (direct contact with HCF, skin disease, ownership of dog/cat, contact with horse farm, use of antibiotics, work in a clinical facility etc.). All tests were two-tailed. A p-value of <0.05 indicated significance.

3.2.3 Genetic analyses of MRSA isolates (Multiplex RT-PCR, PFGE, MLST, DNA microarray)

All confirmed MRSA isolates were subjected to the following genetic analyses:

Multiplex Real-time PCR (RT-PCR)

A multiplex RT-PCR assay was used to confirm the presence of the *mecA* gene and to detect the presence of the *nuc* gene (thermostable extracellular nuclease) according to a previously described method (Costa et al., 2005). RT-PCR for PVL gene determinants were performed as per a previous method (Fey et al., 2003). RT-PCR was performed on five MRSA and 22 MSSA. See Section 2.4.1 for method details.

Macro-restriction pulsed field gel electrophoresis (PFGE)

PFGE of chromosomal DNA was performed on five MRSA and 22 MSSA isolates according to a previously published method (O'Brien et al., 2006) using a contour-clamped homogeneous electric field (CHEF) DRIII system (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). See Section 2.4.2 for method details. The DNA of one livestock associated MSSA ST398 isolate could not be typed using the standardised *SmaI* enzyme, as methylation of the restriction site blocks the activity of this enzyme. This isolate was digested using the *Apa*I enzyme instead, which is effective in cutting the DNA of ST398 strains (Bergstrom et al., 2012, Bens et al., 2006). PFGE data for MSSA isolates are given in Appendix C.

DNA microarray

The *S. aureus*-specific diagnostic DNA microarray assay was performed on five MRSA isolates as previously described (Monecke et al., 2008b). See Section 2.4.3 for method details.

Multilocus sequence typing (MLST)

MLST was performed on five MRSA isolates as previously described (Enright et al., 2000b). See Section 2.4.4 for method details.

3.3 Results

3.3.1 Participants demographic information

From the general community participants (n=123) the majority of the cohort were female (87 vs. 35) and with a median age of 25 years. Demographic data indicated that 3 participants had worked in a clinical setting in the last six months, 74 had direct contact with a healthcare facility (HCF) within the last year, 37 participants had taken antibiotics within the last six months, 51 had close household contacts whom had contact with a HCF in the last year and most participants lived in a household with two or less family members (n=53). In addition, 55 participants owned dogs, 34 owned cats, of which 61 had direct contact with a veterinary facility.

From the University and TAFE staff and students (n=178) the majority of the cohort were female (137 vs. 38) and with a median age of 27 years. Demographic data indicated that 96 participants had worked in a clinical setting in the last six months, 111 had direct contact

with a healthcare facility (HCF) within the last year, 72 participants had taken antibiotics within the last six months, 77 had close household contacts whom had contact with a HCF in the last year and most participants lived in a household with three to five family members (n=85). In addition, 75 participants owned dogs, 54 owned cats, of which 66 had direct contact with a veterinary facility. Table 3.1 details participant demographic information.

Table 3.1 Demographics of community participants, University and TAFE staff and students

Characteristics of participants		General community (n)	University and TAFE (n)
Participants	Total	123	178
Gender	Female	87	137
	Male	35	38
	Not specified	1	3
Age (years)	0 – 20	38	37
	21 – 40	49	100
	41 – 60	30	32
	> 60	3	2
	Not specified	3	7
Clinical placement in last 6 months	Hospital/nursing home/other HCF	3	96
Duration of clinical placement	< 7 Days	1	14
	> 7 Days	2	74
	Not specified	0	8
Direct hospital contact in last 12 months*	Illness	8	20
	Procedure	17	21
	Other e.g. visiting	59	85
Duration of hospital contact	< 7 Days	58	72
	> 7 Days	13	35
	Not specified	3	4
Member of household had contact with healthcare facility in last 12 months	Yes	51	77
Duration that household contact had with healthcare facilitv	< 7 Days	28	42
	> 7 Days	23	33
	Not specified	0	2
Antibiotic use within the last 6 months	Yes	37	72
Plays sport	Yes	11	9
Suffers from skin disease	Yes	10	21
Animal present in household	Dog	55	75
	Cat	34	54
Pet had veterinary visit in last 12 months	Yes	61	66
Number living in household	2 or less	57	77
(excluding participant)	3-5	53	85
	> 6	11	9
	Not specified	2	7

*general community participants with direct personal contact with healthcare facility (HCF) (n=74); please note some participants had direct contact with HCF via visiting as well as illness/procedure

*University and TAFE staff and students with direct personal contact with healthcare facility (HCF) (n=111); please note some participants had direct contact with HCF via visiting as well as illness/procedure

3.3.2 Preliminary screening and carriage prevalence

A total of 32 presumptive MRSA isolates from 301 participants were identified using Brilliance™ MRSA 2 agar. These isolates were then subjected to a series of standard tests to confirm that they were *S. aureus* (Gram stain, catalase test, coagulase test) and subsequent tests (antibiotic susceptibility i.e. FOX positive and M-PCR i.e. *mecA* positive) confirmed 5/32 isolates were MRSA. This indicated that 27/32 isolates selected on the Brilliance™ MRSA 2 agar were false positives. Table 3.2 shows the results for initial MRSA confirmation and Table 3.3 show the results for antibiotic susceptibility of all presumptive MRSA isolates. RT-PCR subsequently confirmed the presence of *mecA* and *nuc* in the five isolates. Presence of PVL was confirmed in one isolate. MRSA carriage was observed to be 1.63% (2/123) in community participants and 1.69% (3/178) in University and TAFE staff and students. Of the five MRSA carriers, four carried MRSA in the nose only and one participant carried MRSA in their throat only.

Table 3.2 MRSA screening and confirmatory tests in community participants, University and TAFE staff and students

Isolates	**Brilliance™ MRSA 2** agar	Gram stain	DNase, catalase test	Coagulase test	M-PCR		Multiplex RT-PCR		
					femA	*mecA*	*nuc*	*mecA*	PVL
Positive	32*	32	32	32	32	5	32	5	1
Negative	0	0	0	0	0	27	0	27	31

M-PCR – multiplex polymerase chain reaction

RT-PCR – real-time polymerase chain reaction

femA - unique to *Staphylococcus aureus*

mecA - alternative penicillin binding protein 2 defines MRSA

nuc – thermostable extracellular nuclease

PVL – Panton-Valentine leukocidin toxin

*presumptive MRSA isolates

Table 3.3 Antibiotic susceptibility of presumptive MRSA isolates by disc diffusion

	FOX*	RD	MUP	W	FD	CN	CIP	E	TE
Resistant	5	0	0	2	1	0	0	5	1
Sensitive	27	32	32	30	31	32	32	27	31

FOX* (30µg) - cefoxitin, correlates to MRSA positive isolates in this study

RD (5µg) - rifampicin

MUP (5µg) - mupirocin

W (5µg) - trimethoprim

FD (10µg) - fusidic acid

CN (10µg) - gentamicin

CIP (5µg) - ciprofloxacin

E (15µg) - erythromycin

TE (30µg) - tetracycline

With regard to the five MRSA carriers, four were women (1n, 2n, 4t, 5n), three had worked in a healthcare facility (1n, 4t, 5n), all five had direct contact with a healthcare facility (illness/procedure/visiting), two had a household contact noted to have been in contact with a HCF (4t, 5n), four participants had taken antibiotics within the previous six months (1n, 2n, 3n, 5n), one participant suffered from a skin disease (2n) and three had direct contact with a veterinary facility (1n, 2n, 4t) (i.e. taking pet to veterinary practice) (see Table 3.4). In addition two of the carriers were members of the same household (1n, 2n). No associations between MRSA carriage and independent variables were identified by Fisher's exact analysis in participants from the general community and in University and TAFE staff and students.

Table 3.4 Characteristics of MRSA positive carriers in the community and in University and TAFE staff and students

Participant	Cohort	Gender	Worked in healthcare facility		Direct personal contact with HCF*		Household contact with HCF		Antibiotic use		Skin disease	Veterinary contact^
		F/M	Yes/No	Duration	Yes/No	Duration	Yes/No	Duration	Yes/No	Type	Yes/No	Yes/No
1n~	U	F	+	>7 days	+	<7 days	-	-	+	CEX	+	+
2n~	C	F	-	-	+	<7 days	-	-	+	MUP	-	+
3n	U	M	-	-	+	<7 days	-	-	+	n/s	-	-
4t	U	F	+	>7 days	+	<7 days	+	<7 days	-	-	-	+
5n	C	F	+	>7 days	+	<7 days	+	>7 days	+	n/s	-	-

*participant had direct contact with healthcare facility as a result of visitation, illness or procedure

^participant had direct contact with veterinary facility as a result of taking companion animal for veterinary visit

cephalexin – CEX

mupirocin – MUP

n=isolated from nose

t=isolated from throat

F/M – female/male

n/s – not specified

HCF – healthcare facility

~members from the same household

C – community participant

U – University participant

3.3.3 Genetic analyses of MRSA isolates

PFGE and MLST results

Macro-restriction pulsed field gel electrophoresis (PFGE) of the five MRSA isolates characterised three unique strains: WA23, Western Samoan Phage (WSPP) and WA44. Table 3.5 shows the MRSA phage type, sequence type, clonal complex type and strain synonyms as determined by PFGE and MLST analysis, and antibiotic susceptibility. Data from PFGE analyses on the 27 meticillin sensitive *S. aureus* (MSSA) isolates is available in Appendix C.

Table 3.5 Characteristics of MRSA isolates in community participants as identified by PFGE, MLST and antibiotic susceptibility

Participant	Sequence type (ST)	Clonal Complex (CC)	Strain synonyms	MRSA type*	Antibiogram resistance
1n, 2n	45	45	WA-MRSA-23	caMRSA	FOX
3n, 4t	30	30	Western Samoan Phage (WSPP)	caMRSA	FOX
5n	72	72	WA-MRSA-44	caMRSA	FOX

n - isolated from nose t - isolated from throat FOX - cefoxitin

* Isolates classified as either caMRSA or haMRSA according to a previously published paper on evolution and genetic diversity of MRSA according to MLST, SCC*mec* type, antibiogram and DNA microarray analyses, specific to Australia (Coombs et al., 2011)

DNA Microarray results

Microarray analysis of five MRSA isolates identified four unique MRSA types in the cohort; isolates 1n and 2n were typed as caMRSA ST45-MRSA-IV, and were isolated from two members in the same family; isolate 3n was typed as CC30-MRSA-IV, a predominant MRSA clonal complex type and was PVL negative; isolate 4t was typed as a caMRSA CC30-MRSA-IV Western Samoan Phage (WSPP) and was PVL positive (*lukF-PV, lukS-PV*); isolate 5n was typed as caMRSA ST72-MRSA-IV, USA700. All isolates carried *cap* 8 (polysaccharide capsule), resistance determinants *mecA, delta_mecR, blaZ,* virulence determinants *egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), hlgA, sak, scn, hla* and adhesion determinants *bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, map, sdrC, sdrD, vwb.*

There were some variations between isolates. Isolates 1n and 2n carried additional virulence and adhesion determinants *seg, ser, chp, cna, fnbB* and *sasG* and isolate 4n carried additional resistance virulence and adhesion determinants *fosB, lukF-PV, lukS-PV, chp, cna* and *fnbB* and isolate 5n carried an additional resistance determinant, *aadD* (aminoglycoside resistance gene), virulence determinants *lukD* and *lukE,* and adhesion determinant *fnbB.* Isolates 1n and 2n possessed *agrI* and *agrIV,* isolates 3n and 4t carried *agrIII* and isolate 5n carried *agrI.* For the complete list of target genes analysed, see Appendix E. Table 3.6 shows DNA microarray genotype profiling (agr type, capsule type, resistance genes, virulence genes and adhesion genes) of MRSA isolates and associated strain assignment.

Table 3.6 Microarray profiling of resistance, virulence and adhesion determinants of MRSA strains in community participants, University and TAFE staff and students

Participant	MLST/SCC*mec*	*agr* type	Capsule type	Resistance genes	Virulence genes	Adhesion genes
1n, 2n	Atypical ST45-MRSA-IV, WA-MRSA-23, "Victorian EMRSA" with SCCmec IV	I, IV	8	*mecA, delta_mecR, blaZ*	*egc-cluster, seg, sei, sej, selm, seln, selo, ser, selu, lukS, lukF, lukS (ST22+ST45), hlgA, sak, chp, scn, hla*	*bbp, clfA, clfB, ebh, ebpS, cna, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
3n	CC30-MRSA-IV	III	8	*mecA, delta_mecR, blaZ*	*egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), hlgA, sak, scn, hla*	*bbp, clfA, clfB, ebh, ebpS, cna, eno, fib, fnbA, map, sdrC, sdrD, vwb*
4t	CC30-MRSA-IV [PVL+], Western Samoan Phage	III	8	*mecA, delta_mecR, blaZ, fosB*	*egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), lukF-PV, lukS-PV, hlgA, sak, chp, scn, hla*	*bbp, clfA, clfB, ebh, ebpS, cna, eno, fib, fnbA, fnbB, map, sdrC, sdrD, vwb*
5n	ST72-MRSA-IV, USA700	I	5	*mecA, delta_mecR, blaZ, aadD*	*egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), lukD, lukE, hlgA, sak, chp scn, , hla*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*

`A complete list of target genes is available in a previously published paper *(Monecke et al., 2008b)*

n - nasal colonisation t - throat colonisation *agr* – accessory gene regulator MLST – multilocus sequence typing SCC*mec* – Staphylococcal cassette chromosome mec

Note: genes have been formatted in bold to highlight main differences between isolates typed in this study

3.4 Discussion

Since their identification in the community in the 1990's, there has been increasing research on novel community-associated MRSA strains (caMRSA), particularly in the US and Europe, where they have been shown to have different characteristics to classical hospital-associated strains. Carriage of MRSA is important because colonised persons are at a greater risk of becoming infected, and can act as potential vectors in the transfer of MRSA to other individuals. Furthermore, haMRSA and caMRSA are known to circulate in both the community and healthcare settings and are no longer isolated within distinct settings. In the present study, based on genotypic analyses, all MRSA isolates were identified as caMRSA strains (5/5), which is suggestive of a community reservoir. However, due to such a low number of positive isolates recovered and the cross-sectional nature of this study it is difficult to speculate on any trend.

Findings in this present study indicate an caMRSA carriage prevalence of 1.63% (2/123) in members of the general community and 1.69% (3/178) in University students, which is within the range of 0.65% to 3% nasal MRSA carriage previously reported in populations of predominantly university students, as well as in general community members (Kuehnert et al., 2006, Tenover et al., 2008, Bearman et al., 2010, Morita et al., 2007, Rackham et al., 2010, Shen et al., 2013). The caMRSA prevalence of 1.63% and 1.69% in this study is however, somewhat higher than a previously reported prevalence rate in Australia, in which caMRSA nasal carriage was reported to be 0.3% (2/699) and haMRSA carriage was 0.4% (3/699) in two Brisbane communities (Munckhof et al., 2009). This finding is expected given the fact that the cohort in the present study was made up of predominantly University students enrolled into a Health Sciences degree (three of five positive participants were students who had worked in a HCF). However, the low prevalence of

caMRSA carriage reported by Munckhof et al (2009) is unexpected as one part of their study assessed carriage in patients presenting to general practices. Another part of their study targeted participants of a higher socioeconomic status, which may account for the low caMRSA prevalence in the study. Hence, based on sampling participants from general practices and sampling a group with a high socioeconomic status the carriage rate reported in their study cannot be representative of the general community.

Previous studies have reported participants in contact with a healthcare setting (including clinical placement) are at an increased risk of becoming MRSA carriers (Verwer et al., 2012, Manzur et al., 2008, Kottler et al., 2010, Hewlett et al., 2009, Cimolai, 2008, Baldwin et al., 2009). Factors associated with increased risk of MRSA carriage were examined in this study by means of a questionnaire; working in or direct contact with a healthcare facility, antibiotic use, skin disease, close contact with person/s with MRSA and contact with a veterinary setting (David and Daum, 2010, Maree et al., 2010, Salgado et al., 2003, Beam and Buckley, 2006). In the present study, of the five MRSA positive carriers, all had contact with a healthcare facility, three of which also had been in contact with a veterinary facility. Due to the small number of positive carriers data analysis examining risk factors could not be performed and significant associations between the risk factors identified and their effect on increased risk of MRSA carriage were unable to be made and this was a limitation of this study. Nevertheless, carriers may increase the likelihood of spreading MRSA between the healthcare setting and the community. Additionally, two participants from the same household were carriers of identical caMRSA strains (1n, 2n), which is consistent with a previous study, in which isolates obtained from family members residing within the same household shared the same strain types and characteristics (Huijsdens et al., 2006c).

Another finding in the present study was the high number of false positive MRSA isolates 84.38% (27/32) identified using the Brilliance™ MRSA 2 agar (see Appendix C). In a recent study evaluating the performance of Brilliance™ MRSA 2 agar, the authors stated a false positive rate of only 12%, with a sensitivity of 100% and specificity of 98% following broth enrichment (Veenemans et al., 2013). On the other hand, detection of MRSA by five different chromogenic agars has been tested and compared by Malhotra-Kumar and colleagues (2010). They compared Brilliance MRSA agar (Oxoid), CHROMagar (CHROMagar Microbiology), ChromID (bioMérieux), MRSASelect (Bio-Rad) and BBL-CHROMagar (BD Diagnostics). In their study they tested a mixture of bacterial colonies on the agars (MSSA, haMRSA, caMRSA, *S. epidermidis, S. warneri, E. coli, E. faecalis, E. faecium, K. pneumoniae, P. aeruginosa* and *Acinetobacter*). The authors reported Brilliance MRSA agar had the highest sensitivity (97.6%) of the five agars but the lowest specificity of the five after 24 hours. This was due to a misinterpretation of a strain of MSSA, one strain of E. coli and four of five methicillin resistant coagulase-negative staphylococci (MRCoNS) strains (Malhotra-Kumar et al., 2010). This could perhaps provide an explanation for the high false positive rate reported in the present study.

The high false positive rate reported in the present study suggests the results obtained with this media should be interpreted as preliminary screening data (as recommended by the manufacturers) for presumptive MRSA isolates rather than as a stand-alone identification method for MRSA. On the other hand identification of MRSA by antibiotic susceptibility testing verified with cefoxitin resistance and detection of the *mecA* and *femA* genes by M-PCR both proved to be robust definitive techniques, in line with other studies that have

used these techniques to accurately distinguish MSSA from MRSA (Jonas et al., 2002, Mohanasoundaram and Lalitha, 2008, Skov et al., 2013).

Isolate characteristics and global isolation

With the detailed information provided by PFGE, MLST and DNA microarray assays it was possible to compare the strains' specific ST types and their genetic characteristics in the present study with strains of the same ST type typically isolated in other countries. By geographic region, the most commonly isolated caMRSA strains reported are ST1, ST30 and ST93 in Australia (Coombs et al., 2013a), ST8 in the USA, ST80 in Europe and the ST59 in Asia (Chua et al., 2011).

Isolates 1n and 2n were atypical WA-MRSA-23 caMRSA strains (ST45, CC45) (Coombs et al., 2012a). The reason these isolates are classified as atypical is due to the unique pattern they yield (i.e. they fail to react with all three *agrD-I* probes, but they are positive with *agrB* and *agrC* probes corresponding to *agr* groups I and IV). This particular ST45 lineage is typically isolated in Australia and Hong Kong (Ip et al., 2005, Monecke et al., 2011a). Both of these isolates carried the same resistance, virulence and adhesion determinants and were isolated from members of the same family. In addition, typical virulence determinants (*sej, ser)* and adhesion determinants (*fnbA, fnbB, sdrD, vwb, sasG)* found in atypical strains of ST45 were present in our isolates.

Isolate 3n was typed as a PVL negative Western Samoan Phage (WSPP) caMRSA strain (ST30, CC30). Panton Valentine Leukocidin negative and *tst1* positive ST30 strains have

been sporadically identified in Australia and Ireland (Monecke et al., 2011a). Typical genetic markers for the ST30 strain include a resistance gene (*fosB),* virulence markers (*egc-cluster, sak, chp, scn)* and an adhesion determinant (*cna).* In 2012 less than 7% (4/60) of WSPP caMRSA strains identified in Australia were PVL negative (Coombs et al., 2013a). Furthermore, this isolate was different to previously identified PVL negative isolates as this isolate also lacked typical resistance gene *fosB* and virulence factors *tst1* and *chp*.

Isolate 4t was typed as a PVL positive Western Samoan Phage (WSPP) caMRSA strain (ST30, CC30). The ST30 strain was originally observed in Samoans living in New Zealand, but has since been found to exist in other parts of the world including Australia, New Zealand, Europe, Hong Kong, Abu Dhabi, Taiwan and the USA (Williamson et al., 2013, Monecke et al., 2007, Monecke et al., 2008a, Takano et al., 2008, Monecke et al., 2011b). Typical genetic markers for the ST30 strain were also present in isolate 4t (Monecke et al., 2011a). Additionally, this isolate was the only strain observed to carry the Panton Valentine Leukocidin (PVL) toxin, which has been implicated in enhancing the virulence of *S. aureus,* due to its detrimental effect on phagocytes, leading to cytolysis or apoptosis of these cells (Dohin et al., 2007, Vandenesch et al., 2010). PVL has also been associated with severe necrotising pneumonia (Labandeira-Rey et al., 2007), although consensus on PVL as a major virulence factor has been highly controversial with conflicting evidence (Tseng et al., 2009).

Isolate 5n was typed as ST72, CC72, USA700, a caMRSA stain. This strain has also been identified in Australia, America, Abu Dhabi, Germany, Czech Republic, Sweden, Portugal

and Asia (Song et al., 2011, Mediavilla et al., 2012). The enterotoxin gene cluster (*egc-cluster*) is a typical virulence determinant found in ST72 and usually present in over half of *S. aureus* strains known to cause invasive infection (Grumann et al, 2013).

This study identified the ST30 (PVL positive and PVL negative strain), ST72 and ST45 (atypical strain) strains; these strains are referred to in the recent report by the Australian Group on Antimicrobial Resistance (AGAR) in which ST30 was the second, ST45-IV was the twenty-sixth and ST72 was the twenty-ninth caMRSA strain in order of predominance of the top thirty-two strain types isolated in Australian healthcare settings (Coombs et al., 2013a). However, a major difference is that the present study investigates MRSA carriage in healthy members of the general community, as well as University and TAFE staff and students, whilst the AGAR study investigates clinical isolates recovered from hospitalised patients. It is noted all isolates in this study were found to possess polysaccharide capsular (*cap*) type 8, albeit one isolate (5n) which carried *cap* type 5; both of which are two predominant *cap* types commonly identified in clinically infectious MRSA strains. This is of relevance as colonisation often precedes infection, and should the participants become infected they are more likely to experience more invasive disease (O'Riordan and Lee, 2004). Based on the findings in the current study, caMRSA does not appear to pose a major threat to general community members and University and TAFE staff and students sampled in Sydney. Further studies on MRSA carriage are required across different Australian regions to assess whether this trend is applicable to other states and not just within one region that has been surveyed.

Chapter 4 Carriage of MRSA in soccer and rugby union players and their genotypes

4.1 Introduction

Within the general community there are sub-groups reported to be at an increased risk of acquiring MRSA, including young children, military recruits, healthcare workers and household contacts of colonised or infected carriers (Rafee et al., 2012, Ho et al., 2007). Another important risk group for caMRSA colonisation and infection is contact sport participants (Kazakova et al., 2005, Creech et al., 2010). Participation in contact sports exposes individuals to an increased risk of acquiring MRSA as these settings provide a number of conditions that are beneficial for the survival and spread of both caMRSA and haMRSA, such as overcrowding, compromised skin integrity and frequent physical contact between players. Contact with contaminated fomites, sharing belongings or sports equipment may also facilitate the spread of this organism (Begier et al., 2004, Nguyen et al., 2005, Cohen, 2005).

The role physical contact plays in the transfer of caMRSA has been described in a number of outbreak studies investigating MRSA in sports teams in the US with colonisation rates as high as 20% reported in some cases (Huijsdens et al., 2006b, Cohen, 2005, Begier et al., 2004, Bowers et al., 2008, Romano et al., 2006, Collins and O'Connell, 2012). In one particular US study a high MRSA carriage was observed in 100 football players, ranging from 4% during the off-season to 19% during the end of playing season (Creech et al.,

2010). In contrast to this finding, other studies have suggested MRSA carriage prevalence in the sport participants is similar to the prevalence observed in the general community (Rackham et al., 2010).

American football and soccer players are reported to be at an increased risk of acquiring MRSA (Begier et al., 2004, Cohen, 2005, Bowers et al., 2008, Romano et al., 2006, Verwer et al., 2012). To date, there have been only four internationally published studies investigating nasal carriage with MRSA in healthy American football players which have not been associated with an outbreak (Lear et al., 2011, Rackham et al., 2010, Creech et al., 2010, Garza et al., 2009). Only one study has examined MRSA carriage in healthy soccer players (n=14) and no studies have examined carriage with MRSA in healthy rugby union players. In addition, no published studies in Australia have examined MRSA carriage in sports participants, including soccer or rugby union players. These groups are of importance as healthy young players can acquire MRSA and in some rare cases this organism has been implicated as a cause of death as a result of contracted infections in contact sports players (Andrews et al., 2007, Mihoces and McLean, 2006).

The current study aims to examine the carriage and strain characteristics of MRSA circulating in people engaged in soccer and rugby in Sydney, Australia.

4.2 Methods

4.2.1 Participants

Initially, prospective sports participants across 20 different soccer and 11 different rugby clubs in Sydney were contacted via the phone. Sports clubs that indicated interest were then sent an email to participate in this study. During the preseason and playing season a total of 181 sports players from four soccer clubs (n=100) and one rugby union club (n=81) were recruited. A response rate of 20% (4/20) was observed for soccer clubs, while a response rate of 9% was observed for rugby union clubs. Participants were required to complete a questionnaire examining demographic and risk factors for MRSA carriage. The University of Sydney Human Ethics Research Committee (HREC) approved the study.

4.2.2 Sample collection and initial screening tests for MRSA identification

Anterior nares and throat swabs were collected by the researcher and used to inoculate nutrient broth (Oxoid Microbiology Products, Australia) containing 4% sodium chloride as described in Section 2.2. Following incubation the cultured broth was used to inoculate chromogenic Brilliance™ MRSA 2 agar (Oxoid Microbiology Products, Australia) and incubated at 37^{o}C for 24 hours. *S. aureus* identification was confirmed by Gram stain, catalase test, DNase test and rabbit plasma tube coagulase test (Becton, Dickinson and Company, Australia). MRSA was confirmed using both antibiotic susceptibility tests and M-PCR. All of the above preliminary identification tests were performed on two MRSA and 10 MSSA isolates as described in Section 2.3 of General methods. MRSA were stored for later use using the Protect bead system (Oxoid - Australia).

Data analysis

Fisher's exact tests were performed on categorical variables where there were less than five cases present in each cell. Tests were performed using IBM SPSS Statistics version 21.0 software (IBM, Australia) to examine associations between the dependent variable (carrier status) and independent variables (direct contact with HCF, skin disease, ownership of dog/cat, contact with horse farm, use of antibiotics, work in a clinical facility etc.). All tests were two-tailed. A p-value of <0.05 indicated significance.

4.2.3 Genetic analyses of MRSA isolates (Multiplex RT-PCR, PFGE, MLST, DNA microarray)

All confirmed MRSA isolates were subjected to the following genetic analyses:

Multiplex Real-time PCR (RT-PCR)

A multiplex RT-PCR assay was used to confirm the presence of the *mecA* gene and to detect the presence of the *nuc* gene (thermostable extracellular nuclease) according to a previously described method (Costa et al., 2005). RT-PCR for PVL gene determinants were performed as per a previous method (Fey et al., 2003). RT-PCR was performed on two MRSA and 10 MSSA isolates, See Section 2.4.1 for method details.

Macro-restriction pulsed field gel electrophoresis (PFGE)

PFGE of chromosomal DNA was performed on two MRSA and 10 MSSA isolates according to a previously published method (O'Brien et al., 2006) using a contour-clamped homogeneous electric field (CHEF) DRIII system (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). See Section 2.4.2 for method details.

DNA microarray

The *S. aureus*-specific diagnostic DNA microarray assay was performed on two MRSA isolates as previously described (Monecke et al., 2008b). See Section 2.4.3 for method details.

Multilocus sequence typing (MLST)

MLST was performed on two MRSA isolates as previously described (Enright et al., 2000b). See Section 2.4.4 for method details.

4.3 Results

4.3.1 Participants demographic information

From the 181 participants the majority of contact sports players were male (136 male vs. 61 female) and played soccer (n=100). The median age for this cohort was 22 years. Demographic data on risk factors indicated that 15 participants had worked in a clinical setting in the last six months, 97 had direct personal contact with a healthcare facility (HCF) within the last year as a result of illness/procedure/visiting, 79 participants had taken antibiotics within the last six months, 55 had close household contacts whom had contact with a HCF in the last year, the majority of participants owned dogs (n=100) and most participants lived in a household with three to five family members. Table 4.1 shows contact sports participant demographic information in more detail.

Table 4.1 Demographics of sports participants

Characteristics of participants		Sports participants (n)
Participants	Total number of participants	181
Type of sport played	Soccer	100
	Rugby union	81
Gender	Female	53
	Male	126
	Not specified	2
Age (years)	0 – 20	
	21 – 40	
	41 – 60	
	> 60	
	Not specified	
Clinical placement in last 6 months*	Hospital	7
	Nursing Home	3
	Other Facility	4
Duration of clinical placement	< 7 Days	8
	> 7 Days	5
Direct hospital contact in last 12 months^	Illness	14
	Procedure	29
	Other e.g. visiting	57
Duration of hospital contact	< 7 Days	58
	> 7 Days	18
	Not specified	7
Member of household had contact with healthcare facility in last 12 months	Yes	49
Duration that household contact had with healthcare facility	< 7 Days	31
	> 7 Days	15
	Not specified	3
Antibiotic use within the last 6 months	Yes	69
Most common antibiotic prescribed	Amoxicillin	8
Animal present in household	Dog	91
	Cat	40
Pet had veterinary visit in last 12 months	Yes	83
Number living in household	≤2	51
(excluding participant)	3-5	90
	> 6	21
	Not specified	19

*13 participants worked in a healthcare facility, please note some worked in both hospital and nursing home

^83 participants had direct personal contact with a healthcare setting; please note some participants had contact via visiting and or procedure/illness

4.3.2 Preliminary screening and carriage prevalence

In total, 7 presumptive MRSA isolates from 181 contact sports participants were identified using Brilliance™ MRSA 2 agar. These isolates were then subjected to a series of standard tests to confirm *S. aureus* (Gram stain, catalase test, coagulase test) and subsequent tests (antibiotic susceptibility and M-PCR) confirmed 2/7 isolates were MRSA. RT-PCR subsequently confirmed the presence of *mecA*, and *nuc* in two isolates from two participants. PVL was absent in all isolates. Hence, of the initial 7 isolates identified as suspect MRSA on the selective Brilliance™ MRSA 2 agar, 5/7 isolates were false positive. MRSA carriage prevalence in contact sports players was 1.66% (2/181). Both MRSA carriers had exclusive nasal carriage. Table 4.2 shows the results for initial MRSA confirmation and Table 4.3 show the results for antibiotic susceptibility testing of all presumptive MRSA isolates.

Table 4.2 MRSA screening and confirmatory tests in soccer and rugby union participants

Isolates	**Brilliance™** MRSA 2 agar	Gram stain	DNase, catalase test	Coagulase test	M-PCR		Multiplex RT-PCR		
					femA	*mecA*	*nuc*	*mecA*	PVL
Positive	7*	7	7	7	7	2	7	2	0
Negative	0	0	0	0	0	5	0	5	7

M-PCR – multiplex polymerase reaction RT-PCR – real-time polymerase reaction

femA - unique to *Staphylococcus aureus* *mecA* - alternative penicillin binding protein 2 defines MRSA *nuc* – thermostable extracellular nuclease

PVL – Panton-Valentine leukocidin toxin *presumptive isolates

Table 4.3 Antibiotic susceptibility of presumptive MRSA isolates by disc diffusion

	FOX*	RD	MUP	W	FD	CN	CIP	E	TE
Resistant	2	0	0	0	1	0	1	1	0
Sensitive	5	7	7	7	6	7	6	6	7

FOX* (30µg) - cefoxitin, correlates to MRSA positive isolates in this study

RD (5µg) - rifampicin MUP (5µg) - mupirocin W (5µg) - trimethoprim FD (10µg) - fusidic acid

CN (10µg) - gentamicin CIP (5µg) - ciprofloxacin E (15µg) - erythromycin TE (30µg) - tetracycline

Of the two positive MRSA soccer carriers, one was a male (1n) and the other was a female (2n), both had direct contact with a healthcare facility lasting less than seven days, and one participant reported to be suffering from a skin disease (1n). Both positive sports participants were members of different clubs. No associations between MRSA carriage and independent variables were identified by Fisher's exact analysis in the sample of soccer and rugby participants. Table 4.4 details the characteristics of MRSA positive carriers.

4.3.3 Genetic analyses of MRSA isolates

PFGE and MLST results

Macro-restriction pulsed field gel electrophoresis (PFGE) of the two MRSA isolates characterised two unique strains: WA3 and WA65. Table 4.5 shows the MRSA phage type, sequence type, clonal complex type, and strain synonyms as determined by PFGE and MLST analysis, and antibiotic susceptibility. PFGE data on the five meticillin sensitive *S. aureus* (MSSA) isolates is available in Appendix C.

Table 4.4 Characteristics of contact sports MRSA carriers

Participant	Gender	Hospital placement		Direct personal contact with HCF*		Household contact with HCF		Antibiotic use		Skin disease»	Veterinary contact^	Sport played
	F/M	Yes/No	Duration	Yes/No	Duration	Yes/No	Duration	Yes/No	Type	Yes/No	Yes/No	Type
1n	M	-	-	+	<7 days	-	-	-	-	+	+	Soccer
2n	F	-	-	+	<7 days	-	-	-	-	-	-	Soccer

* participant had direct contact with healthcare facility as a result of visitation, illness or procedure

^participant had direct contact with veterinary facility as a result of taking companion animal for veterinary visit

»skin disease refers to diseases such as dermatitis or eczema, not applicable to infections

n=isolated from nose F/M – female/male HCF – healthcare facility

Table 4.5 Characteristics of MRSA isolates in contact sports players as identified by PFGE, MLST and antibiotic susceptibility

Participant	Sequence type (ST)	Clonal complex (CC)	Strain synonyms	MRSA type*	Antibiotic resistance
1n	73	5	WA-MRSA-65	caMRSA	FOX
2n	5	5	WA-MRSA-3	caMRSA	FOX

n=isolated from nose FOX - cefoxitin

* Isolates classified as either caMRSA or haMRSA according to a previously published paper on evolution and genetic diversity of MRSA according to MLST, SCC*mec* type, antibiogram and DNA microarray analyses, specific to Australia (Coombs et al., 2011)

DNA Microarray results

Microarray analysis of two MRSA isolates identified one unique MRSA type in contact sports players, being the CC5-MRSA-IV (1n, 2n). Both isolates carried *cap* 5, resistance determinants *mecA, delta_mecR, blaZ, fos B* (metallothiol transferase), virulence determinants *egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), lukD, lukE, hlgA, sak, chp, scn*, and adhesion determinants *bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*. All isolates were PVL negative. Both isolates carried *agrII.*

There were some variations between the two isolates: isolate 1n carried additional resistance determinant *aadD* and virulence determinants *sea* and *seb* enterotoxins, and isolate 2n carried additional virulence determinant *hla*. For the complete list of target genes analysed, see Appendix E. Table 4.6 shows DNA microarray genotype profiling (*agr* type, capsule type, resistance genes, virulence genes and adhesion genes) of MRSA isolates and associated strain assignment.

Table 4.6 DNA Microarray profiling of resistance, virulence and adhesion determinants of MRSA strains in contact sports participants

Participant	MLST/SCC*mec*	*agr* type	Capsule type	Resistance genes	Virulence genes	Adhesion genes
1n	CC5-MRSA-IV, Paediatric clone	II	5	*mecA, delta_mecR , blaZ, aadD, fosB*	*egc-cluster, sea, seb, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), lukD, lukE, hlgA, sak, chp, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
2n	CC5-MRSA-IV, Paediatric clone	II	5	*mecA, delta_mecR , blaZ, fosB*	*egc-cluster, seg, sei, selm, seln, selo, selu, lukS, lukF, lukS (ST22+ST45), lukD, lukE, hlgA, sak, chp, scn, hla*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*

A complete list of target genes is available in the paper published by Monecke and colleagues (Monecke et al., 2008b)

n- nasal colonisation *agr* – accessory gene regulator MLST – multilocus sequence typing SCC*mec* – Staphylococcal cassette chromosome mec

Note: genes have been formatted in bold to highlight main differences between isolates typed in this study

4.4 Discussion

Although MRSA carriage rates of 0% to 4.35% have been observed in soccer and American football players in studies using convenience sampling (Rackham et al., 2010, Garza et al., 2009, Creech et al., 2010, Lear et al., 2011), studies investigating MRSA carriage in healthy soccer and rugby union players are sparse. This study was the first to examine MRSA carriage in the nose and throat of healthy soccer and rugby union participants in Australia, and is the first study internationally to examine MRSA carriage in healthy rugby union players, which contributes to the novelty of the present study. Only one other previous study has detailed nasal carriage of MRSA in a small number of healthy soccer players (n=46) (Rackham et al., 2010), and as such this small number of participants is a limitation of their study. It should be also be noted that DNA microarray analysis of isolates has not previously been performed in any other studies that have assessed caMRSA carriage in healthy soccer and football participants. Whereas, DNA microarray analysis was performed in the current study to gain an insight into the virulence, resistance and cohesion determinants present in the MRSA isolates. As a result strain characteristics of MRSA isolates in the present study cannot be compared to other carriage studies of MRSA in this cohort.

It has been reported direct physical contact is important in the acquisition of MRSA (Bowers et al., 2008, Kazakova et al., 2005), and since physical contact has been implicated as a route of transfer for caMRSA, it would be expected that the rugby union players would have the highest amount of contact and a higher rate of carriage. On the other hand, the soccer players would be expected to have a lesser amount of high physical contact and therefore a lower carriage of MRSA (Cohen, 2005, Begier et al., 2004). This however, was not the case in the present study as none of the rugby union players were

found to be carriers of MRSA in either nose or throat. In the present study of healthy sports participants an overall nasal caMRSA carriage prevalence of 1.66% (2/181) was observed, with 2% (2/100) of soccer participants identified as being MRSA carriers. The two soccer players found to be carriers of MRSA were sampled from two different soccer clubs. The carriage rate of MRSA in the present study is similar to the 1.87% (2/107) carriage rate reported in a previous US study of American football players (Rackham et al., 2010) and is consistent with the range of carriage prevalence observed in members of the general community (Ammons et al., 2010, Tenover et al., 2008, Gamblin et al., 2013, Abudu et al., 2001).

Persons in contact with a healthcare setting are also at an increased risk of becoming MRSA carriers (Verwer et al., 2012, Manzur et al., 2008, Kottler et al., 2010, Hewlett et al., 2009, Cimolai, 2008, Baldwin et al., 2009). This is concerning as MRSA carriers have the potential to spread this organism from the healthcare setting back into the community. In the current study, both of the MRSA positive carriers had direct contact with a healthcare facility. However, the small number of carriers in this study did not allow for statistical analysis to determine risk factors associated with carriage. Another limitation of the present study relates to sampling bias, as the clubs that did participate cannot be regarded as representative of soccer and rugby players across Australia. Additionally, due to the sampling of one rugby union club, as a result of the poor participation response rate (9% participation response rate for rugby union clubs), clustering of participants was observed.

Future surveillance studies investigating caMRSA carriage in sports cohorts would benefit from examination of the role that the environment and close household contacts play in the dissemination of this organism, as a high environmental contamination of 30.56% (33/108) has been previously observed in the locker and weight rooms of American football participants (Oller et al., 2010) and a high MRSA contamination rate of 7.14% (2/14) been observed in the room mates of Danish soccer participants (Huijsdens et al., 2006b). Additionally a higher carriage of MRSA has been reported during follow-up testing of sports participants compared to testing performed at the start of the training season (Creech et al., 2010, Rackham et al., 2010). Creech and colleagues reported 4% (4/100) of American football players were carriers of MRSA at the start of the playing season and 19% (19/100) carried MRSA at the end of the playing season. Longitudinal studies examining colonisation during the playing season and in the off-season, as well as MRSA contamination of soil and the wider training environment may account for other reservoirs of MRSA and may help shed further light on MRSA carriage in this specific risk group.

Isolate characteristics and global isolation

From the two isolates recovered from contact sports participants in the present study, two different MRSA strains were typed by PFGE, MLST and DNA microarray.

Isolate 1n (CC5, ST73, USA800) recovered from a soccer participant is a caMRSA strain that has achieved global spread. A significant virulence determinant, *sea* (enterotoxin) was found in isolate 2n only. The presence of *sea* enterotoxin has been observed to modulate the immune system, which may enhance an isolate's ability to cause severe infection (Dauwalder et al., 2006, Ferry et al., 2006). Typical resistance, virulence and adhesion

determinants carried by ST73 include *aacA-aphD*, *fosB*, *egc-cluster*, *sak*, *chp*, *scn* and *sasG*. Isolate 2n carried all the above typical determinants excluding *aacA-aphD*. However this isolate was found to carry an additional resistance determinant *aadD*, as well as additional virulence determinants (*seb*, *seg*, *sei*, *selm*, *seln*, *selo*, *selu*, *hlgA)* and adhesion determinants (*bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sdrC*, *sdrD*, *vwb)*.

Isolate 2n (ST5, CC5, USA100) recovered from the other soccer participant was identified to be another caMRSA strain due to the presence of SCC*mec* type IV in this isolate, and is sometimes referred to as the Paediatric clone. Isolates of ST5 have been identified globally, and have been classified as both caMRSA (ST5-MRSA-IV) and haMRSA (ST5-MRSA-II) in different studies based on SCC*mec* typing (Coombs et al., 2013a, Coombs et al., 2012a, Monecke et al., 2011a, Kawaguchiya et al., 2013, Sola et al., 2012). Use of SCC*mec* typing alone is not strain discriminating and requires further genetic analyses e.g. PFGE, MLST and DNA microarray. In Australia, the ST5 strain is one of the most commonly reported caMRSA isolates (Coombs et al., 2012a) observed in the clinical setting. Variably associated virulence genes with ST5 include *egc-cluster*, *sea(N315)*, *sed*, *sej* and *ser* (Monecke et al., 2011a, Monecke et al., 2009). This isolate was found to only carry the *egc-cluster*, and lacked the other variably associated genes found in ST5. However this isolate did carry an additional *hla* gene that has been associated with invasive infection. Additionally, all isolates in this study carried *cap* 5; a clinically relevant polysaccharide capsular type present in clinical infections (Rasmussen et al., 2013, Roghmann et al., 2005, Verdier et al., 2007).

The recovered isolates from two soccer players, being ST5 and ST73 were the sixth and seventh most commonly identified strain types Australia wide as reported recently by the AGAR group (Coombs et al., 2013a). As mentioned previously, direct comparison of prevalence is not possible as their study examined only clinical isolates, whilst the present study reports on the carriage prevalence observed in the community. The finding the same caMRSA strains circulating within the sports community setting are also typically identified in the Australian healthcare setting may be suggestive of caMRSA strains becoming better adapted to the clinical environment, and are hence being increasingly disseminated in the healthcare setting (Kouyos et al., 2013). However, as the carriage of caMRSA in the present study was low in soccer players and was absent in rugby union players the researchers are unable to speculate on a trend.

Chapter 5 Assessment of MRSA carriage and potential for transfer between healthy horses and dogs and their respective handlers in the community

5.1 Introduction

Meticillin resistant *Staphylococcus aureus* (MRSA) infections have traditionally been associated with the healthcare setting (haMRSA), especially affecting immunocompromised patients and those undergoing surgery (Alex and Letizia, 2007, Beam and Buckley, 2006, Noskin et al., 2005, Weber, 2005). MRSA isolates are increasingly being reported from animal populations, particularly among dogs, horses and pigs (Bender et al., 2012, Pantosti, 2012, Morris et al., 2012, Weese, 2010). It has been reported humans can act as a potential source of MRSA transmission to their pets (van Duijkeren et al., 2004, Nienhoff et al., 2009, Loeffler et al., 2010b, Weese et al., 2006b). In addition, a companion dog has been found to serve as a reservoir of MRSA transfer to their owners (Manian, 2003). However, the importance of companion animals as a source of MRSA transfer to their owners needs to be studied further (Ferreira et al., 2011).

Individuals who have regular contact with MRSA positive animals outside of a household setting may be at an increased risk of becoming carriers or being infected with MRSA, compared to those who do not have frequent animal contact (Schwaber et al., 2013, Kottler et al., 2010, Weese et al., 2005a, Weese et al., 2006a, Weese et al., 2006b, Graveland et al., 2011). This trend has particularly become evident over the past decade in which higher

carriage and infection rates have been reported among animal handlers, veterinarians and veterinary staff, especially in persons who regularly handle horses (van Duijkeren et al., 2010, Weese et al., 2005a, Voss et al., 2005, Weese, 2010, Weese et al., 2006c, Pantosti, 2012). In one study it was reported that 4.7% of horses and 13% of horse handlers carried MRSA (Weese et al., 2005b).

A significant amount of research, particularly in the last few years, has been undertaken to investigate carriage of MRSA in clinically treated horses and horses attending veterinary clinics, as they represent an animal population in which a high prevalence of MRSA has been observed. Furthermore, MRSA in horses has become increasingly implicated as a cause of both morbidity and mortality (Garcia-Alvarez et al., 2012). In Australia, only one previous study has examined MRSA carriage in horses attending a veterinary clinic. Carriage of MRSA in 216 horses was reported to be 3.7%, which is in line with similar studies in Europe, the USA and Canada, where carriage has been reported to range from 0% to 16% (Weese, 2010). In one study 42% of hospitalised horses were MRSA positive on at least one occasion during weekly sampling over five weeks (van Duijkeren et al., 2010). Such a high carriage rate is concerning as colonisation precedes infection and colonised or infected horses may act as vectors in the spread this organism to other horses as well as their handlers. In contrast to the high carriage of MRSA reported in clinically treated horses, studies investigating MRSA in healthy horses in Canada, Slovakia, the Netherlands, Belgium and Ireland have frequently failed to isolate any MRSA or have observed carriage to be less than 2% (Weese et al., 2005b, Busscher et al., 2006b, Vengust et al., 2006, Van den Eede et al., 2012, Abbott et al., 2010).

Another animal population in which MRSA has been reported is in dogs. Dogs are considered to be an additional reservoir of MRSA and potential vectors in the spread of MRSA in the community (Vanderhaeghen et al, 2011). The majority of studies investigating MRSA carriage in dogs have come from Europe and the US, where the clinically treated dog population and dogs attending veterinary clinics have been the main focus of investigation. For this reason, prevalence rates of MRSA carriage in dogs are varied and have been typically reported to range from 0 to 4%, although carriage rates as high as 20% have been reported during periods of outbreak (Vengust et al., 2006, Gingrich et al., 2011, Pantosti, 2012). Only one Australian study has previously investigated MRSA carriage in both healthy and diseased dogs attending an animal clinic, where no MRSA was found on the skin of healthy dogs (0/51), but was present on the skin lesion of unhealthy dogs (2/141) (Malik et al., 2006a). Contrary to the higher prevalence rates of MRSA in dogs admitted to veterinary clinics reported by some authors, studies investigating the carriage of MRSA in healthy dog populations have failed to isolate any MRSA in this group (Walther et al., 2012, Busscher et al., 2006b, Vengust et al., 2006, Schmidt et al., 2014). To date, only five surveillance studies have examined MRSA carriage in healthy dogs in a community setting in Germany (Walther et al., 2012), Slovenia (Vengust et al., 2006), in the UK (Schmidt et al., 2014) and in the US (Kottler et al., 2010, Hanselman et al., 2009).

As with horses and dogs, horse handlers and dog handlers are another group in which MRSA carriages has been observed. MRSA carriage in horse handlers typically varies from around 2% to as high as 18% (Van den Eede et al., 2013, Weese et al., 2005b). Similarly, MRSA carriage in dog handlers is typically reported to range from 0% to 17.9% (Ferreira et al., 2011, Faires et al., 2009). To date, the majority of MRSA strains isolated

from dogs and horses have been genotyped and confirmed to be of human origin (Walther et al., 2008, Vanderhaeghen et al., 2012, Zhang et al., 2011, Walther et al., 2009, Weese et al., 2006b, Loeffler et al., 2005, Vincze et al., 2013). Strains commonly isolated in dog populations include ST5, ST22, ST45, ST59, ST105 and ST239 (Vanderhaeghen et al., 2012, Kwon et al., 2006, Lin et al., 2011, Walther et al., 2008, Zhang et al., 2011, Loeffler et al., 2005, Strommenger et al., 2006a, Malik et al., 2006a), whilst commonly isolated strains in horses include ST1, ST8, ST22, ST254, ST398 (laMRSA) and ST1173 (Cuny et al., 2008, Cuny et al., 2010, Lin et al., 2011, Walther et al., 2009, Tokateloff et al., 2009, Sieber et al., 2011, Kinnevey et al., 2010, Weese et al., 2005a).

A review of MRSA in companion animals states that even though MRSA has been well documented in animals, the majority of studies examining carriage in companion animals and their close contacts have been in a clinical setting, and research remains sparse on carriage in healthy companion animals and their handlers (Loeffler and Lloyd, 2010, Walther et al., 2012). Globally there have been only three published community studies that have examined MRSA carriage in healthy horses and their respective handlers (Weese et al., 2005b, Busscher et al., 2006b, Van den Eede et al., 2013), and only three published community studies that have examined MRSA carriage in healthy dogs and their respective handlers (Hanselman et al., 2009, Kottler et al., 2010, Walther et al., 2012) not including veterinary personnel. These studies all reported a caMRSA carriage prevalence of less than 5% in these animal handlers (non-healthcare participants) and their healthy pets.

No Australian studies have investigated carriage of MRSA in healthy dogs and horses in a community setting. Furthermore there have been no published studies globally that have examined the link between MRSA carriage in healthy dogs and horses and their respective handlers together in a single community setting. This study aims to determine the carriage of MRSA in the healthy dog and horse populations, and their handlers, in the community and to identify the prevalent sequence types and the genetic characteristics of these isolates.

5.2 Materials and Methods

5.2.1 Participants

Prospective horse handlers, horses, dog handlers and dogs across NSW were recruited via the phone and email. An Internet search engine site was used to identify dog training and horse clubs in Sydney. Thirteen horse-riding schools, farms and stables were initially approached. Horses and their handlers were recruited from three riding schools (two located in Sydney and one in Glenworth Valley), three horse stables (two located in Sydney and one in Camden), one horse-racing stable (located in Menangle Park) and one equine centre (located in Wagga Wagga), a response rate of (61.54%). In total, 310 horses (riding horses n=287, race horses n=23) and their 38 horse handlers (non-professionals) were enrolled. The horses from Glenworth Valley and Wagga Wagga were recruited via the veterinarian performing the swabs of horses in this study. Seven prospective dog training clubs were contacted via phone and email to participate. Dog handlers and their dogs were recruited from two dog-training clubs in the Sydney metropolitan area (a response rate of 28.57%). In total 108 dogs and 94 dog handlers were enrolled in this study; of these dog handlers (n=94), there were 67 single dog handler and dog pairs, 14

dog handlers had themselves and two of their dog's swabbed, three had themselves and their three dogs swabbed and a single dog handler had themselves and their four dogs swabbed. An additional nine dog handlers opted to have swabs collected from only themselves and not their dogs. Animal handlers were required to complete a questionnaire examining demographic factors and risk factors for MRSA colonisation. Participant consent forms, information statements and questionnaires were different for horse handlers, dog handlers, horses and dogs. The questionnaires were created according each group's specific published risk factors. The University of Sydney Human Ethics Research Committee (HREC) and Animal Research Ethics Committee (AREC) approved the study. Questionnaires for these participants are included in Appendix B.

5.2.2 Sample collection and initial screening tests for MRSA identification

Anterior nares and throat swabs from animal handlers were collected by the researcher and used to inoculate nutrient broth (Oxoid Microbiology Products, Australia) containing 4% sodium chloride as described in Section 2.2.

The sampling sites for MRSA carriage in dogs and horses were chosen as per the guidelines of the CLSI (2010) described in Section 2.2.1 (CLSI, 2010). Collection of horse samples was performed by a qualified veterinarian by inserting a swab stick 10-15cm into the nostrils (both sides) and collection of dog samples was performed by the researcher by gently rubbing the swab over the anterior nares (both sides) and slightly inserting the swab stick into the nares. An additional sample was taken from the perineum of dogs. Animal swabs were used to inoculate nutrient broth (Oxoid Microbiology Products, Australia) containing 4% sodium chloride as described in Section 2.2.

Following incubation, the cultured broth was used to inoculate chromogenic Brilliance™ MRSA 2 agar (Oxoid Microbiology Products, Australia) and incubated at 37^{o}C for 24 hours. *S. aureus* colonies were blue denim in colour, whilst *S. pseudointermedius* colonies were light blue on this selective agar. *S. aureus* identification was confirmed by Gram stain, catalase test, DNase test and rabbit plasma tube coagulase test (Becton, Dickinson and Company, Australia). MRSA was confirmed using both antibiotic susceptibility tests and M-PCR. The *femA* gene was used in the M-PCR assay to differentiate *S. aureus* from *S. pseudointermedius.* All of the above preliminary identification tests were performed on three MRSA and 23 MSSA isolates as described in Section 2.3 of General methods. MRSA were stored for later use using the Protect bead system (Oxoid - Australia).

5.2.3 Genetic analyses of MRSA isolates (Multiplex RT-PCR, PFGE, MLST, DNA microarray)

All confirmed MRSA isolates were subjected to the following genetic analyses:

Multiplex Real-time PCR (RT-PCR)

A multiplex RT-PCR assay was used to confirm the presence of the *mecA* gene and to detect the presence of the *nuc* gene (thermostable extracellular nuclease) according to a previously described method (Costa et al., 2005). RT-PCR for PVL gene determinants were performed as per a previous method (Fey et al., 2003). RT-PCR was performed on three MRSA and 23 MSSA isolates. See Section 2.4.1 for method details.

Macro-restriction pulsed field gel electrophoresis (PFGE)

PFGE of chromosomal DNA was performed on three MRSA and 23 MSSA isolates according to a previously published method (O'Brien et al., 2006) using a contour-clamped homogeneous electric field (CHEF) DRIII system (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). See Section 2.4.2 for method details. The DNA of one livestock associated MSSA ST398 isolate could not be typed using the standardised *SmaI* enzyme, as methylation of the restriction site blocks the activity of this enzyme. This isolate was digested using the *Apa*I enzyme instead, which is effective in cutting the DNA of ST398 strains (Bergstrom et al., 2012, Bens et al., 2006). PFGE data for MSSA isolates are given in Appendix C.

DNA microarray

The *S. aureus*-specific diagnostic DNA microarray assay was performed on three MRSA isolates as previously described (Monecke et al., 2008b). See Section 2.4.3 for method details.

Multilocus sequence typing (MLST)

MLST was performed on three MRSA as previously described (Enright et al., 2000b). See Section 2.4.4 for method details.

5.3 Results

5.3.1 Participants demographic information

Horse handlers

Of the 38 horse handlers, the majority of participants were female (26 male vs. 12 female). The median age of horse handlers was 21 years. Demographic data on horse handlers indicated 15 had direct personal contact with a healthcare facility (HCF) within the last year, 13 participants had taken antibiotics within the last six months, 13 had close household contacts who had contact with a HCF in the last year, the majority of participants owned dogs (n=23) and regularly handled less than 20 horses (n=18). In addition, most participants lived in a household with three to five family members and none had worked in a clinical setting within the last six months. Table 5.1(a) details the demographic information provided by horse handlers.

Table 5.1a Demographics of horse handlers

Characteristics of participants		Number (n)
Participant type and total number	Horse handlers	38
Gender	Female	26
	Male	12
Age (years)	18 - 20	15
	21 - 40	9
	41 - 60	5
	> 61	2
	Not specified	7
Clinical placement	Hospital/nursing home/other	0
Direct personal hospital contact*	Illness	5
	Procedure	4
	Other e.g. visiting	10
Duration of hospital contact	< 7 Days	13
	> 7 Days	2
Antibiotic use within the last 6 months	Yes	13
Member of household had HCF contact	Yes	13
Duration that household contact had with	< 7 Days	6
HCF	> 7 Days	7
Animal present in household	Dog	23
	Cat	7
Pet had veterinary visit	Yes	16
Number of horses regularly handled	0-19	18
	>20	12
Number living in household (excluding	≤ 2	10
participant)	≥ 6	6
	Not specified	8

*Please note: 15 horse handlers had direct contact with HCF as a result of a visit, or in some cases a combination of visit, illness and/or procedure

HCF – healthcare facility

Horses

Of the 310 horses, the majority were geldings (187) and the median age of horses was 10 years. Demographic data on the horses indicated 21 had direct contact with a veterinary facility (VF) within the last year, 29 horses had been administered antibiotics within the last six months, the majority of horses were both stabled and kept in a paddock (n=243). The majority of horses lived on premises occupied by more than 41 horses (n=224). Thirty-seven horses had been away from their place of residence for more than seven days in the last six months. The majority of horses in this study were riding horses (n=287) vs. racing horses (n=23). Table 5.1(b) details the demographic information of horses.

Table 5.1b Demographics of horses

Characteristics of participants		Number (n)
Participant type and total number	Horses	310
Gender	Geldings	187
	Entire Male	110
	Filly/Mare	13
Age (years)	0 – 10	215
	11 - 30	95
Contact with veterinary setting	Illness	13
	Procedure	8
Duration of veterinary contact	< 7 days	13
	> 7 days	3
	Not specified	5
Antibiotic use within the last 6 months	Yes	29
Predominantly administered antibiotic	Penicillin	10
Under which conditions the horse is kept	Stable	45
	Paddock	22
	Both	243
How many horses reside at farm/stable	0-10	32
	11-40	54
	41-60	86
	> 61	138
Horse away from place of residence for >7 days	Yes	37
	No	273
Is this horse currently racing	Yes	23
	No	287

Dog handlers

Of the 94 dog handlers the majority of participants were female (66 vs. 28). The median age of dog handlers was 52 years. Demographic data on dog handlers indicated 16 participants had worked in a clinical setting in the last six months, 40 had direct personal contact with a healthcare facility (HCF) within the last year, 24 participants had taken antibiotics within the last six months, 34 had close household contacts whom had contact with a HCF in the last year, 10 had contact with a horse farm and most participants lived in a household with two or less family members. Table 5.1(c) gives details of the demographic information of dog handlers.

Table 5.1c Demographics of dog handlers

Characteristics of participants		Number (n)
Participant type and total number	Dog handlers	94
Gender	Female	66
	Male	28
Age (years)	0 - 20	1
	21 - 40	20
	41 - 60	42
	> 61	21
	Not specified	10
Clinical placement*	Hospital	10
	Nursing Home	4
	Other Facility	7
Duration of clinical placement	< 7 Days	6
	> 7 Days	10
Direct hospital contact^	Illness	4
	Procedure	17
	Other e.g. visiting	26
Duration of hospital Contact	< 7 Days	27
	> 7 Days	12
	Not specified	2
Antibiotic use within the last 6 months	Yes	24
Member of household had contact with HCF	Yes	34
Duration that household contact had with HCF	< 7 Days	20
	> 7 Days	14
Animal present in household	Dog	94
	Cat	12
Pet had veterinary visit	Yes	90
Contact with horse farm	Yes	10
Number living in household	≤2	67
(excluding participant)	3-5	15
	≥6	1
	Not specified	11

HCF – healthcare facility

*16 dog handlers were identified to have clinical placement, some participants a combination of hospital/nursing home/other HCF

^40 participants had direct contact with HCF through either visitation/illness/procedure

Dogs

Of the 108 dogs, the majority of participants were female (59 vs. 49). The median age of dogs was three years. Demographic data on the dogs indicated the majority of participants were solely household pets (n=98, i.e. not involved in shows or used for breeding), 105 had direct contact with a veterinary facility (VF) for vaccination/illness/procedure within the last year, 27 dogs had been administered antibiotics within the last six months, the majority of dogs slept inside the house (n=89) and 21 had briefly stayed in a kennel in the last six months. Table 5.1(d) shows dog participant demographic information from the questionnaire relating to factors associated with increased risk for MRSA acquisition.

Table 5.1d Demographics of dogs

Characteristics of participants		Number (n)
Participant type and total number	Dog	108
Gender	Female	59
	Male	49
Age (years)	2 years or less	50
	3-5	27
	6-10	21
	> 11	8
	Not specified	2
Type of pet	Family pet	98
	Dogs used for breeding	2
	Show dogs	8
Direct veterinary contact*	Dogs who received vaccination	102
	Procedure	29
	Other e.g. illness	21
Duration of veterinary contact	< 7 Days	96
	> 7 Days	7
	Not specified	2
Antibiotic use within the last 6 months	Yes	27
Where does the dog sleep	Inside the house	89
	Kennel	2
	Outside of the house	17
Number of other dogs living in the same household	0	52
	1-2	50
	3-4	6
Dog has been in a kennel in the last 6 months	Yes	21
	No	87
Duration in kennel	< 7 days	5
	> 7 days	17

*105 dogs had direct contact with veterinary facility as a result of either vaccination/procedure/illness

5.3.2 Preliminary screening and MRSA identification

A total of 23 presumptive isolates from 550 participants (horses=310, horse handlers=38, dogs=108, dog handlers=94) were identified using Brilliance™ MRSA 2 agar. These isolates were then subjected to a series of standard tests to confirm *S. aureus* (Gram stain, catalase test, coagulase test) and subsequent tests (antibiotic susceptibility and M-PCR) confirmed 3/23 isolates were MRSA. RT-PCR subsequently confirmed the presence of *mecA*, and *nuc* in three isolates from two horse handlers. None of the isolates were found to carry the PVL toxin. The Brilliance™ MRSA 2 agar gave 20/23 false positive isolates. Table 5.2 shows the results for initial MRSA confirmation and Table 5.3 shows the antibiotic susceptibility of all presumptive MRSA isolates.

Table 5.2 MRSA screening and confirmatory tests

Isolates	**Brilliance™** MRSA 2 agar	Gram stain	DNase, catalase test	Coagulase test	M-PCR		Multiplex RT-PCR		
					femA	*mecA*	*nuc*	*mecA*	PVL
Positive	23*	23	23	23	23	3	23	3	0
Negative	0	0	0	0	0	20	0	20	23

M-PCR – multiplex polymerase reaction RT-PCR – real-time polymerase reaction

femA - unique to *Staphylococcus aureus* *mecA* - alternative penicillin binding protein 2 defines MRSA *nuc* – thermostable extracellular nuclease

PVL – Panton-Valentine leukocidin toxin *presumptive isolates

Table 5.3 Antibiotic susceptibility of presumptive MRSA isolates from animal handlers by disc diffusion

	FOX*	RD	MUP	W	FD	CN	CIP	E	TE
Resistant	3	1	0	2	1	1	1	2	1
Sensitive	20	22	23	21	22	22	22	21	22

FOX* (30µg) - cefoxitin, correlates to MRSA positive isolates in this study

RD (5µg) - rifampicin MUP (5µg) - mupirocin W (5µg) - trimethoprim FD (10µg) - fusidic acid

CN (10µg) - gentamicin CIP (5µg) - ciprofloxacin E (15µg) - erythromycin TE (30µg) - tetracycline

5.3.3 MRSA prevalence and characteristics of horse handler carriers

All three MRSA strains were isolated from two horse handlers (2/38), a carriage prevalence of 5.26%. No MRSA was isolated from horses (0/310), dogs (0/108) or dog handlers (0/94). Amongst the two MRSA carriers, one carried MRSA in the nose and throat and one participant carried MRSA in their throat only. The two participants found to be carriers of MRSA were recruited from two different farms. MRSA was absent in all of the other horse handlers in contact with these MRSA carriers.

Of the two MRSA positive carriers, one had recent contact with a healthcare facility, had taken a course of antibiotics within the last six months of being surveyed, had worked on a horse farm and regularly handled 50 horses. The other MRSA carrier failed to complete the questionnaire. This limited the researcher from performing statistical analysis as a result of incomplete data.

5.3.4 Genetic analyses of MRSA isolates

PFGE and MLST results

Macro-restriction pulsed field gel electrophoresis (PFGE) of the three MRSA isolates characterised two different MRSA types, UK-EMRSA-15 and WA- MRSA-58. Table 5.4 shows the MRSA sequence type, clonal complex type, and strain synonyms as determined by PFGE and MLST analysis, and antibiotic susceptibility. PFGE data on the 20 meticillin sensitive *S. aureus* (MSSA) isolates is available in Appendix 3.

Table 5.4 PFGE, MLST and antibiotic susceptibility characteristics of MRSA isolated from horse handlers

Participant ID	Sequence Type (ST)	Clonal Complex (CC)	Strain Synonyms	MRSA type*	Antibiotic (resistance)
1n, t	22	22	UK-EMRSA-15	haMRSA	FOX, CIP, E
2t	1173	8	WA-MRSA-58	caMRSA	FOX, RD, W, CN, E, TE

n=isolated from nose t=isolated from throat

FOX - cefoxitin E – erythromycin TE - tetracycline W – trimethoprim CIP - ciprofloxacin CN - gentamicin RD - rifampicin

*Isolates classified as either caMRSA or haMRSA according to a previously published paper on evolution and genetic diversity of MRSA according to MLST, SCC*mec* type, antibiogram and DNA microarray analyses, specific to Australia (Coombs et al., 2011)

DNA Microarray results

Microarray analysis of three MRSA isolates identified two unique MRSA types in horse handlers, being haMRSA ST22-MRSA-IV, UK-EMRSA-15 (1n, 1t) and caMRSA CC8-MRSA-IV (2n). All three isolates carried clinically relevant *cap* 5, resistance determinants *mecA, blaZ*, *erm(C)*, virulence determinants *lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA*, *sak* and *hla*, and adhesion determinants *bbp, clfA, clfB, ebpS*, *eno, fib, fnbA*, *map*, *sasG*, *sdrC*, *sdrD*, *vwb.* All isolates were PVL negative.

There were some variations between isolates: isolates 1n and 1t carried additional virulence determinants *egc-cluster, sed, seg, sei, sej, selm, seln, selo, ser, selu, chp*, and adhesin *cna*; isolate 2n carried additional resistance genes *aacA-aphD, dfrS1, tet(M), fosB,* virulence determinants *sea, seb, sek, seq*, and adhesion determinants *ebh* and *fnbB*. Varying *agr* types were also observed; isolates 1n and 1t carried *agr*I and isolate 2n carried *agr*IV. For the complete list of target genes analysed, see Appendix E. Table 5.5 shows the DNA microarray profiles for the three positive MRSA isolates, including, *agr* type, capsule type, resistance determinants, virulence determinants and adhesion factors.

Table 5.5 Microarray profiling of resistance, virulence and adhesion determinants of MRSA strains in horse handlers

Participant	MLST/SCC*mec*	*agr* type	Capsule	Resistance genes	Virulence genes	Adhesion genes
1n,t	ST22-MRSA-IV, Barnim/UK-EMRSA-15	I	5	*mecA, delta_mecR, blaZ, erm(C)*	*egc-cluster, sed, seg, sei, sej, selm, seln, selo, ser, selu, lukS, lukF, lukS (ST22+ST45), lukX, lukY, hlgA, sak, chp, scn, hla*	*bbp, clfA, clfB, ebpS, cna, eno, fib, fnbA, map, sasG, sdrC, sdrD, vwb*
2n	CC8-MRSA-IV	IV	5	*mecA, delta_mecR, blaZ, erm(C), aacA-aphD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, sak, scn, hla*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*

A complete list of target genes is available in a previously published paper (Monecke et al., 2008b)

n- nasal colonisation t- throat colonisation *agr* – accessory gene regulator MLST – multi locus sequence typing SCC*mec* – Staphylococcal cassette chromosome mec

Note: genes have been formatted in bold to highlight main differences between isolates typed in this study

5.4 Discussion

Community-associated MRSA carriage in healthy horses and their handlers

At present, caMRSA carriage data on healthy horses, dogs and their respective handlers in a community setting are limited (Walther et al., 2012). Studies investigating carriage of caMRSA in healthy horses have mainly been carried out in Europe and North America, and most have failed to isolate any MRSA in the healthy horse population (Weese et al., 2005b, Burton et al., 2008, Busscher et al., 2006b, Vengust et al., 2006). In the current study no MRSA was detected in 310 healthy horses. This is consistent with a previous study by Loeffler et al (2010) who examined MRSA carriage within two groups of horses, comprising healthy community horses and horses admitted to clinics. In their study they reported that 1.97% (3/152) of clinically treated horses carried MRSA whereas none of the 296 healthy horses carried this bacterium. This and other studies suggest that the lack of carriage of MRSA in healthy horses may be due to horses not being preferentially colonised with MRSA (Vengust et al., 2006, Burton et al., 2008).

Risk factors reported in the literature to increase caMRSA carriage in horses presenting to veterinary clinics include residing on a MRSA positive farm, receiving antimicrobial therapy and receiving clinical intervention (Weese et al., 2006c, Weese and Lefebvre, 2007). Of the 310 horses in the present study only 29 had received antimicrobial therapy and 21 had received clinical intervention. In addition, none of the farms were MRSA positive and only a small number of horses were found to have demographic factors that have been previously associated with an increased risk of caMRSA carriage. Risk factors for MRSA carriage in this cohort could not be identified due to an absence of MRSA in the

study population. Furthermore, there was an unintentional bias observed in the sampling of horses, due to only 13 of 310 horses sampled identified as being filly/mare.

A recent study of nasal MRSA carriage in healthy horses (2/191) residing in Italy, reported that harness racing horses were significantly more likely to be carriers of MRSA when compared to riding horses and breeding mares (Mallardo et al., 2013). This may explain the absence of MRSA in our study, in which only 23 of the 310 horses sampled were harness racing horses, however this may not hold true. Their study sampled fewer horses than did the present study and convenience sampling was used to sample horses from three locations. In addition risk factors for carriage could not be identified in their study due to the low isolation of MRSA (n=2).

Another explanation for the absence of MRSA in healthy horses in the present study could relate to the differences in the environment and/or housing conditions. In Australia, the environmental conditions horses experience is very different to those in Europe and North America. Horses' residing in Europe and North America experience cold winters and are kept indoors for prolonged periods of time. This is less likely to occur in Australia. The majority of the horses in this study (n=243) were both stabled and kept in a paddock. At each sampling location all horses were kept in a clean environment and had direct exposure to sunlight, which could also explain the absence of MRSA in the present cohort. Natural sunlight has been reported to decrease survival of *S. aureus* (Hobday and Dancer, 2013, Chapple et al., 1992, El-Adhami et al., 1994). Furthermore, it has been reported ultraviolet light has been effective in the inactivation of *S. aureus* (Krishnamurthy et al., 2004). In Australia the UV index ranges from 3 in Winter to 14 in Summer (Lemus-

Deschamps and Makin, 2012). Whereas, in countries where horses and dogs were reported to have higher carriage rates of MRSA, predominantly Europe, America and Canada, the UV index maximum is usually around 5, with occasional increases to around 10 in some areas in the summer months (Lemus-Deschamps and Makin, 2012, He et al., 2013). The potential role UV exposure plays in the sterilisation of bacteria should not be overlooked. However, in the present study UV exposure was not directly measured and therefore this theme is speculative. Future studies on the potential effect UV light from the sun has on inhibiting growth of *S. aureus,* including MRSA, are needed to test this hypothesis.

In contrast to the absence of MRSA in the horse cohort, horse personnel handling these horses had a higher prevalence of MRSA carriage in the present study, with 5.26% (2/38) found to be carriers of MRSA. This prevalence of MRSA in horse handlers is consistent with a previous study in which a MRSA prevalence of 5% (2/41) was reported in horse handlers (Weese et al., 2005b). Other studies have reported MRSA carriage in horse handlers to be 2.38% (1/42) (Busscher et al., 2006b) and 2.4% (4/166) (Van den Eede et al., 2013). In the three studies that have assessed carriage in horses and their handlers two of the studies sampled a small number of horse handlers (<45) (Weese et al., 2005b, Busscher et al., 2006b). This was also a limitation of the present study due to horse handlers handling multiple horses, despite the sampling of a reasonable amount of horses (n=310).

Of the two MRSA positive carriers in the present study, one participant (1n) had been in recent contact with a healthcare facility, used an antibiotic within the last six months of being surveyed and regularly handled 50 horses. The other MRSA carrier (2t) submitted an

incomplete questionnaire. A previous study has reported that the only identifiable risk factor for MRSA carriage in horse handlers is regular direct contact with over 20 horses (Weese et al., 2005b). The low number of positive carriers in the present study did not allow the researcher to identify risk factors for MRSA carriage in horse handlers. The finding that no horses carried caMRSA in the present study, even though two of their horse handlers were carriers, is consistent with two prior studies of horses and their handlers in a community setting (Weese et al., 2005b, Busscher et al., 2006b). In contrast to this finding, targeted horses (46/391) and their handlers (12/66) on farms with prior MRSA notification have been shown to be carriers of the same caMRSA strain type, ST8 (Weese et al., 2005b).

Another finding in relation to MRSA isolates recovered in the horse handler cohort was the greater spectrum of antimicrobial resistance observed in the caMRSA isolate ST1173, CC8 (2t) which was resistant to six antibiotics (cefoxitin, rifampicin, trimethoprim, gentamicin, erythromycin and tetracycline), whilst the pandemic haMRSA isolate ST22, CC22 (1n,t) was resistant to just three antibiotics (cefoxitin, ciprofloxacin and erythromycin). However, it is not possible to draw any conclusions due to there being only one isolate (ST1173) in this cohort. Since there have been no previous studies in Australia that have assessed carriage of MRSA in healthy horses and their handlers in a community setting, the findings in the present study cannot therefore be compared to healthy horses and their handlers in Australia. There has been only one previous Australian study that has assessed MRSA carriage in veterinary personnel (small animal, equine and other veterinarians) (Jordan et al., 2011). Trott (a co-author of the Jordan paper) and colleagues typed all 46 of the MRSA positive isolates collected from the four veterinary conferences (Trott et al., 2013). The authors reported the majority of MRSA isolates from small animal

veterinarians were CC22 (76.9%) and many were resistant to ciprofloxacin. Whereas, CC8 (62.5%) were mainly isolated from equine veterinarians and these MRSA isolates were more often resistant to rifampicin and gentamicin when compared to the CC22 isolates. Strains of ST22 have also previously been identified in veterinary personnel in the UK (Baptiste et al., 2005, Loeffler et al., 2005, Heller et al., 2009, Loeffler et al., 2010b) and in German horses (Walther et al., 2009). Whereas, identification of ST1173 has been previously reported in Canadian (Weese et al., 2005a) and Austrian horses (Kinnevey et al., 2010). The only previous Australian study to assess MRSA carriage in horses presenting to veterinary clinics has identified eight ST612 (CC8) isolates from 216 horses (Axon et al., 2011). From the literature, it appears strains belonging to CC8 have successfully adapted to survival in horses, and researcher's now consider this clonal complex to be horse specific (Weese and van Duijkeren, 2010a, Weese, 2004, Axon et al., 2011).

Compared to all prior published community studies assessing MRSA in healthy horses (Peterson et al., 2012, Van den Eede et al., 2012) and their handlers (Van den Eede et al., 2013, Weese et al., 2005b) in which MRSA was isolated, in depth genotypic analyses utilising DNA microarrays has not been previously performed. The present study is the first to utilise this technique to type the isolates recovered from horse handlers. Had other researchers performed DNA microarrays there would have been information available on the virulence and resistance of isolates circulating in horse handler's and isolate comparison between countries would be possible. This study adds to the current knowledge of strains recovered from horse handlers, and their characteristics. Further studies investigating MRSA carriage at an in depth genetic level in healthy horses and

their handlers have the potential to shed light on strain adaption and evolution in this group.

Isolate characteristics and global isolation

Of the three MRSA isolates from horse handlers, two different MRSA strain types were identified by PFGE, MLST and DNA microarray (ST22 and ST1173).

Isolates 1n and 1t were identical ST22, CC22 strains, commonly known as UK-EMRSA-15, a pandemic haMRSA strain which has been isolated globally (Monecke et al., 2011a). Commonly found genetic markers *erm(C), egc-cluster, cna* and *sasG* in ST22 were also present in this isolate.

Isolate 2t was typed as a ST1173, CC8-MRSA-IV, a caMRSA strain which has been identified in Australia, America, UK, Ireland and Germany (Moremi et al., 2012, Blanc et al., 2007). This strain is typically PVL negative and carries virulence determinants *seb, sek, seq, sak* and *scn*, and resistance determinants *aacA-aphD, dfrS1, tet(M), fos(B), sdrM*. The ST1173 isolate carried all typical virulence and resistance determinants associated with this ST type, with the exception of *erm(A)*. However this isolate was found to carry an additional resistance determinant, *erm(C)* and an additional virulence enterotoxin, *sea.*

Strain ST22 was the most frequently recovered haMRSA clone across healthcare settings Australia wide, as reported in the AGAR study. On the other hand, isolate ST1173 is not

considered to be a predominant caMRSA strain (Coombs et al., 2013a). Rather, ST1173, CC8, whilst not typically observed in the general community, is predominantly identified in horse handlers and in horses residing in America and Canada (Weese et al., 2005a, Weese et al., 2005b, Akridge et al., 2013, Anderson et al., 2008, Moodley et al., 2008b). The fact these strain types were identified in Australian horse handlers in the community setting may be suggestive of international transfer of this particular strain. Another explanation for this finding may be that these horse handlers had travelled to the US or Canada or had indirect contact with persons/horses who did; however participants' recent travel or contact with persons/horses who had travelled recently was not investigated in the questionnaire utilised in the present study.

DNA microarray analyses of these isolates showed there was a greater number of resistance determinants present in the caMRSA isolate, which was identified to carry four additional resistance genes (*aacA-aphD, dfrS1, tet(M), fosB),* compared to the haMRSA isolate. This finding is unexpected and contradicts reports in which haMRSA SCC*mec* types are observed to typically carry a greater number of resistance genes compared to caMRSA SCC*mec* types (Hudson et al., 2013, Chua et al., 2011, Gorwitz, 2008). Such a finding is of concern as caMRSA may cause more severe infections to those of haMRSA infecting strains (Kouyos et al., 2013, Bal et al., 2013). As a consequence, caMRSA may pose an even greater threat than haMRSA in the community and healthcare setting due to the increased virulence of caMRSA (presence of PVL, ACME and PSM's) compared to haMRSA (Okuma et al., 2002, Kouyos et al., 2013, Boyle-Vavra and Daum, 2007, Vignaroli, 2009). Virulence plays a crucial role in immune evasion and damage (Bien et al., 2011), which may lead to opportunistic infection (Yeaman et al., 2014). For example, PVL has been shown to bring about rapid cell death in human and rabbit neutrophils

(Crémieux et al., 2009; Löffler et al., 2010). This is of relevance as neutrophils are predominantly responsible for host defense in humans against *S. aureus*. Contrary to immunocompromised patients being at risk of MRSA infection, these infections now extend to healthy individuals. More studies are needed in healthy horse handlers in Australia to determine the impact caMRSA isolates with increased virulence have on the broader community, and if a similar finding is to be made, as the present study is the first to investigate MRSA carriage in horse handlers (non-professional) in a community setting within Australia.

Lack of MRSA carriage in healthy dogs and their handlers

As was the case with healthy community horses, numerous studies investigating carriage of MRSA in companion animals in many countries have failed to isolate MRSA in healthy community dogs (Schmidt et al., 2014)(Bagcigil et al, 2007; Gingrich et al, 2011; Hanselman et al, 2008; Malik et al, 2006; Vengust et al, 2007; Weese et al, 2005). In the current study, dogs and their handlers were connected; dog handlers surveyed in this study handled the healthy dogs enrolled in this study. No MRSA was present in healthy dogs (0/108) and their handlers (0/94). This finding is consistent with a previously published community study of MRSA carriage in healthy dog handlers and their pet dogs in Germany, in which no MRSA carriage was also observed in either of their study cohorts (Walther et al., 2012). An absence of MRSA in healthy dogs could be due to dogs not being preferentially colonised by *S. aureus* since it has been suggested that infection or carriage of MRSA in dogs is usually the result of transfer from humans (van Duijkeren et al., 2004, Griffeth et al., 2008, Harrison et al., 2014).

On the other hand, *Staphylococcus pseudointermedius* is a common bacterium found in the mucosa and on the skin of dogs and has been misidentified as *S. aureus* in some instances. The reason this bacterium is of relevance in the dog population relates to how similar MRSA and *S. pseudointermedius* appear phenotypically (Bannoehr et al., 2009). In the present study the presence of *femA* was used to confirm *S. aureus* status and differentiate *S. aureus* from other coagulase positive staphylococci (Ishihara et al., 2010). Of further assurance to the researchers is that none of the ST's identified in this study are in the five ST's (ST29, ST68, ST69, ST70, ST71) formally ascribed to MRSP (Bannoehr et al., 2007). It should be noted that assessment of *S. pseudointermedius* carriage in dogs was beyond the scope of the present study and was not investigated. Investigations into the prevalence of MRSP would be a useful addition to future studies investigating MRSA in animals due to the reported prevalence of MRSP increasing in the UK and elsewhere (Stegmann et al., 2010).

Other possible explanations for a lack of MRSA carriage in healthy dogs in the present study may be reflective of the cohort sampled. The majority of dogs were primarily family pets (n=98) attending training and agility clubs with their owners. Also, the majority of dogs were housed inside (n=89), which has been shown to be a significant protective factor against MRSA carriage in this population (Hamilton et al., 2013). In addition, clustering of healthy dogs was observed in the cohort sampled, which was a limitation of this study, and perhaps this determined the results due to sampling participants from two dog clubs as a result of the poor participation rate (29% response rate). Furthermore, a greater power of study is required to determine whether a risk of caMRSA carriage and transfer exists in dogs and horses and their respective handlers if conclusions are to be drawn.

In conclusion, although MRSA is an important concern to public health (Gingrich et al., 2011), findings in the present study indicate healthy dogs and horses did not act as vectors or a true reservoir of caMRSA in this community setting. Further studies specific to healthy animals residing in Australia are needed to elucidate on this trend.

Chapter 6 Carriage of caMRSA by veterinary personnel and veterinarians in Australia and their genotypes

6.1 Introduction

Community-associated meticillin resistant *Staphylococcus aureus* (caMRSA) accounts for a leading burden of illness in humans and is of growing concern due to the wide dissemination of this bacterium in the community and healthcare setting (Weese, 2012). People in certain occupations, such as healthcare workers, and more recently, veterinarians and veterinary staff, are reported to have the highest risk of carriage with this organism (Anderson et al., 2008, Hanselman et al., 2006, Burstiner et al., 2010, Loeffler et al., 2010b). Colonised or infected veterinarians and veterinary personnel may spread this organism to community members and their pets.

The reported prevalence of MRSA varies depending on the geographic region participants have been sampled. MRSA carriage in veterinarians and veterinary personnel has been observed to be as high as 25% in small animal veterinarians in Japan (Ishihara et al., 2010) and 27% in small animal veterinary personnel in the UK (Baptiste et al., 2005). A high MRSA nasal carriage of 20.29% has been reported in equine veterinary personnel in Israel (Schwaber et al., 2013). In Australia, Jordan and colleagues have also reported a high nasal MRSA carriage in veterinary personnel attending a conference, in which equine veterinarians had the highest level of carriage, reported to be 21% (19/89), compared to a

4.8% prevalence in small animal veterinarians and 1.5% in government veterinarians (Jordan et al., 2011).

The high MRSA carriage rate observed in veterinary personnel is of concern as colonisation plays a key role in the pathogenesis of infection, which may then result in significant mortality and morbidity (Cohn and Middleton, 2010, McCarthy et al., 2012). In contrast to the high carriage rates of MRSA reported in veterinarians, a lower carriage prevalence of 5% and below has been reported in other studies examining MRSA carriage in equine and small animal veterinarians and veterinary personnel (Moodley et al., 2008a, Heller et al., 2009, Zemlickova et al., 2009, Boost et al., 2011, Zhang et al., 2011).

Being an employee of a veterinary hospital or a non-clinical laboratory, and contact with an MRSA infected animal are considered to be risk factors associated with MRSA carriage in veterinarians, when compared to veterinary students (Ishihara et al., 2010). Based on carriage studies it has further been reported equine veterinarians have a higher carriage of MRSA than small animal veterinarians (Weese and van Duijkeren, 2010a, Jordan et al., 2011, Weese, 2010). Household contacts of MRSA carriers are also reported to be at increased risk of acquiring MRSA. This trend has been observed in studies investigating MRSA carriage in household contacts of index MRSA infected case patients where carriage rates of around 50% have been reported (Mollema et al., 2010, Rafee et al., 2012).

The household and workspace environments are other significant sources in the transfer of MRSA. Strains of caMRSA are able to colonise inanimate objects and sharing these

objects or belongings may facilitate the spread of MRSA from person to person (Ojima et al., 2002, Miller and Diep, 2008). In one American study examining environmental MRSA contamination within a veterinary setting during a non-outbreak period, an overall MRSA prevalence of 12% (19/157) was detected on the surfaces sampled at the veterinary teaching hospital (Hoet et al., 2011). In that study 16% of small animal treatment areas were contaminated, followed by 12% in equine areas and 0% in food animal areas, with the most commonly isolated strain being the USA100. In a more recent study investigating MRSA contamination of clothing worn by veterinary personnel, it was reported that 3.5% (4/114) of clothing sampled were MRSA positive (Singh et al., 2013). Other studies investigating environmental contamination in veterinary settings have reported contamination with MRSA to be 3.7% (3/81) (Ishihara et al., 2010) and 1.4% (2/140) (Heller et al., 2009).

Further studies are required to address gaps in literature pertaining to the investigation of MRSA prevalence in veterinary professionals, their workspace and household environment, as well as the close household contacts of carriers as at present literature is lacking. The purpose of the current study was to determine MRSA carriage in Australian veterinary personnel and veterinarians, their close household contacts and environment and to identify and characterise the prevalent MRSA sequence types.

6.2 Materials and Methods

6.2.1 Participants

Veterinarians and veterinary personnel were recruited from eight different practices comprising four equine practices and four small animal practices in Sydney. Initially 20

different practices were contacted via the telephone. Practices that indicated interest were sent an email for participation. Of these 20 practices, eight agreed to participate, a response rate of 40%. In total, 108 participants were recruited, comprising equine veterinarians (n=35), equine veterinary nurses (n=13), small animal veterinarians (n=25) and small animal veterinary nurses (n=35). All participants were required to complete a questionnaire examining demographic factors. Participation was voluntary and confidential and each participant was assigned a unique code number. The Human Ethics Research Committee (HREC) of the University of Sydney granted approval.

6.2.2 Sample collection and initial screening tests for MRSA identification

Anterior nares and throat swabs were collected by the researcher and used to inoculate nutrient broth (Oxoid Microbiology Products, Australia) containing 4% sodium chloride as described in Section 2.2. Following incubation the cultured broth was used to inoculate chromogenic Brilliance™ MRSA 2 agar (Oxoid Microbiology Products, Australia) and incubated at 37^{o}C for 24 hours. *S. aureus* identification was confirmed by Gram stain, catalase test, DNase test and rabbit plasma tube coagulase test (Becton, Dickinson and Company, Australia). MRSA was confirmed using both antibiotic susceptibility tests and M-PCR. All of the above preliminary identification tests were performed on 29 MRSA and five MSSA isolates as described in Section 2.3 of General methods. MRSA were stored for later use using the Protect bead system (Oxoid - Australia).

6.2.3 Genetic analyses of MRSA isolates (Multiplex RT-PCR, PFGE, MLST, DNA microarray)

All confirmed MRSA isolates were subjected to the following genetic analyses:

Multiplex Real-time PCR (RT-PCR)

A multiplex RT-PCR assay was used to confirm the presence of the *mecA* gene and to detect the presence of the *nuc* gene (thermostable extracellular nuclease) according to a previously described method (Costa et al., 2005). RT-PCR for PVL gene determinants were performed as per a previous method (Fey et al., 2003). RT-PCR was performed on 29 MRSA and five MSSA isolates. See Section 2.4.1 for method details.

Macro-restriction pulsed field gel electrophoresis (PFGE)

PFGE of chromosomal DNA was performed on 29 MRSA and five MSSA isolates according to a previously published method (O'Brien et al., 2006) using a contour-clamped homogeneous electric field (CHEF) DRIII system (Bio-Rad Laboratories Pty Ltd, Gladesville, Australia). See Section 2.4.2 for method details.

DNA microarray

The *S. aureus*-specific diagnostic DNA microarray assay was performed on 29 MRSA isolates as previously described (Monecke et al., 2008b). See Section 2.4.3 for method details.

Multilocus sequence typing (MLST)

MLST was performed on 29 MRSA isolates as previously described (Enright et al., 2000b). See Section 2.4.4 for method details.

6.2.4 Follow-up sampling for colonised participants, household contacts and environmental contamination

Of the eight initially enrolled clinics, one equine clinic gave consent to have further follow-up swabs performed. From this clinic five participants were identified to be carriers of MRSA. Four of five participants were available for further swabbing. Follow-up swabs from MRSA carriers were collected from the anterior nares only (n=4). Household contacts of carriers had swabs collected from their nares and throat (n=6). Workspace environmental samples from MRSA carriers were taken from the participants' mobile phone (front and rear surface), work telephone (headphone and numbers on machine), computer/laptop keyboards (whole keyboard swabbed), computer mouse (front and rear surface) and light switch (20 environmental samples collected). Household contamination was assessed by collecting swabs from their telephone, TV remote control (front and rear surface), kitchen sink (top surface and taps), toilet flush button and bedroom light switch (20 environmental samples collected). Environmental sites to be sampled were chosen by the researchers, based on frequently touched sites in each environment. All samples from the environment were collected using swabs premoistened in a sterile saline solution as per a previous protocol (Ishihara et al., 2010). Each environmental swab took approximately 30 seconds to collect. MRSA identification was performed as per the above standard procedure (6.2.2 and 6.2.3).

6.2.5 Data analysis

Bivariate analysis was performed on categorical variables in SPSS using Pearson's correlation coefficient (r) to examine associations between the dependent variable (carrier

status) and independent variables (direct contact with HCF, skin disease, participation in contact sport, ownership of dog/cat, contact with horse farm, use of antibiotics, work in a clinical facility etc.) were more than five cases per cell were present. This analysis was used for the veterinarian cohort. All tests were two-tailed. A p-value of <0.05 indicated significance. Strength of correlation was as follows: weak correlation ($0<|r|<0.3$), moderate correlation ($0.3<|r|<0.7$) and strong correlation ($|r|>0.7$). No statistical analysis could be performed for veterinary nurses as only a limited amount were sampled (n=48), and a minimum of 50 participants are required to examine correlations (VanVoorhis and Morgan, 2007).

Binary logistic regression analysis was performed in SPSS on data collected from veterinarians to examine risk factors for MRSA carriage. Positive carriage status was the dependent variable and covariates in this study were risk factors for MRSA carriage identified in the participant questionnaire. Risk factors examined in the veterinarian cohort included age, gender, contact with healthcare facility, recent antibiotic use, owning companion animals and frequency of antibiotic prescription. A p-value of <0.05 was considered to be statistically significant.

6.3 Results

6.3.1 Participants demographic information

Forty-eight veterinary nurses (13 equine and 35 small animal) and 60 veterinarians (35 equine and 25 small animal) were included in this study. Veterinary nurses were predominantly female (n=43) with a median age of 30 years, while veterinarians were also

predominantly female (n=35) with a median age of 32 years. Demographic information provided by veterinary nurses indicate that 30 had direct personal contact with a human healthcare facility (HCF) in the last 12 months, 19 had indirect contact with a HCF, 20 had taken antibiotics within the last six months, and the majority lived in a household with two or less members (n=35) and owned dogs (n=35). Of the veterinarians 13 had direct personal contact with a human healthcare facility (HCF) in the last 12 months, 14 had recently taken antibiotics within the last six months, and the majority owned dogs (n=30). In addition, veterinarians predominantly listed intensive care as their field of specialty (n=43) and indicated that 71-100% of their animal patients were treated with antibiotics, which they had personally prescribed (n=21). Demographic data from veterinary nurses and veterinarians is shown in Table 6.1a and 6.1b.

Table 6.1a Demographics of veterinary nurse participants

Characteristics of participants		Number (n)
Participant type and total number	Veterinary nurses	48
Gender	Female	43
	Male	5
Age (years)	18 – 30	24
	31 – 60	21
	> 61	1
	Not specified	2
Clinical placement in veterinary	Equine veterinary	13
Facility in last 6 months	Small animal veterinary	35
Direct hospital contact in last 12	Illness	10
months*	Procedure	13
	Other e.g. visiting	17
Duration of hospital contact	< 7 Days	23
	> 7 Days	7
Member of household had contact with	Yes	19
Duration that household contact had	< 7 Days	12
with HCF	> 7 Days	6
	Not specified	1
Antibiotic use within the last 6 months	Yes	20
Most common antibiotic prescribed	Cephalexin	4
Animal present in household	Dog	29
	Cat	27
Pet had veterinary visit in last 12 months	Yes	34
Number living in household	2 or less	35
(excluding participant)	3-7	12
	Not specified	1

HCF – Healthcare facility

*30 veterinary nurses had direct personal contact with a healthcare setting; please note some participants had contact via visiting and or procedure/illness

Table 6.1b Demographics of veterinarian participants

Characteristics of participants		Number (n)
Participant type and total number	Veterinarians	60
Gender	Female	35
	Male	25
Age (years)	18 – 30	24
	31 – 60	32
	> 61	3
	Not specified	1
Clinical placement in veterinary facility in last 6 months	Equine veterinary	35
	Small animal veterinary	25
Direct HCF in last 12 months	Illness/Procedure/Other	13
Duration of hospital contact	< 7 Days	12
	> 7 Days	1
Specialisation of Veterinarian	GP	42
	Surgeon	41
	Intensive care	43
	Dentistry	22
	Dermatology	24
Antibiotic use within the last 6	Yes	14
Most common antibiotic used	Cephalexin	3
Animal ownership	Dog	30
	Cat	26
	Horse	13
Percentage of animals treated with antibiotics	0-30%	18
	31-70%	18
	71-100%	21
	not specified	3

HCF – healthcare facility

6.3.2 Initial MRSA identification of strains isolated from veterinarians and veterinary nurses

A total of 26 presumptive MRSA isolates from 108 participants were identified using Brilliance™ MRSA 2 agar. These isolates were then subjected to a series of standard confirmatory tests for *S. aureus* (Gram stain, catalase test, coagulase test). Subsequent tests (antibiotic susceptibility and M-PCR) confirmed 21/26 presumptive isolates were MRSA. This indicated that 5/26 isolates selected on the Brilliance™ MRSA 2 agar were false positives. RT-PCR subsequently confirmed the presence of *mecA*, and *nuc* in the 21 isolates. Presence of PVL was not detected. Table 6.2 shows the results for initial MRSA confirmation and Table 6.3 show the results for antibiotic susceptibility of all presumptive MRSA isolates.

Table 6.2 MRSA screening and confirmatory tests for MRSA isolated from veterinary personnel

Isolates	**Brilliance™** MRSA 2 agar	Gram stain	DNase, catalase test	Coagulase test	M-PCR		Multiplex RT-PCR		
					femA	*mecA*	*nuc*	*mecA*	PVL
Positive	26*	26	26	26	26	21	26	21	0
Negative	0	0	0	0	0	5	0	5	26

M-PCR – multiplex polymerase reaction RT-PCR – real-time polymerase reaction

femA - unique to *Staphylococcus aureus* *mecA* - alternative penicillin binding protein 2 defines MRSA *nuc* – thermostable extracellular nuclease

PVL – Panton-Valentine leukocidin toxin *presumptive isolates

Table 6.3 Antibiotic susceptibility of presumptive MRSA isolates by disc diffusion

	FOX*	RD	MUP	W	FD	CN	CIP	E	TE
Resistant	21	18	0	19	1	18	1	6	15
Sensitive	5	8	26	7	25	8	25	20	11

FOX* (30µg) - cefoxitin, correlates to MRSA positive isolates in this study

RD (5µg) - rifampicin MUP (5µg) - mupirocin W (5µg) - trimethoprim FD (10µg) - fusidic acid

CN (10µg) - gentamicin CIP (5µg) - ciprofloxacin E (15µg) - erythromycin TE (30µg) - tetracycline

6.3.3 Prevalence of MRSA in veterinary nurses, veterinarians, their close household contacts and environment

Prevalence of MRSA in veterinary nurses and veterinarians

Initial sampling of veterinary personnel identified 21 MRSA isolates from 15 participants. Of the 15 MRSA positive carriers, nine carried MRSA in the nose only and six participants carried MRSA in both their nose and throat. Prevalence of MRSA was found to be 28.57% (10/35) in equine veterinarians, 15.38% (2/13) in equine nurses, 8% (2/25) in small animal veterinarians and 2.86% (1/35) in small animal nurses. Overall prevalence for veterinarians and veterinary nurses in this study was found to be 20% and 6.25% respectively. Of the four equine practices and four small animal practices enrolled, veterinary personnel from two equine clinics and two small animal clinics were found to be carriers of MRSA.

Prevalence of MRSA in household contacts of carriers and contamination of MRSA in the workspace and household environment of carriers

Follow-up nasal swabs of four initial carriers in a single equine clinic (three veterinarians and one veterinary nurse) were all found to be positive for MRSA. Of the six close household contacts from four veterinary households who were positive for MRSA, only one household contact of an equine veterinarian was found to be a carrier of MRSA (1/6). This household contact carried MRSA in both the nose and the throat. Workspace MRSA environmental contamination was observed for two of the four MRSA carriers, in which MRSA was isolated from a mobile phone and work telephone. No MRSA household environment contamination was observed. Table 6.4 shows the MRSA prevalence for all participants in this study.

Table 6.4 Total prevalence of MRSA in veterinary nurses, veterinarians, their household contacts and environmental contamination

Veterinary personnel	Number (n)	Prevalence
Equine veterinarians	10/35	28.57%
Equine nurses	2/13	15.38%
Small animal veterinarians	2/25	8%
Small animal nurse	1/35	2.86%
Follow up swabs equine veterinarians	4/4	100%
Household contacts	1/6	16.67%

6.3.4 Statistical analysis for MRSA carriage in veterinarians and veterinary nurses

In veterinarians no associations between MRSA carriage and independent variables were identified by bivariate analysis. Based on binary logistic regression, no factors in this study were found to increase the risk of caMRSA carriage. On the other hand, veterinarians who owned a dog as a family pet were less likely to be carriers of MRSA (p=0.025).

Characteristics of MRSA veterinary carriers are shown in Table 6.5. Of the three veterinary nurses found to be MRSA carriers, one had direct contact with a human healthcare setting (1/3), two had indirect contact with a human healthcare setting (1/3) and two recently used antibiotic (2/3). Of the 12 veterinarians found to be carriers of MRSA, two had direct contact with a human healthcare setting (2/12) and four recently used antibiotic (4/12). Most of the veterinarians who were carriers of MRSA prescribed antibiotics to animal patients on a daily basis (10/12), and all prescribed antibiotics at least

once weekly. Conditions for which antibiotics were most commonly prescribed, included respiratory and skin infections, followed by routine use in surgery. Prescription of antibiotics by veterinarians found to be carrier of MRSA, to their animal patients ranged from 5% to 75% depending on the veterinarian and practice type. Veterinarians found to be carriers of MRSA were primarily general practitioners (7/12), followed by surgeons (3/12) and intensive care veterinarians (2/12).

Table 6.5 Characteristics of veterinary MRSA carriers (veterinarians and veterinary nurses).

Participant	Veterinary placement		Direct contact with HCF*		Antibiotic use		Predominantly prescribed antibiotic	
	Yes/No	Duration	Yes/No	Duration	Yes/No	Type	Type	Frequency
1n	+	>7 days	-	-	-	-	AX	w
2n^°	+	>7 days	+	<7 days	+	n/s	n/a	n/a
3n°, 4n°, 8n, 9n,t, 14n,t	+	>7 days	-	-	-	-	PCN	d
5n	+	>7 days	-	-	-	-	TMPS	w
6n, t°	+	>7 days	+	<7 days	+	CEF	n/a	n/a
7n, t	+	>7 days	-	-	+	DOXY	PCN	d
10n	+	>7 days	-	-	-	-	TMPS	d
11n,t	+	>7 days	+	<7 days	+	CEF	TMPS	d
12n°	+	>7 days	-	-	-	-	n/a	n/a
13n~	+	>7 days	+	<7 days	+	AX	TMPS	d
13HH n,t~	-	-	+	<7 days	-	-	n/a	n/a
15n, t°	+	>7 days	-	-	+	PCN	TMPS	d

*participant had direct contact with human healthcare facility as a result of visitation, illness or procedure

^participant had indirect contact with HC lasting <7 days °participant owned dog

Equine veterinarian Equine nurse Small animal veterinarian Small animal nurse ~close household contact

n - isolated from nose t - isolated from throat F/M – female/male HCF – healthcare facility

CEF - cephalexin PCN - penicillin DOXY - doxycycline AX - amoxicillin TMPS – trimethoprim-sulfamethoxazole

n/a – not applicable n/s – not specified d - daily w - weekly

6.3.5 Antibiotic sensitivity of MRSA isolates

Antibiotic susceptibility of the 29 MRSA isolates from veterinary personnel, close household contact and environmental contamination are included in Table 6.6. MRSA strains isolated from equine veterinarians and equine veterinary personnel were found to be resistant to four or more antibiotics. In comparison MRSA isolates from small animal personnel were resistant to two or fewer antibiotics. Strains isolated from small animal veterinary personnel were typically resistant to cefoxitin, whereas isolates recovered from equine personnel were typically resistant to cefoxitin, rifampicin, trimethoprim, gentamicin and tetracycline (Table 6.6).

Table 6.6 Antibiotic susceptibility profiles of MRSA isolates from veterinary personnel

Participant	Veterinary personnel type	Antibiogram (resistance)
1n	Small animal veterinarian	FOX, CIP
2n	Small animal nurse	FOX
3n, 5n, 7n, 7t, 8n, 10n, 13n, 13n (b)	Equine veterinarians	FOX, RD, W, CN, TE
4n, 11n, 11t, 11n (b), 11e mobile	Equine veterinarians	FOX, RD, W, CN, E,
14n, 14t, 14n (b)	Equine veterinarian	FOX, RD, W, CN, E
9n, 9t	Equine veterinarian	FOX, RD, W, CN
6n, 6t, 12n, 12n (b), 12e telephone	Equine nurse	FOX, RD, W, CN, TE
13 HH n,t	Household contact of equine	FOX, RD, W, CN, TE
15n, 15t	Small animal veterinarian	FOX, W

n - isolated from nose t - isolated from throat HH - household contact colonisation e - environment

(b) - follow-up swab of colonised participant

FOX - cefoxitin E - erythromycin TE - tetracycline W – trimethoprim

CP – ciprofloxacin CN - gentamicin RD - rifampicin

6.3.6 Genetic analyses of MRSA isolates

PFGE and MLST results

Macro-restriction pulsed field gel electrophoresis (PFGE) of the 29 MRSA isolates (21 from initially colonised personnel and eight from follow-up testing) characterised five unique strain types: UK-EMRSA-15 (1), WA-MRSA-2 (2), WA-MRSA-3 (1), WA-MRSA-58 (17) and WA-MRSA-101 (8). One household contact of an MRSA carrier was found to carry two WA-MRSA-58 strains. Of the four initially colonised veterinary personnel, all were found to carry the same strain upon follow up swabbing (WA58 – 13n, 13nb, 14n, 14nb; WA101 – 11n, 11nb, 12n, 12nb). Two isolates of MRSA were recovered from the environment of two initially colonised carriers. An isolate of MRSA was recovered from a mobile phone of one participant and another isolate was obtained from the work telephone of the participant. These strains were typed to be the same as the originally typed isolates from the carriers (WA101 - 11n, 11e mobile, 12n, 12e phone). Table 6.7 shows the MRSA sequence type, clonal complex and clone as determined by PFGE and MLST analysis. All MRSA isolates in this study were classed as caMRSA (28/29) excluding one isolate, UK-EMRSA-15 (1n), which was identified to be a haMRSA strain (1/29). PFGE data on the meticillin sensitive *S. aureus* (MSSA) is available in Appendix C.

Table 6.7 Characteristics of MRSA isolates in veterinary personnel as identified by PFGE and MLST

Participant ID	Veterinary personnel type	Sequence type (ST)	Clonal complex (CC)	Clone	MRSA type*
1n	SAV	22	22	UK-EMRSA-15	haMRSA
2n	SAN	5	5	WA-MRSA-3	caMRSA
3n,4n, 5n, 7n, 7t, 8n, 9n, 9t, 13n, 13n (b), 14n, 14t, 14n (b)	EV	1173	8	WA-MRSA-58	caMRSA
6n, 6t	EN	1173	8	WA-MRSA-58	caMRSA
10n, 11n, 11t, 11n (b), 11e mobile	EV	8	8	WA-MRSA-101	caMRSA
12n, 12n (b), 12 e phone	EN	8	8	WA-MRSA-101	caMRSA
13 HH n, 13 HH t	HH EV	1173	8	WA-MRSA-58	caMRSA
15n, 15t	SAV	78	88	WA-MRSA-2	caMRSA

n - isolated from nose t - isolated from throat HH - household contact colonisation e - environment

(b) - follow-up swab of colonised participant

SAV- small animal veterinarian SN - small animal nurse EV- equine veterinarian EN- equine nurse

*Isolates classified as either caMRSA or haMRSA according to a previously published paper on evolution and genetic diversity of MRSA according to MLST, SCC*mec* type, antibiogram and DNA microarray analyses, specific to Australia (Coombs et al., 2011)

DNA microarray results

Of the 29 MRSA isolates typed by microarray, five unique MRSA types were identified, being the CC5-MRSA-IV (Paediatric clone), CC8-MRSA-IV, CC8-MRSA-IV (Lyon clone/UK-EMRSA-2), CC22-MRSA-IV (UK-EMRSA-15/Barnim EMRSA) and CC88-MRSA-IV (WA-MRSA-2). Resistance genes *mecA*, *blaZ*, *aacA-aphD*, *dfrS1*, *tet(M)* and *fosB* were observed in all isolates with the exception of isolate 1n which lacked *aacA-aphD*, *dfrS1*, *tet(M)* and *fosB*, isolate 2n which lacked *aacA-aphD*, *dfrS1* and *tet(M)* and isolates 15n and 15t which lacked *blaZ, aacA-aphD*, *tet(M)* and *fosB.* Additional resistance gene *aadD* was observed in strains 10n, 11n, 11t, 11 (b) 11e mobile, 12n, 12 (b) and 12e telephone, while an additional resistance gene *erm(C)* was present in isolates 3n, 4n, 11n, 11t, 11 (b) 11e mobile, 13n, 13n (b), 14n, 14t, 14n (b). Predominant virulence enterotoxins (*sea*, *seb*, *sek*, *seq*) were present in isolates 3n-14n (b), with the exception of 1n, 2n and 13HH n,t which lacked *seb, sek* and *seq.* Isolates 15n and 15t did not carry any enterotoxins. Leukocidins (*luk*S, *luk*F, *luk*S-ST22+ST45, *luk*D, *luk*E, *luk*X, *luk*Y, *hlg*A,), hlb-converting phages (*sak, scn*) and presence of haemolysin alpha toxin (hla) were present in all isolates, with the exception of strain 1n, which lacked *lukD* and *lukE* and isolates 15n and 15t which lacked *luk*S-ST22+ST45. Virulence factor *luk*S-ST22+ST45 was ambiguously expressed in isolate 13HH n. Additional virulence genes *chp*, *seg*, *sei*, *selm*, *seln*, *selo*, *egc-cluster* and *selu* were present in 1n and 2n, and additional virulence genes *sea (N315)* and *tst1* were found in isolate 1n, and isolates 15n and 15t carried additional virulence determinant *chp*. All isolates carried adhesion genes *bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sasG*, *sdrC*, *sdrD* and *vwb*, with the exception of strain 1n which lacked *ebh*, strains 5n-9n which lacked *fib* and strains 3n and 4n which lacked *srdD.* Isolate 1n carried an additional adhesion *cna*. Adhesion factor *cna* was only ambiguously expressed in isolate 13 HH t. Isolate 2n carried additional adhesins *ebh* and

fnbB and an additional virulence superantigenic toxin (*tst1*). All isolates carried *agr*I excluding 2n (CC5-MRSA-IV) that carried *agr*II and isolates 15n and 15t carried *agr*III (CC88-MRSA-IV). All isolates were found to carry *cap* 5 (1n-14nb), with the exception of two isolates, which carried *cap* 8 (15n and 15t). Table 6.8 shows the DNA microarray profiles for the 29 MRSA isolates, including *agr* type, capsule type, resistance determinants, virulence determinants and adhesion determinants.

Table 6.8 Microarray profiling of resistance, virulence and adhesion determinants of MRSA strains in veterinary personnel

Participant	MLST/*SCCmec*	*agr* type	Capsule	Resistance genes	Virulence genes	Adhesion genes
1n	CC22-MRSA-IV, UK-EMRSA-15/Barnim EMRSA	I	5	*mecA, delta_mecR, blaZ*	*seg, sei, selm, seln, selo, egc-cluster, selu, lukS, lukF, lukS (ST22+ST45), lukX, lukY, hlgA, hla, sak, scn, chp*	*bbp, clfA, clfB, cna, ebpS, eno, fib (MRSA252), fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
2n	CC5-MRSA-IV, Paediatric clone	II	5	*mecA, delta_mecR, blaZ, fosB*	*tst1, sea (N315), seg, sei, selm, seln, selo, egc-cluster, selu, lukS, lukF, lukS (ST22+ST45), lukD, lukE, hlgA, hla, sak, scn, chp*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
5n, 6n, 6t, 7n, 7t, 8n, 9n, 9t	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, aacA-aphD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
10n, 12n, 12n (b), 12e telephone	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, aacA-aphD, aadD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
11n, 11t, 11n (b), 11n mobile	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, erm(C), aacA-aphD, aadD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
3n, 4n	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, erm(C), aacA-aphD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, vwb*
13n, 13n (b), 14n, 14t, 14n (b)	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, erm(C), aacA-aphD, dfrS1, tet(M), fosB*	*sea, seb, sek, seq, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*

Table 6.8 Microarray profiling of resistance, virulence and adhesion determinants of MRSA strains in veterinary personnel

continued…

Participant	MLST/*SCCmec*	*agr* type	Capsule	Resistance genes	Virulence genes	Adhesion genes
13HH n	CC8-MRSA-IV, Lyon clone/UK-EMRSA-2	I	5	*mecA, delta_mecR, blaZ, aacA-aphD, dfrS1, tet(M), fosB*	*sea, lukS, lukF, lukS (ST22+ST45)*, lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
13HH t	CC8-MRSA-IV	I	5	*mecA, delta_mecR, blaZ, aacA-aphD, dfrS1, tet(M), fosB*	*sea, lukS, lukF, lukS (ST22+ST45), lukD, lukE, lukX, lukY, hlgA, hla, sak, scn*	*bbp, clfA, clfB, cna*, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*
15n, 15t	CC88-MRSA-IV, WA-MRSA-2	III	8	*mecA, delta_mecR, dfrS1*	*lukS, lukF, lukD, lukE, lukX, lukY, hlgA, hla, sak, chp, scn*	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sasG, sdrC, sdrD, vwb*

A complete list of target genes is available a previously published paper *(Monecke et al., 2008b)*

n- nasal colonisation t- throat colonisation *agr* – accessory gene regulator MLST – multilocus sequence typing *SCCmec* – Staphylococcal cassette chromosome mec

*gene ambiguously expressed

Note: genes have been formatted in bold to highlight main differences between isolates typed in this study

6.4 Discussion

Veterinarians and veterinary personnel with close animal contact are known to be a risk group for MRSA carriage, especially those caring for equine patients (Jordan et al., 2011, Hanselman et al., 2006, van Duijkeren et al., 2010, Weese and van Duijkeren, 2010a). Findings in the present study show that veterinarians and veterinary nurses had a higher prevalence of MRSA carriage compared to persons in the general community. Carriage of MRSA was 28.57% (10/35) in equine veterinarians, 15.38% (2/13) in equine nurses, 8% (2/25) in small animal veterinarians and 2.86% (1/35) in small animal nurses. These findings are comparable to previous studies (Jordan et al., 2011, Hanselman et al., 2006). An MRSA prevalence of 15.6% (15/96) has been observed in large animal veterinarians and 4.4% (12/271) in small animal veterinarians in the US (Hanselman et al., 2006). A high MRSA carriage has also been previously reported in Australia in which 21.35% (19/89) of equine veterinarians and 4.80% (12/250) of small animal veterinarians were found to be carriers (Jordan et al., 2011). The relatively high prevalence of MRSA in equine veterinarians compared to small animal veterinarians is of concern as they may act as potential vectors in the spread of this organism to their close household contacts and animal patients (Weese et al., 2005a, Schwaber et al., 2013, van Duijkeren et al., 2010) which in turn has the potential to exacerbate the spread of MRSA to the wider community and human and animal healthcare settings.

Based on published literature, the present study was the first to assess colonisation (collection of swabs from the same equine veterinary personnel and veterinarians who were identified as carriers of MRSA on two sampling occasions), which included a pre-enrichment step, follow-up sampling of close household contacts of MRSA carriers and collection of swabs from the household and workspace environment of

carriers. All four of the initial equine veterinary MRSA carriers in the current study, were found to carry the same strain upon follow-up testing. The present study suggests colonisation in this cohort may be persistent, however additional sampling of the same participants is required and the researchers cannot draw conclusions based on the collection of swabs with only one follow-up. Further studies are needed if this finding is to be confirmed. Internationally, there has been only one previous study that has formally assessed colonisation (collection of swabs from small animal veterinarians, veterinary staff and students on two separate occasions) in Japan (Ishihara et al., 2010). However their study fails to account for whether the same veterinarians were swabbed consecutively, as in 2007 the researchers sampled 20 participants and in 2008 sampling of 34 participants occurred.

Carriage of MRSA in household contacts of MRSA carriers was also investigated in the present study. In this study, one of six household contacts swabbed was found to be a carrier of MRSA (16.68%). A similar carriage prevalence of MRSA has been reported in previous studies, with carriage rates of 12% (82/712) (Nerby et al., 2011), 22% (177/812) (Miller et al., 2012) and 23% (18/77) (Rafee et al., 2012). However as only a small number of household contacts of MRSA carriers responded and were sampled, direct comparison between studies cannot be made. The household contact (13 HH) found to be a carrier of MRSA carried in their throat the same strain as their veterinarian household contact (CC8-MRSA, USA500), but carried a different MRSA strain in their nose (Lyon clone/UK-EMRSA-2) as typed by DNA microarray. Further studies with larger cohorts specifically investigating MRSA carriage in household contacts of veterinary MRSA carriers may account for the expanding reservoir of MRSA (Morris et al., 2010, Nerby et al., 2011, Miller et al., 2012, Rafee et al., 2012).

Another aspect of this study examined the role the environment plays as a reservoir for MRSA. In the present study environmental contamination was observed in the workspace (on a mobile phone and on a work telephone) of two equine employees, a contamination prevalence of 10% (2/20). Strain typing of human and contaminated surfaces revealed identical strain types. A similar contamination prevalence of 10% (3/30) has been observed in an earlier study of MRSA environmental contamination in a small animal referral hospital (Loeffler et al., 2005). Other studies which have examined environmental contamination with MRSA in the veterinary workspace environment have reported contamination rates to range from as low as 1.4% (2/140) (Heller et al., 2009) during absence of MRSA infection in animals admitted to a small animal veterinary hospital to as high as 53% (19/36) (van Duijkeren et al., 2010) during periods of outbreak in an equine setting. Contrary to the higher contamination rate of MRSA in equine clinics, no MRSA contamination was found to be present in the household environment of the four initially colonised veterinary carriers. A possible explanation for this could be perhaps a more thorough cleaning of the household environment in which a known positive MRSA carrier resides, as regular disinfection of fomites has been shown helpful in reducing contamination with MRSA (Patel et al., 2007).

Isolate characteristics of veterinary MRSA and their relevant genomic markers

Genotypic analyses by PFGE, MLST and DNA microarray assays identified five unique MRSA strain types (ST5, ST8, ST22, ST78 and ST1173) from the 29 MRSA isolates. Twenty-eight isolates were classified as caMRSA and one isolate was confirmed to be haMRSA based on genotypic analyses. Isolate 1n was the global pandemic haMRSA strain

UK-EMRSA-15 (ST22, CC22) (Monecke et al., 2011a). Commonly found genetic markers *egc-cluster*, *cna* and *sasG* were present in this isolate, however the commonly found resistant determinant *erm(C)* was absent in this strain. Isolate 2n was the global pandemic USA100 (ST5, CC5) MRSA strain, sometimes referred to as the Paediatric clone. In Australia, the ST5 strain is one of the most commonly reported caMRSA isolates (Coombs et al., 2012a). Changeably associated virulence genes in ST5 include *egc-cluster*, *sea(N315)*, *sed*, *sej*, and *ser* (Monecke et al., 2011a, Monecke et al., 2009). Isolate 2n was found to carry the *egc-cluster* and *sea(N315)* virulence determinants, and lacked the other changeably associated genes found in this strain. Isolate 2n also carried *sasG*, an adhesion genomic marker for this strain, and an additional *tst1* gene that is associated with toxic shock syndrome. Isolates 3n-9t and 13n-14n (b) (excluding 13HHn) were typed as community strains (ST1173, CC8-MRSA-IV). Strains of ST1173 have been identified in Australia, America, UK, Ireland and Germany. This strain has further been identified in horses and from humans who have close contact with horses (Kinnevey et al., 2010, Weese et al., 2005a). The findings presented in this study support the association of strain ST1173 with persons who have regular contact with horses. All ST1173 isolates in this study carried the virulence factors *seb*, *sek* and *seq* typically carried by this strain and all were also PVL negative. These isolates also carried an additional virulence enterotoxin *sea*, with the exception of isolates 13HHn,t that lacked *seb*, *sek* and *seq*. Isolates 10n-12e telephone were typed as WA-MRSA-101 strains (a USA500 clone). Typical ST8 virulence determinants *seb*, *sek* and *seq* were also present in our isolates. The ST8 strain has been identified previously in Australia, Ireland, the UK, the US, Germany and South Africa, and has been observed in horse handlers as well as horses (Monecke et al., 2011a, Weese and van Duijkeren, 2010b). Isolate 13HHn was typed as a CC8-MRSA-IV, (Lyon clone/UK-EMRSA-2). The Lyon clone has been isolated in Australia, UK, Ireland, Germany, France,

Norway and the Netherlands (Kinnevey et al., 2010, Dauwalder et al., 2008, Monecke et al., 2011a). Typically identified virulence determinants *sea, sak* and *scn* and variable resistance determinants *aacA-aphD, dfrS1, erm(C)* and *tet(K)* in the Lyon clone were also present in this isolate. Isolates 15n and 15t were typed as ST78, a frequently identified PVL negative strain in Australia (Monecke et al., 2011a). Strains of ST78 have been less frequently identified in Japan, Israel, Europe and Africa (Chua et al., 2011, Biber et al., 2012, Hisata et al., 2005, Monecke et al., 2011a). Resistance determinants *blaZ* and *erm(A)* and virulence determinants *sec* and *sel* typically found in strains of ST78 were absent in our isolates, however typical virulence determinants *sak, chp* and *scn* were present. All isolates carried the alpha toxin (*hla*), which has been associated with increased pathogenesis in invasive isolates (Kobayashi et al., 2011).

In the present study ST5 (CC5), ST22 (CC22) and ST78 (CC88) were isolated from a small animal nurse and two small animal veterinarians. These ST types are considered to be characteristically human MRSA strains (Coombs et al., 2013a). All equine nurse and equine veterinarian isolates were typed as equine associated ST8 (CC8) and ST1173 (CC8) strains, regardless of their isolation location in Sydney. Our results are consistent with a previous Australian study in which small animal veterinarians were predominantly found to carry CC22 and equine veterinarians were found to predominantly carry CC8 (Jordan et al., 2011, Trott et al., 2013). However, DNA microarray analyses of the recovered isolates in their study were not performed, hence limiting comparison of any unique strain characteristics between their study and the present study. Axon and colleagues add further support to these findings in their study of horses admitted to an Australian equine hospital in which only CC8 (ST612) was recovered (Axon et al., 2011). Therefore, identification of animal practice specific strains in the present study is in accordance with previous studies,

with the exception of ST78 (CC88), which was identified in small animal practice. Although, the ST78 is the fifth predominant caMRSA strain identified in human healthcare settings across Australia (Monecke et al., 2011a, Coombs et al., 2013a, Weese and van Duijkeren, 2010b, Jordan et al., 2011). The participant who was found to carry ST78 in the present study reported they did not have direct contact with a healthcare facility. This finding suggests this strain was introduced to the carrier via indirect transfer.

Factors associated with MRSA carriage in veterinarian cohort

Being an employee of a veterinary hospital (particularly in equine practice) or a non-clinical laboratory and contact with or treatment of an identified animal MRSA case have been reported to increase the risk of MRSA carriage (Ishihara et al., 2010, Anderson et al., 2008, Jordan et al., 2011, van Duijkeren et al., 2010, Weese et al., 2005a, Voss et al., 2005, Weese, 2010, Weese et al., 2006c, Pantosti, 2012). In the present study the researchers failed to identify any factors associated with increased risk of MRSA carriage. Furthermore, in this study the ownership of horses by equine veterinarians did not have an effect on carriage status. On the other hand, veterinarians who owned dogs were at a decreased risk (OR 0.223; p=0.025) of being an MRSA carrier. The association of dog ownership with the lowered risk of MRSA carriage in veterinarians is novel and needs to be elucidated further. If this finding is to be further explored, future studies on risk factors, as well as protective factors, for MRSA carriage in this cohort could survey a larger sample of veterinarians who own dogs and include a control group of veterinarians, then run statistical analysis comparing the two groups. In addition, result bias is present in this study due to the small number of MRSA positive clinics. Of the eight sampled clinics MRSA was present in three, which may have led to an over estimation of MRSA carriage

in the veterinary cohort and such a prevalence can not be considered as representative of the veterinary population in Australia.

Antibiotic use in veterinary practice

In veterinary medicine antibiotics are frequently prescribed, which is considered to have contributed to an increase in antibiotic resistance (Garcia-Alvarez et al., 2012). In the present study, 10 of 12 veterinarian carriers prescribed antibiotics on a daily basis. Of the 12 carriers, seven reported prescribing antibiotics to 50% or more of their animal patients to treat either infections or for prophylaxis. Veterinarians most commonly prescribed penicillin (6) and trimethoprim-sulfamethoxazole (5), drugs considered less likely to exert selective pressure. Based on these prescription patterns, equine veterinarians would be expected to have a lower carriage of MRSA. However, contrary to this assumption, equine veterinarians were found to have the highest MRSA carriage and the isolates recovered from equine veterinarians were more resistant to antibiotics tested compared to small animal MRSA isolates. This observation is consistent with a previous study of MRSA carriage in veterinary personnel (Jordan et al., 2011, Trott et al., 2013). Our results are consistent with a previous Australian study in which small animal veterinarians were predominantly found to carry CC22 and equine veterinarians were found to predominantly carry CC8 (Jordan et al., 2011, Trott et al., 2013). Additionally of the 45 MRSA isolates recovered from small animal and equine veterinarians in their study, antibiotic resistance to gentamicin and rifampicin was frequently observed in equine veterinarian isolates, whereas resistance to ciprofloxacin was frequently observed in companion animal veterinarian isolates. Consistent with that study, all isolates recovered from equine veterinarians in the present study were resistant to gentamicin and rifampicin. Of the

isolates recovered from three small animal veterinarians, resistance to ciprofloxacin was observed in only one of these isolates.

Future directions

At present, MRSA is an important occupational health issue in Australian veterinary medicine and further research and policy development are required (Jordan et al., 2011). Until recently, Australian-specific guidelines for MRSA in veterinary practice were lacking (Attard et al., 2012, Gosbell, 2011). Evidence-based policy development is needed for antibiotic stewardship in veterinary medicine (Gosbell, 2011), as the control of antibiotic use appears to be one of the only plausible ways in which to decrease resistance of MRSA strains to antibiotics and to reduce the spread and transmission of MRSA (Carlet et al., 2012, Lee et al., 2013). Studies examining the effect of prudent antibiotic use by veterinarians to treat infections may in turn help reduce the spread of this organism in veterinary settings. As the majority of veterinarians sampled prescribed antibiotics daily in the present study, it would have been informative to investigate the effect the prescribing patterns of these veterinarians had on antimicrobial resistance; however, this was beyond the scope of this study.

In the present study the high carriage of MRSA observed in equine veterinarians (28.57%) and equine nurses (15.38%) is concerning. In order to gain a better understanding of the caMRSA situation in Australia and account for expanding reservoirs of caMRSA future studies could investigate a broader population of Australian veterinarians and personnel with close animal contact, including livestock veterinarians, as well as their animal

patients. With better control, the negative socioeconomic impact MRSA places on both the public healthcare system and veterinary healthcare can be reduced.

Chapter 7 General Discussion

Recent research, as outlined in chapter 1, has established that caMRSA poses a potential threat to individuals in the community, and in healthcare and veterinary settings. However, relatively few studies have reported on the prevalence of caMRSA carriage in healthy people and animals in Australian communities, and elsewhere in the world. In the studies, which have been published in Australia, all of them have been cross-sectional, and use of convenience sampling to enrol participants is commonplace.

In the present study, caMRSA carriage was observed to be low in members of the general community and in University and TAFE staff and students. This finding is consistent with previous research on caMRSA carriage in healthy community members (Abudu et al., 2001, Munckhof et al., 2009, Gamblin et al., 2013, Ammons et al., 2010, Bearman et al., 2010, Rackham et al., 2010, Walther et al., 2012). The only other Australian study to report on carriage in the Australian community was published in 2009 (Munckhof et al., 2009). However, participants attending general practices were included in the Munckhof study, and as a result limited the researchers ability to generalise their findings as being representative of the general community. Whereas, the present study recruited members of the general community and included an additional recruitment of University and TAFE staff and students, which could also be considered as atypical of the general community. Additionally, carriage of caMRSA in the present study was reported to be higher (1.69% in University and TAFE staff and students; 1.63% in general community participants) than

the prevalence of caMRSA (0.4%) in the Munckhof study (2009), despite the authors sampling participants from general practice.

Carriage of caMRSA was also observed to be low in contact sports participants, with none of the rugby union players found to be carriers of MRSA (0/81) and with only two soccer players identified as being MRSA carriers (2%; 2/100). The assessment of caMRSA carriage in rugby union players is novel and has not been reported elsewhere prior to the current study. More studies are required to test this finding in the current study in order to draw comparisons between the MRSA carriage reported in healthy Australian rugby union players and their American football counterparts.

There have been no previous international or national studies that have assessed MRSA carriage in both horses and dogs and their respective handlers in the same study. The present study assessed carriage in this cohort and found MRSA to be absent in horses (0/310), dogs (0/108) and dog handlers (0/94). This finding is consistent with previous studies investigating the carriage of caMRSA in healthy dogs and horses in other community settings (Burton et al., 2008, Busscher et al., 2006b, Walther et al., 2012, Vengust et al., 2006, Schmidt et al., 2014). Whilst this finding is encouraging, it is still useful to monitor the prevalence of MRSA, as the incidence of MRSA is ever changing (Loeffler et al., 2011, van Balen et al., 2013, Köck et al., 2010a). On the other hand, MRSA was present in the horse handlers sampled (2/38), which is also consistent with previous studies (Busscher et al., 2006a, Van den Eede et al., 2013). This finding suggests horse handlers may represent a reservoir of MRSA in the community, however due to the low number of horse handlers enrolled no conclusions can be made.

In Australia, Jordan and colleagues (2011) have published the only previous study of MRSA carriage in veterinary personnel attending four conferences. The idea to assess caMRSA carriage in Australian veterinarians was novel up until then. Despite this, the present study of veterinary personnel does contain novel aspects compared to international literature. The present study was the first to longitudinally assess carriage of MRSA in veterinary personnel, their close household contacts and their household and workspace environment in the same study. In the present study caMRSA carriage in Australian veterinarians, especially those in equine practice, was found to be higher than that of veterinarians in small animal practice and in general members of the community. This confirms the findings of previous studies that have investigated these groups (Jordan et al., 2011, Ishihara et al., 2010). Of interest was the finding dog ownership among veterinarians was associated with a lowered risk of caMRSA carriage in the current study. This finding has not previously been reported. However, this finding is inconclusive as a clustering effect within clinics was observed and dog ownership as a factor that reduces risk of caMRSA carriage cannot be representative of the entire population of veterinarians in Australia. Further studies are needed to elucidate on whether owning a dog reduces the risk of carriage.

The current study also contributes to the knowledge of caMRSA carriage and the isolates genetic characteristics in community members and in groups suspected to be at an increased risk for MRSA acquisition in community and veterinary settings in Australia, as to date none of these Australian studies have previously examined virulence, resistance and adhesion characteristics utilising DNA microarrays.

Overall MRSA prevalence, strain types and sites of isolation

In this study, 24 human carriers of MRSA were identified. A total of 31 MRSA isolates were recovered from all cohorts primarily sampled, 28 of which were caMRSA (90.32%) and three of which were haMRSA (9.68%). Twenty-two isolates were recovered from the nose and nine isolates were recovered from the throat of carriers. The finding the majority of MRSA isolates were recovered from the nose is consistent with the bulk of current literature, where the nares have been identified as the primary site of MRSA carriage in humans (Lautenbach et al., 2009, Mermel et al., 2011). However, the nares should not be regarded as the only important site as some participants were found to be exclusive throat carriers. Therefore sampling the nares only may underestimate the prevalence of carriage in the cohorts examined. This finding is consistent with a previous study which has examined nasal and throat carriage of MRSA (Hamdan-Partida et al., 2010). The researcher's reported that had they only sampled the nares, 38% of throat carriers would have been missed.

Follow-up nasal sampling of the four veterinary carriers revealed all were colonised with their original strain of caMRSA. Carriage status could not be assigned as being persistent due to the researchers attempting only one follow-up swabbing of these carriers. Of their household contacts, one was found to be a nasal and throat carrier of caMRSA. Two of the isolates recovered from the workspace environment of a veterinarian and veterinary nurse were identified to be caMRSA. Isolates from veterinary MRSA carriers were identical to isolates recovered from their workspace environment (ST8, WA-MRSA-101). This is consistent with a previous study in which the majority of MRSA strains isolated from

equine veterinary personnel and their environment were found to be identical (Schwaber et al., 2013).

Overall, 39 MRSA isolates were recovered in this study from participants primarily sampled and follow-up swabs of veterinary personnel identified to be MRSA carriers, and their household contacts and workspace environment, with the majority of strains identified to be caMRSA (92.31%; 36/39). This finding is expected as all specimen collection was performed in the community setting, with the exception of veterinary personnel (n=108), where specimen collection was performed in a veterinary setting.

Sequence types (ST's) and clonal complexes (CC'S) identified

In the present study, nine sequence types (ST5, ST8, ST22, ST30, ST45, ST72, ST73, ST78, ST1173) belonging to seven different clonal complexes (CC5, CC8, CC22, CC30, CC45, CC72, CC88) were identified. The predominant clonal complexes that have been isolated in other countries, comprising CC5, CC8, CC22, CC30 and CC45 (Campanile et al., 2010, Stefani et al., 2012), were also isolated in the present study (Chapters 3 to 6). The other two strains that were found in this study, CC72 and CC88, are less prevalent globally (Monecke et al., 2011a), although CC88 is commonly found in Australian healthcare settings (Coombs et al., 2013a). Whereas, CC72 is less prevalent in Australia (Coombs et al., 2013a, Monecke et al., 2011a), it has been previously isolated from humans in America, Abu Dhabi, Germany, Czech Republic, Sweden and Portugal (Song et al., 2011, Mediavilla et al., 2012). More recently, CC72 (ST72) was isolated from a dog presenting to a small animal veterinary clinic in the US (Hamilton et al., 2013).

Results from the present study support the use of genotypic techniques (RT-PCR, PFGE, SCC*mec* typing, MLST, DNA microarrays) to definitively distinguish between community-associated and hospital-associated strain types at a strain and gene level. For example, two atypical ST45 strains, a commonly identified PVL positive ST30 strain and less frequently identified PVL negative ST30 strain was typed. For all other identified ST types in this study, including ST30 and atypical ST45 strains, it was possible to compare resistance, virulence and adhesion determinants to previously identified strain types in other geographic regions. However comparison of strains recovered in this study to other strains previously isolated in the cohorts tested within Australia were limited due to the scarcity of studies that exist in this country.

Participant recruitment

All participant recruitment in the present study relied on convenience sampling, albeit the sampling of general community members, which were selected at random. The reason convenience sampling was used in all other cohorts was due to the study targeting specific groups reported to be at risk for MRSA carriage, and the researchers aimed to recruit as many subjects into the study as possible due to the low prevalence of caMRSA reported in previous studies (Rackham et al., 2010, Munckhof et al., 2009, Vengust et al., 2006). A difficulty in obtaining participants across all cohorts, including follow-up consent from close household members of those initially found to be carriers of MRSA was encountered throughout this study. Possible reasons for hesitation in participating have been suggested in the literature. These include the limited knowledge community members may have had about the study, the perceived time commitment, privacy concerns, distrust of medical

research, a fear of having samples collected and a fear of being stigmatised should they be found to be a carrier (Loeffler et al., 2010b, Kerath et al., 2013, Trauth et al., 2000, Limkakeng et al., 2013, Corbie-Smith et al., 1999). In this study, one of the veterinary clinics that chose to participate was better informed due to the fact they had a resident microbiologist who recognised MRSA as a problem in their field of practice. When members of staff were found to be carrier of MRSA, the practice responded by reviewing and implementing stricter disinfection protocols to try to eradicate MRSA in the work environment.

Participant recruitment was constrained by ethical approval guidelines that required all initial recruitment to be indirect using an opt-in approach (which relies on active participation) in the present study. Direct participant recruitment was not permitted in order to ensure participation was voluntary. In a recent systematic review and meta-analysis and in research investigating methods to improve recruitment of participants into studies it was reported an opt-out approach (which was defined as non-response participants followed up repeatedly unless they signalled they did not wish to participate), cash incentives, paid participation, telephone contact, verbal education and video presentation with written information increased recruitment (Treweek et al., 2013, Junghans et al., 2005, Hunt et al., 2013, Green et al., 2013). In addition, as newer technologies emerge, collection of consent permission and data electronically is shown to be a useful tool in participant recruitment and in the improved organisation of large amounts of participant data making research more efficient (Sanderson et al., 2013). Based on these reports we recommend future research utilise, subject to ethical approval, an opt-out approach, paid participation and collection of data electronically to maximise study participation.

Statistical analysis

The small numbers of MRSA carriers in the present study prevented the performance of statistical analyses to assess the risk factors associated with carriage in the majority of the cohorts (Chapters 3 to 5). With the exception being the veterinarian cohort (Chapter 6) who was noted to have the highest prevalence of MRSA compared to all other groups. This is consistent with a previous study reporting that veterinarians and veterinary personnel are at an increased risk for the acquisition of MRSA as an occupational hazard (Ishihara et al., 2010). A previous study has reported that equine veterinarians were found to have a higher carriage of MRSA than their small animal veterinarian counterparts (Jordan et al., 2011), which is consistent with this study. The high carriage prevalence in this group has significant implications (e.g. expanding reservoir and potential infection) not only for the veterinarians themselves, but also for their household contacts and their animal patients. However, in the present study no risk factors for MRSA carriage were identified. Sampling a larger cohort may overcome this issue and identify risk factors for carriage.

Future directions

Although a low caMRSA carriage was observed in the community and sports participant cohorts in the present study, the potential threat caMRSA poses to healthy community members should not be overlooked (WHO, 2013). Further studies investigating caMRSA carriage in contact sport participants are warranted, and could follow participants longitudinally, as a previous study has identified carriage of MRSA to be higher in sports participants (a cohort of American football players) at the conclusion of the playing

season, with 4% (4/100) carrying MRSA at the start of playing season compared to 19% (19/100) at the end of playing season (Creech et al., 2010). An investigation of soil and turf surfaces as reservoirs for MRSA contamination may also be warranted, as artificial turf has been identified to be a source of MRSA, on which the organism was able to survive for up to one month (Waninger et al., 2011).

At present, there are few studies specifically investigating caMRSA carriage in non-professional animal handlers in the community. The lack of MRSA carriage in animals and their handlers in the present study seems consistent with the generally low caMRSA carriage prevalence in an Australian communities (Munckhof et al., 2009). This finding should not lead to complacency; rather continual monitoring is warranted to facilitate the early detection of an increase in caMRSA in the community setting. Further studies could examine the role the environment and close household contacts of companion animals and their handlers play as reservoirs in proliferating caMRSA, as a lack of community specific caMRSA prevalence data is available for the environment (Scott et al., 2008, Peterson et al., 2012) in which healthy dogs and horses reside. There is also limited information on household contacts of companion animals and their handlers (Kottler et al., 2010).

Further investigation into carriage factors in at-risk groups for the acquisition of caMRSA, particularly veterinary professionals, is warranted in order to aid infection control measures in community settings. For veterinary practices identified to be positive for MRSA, on-going follow-up testing in the veterinary cohort could focus on investigating the effect astute use of antibiotics has in the reduction of MRSA, as well as the effect disinfecting of hands, their practice environment, and disinfecting personal communication

devices, such as mobile and telephones, has on the elimination of this organism. Implementation of MRSA control programs may be of further benefit in Australian practice. These combined efforts have the potential to minimise dissemination and cross contamination of MRSA between healthcare, veterinary and community settings (Humphreys et al., 2009). Previous studies have reported a reduction of MRSA in healthcare settings following the implementation of control programs (Schelenz et al., 2005, Jurke et al., 2013, van Rijen and Kluytmans, 2009).

Finally, in order to account for the expanding reservoirs of MRSA in the community future studies should explore the roles household contacts and the household environment play in the spread of MRSA, as high carriage rates of 12% (82/712) to 23% (18/77) have been observed in household contacts of colonised MRSA carriers (Rafee et al., 2012, Nerby et al., 2011) and high contamination MRSA rates of 12% (10/85) to 53% (19/36) have been observed in the household and workplace environment of participants (Roberts et al., 2011b, van Duijkeren et al., 2010). In the present study one household contact of a veterinary carrier was also found to carry MRSA (1/6), and MRSA was present in the environment of two of the four veterinarians and veterinary nurses who had subsequent follow-up testing performed. An enhanced understanding of MRSA reservoirs has the potential to improve monitoring and aid in the control of this organism in community and veterinary settings.

Conclusion

Findings in the present study indicate MRSA carriage in the cohorts sampled are similar to carriage rates observed in general community settings in countries in which MRSA carriage has been investigated. Veterinary personnel were an exception to this trend and were found to have high carriage rates of MRSA. This study further reports that caMRSA was absent in horses, dogs and dog handlers. Investigations of caMRSA carriage, in Australia, in relation to these cohorts sampled have previously been scarce or lacking. In conclusion, findings from the present study have added to the general knowledge of caMRSA carriage and genetic characteristics of these strains, with particular relevance to the Australian context.

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Appendix A

A1 General community and sport participant questionnaire

1. ID #

2. Name

Email address or postal address:

3. Age (at 1 January 2012):	years		4. Gender:	Female	☐ Male	☐
5. Within the last 6 months have you worked in:		Hospital?	Yes	☐	No	☐
6.		Nursing home?	Yes	☐	No	☐
7.		Other healthcare facility?	Yes	☐	No	☐
8. If yes, how long for?			< 7 days	☐	> 7 days	☐
9. Within the last year have you been in hospital for:		Illness?	Yes	☐	No	☐
10.		A medical procedure?	Yes	☐	No	☐
11.		Other reason (e.g. visiting)?	Yes	☐	No	☐
12. If yes, how long for?			< 7 days	☐	> 7 days	☐
13. Have you taken any antibiotics within the last 6 months?			Yes	☐	No	☐
					Don't know	☐
14. If yes, what type?			Don't know	☐		
			Yes	☐	No	☐
16. If yes, how long for?			< 7 days	☐	> 7 days	☐
17. Do you currently suffer any type of skin disease?			Yes	☐	No	☐
If yes; what type of skin disease do you suffer from?					Don't know	☐
18. a. Are you currently competing in any contact sports?			Yes	☐	No	☐
b. If yes, please indicate which sport(s) played.						
c. In the last 6 months, have you shared:		Sports equipment (e.g. shin pads)	Yes	☐	No	☐
d.		Sports clothing (e.g. boots, socks)	Yes	☐	No	☐
e.		Drink bottles	Yes	☐	No	☐
Do you own a cat or dog?			19. Dog	☐	20. Cat	☐
21. Has your pet been in contact with a veterinary practice within the last year?			Yes	☐	No	☐
22. Have you been in recent contact with a horse or horse farm?			Yes	☐	No	☐
23. How many people do you share a house with?						
24. a. What relationship does each of these people have to you?		Family	Yes	☐	No	☐
b.		Flatmate	Yes	☐	No	☐
c.		Friend	Yes	☐	No	☐
d.		Other	Yes	☐	No	☐

Thank you for your participation in this study.

A2 Horse personnel questionnaire

1. ID #

2. Name

Email address or postal address:

3. Age (at 1 January 2012): years		4. Gender:	Female ☐	Male	☐
5. Within the last 6 months have you worked in:	Hospital?	Yes	☐	No	☐
6.	Nursing home?	Yes	☐	No	☐
7.	Other healthcare facility?	Yes	☐	No	☐
8. If yes, how long for?		< 7 days	☐	> 7 days	☐
9. Within the last year have you been in hospital for:	Illness?	Yes	☐	No	☐
10.	A medical procedure?	Yes	☐	No	☐
11.	Other reason (e.g. visiting)?	Yes	☐	No	☐
12. If yes, how long for?		< 7 days	☐	> 7 days	☐
13. Have you taken any antibiotics within the last 6 months?		Yes	☐	No	☐
				Don't know	☐
14. If yes, what type?		Don't know	☐		
		Yes	☐	No	☐
16. If yes, how long for?		< 7 days	☐	> 7 days	☐
17. Do you currently suffer any type of skin disease?		Yes	☐	No	☐
If yes; what type of skin disease do you suffer from?				Don't know	☐
18. Are you currently competing in any contact sports?		Yes	☐	No	☐
If yes, please indicate which sport(s) played.					
21. Has your pet been in contact with a veterinary practice within the last		Yes	☐	No	☐
23. What relationship does each of these people have to you? (e.g. family,		Yes	☐	No	☐
24. Have you been in contact with a veterinary facility in the last 6 months?		Yes	☐	No	☐
25. If yes, how long for?		< 7 days	☐	> 7 days	☐

26. How many horses do you regularly handle?

27. For how many stables do you regularly handle horses?

28. What other animal species are you regularly in contact with?

29. What is your main occupation?

A3 Horse questionnaire

ID number:		Sampling Date:	
Sampling Location:			
Trainer's Name:			
Trainer contact details:			
Age of horse:			

Gender (please circle)	Gelding	Entire Male	Filly/Mare
Has this horse been in a veterinary hospital within the last year? (please circle)	For illness?	Yes	No
	For surgery?	Yes	No
	If yes, how long for?	Less than 7 days	More than 7 days
Has this horse received any antibiotics within the last 6 months?	Yes	No	Don't know
Specify type if known			Not known

Under what conditions is this horse kept?	Stable	Paddock
How many horses are kept at this farm or stable?		
In the last 6 months has the horse been away from the home stable for more than 7 days?	Yes	No

FOR HORSES SAMPLED AT STABLE ONLY		
Is this horse currently racing or in full work?	Yes	No
If no, how long since the horse raced or been in full work?		

Thank you for your participation in this study

A4 Dog handler questionnaire

1. ID #						
2. Name:						
3. Email address or postal address:						
4. Age (at 1 January 2012): years		5. Gender:	Female ☐		Male	☐
6. Within the last 6 months have you worked in:	Hospital?	Yes	☐		No	☐
7.	Nursing home?	Yes	☐		No	☐
8.	Other healthcare facility?	Yes	☐		No	☐
9. If yes, how long for?		< 7 days	☐		> 7 days	☐
10. Within the last year have you been in hospital for:	Illness?	Yes	☐		No	☐
11.	A medical procedure?	Yes	☐		No	☐
12.	Other reason (e.g. visiting)?	Yes	☐		No	☐
13. If yes, how long for?		< 7 days	☐		> 7 days	☐
14. Have you taken any antibiotics within the last 6 months?		Yes	☐		No	☐
					Don't know	☐
15. If yes, please specify type (if known):					Don't know	☐
16. Do you currently suffer any type of skin disease?		Yes	☐		No	☐
17. If yes; what type of skin disease do you suffer from?					Don't know	☐
18. Are you currently competing in any contact sports?		Yes	☐		No	☐
19. If yes, please indicate which sport(s) played.						
20. In the last 6 months, have you shared:	Sports equipment (e.g. shin pads)	Yes	☐		No	☐
21.	Sports clothing (e.g. boots, socks)	Yes	☐		No	☐
22.	Drink bottles	Yes	☐		No	☐
23. How many people do you share a house with?						
24. What relationship does each of these people have to you?	Family	Yes	☐		No	☐
25.	Flatmate	Yes	☐		No	☐
26.	Friend	Yes	☐		No	☐
27.	Other	Yes	☐		No	☐
28. Within the last year, have any of your close household contacts been in a healthcare facility?		Yes	☐		No	☐
29. If yes, how long for?		< 7 days	☐		> 7 days	☐
30. Do you own a cat or dog?		Dog	☐		Cat	☐
31. Has your pet been in contact with a veterinary practice within the last year?		Yes	☐		No	☐
32. Have you been in recent contact with a horse or horse farm?		Yes	☐		No	☐

A5 Dog questionnaire

1. ID#:			
2. Name of dog:			
3. Breed of dog:			
4. Age of dog:			
5. Gender of dog:		Female ☐	Male ☐
Is this dog *primarily*: (Please select the *most* applicable to this dog)			
6.	A family pet?	Yes ☐	No ☐
7.	For breeding?	Yes ☐	No ☐
8.	A Show dog?	Yes ☐	Yes ☐
9. In the past 6 months, how many Shows has this dog entered?			Not applicable ☐
10. In the past 6 months, how many Agility or Obedience trials has this dog entered?			Not applicable ☐
Within the last year has this dog been in a veterinary clinic or hospital for:			
11.	Vaccination?	Yes ☐	No ☐
12.	A medical procedure?	Yes ☐	No ☐
13.	Illness?	Yes ☐	No ☐
	Other (please specify)		
14. If yes, how long for?		< 7 days ☐	> 7 days ☐
15. Has this dog received any antibiotics within the last 6 months?		Yes ☐	No ☐
			Don't know ☐
16. If yes, please specify type (if known):			Don't know ☐
Where does this dog normally live?			
17.	Kennel	Yes ☐	No ☐
18.	Outside the house	Yes ☐	No ☐
19.	Inside the house	Yes ☐	No ☐
20.	Other (please specify)		
21. With how many other dogs does this dog normally live?			
22. Has this dog been boarded in kennels within the last 6 months?		Yes ☐	No ☐
23. If yes, how long for?		< 7 days ☐	> 7 days ☐

A6 Veterinarian questionnaire

Name: _____ Email: _____

Age: _____ yrs Gender: Female ☐ Male ☐

Number of days spent as a hospital patient in the last 12 months: _____ days

Antibiotics taken in the last 6 months: _____

Number of people you live with: _____

Number of animals you own: dogs ___ cats ___ horses___

Your veterinary practice details

Principal practice type:

Hospitalised patients: ☐ Out patients: ☐

Ambulatory care: ☐

Species you treat:

Dogs	Daily ☐	Weekly ☐	Monthly ☐
Cats	Daily ☐	Weekly ☐	Monthly ☐
Horses	Daily ☐	Weekly ☐	Monthly ☐

Main areas of clinical care:

Please place a number in each relevant box – '1' for most often, '2' for next most often etc.

General practice	☐	Dentistry	☐	Surgery	☐
Dermatology	☐	Intensive care	☐		

What percentage of cases do you see that you would consider critical (life threatening)? ___ %

What are the 3 most common antibiotics you prescribe, and how often do you prescribe them?

1. _____	Daily ☐	Weekly ☐	Monthly ☐
2. _____	Daily ☐	Weekly ☐	Monthly ☐
3. _____	Daily ☐	Weekly ☐	Monthly ☐

What percentage of animals you see are treated with antibiotics? _____ %

What are the most common conditions for which you prescribe antibiotics? _____

A7 Veterinary nurse questionnaire

1. ID #						
2. Name:						
3. Email address or postal address:						
4. Age (at 1 January 2012): years		5. Gender:	Female	☐	Male	☐
6. Within the last 6 months have you worked in:	Hospital?	Yes	☐		No	☐
7.	Nursing home?	Yes	☐		No	☐
8.	Other healthcare facility?	Yes	☐		No	☐
9. If yes, how long for?		< 7 days	☐		> 7 days	☐
10. Within the last year have you been in hospital for:	Illness?	Yes	☐		No	☐
11.	A medical procedure?	Yes	☐		No	☐
12.	Other reason (e.g. visiting)?	Yes	☐		No	☐
13. If yes, how long for?		< 7 days	☐		> 7 days	☐
14. Have you taken any antibiotics within the last 6 months?		Yes	☐		No	☐
15. If yes, please specify type (if known):					Don't know	☐
16. Do you currently suffer any type of skin disease?		Yes	☐		No	☐
17. If yes; what type of skin disease do you suffer from?					Don't know	☐
18. Are you currently competing in any contact sports?		Yes	☐		No	☐
19. If yes, please indicate which sport(s) played.						
20. In the last 6 months, have you shared:	Sports equipment (e.g. shin pads)	Yes	☐		No	☐
21.	Sports clothing (e.g. boots, socks)	Yes	☐		No	☐
22.	Drink bottles	Yes	☐		No	☐
23. How many people do you share a house with?						
24. What relationship does each of these people have to you?	Family	Yes	☐		No	☐
25.	Flatmate	Yes	☐		No	☐
26.	Friend	Yes	☐		No	☐
27.	Other	Yes	☐		No	☐
28. Within the last year, have any of your close household contacts been in a healthcare facility?		Yes	☐		No	☐
29. If yes, how long for?		< 7 days	☐		> 7 days	☐
30. Do you own a cat or dog?		Dog	☐		Cat	☐
31. Has your pet been in contact with a veterinary practice within the last year?		Yes	☐		No	☐
32. Have you been in recent contact with a horse or horse farm?		Yes	☐		No	☐

Thank you for your participation in this study

A8 Human Participant Information Statement

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Prevalence of community-associated meticillin-resistant *Staphylococcus aureus* (caMRSA) colonisation in the general community and specific risk groups in Australia, and the characterisation of predominant genotypes and sequence types

We would like to invite you to take part in our research study into the prevalence of community-associated meticillin-resistant *Staphylococcus aureus* (caMRSA) colonisation in the general community and specific risk groups in Australia, and the characterisation of predominant genotypes and sequence types. Community-associated meticillin resistant *Staphylococcus aureus* (caMRSA) is a global problem. The role of this organism as a cause of infection has risen significantly in recent years. Also, normal, healthy people may carry this organism. This study aims to assess the prevalence of caMRSA carriage in healthy individuals in the community, as well as specific risk groups. The study is being conducted by Dr. Elizabeth Hegedus, Dr. Diana Oakes, Dr. Gary Lee and Danijela Stancic, as part of the latter's PhD project.

Attached is a questionnaire for you to complete. Personal information will be gathered in relation to your hospitalisation history, frequency of antibiotic use, sporting activities and any family pets you may have. Should you choose to participate in this study, swabs will be taken from specific body sites (nose and throat) and cultured on microbiologic media to identify caMRSA positive strains.

If found to be colonised with caMRSA you will be made aware of this and advised to consult with your doctor if you are concerned. Colonised participants will be contacted in 3 months time for follow-up swabs to determine whether they are intermittent or persistent carriers of this organism.

Participation in this study is entirely voluntary. Please understand that you are not obliged to participate and whatever your decision, it will not affect your relationship with the University of Sydney in any way.

If you would like to participate, then please complete the participant consent form and fill out the subject questionnaire. We estimate that the questionnaire will take less than 5 minutes to complete.

All aspects of the study, including results, will be strictly confidential and only the researchers will have access to information collected from participants.

When you have read this information, Elizabeth, Diana, Gary or Danijela will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact Elizabeth on 9351 9136 or elizabeth.hegedus@sydney.edu.au, or Danijela on dsta3054@uni.sydney.edu.au.

Any person with concerns or complaints about the conduct of a research study can contact the Deputy Manager, Human Ethics Administration, University of Sydney on +61 2 8627 8176 (Telephone); +61 2 8627 8177 (Facsimile) or ro.humanethics@sydney.edu.au (Email).

This information sheet is for you to keep

A9 Human Participant Consent Form

THE UNIVERSITY OF SYDNEY

ABN 15 211 513 464

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Discipline of Biomedical Sciences
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Email: elizabeth.hegedus@sydney.edu.au

Participant Consent Form

I, ... (Name), give consent to my participation in the research project:

Prevalence of community-associated meticillin-resistant *Staphylococcus aureus* (caMRSA) colonisation in the general community and specific risk groups in Australia, and the characterisation of predominant genotypes and sequence types

In giving my consent to participate in this research study, I acknowledge that:

1. The procedures required for the project and the time involved has been explained to me, and any questions I have about the project have been answered to my satisfaction. The procedures involve the completion of a subject questionnaire and the collection of swabs from my nose and throat by the researcher.

2. I have read the Participant Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.

3. I understand that I can withdraw from the study at any time, without affecting my relationship with the researchers now or in the future.

4. I understand that my involvement is strictly confidential and no information about me will be used in any way that reveals my identity.

Signed: .....

Name (please print): .....

Date: .....

A10 Information Statement for Dogs

THE UNIVERSITY OF SYDNEY

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Prevalence of community-associated meticillin-resistant *Staphylococcus aureus* (caMRSA) colonisation in the general community and specific risk groups in Australia, and the characterisation of predominant genotypes and sequence types

Community-associated meticillin resistant *Staphylococcus aureus* (caMRSA) is a specific type of bacteria that has become a global problem and a significant cause of infection in humans as well as animals. This study aims to determine the percentage of companion animals, including dogs that are carriers of this organism. Previous studies have shown that MRSA can be spread from animals to humans. At present studies on this organism in companion animals are lacking in Australia. It is hoped that this study will improve the control of MRSA and reduce its spread in community and veterinary settings. The study is being conducted by *Danijela Stancic (PhD student) and* will form the basis for the degree of *PhD* at The University of Sydney under the supervision of *Dr. Elizabeth Hegedus, Dr Diana Oakes and Dr Gary Lee.* We will collect a swab from the dog's nostrils and perineum and we will ask you to complete a short questionnaire. A swab is a standard, non-invasive, clinical procedure that causes minimal distress to the animal.

The collection of each swab will take a maximum of 30 seconds and the questionnaire will take approximately 2 minutes.

Being in this study is completely voluntary - you are not under any obligation to consent and - if you do consent - you can withdraw, yourself and your animal, at any time without affecting your relationship with the University of Sydney. All aspects of the study, including results, will be strictly confidential and only the researchers will have access to information on your animal. A report of the study may be submitted for publication, but individual participants and animals will not be identifiable in such a report. The study will not benefit your animal, but if your dog is found to be carrier of caMRSA you will be made aware of this by email. You are free to tell others about this study and may direct them to the contact details below should they also be interested in participating in the study. When you have read this information, *Dr. Elizabeth Hegedus and Danijela Stancic* will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact *Dr. Elizabeth Hegedus (Elizabeth.hegedus@sydney.edu.au) or Ms Danijela Stancic (dsta3054@uni.sydney.edu.au) via the email addresses provided.*

Any person with concerns or complaints about the conduct of a research study can contact the Animal Secretariat, University of Sydney on (02) 8627-8175 (Telephone); (02) 8627 8180 (Facsimile).

This information sheet is for you to keep

A11 Information Statement for Horses

THE UNIVERSITY OF SYDNEY

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(1) What is the study about?

Community-associated meticillin resistant *Staphylococcus aureus* (caMRSA) are specific type of bacteria which are mostly carried by healthy humans and animals, but which sometimes can cause infection in humans.

This study aims to determine the percentage of horses that are carriers of this organism. Previous studies have shown that MRSA can be spread from horses to humans, and vice versa. At present, studies on this organism in horses are lacking in Australia. It is hoped that this study will improve the control of MRSA and reduce its spread in community and veterinary settings.

This study has been approved by the University of Sydney Animal Ethics Committee.

(2) Who is carrying out the study?

The study is being conducted by *Danijela Stancic (PhD student) and* will form the basis for the degree of *PhD* at The University of Sydney under the supervision of *Dr. Gary Lee (Senior Lecturer) and Dr. Peter Knight (Deputy Head of Discipline).*

(3) What does the study involve?

We will collect a nasal swab from the horse at its home stable. The swab will be collected by an experienced veterinarian. We will ask you to complete a short questionnaire about the horse.

A nasal swab is a standard clinical procedure that causes minimal distress to horses.

(4) How much time will the study take?

The collection of each swab will take a maximum of 30 seconds.

(5) Can I withdraw this horse from the study?

Being in this study is completely voluntary - you are not under any obligation to consent and - if you do consent - you can withdraw your horse, at any time without affecting your relationship with the University of Sydney.

(6) Will anyone else know the results?

All aspects of the study, including results, will be strictly confidential and only the researchers will have access to information on your horse. A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.

(7) Will the study benefit my horse?

No, results will be sent by email to manager/trainer of the horse if their horse is found to be a carrier of MRSA.

(8) Can I tell other people about the study?

Yes, if you wish.

(9) What if I require further information?

When you have read this information, *Dr Gary Lee*, *Dr. Peter Knight and Danijela Stancic* will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact *Dr Gary Lee (gary.lee@sydney.edu.au), Dr. Peter Knight (p.knight@sydney.edu.au) or Danijela Stancic (dsta3054@uni.sydney.edu.au) via the email addresses provided.*

(10) What if I have a complaint or concerns?

Any person with concerns or complaints about the conduct of a research study can contact the Animal Secretariat, University of Sydney on (02) 8627-8175 (Telephone); (02) 8627 8177 (Facsimile).

This information sheet is for you to keep

A12 Consent Form for Dogs and Horses

THE UNIVERSITY OF SYDNEY

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Email: elizabeth.hegedus@sydney.edu.au

In giving my consent I acknowledge that:

1. The procedures required for the project and the time involved have been explained to me, and any questions I have about the project have been answered to my satisfaction.

2. I have read the Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.

3. I understand that I can withdraw this/these dog(s) from the study at any time, without affecting my relationship with the researcher(s) or the University of Sydney now or in the future.

4. I understand that my involvement is strictly confidential and no information about this/these dog(s) will be used in any way that reveals my identity.

5. I understand that being in this study is completely voluntary – I am not under any obligation to consent.

I, Mr/Mrs/Ms _____ [NAME OF OWNER]

agree to permit the dogs listed below to participate in the study.

_____ [ANIMAL'S NAME/ IDENTIFIER]

_____ [ANIMAL'S NAME/ IDENTIFIER]

_____ [ANIMAL'S NAME/ IDENTIFIER]

I have read and understood the information statement on the above named research study and I have discussed it with the researchers.

Signed: .....

Name (Please print): .....

Date: .....

Appendix B

Primer sequences used in M-PCR, multiplex RT-PCR and MLST

Appendix B1. Primer sequences for *mecA and femA* genes sourced from previously published paper (Al-Talib et al., 2009)

Primer	Oligonucleotide sequence	PCR amplicon length (bp)
*mecA*2 F	5' ACG AGT AGA TGC TCA ATA TAA 3'	293
*mecA*2 R	5' CTT AGT TCT TTA GCG ATT GC 3'	
femA F	5' CGA TCC ATA TTT ACC ATA TCA 3'	450
femA R	5' ATC ACG CTC TTC GTT TAG TT 3'	

Appendix B2. Primer and probe sequences for *mecA, nuc* and PVL gene sourced from previously published papers (Costa et al., 2005, Fey et al., 2003)

Primer	Oligonucleotide sequence	PCR amplicon length (bp)
*mecA*2 F	5' GAT AAA AAA GAA CCT CTG CT 3'	273
*mecA*2 R	5' ACT GCC TAA TTC GAG TG 3'	
*mecA*2 FL*	5' GTG GTA AAT GGT AAT ATC GAC TTA AAA CA X 3'	
*mecA*2 640*	5' GCA ATA GAA TCA TCA GAT AAC ATT TTC TTT GC P 3'	
*nuc*274 F	5' GAT TGA T GGTGG TGA TAC GGT 3'	274
nuc 274 R	5' CAA GCC TTG ACG AAC TA 3'	
*nuc*274 FL*	5' GTT TGA CAA AGG TCA AAG AAC TGA TAA T X 3'	
*nuc*274 705*	5' TGG ACG TGG CTT AGC GTA TAT TTA T P 3'	
LuKS-F	5' GGC CTT TCC AAT ACA ATA TTG G 3'	1554
LukS-R	5'CCC AAT CAA CTT CAT AAA TTG 3'	
PVL- FL*	5' CAA CAC ACT ATG GCA ATA GTT X 3'	
PVL- 610*	5' TTT AGA AGG ATC TAG AAT ACA CAA GGC P 3'	

*Probes X - fluorescein P - phosphorylated

Appendix B3. Primers sequences for housekeeping genes *arc, aro, glp, gmk, pta, tpi* and *yqi* sourced from previous study (Enright et al., 2000b)

Primer	Primer sequence	PCR amplicon length (bp)
*arc*F	5' TTG ATT CAC CGC GTA TTG TC '3	456
*arc*R	5' AGG TAT CTG CTT CAA TCA GCG '3	
*aro*F	5' ATC GGA AAT CCT ATT TCA CAT TC '3	456
*aro*R	5' GGT GTT GTA TTA ATA ACG ATA TC '3	
*glp*F	5' CTA GGA ACT GCA ATCA TTA ATC C '3	465
*glp*R	5' TGG TAA AAT CGC ATG TCC AAT TC '3	
*gmk*F	5' ATC GTT TTA TCG GGA CCA TC '3	429
*gmk*R	5' TCA TTA ACT ACA ACG TAA TCG TA '3	
*pta*F	5' GTT AAA ATC GTA TTA CCT GAA GG '3	474
*pta*R	5' GAC CCT TTT GTT GAA AAG CTT AA '3	
*tpi*F	5' TGC TTC ATT CTG AAC GTC GTG AA '3	402
*tpi*R	5' TTT GCA CCT TCT AAC AAT TGT AC '3	
*yqi*F	5' CAG CAT ACA GGA CAC CTA TTG GC '3	516
*yqi*R	5' CGT TGA GGA ATC GAT ACT GGA AC '3	

Appendix C

Typing of meticillin susceptible *Staphylococcus aureus* (MSSA) isolates recovered in this study (PFGE and antibiotic susceptibility)

In total 60 false positive MRSA isolates were recovered from all participants in this study due to initial screening on Brilliance™ MRSA 2 agar, termed MSSA. All MSSA isolates had antibiotic susceptibility testing and macro-restriction pulsed field gel electrophoresis (PFGE) assays performed on them. Appendix C gives the results for these MSSA isolates. From the 60 MSSA isolates recovered two isolates could not be assigned a clonal complex (CC) and two isolates were non-typeable by PFGE and were classified as new strain types. Tables C1 to C4 show the antibiotic susceptibilities, strain sequence type (ST) and CC of MSSA isolates as typed by PFGE in this study.

Appendix C1. Characteristics of the 27 MSSA strains isolated in the community and in University and TAFE staff and students (MSSA type, ST, CC, antibiotic susceptibility)

Participant	Sequence type (ST)	Clonal Complex (CC)	Antibiotic resistance
General community			
1n	15	15	E
2t, 18n	96	Unknown*	-
3n, 10n	15	15	-
4n, 17n	8	8	W
5t, 6n, 23n	188	188	-
7n, 8na	81	1	-
8nb, 9n	22	22	-
10t	5	5	-
11n, 11t	8	8	-
12n	1	1	FD
13n	12	12	-
14t	239	8	-
15n, 26t	88	88	E
16n	88	88	-
19n	398	398	E, TE
26n	109	9	E
27t	20	20	-
28n	34	30	-

n - isolated from nose t - isolated from throat *unknown – isolate ST possesses unknown clonal complex

E - erythromycin W - trimethoprim TE - tetracycline FD – fusidic acid

Appendix C2. Characteristics of the 5 MSSA strains isolated in soccer and rugby players (MSSA type, ST, CC, antibiotic susceptibility)

Participant	Sequence type (ST)	Clonal Complex (CC)	Antibiotic resistance
Contact sports participants			
20n	88	88	-
21n	15	15	-
22n	1	1	FD, E
24n	5	5	-
25n	188	188	-

n - isolated from nose t - isolated from throat

E – erythromycin FD - fusidic acid RD – rifampicin

Appendix C3. Characteristics of the 23 MSSA strains isolated in dogs, horses and their respective handlers (MSSA type, ST, CC, antibiotic susceptibility)

Participant	Sequence type (ST)	Clonal Complex (CC)	Antibiotic resistance
Horses			
29n	30	30	-
30n	5	5	-
Horse handlers			
31n, 32n	30	30	-
31t	188	188	-
33n, 33t, 34n, 35n	97	97	-
36t	1	1	FD
36n, 37n, 37t, 38n	7	7	-
39n	398	398	-
40n	81	1	-
Dogs			
41n	unique type	unique type	-
42p	unique type	unique type	W
Dog handlers			
43n	81	6	-
44t	15	15	-
45n, 45t	199	1	-
46n	15	15	-

n - isolated from nose t - isolated from throat

FD - fusidic acid W - trimethoprim unique type – novel type of MSSA not seen at ACCESS with unique ST and CC

Appendix C4. Characteristics of the 5 MSSA strains isolated in small animal veterinary nurses (MSSA type, ST, CC, antibiotic susceptibility)

Participant	Sequence type (ST)	Clonal Complex (CC)	Antibiotic resistance
Veterinary Nurses			
47n	630	8	-
48n	88	88	-
49na	88	88	TE
49nb	97	97	W, CN
50n	188	188	E

n - isolated from nose

na/nb – two different strains isolated from the nose of the same person

E - erythromycin

W - trimethoprim

TE - tetracycline

CN - gentamicin

Appendix D

DNA microarray gene profile

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene targets

Gene	Gene product /function
aacA-aphD	bifunctional enzyme Aac/Aph, gentamicin resistance
aadD	aminoglycoside adenyltransferase, tobramycin resistance
agrB	accessory gene regulator B
agrC	accessory gene regulator C
agrD	accessory gene regulator D
aphA3	3'5'-aminoglycoside phosphotransferase, neo-/kanamycin resistance
arcA	ACME-locus
arcB	ACME-locus: ornithincarbamoyltransferase
arcC	ACME-locus: carbamatkinase
arcD	ACME-locus: arginine/ornithine-antiporter
aur	aureolysin
bap	surface protein involved in biofilm formation
bbp	bone sialoprotein-binding protein
blaI	beta lactamase repressor (inhibitor)
blaR	beta-lactamase regulatory protein
blaZ	beta-lactamase
capH	capsular polysaccharide synthesis enzyme CapH of capsule types 1, 5, and 8
capI	capsular polysaccharide biosynthesis protein CapI
capJ	O-antigen polymerase CapJ of capsule types 1, 5, and 8
capK	capsular polysaccharide biosynthesis protein CapK of capsule types 1, 5, and 8
cat	chloremphenicol acetyltransferase
ccrA	cassette chromosome recombinase A
ccrB	cassette chromosome recombinase B
ccrC	cassette chromosome recombinase
cfr	23S rRNA methyltransferase
chp	chemotaxis-inhibiting protein (CHIPS)
clfA	clumping factor A

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene **targets continued…**

Gene	Gene product /function
clfB	clumping factor B
cna	collagen-binding adhesin
coA	coagulase
dcs-Q9XB68	hypothetical protein from SCCmec elements
dfrA	dihydrofolate reductase type 1
ebh	cell wall associated fibronectin-binding protein
ebpS	cell surface elastin binding protein
edinA	epidermal cell differentiation inhibitor precursor
edinB	epidermal cell differentiation inhibitor B
edinC	epidermal cell differentiation inhibitor C
eno	enolase
erm(A)	rRNA adenine N-6-methyltransferase, erythromycin/clindamycin resistance
erm(C)	erythromycin/clindamycin resistance
etA	exfoliative toxin serotype A
etB	exfoliative toxin serotype B
etD	exfoliative toxin D
far1	fusidic acid resistance
fexA	chloramphenicol/florfenicol exporter
fib	fibrinogen binding protein (19 kDa)
fnbA	fibronectin-binding protein A
fnbB	fibronectin-binding protein B
fosB	metallothiol transferase
gapA	glyceraldehyde 3-phosphate dehydrogenase, locus 1
hl	putative membrane protein
hla	haemolysin alpha
\hlb	haemolysin beta
hld	haemolysin delta
hlgA	haemolysin gamma, component A
hlIII	putative membrane protein
hsdS1	type I site-specific deoxyribonuclease subunit, 1st locus
hsdS2	type I site-specific deoxyribonuclease subunit, 2nd locus
hsdS3	type I site-specific deoxyribonuclease subunit, 3rd locus

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene **targets continued…**

Gene	Gene product /function
hsdSx	type I site-specific deoxyribonuclease subunit, unknown locus
hysA1/2	hyaluronate lyase, first / second locus
hysA2	hyaluronate lyase, second locus
icaA	intercellular adhesion protein A
icaC	intercellular adhesion protein C
icaD	biofilm PIA synthesis protein D
isaB	immunodominant antigen B
isdA	transferrin-binding protein
katA	katalase A
kdpA	potassium-translocating ATPase A, chain 2
kdpB	potassium-transporting ATPase B, chain 1
kdpC	potassium-translocating ATPase C, chain 2
kdpD	sensor kinase protein
kdpE	KDP operon transcriptional regulatory protein
linA	Lincosaminid-Nucleotidyltransferase
lmrP	hypothetical protein, similar to integral membrane protein LmrP
lukD	leukocidin D component
lukE	leukocidin E component
lukF-hlg	haemolysin gamma / leukocidin, component B
lukS-hlg	haemolysin gamma / leukocidin, component C
lukF-PV	Panton Valentine leukocidin F component
lukS-PV	Panton Valentine leukocidin S component
lukF-PV83	F component from hypothetical leukocidin from ruminants
lukM	S component from hypothetical leukocidin from ruminants
"lukX"	leukocidin/haemolysin toxin family protein
"lukY"	leukocidin/haemolysin toxin family protein
map	Major histocompatibility complex class II analogue protein (=Extracellular adherence protein, eap)
mecA	penicillin binding protein 2, beta-lactam resistance defining MRSA
mecI	meticillin-resistance regulatory protein
mecR1	signal transducer protein MecR1
mefA	macrolide efflux protein A
merA	mercury-reductase

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene **targets continued…**

Gene	Gene product /function
merB	mercuric resistance operon regulatory protein
mph(BM)	probable lysylphosphatidylglycerol synthetase
mprF	defensin resistance protein
msr(A)	energy-dependent efflux of erythromycin
mupR	mupirocin resistance protein
nuc1	thermostable extracellular nuclease
ORF CM14	enterotoxin-like protein ORF CM14
pls-SCC	plasmin-sensitive surface protein
Q2FXC0	hypothetical protein, located next to serine protease operon
Q2YUB3	Unspecific efflux/transporter
Q6GD50	hypothetical protein associated with fusidic acid resistance
Q7A4X2	hypothetical protein
qacA	quaternary ammonium compound resistance protein A
qacC	quaternary ammonium compound resistance protein C
rrn STAU	Ribosomal sequence from S. aureus (genus specific positive control)
saeS	histidine protein kinase, sae locus
sak	staphylokinase
sarA	staphylococcal accessory regulator A
sasG	*Staphylococcus aureus* surface protein G
sat	streptothricine-acetyltransferase
sbi	IgG-binding protein
scn	staphylococcal complement inhibitor (SCIN)
sdrC	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein C
sdrD	Ser-Asp rich fibrinogen-/bone sialoprotein-binding protein D
sea	enterotoxin A
seb	enterotoxin B
sec	enterotoxin C
sed	enterotoxin D
see	enterotoxin E
seg	enterotoxin G
seh	enterotoxin H
sei	enterotoxin I
sej	enterotoxin J

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene **targets continued…**

Gene	Gene product /function
sek	enterotoxin K
sel	enterotoxin L
sem	enterotoxin M
sen	enterotoxin N
seo	enterotoxin O
seq	enterotoxin Q
ser	enterotoxin R
"setB1"	staphylococcal exotoxin-like protein, second locus
"setB2"	staphylococcal exotoxin-like protein, second locus
"setB3"	staphylococcal exotoxin-like protein, second locus
"setC"	staphylococcal exotoxin-like protein
seu / sey	Enterotoxin U and/or Y
spa	Staphylococcus protein A
splA	Serinprotease A
splB	Serinprotease B
splE	Serinprotease E
ssl01	staphylococcal superantigen-like protein 1
ssl02	staphylococcal superantigen-like protein 2
ssl03	staphylococcal superantigen-like protein 3
ssl04	staphylococcal superantigen-like protein 4
ssl05	staphylococcal superantigen-like protein 5
ssl06	staphylococcal superantigen-like protein 6
ssl07	staphylococcal superantigen-like protein 7
ssl08	staphylococcal superantigen-like protein 8
ssl09	staphylococcal superantigen-like protein 9
ssl010	staphylococcal superantigen-like protein 10
ssl011	staphylococcal superantigen-like protein 11
sspA	glutamylendopeptidase
sspB	staphopain B, Protease
sspP	staphopain A (staphylopain A), Protease
"tetEfflux"	Transport-/Efflux protein
tet(K)	tetracycline-resistance
tet(M)	tetracycline-resistance

Appendix D1. DNA microarray regulatory, resistance, virulence and adhesion gene targets co**ntinued…**

Gene	Gene product /function
tst1	toxic shock syndrome toxin 1
ugpQ	glycerophosphoryl diester phosphodiesterase, associated with *mecA*
vanA	vancomycin resistance gene
vanB	vancomycin resistance gene from enterococci and Clostridium
vanZ	teicoplanin resistance gene from enterococci
vatA	virginiamycin A acetyltransferase
vatB	acetyltransferase inactivating streptogramin A
vga	ATP binding protein, streptogramin-A-resistance
vgb	virginiamycin B hydrolase
vraS	sensor protein
vwb	van Willebrand factor binding protein
xylR	homolog of xylose repressor, associated with SCCmec-elements

Adapted from previously published papers (Monecke et al., 2008a, Monecke et al., 2008b, Coombs et al., 2011)

Appendix E

Appendix E1. Gel image of M-PCR assay

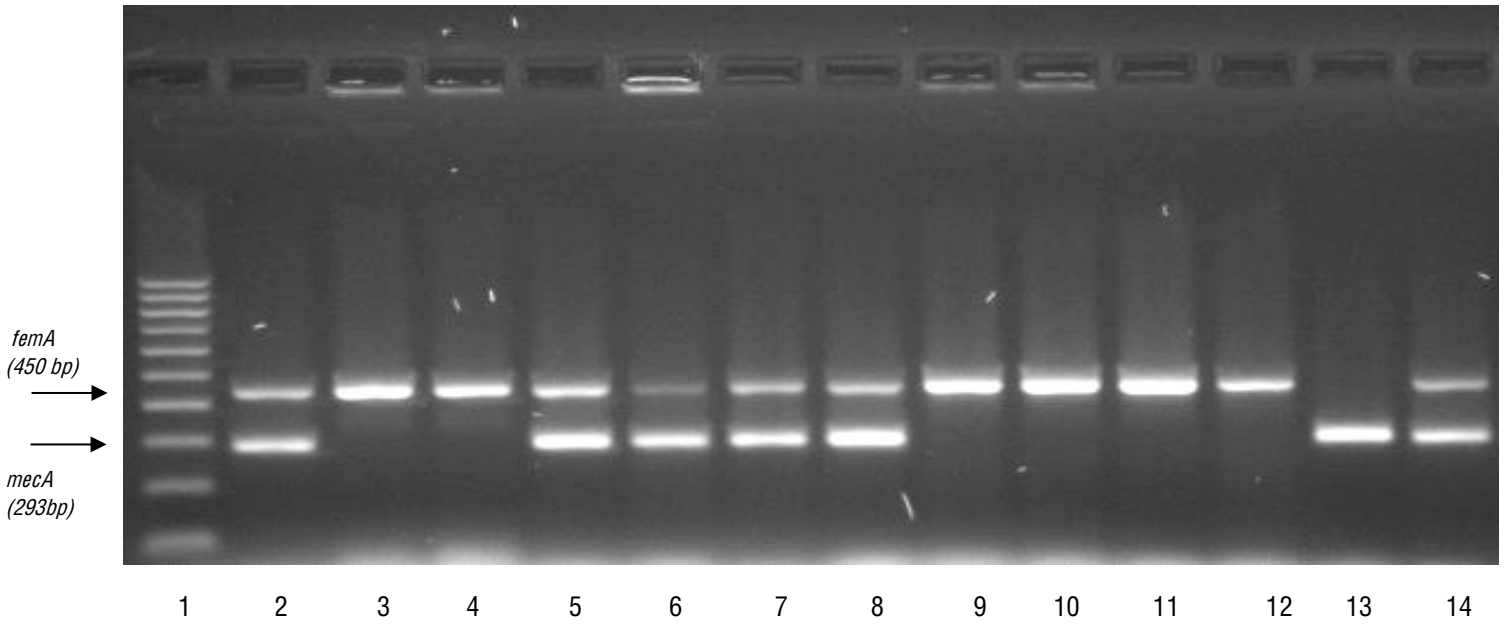


Figure 2.3 Gel image of M-PCR assay for mecA (293bp) and femA (450bp) genes in MRSA and MSSA isolates recovered from community participants.

Lane 1, DNA ladder (100bp increments); Lane 2, MRSA control (NCTC 10443); Lane 3, Staphylococcus aureus control strain (ATCC8235); Lane 4, MSSA control (clinical isolate); Lane 5, MRSA control (clinical isolate); Lane 6, Isolate 1n MRSA; Lane 7, Isolate 2n MRSA; Lane 8, Isolate 3n MRSA; Lane 9, MSSA isolate 5t; Lane 10, MSSA isolate 6n; Lane 11, MSSA isolate 7n; Lane 12, MSSA isolate 8n; Lane 13, meticillin resistant coagulase negative Staphylococcus control; Lane 14, Isolate 4t MRSA.

Note: image obtained after M-PCR assay performed on isolates in community study (see Chapter 3).

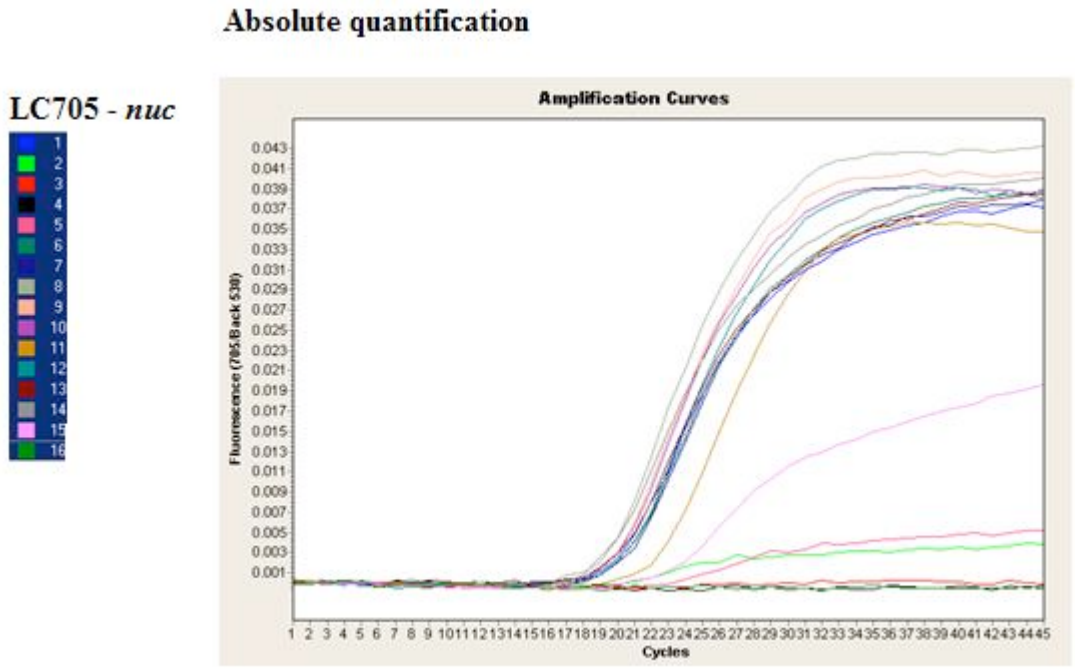
n – isolated from nose t – isolated from throat

Appendix E2. Multiplex RT-PCR amplification curves

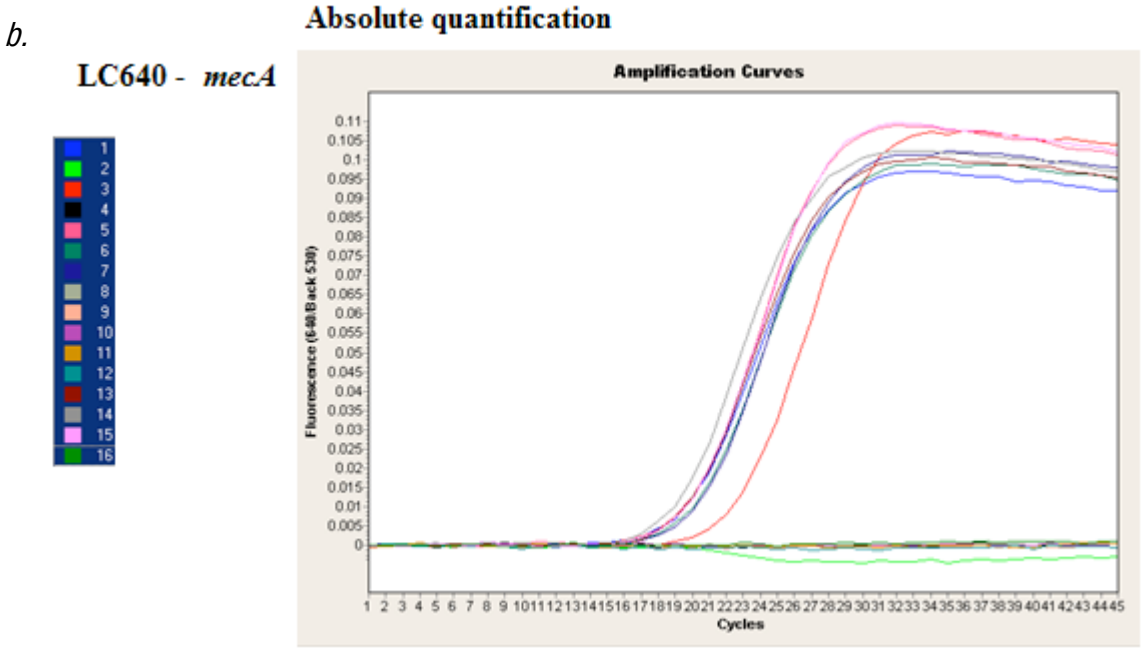
Figure 2.4 Representative multiplex Real-time PCR (nuc, mecA, PVL gene) amplification curves for MRSA and MSSA isolates recovered in this study

PCR data for nuc (2.4a), mecA (2.4b) and PVL gene (2.4c) obtained from MSSA and MRSA isolates from members of the community were analysed using the absolute quantification method. Real-time PCR crossing point (Cp) data points were obtained and analysed using the Roche LightCycler 2.0 Instrument software.

a.

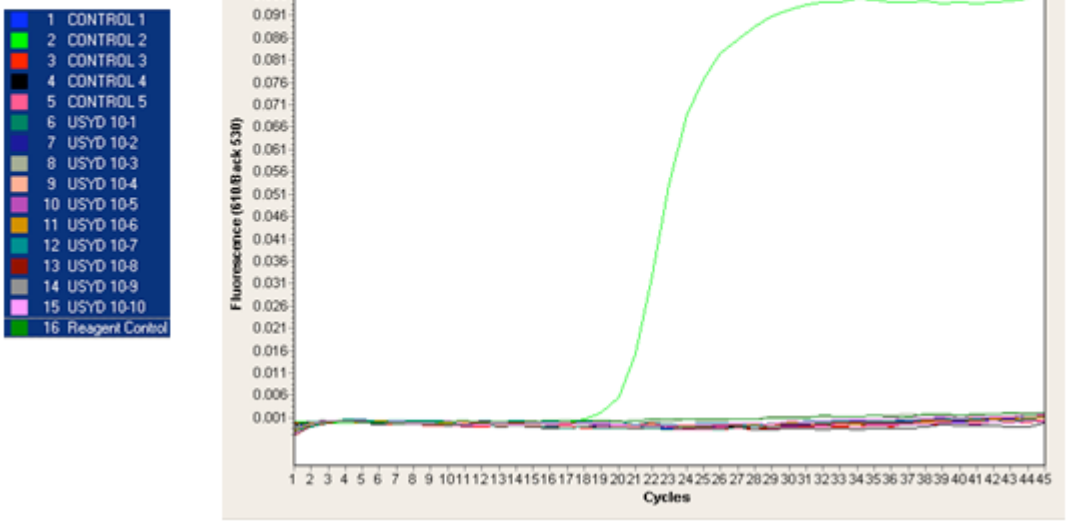


b.



Absolute quantification

LC610 - PVL



Appendix E3. Gel image of PFGE and representative table of PFGE results

Table 2.3 PFGE results and corresponding participants

Lane	Participant	Cohort	MRSA/MSSA type
1	5t	Community	ST188-MSSA
2	7n	Community	ST81-MSSA
3	8na	Community	ST81-MSSA
4	Control	*Staphylococcus aureus* NCTC8325	*S. aureus*
5	8nb	Community	ST22-MSSA
6	9n	Community	ST22-MSSA
7	1n	Community	WA-MRSA-23
8	2n	Community	WA-MRSA-23
9	3n	Community	Non typeable

MRCN - meticillin resistant coagulase negative *Staphylococcus*

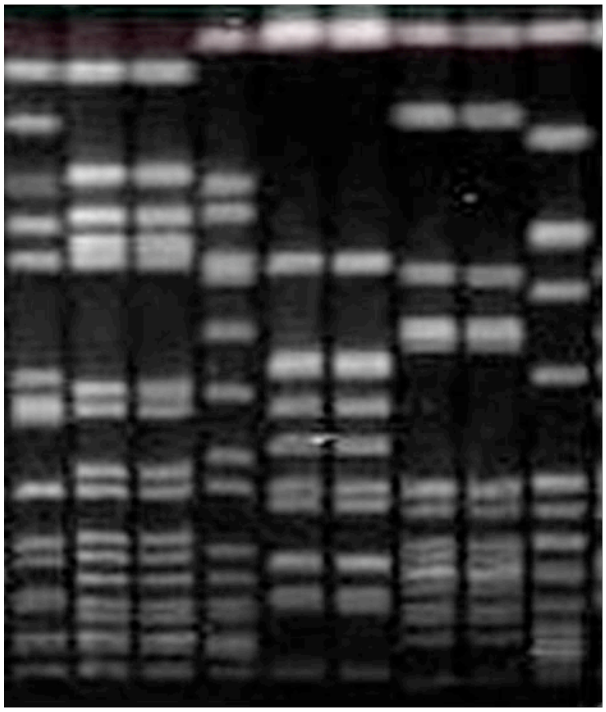
MSCN - meticillin sensitive coagulase negative *Staphylococcus*

MSSA - meticillin sensitive *Staphylococcus aureus*

MRSA - meticillin resistant *Staphylococcus aureus*

n- isolated from nose t – isolated from throat

na/nb – two different isolates recovered from nose of same participant



1 2 3 4 5 6 7 8 9

Figure 2.5 Gel image of macro-restriction PFGE patterns of MRSA and MSSA isolates recovered from community participants

MRSA isolates run on 1.0% agarose gel following macrorestriction with SmaI enzyme. Chromosomal patterns were visually examined and scanned with a Quantity One device.

Appendix E5. Dendogram of PFGE patterns

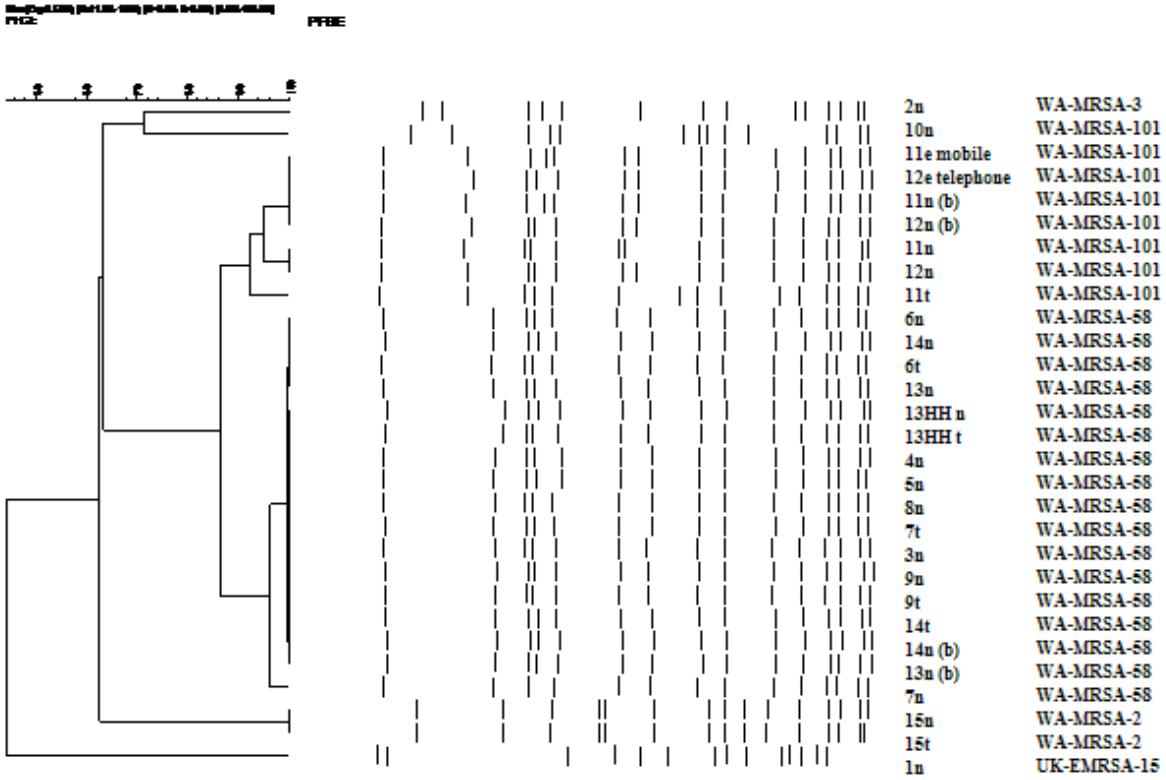


Figure 2.6 Representative Dendrogram of caMRSA PFGE patterns.

PFGE patterns of MRSA isolates recovered from the veterinary cohort (Chapter 6) were compared with a database of fully characterised Australian MRSA and MSSA isolates (MLST, SCCmec for MRSA, DNA microarray for MRSA and spa typing). FPQuest software was used to digitally analyse the electrophoresis patterns of these MRSA isolates. CHEF patterns were interpreted according to the criteria of Tenover et al (1996). Patterns 100% related by dendrogram to another subtype were assigned the same name. PFGE patterns of isolates tested were characterised to be WA-MRSA-2, WA-MRSA-3, WA-MRSA-58 and WA-MRSA-101 caMRSA strains and haMRSA strain UK-EMRSA-15.

Appendix E6. Image of representative DNA microarray chip

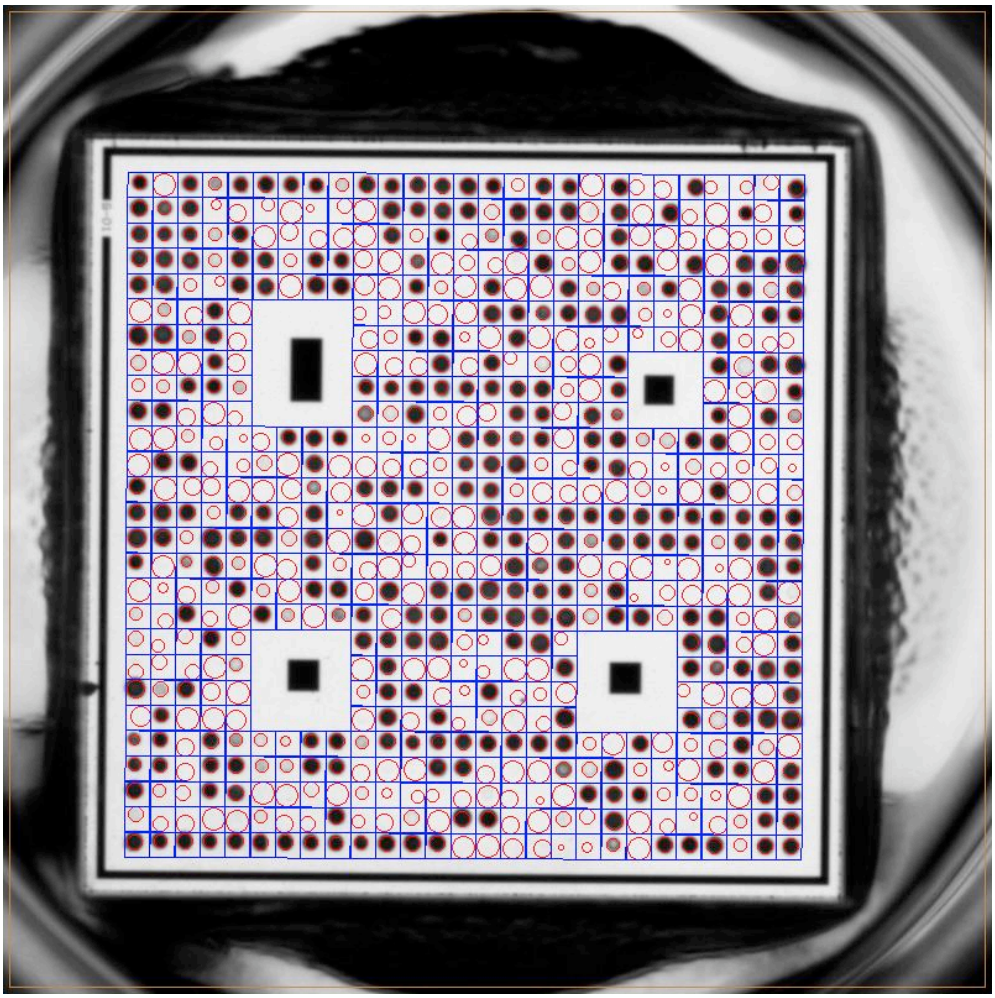


Figure 2.7 Representative DNA microarray chip image of MRSA.

A representative image of the DNA microarray array chip (StaphyType system by Alere Technologies GmbH, Jena, Germany) used in this study was captured for isolate 14n in the veterinary cohort (Chapter 6). This image was interpreted using the CLONDIAG® reader and software system (CLONDIAG® Chip Technologies GmbH, Jena, Germany). Isolate 14n was typed as CC8-MRSA-IV (USA500).

Legend key of DNA hybridisation:

Gene expressed (positive hybridisation)

Gene expressed ambiguously (ambiguous hybridisation)

Gene not expressed (negative hybridisation)