Epidemiology and molecular characterisation of methicillin resistant *Staphylococcus aureus* in the Australian pig industry

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I  

**Shafi ullah Sahibzada**

Hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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* Subject to confidentiality provisions as approved by the University
Author’s contributions to research and publications

Articles and abstracts published in journals and conference proceedings from this thesis:


**Shafiullah, S., Hernandez-Jover, M., Jordan, D. & Heller, J. Prevalence and risk factors associated with MRSA carriage in humans working at a pig farm OHEH, 2016 Melbourne, Australia.**


* Please note that in some publications my first name (Shafiullah) appeared as surname due to miss documentation that was corrected in 2017
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## Abbreviations

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<th>Description</th>
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<tr>
<td>ABARES</td>
<td>Australian Bureau of Agricultural and Resource Economics and Sciences</td>
</tr>
<tr>
<td>AIAO</td>
<td>All-in-all-out farming system</td>
</tr>
<tr>
<td>ABS</td>
<td>Australian Bureau of Statistics</td>
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<tr>
<td>ACME</td>
<td>Arginine catabolic mobile element</td>
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<td>ACSQHC</td>
<td>Australian Commission on Safety and Quality in Health Care</td>
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<tr>
<td>AGAR</td>
<td>Australian Group on Antimicrobial Resistance</td>
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<tr>
<td>AMC</td>
<td>Amoxycillin/clavulanic acid</td>
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<td>APL</td>
<td>Australian pork limited</td>
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<tr>
<td>CA-MRSA</td>
<td>Community-associated methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>CAZ</td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>CC</td>
<td>Clonal complex</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
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<tr>
<td>CEF</td>
<td>Cephalothin</td>
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<tr>
<td>CEP</td>
<td>Cefepime</td>
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<tr>
<td>CER</td>
<td>Ceftiofur</td>
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<tr>
<td>CHL</td>
<td>Chloramphenicol</td>
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<tr>
<td>CI</td>
<td>95% Confidence interval</td>
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<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
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<tr>
<td>CLI</td>
<td>Clindamycin</td>
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<tr>
<td>DANMAP</td>
<td>Danish Integrated Antimicrobial Resistance Monitoring and Research Programme</td>
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<tr>
<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<td>ENR</td>
<td>Enrofloxacin</td>
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<td>ERY</td>
<td>Erythromycin</td>
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<tr>
<td>FOX</td>
<td>Cefoxitin</td>
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<tr>
<td>FUS</td>
<td>Fusidic acid</td>
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<tr>
<td>GEN</td>
<td>Gentamicin</td>
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<tr>
<td>HACO-MRSA</td>
<td>Healthcare-associated community-onset</td>
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<tr>
<td>HA-MRSA</td>
<td>Hospital-associated methicillin resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>IEC</td>
<td>Human immune evasion gene cluster</td>
</tr>
<tr>
<td>LA-MRSA</td>
<td>Livestock-associated methicillin resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>LNZ</td>
<td>Linezolid</td>
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<tr>
<td>MALDI-TOF MS</td>
<td>Matrix-assisted laser desorption ionization-time of flight mass spectrometry</td>
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Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands

MDR  Multi-drug resistance
MLST  Multi-locus sequence typing
MRSA  Methicillin resistant *Staphylococcus aureus*
MUP   Mupirocin
MXF   Moxifloxacin
NAL   Nalidixic acid
NEO   Neomycin
NIT   Nitrofurantoin
NOV   Novobiocin
NSW   New South Wales
OR    Odds ratio
PEN   Penicillin
PFGE  Pulsed-field gel electrophoresis
PPE   Personal protective equipment
PVL   Panton-valentine leukocidin
QAC   Quaternary ammonium compounds
QD    Quinupristin-dalfopristin
RIF   Rifampin
SCCmec Staphylococcal cassette chromosome *mec*
SNP   Single nucleotide polymorphism
spa   *Staphylococcus aureus* protein A
SSTIs Skin and soft tissue infections
ST    Sequence type
SXT   Trimethoprim /Sulfamethoxazole
TEI   Teicoplanin
TEL   Telithromycin
TET   Tetracycline
USA   The United States of America
VAN   Vancomycin
WA    Western Australia
WGS   Whole genome sequence
WHO   World Health Organisation
Thesis abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen of humans and some livestock that causes skin and soft tissue infections with potential to progress to fatal bacteraemia. MRSA poses a potent threat to human and animal health because of its resistance to many common antibiotics. Pigs are a notable reservoir for MRSA due to their asymptomatic carriage of particular sequence types and a demonstrated capacity to transfer these to humans involved in pig farms. In the last decade, MRSA has emerged as a challenge in many pig-producing regions, especially across Europe and North America, and is traditionally known as livestock-associated MRSA (LA-MRSA). In Australia, LA-MRSA isolates appear to represent a smaller proportion of invasive *S.aureus* isolates obtained from humans than observed in Europe and this may explain why epidemiological aspects of LA-MRSA in the Australian pig industry have not been well described. However, in 2014, MRSA was implicated as the cause of an ongoing outbreak of staphylococcal disease amongst workers at a pig farm in New South Wales.

The overall goal of this research was to advance the understanding of MRSA in pigs in Australia. The first objective of this study was to explore a unique outbreak of MRSA in piggery workers and to elucidate the potential role of the pigs in this outbreak, including identification of the potential risk factors for MRSA carriage associated with the occupational activity. The second objective of this study was to determine the prevalence, distribution, and antibiotic susceptibility of MRSA in Australian pig herds.

The outbreak investigation revealed occurrence of bidirectional transmission of MRSA between pigs and humans. A high prevalence of asymptomatic carriage was found in pigs (75.2%; 95% CI 71.8-78.6) and humans (59.6% CI 46.1-71.8) with two distinct MRSA lineages present. One comprised ST93 - regarded as a highly-virulent form of community-associated (CA) MRSA in humans in Australia. The second was ST398 - a pig-adapted strain of LA-MRSA that is thought to have a global distribution. The odds of MRSA carriage for persons working in direct contact with pigs was 24 times that for persons with no pig contact and a dose-response relationship was found between carriage of workers and the number of hours of exposure to pigs. Moreover, this association was observed both for the LA-MRSA ST398 and the human-derived CA-MRSA ST93, therefore, reporting for the first time ST93 as a potential occupational-risk for piggery workers. In a subsequent national survey of commercial pig herds, MRSA was found on 54% (CI 35.5-71.2) of the 26 study farms, with pig-level prevalence ranging from 1.6% to 100% within infected farms. All MRSA collected in the national herd survey were identified as ST398. No ST93 or any other CA-MRSA were detected on any other farm. This study also indicates that MRSA carriage and infection in piggery workers could potentially
cause a significant economic impact to the Australian pig industry, and the emergence of new MRSA strains like ST93 amongst piggery workers could pose a major economic challenge. The outcomes of these studies indicate that in Australia, and probably elsewhere, greater scrutiny of staphylococcal infections in humans and animals is warranted, in the form of coordinated investigation of outbreaks to identify the factors predisposing each host species to infection and emergence of virulent pathogens with novel resistance traits. The risk of MRSA carriage is greatest when working with direct pig contact; therefore, emphasis is required on personal protective equipment. Findings and recommendations outlined in this thesis could be used to develop relevant strategies and interventions to limit the MRSA spread among pigs and piggery workers.
Chapter 1.

Introduction
1.1 Background information

*Staphylococcus aureus* is a commensal bacterium which causes opportunistic infections, in humans and in some animal species. *S. aureus* strains that are resistant to the antibiotic cefoxitin or oxacillin (used as a surrogate for methicillin) are known as methicillin resistant *S. aureus* (MRSA) (CLSI, 2014). In humans, MRSA often causes skin infections; however, it also has potential to cause bacteraemia and other systemic infections (Coombs et al., 2013a, Dantes et al., 2013). Antimicrobial resistance exacerbates the burden of disease attributable to *S. aureus* through longer hospitalisations and increased morbidity and mortality due to limited antibiotic options (Filice et al., 2010, Uematsu et al., 2016). MRSA has been reported across the world with varying proportions of staphylococcal morbidity in humans (WHO, 2014). In Europe, the percentages of MRSA amongst clinical *S. aureus* isolates collected from humans in 30 countries in 2015, ranged from zero percent in Iceland to 57% in Romania (ECDC, 2017). In the USA *S. aureus* is reported as the most common pathogen causing hospital-associated infections in humans, with 43-58% of these recorded as MRSA (Sievert et al., 2013). In Australia, 19% of clinical *S. aureus* isolates from humans are reported as MRSA (Coombs et al., 2016).

Traditionally, MRSA isolates have been divided into three major groups based on molecular characteristics and the ecological setting from which they originate, being labelled as either: hospital-associated (HA-MRSA), community-associated (CA-MRSA) or livestock-associated (LA-MRSA). HA-MRSA usually affects people who have had exposure to health-care environments whereas CA-MRSA is reported in people with no links to a hospital or recent history of hospitalisation (CDC, 2015). MRSA is also classified as community-associated when the culture has been obtained during an outpatient visit or within 48 hours of hospital admission, but the patient has no hospital associated risk factors, such as surgery, haemodialysis or other history of hospitalisation (Lee et al., 2013, Uhlemann et al., 2014). LA-MRSA normally circulates amongst livestock species and people working with livestock (EFSA, 2012). There are numerous strains associated with the three categories of MRSA, and some strains are predominantly associated with one type of MRSA. However, the three types of MRSA based on ecological origin are somewhat fluid, as exchange of clones between different ecological settings has been reported.

Many parts of Europe are recognised for having a lower proportion of HA-MRSA and CA-MRSA amongst human *S. aureus* isolates compared to the rest of the world, including Australia (Albrich and Harbarth, 2008, Stefani et al., 2012, Sowash and Uhlemann, 2014, Uhlemann et al., 2014). In the last decade LA-MRSA has emerged as a challenge in some domestic animals such as horses (Guérin et al., 2017), cattle (Graveland et al., 2012), and pigs (Reynaga et al., 2016). Pigs are the most commonly reported amongst livestock for LA-MRSA carriage that could transfer MRSA to people in direct
Therefore, LA-MRSA is reported as an occupational health-hazard in many countries, especially those where pig production is common. A prevalence of LA-MRSA carriage as high as 86% has been reported in pigs in the Netherlands (Morcillo et al., 2015), and a similar proportion of carriage has also been reported in people working in direct pig contact in Germany (Cuny et al., 2009).

It is well established that in Australia, a greater proportion of S. aureus infections in humans are either CA- or HA-MRSA, compared to other pig-producing countries in Europe (Coombs et al., 2016, ACSQHC, 2017). While LA-MRSA has been found in pigs and reported as an occupational health issue for piggery workers across the globe, especially in Europe, the epidemiology and distribution of MRSA has not been investigated in the Australian pig industry. In Australia, only one study isolated LA-MRSA from a pig production environment, this being from three pigs on a single farm (Groves et al., 2014). A second study isolated LA-MRSA on a single occasion from a veterinarian servicing the pig industry (Groves et al., 2016). Thus, while it is known that LA-MRSA is present in Australia, the extent of the impact this will have on public health and pork industry is unclear.

Despite the rare isolation of LA-MRSA in Australia, in 2014, the School of Animal and Veterinary Sciences at Charles Sturt University was contacted in relation to a pig farm that had been identified by the New South Wales Department of Health for having a recurrent outbreak of MRSA in workers over the preceding three-year period, requesting further investigation.

1.2 General aims and objectives

The overall goal of this research was to advance the understanding of MRSA in pigs in Australia. The first objective of this study was to explore a unique outbreak of MRSA in piggery workers and to elucidate the potential role of the pigs in this outbreak, including identification of the risk factors for MRSA carriage associated with the occupational activity, and its potential economic impact on the pig industry. The second objective of this study was to improve the broader understanding of MRSA in pigs in Australia by describing the prevalence of carriage, distribution, and antibiotic susceptibility of isolates present in a national survey.

1.3 Thesis structure

The overall goal and research aims of this thesis are addressed across multiple experimental chapters (Chapters 3 to 7). Each chapter contains a custom set of aims, which each contributes to the goal and are briefly explained in the following paragraphs. The first objective of this thesis corresponds to the experimental chapters three, four, and five. The second objective of the thesis is addressed in chapter six and seven. All the experimental chapters of this thesis follow a thesis by publication format, and
are presented in the format that they have been or will be submitted for publication. Chapter 3 has been published and Chapter 5 has been submitted for publication. The other experimental chapters are in the format to allow them to be submitted for publication. In order to produce a thesis with consistency in presentation, the entire document is referenced in accordance with the Harvard style referencing, and the bibliography for all chapters has been placed at the end of the thesis. A conclusions chapter reflects on the findings of each of the chapters which present results as to how they have met the objectives of the thesis as a whole.

1.4 Introduction to the thesis chapters

Chapter 2: Review of the literature

Chapter 2 provides a revision of the literature regarding MRSA in general and LA-MRSA ST398 in particular.

Chapter 3: Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia

The third chapter focuses on the detection of MRSA in pigs and people working on a piggery that had been identified to have an ongoing MRSA outbreak affecting humans. This chapter reports the prevalence and molecular characteristics of all MRSA isolates collected in humans and a subset of MRSA isolated in pigs and the environment. Genotypic and phylogenetic analyses are also reported. This chapter has already been published as follows:


Chapter 4: Prevalence and antibiotic resistance of MRSA across different pig age groups in an intensive pig production system in Australia

Chapter four reports the prevalence and antibiotic resistance phenotype of MRSA amongst pigs of different production stages within the piggery described in Chapter 3. This study follows on from the results reported in Chapter 3 and describes the typing and antibiotic susceptibility of the entire set of MRSA isolates collected in pigs and environment on the piggery associated with the ongoing MRSA outbreak. Parts of this chapter have been published in conference proceedings, as follows:

Chapter 5: Emergence of highly prevalent CA-MRSA ST93 as an occupational risk in people working on a pig farm in Australia

Chapter 5 investigates the risk factors associated with MRSA carriage (overall and strain-specific) amongst staff members working on the outbreak farm. Potential risk factors associated with MRSA carriage in workers, such as pig exposure, type of role and occupational activities, health factors, and participants perceptions were explored. Parts of this study has been presented at international conferences and have been published in proceedings, as follows:


Chapter 6: MRSA prevalence amongst commercial pig herds in Australia

Chapter 6 presents the prevalence and distribution of MRSA in a sample of commercial piggeries in Australia. This study also reports the results of an associated questionnaire study regarding antibiotics use, information on farm characteristics, farm hygiene and biosecurity practices on the sample farms.

Chapter 7: Assessment of the potential economic impact of MRSA on the Australian pig industry

Chapter 7 simulates the potential economic impact of MRSA carriage among people working on commercial piggeries in Australia, depending on the type of circulating MRSA. A Monte Carlo simulation approach was used to assess different situational scenarios depending on MRSA strain carriage in piggery workers. Results of this study have also been presented at a conference.

Chapter 8: General Discussion

The major findings of this thesis are summarised and discussed in chapter 8.
Chapter 2.

Review of the literature
2.1 Antimicrobial resistance

Antimicrobial resistance (AMR) is the ability of a microorganism such as bacteria to resist the effects of drugs or others microbial toxins and may be intrinsic (natural) or acquired. Intrinsic resistance existed even before the use of antimicrobials in therapeutics (D’Costa et al., 2011), whereas acquired resistance is the capacity of a microorganism or pathogen to survive exposure to a defined concentration of an antimicrobial substance to which it was originally sensitive (EFSA, 2009b, CLSI, 2007). Antimicrobial resistance is an increasing global issue which threatens to result in a post-antibiotic era, in which common infections and minor injuries that are currently treatable with antibiotics can once again cause deaths (WHO, 2012). The development of resistance in bacteria is a complex mechanism. While little is known about the process that triggers AMR in microbes, antimicrobial usage is reported as the most important factor for development of resistance in bacteria common in humans as well as in animals (Davies and Davies, 2010, Catry et al., 2014, Broens et al., 2011b). The imprudent use of antibiotics results in excessive selective pressure for the survival of bacteria that can resist the antimicrobials and thereby increases the spread of resistant bacteria. Once a microbe has developed resistance to one or multiple drugs it may persist even in the absence of further selection pressure and has the potential to spread to other microbes through processes such as transduction (through transfer of bacteriophage), transformation (acquiring DNA from the surrounding environment), and conjugation (transmission of DNA from donor to recipient) (Davies and Davies, 2010). AMR is observed in most of the commonly reported bacterial pathogens, and Staphylococcus aureus is one of the most frequently isolated bacteria amongst all reported pathogenic infections in humans that has greater ability to acquire resistance to different antimicrobials (Colomb-Cotinat et al., 2016, ECDC, 2009).

2.2 Staphylococcus aureus and MRSA

Staphylococcus aureus, commonly known as staph or “golden staph”, is a commensal bacterium of skin and mucus membranes. This bacterium has been isolated from multiple body sites including axilla, groin, inguinal region, throat, and nasal passage. Nasal carriage is reported as the most frequent site for S.aureus carriage (Wertheim et al., 2005). One-third of the healthy population is reported to carry S.aureus asymptomatically (Graham et al., 2006, Gorwitz et al., 2008) with no apparent ill effects (EFSA, 2009d). S.aureus is an opportunistic bacterium that may cause skin and soft tissue infections (SSTIs); however, infection with this pathogen may also result in systemic infections and bacteraemia (Coombs et al., 2013a).

S.aureus develops resistance through mutation or simply acquiring resistance genes from other bacteria. Antibiotics resistance to the betalactam group of antibiotics (i.e. penicillins, cephalosporins,
carbapenems) is the most commonly observed resistance in *S. aureus* and this results from the acquisition of the *mecA* gene which encodes for an alternative penicillin-binding protein (PBP2a) (EFSA, 2009a). The *mecA* gene is carried on a large mobile genetic element, the staphylococcal cassette chromosome *mec* (SCCmeC), and *S. aureus* bacteria that carry this gene are known as methicillin resistant *S. aureus* (MRSA). Although methicillin is not used in therapy anymore, the term methicillin-resistant continues to be used to describe *S. aureus* that is resistant to betalactams. These days, cefoxitin or oxacillin are used as surrogate markers for the detection of methicillin resistance; however, cefoxitin is preferred over oxacillin in disk diffusion methods due to its high sensitivity for methicillin resistance detection (CLSI, 2014, Farahani et al., 2013). *S. aureus* that is methicillin susceptible is known as methicillin susceptible *S. aureus* (MSSA) although these bacteria can be resistant to other antimicrobials (Chao et al., 2013). MRSA has the ability to acquire resistance to virtually any antibiotics that it is exposed to. Resistance is often carried on plasmids which are spread through horizontal gene transmission; however, chromosomal mutations also contribute to the resistance to some antibiotics (Kim et al., 2013, Foster, 2017). Resistance in MRSA is usually associated with the origin of isolates, for example, MRSA isolates collected in hospitals are frequently resistant to antibiotics commonly used in these institutions, such as fluoroquinolones (Lindsay, 2013, Couderc et al., 2014). Similarly, isolates collected from livestock show a high prevalence of resistance (up to 100%) to tetracycline, which is frequently used in this sector (Conceição et al., 2017, Alt et al., 2011). Resistance to linezolid and vancomycin, used as last resorts against MRSA in human medicine, has also been reported in different parts of the world although they remain at a low prevalence (ECDC, 2017).

Nasal MRSA carriage in humans is known to be a risk factor for the development of subsequent clinical infection (Corne et al., 2005, Rahimian et al., 2007, Fritz et al., 2009). One study that screened patients for MRSA on admission to a trauma intensive care unit found a significantly (*P* < 0.001) higher proportion of subsequent infections in patients with MRSA carriage (33.3%) compared to those with no carriage (6.6%) (Croft et al., 2009). Other studies have reported higher rates of MRSA infections in children who carried MRSA compared to those who did not (Huang et al., 2006, Fritz et al., 2009). Similarly, a meta-analysis investigating *S. aureus* carriage as a risk factor for skin and systemic infections in the community found greater odds of having the disease (OR 7.06, 95%CI, 4.60–10.84) among people positive for MRSA carriage compared to MSSA carriage (Kim et al., 2018). However, a number of factors have been reported to be associated with subsequent infection in a person who is positive for nasal MRSA carriage, and these depend on the predisposing factors of a person and the MRSA strain involved (David and Daum, 2010).
While there is much literature that presents risk factors for carriage and colonisation with MRSA, there is a confusion within some of those papers about the terms colonisation and carriage, with these terms used interchangeably on occasions (Monaco et al., 2013, Goerge et al., 2017). However, these terms serve a different purpose. The term colonisation refers to when MRSA adhere asymptotically to epithelial cells like mucosal membranes by establishing a balance with the host immune system, can multiply within the host without resulting in symptoms of disease and the presence is confirmed over time (Ryu et al., 2014, Sollid et al., 2014). Similar to colonisation, carriage is identified when a host carries MRSA asymptotically in the nose, on the skin or in the throat (EFSA, 2009d), however, it has only been isolated on a single occasion. In point prevalence studies, mostly a single swab is used to determine the presence of bacteria on the host. The term carriage does not imply whether bacterial presence is due to transient contamination of the host sites during short exposure to an MRSA positive source that may suddenly disappear, or due to long-term colonisation. As an example, Angen et al. (2017) investigated MRSA carriage among 34 volunteers who were tested for MRSA carriage before visiting an MRSA-positive piggery, and they all were negative. After exposure to pigs for an hour, 94% of the participants were positive for nasal MRSA carriage on leaving the farm; however all except one cultured negative in subsequent sampling, collected 2 hours after leaving the farm, and the single positive person was confirmed to have a negative culture two weeks after leaving the farm. This result suggests that short-term exposure often leads to nasal contamination. Carriage can be persistent or intermittent. Persistent carriage can be defined as the isolation of bacteria from a series of samples collected from a single person at different longitudinal time points; whereas, intermittent carriers are those identified as positive on at least one occasion over a series of sampling events (van Cleef et al., 2014). Confusingly, some cross-sectional studies use the term colonisation when they are actually reporting carriage. In contrast to carriage and colonisation, infection is the condition when MRSA evade of the host immune system and leads to disease symptoms (Chan et al., 2017), such as boils, septicaemia and toxic shock syndrome.

MRSA does not generally differ in virulence to MSSA, although infections caused by MRSA are more difficult to treat due to fewer options for antibiotics. However, some strains are reported to have increased virulence and pathogenesis (Chua et al., 2011b). In 2013, the Centre for Disease Control and Prevention (CDC) categorised drug-resistant threats based on the level of concern as either ‘urgent’, ‘serious’, and ‘concerning’, with MRSA being considered as a serious threat (CDC, 2013). The major concern with respect to MRSA is the increasing frequency of multi-drug resistance (MDR) reported globally in humans as well as in animals (Ge et al., 2017, Wendlandt et al., 2015). The term MDR is used for bacteria that show resistance to at least three different classes of antibiotics (Wendlandt et al., 2015). The prevalence of nasal carriage with MRSA in the general population is
reported to be 1.5% (Gorwitz et al., 2008). MRSA carriage and subsequent infection have been challenging issues for healthcare environments. Studies have observed the length of hospital stays in patients infected with MRSA to range from 10-90 days with a median of 18 days in Germany (Wernitz et al., 2005), 50 days in The Netherlands (Nulens et al., 2008), 21 days in Japan (Uematsu et al., 2016), 24 in Canada (Papia et al., 2015), and 17 days in the USA (Tejiram et al., 2017). In Australia, 29 days has been reported as the mean length of stay for people with MRSA infections (The Expert Working Group, 2001). Different studies have estimated varied cost of hospitalisation associated with cases of MRSA infection. In Israel, costs were estimated to be equivalent to AU$33,848 (calculated using exchange rate identified in March 2018 (www.xe.com)) (Cosgrove et al., 2005), AU$14,536 in France (Chaix et al., 1999), AU$45,948 in Netherlands (Nulens et al., 2008), AU$12,175 in Germany (Wernitz et al., 2005), AU$4,279 in Spain (Gavaldà et al., 2016), AU$11,197 in Japan (Uematsu et al., 2016), AU$5,252 in Canada (Papia et al., 2015), AU$44,393 in the US (Filice et al., 2010), and AU$3,200 in Australia (The Expert Working Group, 2001).

2.3 MRSA typing

MRSA can be typed by several different genetic typing methods, including Multi-locus sequence typing (MLST), S. aureus protein A (spa) gene typing, SCCmec typing, and macro-restriction pulsed-field gel electrophoresis (PFGE) (EFSA, 2012). Molecular typing helps in the understanding of the epidemiology of MRSA aiding to study the molecular evolution of MRSA. Typing also help in defining and comparing local or regional epidemics and potential sources of MRSA spread. Each typing method has advantages and disadvantages. Usually, MRSA is classified as the sequence type (ST) ST-SCCmec type and add the corresponding clonal complex (CC) which is explained in the following paragraphs.

MLST is currently considered to be the gold standard in S. aureus molecular typing and is the preferred method for epidemiological studies because of harmonised protocols and nomenclature that allows high inter-laboratory reproducibility, and facilitates the comparison of a large number of STs globally (Kinross et al., 2017, Williamson et al., 2015). MLST classifies S. aureus on the basis of nucleotide allelic variation in the seven housekeeping genes, and identical isolates are assigned a ST (http://saureus.mlst.net). Closely related STs are grouped in a CC (Enright et al., 2000) such as CC398. Although MLST is a widely used method, it may not be useful to investigate local outbreaks due to a lack of discriminatory power within the strains.

Spa typing is another powerful technique used in epidemiological investigations. This typing is based on sequencing a single gene, S. aureus protein A gene, which is a highly polymorphic X-region containing different short tandem repeats whose combination results in different spa types (Harmsen
et al., 2003). Different spa types can also be grouped within CCs. The method is supported by a central spa server (http://www.seqnet.org) which hosts thousands of spa types and allows to compare local MRSA epidemiology in relation to other regions and countries. Spa typing is thought to be a more useful tool for epidemiological investigation compared to MLST as it is more discriminator, for example, a single ST398 contains different spa types like t011, t034, t108, and t01254 (Ballhausen et al., 2014). However, CC cannot always be deduced from spa type results because the same spa type can belong to unrelated lineages (Cuny et al., 2010).

The SCCmec is another way to group MRSA that is based on the variation across different SCCmec elements (http://www.sccmec.org). The SCCmec cassette is a mobile genetic element consisting of the mec gene complex, the cassette chromosome recombinase (ccr) gene complex (i.e. ccrA, ccrB or ccrC), and the junkyard regions (j-region) (Zhang et al., 2005). Based on the variability and combinations of these genetic elements, molecular typing of MRSA strains has revealed up to eleven distinct SCCmec types (I–XI) (Liu et al., 2016) which differ from each other in antibiotic resistance markers. The SCCmec typing is of minimal epidemiological importance as they are unable to detect a single-nucleotide polymorphism that helps to distinguish the major type of MRSA, for example, LA-MRSA vs CA-MRSA. Therefore, SCCmec typing is usually used in conjunction with MLST such as ST398-V, ST93-IV.

PFGE is a typing technique used for the separation of large DNA molecules using rare-cutting restriction enzymes, such as SmaI and Cfr9I, that produce different banding patterns on agarose gel in the presence of electric field with alternative voltage gradient (Bosch et al., 2010). PFGE can be a useful tool in a local outbreak investigation because of high dissimilatory power (Argudín et al., 2010); however, it has limited use in epidemiological studies. As protocols and nomenclature are not harmonised for PFGE, it makes it difficult to compare inter-laboratory results (EFSA, 2012). Although PFGE typing is still used in the USA to describe the major MRSA clones (e.g. USA100, USA300); spa and MLST typing are now the most commonly used methods in Europe and other parts of the world (EFSA, 2012, Kinross et al., 2017).

Whole genome sequencing (WGS) is a technique used for typing of bacteria including S. aureus. This technique is gaining popularity in epidemiological investigations. It is more rapid, accurate, and low cost compared to other typing techniques (Salipante et al., 2015). WGS elucidates the molecular epidemiology of strains, which provides the greatest resolution and can resolve ST and spa gene type and virulence genes associated with ‘human’ or ‘animal’ clades of ST398 for example.

MRSA can be classified into three major types based on genetic characteristics, and the setting that infections are associated with: hospital-associated (HA-MRSA), community-associated (CA-
MRSA), and livestock-associated (LA-MRSA). HA-MRSA was first reported in the early 1960s in a human hospitalised patient in the United Kingdom (Jevons, 1961) and initially there was a limited concern because of the relatively low incidence of disease. MRSA from this setting was later termed as HA-MRSA. Before the 1990s MRSA was principally considered to be a nosocomial pathogen; however, later on, a new lineage of MRSA emerged in people with no prior hospital association, and this was termed CA-MRSA (Udo et al., 1993). Another major epidemiological shift occurred in mid-2000 when a new lineage of MRSA known as LA-MRSA was isolated from a pig farmer in France (Armand-Lefevre et al., 2005). Since then, LA-MRSA has been reported throughout the world in different livestock species (Cuny et al., 2010), and people with livestock contact (Deiters et al., 2015, Liu et al., 2015).

### 2.4 Difference between the three MRSA types

Commonly, MRSA is referred as HA-MRSA if it is isolated from a person working in a hospital or nursing home, or patients with 48 hours (72 hours in some studies) of hospital admission (Dantes et al., 2013, Heckel et al., 2017). In addition, HA-MRSA includes those isolates that are collected from a person with healthcare-associated risk factors including haemodialysis, surgery, and indwelling medical devices (Peters et al., 2017, CDC, 2015). Although SCCmec-IV has been reported in HA-MRSA, this lineage usually harbours SCCmec I, II, or III (Uhlemann et al., 2014). They are normally resistant to a large number of antibiotic classes and classified as MDR, (Lindsay, 2013). HA-MRSA is lacking Panton–Valentine leukocidin (PVL) which is composed of two components, the lukF-PV and lukS-PV, encoded for the toxin that causes tissue necrosis (Coombs et al., 2012, Shallcross et al., 2013). HA-MRSA is mainly comprised of five different CCs: CC5, CC8, CC22, CC30, and CC45 (Lindsay, 2013, Monaco et al., 2017). It is believed that major MRSA strains have emerged globally from the abovementioned clonal complexes (Robinson and Enright, 2003). A sub-category of HA-MRSA known as “healthcare-associated community-onset” (HACO-MRSA) is also being used to define cases that would be HA-MRSA infections by the history of health care exposure but have their onset in the community (CDC, 2014). HA-MRSA is more likely to affect people with predisposing factors such as elderly age (Coombs et al., 2013b), impaired immune systems, history of hospitalisation (Deiters et al., 2015), antibiotic use (Catry et al., 2014), and type of gender (i.e. male) (Peters et al., 2017).

CA-MRSA is defined as the sample obtained during an outpatient visit or within 48 hours of hospital admission, and the person should not belong to any nursing home or long-term care facility and have no recent history of hospitalisation (Lee et al., 2013, Uhlemann et al., 2014). Other criteria to define CA-MRSA are related to relevant isolate characteristics such as susceptibility pattern and presence
of specific SCCmec types and genes. CA-MRSA has been reported to be resistant to fewer antibiotic classes (Lim et al., 2016) compared to HA-MRSA. Moreover, they carry additional virulence factors such as PVL genes and the arginine catabolic mobile element (ACME) that provide a major selective advantage during skin infections and colonisation. These extra virulence factors enhance the ability of CA-MRSA to evade the host immune response, contributing to the evolutionary success of CA-MRSA (Coombs et al., 2011, Planet et al., 2013, Otto, 2013). The presence of PVL is typically associated with SSTIs but can also be present in MRSA that result in systemic infections and bacteraemia (Coombs et al., 2012, Shallcross et al., 2013). CA-MRSA harbors SCCmec IV or V, which are smaller and much more mobile than the SCCmec I–III present in HA-MRSA, therefore easily transferred between MRSA strains (Stryjewski and Corey, 2014, Coombs et al., 2011). CA-MRSA consists of CCs such as CC1, CC8, CC22, CC30, CC45, CC59, CC80, CC88 (Mediavilla et al., 2012, Monaco et al., 2017). Amongst these clonal complexes, CC8 and CC30 are considered as pandemic globally (Mediavilla et al., 2012). CA-MRSA is reported to infect healthy and young individuals unlike HA-MRSA that normally affects people with predisposing factors (mentioned earlier) (Agostino et al., 2017). However, certain community cohorts are reported to be at high risk of CA-MRSA carriage and infections such as indigenous people (Harch et al., 2017), prisoners (Mukherjee et al., 2014), players of contact sports (Jiménez-Truque et al., 2017), and children (Vaska, 2016).

LA-MRSA was formerly known as non-typeable (NT-MRSA) using PFGE, as the restriction enzyme SmaI could not digest the DNA of this lineage (Voss et al., 2005) and subsequently typed as ST398. LA-MRSA usually carry SCCmec type IV or V and often exhibit co-resistance to other non-betalactams that have commonly been used in animal production, such as tetracyclines (Mutters et al., 2016). Although it has been reported that LA-MRSA carry more resistance genes than human strains, it also carries fewer virulence genes (Mutters et al., 2016) such as PVL, tst1(toxic shock syndrome toxin-1) and other enterotoxin genes (Ballhausen et al., 2017). Therefore, LA-MRSA appears to be less likely to cause diseases in humans compared to CA- and HA-MRSA (Grundmann et al., 2010). LA-MRSA consists of clonal complexes such as CC398, CC5, CC9, CC30, CC97 (Butaye et al., 2016, Crombé et al., 2013).

Caution is required in use of the above epidemiological distinctions, as MRSA lineages are in continuous evolution, and exchange and spreading of clones and lineages between different settings have been reported. In assertion to this, clones with a typical hospital-acquired genetic background are being reported within the community (Bygott et al., 2008, Jeong et al., 2007). Similarly, typical clones with community-acquired genetic background are circulating in health care facilities (Nimmo et al., 2013). Also, LA-MRSA has been isolated from persons with no livestock contact (Larsen et
Therefore, the distinction between different types of MRSA requires more than one criteria including the setting MRSA is found in, predisposing factors and molecular typing.

2.5 Global epidemiology of different MRSA strains

MRSA represents a global issue and has been isolated in almost every country (Monaco et al., 2017). The number of infections due to MRSA is reported to be the highest among all antimicrobial-resistant bacteria (CDC, 2013). Different nomenclature is used in different countries which makes it difficult for international comparison of MRSA strains. For example, PFGE typing is used in the United States; whereas MLST typing (STs and CCs), is commonly used in Europe and other parts of the world. In some countries, MRSA is also described by the geographical area where the clones were first recovered such as the ‘Queensland clone’ which was first reported in south-east Queensland in Australia (Munckhof et al., 2003). However, as mentioned earlier the local nomenclature is being replaced by the most universally used typing such as STs, CCs, and spa (Monaco et al., 2017). MRSA is endemic worldwide with varying prevalence across the world, and several well-characterised strains predominate in different regions which have been described in the following sections.

2.5.1 HA-MRSA

A wide range of prevalence is reported for carriage of HA-MRSA in hospital staff and patients. Although epidemiological data from separate studies are often not comparable due to differences in study design and populations sampled, a trend in the proportion of MRSA amongst S. aureus isolates can be seen in the literature. Apparently, Europe has a lower proportion of HA-MRSA amongst S. aureus isolates collected in humans compared to the rest of the world (Albrich and Harbarth, 2008, Stefani et al., 2012). In line with this, a review article by Albrich and Harbarth (2008), which reported on 127 studies of carriage and infection of MRSA amongst health-care workers, reported an overall MRSA carriage rate of 4.6% among 33,318 screened health-care workers, with different prevalence ranges for different countries. For example, America had an MRSA prevalence ranging from 4.2% in North America to 6.5% in South America, while for Europe 1.6% (East Europe) to 5.3% (North Europe). Prevalence up to 15% was reported for African and Asian countries. Australia and New Zealand were identified to have 9.7% prevalence. Another systematic review also reported a lower MRSA carriage among healthcare workers in Europe (1.8%-4.4%) compared to the United States 6.6% (Dulon et al., 2014).

A few distinct MRSA strains are commonly associated with HA-MRSA globally such as ST36, ST22, ST5, and ST239. In Europe, the two most successful HA-MRSA strains reported in hospitals over the past 20 years are ST36 which belongs to CC30, also known as EMRSA-16 or USA200, and ST22,
also known as EMRSA-15 (Uhlemann et al., 2014). ST22 is currently the predominant strain in Europe (Monaco et al., 2017). ST5 (New York/Japan clone or USA100) is another major HA-MRSA strain, isolated from various parts of the world including America and Europe (Bal et al., 2016). A recent survey in the United States that investigated clinical S.aureus isolates collected from different medical centres found ST5 as the most isolated HA-MRSA, representing 18% of the total MRSA (Diekema et al., 2014). In Asia, ST239 is the predominant HA-MRSA strain and this strain is ranked second in Australia after ST22 (Coombs et al., 2016, Chen et al., 2009, Shang et al., 2016).

2.5.2 CA-MRSA

CA-MRSA is reported worldwide, and a global increase in the proportion of CA-MRSA amongst S.aureus isolates has been noticed in recent years (Sowash and Uhlemann, 2014, Uhlemann et al., 2014). In the USA, CA-MRSA is endemic in nature, and reported to represent up to 54% of the total S.aureus and up to 80% of the total MRSA isolates (Diekema et al., 2014, Dukic et al., 2013). Although the USA is recognised for a higher CA-MRSA prevalence compared to Europe and Asia (Li et al., 2014), there is marked heterogeneity between studies and limited epidemiological study being done in some parts of the world such as Africa, Asia, and the Middle East. Therefore, a direct comparison between countries for CA-MRSA prevalence is difficult.

The prevalence of CA-MRSA strains is geographically diversified. In the USA, ST8 (USA300) is the predominant strain of CA-MRSA which has also been reported in other countries in addition to the USA. (Glaser et al., 2016, Reyes et al., 2009, Alam et al., 2015, Tenover and Goering, 2009, Liu et al., 2008). Besides ST8, other strains such as ST1 (WA-1 or USA400) and ST30 have also been reported in the USA (Golding et al., 2011, Monaco et al., 2017). In Europe, CA-MRSA with ST80, so-called European strain, is a predominant lineage (Junie et al., 2018); other reported strains include ST8, ST59, and ST30 (Otter and French, 2010). CA-MRSA is apparently less prevalent in Europe than that reported in the USA (Otter and French, 2010). In Asia, ST59 (Taiwan clone or USA1000), ST30 (South West Pacific [SWP] clone or USA1100), and ST72 are the major CA-MRSA strains (Chen and Huang, 2014, Bal et al., 2016). In Australia, ST93 (Queensland clone) is the predominant CA-MRSA (Coombs et al., 2016) which is reported to be the most virulent strain among the most frequently reported CA- and HA-MRSA (ST8, ST1, and ST239) investigated in wax moth larvae and mouse skin infection models (Chua et al., 2011b). However, this strain has not spread on a large scale to other parts of the world though it has occasionally been isolated in other countries (Mediavilla et al., 2012).
2.5.3 LA-MRSA

LA-MRSA has been reported mainly in countries where livestock production is intensive. It is postulated that LA-MRSA originated as a human MSSA strain which transferred to pigs and evolved as a new lineage of MRSA by acquiring SCC\textit{mec} (Price et al., 2012). As already mentioned, ST398 belongs to LA-MRSA, is most frequently found in pigs and people working on pig farms, and thus is also referred to as porcine MRSA (Butaye et al., 2016, Crombé et al., 2013). In most European countries, ST398 is the dominant strain of LA-MRSA. In some countries (e.g. Denmark), ST398 represents the highest proportion (40%) of the total MRSA isolates collected in humans through screening and clinical samples (DANMAP, 2016). In the USA and Canada ST398 is the most commonly identified type of LA-MRSA, though other strains such as ST97 (Sato et al., 2015), ST30 (Lahuerta-Marí et al., 2016), and ST5 (Hau et al., 2015) have also been reported. Whereas in Asia, ST9 appears to be the predominant strain of LA-MRSA isolated from pigs and pig farmers (Fang et al., 2014, Ye et al., 2016). Prevalence of LA-MRSA carriage among pigs and pig workers found in different countries across the globe is presented in Table 2.1 and Table 2.2.

A decrease in the overall proportion of MRSA amongst \textit{S. aureus} isolates has been observed in Europe from 16\% in 2010 to 10\% in 2015 (Walter et al., 2017); however, an increase in LA-MRSA is reported in most European countries (Kinross et al., 2017). This increase may reflect an increase in MRSA screening at hospitals, which has become mandatory in some European hospitals (Kaïret et al., 2017, van Cleef et al., 2013) or it may be due to an improvement in diagnostic techniques for LA-MRSA that has been observed in the last few years (Williamson et al., 2015). There are also possibilities that this may be a real increase in the occurrence since intensive pig production is increasing in those countries and LA-MRSA is associated with intensive production enterprises (ESVAC, 2017). In contrast to Europe, the prevalence of LA-MRSA is apparently lower in North America. However, there is no surveillance system in those countries that could identify the overall trend in the LA-MRSA. Some studies in the USA suggest a decreasing trend in the prevalence. In line with this, a study in 2009 reported 49\% MRSA carriage among pigs swabbed at two different farms (Smith et al., 2009). However, subsequent studies in the USA investigating up to 45 piggeries found less than 20\% carriage in pigs (Smith et al., 2013, Frana et al., 2013). Moreover, a recent study that collected 20 nasal swabs per herd from 37 pig farms, including one MRSA positive control farm (previously identified for MRSA infection), found no MRSA positive herds other than the positive control (Sun et al., 2015).
2.6 Prevalence of LA-MRSA ST398 carriage and infection in humans

LA-MRSA carriage is frequently reported among at-risk groups, people with regular pig exposure, including veterinarians (Wulf et al., 2008b), pig transporters (van Cleef et al., 2010a), abattoir workers (Gilbert et al., 2012), and piggery workers (Dorado-Garcia et al., 2015). In the countries where ST398 is the most common LA-MRSA found in pigs, a prevalence of carriage in people with pig exposure as high as 86% has been reported (Cuny et al., 2009). Despite few studies reporting human infections in farmers due to ST398, it remains unclear whether nasal carriage of ST398 is a risk factor for subsequent infection. One study that collected nasal swabs from 281 pig farmers on 49 piggeries reported 34% persistent MRSA carriage on six sampling moments over a year (van Cleef et al., 2016). In the study 11% (23/198) of respondents reported infections (mostly skin infections) on questionnaires; however, no typing information was available for the strain involved in infections to identify whether ST398 was the cause of infection. Moreover, the study concluded that LA-MRSA carriage among healthy pig farmers have has limited effects on their health and wellbeing. As mentioned earlier, ST398 are less infectious and pathogenic compared to non-ST398, and consequently, this strain is infrequently reported to be associated with infections compared to CA- and HA-MRSA. In line with this, a survey conducted in 26 European countries to investigate invasive S.aureus infections in humans found only 0.4% of the 2890 isolates to be ST398, and all of these were MSSA (not MRSA), suggesting that this lineage rarely causes systemic infections (Grundmann et al., 2010). Similarly, in Germany, LA-MRSA is reported to represent less than 3% of the total MRSA isolated from nosocomial infections (Cuny et al., 2015b). Furthermore, ST398 has now been further classified as belonging to a human clade or animal clade by differences of a few SNPs and the presence/absence of certain genes, for example the human clade of ST398 usually carries human immune evasion genes (IEG), also known human immune evasion cluster (IEC), and is normally absent of tet(M) resistance genes (Stegger et al., 2013, Price et al., 2012, Cuny et al., 2015a). The IEC is carried on the prophage φ3 encoding proteins such as staphylokinase (sak), staphylococcal complement inhibitor (scn), and chemotaxis inhibitory protein (chp) that enhance the virulence and adherence ability of ST398 in humans. Besides IEG carriage, a human-adapted clade of ST398 is also documented for the presence of PVL (Cuny et al., 2015a). Therefore, it is important to consider whether infections are associated with the human or animal clade of ST398.

Although a few studies claim high numbers of bacteraemia and systemic infections due to ST398 (Köck et al., 2013, van Alen et al., 2017), they should be interpreted with caution. Because in these previous studies, the majority of ST398 are identified through screening, and ST398 represent only a small proportion of the total MRSA isolated from infections. Besides, in most cases, the studies have reported ST398 infections without consideration of whether these are caused by the human or animal
associated clade. Moreover, studies reporting ST398 infections are mostly carried out on clinical isolates collected in hospitals where the isolates are likely to have originated from clinically affected individuals; therefore, a high prevalence can be expected. The results might not be the same if the investigation is carried at the farm level. For example, one survey in Germany that investigated MRSA carriage and infections in humans in livestock populated areas, reported ST398 as the most frequently isolated strain with the overall proportion of 19% \((n = 2607)\) amongst collected MRSA isolates \((n = 1406)\) (Köck et al., 2013). The study further reported up to 14% of ST398 associated with systemic infections and concluded that LA-MRSA was a major cause of human infection. However, in fact, the proportion of MRSA ST398 infection among all MRSA cases was only 3.1% \((n = 436/14036)\), over one half of the infected ST398 \((n = 239)\) were isolated from skin infections (wound/abscess/catheters), only 16 isolates were associated with bacteraemia, representing only 0.1% of the total MRSA, and only a very small proportion of ST398 (0.6%) were associated with systemic infections, with isolation predominantly from urine and respiratory fluids. Another study conducted in North Germany in a hospital found 24% of 6,555 MRSA isolates collected over 15 years belonged to ST398, and claimed 8% \((124/1591)\) of the total ST398 were derived from clinical specimens; however, only a small number \((n = 7)\) were associated with bacteraemia (van Alen et al., 2017). Although the proportion of ST398 among MRSA isolates causing human infections are lower than non-ST398, a slight increase in the reported number of infections has been observed in the last few years. For example, in Denmark, cases of infection are reported to have increased from 13 cases in 2009 (92 in 2012, 157 in 2013) to a total of 208 cases in 2015 (DANMAP, 2014, DANMAP, 2016, DANMAP, 2010).

Outbreaks of human infections due to CA- and HA-MRSA strains are commonly reported, whereas in contrast, ST398 has rarely been reported to cause outbreaks on a large scale. One study reported an outbreak of MRSA infections due to ST398 a decade ago in a hospital (Wulf et al., 2008a). However, the report did not differentiate between infection and carriage, as only one patient’s culture obtained from a foot ulcer returned positive for ST398, the additional four patients and five health workers were positive for carriage only. Nevertheless, future outbreaks in hospitals are possible as ST398 changes to acquire human pathogenic genes. Given that the likelihood of hospital outbreaks due to ST398 is very low, the estimated probability of community outbreaks is even less likely than hospital outbreaks (Cox and Popken, 2014).

### 2.7 Risk factors for LA-MRSA ST398 carriage in humans

LA-MRSA strains such as ST398 carriage in people at-risk is associated with a number of factors such as pig contact, contact intensity (number of hours working in direct pig contact), contact with
the type of pig age group, MRSA prevalence in pigs, pig stocking density, and other individuals risk factors which are explained as follows.

2.7.1 ST398 carriage in people with direct pig exposure

MRSA ST398 transmission and the extent of its ability to persist in humans are not fully understood; however, occupational pig contact with MRSA-positive pigs is an important risk factor for nasal carriage (Wardyn et al., 2015). LA-MRSA has rarely been isolated from people without pig exposure. In line with this, a cross-sectional study that collected nasal swabs from school children \( n = 462 \) in a pig populated area in Germany recovered ST398 only from three children, and all were living on a pig farm (Cuny et al., 2009). Although some studies have also linked occupational cattle contact with MRSA carriage in people (Graveland et al., 2010), numerous studies have established pig contact as the most important risk factor (Liu et al., 2015, Crombé et al., 2013). A case-control study that assessed 384 patients from four different hospitals in a livestock dense region in Germany found 21% carriage with ST398 and 62% \( n = 34 \) of those reported livestock contact, including pigs \( n = 27 \) (Deiters et al., 2015). The study found an increased odds ratio for people living directly on a pig farm \( \text{OR} \ 12.8, \ CI \ 2.4–67.1 \). Another study by Voss and colleagues demonstrated a 760-fold MRSA carriage rate among pig farmers compared to the general Dutch population (Voss et al., 2005). A systematic review that examined 33 studies found animal contact, especially pig contact \( \text{OR}, \ 5.9, \ CI \ 4.8-7.2 \) as the most important risk for MRSA carriage in people (Liu et al., 2015). The study found a higher prevalence of MRSA carriage in farmers \( 18.2\%; \ 95\%CI \ 9.3-29.0\% \) compared to veterinarians \( 9.4\%; \ 3.5-17.6\% \), slaughterhouse workers \( 2.6\%; \ 0.8-5.4\% \), or butchers \( 5.7\%; \ 3.3-8.9\% \). The high proportion of carriage in farmers compared to other professionals reported by Liu et al. (2015) indicates that the risk of MRSA carriage might have been associated with the nature of work, in addition to pig contact, as farmers come into pig contact more often and work in constant close contact with pigs. But, the previous study reported no typing data for MRSA carriage. However, other studies with typing information have found a positive association between ST398 carriage in people and intensity of pig contact, for example, a study by Dorado-Garcia et al. (2015) reported that the odds for ST398 carriage increased 1.8 times for each 10-hour increase in pig contact. These studies indicate that people in close contact with pigs had an increased risk of MRSA carriage, and the probability of MRSA detection in pig farmers is positively associated with the number of hours work in pig contact.
2.7.2 ST398 carriage in people with no direct pig exposure

There are a mixture of reports on LA-MRSA carriage in people with absence of pig contact. Although a few studies have claimed ST398 carriage and infections with no occupational pig exposure (Larsen et al., 2017, Bosch et al., 2016, Zomer et al., 2017), the majority of studies have found direct pig contact as a risk factor for LA-MRSA carriage, as mentioned earlier. In addition, the studies that have reported ST398 carriage and infection in people in the absence of pig exposure need to be interpreted with caution, as it is not clear how reliably the information was obtained, and whether the information was validated. For example, a Dutch study by Zomer et al. (2017), found LA-MRSA carriage in a very small proportion (0.4%, n = 10/2492) of the target population in a rural area and reported that persons positive for LA-MRSA lived in closer proximity to a pig farm (400m away from a farm) compared to persons negative for MRSA (700m). Although this previous study found only a small proportion of LA-MRSA carriage with only 200m differences in distance to a pig farm, the study claimed that living in the proximity of livestock farms, including pig farms, was a risk factor for MRSA carriage and infections, irrespective of occupational contact. In contrast, a recent study investigating distance to pig farms as a risk factor for MRSA carriage in the general population, examined 11,174 MRSA cases, with 24% being ST398, and found no significant association between ST398 carriage and distance using spatial measurement and statistics (Anker et al., 2018). LA-MRSA ST398 is reported to be a poor human coloniser as explained earlier in this chapter, and it has low potential for human to human transmission (Price et al., 2012, Jamrozy et al., 2012, van Cleef et al., 2011b, Wassenberg et al., 2011), thereby, living in the proximity of livestock farms was not a risk factor for persistent carriage. Food has also not been considered an important source of MRSA carriage or infection in humans. No study has established pig meat as a source of MRSA carriage. Supporting this, De Jong et al. (2010) found no MRSA carriage in 95 participants working in the cold meat processing industry and institutional kitchens, despite the fact that MRSA was present in some meat samples. This study suggests minimum cross-contamination of carcasses during the slaughter process, despite high nasal carriage in the nasal cavity of incoming pigs (Narvaez-Bravo et al., 2016).
2.7.3 ST398 carriage association with pig age group

MRSA carriage in piggery workers has also been linked with working with particular pig age groups. Dorado-Garcia et al. (2015) found exposure to the farrowing group (piglets) as significantly associated with MRSA carriage in people (OR 2.21, CI 1.16–4.22, $P = 0.02$). Another study has also found a significantly higher odds ratio for MRSA carriage in farmers that have assisted sows at farrowing (OR 2.26, CI 1.10–4.67, $P = 0.03$) (van Cleef et al., 2014). Although it is not clear why contact with farrowing sows and piglets increases the risk of MRSA carriage in people with exposure, it might be due to the nature of contact (frequent and intense) required working with this age group.

2.7.4 ST398 carriage association with MRSA prevalence in pigs and stocking intensity

There are reports that MRSA carriage in humans is also associated with MRSA prevalence in animals on a farm. A cross-sectional study found MRSA carriage in humans on 30% (15/50) of the MRSA positive piggeries whereas human carriage was not found for MRSA negative piggeries (van Den Broek et al., 2009). Similarly, a study conducted in Canada found a significant association ($P < 0.003$) between the presence of MRSA in pigs and carriage in workers with 20% of workers carrying MRSA on farms having MRSA positive pigs but no MRSA carriage in humans on negative farms (Khanna et al., 2008). A positive association between MRSA carriage in workers and pig stocking density has also been revealed with an increased risk of MRSA carriage on farms with high stocking densities (van Den Broek et al., 2009). Reynaga et al. (2016) found a significantly ($P < 0.001$) higher percentage of MRSA ST398 carriage amongst farmers in piggeries with more than 1250 pigs (76%) than those farms with less than 1250 pigs (42%). This association might be explained by the MRSA prevalence in pigs as larger farms are recognised for a higher MRSA prevalence in pigs compared to smaller farms (Fromm et al., 2014, Fang et al., 2014), thereby, increase the risk of MRSA carriage in people working with direct pig contact on these farms.

2.7.5 ST398 carriage in workers and the use of Personal Protective Equipment (PPE)

The use of proper PPE is a promising measure to reduce the risk of MRSA carriage and infections. van Cleef et al. (2014) found a significantly lower risk of MRSA carriage in pig farmers who wore a mask continuously (OR 0.13, CI 0.02–0.76) compared to those who did not always wear one. Although MRSA carriage among pig farmers has been reported despite using PPE in a few surveys like Huang et al. (2014), it can reduce the risk of the carriage (van Cleef et al., 2014). There are chances that respondents might have deliberately picked the best answer on a questionnaire about their hygiene practices at work, resulting in bias in the study that reported a negative association for MRSA carriage with PPE.
2.7.6 Individual risk factors for ST398 carriage

Some studies have also linked LA-MRSA carriage in the at-risk group with individual risk factors such as age, gender, education, and history of hospitalisation (Liu et al., 2015, Deiters et al., 2015, Köck et al., 2009). However, other studies have found no significant association between MRSA carriage in the at-risk group and individual level risk factors (Zomer et al., 2017, Reynaga et al., 2016, Smith et al., 2013), possibly due to the strong association of pig contact masking the effects of the other explanatory variables, and hence inability to assess their importance.

Elderly age and male gender are positively associated with the majority of studies for all three types of MRSA (CA, HA, and LA). For example, Coombs et al. (2013b) reported that being aged over 40 years is a significant risk for carriage and infection with CA- and HA-MRSA (OR 1.8, CI 1.3-1.5). Similarly, a systematic review investigating MRSA carriage in people with livestock contact estimated an increasing odds of 1.34 times for every ten years increased in age (OR 1.34, CI 1.18-1.52) (Liu et al., 2015). Another study found a higher risk of MRSA carriage among pig farmers over 40 years of age compared to farmers aged 17-39 (van Cleef et al., 2014). Often, when people age, they have comorbid conditions, and low immune response may predispose them to carriage and infection. There are mixed reports on the association between ST398 carriage in humans and gender. Some studies have reported higher odds ratios of MRSA carriage in males compared with females (Verkade et al., 2014, van Cleef et al., 2016, van Den Broek et al., 2009), others have found no significant association with gender (Smith et al., 2013, Garcia-Graells et al., 2013, van Cleef et al., 2010a). Although the reason for gender differences regarding MRSA carriage reported in some studies is not known, these differences may reflect a higher male to female ratio in these studies and, moreover, compliance with proper use of PPE can also vary between genders. Although no study has investigated the compliance of PPE by gender and its association with MRSA carriage in piggeries, a review investigating gender differences in hygiene behaviour among healthcare staff reported that males are less frequently compliant with hygiene compared to females which might predispose them to higher MRSA carriage (Humphreys et al., 2015). History of hospitalisation (within six months) has also been positively associated with ST398 carriage (Deiters et al., 2015, Köck et al., 2009).

2.8 Prevalence of ST398 carriage in pigs

The prevalence of ST398 carriage among pigs in several European countries ranges from zero in Sweden (Unnerstad et al., 2017) to 86% in The Netherlands (Morcillo et al., 2015), and over 80% of piggeries have been reported to be contaminated with MRSA ST398 in Germany (Cuny et al., 2009).
Although varied prevalence of LA-MRSA has been reported between-herds and within-herds across the globe, these results need to be compared with caution because a number of factors can affect the detection of MRSA. For example, sampling techniques used during the investigation and whether sampling was done in pigs or the environment. Pig nasal swabs have higher sensitivity for detection of MRSA compared to environmental samples (Broens et al., 2011a). Similarly, the steps used in analysing the samples in the laboratory are very important. The most commonly used steps for isolation of MRSA are the double broth enrichment followed by inoculation on chromogenic MRSA agar and blood agar in conjunction with biochemical testing (i.e. agglutination) as described by the European Food Safety Authority (EFSA) and the European Union Reference Laboratory for AMR (EURL-AMR) for the testing of MRSA in food-producing animals and food samples (EURL-AMR, 2009, EFSA, 2012). Subsequent testing of the presumptive MRSA isolates on Mueller-Hinton agar for antibiotic susceptibility using disc diffusion method is recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014). Similarly, it is important to consider whether individual or pooled sampling is used in a study as pooling reduces the likelihood of detection of positive samples (Broens et al., 2011a, EFSA, 2012, Grmek-Kosnik et al., 2005). Type of target population, (farm or abattoir level) also reflects variation in prevalence of MRSA. Studies have suggested that high density during transportation and at the abattoir house increases MRSA carriage in pigs and there is a high chance that MRSA negative pigs will become positive during the transportation process (Broens et al., 2011c). Although estimates from the abattoir might not reflect the true farm level prevalence (EFSA, 2014), some studies have used sampling in pigs at the abattoir and extrapolated prevalence to the respective farms from which they originated. For example, a study by Agersø et al. (2012) sampled pigs at an abattoir, but the information was collected from individual piggeries where pigs were sourced to investigate the associated risk factors. Other management practices on farm can also affect MRSA prevalence in pigs such as antibiotic usage, size of farm (small or large production system) (Alt et al., 2011, Fang et al., 2014), type of farm and system in place (intensive, extensive, closed, or open) (Dorado-Garcia et al., 2015, Porrero et al., 2012), and stage of pig production (dry sow, farrowing sows, weaner, grower, or finisher) (Dorado-Garcia et al., 2015). Some of the farm-related factors associated with MRSA carriage in pigs are discussed in the following paragraphs.

2.9 Risk factors for ST398 carriage in pigs

2.9.1 Antibiotic usage

Antibiotic use has been suggested to contribute to the emergence and dissemination of LA-MRSA including ST398 within the pig environment (Smith et al., 2013, Dorado-Garcia et al., 2015), and most ST398 isolates are resistant to antibiotics commonly used in pigs (Dorado-Garcia et al., 2015,
EFSA, 2012, Pyörälä et al., 2014). A study that collected pig nasal swabs from 45 farms comprising 21 antibiotic-free and 24 conventional herds in the USA found no MRSA carriage in antibiotic-free farms while 8.5% pigs were positive on conventional farms (Smith et al., 2013). Antibiotics are routinely used on conventional piggeries as prophylactic (preventive) measure, and this could possibly be one of the reasons that MRSA positive pigs were more likely to be found on conventional farms compared to antibiotic free farms in the previous study. A positive association between the quantity of antibiotic usage in pigs and MRSA carriage on piggeries has already been established. Confirming this assertion, Dorado-Garcia et al. (2015) found 1.2 times increased odds of MRSA carriage for each unit increase in the number of days of antimicrobial use per animal per year (OR 1.16, CI 1.02-1.33). The use of some antibiotics has been identified to be strongly associated with MRSA carriage in pigs, including betalactams and tetracyclines (Dorado-Garcia et al., 2015). Tetracyclines and betalactams are reported to be the most commonly used antibiotics in pigs in 30 European countries representing 33% and 25% respectively of the total antimicrobials used (ESVAC, 2017). Since the betalactam resistance genes (meca) and tetracycline resistance genes (tet) are present in all MRSA ST398, the use of these drugs in pigs selects for this strain. Broens et al. (2012a) found higher MRSA transmission rates in pigs treated with tetracyclines and betalactams compared to untreated pigs. Similarly, Moodley et al. (2011b) found a higher nasal ST398 load in pigs treated with tetracyclines compared to the non-treated pigs in an experimental study. Apart from betalactam and tetracycline usage, other antibiotic commonly used in pigs such as macrolides and lincosamides could also promote the emergence of ST398 (Pyörälä et al., 2014). Marked resistance in pigs to these antibiotics has been reported in the literature, with resistance to tetracycline present in up to 100% of isolates, and up to 95% of isolates are resistant to macrolides and lincosamides (Conceição et al., 2017, Broens et al., 2011d, EFSA, 2014). However, resistance to fluoroquinolones, aminoglycosides and oxazolidones is infrequently reported (EFSA, 2014) because these antibiotic classes are not commonly used in pig production (ESVAC, 2017). These studies imply that antibiotic usage in pigs is a risk factor for the emergence and spread of MRSA ST398 due to selection pressure.

The route of antibiotic administration is also considered an important factor associated with MRSA prevalence in pigs. In intensive farming treating an individual animal with an antibiotic is a challenge because it is labour intensive and not economical. Therefore, animals are usually treated in groups with drug supplementation in water and feed that may result in over- or under-dosage (Callens et al., 2012) as animals can choose the dose of drug administered by the quantity of feed or water consumed (Love et al., 2011). Oral administration of drugs is linked to an increased risk of antibiotic resistance in bacteria (Burow et al., 2014). According to an estimate, 99% of therapeutic antimicrobials in Australia are used orally in pigs (APVMA, 2014).
Antibiotics are used at subtherapeutic levels for the purpose of growth promotion which has been associated with emergence of resistance in pigs (EFSA, 2009b). However, there is also evidence that the use of antibiotics as prophylactic or metaphylactic measures could lead to spread of resistance. Prophylaxis use is defined as the group treatment of healthy animals for prevention of diseases for an excessive duration, whereas metaphylactic use is defined as the group treatment of sick animals along with healthy ones (APUA, 2010, Callens et al., 2012). A Belgian survey investigating antimicrobial use on 50 pig farms retrospectively revealed that 93% of group treatments were purely prophylactic, involving no disease diagnosis (Callens et al., 2012).

Besides antibiotics, heavy metal usage in pig feed such as zinc and copper have also been shown in experimental and observational studies to co-select for ST398 (Slifierz et al., 2015a, Amachawadi et al., 2015, Yazdankhah et al., 2014). One experimental study found a significantly ($P = 0.015$) higher nasal ST398 load in pigs treated with zinc compared to the non-treated pigs (Moodley et al., 2011b). Similarly, other experimental studies found a significant association between MRSA carriage in pigs and the level of zinc supplementation in pig feed (Slifierz et al., 2015a, Amachawadi et al., 2015). Heavy metal usage in pig feed has increased in Europe after the ban on antibiotic use for growth promotion. However, $czerC$ gene that collocated with the $mecA$ gene and confer resistance to zinc has recently emerged in MRSA ST398 (Hau et al., 2017, van Alen et al., 2018), thereby, the use of zinc in feed may play a role in co-selection of ST398 in pigs. However, The EU has now recommended banning zinc in animal feed due to its association with the emergence of resistance in bacteria and the tendency to accumulate in soil leading to environmental pollution (Jensen et al., 2016, Bernhoft et al., 2014).

Although antibiotic use in pigs has been established as an important driving force for the emergence and spread of MRSA, other husbandry practices such as pig housing, biosecurity and hygiene measures on farms are also reported to be associated with MRSA carriage in pigs which are described in the following paragraphs.

### 2.9.2 Pig stocking density

A meta-analysis that analysed data of 400 pig herds, found smaller pig farms (pigs <500) to be less likely MRSA positive compared to pig farms with more than 500 sows (OR from 3.6 to 5.4) (Fromm et al., 2014), a similar magnitude of risks than those described by Alt et al. (2011). Similarly, Fang et al. (2014) reported significantly ($P < 0.001$) higher MRSA prevalence (34.3%) in pigs raised on a large scale (>10,000 pigs) than among pigs raised in smaller farms (7%). The association of herd size with the proportion of MRSA-positive pigs in larger herds may reflect the crowding stress that leads to antibiotic usage, as crowded sheds in intensive farming may result in stress in pigs, which
could negatively impact pig health and make them sensitive to bacterial infections (Moinard et al., 2003, Oh et al., 2010). The increased antibiotic usage could then select for MRSA. In line with this, a Dutch study has reported a positive association between the number of pigs on farms and the use of antimicrobials, with larger farms tending to use more antimicrobials than smaller farms (van der Fels-Klerx et al., 2011). However, a German study claimed no association between antibiotic treatment frequency and farm size (van Rennings et al., 2015), unlike the previous research. These studies categorized herd sizes differently which could influence the results obtained.

The above-stated studies suggest that reducing stocking densities might be helpful in controlling MRSA emergence and spread in pigs; since increasing space allowance causes a marked reduction in aggression and physiological stress (Remience et al., 2008). However, increasing the space allocation within a pig facility might result in a lowest asset turnover ratio; therefore, economic decisions sometimes dominate over the pig performance and welfare (Brumm, 2006).

### 2.9.3 Pig age group

In intensive pig production, pigs are usually categorised into five different groups: dry sows, farrowing sows, weaners, growers, and finishers. These five groups are normally raised in separate units/sheds. Dry sows are also known as breeding sows or pregnant sows, and are a group of female pigs kept on a farm for breeding purposes. Dry sows are transferred to farrowing crates 3-5 days before farrowing and remain there until the piglets are weaned. Piglets are normally weaned at the age of three to four weeks and weaners stay in this production category until 8-10 weeks of age when they weigh about 20kg. Weaners are then transferred to another shed where they are termed ‘growers’ and stay here until around 100 days of age and achieve 40kg to 70kg in live weight. Growers are then moved to a finisher shed, and are subsequently sent for slaughter at around 170 days of age when they weigh around 100kg.

Some studies have associated MRSA carriage in pigs with age. Dry sows are regarded as least likely carriers of MRSA amongst all production stage groups (Dorado-Garcia et al., 2015, Broens et al., 2012a). In comparison with dry sows, Dorado-Garcia et al. (2015) found a significantly ($P < 0.001$) higher risk of MRSA carriage for suckling piglets (OR 3.87, CI 2.34–6.39), weaners (OR 9.89; CI 5.96–16.39), gilts (OR 1.08, CI 0.65–1.80), and finishers (OR 4.09, CI 2.30–7.25). Usually, weaners are recognised to have a high proportion of MRSA carriage with up to 100% MRSA prevalence recorded for this age group (Smith et al., 2009). Many factors trigger the emergence and spread of MRSA in weaners, but antibiotic usage is an important potential factor (Postma et al., 2016, van Rennings et al., 2015). Weaning is recognised as the most stressful event in a pig’s life due to a series of demanding changes, including nutritional, social (mother separation), and physiological factors,
which can negatively impact pig health (Campbell et al., 2013). These events that make pigs sensitive to bacterial infections subsequently attract the most antimicrobial usage compared to any other stage (Sjölund et al., 2016). In Denmark, 42% of the total antimicrobials sold for pig production in 2015 were used in weaners only (DANMAP, 2016). Similarly, early weaning has also been associated with MRSA carriage in this age group. In most intensive production systems piglets are weaned at 21-26 days. However, in some cases it occurs as early as two weeks. Lower weaning age leads to weaker pigs that are more susceptible to infections, therefore attracting high antibiotic usage. In line with this, a study conducted on 227 pig farms in four European countries reported a significantly higher antimicrobial usage in weaning age which was reported to be associated with early weaning practices (Sjölund et al., 2016). Therefore, increasing weaning age from up to 35 days can markedly reduce antimicrobial usage at weaning stage (Sjölund et al., 2016) and, subsequently, selection pressure.

### 2.9.4 Biosecurity and hygiene

Pig to pig contact is the most likely route of MRSA transmission between pigs and experimental studies have estimated the basic reproduction ratio ($R_o$) between 3.7 to 52.54 (Broens et al., 2012b, Broens et al., 2012a, Crombé et al., 2012a). The $R_o$ is defined as the average number of secondary infections in a susceptible population caused by a single infected individual over the course of its infectious period (Heffernan et al., 2005). The high $R_o$ suggests that ST398 can easily spread among pigs once it gets introduced into a farm, stressing the importance of biosecurity. Biosecurity practices on pig farms such as the type of production system (open/closed), isolation of incoming animals and shed disinfection have also been linked with MRSA in pigs. Closed farms are defined as farms who solely breed and raise their own pigs on site without introducing replacement pigs from outsources, unlike open farms. Pig farms with closed farming systems are reported to have a lower prevalence of MRSA carriage compared to open systems (Crombé et al., 2012b, Dorado-Garcia et al., 2015). In line with this, previous studies have identified farms who purchase pigs from outside sources to be at a high risk for MRSA carriage, compared to farms who bred their own pigs or outsourcing from limited suppliers (Fromm et al., 2014, Alt et al., 2011, Grøntvedt et al., 2016), with the MRSA status of the supplying herds being of great importance. A study found significantly higher odds ratio for MRSA detection in farms sourcing pigs from MRSA positive herds compared to those outsourcing their pigs from MRSA-negative suppliers (OR 10.8, CI 1.5–110.1) (Broens et al., 2011d). In addition, piggeries that have a continuous farming system in place are more likely positive for MRSA compared to farms practicing all in and all out (AIAO) (Alt et al., 2011), emphasizing the importance of disinfection practices.
Besides pig to pig contact, the environment also plays a role in the transmission of MRSA in farms. Several studies have reported MRSA contaminated environments in association with MRSA carriage in pigs (Espinosa-Gongora et al., 2012b, EFSA, 2009d). An experimental study showed that CA-MRSA like ST8 could dominate other established porcine-MRSA like ST398 and also revealed that decolonisation of nasal carriage occurred upon transferring into a clean house (Moodley et al., 2012) which indicates the importance of shed cleaning and hygiene. Some studies have linked disinfectant use with increased MRSA carriage (Fromm et al., 2014, Alt et al., 2011). Although the reason for the association with disinfectant use is not clear, this could possibly be due to co-selection associated with resistance genes such qac gene, quaternary ammonium compounds resistance gene, that have been identified in ST398 isolates (Argudín et al., 2016, Slifierz et al., 2015b). There are also possibilities that cleaning and disinfection procedures might not be carried out appropriately on the study farms where an association between MRSA and disinfectant is found. ST398 is shown to be susceptible to a larger number of antiseptics and disinfectants despite the emerging reports of qac genes (Espigares et al., 2017). In addition, disinfection alone might not be helpful in controlling MRSA without improving husbandry practices.

Inappropriate biosecurity and hygiene measures on farm have also been associated with high antibiotic usage in pigs, which is one of the most important risk factors for MRSA emergence and spread in piggeries, as mentioned earlier. An interventional study conducted in Belgium on 61 commercial pig herds revealed that improving farm biosecurity measures and regular vaccination resulted in 52% reduction in antimicrobial use on farms in just eight months (Postma et al., 2017). Some of the interventions adopted in the previous study regarding antimicrobial usage included a complete elimination of prophylactic usage in all age groups, use of narrow-spectrum instead of broad spectrum, parenteral antimicrobial usage instead of oral, no antimicrobial at castration, pigs treated as individuals or in small age groups targeting a specific disease, and regular diagnostic testing for antibiotic susceptibility. Another study using quasi-experimental intervention approach assessed the economic impact of reducing antimicrobial use coupled with improved biosecurity and vaccination measures on 48 piggeries showed that producers could save a median equivalent to AU$4.2/sow/year (calculated using online exchange rate at www.xe.com identified in March 2018) even after spending a median of AU$6.3/sow/year on improving biosecurity measures (Rojo-Gimeno et al., 2016). The study showed that the new management strategies not only reduced the cost of antibiotics (AU$12.0/sow/year) but also significantly reduced mortality rate in pigs by 1.1%, compared to the status of the farms before the interventions. However, there was a greater variation in the cost reduction of antimicrobial usage (±AU$47.0). Moreover, a single cost of adopting biosecurity measures on a farm cannot be generalised to all piggeries due to factors that vary amongst farms, such
as existing biosecurity facilities, type of production system, farm size and geographical location of the farm. Although the results of the studies reported may not be generalisable to all establishments, they suggest that improved biosecurity is a prime tool for achieving pig health and reducing antibiotic usage, ultimately minimising the emergence and spread of resistance.

2.10 MRSA in the Australian context

Australia has played an important role in the global history of *S. aureus*. The first pandemic strains of MSSA that was penicillin-resistant caused infections in healthcare throughout the world in the 1950s had first been isolated in Australia from infected babies in a Sydney maternity hospital. This was later termed as “phage type 80/81” (Isbister et al., 1954), also known as the Southwest Pacific clone subsequently characterised as ST30 (DeLeo et al., 2011). Similarly, the first ever CA-MRSA, has been reported in Australia in the Kimberley region (Udo et al., 1993) which was subsequently identified as ST1 (Coombs et al., 2004). Since then, CA-MRSA has been reported from all over the world (Mediavilla et al., 2012). In the early 2000s, ST1 was the most common CA-MRSA in Australia followed by ST30 (Coombs et al., 2004) which has now been replaced by ST93 in the last few years (Coombs et al., 2016, ACSQHC, 2017). A study conducted in 2013 also suggest that ST93 is an emerging cause of human infections in hospitals (ACSQHC, 2017, Nimmo et al., 2013). ST1 and ST30 that were first isolated in Australia are among the five predominant CA-MRSA strains reported around the world; the remaining three are ST8, ST59, and ST80 (Mediavilla et al., 2012).

The ongoing emergence, prevalence and dissemination of MRSA is still considered a serious challenge in Australian health settings and communities as it is associated with a mortality of up to 19% in infected persons (ACSQHC, 2017). The HA-MRSA isolated in Australia are classified as MDR and display resistance to a number of non-beta-lactam antibiotics such as ciprofloxacin, macrolides, lincosamides, gentamicin, and tetracycline (Coombs et al., 2015, Coombs et al., 2016). In contrast, CA-MRSA in Australia is usually non-MDR (Coombs et al., 2016, Coombs et al., 2013b).

The overall MRSA proportion amongst *S. aureus* isolates in Australia is apparently higher compared to Europe but lower than the USA (Coombs et al., 2016, ACSQHC, 2017). A recent result from the Australian Group on Antimicrobial Resistance (AGAR) reported 19% of the 2,206 *S. aureus* isolates as MRSA, and 60% of MRSA were due to community-associated strains (Coombs et al., 2016). In this report, the MRSA proportion amongst invasive *S. aureus* isolates was reported to be higher than in Europe. AGAR is the only source of information that allows monitoring of trends in susceptibility and molecular characteristics of *S. aureus* in Australia. AGAR collects the first 100 consecutive non-duplicate *S. aureus* isolates of blood culture from 27 participating laboratories across Australia every year. Recently in Europe, a study investigating *S. aureus* isolates collected from blood and
cerebrospinal fluid in 30 countries found 17% to be MRSA, however, with a great variation across countries, ranging from 0-57% (ECDC, 2017). Although 17% does not appear greatly different from 19% reported in the AGAR survey, one-third of European countries reported a proportion less than 10%, and only ten European countries have an MRSA proportion over 19% (ECDC, 2017). A survey in the USA which collected 100 consecutive unique clinical *S.aureus* isolates of different infection sites including blood culture from 43 medical centres found 51% MRSA among 4,131 isolates with 61% of these classed as CA-MRSA (Diekema et al., 2014). Similarly, another study investigated MRSA proportion amongst 1,015 *S.aureus* isolated of bloodstream infections in a Chicago hospital identified 36% as MRSA (Rhee et al., 2015).

Although Australia is well recognised for a high proportion of CA- and HA-MRSA amongst *S.aureus* compared to pig producing countries in Europe, no study has observed the prevalence and distribution of MRSA on a large scale in commercial piggeries in Australia to date. Anecdotally, LA-MRSA is less common in Australia, as this pathogen has been isolated on only two occasions in Australia. One study that collected 324 samples from five commercial and one feral pig herd in 2009 found only three MRSA positive samples on a single farm, that were further typed as ST398 (Groves et al., 2014). Another cross-sectional study examined nasal MRSA carriage among veterinarians at different conferences in Australia in 2011, and isolated a single MRSA ST398 from a pig veterinarian (Jordan et al., 2011). In contrast, studies conducted in other countries have found an increased risk of ST398 carriage in veterinarians working with pigs than other livestock, and up to 50% of carriage is reported among participating veterinarians (Verkade et al., 2013, Garcia-Graells et al., 2012). There is a knowledge gap regarding the prevalence and distribution of MRSA in the Australian pig industry.

### 2.11 Summary of the literature review

*S.aureus* is a commensal bacterium which colonises different body parts of humans and animals. MRSA is a *S.aureus* that shows resistance to different antibiotics, including methicillin. MRSA is an opportunistic bacterium that can cause infections in humans and some livestock species and is categorised into three types: HA-MRSA, CA-MRSA, and LA-MRSA. HA-MRSA primarily causes infections in hospital settings and carries resistance to a larger number of antibiotics on a mobile genetic elements SCC*mec* (types I-III) and lacking PVL genes. The CA-MRSA are resistant to fewer antibiotic classes, and the resistance is carried on the short SCC*mec* elements types IV and V; however, this carries extra human virulence genes, such as PVL and ACME. LA-MRSA is normally found in pigs and people with pig exposure. Pigs carry LA-MRSA asymptptomatically, which could transfer to humans and potentially cause infections. In this review, we found evidence that direct contact with MRSA positive pigs and contact intensity are the most important risk factors for LA-
MRSA carriage in humans. However, studies have shown that LA-MRSA is less pathogenic and transmissible in humans compared to non-LA-MRSA. Still, LA-MRSA has the potential to acquire further virulence and resistance genes to evolve more successful and virulent strains. ST398 and ST9 are the predominate LA-MRSA strains reported in Europe and Asia, respectively. LA-MRSA, such as ST398 shows high resistance to antibiotics that are commonly used in pigs. Research indicated that LA-MRSA, such as ST398, has a high potential of transmission within and between pig farms, and MRSA carriage in pigs is associated with a variety of pig husbandry practices, such as pig stocking density, farm biosecurity and hygiene, and production age group. Europe is recognised for a lower prevalence of HA- and CA-MRSA amongst isolates compared to any other parts of the world; however, the prevalence of LA-MRSA is reported to be higher in the European pig industry compared with the rest of the world.

In Australia, the overall proportion of MRSA among *S.aureus* isolates in humans is higher compared to other pig producing countries in Europe. However, limited studies on MRSA carriage in pigs and people with pig exposure have been conducted in Australia. To date, MRSA was considered less prevalent in the Australian pig industry compared to other parts of the world, as LA-MRSA ST398 has been reported on only two occasions, once isolated from a single pig veterinarian, and once on three pig samples from a single pig farm. However, as a precedent of this thesis, in 2014, the School of Animal and Veterinary Sciences at Charles Sturt University was contacted in relation to a pig farm that had been identified by the New South Wales Department of Health for having a recurrent outbreak of MRSA in workers over the preceding three-year period, requesting assistance in further investigation.

Table 2.1 Overview of LA-MRSA prevalence on herd and pig level in various part of the world

<table>
<thead>
<tr>
<th>Herd prevalence</th>
<th>Animal prevalence</th>
<th>MLST</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>68% (34/50)</td>
<td>44.2% (663/1500)</td>
<td>ST398</td>
<td>Belgium</td>
<td>(Crombé et al., 2012b)</td>
</tr>
<tr>
<td>45% (9/20)</td>
<td>24.9% (71/285)</td>
<td>ST398, ST5</td>
<td>Canada</td>
<td>(Khanna et al., 2008)</td>
</tr>
<tr>
<td>61.9% (410/662)</td>
<td>ST398, ST5</td>
<td>Canada</td>
<td>(Narvaez-Bravo et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>41.9% (13/31)</td>
<td>11.4% (58/509)</td>
<td>ST9, ST912</td>
<td>China</td>
<td>(Cui et al., 2009)</td>
</tr>
<tr>
<td>74% (230/311)</td>
<td>ST398</td>
<td>Denmark</td>
<td>(Espinosa-Gongora et al., 2012b)</td>
<td></td>
</tr>
<tr>
<td>Abattoir</td>
<td>12.8% (101/789)</td>
<td>ST398, ST1, ST30</td>
<td>Denmark</td>
<td>(Agersø et al., 2012)</td>
</tr>
<tr>
<td>35.3% (6/17)</td>
<td>Dust samples</td>
<td>ST398</td>
<td>Germany</td>
<td>(Dahms et al., 2014)</td>
</tr>
<tr>
<td>82.5% (47/57)</td>
<td></td>
<td>ST398</td>
<td>German</td>
<td>(Cuny et al., 2009)</td>
</tr>
<tr>
<td>Sample Type</td>
<td>ST Type(s)</td>
<td>Source</td>
<td>Percentage</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>--------</td>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Dust samples</td>
<td>ST398</td>
<td>Germany</td>
<td>52% (152/290)</td>
<td>(Alt et al., 2011)</td>
</tr>
<tr>
<td>Abattoir</td>
<td>ST398</td>
<td>Germany</td>
<td>58.1% (596/1026)</td>
<td>(Tenhagen et al., 2009)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398, ST5, ST1</td>
<td>Netherlands</td>
<td>67.8% (137/202)</td>
<td>(Morcillo et al., 2015)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>Netherlands</td>
<td>77.8% (28/36)</td>
<td>(Dorado-Garcia et al., 2015)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>The Netherlands</td>
<td>56.2% (27/48)</td>
<td>(Broens et al., 2011d)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>Netherlands</td>
<td>20/20</td>
<td>(Reynaga et al., 2016)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>Sweden</td>
<td>0/92</td>
<td>(Unnerstad et al., 2017)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>Taiwan</td>
<td>14.3% (5/35)</td>
<td>(Fang et al., 2014)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>USA</td>
<td>50% (1/2)</td>
<td>(Smith et al., 2009)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>USA</td>
<td>8.9% (4/45)</td>
<td>(Smith et al., 2013)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398, ST5</td>
<td>USA</td>
<td>30% (12/40)</td>
<td>(Frana et al., 2013)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398, ST9</td>
<td>USA</td>
<td>50% (5/10)</td>
<td>(Molla et al., 2012)</td>
</tr>
<tr>
<td>Pooled sample</td>
<td>ST398</td>
<td>USA</td>
<td>0% (0/36)</td>
<td>(Sun et al., 2015)</td>
</tr>
</tbody>
</table>
Table 2.2 Overview of LA-MRSA prevalence reported in pig farmers in various parts of the world

<table>
<thead>
<tr>
<th>MRSA prevalence</th>
<th>MLST</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.8 (48/127)</td>
<td>ST398</td>
<td>Belgium</td>
<td>(Denis et al., 2009)</td>
</tr>
<tr>
<td>20 (5/25)</td>
<td>ST398, ST5</td>
<td>Canada</td>
<td>(Khanna et al., 2008)</td>
</tr>
<tr>
<td>6.7 (3/45)</td>
<td>ST9 and other</td>
<td>China</td>
<td>(Ye et al., 2015)</td>
</tr>
<tr>
<td>15.4 (2/13)</td>
<td>ST9</td>
<td>China</td>
<td>(Cui et al., 2009)</td>
</tr>
<tr>
<td>62.7 (69/110)</td>
<td>ST398</td>
<td>Netherlands</td>
<td>(van Cleef et al., 2014)</td>
</tr>
<tr>
<td>85.8 (97/113)</td>
<td>ST398</td>
<td>Germany</td>
<td>(Cuny et al., 2009)</td>
</tr>
<tr>
<td>45 (9/20)</td>
<td>ST398</td>
<td>Germany</td>
<td>(Smith et al., 2009)</td>
</tr>
<tr>
<td>55.6 (20/36)</td>
<td>ST398</td>
<td>Germany</td>
<td>(Dahms et al., 2014)</td>
</tr>
<tr>
<td>79.4 (27/34)</td>
<td>ST398</td>
<td>Germany</td>
<td>(Deiters et al., 2015)</td>
</tr>
<tr>
<td>77.1 (27/35)</td>
<td>ST98</td>
<td>Germany</td>
<td>(Köck et al., 2012)</td>
</tr>
<tr>
<td>55.6 (20/36)</td>
<td>ST3988</td>
<td>Germany</td>
<td>(Dahms et al., 2014)</td>
</tr>
<tr>
<td>84.6 (11/13)</td>
<td>ST398</td>
<td>Netherlands</td>
<td>(van Cleef et al., 2010b)</td>
</tr>
<tr>
<td>28.6 (28/98)</td>
<td>ST398</td>
<td>Netherlands</td>
<td>(van Den Broek et al., 2009)</td>
</tr>
<tr>
<td>57.9 (81/140)</td>
<td>ST398</td>
<td>Spain</td>
<td>(Reynaga et al., 2016)</td>
</tr>
<tr>
<td>9.3 (5/54)</td>
<td>ST398</td>
<td>Spain</td>
<td>(Morcillo et al., 2015)</td>
</tr>
<tr>
<td>19.2 (10/52)</td>
<td>ST30, ST9, ST59</td>
<td>Taiwan</td>
<td>(Fang et al., 2014)</td>
</tr>
<tr>
<td>22.2 (2/9)</td>
<td>ST5, ST36</td>
<td>USA</td>
<td>(Osadebe et al., 2013)</td>
</tr>
<tr>
<td>45 (9/20)</td>
<td>ST398</td>
<td>USA</td>
<td>(Smith et al., 2009)</td>
</tr>
</tbody>
</table>
Chapter 3.

Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia

This chapter has been published:

Abstract

Pigs have been recognised as a reservoir of livestock associated methicillin-resistant \textit{Staphylococcus aureus} (LA-MRSA) in Europe, Asia and North America. However, little is known about the presence and distribution of MRSA in the Australian pig population and pig industry.

This study describes the presence, distribution and molecular characteristics of the human adapted Australian CA-MRSA ST93 isolated from pigs, people, and the environment within a piggery. Isolates were subjected to antibiotic susceptibility testing, DNA microarray, whole genome sequencing, multi locus sequence typing, virulence and resistance gene characterization and phylogenetic analysis.

MRSA were isolated from 60\% \((n = 52)\) of farm workers where 84\% of isolates returned ST93 and the rest ST398. Of the thirty-one pig isolates tested further, an equal number of ST398 and ST93 (15 each) and one as ST30-V were identified. Four of six environmental isolates were identified as ST93 and two as ST398.

This study has identified for the first time in Australia the occurrence of CA-MRSA ST93 and LA-MRSA ST398 amongst farm workers, pigs, and the farm environment. Comparative genome analysis indicates that ST398 is likely to have been introduced into Australia from Europe or North America. This study also reports the first linezolid resistant MRSA isolated in Australia.
3.1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), in addition to being resistant to most betalactams, are typically resistant to multiple classes of antibiotics. First reported in a human hospitalised patient in the United Kingdom (Jevons, 1961), MRSA are now frequently isolated worldwide. Although MRSA infections were initially exclusively associated with the hospital setting, a change in epidemiology occurred in the 1990s when infections began to emerge amongst people who had no prior hospital association (Udo et al., 1993). These isolates were categorised as community-associated MRSA (CA-MRSA), and based on their accessory genome, staphylococcal cassette chromosome *mec* (SCCmec) and epidemiology were easily distinguishable from hospital-associated MRSA (HA-MRSA) (Enright et al., 2002, Vandenesch et al., 2003). Clinically, CA-MRSA infections are typically skin and soft tissue-related and occur in community members who do not have traditional health care risk factors. In contrast, HA-MRSA are more frequently associated with surgical and systemic infections in the hospital setting (Uhlemann et al., 2014). CA-MRSA typically harbors a SCCmec IV or V element and additional virulence factors such as Panton-Valentine leukocidin (PVL). Although resistant to betalactams, CA-MRSA are generally susceptible to most antimicrobial classes (David and Daum, 2010). In contrast, HA-MRSA usually harbour a SCCmec I, II, or III element, are PVL negative and are normally resistant to two or more non-betalactam antimicrobial classes (Lindsay, 2013, Hiramatsu et al., 2001). However, the distinction between CA-MRSA and HA-MRSA has become blurred with HA- and CA-MRSA crossing environmental boundaries, and in some cases exchanging genetic material (Boakes et al., 2011). CA-MRSA ST93-IV is the most common CA-MRSA in Australia and it is the second most common strain to cause infection in people, following HA-MRSA ST22-IV (Coombs et al., 2016).

A third category of MRSA, known as livestock-associated MRSA (LA-MRSA), was reported in the last decade (Huijsdens et al., 2006). LA-MRSA emerged independently from CA-MRSA and HA-MRSA with the most frequently isolated LA-MRSA belonging to clonal complex 398 (CC398). CC398 MRSA, first identified in pigs and pig farmers in 2005 (Armand-Lefevre et al., 2005), has been reported worldwide in a variety of food-animal species (Cuny et al., 2010) and is the predominant lineage of LA-MRSA in Europe and North America. Although CC398 has been isolated in Asia, LA-MRSA ST9 predominates in pigs in the Asian region (Larsen et al., 2012). CC398 MRSA has been isolated from up to 50% of pigs in some European countries (EFSA, 2010) and in up to 86% of pig workers (Cuny et al., 2009, EFSA, 2010, van Cleef et al., 2014). While CC398 is frequently reported to be associated with nasal carriage or colonization, infections resulting from this strain in humans, although rare, may also occur (Köck et al., 2013). Consequently, CC398 MRSA is
recognised as an occupational hazard for people working in the pig industry, including farm workers, veterinarians and abattoir workers (Wardyn et al., 2015, Nadimpalli et al., 2015).

LA-MRSA transmission and the extent of its ability to persist in pigs and humans are not fully understood. However, it is known that direct animal contact plays an important role in human carriage (Wardyn et al., 2015). A study by Voss and colleagues demonstrated a 760-fold higher MRSA carriage rate among pig farmers compared to the general Dutch population (Voss et al., 2005). Duration and intensity of animal contact and the number of MRSA positive animals on a farm have been linked with human CC398 carriage and infection (Köck et al., 2013, Graveland et al., 2011). Furthermore, as CC398 has the ability to survive in the environment, environmental contamination may contribute toward further dissemination. Although occupational exposure to pigs is a risk factor for carriage, compared to CA-MRSA and HA-MRSA, CC398 is not easily transmissible from human to human, and has few virulence-associated genes (Price et al., 2012, Jamrozy et al., 2012, van Cleef et al., 2011b). Furthermore, the prevalence of CC398 has been shown to rapidly decrease during the absence of animal contact (van Cleef et al., 2011b, Graveland et al., 2011, Jamrozy et al., 2012). While colonization with CC398 rarely results in human infection (Monaco et al., 2013), various forms of skin and soft tissue infections, usually minor or localized, have been reported (Köck et al., 2013).

In contrast to the knowledge about HA- and CA-MRSA in Australia (Coombs et al., 2014a), little is known about the presence and distribution of MRSA in the Australian pig population and in the Australian pig industry. One cross-sectional study in 2011 examined nasal carriage of MRSA among veterinarians (Jordan et al., 2011). A single ST398 MRSA from a pig veterinarian was identified (Groves et al., 2016). A second Australian study in 2014 isolated CC398 from less than 1% of 324 pigs sampled from five different commercial pig farms and one feral herd located across Australia (Groves et al., 2014).

Despite the limited documentation of LA-MRSA in pigs and pig professionals in Australia, an Australian pig farm was recently identified as being the focus of ongoing MRSA infections amongst its piggery employees over a three-year period. This study aims to describe the presence, distribution and molecular characteristics of MRSA isolated from the people, pigs and environment of the pig production facility where an ongoing outbreak in humans was occurring.

### 3.2 Methods and methodology

Approval for the recruitment of human participants into this study was granted by the Charles Sturt University Human Research Ethics Committee (Protocol number 2015/016). Approval for the sample collection from pigs was obtained from the Charles Sturt University Animal Care and Ethics
Committee (Protocol number 14/096). All methods were performed in accordance with the relevant guidelines and regulations.

### 3.2.1 Sampling

A cross-sectional study was performed at a commercial pig enterprise in Australia to detect the presence or absence of MRSA in people, pigs and the environment. The facility was identified by the farm’s jurisdictional health department due to the recurrent detection of MRSA amongst farm workers with clinical staphylococcal disease. The farm consisted of two sites (designated site-A and site-B) geographically separated by approximately 40 km. Sampling at each site occurred over a two-day period, with site-A visited in May and site-B in August 2015.

All farm workers (n = 52) were approached to participate in the study. Participation was voluntary, and signed informed consent was obtained from each participant. A sterile cotton applicator swab (Liquid Amies Elution Swab, Copan ESwab™) was provided and each participant was instructed to rotate the swab in both nostrils for five seconds, before placing the swab in a covered sterile transport tube.

On both farms, pigs were housed in separate sheds based on their age group and class (i.e. dry sows, farrowing, weaners, growers, and finishers). On site-A, to optimise sensitivity of the testing procedure, swabs from pigs were collected from the skin caudal and adjacent to the pinnae and from the external nares (Pletinckx et al., 2012). With the aim of determining if MRSA was present in the pigs on site-A, a previously described pooled sampling method was used: to detect MRSA carriage at a prevalence of at least 2% and assuming 90% test sensitivity with 95% confidence the minimum number of swabs per pool and the minimum number of pools required was calculated to be 10 and 17 respectively, using Epitools online (Sergeant, 2015) [http://epitools.ausvet.com.au]. In addition, in order to obtain estimates of prevalence specific for each shed, six pools (60 swabs) were collected from each shed (Broens et al., 2011b, EFSA, 2007). From the seven sheds on site-A, 420 swabs were collected, providing 42 pools. Animals were randomly selected by farm workers and animals were marked after sampling so re-sampling of the same animals could not occur.

As a large number of MRSA positive pools were found on site-A, the sampling procedure was refined for site-B. Swabs were collected from the external nares only and samples were processed individually, rather than being pooled. As the prevalence was unknown (and therefore set at 50%) and considering a precision of 5% and 95% confidence, a minimum sample size of 385 swabs was required to determine the prevalence of MRSA in the pigs on this farm. Subsequently, nasal swabs were collected from 408 pigs of different stages of production housed in 13 sheds.
Using established protocols for dust sampling, environmental samples were collected and pooled (five swabs in each pool) from all sheds and effluent collection ponds on both farms (EFSA, 2009d). Samples were collected from inside each shed and included walk ways, pens floors, feeders, fences, and walls. Swabs were also collected from the human environment (offices, showers, toilets, and kitchens/sitting rooms).

### 3.2.2 Laboratory analysis

All swabs were transported to the microbiology laboratory on ice within 12 hours of collection and immediately refrigerated upon arrival. Swabs were processed within 24 hours of arriving in the laboratory using the isolation method recommended by the European Union Reference Laboratory for AMR (EURL-AMR) for the testing of MRSA in food-producing animals and food samples (EURL-AMR, 2009, EFSA, 2012).

#### 3.2.2.1 MRSA isolation

Swabs were pre-enriched in Mueller-Hinton broth (BD™ Difco™) containing 6.5% sodium chloride for 24 hours at 37 °C. Post incubation selective enrichment was performed by transferring one ml of pre-enriched broth into Tryptone Soy Broth (CM0129, Oxoid™) containing 3.5 mg/L cefoxitin and 75 mg/L aztreonam and incubating overnight at 37 °C. Following incubation, a loopful of the selective enriched cultured was inoculated onto chromogenic MRSA agar (CHROMagar™ MRSA) and incubated for 24 hours. As per the manufacturer’s instructions, rose to mauve coloured round colonies were preliminarily identified as MRSA. A single presumed MRSA colony from chromogenic agar was plated onto 5% sheep blood agar and incubated for 24 hours at 37 °C. Cultures were subsequently identified as *S. aureus* based on the Gram stain, catalase production and the *S. aureus* Protein-A latex agglutination test (staphylase, Oxoid™). *S. aureus* ATCC 29213 was used as the control strain.

#### 3.2.2.2 Antimicrobial Susceptibility Testing

Antimicrobial disc diffusion susceptibility testing was performed on all 400 *S. aureus* identified. Clinical Laboratory Standards Institute (CLSI) disc concentrations and interpretive criteria were used (CLSI, 2014). Antimicrobials tested were: cefoxitin, tetracycline, erythromycin, amoxicillin-clavulanate, ceftiofur, gentamicin, penicillin, neomycin, ciprofloxacin, chloramphenicol, linezolid, trimethoprim/sulfamethoxazole, vancomycin, teicoplanin, clindamycin, quinupristin-dalfopristin, rifampin, and mupirocin.
Linezolid non-susceptibility was confirmed by minimum inhibitory concentration (MIC) testing using Etest® strips according to the manufacturer's recommendations (bio Mérieux).

3.2.3 Molecular characterisation

Molecular testing was performed on 68 MRSA isolates which included the 31 MRSA isolated from humans, and a subset of the pig (n = 31) and environmental (n = 6) MRSA isolates. Pig and environmental MRSA isolates were selected based on their phenotypic antimicrobial susceptibility profiles to include as much phenotypic diversity as possible. The pig isolates were selected to include at least two isolates from each age group on both sites.

3.2.3.1 nuc and mecA characterization

*S. aureus* species and methicillin resistance were confirmed by the detection of *nuc* (thermostable extracellular nuclease) and *mecA* (methicillin resistance) genes respectively using multiplex PCR (Costa et al., 2005).

3.2.3.2 DNA microarray

Isolates were initially characterised using a *S. aureus* DNA microarray (Alere Technologies, Jena Germany). Arrays and reagents were obtained from Alere Technologies, Jena Germany. The principle of the assay, related procedures, and a list of targets have been described previously (Monecke et al., 2011). The microarray was used to detect the presence of virulence and antimicrobial resistance genes. Probes for *mecA*, *ugpQ*, *xylR*, and two probes for *mecR* were used for SCCmec typing. The last two probes allowed detection and discrimination for untruncated *mecR* and *mecR*, respectively. Probes for the recombinase genes *ccrA1*, *ccrB1*, *ccrA2*, *ccrB2*, *ccrA3*, *ccrB3*, *ccrA4*, *ccrB4* and *ccrC1*; the fusidic acid resistance marker Q6GD50; and the J region proteins, *dcs, plsSCC* and the *kdp*-operon also were included. Ambiguous array results were considered negative.

3.2.3.3 Whole genome sequencing (WGS)

Whole genome sequencing was performed using Illumina MiSeq. Genomic DNA libraries were prepared using the Nextera XT kit (Illumina) and sequenced on the 300-bp pair-ended chemistry. Raw sequences reads were *denovo* assembled using CLC genomics workbench (8.5.1).
3.2.3.4 Multilocus sequence typing (MLST)

DNA sequences collated at http://saureus.mlst.net/ belonging to 3,044 sequence types (ST) were downloaded in FASTA format and used as the database in the SRST2 pipeline to match the corresponding MLST profiles to the Illumina sequence data (Inouye et al., 2014).

3.2.3.5 In-silico virulence and resistance gene characterization

The presence of virulence and resistance genes were confirmed using VirulenceFinder, ResFinder tools from Centre for Genomic Epidemiology (CGE) website (www.genomicepidemiology.org) from the FASTA files generated using Illumina sequencing. Additional virulence factors, resistance gene determinants, beyond those detected by VirulenceFinder and ResFinder were determined using CLC Genomics Workbench.

3.2.3.6 Comparative Genomics

The MRSA identified in this study were compared to previously published MRSA strains. For ST93 the reference genome JKD6159 (NC_017338) was used as a template and aligned against all contigs to determine SNP locations (Chua et al., 2010). The comparative genomic analysis was performed using a comprehensive Australian collection of MRSA ST93 using the 519 SNPs previously reported (Stinear et al., 2014). The comparative genome analysis of MRSA ST398 was performed by aligning the ST398 genomes sequenced in this study against the reference genome HO 5096 0412 (HE681097) (Holden et al., 2013). The reference genome was modified to exclude all phage and intergenic regions. An international collection of MRSA ST398 from a previous study was utilised to perform comparative genomics (Price et al., 2012). SNPs were obtained using Panseq (Laing et al., 2010).

Maximum parsimony phylogenetic trees, based on the SNPs, were generated for both strains using MEGA (V6.06) (Kumar et al., 2016).

3.3 Statistical Analysis

All analyses were performed using the R statistical package (R Core Team, 2015). Data on inhibition zone size for each drug were converted to dichotomous classification of resistant or susceptible based on inhibition zone diameters recommended in the CLSI documents M100-S24 (CLSI, 2014) and VET01-S2 (CLSI, 2013). All intermediate resistance isolates were considered as susceptible. Confidence intervals (CI) were calculated at the 95% level of significance.
3.4 Results

3.4.1 Isolation of MRSA from farm workers, pigs and the environment

MRSA were isolated from 31 (60%) of the 52 farm workers who participated in the study: 10/19 (53%) on site-A and 21/33 (64%) on site-B.

Forty of the 42 pools of pig samples from site-A were MRSA positive. On site-B, the prevalence of MRSA-positive pigs was estimated to be 74% (302/408; 95% CI 70–78%).

Twenty five of the 40 pooled environmental samples were MRSA positive (63%; 95% CI 47–76%). MRSA was isolated from 18 of the 20 sheds (90%; 95% CI 70–97%). All seven sheds on site-A were positive while 11 were positive on site-B. Apart from sheds, the farms’ toilets (2/5), showers (2/4), and kitchens/sitting areas (2/3) were also positive for MRSA. None of the effluent collection ponds or farm-offices grew MRSA.

3.4.2 Molecular characterization of MRSA

The 68 MRSA selected for molecular characterization were mecA and nuc gene positive. Initial characterization of the isolates by DNA microarray identified three MRSA strains: ST93 \( (n = 45) \), ST398 \( (n = 22) \) and ST30 \( (n = 1) \). Forty-four of the 45 ST93 isolates carried a SCCmec IV element. A SCCmec V element was identified in one ST93 and in the ST30 and ST398 isolates.

The 31 human MRSA were identified as either ST93-IV (26 isolates) or ST398-V (5 isolates). Of the 31 pig isolates typed further, 15 were identified as ST398-V, 15 as ST93 (14 harbouring SCCmec-type IV and one with SCCmec-type V), and one as ST30-V. ST93-IV (4 isolates) and ST398-V (2 isolates) were isolated in the environment.

On site-A, all ten MRSA (100%) isolated from the human participants were characterised as ST93. The pig isolates were characterised as ST93 \( (n = 5) \) and ST398 \( (n = 3) \). ST93 and ST398 were also isolated in the environment.

On site-B, 16 of 21 MRSA (76%) isolated from the human participants were characterised as ST93-IV. The remaining five isolates were characterised as ST398-V (24%). Amongst the pig isolates four different MRSAs were characterised: 12 ST398-V, nine ST93-IV, and single isolates of ST93-V and ST30-V. MRSA ST398-V and ST93-IV were also identified in the environment.
3.4.3 Antimicrobial resistance phenotypes and genotypes

The antimicrobial resistance phenotype and clonal association are described in Table 3.1. In addition to the β-lactams, in ST93 and ST398 chloramphenicol, clindamycin, erythromycin, neomycin, and tetracycline, resistance was detected. Quinupristin-dalfopristin and linezolid resistance were also detected in ST398. The ST30 isolate was only resistant to the β-lactams and tetracycline.

All ST93 MRSA carried the chloramphenicol fexA resistant determinant (Table 3.2). Erythromycin (n = 35) and clindamycin (n = 37) resistance was conferred by ermC. For the 12 tetracycline resistant isolates 10 carried either tetL (9 isolates) or tetK (1 isolate).

A large proportion of ST398 were co-resistant to tetracycline, erythromycin, and clindamycin (Table 3.2). Resistance to erythromycin (n = 18) and clindamycin (n = 19) was conferred by ermC (19/22). Tetracycline resistance (n = 22) was conferred by the carriage of the tetM, tetK, and tetL genes. Ten isolates carried the vgaA gene which encodes resistance to lincosamides, pleuromutilins and streptogramin A. The lincosamides resistant isolates also carried the InuB gene. Chloramphenicol resistance (10 isolates) was conferred by the fexA gene and aminoglycoside resistance (11 isolates) by the aadD (2 isolates) and aadE (9 isolates) genes. Three ST398 MRSA harboured the cfr gene cassette; two from the pig samples and one from a human participant. Only the human isolate was phenotypically linezolid resistant (MIC 32 mg/L). Although the dfrG gene was identified in nine isolates, none were phenotypically co-trimoxazole (trimethoprim/sulfamethoxazole) resistant.

The ST30-V isolate was found to carry tetK, tetL and vgaA antimicrobial resistance genes.

3.4.4 Virulence gene data

The carriage of virulence genes was influenced by the MRSA type (Table 3.2). Thirty of the 45 ST93 isolates carried the luk-F-PV/lukS-PV PVL associated genes: Sixteen of the 26 ST93 human isolates; 12 of the 15 pig isolates; and two of the four environmental isolates. Fourteen ST93 (11 from the human participants and three from pigs) also carried the type B (sak+chp+scn) human immune evasion gene cluster (IEC) genes. All ST93 isolates carried enterotoxin-like ORF CM14 gene. Enterotoxins sed (n = 6), seg (n = 7), seh (n = 1) and the enterotoxin gene cluster [egc] (n = 4) was also detected.

None of the ST398-V isolates carried lukf-PV/lukS-PV or IEC genes. Enterotoxins sea (n = 2), sed (n = 4), and toxic shock syndrome toxin-1 tst1 (n = 1) genes were detected.

The ST30-V isolate harboured the egc complex. The isolate was negative for the PVL and IEC associated genes.
3.4.5 ST93 phylogeny

Between the ST93 MRSA isolates very few SNP mutations were detected. The isolates were closely related to the reference genome JKD6159 (NC_017338) and shared nine common SNP markers. Based on the SNPs the isolates could be classified into three clusters (Figure 3.1). There was no relation between the source of the isolates and the cluster.

3.4.6 ST398 phylogeny

The maximum parsimony tree constructed from ST398 isolates showed three clusters suggesting either multiple introductions of ST398 or an ongoing evolution of the strain. When compared to the international collection of ST398 MRSA genomes, the Australian ST398 isolates from the farmworkers and pigs were similar to the ST398 isolates from Europe and North America (Figure 3.2).

3.5 Discussion

This study has investigated the carriage and the molecular characteristics of MRSA isolated from an Australian pig farm spanning two sites that had on-going clinical MRSA infections amongst its farm workers.

The study has shown concurrent carriage of MRSA in humans and pigs on both farms with two predominant strains; the Australian CA-MRSA ST93-IV and the international LA-MRSA ST398-V. Furthermore, whole genome sequencing suggests not only has anthropozoonotic and zoonotic transmission occurred, the movement of ST93-IV from human to pig and then back into humans in the presence of the multi-resistant ST398-V has resulted in the acquisition of multiple antimicrobial resistance determinants, such as those encoding for resistance to tetracycline, clindamycin and chloramphenicol, that are not normally found in ST93-IV.

The three major findings arise from this study are as follow: First, we have identified for the first time in Australia the dissemination of CA-MRSA ST93-IV and LA-MRSA ST398-V amongst farm workers, pigs and the farm environment; Second, we have found, using comparative genome analysis, that ST398-V was likely to have been introduced into Australia from Europe or North America and the ST93-IV pig MRSA isolates came from humans with subsequent pig adaptation; and third, to the best of our knowledge we report the first linezolid resistant MRSA isolated in Australia.

ST93-IV, colloquially known as “Queensland CA-MRSA” was first described in the early 2000s in a population of Caucasian humans in Queensland (Munckhof et al., 2003). Typically, ST93-IV has been associated with a range of skin and soft tissue infections in humans only. A singleton by MLST
eBURST analysis, ST93-IV has been shown to be highly virulent compared to other well-characterised Australian MRSA, including ST1-IV [2B], ST30-IV [2B] and ST239-III [3B], and the epidemic North American strain, USA300 (Chua et al., 2011a). Since 2014 ST93-IV has become the dominant CA-MRSA across Australia (Coombs et al., 2014a), accounting for approximately 40% of CA-MRSA, 25% of MRSA and 5% of S. aureus community-onset infections (Coombs et al., 2012). Consequently, the acquisition of ST93-IV in pigs is likely to have been anthropozoonotic (i.e. human to animal). Although comparative genome analysis showed the porcine and human ST93-IV isolates in our study were closely related to ST93-IV previously characterised in the region (Figure S. 3.1, supplementary data), the isolates had a lower than expected carriage of the bacteriophage encoded PVL-associated luk-F PV and lukS-PV genes. In previous studies up to 100% of ST93-IV have been identified as PVL positive (Coombs et al., 2016, Coombs et al., 2013a). In contrast, one-third of ST93-IV isolates in our study were PVL negative; 39% of farmworker isolates and 20% of pig isolates. Similarly, a lower than expected carriage of the φSa3 prophage-mediated type B human invasion gene cluster (IEC; sak/scn/chp) was observed. Unlike previous studies, which have shown 100% of ST93-IV harbor a type B IEC, the majority of the pig (80%) and the farmworker (58%) ST93-IV isolates lacked an IEC. The loss of the human associated virulence lukF-PV and lukS-PV and sak/scn/chp genes in a large proportion of the ST93-IV isolates suggests the strain has successfully adapted to the porcine host. A similar adaption for S. aureus moving from the human to porcine host has been reported in CC398 (Price et al., 2012).

Contrary to the loss of virulence factors, the ST93-IV identified in our study were phenotypically multi-resistant (including chloramphenicol, clindamycin, erythromycin, neomycin and tetracycline) and harboured multiple resistance genes. Typically, ST93-IV is only betalactam resistant with only up to 20% and 2% of isolates resistant to erythromycin and ciprofloxacin respectively (Coombs et al., 2009, Coombs et al., 2014b, Coombs et al., 2006). It is possible that the observed elevated frequency of resistance to non-betalactam antimicrobials among the ST93-IV isolates in our study is due to selection within the pig environment, linked to antimicrobial use on the pig farm. The anthropozoonotic transmission of the highly successful CA-MRSA ST93-IV in pigs and the acquisition of additional antimicrobial resistance genes and loss of human associated virulence genes indicate the strain has successfully evolved in the porcine host. This successful evolution may lead to the co-selection, long term maintenance, and further adaptation of ST93-IV in pigs and in the piggery environment (Dahms et al., 2014).

Prior to our study, ST398-V has only been reported in Australia on two occasions (Groves et al., 2016, Groves et al., 2014). In our study, 22 isolates were identified as ST398-V (representing 23% of MRSA isolates from farmworkers and also isolated from pigs and the environment). Previous
studies have distinguished the livestock-associated \textit{S. aureus} ST398 clade from the human clade by the presence of the \textit{tetM} gene and the absence of IEC (Stegger et al., 2013). As the ST398-V isolated in our study lacked an IEC and a high proportion carried the \textit{tetM} gene it can be assumed the isolates belong to the livestock clade (Stegger et al., 2013). Comparative phylogenetic analysis suggests the ST398-V was introduced into Australia from Europe or North America (Stegger et al., 2013). The identification of three ST398-V clusters suggests either multiple introductions into Australia have occurred or the strain has diverged within the farm environment. However, as one of the clusters (cluster 3 versus clusters 1 and 2) differed by more than 34 SNPs over a short period it is more likely that both events have occurred (Figure S. 3.2, supplementary data).

The importation of CC398 into a country previously free of CC398 and the subsequent transmission into the country’s pig population followed by zoonotic spread is not unique (Cuny et al., 2015c). In Europe, the spread of LA-MRSA between countries is often mediated by animal trading of piglets sold by specialised producers (Broens et al., 2011d). However, in Australia, the importation of live pigs ceased over 40 years ago. Consequently, it is possible that the introduction of ST398-V into Australia has occurred from pig farmers or pig veterinarians who have had contact with European or North American pig farms, or through the entry of other animal species, such as horses, where the same movement restrictions are not present.

Although \textit{S. aureus} does not cause much illness in pigs, CC398 MRSA does cause a wide spectrum of infections in humans, ranging from relatively minor or localised infections to more serious or invasive infections (Verkade and Kluytmans, 2014). Furthermore, nosocomial infections and outbreaks have occurred associated with this MRSA. However, similar to other \textit{S. aureus}, transmission of CC398 MRSA is primarily mediated by physical contact and overall relatively few cases of CC398 MRSA have occurred in people who are not directly involved in livestock production. The low prevalence of CC398 MRSA in people not associated with pig farming is probably due to the low transmissibility of the organism (Verkade et al., 2014). Consequently, the clinical significance of CC398 MRSA within the community is thought to be low. However, the emergence of ST398 in the Australian pig industry is a public health concern for those working within the industry. Although ST398 MRSA is less virulent and less transmissible compared to other \textit{S. aureus}, the organism exhibits co-resistance to many non-β-lactam antimicrobials used in medical and veterinary practice including macrolides, tetracycline, trimethoprim, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, lincosamides and streptogramin B (Cuny et al., 2015c, Verkade and Kluytmans, 2014). Although ST398 remains susceptible to the glycopeptides, daptomycin, tigecyclines, fusidic acid and rifampicin, isolated cases of linezolid resistance have been reported (Cuny et al., 2015c). In ST398 MRSA, linezolid resistance is due to the acquisition of a plasmid harbouring the \textit{cfr} gene.
which in addition to linezolid resistance mediates resistance to lincosamides, fencols and pleuromutilins (Witte and Cuny, 2011). The selective pressure in favour of the spread of the cfr containing plasmid may be influenced by the use of linezolid in human medicine and florfenicol and tiamulin in veterinary medicine. The linezolid resistance identified in a ST398 MRSA isolated from a human in this study is the first instance of linezolid resistance in MRSA to be reported in Australia. Although multidrug resistance may compromise the treatment of ST398 MRSA infections, the reservoir of resistance genes harboured by ST398 MRSA is more concerning from a clinical standpoint. This concern was recently highlighted by Brennan et al. following the introduction of CC398 into the Republic of Ireland’s pig industry who suggested that the reservoir of resistance genes harboured by the less virulent and less transmissible CC398 MRSA could potentially spread to other animal and human strains (Brennan et al., 2016). Unfortunately, this may have occurred in Australia.

It appears from our study the movement of the highly virulent and highly transmissible ST93-IV CA-MRSA from humans to pigs and the importation of ST398-V LA-MRSA into the Australian pig farms has resulted in the emergence of a multi-resistant PVL-positive ST93-IV strain which continues to harbour a type B IEC. However, a comprehensive molecular comparison of the antimicrobial resistance genes and the associated mobile genetic elements found in the ST93-IV and ST398-V is required. Furthermore, the fitness cost to ST93-IV following the acquisition of the additional genetic material will need to be determined in order to assess the human health risk, if any, to those animals and humans not exposed to the particular pig herds investigated in this study.

### 3.6 Conclusion

In conclusion, our study has demonstrated for the first time the widespread occurrence of the highly virulent Australian ST93-IV CA-MRSA and the international ST398-V LA-MRSA strains in an Australian piggery. Furthermore, the evidence suggests that anthropozoonotic and zoonotic transmission may have occurred. The presence of multiple antimicrobial resistance determinants that are not usually found in ST93-IV could be a consequence of the transmission of ST93-IV from humans to pigs and then back into humans in the presence of the multi-resistant ST398-V. The outcomes of this study indicate that in Australia, and probably elsewhere, greater scrutiny of staphylococcal infections in humans and animals is warranted in the form of coordinated investigation of outbreaks to identify the factors predisposing each host species to infection and emergence of virulent pathogens with novel resistance traits.
Table 3.1 Number and proportion of each methicillin-resistant *Staphylococcus aureus* clone resistant to non-β-lactam antimicrobials

<table>
<thead>
<tr>
<th>Clone</th>
<th>CHL</th>
<th>CIP</th>
<th>CLI</th>
<th>ERY</th>
<th>GEN</th>
<th>LNZ</th>
<th>MUP</th>
<th>NEO</th>
<th>QD</th>
<th>RIF</th>
<th>SXT</th>
<th>TEC</th>
<th>TET</th>
<th>VAN</th>
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<tbody>
<tr>
<td>ST398</td>
<td>10(45.45)</td>
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<td>19(86.36)</td>
<td>18(81.82)</td>
<td>0(0)</td>
<td>1(4.54)</td>
<td>0(0)</td>
<td>1(4.54)</td>
<td>8(36.36)</td>
<td>0(0)</td>
<td>0(0)</td>
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<td>22(100)</td>
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<tr>
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<td>35(77.78)</td>
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CHL (chloramphenicol), CIP (ciprofloxacin), CLI (clindamycin), ERY (erythromycin), GEN (gentamicin), LNZ (linezolid), MUP (mupirocin), NEO (neomycin), QD (quinupristin-dalfopristin), RIF (rifampin), SXT (trimethoprim/sulfamethoxazole), TEC (teicoplanin), TET (tetracycline), VAN (vancomycin)
Table 3.2 Molecular characteristics and phenotypic antimicrobial resistance profile of MRSA isolated from farmworkers, pigs, and environment

<table>
<thead>
<tr>
<th>No</th>
<th>Resistance genes</th>
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<th>IEC</th>
<th>PVL</th>
<th>Phenotypic antimicrobial Resistance</th>
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<tr>
<td><strong>Human Isolates (31 isolates)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>ST398-V (5 isolates)</td>
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<td>blaZ,ermC,tetK,tetM,norA</td>
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<td>-</td>
<td>-</td>
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<td>H23</td>
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<td>-</td>
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<td><strong>ST93-IV (26 isolates)</strong></td>
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<td>ORFCM14</td>
<td>-</td>
<td>+</td>
<td>BLA,ERY,CHL,CLI</td>
</tr>
<tr>
<td>H3</td>
<td>blaZ,ermC,fexA,norA</td>
<td>ORFCM14</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
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<td>+</td>
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<tr>
<td>H14</td>
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<td>ORFCM14</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>+</td>
<td>-</td>
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H24  \(\text{blaZ,ermC,fexA,fosB,norA,tetL}\)  seg, egc ORFCM14  +  +  BLA,TET,ERY,CHL,CLI
H25  \(\text{blaZ,ermC,fexA,norA}\)  ORFCM14  -  -  BLA,ERY,CHL,CLI
H26  \(\text{blaZ,ermC,fexA,norA}\)  ORFCM14  +  -  BLA,ERY,CHL,CLI
H27  \(\text{blaZ,ermC,fexA,norA}\)  ORFCM14  -  +  BLA,ERY,CHL,CLI
H29  \(\text{ermC,fexA,norA}\)  ORFCM14  +  +  BLA,ERY,CHL,CLI
HW-24  \(\text{blaZ,fexA,norA}\)  ORFCM14  +  +  BLA,CHL
H31  \(\text{blaZ,fexA,norA}\)  ORFCM14  +  -  BLA,ERY,CHL,CLI

**Pig Isolates (31 isolates)**

**ST30-V(1 isolate)**

W1Bb-25  \(\text{blaZ,norA,vgA,tetL,tetK}\)  seg, sei, sem, sen, seq, seu, egc  -  -  BLA,TET

**ST389-V (15 isolates)**

PTDrAP2  \(\text{aadD,blaZ,norA,vgA,ermC,lnuB,cfr,fexA,tetK,tetM,dfrG}\)  -  -  -  BLA,TET,ERY,CHL,CLI,QD
PTWeP5  \(\text{aadE,blaZ,norA,ermC,tetM,tetK,tetL}\)  sed  -  -  BLA,TET,ERY,NEO,CLI
PTGrBP1  \(\text{aadE,norA,lnuB,cfr,vgA,ermC,fexA,tetM,tetK,dfrG}\)  -  -  -  BLA,TET,ERY,CHL,CLI,QD
W1FPa-4  \(\text{blaZ,norA,ermC,tetK,tetM}\)  -  -  -  BLA,TET
W1FSpb-2  \(\text{blaZ,norA,tetK,tetM}\)  sed  -  -  BLA,TET
W1FPPB-20  \(\text{aadE,blaZ,norA,ermC,lnuB,vgA,fexA,tetK,tetM,dfrG}\)  -  -  -  BLA,TET,ERY,CHL,CLI,QD
W1Gr-12  \(\text{blaZ,norA,ermC,tetK,tetM}\)  sed  -  -  BLA,TET,ERY,CLI
WweU-9  \(\text{aadE,blaZ,norA,ermC,lnuB,vgA,fexA,tetK,tetM,dfrG}\)  -  -  -  BLA,TET,CHL
P216  \(\text{ermC,vgaA,tetK,lnuB}\)  -  -  -  BLA,TET,ERY,CHL,CLI,QD
P221  \(\text{blaZ,ermC,tetK,tetM,norA}\)  -  -  -  BLA,TET,ERY,CLI
P223  \(\text{blaZ,ermC,tetK,tetM,norA}\)  sea  -  -  BLA,TET,ERY,CLI
P236  \(\text{tetK,tetM,norA}\)  sea  -  -  BLA,TET,ERY,CLI
P244  \(\text{blaZ,ermC,vgaA,tetK,tetM,fexA,norA,aadE,dfrG,lnuB}\)  -  -  -  BLA,TET,ERY,CHL,CLI,QD
<table>
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<td>-</td>
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<td>P300</td>
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<td>-</td>
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**ST93-IV (14 isolates)**

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<td>PTGpP1</td>
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<td>sed, seg ORFCM14</td>
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**ST93-V (1 isolate)**

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**Environmental Isolates (6 isolates)**

**ST93-IV (4 isolates)**

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<tr>
<td>E021</td>
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**ST398-V (2 isolates)**

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<th>seg, egc ORF CM14</th>
<th>BLA,CHL,CLI</th>
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</table>
Figure 3.1 Phylogenetic tree constructed by core genome SNPs of MRSA ST93 isolated from humans, pigs and piggery environment. Each circle represents individual MRSA clone. Isolates from humans, pigs, and environment were identified by green, red and purple colours respectively. Isolates that did not carry pvl gene were notified by a black bar in the centre.
Figure 3.2 Phylogenetic tree constructed by core genome SNPs of MRSA ST398 isolated from humans, pigs, and piggery environment from this study compared to isolates from different parts of the world. Each circle represents individual MRSA clone. Isolates from pigs, human, turkey meat, cattle, environment and horses identified by green, red, purple, orange, blue and dark green colours respectively. Isolates were coded by the country of origin and year in which they were isolated. Isolates from this study is coded as AUS (2015).
Supplementary data

Figure S. 3.1 Phylogenetic tree constructed by core genome SNPs of MRSA ST93 isolated from humans, pigs, and piggery environment from this study compared to isolates from different parts of Australia. Isolates form this study is represented by a red circle.
Figure S. 3.2 Phyllogenetic tree constructed by core genome SNPs of Australian MRSA ST398 isolated from humans, pigs and piggery (blue) and compared to international isolates. Isolates are divided into 3 clusters designated as I, II and III.
Acknowledgements

This study was funded by Australian Pork Limited (Project number 2015/013). The authors would like to acknowledge Mrs Michelle Ayton, Senior Technical Officer at Charles Sturt University and other laboratory members for their tremendous help in microbiological analysis. We are indebted to the producer who allow us to collect swabs from their farm. The authors would like to thank the workers who participated in this study. The authors would also like to acknowledge Ms Sharon Nielsen and Dr David Luckett for their assistance in R. We are grateful to Dr Muhammad Shoaib Tufail for his help in sampling.

Author contributions statement

S.S. designed the study and conceived the experiments, analysed the data and results. J.H., M.H., and D.J., designed and supervised the whole study. S.A., S.P., and G.C conducted the molecular work. S.S., S.A., G.C., and J.H. drafted the manuscript. All authors reviewed and approved the final manuscript.

Competing financial interests

The authors declare no competing financial interests. All authors: No potential conflicts of interest.
Chapter 4.

Prevalence and antibiotic resistance of MRSA across different pig age groups in an intensive pig production system in Australia
Abstract

Aim

This study aims to determine MRSA prevalence at the pig level and within different age cohorts of pigs at two sites of a pig enterprise which was the subject of an MRSA outbreak in piggery workers. This study also investigates the association of MRSA strain carriage and antibiotic resistance with different pig age groups.

Methods

A total of 658 samples were collected from pigs (n = 618) and from pig the environment (n = 40) on two sites (site-A and site-B) of a single pig enterprise in 2015. The pig samples were collected at both sites from five different pig age groups. Presumptive MRSA isolates were subjected to sensitivity of 18 different antibiotics using disc diffusion method. Presumptively identified MRSA were also subject to MALDI-TOF to confirm their identification. Confirmed S. aureus isolates with presumptive methicillin resistance were subjected to RT-PCR for the presence of the meca gene, PVL genes, and sequence types (ST398 and ST93).

Results

Three quarters (75.2%) of samples collected in pigs were identified as MRSA, and 71% of the MRSA isolates returned as Community-Associated (CA)-MRSA ST93 and 29% as Livestock-Associated (LA)-MRSA ST398. Amongst environmental isolates only 20% were typed as ST398 and the remainder were ST93. All MRSA isolates from pigs and the environment were susceptible to ciprofloxacin, gentamicin, linezolid, mupirocin, rifampicin, sulfamethoxazole-trimethoprim, teicoplanin, and vancomycin. Resistance to penicillin (100%), clindamycin (97.6%), erythromycin (96.3%), ceftiofur (93.7%), chloramphenicol (81.2%), tetracycline (63.1%), and amoxicillin-clavulanate (AMC) (63.9%) were common among the MRSA isolates. A low prevalence of resistance (9.2%) was observed against neomycin and quinupristin-dalfopristin (QD). Multi-drug resistance was observed in 98% (481/490) of the MRSA isolates. A significantly higher resistance was observed for ST93 in comparison with ST398, against AMC, ceftiofur, chloramphenicol, erythromycin, and neomycin; but lower resistance was observed to QD, and tetracyclines.

The probability of MRSA carriage was found to be significantly higher ($P < 0.001$) in farrowing sows (76.8%), weaners (97.8%), growers (94.2%), and finishers (98.3%) when compared to dry sows (42.2%). Amongst different production age groups, a significant difference was also found in
antibiotic resistance for AMC, neomycin, chloramphenicol, and tetracycline, with weaner pigs showing higher probability of exhibiting resistance against these antibiotics than other age groups. Weaners were identified as having a significantly higher ($P < 0.001$) carriage of ST93 compared to any other age group.

**Conclusion**

This study identifies a high prevalence of CA-MRSA ST93 carriage in pigs in an establishment where MRSA disease was occurring in humans. A significant association exists between pig age-group and MRSA carriage. An association between pig age groups and antibiotic resistance was also observed which is possibly linked to management practices including antimicrobial use in different pig production stages.
4.1 Introduction

*Staphylococcus aureus* is an opportunistic pathogen of humans and some livestock that causes skin and soft tissue infections with potential to progress to fatal bacteraemia. Methicillin resistant forms of *S. aureus* (MRSA) have become increasingly common in humans and are regarded as a serious threat to the health of individuals and the community. Presence of mechanisms coding for methicillin resistance in *S. aureus* is unwanted because this also prevents all other betalactam drugs from exerting their lethal effect; thus requiring reliance in human medicine on higher-importance drugs such as vancomycin. MRSA can also be carried by livestock species, including pigs, and is commonly referred to as Livestock Associated (LA) MRSA. Clonal complex (CC) 398 is the most frequently documented LA-MRSA (Butaye et al., 2016, Crombé et al., 2013). The majority of strains in CC398 belong to ST398 and comprise different *spa* types such as t011, t034, and t108 (Köck et al., 2013). ST398 is reported to be resistant to fewer non-betalactam antibiotics compared to non-ST398 strains (Mutters et al., 2016), though a higher resistance to macrolides, lincosamides and tetracycline is reported (Conceição et al., 2017).

Recently, a high carriage of MRSA was identified on two different sites of a single pig enterprise in Australia associated with an ongoing MRSA outbreak in piggery workers (Chapter 3, referred here and onward as Sahibzada et al., 2017). In this investigation, two predominant MRSA strains, ST398 and ST93, were identified in pigs and humans. ST93 is a highly prevalent form of Community Associated (CA) MRSA in Australia that had not previously been reported in pigs. The initial investigation of the outbreak involved the study of only a subset of isolates from pigs (*n* = 31) and the environment (*n* = 6) and did not attempt to investigate in detail the features of the epidemiology of MRSA in the affected herd. Thus, the current study represents an expansion of the initial investigation and aims to examine the prevalence of MRSA and specific strain carriage in different age groups of pigs at the two sites.

4.2 Methods

4.2.1 Farm topography and sampling

The pig enterprise had two sites referred herein as site-A and site-B. The two sites were geographically separated by approximately 40 km with workers and pigs moving between sites. Replacement pigs are usually brought in to site-A; however, they are also move to site-B. The distribution of pigs was similar on both sites, and the number of animals varied between 1,000 and 3,000 pigs per shed. Pigs were housed in separate sheds based on their age group and were classed as dry sows (breeding stock), farrowing sows, weaners, growers, and finishers. There were a total of 20
sheds across both sites. Site-A had seven sheds: two housed dry sows, one farrowing sows, one weaners, two growers, and one finishers. Site-B had total of 13 sheds: four housed dry sows, four farrowing sows, two weaners, two growers and one finishers. In all sheds, the pigs were randomly chosen in conveniently selected pens, and animals that had been sampled were identified with a coloured marker to avoid re-sampling. In the farrowing sheds, a sow from a randomly selected crate was swabbed, and at the same time, nasal swabs were collected from a randomly chosen piglet in the same crate.

In the study reported previously, different sampling techniques were used on these sites as explained in Sahibzada et al. (2017). In summary, a pooled sampling method was used on site-A, whereas individual animal samples were processed in the laboratory for site-B. In the current study, the entire set of individual pig samples collected during the initial investigation from site-B were included while all individual samples were processed for site-A. On site-B, a total of 408 samples were collected from 13 sheds with 30 samples per shed except for a farrowing sow shed where 48 samples were collected as explained in Sahibzada et al. (2017). This particular farrowing sow shed had eight different farrowing rooms, so six nasal swabs from pigs were collected in each room resulting in 48 swabs. For site-A, the swabs were collected in duplicate from 60 individual animals in seven different sheds. One set of swabs were analysed in the lab with in 24 hours of collection while the second set of samples were stored at -80 °C. Later on, a total of 30 samples out of the 60 nasal swabs were systematically chosen (samples with odd numbers) from each shed, resulting 210 samples for site-A.

4.2.2 Laboratory analysis

MRSA were isolated using the method previously described by Sahibzada et al. (2017). In brief, pig samples were pre-enriched in Mueller-Hinton broth containing 6.5% sodium chloride for 24 hours at 37°C. The five environmental samples per shed were pooled in 50 ml of the pre-enriched broth. Afterwards, one ml broth from the pre-enriched culture was inoculated into nine ml Tryptone Soy Broth supplemented with 3.5mg/l cefoxitin and 75mg/l aztreonam and incubated overnight at 37°C. Subsequently, a loopful was streaked onto chromogenic MRSA agar and incubated for next 24 hours at 37°C. Ultimately, presumptive MRSA colonies were transferred on to sheep blood agar plates, and a latex agglutination test was performed with Staphylase (Oxoid™) kit. S.aureus ATCC 29213 was used as the reference strain. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was also performed on all isolates to confirm the S.aureus species identification. For MALDI analysis a pin point colony of a fresh overnight culture grown on sheep blood agar at 37°C was spotted on a steel MALDI plate (MSP 96 target polished steel BC, Bruker Daltonics) to make a thin smear. Afterwards, a drop of one µl of the MALDI matrix was deposited
onto each sample spot of the MALDI target plate and allowed to air dry. The MALDI matrix was prepared as per manufacturer instruction by dissolving one µg of the HCCA (α- Cyano- 4-hydroxycinnamic acid) per ml of solution of acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%. A comparative analysis of *Staphylococcus aureus* was performed on microflex LT (Bruker Daltonics) using MALDI BioTyper software and a score of >2 generated by the MALDI was considered as confirmed species.

Confirmed *S.aureus* isolates with presumptive methicillin resistance were subjected to antibiotic susceptibility testing by the disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) protocols (CLSI, 2014), and involved the following 18 antibiotics: amoxicillin-clavulanate (AMC), ceftiofur, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, mupirocin, neomycin, penicillin, quinupristin-dalfopristin (QD), rifampin, tetracycline, teicoplanin, sulfamethoxazole-trimethoprim (SXT), and vancomycin.

### 4.2.3 Molecular typing

#### 4.2.3.1 DNA extraction

DNA was extracted using 6% chelex as previously described in Abraham et al. (2012) with a minor modification. Briefly, a loop full of bacteria taken from freshly cultured blood agar was suspended in 100µl of molecular grade water and centrifuged at 5000RPM for two minutes. The supernatant was removed followed by addition of 100µl of 6% chelex matrix. Subsequently, the chelex mixture with bacterial colonies was incubated at 56°C for 20minutes then 100°C for 5minutes. The mixture was spun at 2040g (RCF) for five minutes at room temperature, and the supernatant was used in the PCR amplification. DNA quantification was performed using a Qubit HS DNA assay kit (Invitrogen) on a Qubit-3 Fluorometer.

#### 4.2.3.2 Performing real-time PCR

All MRSA isolates were screened for *mecA* and PVL (Panton-Valentine Leukocidin) genes by singleplex real-time polymerase chain reaction (RT-PCR). A lineage-specific singleplex RT-PCR was also used for detection of ST93 and ST398. A list of primers and probes used in this study is given in Table 4.1. TaqMan® Fast mastermix probe-based PCR reagents were used for detection of *mecA* genes and strain type ST398 whereas SYBR® Green reagents assay based PCR were used for the detection of PVL gene and strain type ST93. The primers for the ST93 assay consists of two reverse primers aroE252G and aroE252T differing by only one nucleotide at the 3’ end. Using both reverse primers with the same forward primer in parallel allow differentiation between ST93 and non-
ST93 isolates. The aroE252G is an ST93 specific primer that appears at least ten cycles earlier than non-ST93 isolates on the amplification curve when compared with the aroE252T amplification curve. The MRSA isolates that were previously characterised by whole-genome sequencing for strain typing and detection of PVL genes by Sahibzada et al. (2017) were used as a control for comparison.

The RT-PCR SNP assay was made to a total volume of 10 µl and was amplified on the QuantStudio™ 6 Flex Real-Time PCR system using 96-well plate template setup. For each reaction the assay contained forward and reverse primer at a concentration of 0.5 µM, 2 µl of nuclease-free water, 5 µl master mix (PowerUp SYBR™ Green Master Mix), and 2 µl of DNA template. The ST398 and mecA assays were probe based and consisted of primers at a final concentration of 0.9 µM, 1 µl of nuclease-free water, 5 µl master mix (TaqMan® Fast Advanced Master Mix), probe at a final concentration of 0.2 µM, and 2 µl of DNA template.

The PCR condition for SYBER green assay was as follows. The initial hold stage was set for 2 min at 50 °C followed by another 2 min at 95 °C (for enzyme activation). The PCR stage was setup for 40 cycles of three-temperature cycling consisting of 95 °C for 15 sec (for denaturation), 50 °C for 15 sec and 72 °C for one min (for annealing and polymerization). The third stage (melt-curve) was used to confirm the specificity of the amplification products. The melt-curve stage was set at 90 °C for 15sec, 60 °C for 1min, and 95 °C for 15sec.

The PCR conditions for the probe based assay consisted of an initial hold stage of 2 min at 50 °C and 20 sec at 95 °C (polymerase activation), 40 cycles with one sec at 95 °C (denature) followed by 20 sec at 60 °C (annealing/extension).

**4.2.3.3 Staphylococcus Protein A typing**

A subset of MRSA isolates \( n = 11 \) were characterised by the *Staphylococcus Protein A* (*spa*) gene typing as previously described (Harmsen et al., 2003) using whole gene sequence data. The *spa* typing was performed by amplifying and sequencing the variable repeat region of the *spa* gene, which was then compared with the *spa* sequences according to the Ridom web server (http://www.spaserver.ridom.de/).

**4.2.4 Statistical analysis**

All analyses were performed using the R statistical package (R Core Team, 2017). Data on inhibition zone size for each drug were converted to dichotomous classification of resistant (R) or susceptible (S), as recommended in the CLSI documents M100-S24 (CLSI, 2014) and VET01-S2 (CLSI, 2013). All intermediate resistance isolates were considered as susceptible. Isolates resistant to at least one
betalactam and two non-betalactam antibiotic classes were categorized as multidrug-resistant (MDR). Kruskal-Wallis test was used to assess significant differences between MDR and pig age groups. Mixed GLM models were used to assess associations between MRSA carriage (modelled as dependent variable) and pig age groups (independent variable) with site included as a random effect. The association between antibiotic resistance and pig age group was assessed by including each phenotype as a dependent variable and using separate univariable GLMs. The odds ratio and confidence intervals for each antibiotic resistance was calculated from GLM models. Plotting was done in the statistical environment R using the package ggplot2 for a graphical display (Wickham, 2016).

4.3 Results

4.3.1 Overall MRSA prevalence

Overall, MRSA was isolated from 490 out of 658 samples from pigs and the environment. In pigs, a prevalence of 75.2% (465/618; 95% CI 71.8-78.6) was found across both sites. There was no significant difference ($P = 0.27$) in proportion of MRSA positive pigs between sites; there being 77.6% (163/210; 95% CI 71.9-83.2) positive on site-A and 74.0% (302/408; 95% CI 69.7–78.2) on site-B. A majority of MRSA isolates ($n = 351/490, 71.6\%$) were found to be ST93, and 138 were identified as ST398, on RT-PCR. Only one isolate from site-A could not be typed using the methods outlined in this study but was identified as ST30 using whole genome sequencing in the previous study (Sahibzada et al., 2017). Most of the ST93 isolates (68.9%) were positive for the PVL gene while all ST398 were negative. No significant difference ($P = 0.5$) was found between sites for PVL carriage amongst ST93. A higher proportion of ST93 ($n = 130, 79.8\%$) was found on site-A compared to site-B ($n = 201, 66.6\%$). Using the univariable model the odds of ST93 carriage amongst pigs was estimated significantly higher for site-A compared to site-B (OR 1.93, CI 1.25-3.02, $P = 0.003$).

Four different spa types were identified amongst 11 selected MRSA isolates. The spa types were t011 ($n = 4$), t2510 ($n = 2$), t202 ($n = 4$) and one unknown type. The spa type t202 belonged to ST93, and the remainder were associated with ST398. The typing performed on the 25 environmental isolates identified twenty ST93 and five ST398.

4.3.2 MRSA prevalence amongst different pig production age groups

MRSA was isolated from all pig age groups and shed level prevalence estimates ranged from 42.0% to 98.0% on both sites. The distribution and proportion of overall MRSA and the strain carriage across five different age-groups is shown in Figure 4.1. A significant association between MRSA prevalence and pig age-group was identified ($P <0.001$). Overall, for both sites, the lowest proportion of MRSA
carriage was identified in dry sows (42.2%) when compared with farrowing sows (76.8%), weaners (97.8%), growers (94.2%), and finishers (98.3%). A significant association was also found between pig age group and MRSA strain type (P < 0.001). In comparison with the weaner group (within which 89% of the MRSA was typed as ST93) a lower risk of ST93 carriage was found amongst dry sows (60%, OR 0.19, CI 0.08-0.42), farrowing sows (67%, OR 0.26, CI 0.12-0.53), growers (69%, OR 0.29, CI 0.13-0.60), and finishers (75%, OR 0.38, CI 0.15-0.90). Within the farrowing group, there was no significant difference (P = 0.38) of MRSA carriage between the farrowing sows and piglets. No statistical difference was noted between sites and overall MRSA or strain carriage in different age groups. The distribution of MRSA carriage in different age groups on each site is shown in Supplementary Data Figure S. 4.1.

4.3.3 Overall antibiotic resistance profile

None of the 490 MRSA isolates were resistant to ciprofloxacin, gentamicin, linezolid, mupirocin, rifampicin, trimethoprim/sulamethoxazole, teicoplanin, and vancomycin. A low frequency of resistance was identified to neomycin (9.1% CI, 6.5-11.7) and QD (9.3% CI, 6.7-11.9). Two thirds of the MRSA isolates were resistant to AMC (63.8% CI, 59.5-68.0) and tetracycline (63.8% CI, 59.5-68.0). Chloramphenicol resistance was observed in 80.9% (CI 77.4-84.4) of the isolates. Over 90% of the total MRSA isolates were non-susceptible to ceftiofur (93.6% CI, 91.4-95.8), erythromycin (96.5% CI, 94.8-98.1), clindamycin (97.7% CI, 96.4-99.0), and penicillin (100%).

The proportion of resistance amongst ST398 and ST93 is plotted in Figure 4.2. A significantly greater proportion of resistance was observed for ST93 in comparison with ST398 against AMC (P < 0.001), ceftiofur (P < 0.001), chloramphenicol (P < 0.001), erythromycin (P = 0.03), and neomycin (P = 0.03). In contrast, a lower proportion of resistance was observed against QD (P < 0.001), and tetracyclines (P < 0.001) for ST93 compared with ST398. No significant difference was found for antibiogram profile between pig and environmental isolates (shown in Supplementary Data Figure S. 4.2). The distribution of antibiotic phenotype amongst MRSA isolates for both sites is displayed in Figure S. 4.3.

A total of 98% (481/490) of the MRSA isolates were observed to exhibit MDR. A total of 11 (2.4%) isolates were resistant to one or two non-beta-lactam antibiotics, 267 (54.5%) to three, 128 (26.1%) to four, 79 (16.1%) to five, and four (0.8%) isolates were resistant to six of the non-beta-lactam drugs in the test panel. Eleven different resistance patterns to multiple antibiotics were obtained among MRSA isolates as shown in Table 4.2. A significant difference (P < 0.001) was found between MDR pattern and pig age-group. On average, weaner isolates were significantly (P < 0.001) more likely to be resistant to a larger number of non-beta-lactam antibiotics (median=4, ranging 1-6) compared to the
other production age groups. Amongst ST93 isolates, the most common pattern of multiple resistance (50.1%) to non-beta-lactam group was a combination of chloramphenicol, clindamycin, and erythromycin. The majority of ST398 isolates (60.1%) were resistant to a combination of tetracycline, clindamycin, and erythromycin.

Amongst different production age groups, a significant difference ($P < 0.001$) in antibiotic resistance was found for AMC, chloramphenicol, neomycin, and tetracycline. Weaners in comparison with other age groups presented a higher risk for exhibiting resistance against these antibiotics as shown in Table 4.3.

4.4 Discussion

4.4.1 Overall MRSA prevalence

This is the first comprehensive study describing the distribution and frequency of MRSA prevalence, including different strain carriage and antibiotic resistance in different pig age groups in a piggery in Australia. A major strength of this study is the extensive sampling in pigs of all ages raised under the same management practices, ensuring minimal variability of MRSA carriage due to management factors. Additionally, the work includes extensive data on the behaviour of a common CA-MRSA that, in this instance, has moved from humans into pigs and this information appears to be unique in the literature. The overall prevalence of MRSA carriage in pigs was just over 75%, which is consistent with reports from European and USA studies, which have identified up to 85% MRSA carriage in pigs (Morcillo et al., 2015, Dewaele et al., 2011, Smith et al., 2009), although that is typically only involving LA-MRSA In Australia, MRSA in pigs was first reported in 2014 (Groves et al., 2014), where 324 samples were collected from five different commercial pig farms and one feral herd, resulting in only three samples from a single farm being MRSA positive (and further typed as ST398). The majority of the MRSA isolates found in the current study (71.6%) were typed as ST93. The ST93 is the predominant CA-MRSA in Australia, which has been reported to be to the most virulent strain amongst CA-MRSA, including ST8 (USA300) (Chua et al., 2011b). One third of the ST93 (31%) isolates did not have PVL encoding genes. In previous reports involving isolates procured directly from human cases, up to 100% of ST93 has been reported as PVL positive (Coombs et al., 2016, Harch et al., 2017). The presence of PVL, regarded as a virulence factor in human isolates, is used as a marker to distinguish CA-MRSA from other clades. The loss of PVL among a large proportion of ST93 in this scenario indicates potential adaptation of this clone to the new porcine host and its environment – a phenomenon which is also previously described by Sahibzada et al. (2017).
The *spa* typing performed on selected MRSA isolates shows three different types. For ST93, only t202 was found, which is the predominant *spa* type hitherto associated with this clone in Australia (Coombs et al., 2012). In ST398, two different *spa* types (t011, t2510) and an unknown type were found. Both t011 and t2510 are associated with ST398 in Europe (EFSA, 2009a), with t011 being one of the most frequently reported (Broens et al., 2011b, Crombé et al., 2012b). The *spa* type t011 found in this study is considered to have decreased affinity for carriage and infection in humans (Ballhausen et al., 2014).

### 4.4.2 Prevalence in different production age

Our results show that dry sows are less likely to carry MRSA (42.2%) compared to the other stages of production. Broens et al. (2012a), observed similar results, when conducting a longitudinal study aiming to estimate MRSA transmission rates using six pig herds in two different countries. In the study published by Broens and colleagues (2012) in Germany, a lower carriage in dry sows (33.3%) compared to farrowing sows (77.3%), weaners (79.6%), growers (86.6%) and finishers (69.6%) was observed. Similarly, Dorado-Garcia et al. (2015) found a significantly (*P* < 0.001) lower risk of MRSA carriage among dry sows compared to other age groups. The association of MRSA carriage with age observed in this study and other reports could be due to specific management factors. For example, dry sows, recognised for the lowest MRSA carriage are usually maintained at low stocking density with less antibiotic exposure (Postma et al., 2016, van Rennings et al., 2015) compared to other pig age groups, which might contribute to the lower carriage of MRSA in this production pig group. In contrast, weaners and growers were found to have a high MRSA carriage in this and other studies. While no data are available for comparison on antibiotic usage in different production stages in Australia, a study from Germany, reported a much higher proportion of total antibiotic usage among weaners (32%) and growers (40%) in comparison with piglets (16%) and dry sows (12%) (van Rennings et al., 2015). Weaners are likely to carry MRSA from the suckling stage that they acquired either from contaminated environment or from their MRSA positive mother (horizontally as well as vertically) (Verhegghe et al., 2013, Moodley et al., 2011a) with a tendency to carry onto the next production stages (Weese et al., 2011). In addition, weaning is recognised as the most stressful event in a pig’s life due to a series of demanding changes, including nutritional, social (mother separation), and physiological factors, which can negatively impact pig health, growth and feed intake (Campbell et al., 2013). These events that make pigs sensitive to bacterial infections subsequently attract the most antibiotic usage compared to any other stage (Sjölund et al., 2016).

Assessing the relationship between strain carriage and different age groups was conducted in the current study and weaners were found to be more likely to carry ST93 in comparison with the other
production age groups. Although the reason for this association is not clear, it could be due to selection pressure exerted by pattern of antibiotic use in this cohort. For example, a study analysing the genomic and phenotypic traits of a highly prevalent HA-MRSA strain ST239 in Australia revealed that the usage of glycopeptide and daptomycin in hospitals readily favour the selection of this clone (Baines et al., 2015). A similar association of strain survival in the presence of fluoroquinolones usage in the United Kingdom has also been described for another HA-MRSA strain ST22 (Knight et al., 2012). The overall weaning stress and widespread use of antibiotics in weaners could have contributed to the strain carriage and antibiotics resistance in this stage of production, but there is no conclusive evidence to support this notion. More work is needed towards comprehensive understanding of the emergence and successful adoption of CA-MRSA in the pig environment.

4.4.3 Antibiotic resistance

Despite a high percentage of isolates in our study found to be resistant to antibiotics commonly used in pigs, they were also susceptible to a number of non-betalactam antibiotics. All MRSA isolates in this study exhibited no resistance to ciprofloxacin (a fluoroquinolone) and vancomycin (a glycopeptide), which are classified as critically or highly important in humans (WHO, 2017). Vancomycin is particularly important in treatment of staphylococcal disease in humans because it is a last line of defence drug. A number of studies performed in Europe have found high percentages of resistance in pig isolates against quinolones (over 30%) (Crombé et al., 2012b), aminoglycosides (up to 70%) (Conceição et al., 2017) and streptogramins (up to 40%) (Boost et al., 2015). Australia has not only restricted the use of quinolones and glycopeptides in humans through its national pharmaceutical subsidy scheme, but has also not permitted the use of these drugs in food-producing animals (Cheng et al., 2012). Avoparcin (a glycopeptide) was used extensively in chicken production in Australia during the 1990s. However, this drug was voluntarily withdrawn from the market in 1999 after concerns that their in-feed usage might lead to bacterial resistance (APVMA, 2004). Such strict control and successful regulation might be the reasons that no resistance was observed against these antibiotics in our study. In humans, fluoroquinolones are mainly reserved for infections caused by resistant bacteria, and a greater decline in its use has also been observed in the last few years. Such restriction has greatly assisted in keeping rates of resistance to this antibiotic class low in humans as well (ACSQHC, 2017). Australia is one of the countries with the strictest regulatory measures in place when it comes to use of certain antibiotics in food animals that are critically important in human medicine with fluoroquinolone being one of them.

The current study identified a significant association between antibiotic resistance and age-group of the pig. MRSA isolates collected in weaners showed significantly greater odds of resistance for
chloramphenicol, neomycin, AMC, and tetracycline. Although chloramphenicol is not used in pigs in Australia, resistance to this antibiotic observed in this study might be due to cross-resistance of florfenicol; belonging to the same class of antibiotics used in pigs. Similarly, the use of neomycin, amoxicillin, and tetracycline is commonly used on piggeries and the usage of these antibiotics is not uncommon in weaners to prevent diseases (Jordan et al., 2009, van Rennings et al., 2015). It is recognised that a high proportion of the overall antimicrobial use amongst all pig production stages occurs in weaners (DANMAP, 2016, Sjölund et al., 2016). In Denmark, 42% of the total antimicrobials sold in 2015 were reported to be used only in the weaning stage of production (DANMAP, 2016). The high MRSA prevalence and resistance found in weaners in this study is likely to be driven by the high antimicrobial usage as an association between antimicrobial use and resistance among MRSA isolated from pigs; as has already been established in the literature (Dorado-Garcia et al., 2015). However, other management practices such as biosecurity and farm hygiene also play an important role in the prevalence and spread of MRSA in pigs (Alt et al., 2011, Dorado-Garcia et al., 2015). Further investigation is required to study the risk factors such as antibiotic usage, biosecurity practices, and hygiene practices that prevail in Australian piggeries and are putatively able to be varied, that could potentially be associated with the MRSA emergence in pigs.

This study has also shown that within a pig herd affected with an outbreak of MRSA, particularly when a CA-MRSA strain is present, the resistance and virulence traits do not appear to be stable across the population of isolates present. This has substantial ramifications for studies seeking to describe the epidemiology and ecology of CA MRSA in pigs given that a small number of isolates clearly does not accurately characterise the outbreak. Similarly, the need for a “large sample” and “designed study” approach to understanding MRSA in pigs is also emphasised by the differences in sample prevalence amongst different age-groups of pigs.

4.5 Conclusion

This study observed the widespread carriage of CA-MRSA ST93 among pigs and the piggery environment on a piggery in Australia. The study shows that antibiotic resistance, including resistance to the non-betalactam group is commonly present amongst pig isolates in both strains (ST398 and ST93). An association between pig age and MRSA carriage as well as antibiotic resistance was found, with lower carriage in dry sows compared to the other age groups, which is most likely to be due to the differences in management practices. In addition, weaners were identified for increased risk of carriage and presence of antibiotic resistance compare to other age groups. Further investigation is warranted to determine management factors linked to MRSA carriage and resistance determinants in pigs to identify potential control strategies to minimize it.
Table 4.1 List of the primers and probes used for the detection of mecA, PVL, ST398 and ST93 amongst MRSA isolates collected on two sites of a piggery in Australia

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer and probe</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>MECA-F</td>
<td>5’-TGGTATGTGGAAGTTAGATTGGGAT</td>
<td>(Nakagawa et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>MECA-R</td>
<td>5’-CTAATCTCATATGTGTTCTCTGTATTGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECA-P1 (FAM)</td>
<td>5’-TTCCAGGAATGCGAAAGACCAAAGCA</td>
<td></td>
</tr>
<tr>
<td>pvl</td>
<td>PVL-F</td>
<td>5’-AAGGCTCAGGAGATAACAAGTG</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>PVL-R</td>
<td>5’-TCACTTCTATTAACTTGGAT</td>
<td></td>
</tr>
<tr>
<td>ST398</td>
<td>tmST398-F</td>
<td>5’-CATTCATCACACGTATATTCTATAGGTTCC</td>
<td>(van Meurs et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>tmST398-R</td>
<td>5’-TAAGAAATCTGTATTTAATTCAGATGGTCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tmST398 (FAM)</td>
<td>5’-ACCGCAATTCATACGAC</td>
<td></td>
</tr>
<tr>
<td>ST93</td>
<td>aroE252-F</td>
<td>5’-ACCTGCGCCCCAAAAATTAAAA</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>aroE252G-R*</td>
<td>5’-GGTATAATACAGATGGTATCGGTATG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>aroE252T-R*</td>
<td>5’-GGTATAATACAGATGGTATCGTTATG</td>
<td></td>
</tr>
</tbody>
</table>

* Primer used for detection of ST93
* Primer used for detection of non-ST93

Table 4.2 Multiple drug resistance phenotypes of MRSA isolates (n = 490) collected in pigs and environment on a single pig enterprise in Australia. The table shows resistance pattern individually for each strain, ST398 and ST93, as well as for MRSA isolates irrespective to strain.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>MRSA n(%)</th>
<th>ST398 n(%)</th>
<th>ST93 n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla, tet</td>
<td>5(1.02)</td>
<td>4(2.9)</td>
<td>0</td>
</tr>
<tr>
<td>bla, chl</td>
<td>4(0.82)</td>
<td>0</td>
<td>4(1.14)</td>
</tr>
<tr>
<td>bla, chl, tet</td>
<td>3(0.61)</td>
<td>0</td>
<td>3(0.85)</td>
</tr>
<tr>
<td>bla, chl, cli, tet</td>
<td>6(1.22)</td>
<td>5(3.62)</td>
<td>1(0.28)</td>
</tr>
<tr>
<td>bla, chl, cli, ery</td>
<td>177(36.12)</td>
<td>1(0.72)</td>
<td>176(50.14)</td>
</tr>
<tr>
<td>bla, cli, ery, tet</td>
<td>84(17.14)</td>
<td>83(60.14)</td>
<td>1(0.28)</td>
</tr>
<tr>
<td>bla, chl, cli, ery, tet</td>
<td>125(25.51)</td>
<td>2(1.45)</td>
<td>123(35.04)</td>
</tr>
<tr>
<td>bla, chl, cli, ery, neo, tet</td>
<td>3(0.61)</td>
<td>3(2.17)</td>
<td>0</td>
</tr>
<tr>
<td>bla, chl, cli, ery, qd, tet</td>
<td>41(8.37)</td>
<td>37(26.81)</td>
<td>4(1.14)</td>
</tr>
<tr>
<td>bla, chl, cli, ery, neo, qd, tet</td>
<td>38(7.76)</td>
<td>1(0.72)</td>
<td>37(10.54)</td>
</tr>
<tr>
<td>bla, chl, cli, ery, neo, qd, tet</td>
<td>4(0.82)</td>
<td>2(1.45)</td>
<td>2(0.57)</td>
</tr>
</tbody>
</table>
Table 4.3 Risk of detecting resistance amongst different pig age group against. The odds ratios and $P$.values were calculated by univariable analysis for each antibiotics using pig age group as a predictor and antibiotics resistance (yes/no) as an explanatory variable for all pig isolates ($n = 465$) collected at different age groups on a piggery in Australia.

<table>
<thead>
<tr>
<th>Pig age group</th>
<th>amoxicillin-clavulanate</th>
<th>Tetracycline</th>
<th>Chloramphenicol</th>
<th>Neomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry sows</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Farrowing</td>
<td>1.00 (0.48-1.48)</td>
<td>0.47 (0.27-0.82)</td>
<td>0.94 (0.49-1.79)</td>
<td>0 (inf)</td>
</tr>
<tr>
<td>Weaner</td>
<td>2.97 (1.49-6.1)</td>
<td>7.0 (14.39-1264)</td>
<td>6.77 (2.41-24.25)</td>
<td>30 (6-500)</td>
</tr>
<tr>
<td>Grower</td>
<td>0.85 (0.47-1.51)</td>
<td>2.07 (1.14-3.79)</td>
<td>1.15 (0.58-2.25)</td>
<td>10 (2-200)</td>
</tr>
<tr>
<td>Finisher</td>
<td>1.3 (0.65-2.63)</td>
<td>1.2 (0.61-2.37)</td>
<td>1.59 (0.69-3.84)</td>
<td>3 (0.3-60)</td>
</tr>
</tbody>
</table>

Figure 4.1 Distribution and proportion of MRSA pig isolates ($n = 465$) across different pig age groups in a single pig enterprise in Australia. The bars with errors show total percentages of MRSA across pig age groups while the different colours representing the proportion of strains.
Figure 4.2 Proportion of resistance found amongst total ST398 and ST93 isolates collected from pigs and environment on a piggery in Australia.

All MRSA isolates were susceptible to ciprofloxacin, gentamicin, linezolid, mupirocin, rifampicin, trimethoprim/sulfamethoxazole, teicoplanin, and vancomycin.

*amc (Amoxycillin/ clavulanic acid), cer (Ceftiofur), chl (Chloramphenicol), cli (Clindamycin), ery (Erythromycin), fox (cefoxitin), neo (Neomycin), pen (Penicillin), qd (Quinupristin-dalfopristin), tet (Tetracycline)
Supplementary data

Figure S. 4.1 Distribution and prevalence of MRSA carriage in different stage of production across two sites of a single pig enterprise in Australia.

Figure S. 4.2 Distribution of antibiotic phenotype amongst MRSA isolates collected in pigs and their environment in two different sites of a piggery in Australia.

All MRSA isolates were susceptible to ciprofloxacin, gentamicin, linezolid, mupirocin, rifampicin, trimethoprim/sulfamethoxazole, teicoplanin, and vancomycin
*amc (Amoxycillin/ clavulanic acid), cer (Ceftiofur), chl (Chloramphenicol), cli (Clindamycin), ery (Erythromycin), fox (cefoxitin), neo (Neomycin), pen (Penicillin), qd (Quinupristin-dalfopristin), tet (Tetracyline)
Figure S. 4.3 Distribution of antibiotic phenotype amongst MRSA isolates collected from two different sites of a single piggery in Australia. All MRSA isolates were susceptible to ciprofloxacin, gentamicin, linezolid, mupirocin, rifampicin, trimethoprim/sulfamethoxazole, teicoplanin, and vancomycin
*amc (Amoxycillin/ clavulanic acid), cer (Ceftiofur), chl (Chloramphenicol), cli (Clindamycin), ery (Erythromycin), fox (cefoxitin), neo (Neomycin), pen (Penicillin), qd (Quinupristin-dalfopristin), tet (Tetracycline)
Emergence of highly prevalent CA-MRSA ST93 as an occupational risk in people working on a pig farm in Australia

This chapter has been submitted and is currently under review:
Abstract

The occurrence of livestock-associated (LA) MRSA (ST398) in pig herds has emerged as a threat to occupational safety in many parts of the world. Recently, an outbreak of skin lesions due to MRSA occurred in workers at a pig farm in regional Australia and both the humans and pigs were shown to have a high prevalence of carriage of either the human-strain ST93 or porcine strain ST398. This study closely scrutinises this outbreak to determine factors associated with MRSA carriage amongst the workers. Information on potential risk factors was collected from employees by means of a questionnaire. The carriage status of MRSA by workers was assessed by nasal swabs processed using standard laboratory techniques with confirmed isolates subjected to sequence typing. Associations between MRSA carriage in workers and their questionnaire responses were investigated using univariable and multivariable logistic regression.

Nasal carriage of MRSA was identified in 60% (31/52) of participants. Workers having contact with pigs had 24 times the odds of MRSA carriage compared to workers with no direct contact (OR 23.6; CI 5.2-172.8). In addition, the probability of MRSA carriage in workers was significantly ($P < 0.001$) associated with the number of hours in contact with pigs and each hour of contact-time per day increased the risk of MRSA carriage by 1.44 times (CI 1.14-1.96). These associations were significant ($P < 0.001$) for both strains, ST398 and ST93, present on this farm. Using a multivariable logistic regression model that incorporated human exposure to five different pig age groups (dry sows, farrowing, weaner, grower, and finisher) as fixed effects, a significant ($P = 0.027$) increased odds of MRSA carriage was found for persons working with farrowing sows compared with those who did not (OR 6.39, CI 1.23-39.36).

This study shows that workers in close contact with pigs on a pig farm where MRSA is present had a higher risk of MRSA carriage as the number of hours of direct contact with pigs increased. Since a significant association for the human-derived CA-MRSA ST93 has been detected in this study, similar to the pig-adapted LA-MRSA ST398, thereby, ST93 is considered as a potential occupational risk for piggery workers. The risk of MRSA carriage is greatest when working with the farrowing group; therefore, an emphasis is required on personal protective equipment while working in the farrowing house. The study has ramifications for the conduct of surveillance for MRSA in people exposed to pigs.
5.1 Introduction

*Staphylococcus aureus* is a component of the normal flora of humans and some livestock. The organism is a common cause of skin and soft tissue infections, ranging from mild to invasive and life-threatening. *S. aureus* strains continuously evolve and adapt to new environments by exchanging resistance and virulence genes (Xia and Wolz, 2014). Methicillin resistant forms of *S. aureus* (MRSA) rose to prominence in the 1960s, as the cause of nosocomial infections unresponsive to standard therapy and capable of being carried by hospital staff (Jevons, 1961). So called “hospital-acquired MRSA” (HA-MRSA) infections have now evolved to be both highly infectious and resistant to a large number of antibiotic classes (Lindsay, 2013). In the 1980s, MRSA were isolated from cases without any recent exposure to health care environments and were termed “community-associated MRSA” (CA-MRSA) (Saravolatz et al., 1982, Udo et al., 1993). Although CA-MRSA are typically resistant to fewer antibiotic classes than HA-MRSA, they can carry genes encoding for virulence factors resulting in occurrence of severe disease in otherwise healthy people (Otto, 2013). In 2005, a new MRSA lineage known as “livestock-associated” MRSA (LA-MRSA) was isolated from pig farmers. Since then, the frequency of reports of LA-MRSA carriage has dramatically increased in certain classes of livestock (especially in pigs) and exposed humans. Although transmission to humans, resulting in carriage and infections, has been reported for LA-MRSA (Goerge et al., 2017), it appears to be less likely to cause disease compared to human-adapted strains (Graveland et al., 2010). LA-MRSA is presently recognised to be comprised of several clonal complexes including CC8, CC9, CC97, CC5 and CC398 (Butaye et al., 2016) with the latter the most frequently isolated of which ST398 is the most common strain type. Europe has been recognised as having a higher ST398 prevalence in people with livestock contact compared to other parts of the world (Liu et al., 2015). In Asia ST9 is the predominant LA-MRSA although ST398 is also present (Chuang and Huang, 2015).

ST398 has been isolated from a variety of animals, and its carriage has been observed in persons in close contact with MRSA infected or carrier animals. However, this strain is most commonly found in pigs and people working on pig farms and is thus also referred as porcine MRSA (Butaye et al., 2016, Crombé et al., 2013). The majority of studies have reported that simply having pig contact and living or working on a pig farm are important risk factors for carriage of ST398 (Liu et al., 2015, Crombé et al., 2013). Moreover, the probability of human carriage of porcine MRSA has been shown to increase with increasing frequency of pig contact, stocking density of pigs, and MRSA prevalence in pigs (van Den Broek et al., 2009, van Cleef et al., 2015). Recently, a high prevalence of MRSA carriage has also been found in pigs and people working on a pig farm in regional Australia, where two different strains, ST93 and ST398, were identified in people and pigs (Chapter 3). The farm had
been recognised as it had experienced recurrent clinical MRSA infections among employees over a three-year period and because of an unusual mixture of MRSA clones present. This study investigates the factors associated with MRSA carriage (overall and strain specific) amongst staff members working on the outbreak farm which represents a unique natural experiment for comparing and contrasting the behaviour of human- and pig- adapted forms of the organism in human host.

5.2 Ethics approval

Participation was voluntary, and signed informed consent was obtained from each participant. Approval for the recruitment of human participants into this study was granted by the Charles Sturt University Human Research Ethics Committee (Protocol number 2015/016). Approval for the sample collection in pigs was obtained from the Animal Care and Ethics Committee in Charles Sturt University (Protocol number 14/096).

5.3 Methods and methodology

5.3.1 Data collection

The pig-production enterprise involved in this study was identified as the focus of an MRSA outbreak by the New South Wales Department of Health due to the recurrent detection of MRSA in farm workers affected with clinical staphylococcal disease. A cross-sectional study was commenced in 2015 investigating microbiological and epidemiological aspects of MRSA carriage among piggery employees was conducted in 2015. Recently, a high MRSA carriage has been reported amongst workers on this facility with a mixture of sequence types being present and some novel antibiograms (Sahibzada et al., 2017). This study, focusing on the epidemiology of the MRSA outbreak, examines the risk factors associated with carriage of MRSA in staff members.

The pig enterprise had two sites (site-A and site-B) geographically separated by approximately 40 km, with a total workforce of 52 employees and with workers moving between both sites. All workers at each site were approached to participate in the study. Participation was voluntary, and informed consent was obtained from all participants. Participation involved collecting a single swab from the external nares and completing a questionnaire.

The questionnaire addressed individual information such as age, sex, level of education, ethnicity, number of years working with pigs, number of months working on the current piggery, main role within the piggery, number of hours working with pigs per week, age group of pigs in contact, and contact with another animal species. The questionnaire also collected self-reported medical history, such as previous and current MRSA infections, hospitalisation, and current or previous antibiotic
treatment. Questions regarding personal hygiene, knowledge and behaviour with respect to antibiotic use and attitude regarding the potential for MRSA emergence were also asked. The questionnaire was completed individually, with a member of the research team available for clarifications. The questionnaire is available from the corresponding author upon request (attached as Annex-2 with this thesis).

Nasal swabs were collected from all participants on the pig farm followed by isolation of MRSA in the laboratory as described in Sahibzada et al. (2017). In short, MRSA was isolated using the method recommended by the European Union Reference Laboratory for AMR and screened for various antibiotic resistance attributes following the Clinical and Laboratory Standards Institute protocols (CLSI, 2014). Species identification was performed by matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Subsequently, all presumptive-MRSA isolates were further confirmed by PCR for identification of nuc and mecA genes. In addition, DNA microarray, whole genome sequencing, and multi locus sequence typing (MLST) were performed on all MRSA isolates.

5.3.2 Data analysis

Data were entered into Microsoft Excel, and descriptive and statistical analysis were performed with the statistical package R (R Core Team, 2017) and the package ggplot2 used for graphical display of data (Wickham, 2016). The associations between MRSA carriage and factors were investigated using univariable and multivariable logistic regression models. An alpha level of 0.05 was used as a significance criterion for all statistical tests. The overall model fit was assessed using a likelihood ratio test that derives the P-values using a $\chi^2$ distribution. Odds ratios (OR) and confidence intervals (CI) for potential risk factors were calculated from the logistic regression models by exponential transformation of the coefficients and its intervals using commands `coef` and `cofint`.

Initially, associations between the different potential risk factors with the presence of MRSA carriage were investigated using univariable logistic regression. The following risk factors were assessed: pig contact (yes/no), intensity of contact (hours per week in direct pig contact), total number of years working with pigs, number of months working on the current study farm, and working on either site-A or site-B (mutually exclusive).

The relationship between MRSA carriage and a participant’s main role on the farm was also assessed. The main role variable had five levels classified as administrative, pastoral, feedmill, maintenance and pig shed workers. An administrative worker was someone dedicated to working in an administration building situated at least 200 m away from the pig sheds. Pastoral workers were those...
working in the agriculture and cropping section. The feedmill workers only worked in the feedmill. None of administrative, pastoral or feedmill workers reported coming into direct contact with pigs. Maintenance workers were those performing maintenance jobs inside as well as outside the pig sheds, such as fixing fences, water troughs, feed lines and feeders. Some maintenance workers also reported on functioning of the effluent treatment system and helped to move pigs between sheds. Pig shed workers were those who worked directly with pigs in different sheds categorised for different pig age groups. Separate univariable models were also fitted to determine relationship of MRSA in piggery workers that perform a variety of jobs (using each type of job as a separate binomial predictor) including cleaning/washing sheds, moving pigs between sheds and for transportation, feeding, vaccinating, medicating, marking piglets, and assisting farrowing. Pigs were raised in separate sheds based on their production stage: dry sow, farrowing, weaner, grower, and finisher. Relationships between MRSA carriage in workers and working with certain pigs’ age groups were also examined considering each age group as separate binomial predictors (yes/no). Relationships between pig contact and carriage of a specific strain (ST93 and ST398) were also assessed using separate univariable analyses. Firstly, the association was determined for one strain (i.e. ST93) by excluding positive records from the counter strain (i.e., ST398) in the baseline model and then comparing the carriage of that strain with MRSA non-carriage. Then the same process was repeated for the second strain.

Participants’ health-related factors were also assessed for association with MRSA carriage which included the history of MRSA diagnosis, hospitalisation in the last twelve months, chronic disease, smoking, consumption of alcohol and personal hygiene. The factors related to the participants’ demography, knowledge and perception toward antibiotic usage and MRSA infection were also included in the analysis.

In addition, two multivariable nested models with predictor variables nested within a single factor ‘pig-contact’ were used to assess the association with MRSA carriage in people. In one model, working (yes/no) with any of the five pig age groups (used as five separate factors in the model) was assessed, these being nested within ‘pig-contact’ (yes/no). In another model, worker participation in various activities as separate binary variables (yes/no) were assessed, namely feeding, medicating, vaccinating, performing artificial insemination, marking piglets, assisting farrowing, cleaning/washing sheds, treating effluent, and moving pigs between sheds, all these being nested within the ‘pig-contact’ status. For both nested models, all selected variables were entered in the model first, and then a backwards elimination procedure based on P-value associated with the Wald statistic was used. The likelihood ratio chi-square test in conjunction with Akaike's information criteria (AIC) was used for checking the actual model fit on each step. In addition, these multivariable
models were compared with forward selection, and this resulted in the same variables being selected as in the backward elimination method.

5.4 Results

A total of 52 pig workers (all workers at either site) participated in the study, including 77% male and 23% female workers. The majority identified themselves as Caucasian (61%) followed by Indigenous Australian (22%), and Asian (12%) background. Approximately half of the participants \((n = 27)\) were over 40 years of age. In relation to education, 52% of participants had completed tertiary education such as vocational TAFE (Technical and Further Education) or a University degree. Just over 44% had worked in the pig industry for at least five years. The majority (67%) reported working on the current study farm for at least twelve months. All workers but one reported that they worked for at least five days a week and at least seven hours a day on the study farm.

The univariable analyses identified no significant associations with MRSA carriage for gender, age, ethnicity, number of years working with pigs, number of months working on the current study farm, working on either site-A or site-B, smoking, alcohol consumption, history of hospitalization in the last twelve months, chronic disease, and previous MRSA diagnosis (Suplementary data Table S. 5.1). There was also no association detected between MRSA carriage and occupational hygiene practices, individual’s knowledge and perception of MRSA emergence on the farm (data not shown). The risk factors that have been found significantly associated with MRSA carriage are explained in the following sections.

5.4.1 Work related exposure factors

5.4.1.1 Pig exposure

Nasal MRSA carriage was identified in 31 of the 52 (60%) participants. The typing results revealed two strain types: ST398 (16.13%) and ST93 (83.87%). On site-A only ST93 was found, whereas both ST93 and ST398 were found on site-B (Figure 5.1).

Firstly, it was investigated if pig exposure was associated with MRSA carriage in people working on the farm. A univariable logistic regression analysis identified a significant \((P<0.001)\) association between pig contact and MRSA carriage in farm workers. There was almost 24 times increased odds of MRSA carriage for those working in direct contact with pigs compared to those with no pig contact \((OR 23.6; CI 5.2-172.8)\). Similarly, the number of hours worked with pig exposure was significantly \((P < 0.001)\) associated with MRSA carriage in workers with an increasing odds of 1.5 times for each hour increase in a day of direct pig contact \((OR 1.44, CI 1.14-1.96)\). The association between MRSA
carriage and contact intensity amongst pig workers is illustrated in Figure 5.2 (Both strains). The association between pig exposure and carriage of MRSA strains was also explored in piggery workers. Using univariable models, an increase in odds for carriage of either strain carriage in pig workers was observed in relation to increase in each hour pig contact in a week as shown in Figure 5.2 (ST93 and ST398).

5.4.1.2 Role and performing specific activities

A significant ($P < 0.001$) univariable association was also found between the main role of piggery workers within the piggery and MRSA carriage. While the true effects cannot be accurately estimated for each specific role, due to low numbers of observations in some groups, the prevalence of MRSA carriage was higher (85.2%) for persons working in pig sheds compared to people with other roles (Figure 5.3).

All workers except people in administrative or pastoral roles performed various activities involving pig contact. It was also assessed whether the type of work they were performing had any association with MRSA carriage using univariable models. Except for artificial insemination and effluent treatment, all the activities performed in direct pig contact were significantly associated ($P < 0.05$) with MRSA carriage (details are given in the Supplementary data in Table S. 5.1). Subsequently a multivariable model was fitted using all the significant associated activities involved, namely cleaning/washing sheds, moving pigs between sheds and for transportation, feeding, vaccinating, medicating, marking piglets, and assisting farrowing. In the model, the activities were nested within pig contact and assisting farrowing was identified as the only significant ($P = 0.003$) risk factor associated with MRSA carriage in people.

5.4.1.3 Working with a specific pig age group

MRSA carriage in workers was also associated with exposure to a specific age group of pigs. Using univariable analysis a significant association was identified between MRSA carriage and all pig age groups (production pig groups) except for dry sows. The univariable models produced the highest odds ratio for people working with farrowing sows (OR 17.25; CI 4.47-89.2; $P < 0.001$). Significantly higher odds of MRSA carriage were also identified for piggery workers working with finishers (OR 4.94; CI 1.33-24.25; $P < 0.001$), grower (OR 4.53; CI 1.32-18.62; $P = 0.02$), and weaner pigs (OR 4.53; CI 1.32-18.62; $P = 0.02$). A multivariable nested model was used in order to assess the risk posed by each pig age group, when adjusted by the other age groups. After backwards elimination, the model returned only one factor, namely working with farrowing sows, as a significant predictor of MRSA carriage. The model revealed that amongst piggery staff working in pig contact, a person
working in the farrowing shed is approximately six times more likely to carry MRSA compared to those not working in the farrowing shed (OR 6.39, CI 1.23-39.36, \( P = 0.027 \)).

### 5.4.2 Demographic, health, hygiene and perception exposure factors

Using univariable models the association was explored between MRSA carriage and demographic, health, hygiene practices, and attitudes and perceptions in relation to MRSA emergence and transmission on a pig farm. Level of education was the only demographic factor that was significantly \( (P = 0.02) \) associated with MRSA carriage. In a multivariable model, after controlling for pig contact, education continued to be significantly associated with MRSA carriage in workers. The estimated odds ratio for MRSA carriage in workers who had high school or lower level of education was 6.36 (CI 1.38-45.94, \( P = 0.02 \)) when compared with those with tertiary education. The proportion of people who had pig contact was almost similar in both education levels, tertiary 49\% \((n = 18)\) and high school 51\% \((n = 19)\).

A total of 30\% \((n = 16)\) of participants reported that they had been diagnosed with MRSA infections on the farm since 2011 resulting a mean number of 18 days off work. In addition, one participant reported being diagnosed in 1993 but was not working on the same farm at the time. The majority of those participants previously diagnosed with MRSA infections on this farm \((13/16)\) were also positive for MRSA carriage in the current study, and most of those \((11/13)\) were carrying ST93. All of those previously diagnosed with MRSA on the study farm reported having had pig contact at the time of diagnosis. A total of 31\% of those diagnosed with MRSA on the farm required hospitalisation.

Participants were asked to report the confidence surrounding their personal hygiene and co-workers’ hygiene practices at the work place. Only one third (34\%) of participants reported being very confident about their own personal hygiene and their co-workers’ hygiene practices. Most respondents (82\%) reported an improvement in their personal or co-workers’ hygiene on the farm after the MRSA outbreak. When participants were asked to rate on a Likert-scale from 0 (minimum) to 5 (maximum) their current level of knowledge about occupational hygiene practices, in terms of reducing the risk of zoonotic transmission of MRSA, the median score was four. Figure 5.4 shows that the majority agreed that using proper personal protective equipment like disposable gloves, coveralls and masks could help preventing zoonotic infections. However, some workers (as shown in Figure 5.4) believed that such practices had no role in preventing zoonotic infections. Less than half (47\%) of participants believed that antibiotic use as growth promotion or prophylactic could potentially increase the risk of MRSA emergence on a farm Figure S. 5.1.
Over two thirds (69%) of respondents showed concern about working on a pig farm and being exposed to zoonoses. Interestingly, a higher proportion (80%) were concerned about their family members getting a zoonotic infection through them than were concerned about acquiring infection themselves. Furthermore, 82% were concerned about their coworkers’ potential exposure to zoonotic diseases. However, when they were asked about the potential for themselves to transmit infections to the pigs, just over half (56%) showed some level of concern, with the rest not being concerned at all (Figure S. 5.2).

In relation to participants’ perception of risky occupational places for MRSA carriage, working in public hospitals and aged care facilities were perceived to pose a very high risk of exposure, followed by working in veterinary clinics and piggeries. Dairy, sheep and beef farms were ranked the least risky places for occupational MRSA carriage in people (Supplementary data Figure S. 5.3).

5.5 Discussion

5.5.1 Pig contact and intensity of contact

Pig exposure was an important determinant for MRSA carriage in this study. We found a 24-fold increase in odds of MRSA carriage in people with direct pig contact, compared to those without direct contact within the study piggery. Direct contact with MRSA-positive pigs has previously been reported as a major risk for MRSA carriage in farmers (van Cleef et al., 2015, Cuny et al., 2009). In a systematic review that examined 33 studies, animal contact, especially pig contact (OR, 5.91, CI 4.84-7.24), was found to be the most important risk for MRSA carriage in people (Liu et al., 2015).

Similarly, in a retrospective case control study that assessed 100 patients in each group carrying ST398 and non-ST398 MRSA, pig contact was found to be an important determinant for ST398 carriage (OR 20.46, CI 7.83–64.39, P < 0.001), accounting for age, type of infection, hospitalisation, pig contact, and contact with other livestock (Köck et al., 2009). However, in contrast to the current study, prior studies have attempted to identify risk factors for MRSA carriage amongst people on a multi-farm level, and carriage under the same environmental condition has rarely been explored.

A dose-response association between pig contact and MRSA carriage was found in this study, with the odds of MRSA carriage increasing by 1.44 times for every hour per day increase in direct pig contact. Dorado-Garcia et al. (2015) reported a similar quantitative association between the number of hours worked in pig contact and MRSA carriage in farm workers in a longitudinal study conducted over 18 months on 36 piggeries. On univariable analysis, they reported an odds ratio of 1.82 (1.58–2.06; P < 0.001) for each 10 hours per week increase in work. Another study reported an odds ratio of 2.13 (1.65-2.74) for each hour per day increase in pig contact (Ye et al., 2015), however, it was
possible for workers to have other livestock and poultry contact simultaneously with pig contact. Similarly, this study identified a positive association between MRSA strain carriage status and contact intensity (number of hours) for both strains (ST398, ST93).

A significant positive association between hours of pig contact and ST398 carriage has been established (Dorado-Garcia et al., 2015, Ye et al., 2015). However, such a relationship has never been reported for a non-LA-MRSA. The association found in this study between the CA-MRSA ST93 and contact intensity shows an even stronger and steeper relationship compared to the LA-MRSA ST398 as shown in Figure 5.2 (ST93 and ST398). ST93 is the most prevalent CA-MRSA in Australia and is frequently isolated from people in community and hospital setups (Coombs et al., 2015, Brennan et al., 2013, Munckhof et al., 2009). The genetic and antibiotic resistance profile amongst ST93 isolates found on this farm has been described previously (Sahibzada et al., 2017) and strongly suggests the strain may have become adapted to their new porcine host. Since the likelihood of ST93 carriage found in this study increases as the number of hours in direct pig contact increases on this farm, like other porcine MRSA, this strain is classed as an occupational risk, when present in a piggery setting. While ST93 is already a major cause of community-acquired infections in Australia, the adaptation of ST93 to pigs may serve as a reservoir for this strain in isolated cases and may represent a challenge for the pig industry with respect to occupational risk. In this study, ST398 was found only in workers who had direct pig contact, which supports findings of other studies that identifies transmission between humans without pig contact seems to be of minor importance for ST398 (Bisdorff et al., 2012, Price et al., 2012, van Cleef et al., 2011b).

5.5.2 Working with a specific pig age group

Amongst those working in direct pig contact, MRSA carriage was influenced by exposure to different pig age groups/types of production group. Comparing the odds ratios of working with different age groups produced by univariable models, the highest odds ratio was generated for working with the farrowing group (OR = 17.3). Even in a multivariable model that accounts for working in all five sheds and pig contact, working in the farrowing shed continued to have a strong association with MRSA carriage in piggery workers (OR = 6.4). In another multivariable model that was used to identify the most important risky activities performed by piggery workers, assisting farrowing was recognised as the only significant factor associated with MRSA carriage and this activity is performed only in a farrowing shed. All these analyses indicate that working with farrowing is the most important risk factor when compared to the other age groups. Although the reason for this association is not clear, it could be due to the intense pig contact, since pigs and piglets require intensive and regular handling (birth assistance, tail docking, castration, cutting teeth) and workers are likely to be
in much closer contact for longer periods within the working hour with farrowers than with other categories of pigs.

5.5.3 Knowledge and perception

Participants reported that they have mixed knowledge and perception about the risk factors related to the emergence of MRSA on farms. Almost half of the respondents believed that antibiotic use as growth promotants or for prophylactic treatment have no role in the emergence of resistance bacteria. Numerous studies have found a strong correlation between antibiotic usage as growth promotants and the rise of resistance in bacteria on farms (Aarestrup et al., 2001, Funk et al., 2006) that subsequently could transfer to humans.

Overall, attitudes towards control of zoonotic disease were positive, but there were some respondents who were not in agreement that using proper personal protection could help prevent infectious agents transmitting from animals to workers. The workers were intuitively concerned while working with pigs since the majority perceived pig farming as the riskiest occupational place amongst all livestock farming (dairy, beef, sheep) for MRSA exposure and other zoonotic infections. However, the level of concern was comparatively low regarding reverse zoonosis as almost half (44%) showed no concerns about transferring diseases to pigs.

Overall, the knowledge and perception results show a moderate degree of risk awareness and responsibility among participants. However, these responses cannot be directly extrapolated to other commercial piggeries because such response from the participants on this farm might be biased towards knowledge and awareness as, during the course of the outbreak, the majority of them had participated in seminars and training arranged by the government health department. A subsequent survey is required that collects information from commercial piggeries on a larger scale to explore producers’ and workers’ knowledge and perceptions regarding the risk of MRSA emergence and risk management strategies.

5.6 Conclusion

Workers in close contact with pigs had an increased risk of carriage of MRSA, and the likelihood of MRSA carriage increases with the number of hours in direct pig contact. Importantly, these associations have been shown for both the pig-adapted (ST398) and community acquired (ST93) strains of MRSA within this establishment. Therefore, ST93 can be considered as a potential occupational hazard ST93 equal to ST398.
Moreover, exposure of workers to farrowing sows is associated with a higher risk of MRSA carriage, compared with exposure to other types of pigs. The piggery workers are encouraged to use appropriate personal protective equipment and good hygiene practices while working in direct pig contact especially in farrowing house. Greater understanding of the occurrence of human-adapted strains of MRSA in pigs in Australia based on studies of multiple herds is needed.
Figure 5.1 Distribution and frequency of ST93 and ST398 carriage amongst piggery staff on two sites (site-A, site-B) of a single pig enterprise in Australia with a recurrent MRSA outbreak in humans.

Figure 5.2 Logistic regression model predictions (and 95% CI) of the probability of MRSA carriage (both strains, ST93, and ST398) in piggery staff for the number of hours per week spent in direct pig contact in a piggery in Australia with a recurrent MRSA outbreak in humans.
Figure 5.3 Distribution and frequency of carriage of different strains of MRSA amongst piggery workers performing different roles in a piggery in Australia with a recurrent MRSA outbreak in humans.
* Those who come in direct pig contact.

Figure 5.4 Perception of piggery staff working in a pig enterprise in Australia towards adoption of actions preventing MRSA on a pig farm.
The negative percentages (left of the vertical black line) represent percent responses indicating the level of disagreement (‘slightly disagree’ to ‘strongly disagree’, and ‘don’t know’) whereas positive percentages at the right of the vertical black line indicate levels of agreement.
Supporting information

Table S. 5.1 Outcomes of univariable regression analysis investigating the association of characteristics and practices of piggery workers with carriage of MRSA on a pig farm in Australia with recurrent MRSA infections in humans.

<table>
<thead>
<tr>
<th>Factors</th>
<th>MRSA+ n (%)</th>
<th>OR (CI)*</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (57.5)</td>
<td>1</td>
<td>0.57</td>
</tr>
<tr>
<td>Female</td>
<td>8 (66.67)</td>
<td>1.48 (0.4-6.29)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>20s</td>
<td>8 (57.1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>30s</td>
<td>6 (54.5)</td>
<td>0.9 (0.18-4.52)</td>
<td></td>
</tr>
<tr>
<td>40s+</td>
<td>17 (63)</td>
<td>1.28 (0.33-4.79)</td>
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</tr>
<tr>
<td>Ethnicity</td>
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</tr>
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<td>Indigenous</td>
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<tr>
<td>Asian</td>
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<td>Caucasian</td>
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<td>Education</td>
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<tr>
<td>Tertiary</td>
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<td></td>
</tr>
<tr>
<td>High school</td>
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<td>3.96 (1.25-13.84)</td>
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<td>Pig contact</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2 (13.33)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (78.38)</td>
<td>23.56(5.20-172.75)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Perform Cleaning (yes)</td>
<td>5.82 (1.77-21.97)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Perform Vaccination (yes)</td>
<td>8.91 (2.1-62.13)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Perform medication (yes)</td>
<td>4.53 (1.32-18.62)</td>
<td>0.015</td>
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<tr>
<td>Perform feeding (yes)</td>
<td>10.39 (2.95-44.59)</td>
<td>&lt;0.001</td>
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<td>Perform moving pigs (yes)</td>
<td>13.33 (3.74-56.46)</td>
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<tr>
<td>Perform artificial insemination (yes)</td>
<td>2.34 (0.66-9.68)</td>
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<td>Perform farrowing assistance (yes)</td>
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<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Perform pig marking/docking (yes)</td>
<td>∞</td>
<td>&lt;0.001</td>
<td></td>
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<td>Perform effluent treatment (yes)</td>
<td>2.68 (0.77-11.07)</td>
<td>0.12</td>
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</tr>
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<td>Long-term (lifelong) duration of pig contact (years)</td>
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<td>0.07</td>
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<td>Number of hours per week work with dry sows</td>
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<td></td>
</tr>
<tr>
<td>Number of hours per week work with farrow</td>
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<tr>
<td>Number of hours per week work with weaner</td>
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<tr>
<td>Number of hours per week work with grower</td>
<td>1.28 (1.00-1.76)</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>Number of hours per week work with finisher</td>
<td>1.86 (1.10-4.62)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Number of hours work per week and ST93 carriage</td>
<td>1.07 (1.02-1.14)</td>
<td>&lt;0.001</td>
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</tbody>
</table>
Number of hours work per week and ST398 carriage & 1.08 (1.02-1.21) & 0.004 \\
History of MRSA diagnosis & 0.08 \\
No & 18 (51.43) & 1 \\
Yes & 13 (76.47) & 30.7 (0.89-12.61) \\
Chronic disease & 0.17 \\
No & 22 (55) & 1 \\
Yes & 5 (83.33) & 4.09 (0.59-82.18) \\
History of hospitalisation (last 12 months) & 0.34 \\
No & 23 (62.16) & 1 \\
Yes & 3 (42.86) & 0.46 (0.08-2.36) \\
Smoking & 0.29 \\
No & 22 (66.67) & 1 \\
Yes & 7 (50) & 0.5 (0.14-1.8) \\
Drinking alcohol & 0.69 \\
No & 6 (66.67) & 1 \\
Yes & 22 (59.46) & 0.73 (0.14-3.25) \\
*The odds ratio and P-values were calculated using univariable logistic regression analysis

<table>
<thead>
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<th>Activity</th>
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<tr>
<td>Washing animals with water</td>
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<td>1</td>
</tr>
<tr>
<td>Soapy water for cleaning</td>
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<td>1</td>
</tr>
<tr>
<td>Low quality concentrate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*abs as prophylactic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*abs as growth promotant</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*abs in treatment</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*abs in cleaning</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure S. 5.1 Do you think the following activities are likely to increase the occurrence of MRSA on farm (*abs = antibiotics use)
Figure S. 5.2 As you are working with pigs, how concerned are you that …….

Figure S. 5.3 How likely do you think it would be that working at the following workplaces could increase the level of risk of exposure to MRSA? 0 no risk, 5 maximum risk

Figure S. 5.4 Venn diagram showing the overlap of number of piggery workers in different roles in relation to pig age groups and corresponding sheds in a piggery in Australia with a recurrent MRSA outbreak in humans.
Figure S. 5.5 Confidence level of piggery workers about their own and co-workers’ hygiene and protection when working on the farm.

Figure S. 5.6 Distribution and frequency of MRSA strains found amongst piggery staff working with different pig age groups on two different sites (site-A, site-B) of a piggery in Australia with a recurrent MRSA outbreak in humans.
Chapter 6.

MRSA prevalence amongst commercial pig herds in Australia
Abstract

Introduction

Recently, a high prevalence of community-associated MRSA was found on a single commercial piggery in Australia which raised concerns that the molecular epidemiology of MRSA in pigs might be different in Australia compared to the rest of the world (Sahibzada et al., 2017).

Aims

This study aimed to investigate the presence, prevalence and distribution of MRSA in commercial piggeries in Australia with a focus on identifying which sequence types are present. This study also determined the phenotypic characteristics of the MRSA isolates detected and examined patterns of antibiotic use amongst the participating piggeries.

Methods

A cross-sectional study was performed in the Australian national pig herd in 2017. Five environmental and 60 nasal swabs from weaners were collected from each of 26 farms. MRSA was isolated in the laboratory using standard techniques, and antibiotic susceptibility testing was performed using the disc diffusion method against 12 different antibiotics. MALDI-TOF was performed for species identification, and the presence of meca and PVL genes were confirmed using RT-PCR. Information related farm management practices was collected by means of a questionnaire.

Results

MRSA was identified in 53.9% (14/26) of the study farms and prevalence ranged between 1.6% and 100% on infected farms. On average the prevalence of MRSA carriage in pigs on MRSA affected farms was 40.28% (95% CI 27.03-53.54%). MRSA was found in 36.6% (n = 45/130) of the total environmental isolates. All MRSA positive isolates possessed meca, were typed as ST398, and lacked the lukF-lukS genes encoding Panton-Valentine leukocidin. A high prevalence of resistance was observed for tetracycline (100%, CI 99.6-100), clindamycin (94.6%, CI 92.5-96.1), erythromycin (76.7%, CI 73.2-79.9), and amoxicillin-clavulanate (68.3%, CI 64.5-71.9). A lower prevalence was noted for resistance to chloramphenicol (30.5%, CI 27-34.3), ciprofloxacin (25.8%, CI 22.5-29.4), and Quinupristin-dalfopristin (17.2%, CI 14.5-20.4). Only 4.1% (CI 2.8-6.0) of isolates showed resistance to gentamycin and neomycin. Resistance to sulfamethoxazole-trimethoprim was only 1.6% (CI 0.9-3.0). All isolates were susceptible to linezolid.

The most commonly used antibiotics among study piggeries were reported to be amoxicillin (92.3%), penicillin (92.3%), tetracycline (80.8%), tylosin (80.8%), sulfamethoxazole-trimethoprim (69.2%),
and lincomycin (53.8%). Ceftiofur was also used on 30.8% of the piggeries. Heavy metal use such as zinc and copper was reported on 65.4% of the piggeries. The majority of the farms (58%) had an open herd system in place and half of them were outsourcing their replacement pigs from breeding companies. Quarantine practices for replacement pigs were placed in only two thirds of these farms. One quarter (23%) never disinfect sheds following stock removal.

**Conclusion**

This study found evidence of wide spread presence of LA-MRSA ST398 amongst commercial piggeries in Australia, similar to what is reported in other parts of the world. However, a lower proportion of resistance was noticed for most of the non-beta-lactam antibiotics tested in comparison with other study findings. No ST93 was found in this study. The information collected by questionnaire revealed that biosecurity and hygiene practices on farm level can be further improved. Moreover, strengthening of antibiotics stewardship might help to reduce the usage of antibiotics and heavy metals that select for resistance.
6.1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a resistant bacterium that evolved from methicillin-susceptible *S. aureus* by acquiring the *mecA* gene on plasmids, which codes for resistance against betalactam antibiotics. Livestock-associated MRSA (LA-MRSA) has been isolated from a variety of food animals, but pigs are considered to be the primary reservoir (EFSA, 2012). Pigs carry LA-MRSA asymptotically, but these bacteria may potentially transfer to people via occupational exposure and can result in infection in occupationally at-risk individuals (Chapter 4). A number of studies have established a strong association between MRSA prevalence in pigs and transmissibility to humans. MRSA carriage in pigs is influenced by many factors: including pig stocking density (Broens et al., 2011b), farm hygiene, biosecurity, and antibiotic usage (Dorado-Garcia et al., 2015). Antimicrobial usage has been identified as an important factor for the selection and dissemination of MRSA (Broens et al., 2012a, Dorado-Garcia et al., 2015). LA-MRSA are genetically distinct from Community-Associated (CA) and Hospital-Associated (HA) strains, as they lack genes such as *lukf-PV/lukS-PV* genes which encode for Panton-Valentine leukocidin (PVL) toxin and human immune evasion gene cluster (IEC) (Sahibzada et al., 2017, Mutters et al., 2016). This is possibly why LA-MRSA is regarded as having low infectivity for humans (van Cleef et al., 2011a, Jamrozy et al., 2012). LA-MRSA shows resistance to all betalactam antibiotics, and an additional resistance to extended spectrum and some non-betalactam antibiotics has also been reported (EFSA, 2014). In addition, they also carry genes that make them resistant to heavy metals commonly used in piggeries (Slifjerz et al., 2015a, Amachawadi et al., 2015).

The predominant strain of LA-MRSA isolated in pigs from Europe and North America is ST398 (Alt et al., 2011) and North America (Smith et al., 2009) while ST9 is commonly reported in Asia (Chuang and Huang, 2015). In the countries with ST398 a predominant LA-MRSA in pigs, a prevalence as high as 86% is reported in pigs and up to 100% herds (Morcillo et al., 2015). The prevalence of ST398 carriage in pigs has been linearly associated with the quantity of antimicrobial use in pigs (Dorado-Garcia et al., 2015), especially with tetracyclines, ceftiofur, and also with heavy metals, such as zinc (Dorado-Garcia et al., 2015, EFSA, 2009d, Moodley et al., 2011b). There is currently no surveillance system for antibiotic resistance and usage in pigs at the national level in Australia. Recently, a high carriage of MRSA (75%) in pigs was identified on a single pig enterprise in Australia with the majority of the MRSA identified as CA-MRSA ST93 (72%) and the minority ST398 (28%) (Chapter 3). In a subsequent study on the same farm ST93 was identified as an occupational health issue (Chapter 4 and 5). Given that ST398 and ST9 were the predominant LA-MRSA in other parts of the world, and ST93 had never been reported as an occupational health issue, it was hypothesised that the molecular epidemiology of MRSA in the Australian pig industry might be different compared to
the rest of the world. In this study, it was aimed to investigate the prevalence and distribution of MRSA in commercial piggeries at the national level in Australia. This study also investigates the antibiotic usage among Australian piggeries and determines the phenotypic characteristics of the MRSA isolates.

6.2 Methods

6.2.1 Study design and sampling

This study is based upon voluntary participation of farmers and veterinarians servicing piggeries in Australia. The study was approved by the Charles Sturt University Human Research Ethics Committee (Protocol number 2015/016) and Animal Care and Ethics Committee (Protocol number A16049).

A cross-sectional prevalence survey was conducted during the period January–October 2017. All commercial farms in Australia that had at least 300 breeding sows and were registered with Australian Pork Limited (APL) were considered for inclusion in the study. APL is a rural industry service body for the Australian pork industry. It is a producer-owned company delivering integrated services to a large number of Australia’s pig producers. Over 90% of the piggeries producing pork on commercial level in Australia are registered with APL. According to APL estimates, there are 200 piggeries with over 300 breeding sows, representing 90% of the total pig production in Australia (LK. Van Breda, personal communication, December 21, 2017). Pig producers and veterinarians were encouraged to participate in the survey via information distributed through APL. At least three reminder emails were sent out to the enterprises and pig veterinarians working with the target piggeries. To detect pig level prevalence, the sample size was calculated with an expected 15% pig prevalence, a population of 10,000 or over on a farm, and assumed test sensitivity and specificity of 100% (EFSA, 2009a). With 95% confidence and a desired precision of 10%, it was identified that 49 animals were required per farm. However, 60 animals as a standard sample size was adopted for each farm in line with other studies and the EU-wide baseline survey that have collected 60 nasal swabs in pigs for investigating herd level prevalence (Broens et al., 2011b, EFSA, 2009a).

The piggeries willing to participate that were located within a 300 km range from the university where researchers were based, were swabbed by the research team. Piggeries that were geographically outside this range were swabbed by the consulting veterinarian. Sample collection kits were mailed to those farms or directly to the consulting veterinarian. Such kits consisted of sterile cotton applicator swabs (Liquid Amies Elution Swab, Copan ESwab™), questionnaire, MRSA information sheet, and sampling instructions. Written instructions as well as video link instructions were sent to demonstrate
the procedure for collection of samples in pigs and the environment within the pig shed. According to the instructions provided, the research team or the farm veterinarian collected nasal swabs from sixty randomly selected weaners at each farm. Five environmental samples were also collected from inside each shed and included a walkway, pens floor, feeder, fence and wall. At the same time, a questionnaire was completed by the farm veterinarian or manager. The questionnaire was divided into three main sections that collected information regarding: 1) farm characteristics, hygiene and biosecurity, 2) antibiotic usage, 3) participants’ perceptions. In the first section, questions were asked about the number of different aged pigs on the farm, the production system in practice, sources of replacement pigs, on-farm quarantine practices, and information about cleaning and disinfecting the weaner sheds. In the second section, open-ended questions were asked to collect information relating to antibiotic usage (oral and injectable) in weaners where the swabs were collected and elsewhere on the piggery in the past 12 months. In the third section, questions about MRSA infections in piggery workers on the farm and participant perceptions questions were asked. The participant information sheet and questionnaire is attached as Annex-3 and 4, respectively, with this thesis.

6.2.2 Laboratory analysis and molecular typing

All swabs collected by veterinarians along with the completed questionnaire were sent by courier using an overnight service to the Charles Sturt University diagnostic laboratory. In the laboratory, the samples were stored at 4 °C on arrival and processed within a week. MRSA was isolated following the protocol described by Sahibzada et al. (2017). Briefly, swabs were enriched in Mueller Hinton Broth with 6.5% NaCl and Tryptone Soya Broth with 4 mg/L cefoxitin and 75 mg/L aztreonam followed by streaking on chromogenic agar and blood agar plates. Latex agglutination tests were performed on all presumptive MRSA isolates. Then they were tested for 12 different antibiotics: amoxicillin-clavulanate (AMC), cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, neomycin, quinupristin-dalfopristin (QD), tetracycline, and trimethoprim-sulfamethoxazole (SXT). Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was also performed on all isolates for the Staphylococcus aureus species identification as described in Chapter 3.

DNA was extracted using 6% chelex and molecular typing was performed on all isolates using the Real-Time Polymerase Chain Reaction (RT-PCR) as explained in Chapter 3. Isolates were also screened for the presence of mecA and PVL genes following the protocols described in Chapter 3.
6.2.3 Data analysis

All statistical analysis were performed using the professional statistical software R (R Core Team, 2017). Confidence intervals were calculated using the prevalence package built in R (Devleesschauwer et al., 2015). Data on inhibition zone size for each drug were converted to the dichotomous classification of resistant (R) or susceptible (S) as recommended in the CLSI documents M100-S24 (CLSI, 2014) and VET01-S2 (CLSI, 2013). All intermediate resistance isolates were considered as susceptible for the purpose of prevalence evaluation. Isolates resistant to at least one betalactam and two non-betalactam antibiotics were classed as multidrug-resistant (MDR). The association of MRSA carriage in pigs with the farm biosecurity and hygiene practices such as type of farming system, quarantine in place, and disinfecting weaners sheds were investigated using generalised logistic regression models (GLM) involving proportional response variables. The GLM models were evaluated, and the models were specified with quasibinomial family for error distribution by taking into account the overdispersion concerns. No multivariable models were used because of a low number of observations in this study.

6.3 Results

6.3.1 MRSA prevalence

A total of 1,560 samples from pigs and 130 samples from the piggery environment were collected from 26 herds in four states of Australia. A farm was considered to be MRSA positive when at least one MRSA isolate was detected in pig or environmental samples. Of 26 commercial herds, 14 were found to be MRSA positive (53.9%; 95%CI 35.5-71.2). On average 40.28% (CI 27.03-53.54) of the pig samples collected on MRSA affected farms tested positive for MRSA. The prevalence of MRSA within affected farms ranged from 1.6% to 100% (shown in Figure 6.1). The prevalence of pig MRSA varied at the state level between 11% and 56% (data is not shown to preserve the anonymity of the study piggeries). All MRSA isolates were characterised as Staphylococcus aureus on MALDI-TOF and tested meCA positive and PVL negative on RT-PCR. All isolates were typed as ST398.

A total of 36.6% (n = 45/130) of the environmental isolates returned positive for MRSA. Amongst the environmental samples, 38.5% (10/26) of the samples collected from pen floors were contaminated with MRSA. A similar proportion (34.6%) of MRSA contamination was observed across drinkers, feeders and entrances. Of the walls sampled, 15.6% were positive for MRSA. Environmental samples were found to be MRSA positive in most farms where pig samples were positive, except in three farms, where there was a low MRSA prevalence in pigs (< 13%). All piggeries that were negative for MRSA in pigs were also negative for the environmental isolates.
6.3.2 Antibiotic resistance

No resistance to linezolid was observed among the MRSA isolates. Only a small number of isolates (< 5%) showed resistance against SXT, gentamycin, neomycin and QD. All isolates were resistant to tetracycline. The frequency of antibiotic resistance amongst MRSA isolates is shown in Figure 6.2. No significant difference of antibiotic resistance was found between pig and environmental isolates (shown in Figure 6.3). A total of 94.7% of the isolates were found to be MDR. The most common pattern of resistance in MRSA isolates (47.3%) was found against a combination of non-betalactam antibiotics: clindamycin, erythromycin, and tetracycline (shown in Table 6.1).

6.3.3 Antibiotic usage

On the study farms, amoxicillin and penicillin were the most commonly used antibiotics (both used on 92.3% of farms), followed by tylosin (80.8%), tetracyclines (80.8%) and SXT (69.2%). Ceftiofur was also used on 30.8% of the piggeries. None of the study farms reported the use of erythromycin, avoparcin or virginiamycin. The antibiotics and heavy metals used in piggeries over a 12-month period before the survey is shown in Figure 6.4. When participants were asked to rate the usage of particular antibiotics on their farm on a Likert-scale from never used (0) to frequently used (4), amoxicillin and penicillin were reported to be the most frequently used antibiotics in injectable form, with a median treatment frequency of three times per year, followed by tylosin (median = 2) and SXT (median = 1.5). In oral form, (mixed in feed or water) zinc and amoxicillin were the most commonly used products (median = 3) followed by tylosin (median = 2). The proportion of farms with frequencies of injectable and oral antibiotic usage can be found in Figure 6.5 and Figure 6.6. Based on antibiotic classes, betalactams (amoxicillin, penicillin, ceftiofur) were used in 100% of participating piggeries, macrolides (kanamycin, tilmicosin, tulathromycin, tylosin) in 88.5%, tetracyclines (tetracycline, oxytetracycline) in 80.8%, and aminoglycosides (neomycin, apramycin, spectinomycin) in 61.5%. Heavy metals (zinc, copper) were used in 65.4% of the piggeries. Amongst the weaners that were swabbed for MRSA investigation on study farms, amoxicillin was reported to be used the most (57.7%) followed by SXT (30.8%), florfenicol (19.2%), and a similar proportion of usage (15.4%) was reported for lincomycin, neomycin, tulathromycin, and tylosin.

6.3.4 Herd characteristics

The number of breeding sows per herd ranged from 260 to 7,500, with up to 52,000 total pigs including growers and finisher on individual farms. Only 42% of the farms had a closed herd system in place, with the rest of the farms having open systems. Open systems tended to have more MRSA
carriage compared to closed systems (Figure 6.8). However, due to low statistical power the association was not significant ($P = 0.19$).

Approximately half (53%) of the piggeries with open herds were outsourcing their replacement pigs from breeding companies, and 71% of these reported that they never quarantine replacement pigs. Only a small proportion of study farms (11.5%) were practicing all-in-all-out (AIAO). Using univariable analysis, a significantly higher ($P = 0.04$) proportion of MRSA carriage in pigs was identified on farms with continuous systems compared to all-in-all-out system. Although an increased number of MRSA carriage in pigs was found in piggeries failing to quarantine replacement pigs compared to those that practice quarantine regularly (Figure 6.9), the association was non-significant ($P = 0.08$). The majority of farms (77%) reported disinfecting the weaners’ sheds every time following the stock removal. The distribution of MRSA carriage found in pigs for the farms that never disinfect the weaners’ sheds and those that always do is shown in Figure 6.10. Chloride, virkon and quaternary ammonium chloride (QAC) were the most commonly used disinfectants in piggeries (shown in Figure 6.6).

The majority of the participants (65.5%) perceived antibiotic resistance as an important issue in pigs. The rest of the respondents either reported not knowing (11.5%) or disagreed (23%) with the statement that resistance was an issue. Most participants (76.9%) were in favour of reducing antibiotic usage. The willingness to reduce antibiotics were shown both by those who considered antibiotic resistance as an issue (76.5%) in pigs as well as those who didn’t consider resistance as an issue (83.3%). Some participants (15.4%) showed willingness to reduce antibiotic usage only if it would not affect the overall farm productivity. Only a small proportion of participants (7.7%) did not show a willingness to reduce antibiotic usage. All participants indicated that they either always (60%) or sometimes (40%) ask the consulting veterinarians’ opinion about the risk of antibiotic use on their farms. And the participants who did consult veterinarians sought alternatives to antibiotics always (68%) or sometimes (32%).

6.4 Discussion

6.4.1 Prevalence

In this study, 54% of a sample of Australian piggeries were found to have presence of MRSA on their piggery and within the affected piggeries on average two-fifths (40.28%) of pigs were carrying MRSA. The prevalence found in this study is in line with the prevalence reported in the studies conducted in Europe and other parts of the world where a variable prevalence of MRSA carriage in pigs ranging from 0 to 85% has been reported (Espinosa-Gongora et al., 2012b, Cuny et al., 2009,
Morcillo et al., 2015). A study that collected 60 nasal and five environmental samples in different pig herds in the Netherlands found 71% MRSA positive herds with 54% of pigs positive (Broens et al., 2011b). However, the study pooled nasal swabs which is likely to have affected the sensitivity of MRSA detection (Grmek-Kosnik et al., 2005). Another study in the Netherlands collected 60 nasal swabs in each of 36 piggeries, and found 78% of farms infected with MRSA ST398 (Dorado-Garcia et al., 2015). Similarly, in Belgium, 68% (34/50) of randomly selected piggeries were identified MRSA positive, with 44% (663/1500) of pigs carrying nasal MRSA (Crombé et al., 2012b). Although prevalence of carriage in pigs in most Asian counties is reported to be less than twenty percent (Chuang and Huang, 2015), herd level prevalence has been reported as high as 59%, with a common sequence type ST9 (Fang et al., 2014). A recent study in the USA that collected 20 nasal swabs per herd from 37 pig farms, including one MRSA positive control farm (previously identified for MRSA infection), found no MRSA positive herds other than the positive control (Sun et al., 2015). However, a previous study reported up to 30% of farms positive for MRSA in the USA and pig level prevalence to be 18% with two MLST types, ST398 and ST5 (Frana et al., 2013).

Recently, a high prevalence of carriage of highly infectious and the most prevalent CA-MRSA strain in Australia, ST93, was reported in pigs and piggery workers on a single Australian pig establishment which had been identified as the focus of ongoing MRSA infections among piggery employees over a three-year period (Sahibzada et al., 2017). Finding a high carriage of ST93 in pigs and piggery workers in the previous study gave rise to concern that the molecular epidemiology of MRSA in the Australian pig industry might be different from the rest of the world, where LA-MRSA is predominantly reported. However, the current study found ST398 as the only strain within the national pig herd, as sampled, and indicated that Australia is similar to other pig producing countries in this respect. The survey also collected information about human infection on study farms, and none of the farms sampled reported human infection due to MRSA. Finding no ST93 on any tested farm with no reported infection in piggery workers implies that this strain may not be established in the commercial piggeries on a large scale at this point or might be present at undetectable levels that our sample size and choice of production stage (weaner) may have not allowed the detection. In future studies, sampling additional farms including small and medium size and different production stage animals is required to confirm the absence of this pathogen across the industry. In addition, sampling across different size of piggeries can assist in benchmarking between the farms to identify potential risk factors. Although LA-MRSA carriage in pigs has been reported as an occupational risk (van Cleef et al., 2015), there is no evidence of risk to the wider public. In addition, LA-MRSA ST398 is reported to be a less likely cause of human infections, compared to the human-adapted strains (Graveland et al., 2010).
In the current study convenience sampling of pigs within the weaning stage of production was performed. The MRSA status of weaners is reported to have ramifications for the MRSA status of the remainder of the herd, as these animals can sustain the carriage status in onward production stages. For example, herds with high prevalence in the weaning stage are likely to maintain this level of carriage in subsequent production stages (i.e. growing and finishing) (Weese et al., 2011). In addition, weaner pigs have been reported to have higher MRSA prevalence than pigs in other stages of production (Dorado-Garcia et al., 2015; Chapter 4). As such, the MRSA carriage prevalence presented in the current study might be overestimating the individual level carriage for the farm when other age groups are accounted for.

In this study, the environmental samples were negative on farms where no MRSA carriage was observed in pigs and positive on all farms where MRSA prevalence in pigs was greater than 13%. This indicates that sampling from the pig environment could be used for a routine assessment of MRSA presence amongst pig farms in the case of limited resources and logistics; however, the low sensitivity of detecting MRSA must be taken into account. Other studies have also used only environmental samples for investigating prevalence of MRSA across different regions; for example Alt et al. (2011) in Germany collected five dust samples in each piggery with a total of 290 farms to investigate potential risk factors associated with ST398 detection on piggeries; similarly, another study has also used five dust samples per farm for detection of MRSA (Dahms et al., 2014).

6.4.2 Antibiotic usage and resistance

Intensive farming is reported as an important driving force for antimicrobial usage in food animals, and pig farming has been reported to have the highest antimicrobial usage amongst livestock and poultry farming in 26 countries in Europe (ESVAC, 2017). There is currently no surveillance system for antibiotic resistance and usage in pigs at the national level in Australia. Recently, Australian Pesticides and Veterinary Medicines Authority (APVMA) has collected information on the total veterinary antimicrobials sold for usage in food animals (APVMA, 2014) and pigs were reported to represent 31% of the total sold antimicrobials (89.4/288 tonnes). In addition, excluding polypeptides and oligosaccharides from the total usage (not used in pigs), pigs represented one half of the total therapeutic veterinary drug sold in 2010 (170/288 tonnes). Nevertheless, details on quantities used in different livestock species were not available for all products, therefore, making a comparison between species is not possible. The total usage in food animals cannot be generalised for pigs as drug usage varies between species. As an example, pigs represented only 1% of the total betalactam but 99% of total lincosamides sold in 2010 in Australia (APVMA, 2014). Only a single study has previously collected information on antimicrobial usage in piggeries in Australia (Jordan et al., 2009).
However, this previous study reported qualitative usage based on antimicrobial classes; and individual antibiotics of all registered classes were not studied. The current study provides an insight into antimicrobial usage in pigs in Australia, reporting information on usage of antibiotic agents that are registered and allowed for extra-label use in pigs. The current study shows that antibiotic classes such as betalactam, macrolides, tetracycline, sulphonamides, aminoglycosides, and lincosamides, respectively, were commonly used antibiotics amongst the study farms. Comparing the usage of these classes of antibiotics with the previous survey indicates a slight increase, but this difference might be due to differences in the sample size and the target population. Over the same time, statistics show an approximate 16% drop in pig population between 2006 and 2010 (ABARES, 2013) but no reduction in the overall therapeutic use of antibiotics in pigs has been seen, rather a slight increase from 89 to 89.4 tonnes is reported (APVMA, 2014) which points to an overall increase in antibiotic usage in pigs in Australia for the last few years. Since the emergence of LA-MRSA in pigs, subsequent studies established its association with the quantitative use of antimicrobials (Dorado-Garcia et al., 2015, Pyörälä et al., 2014, Smith et al., 2013) resulting in a marked reduction in antimicrobial usage in pigs in Europe. In some European countries, for example Netherlands, antimicrobial usage has been reduced by 50% since 2009 (SDa, 2016). Moreover, a clear reduction in the resistance in bacteria including MRSA has been noticed in some European countries like in the Netherlands (MARAN, 2016). Therefore, reduction in antibiotic use in Australian pig industry can assist in reducing the MRSA load. Developing a strategy for monitoring antibiotic consumption at the national as well as farm level will allow the pig industry to be benchmarked and compared with other countries that have benefited from reducing the overall antibiotic consumption.

6.4.2.1 Association between antibiotic usage and resistance in MRSA

In this study, a high prevalence of resistance is noted for antibiotics that are commonly reported to be used on the surveyed piggeries. As an example, 100% resistance against tetracycline was observed in the current study and the usage of tetracycline was reported in the majority of the study piggeries. Similarly, a high proportion of MRSA isolates were resistant to macrolides (i.e. erythromycin 76.7%) and lincosamides (i.e. clindamycin 94.6%), and the majority of the piggeries reported its usage. Studies have found a high ST398 transmission rate in farms when tetryclines and betalactam were used (Broens et al., 2012a, Dorado-Garcia et al., 2015). Similarly, the high usage of macrolides and lincosamides in pig producing countries is reported to be linked with the high resistance of these antibiotics in ST398 (Pyörälä et al., 2014). These antibiotics target the 50S subunit of the bacterial ribosomes, therefore, resistance in bacteria is often linked. In addition, the resistance to any of these two class of antibiotics lead to resistance against streptogramin B which is classified as highly important for human medicine; therefore, resistance in bacteria is often referred as MLSB (EFSA,
Macrolides are antibiotics classified as critically important in human medicine. A number of macrolide antibiotics are registered for pig use in Australia: erythromycin, kanamycin, tulathromycin, tilmicosin, and tylosin. All are all classified as critically important (WHO, 2017) and the survey indicated that the usage is not uncommon in pigs. However, macrolides, lincosamides, and tetracyclines are the first line antibiotics used in pigs in many countries, with a resistance of over 90% has been reported (Broens et al., 2011d, Conceição et al., 2017).

Since all MRSA, including ST398, are resistant to betalactam antibiotics including penicillins, amoxicillin and cephalosporin, the use of these antibiotics in pigs increases selection pressure. Ceftiofur is a third generation cephalosporin classified as a critically important drug in human medicine (WHO, 2017). In pigs, ceftiofur use has been linked with MRSA carriage on farms (Dorado-Garcia et al., 2015). In Australia, this drug is not registered for pig use; however it is prescribed as extra-label in pigs. It is evident from this survey that a considerable proportion (30.8%) of the study farms reported to have used ceftiofur in pigs within the 12-month period prior to the survey. However, it is reported to be used in injectable form only and the frequency of usage was quite low as often or frequent usage was reported in only 7.6% of the participants. In a previous survey, ceftiofur use was reported in a relatively lower proportion of study piggeries in Australia with one quarter (25%) of the farms reporting the usage (Jordan et al., 2009). In addition, a slight increase of cephalosporins from 0.02 tonnes to 0.3 tonnes has also been observed by APVMA (2014) over a 5 year period (2006-2010). The Food and Drug Administration (FDA) in America has prohibited the extra-label use of cephalosporin in food animals since 2012 because of its potential risk of developing resistance to zoonotic pathogens.

One-half of the piggeries (46.2%) in this survey reported having used tiamulin in pigs. Tiamulin is a pleuromutilin used mainly in veterinary medicine, except retapamulin use topically in humans, is not rated either critical or high (WHO, 2017). However, tiamulin is claimed to trigger resistance caused by vga and cfr genes confer a combined resistance to pleuromutilin, lincosamides, and streptogramin agents (van Duijkeren et al., 2014, Kadlec et al., 2010). This gene is also associated with linezolid resistance (Sahibzada et al., 2017, Paridaens et al., 2017). In a previous investigation by Sahibzada et al. (2017) a novel resistance to linezolid was identified in ST398 isolated from a piggery worker; however, in this study no resistance was found amongst ST398.

In the current study, zinc was used in pig feed in 65.4% of the herds. Likewise, antibiotics, heavy metals (such as zinc and copper) usage in animal feed are also demonstrated to select for ST398 in pigs due to the co-location of resistance determinants (Hau et al., 2017, Slifierz et al., 2014). The EU has now recommended banning zinc in animal feed in like manner tylosin, avoparcin, spiramycin, bacitracin and virginiamycin.
The information collected on antibiotic usage in this study provide a good understanding of the overall antibiotic usage practice in Australian pig farms; however, gathering information on usage in the previous 12 months might be subject to recall bias, if this information was not accurately recorded on the farm.

### 6.4.2.2 Low resistance to restricted antibiotics

This study identified resistance to chloramphenicol (30.5%), ciprofloxacin (25.8%), QD (17.2%), and gentamycin (4.1%). These drugs, along with other antibiotic classes rated as critical or high in humans (such as all streptogramins, fluoroquinolones, fusidanes, glycopeptides, and polypeptides) are restricted in pigs in Australia. Such restrictions might have resulted in a low resistance in contrast to other countries (EFSA, 2014). Previously, avoparcin, a glycopeptide, and virginiamycin, a streptogramin, were allowed to be used in pigs in Australia. However, these antibiotics were voluntarily withdrawn from the market after concerns that their in-feed usage might lead to bacterial resistance (APVMA, 2004) against antibiotics classified as highly and critically important in humans, such as QD and vancomycin (WHO, 2017). Given zero use of these antibiotics was reported in the study farms, this validates that these are not in use anymore. Despite the usage restriction, expression of resistance to these antibiotics was found in this study. The reason for this finding is unknown but might be due to the cross-resistance corresponding to the use of different antibiotics belonging to the same class or carrying a gene encoded for multiple resistances. For example, *erm* genes confer cross-resistance to all macrolides as well as to lincosamides and streptogramins. Similarly, another gene, *cfr*, causes resistance to several antibiotics including amphenicols, lincosamides, pleuromutilins, streptogramin A, and some macrolides, as well as linezolid. The *erm* and *cfr* gene has previously been identified in ST398 isolates in Australia (Sahibzada et al., 2017). Although chloramphenicol and gentamycin are prohibited for use in pigs, other antibiotics of the same classes are registered, such as florfenicol, neomycin, spectinomycin, and apramycin. In contrast, fluoroquinolones have never been approved for use in food animals in Australia. Fluoroquinolone resistance occurs as a point mutation in *Staphylococcus aureus* resulting alteration in the DNA gyrase enzymes (Holden et al., 2010, Lee et al., 2017). Therefore, fluoroquinolone resistance usually spreads vertically in bacteria; however, horizontal transmission through generalized transduction is also possible. Since fluoroquinolone has never been permitted in food animals in Australia, there are chances that such resistant genes may have been imported along with this strain when introduced into Australia. In some European countries, fluoroquinolones are used in production animals, and up to 43% resistance has been recorded in MRSA isolated in pigs in these regions (EFSA, 2014).
6.4.2.3 Subtherapeutic oral use of antibiotics

Antibiotics are important for animal health, welfare, and production; however, imprudent usage at a subtherapeutic level for growth promotion and group treatment with the intention of prophylactic use leads to resistance in bacteria. Continuous exposure to antibiotics, in particular, those used in oral form for longer periods, provide the strongest selection pressure for bacteria including MRSA (Burow et al., 2014). The oral administration of group treatment usually leads to the improper dosage of drugs in pigs. A study that collected information on therapeutic antimicrobial treatment in 50 pig herds in Belgium reported oral administration as under-dosed 47% of the times the drugs were administered, and overdosed 23% of the times (Callens et al., 2012). In the current study betalactam and macrolides were the most commonly used oral antibiotics followed by tetracyclines and lincosamides; and a high resistance was identified to these antibiotics in this study. However, oral administration is a common way of using antimicrobials in intensive piggeries with up to 99% administered in oral form (APVMA, 2014) because treating an individual animal can be labour intensive and uneconomical.

Although the type of antibiotics, the quantity and the routes of administration are all important in relation to resistance in pigs, MRSA carriage has also been observed in antibiotic free piggeries (Smith et al., 2013). Moreover, experimental studies have found MRSA transmission in pigs even in the absence of antimicrobial use (Weese et al., 2011, Broens et al., 2012b, Crombé et al., 2012a) indicating the importance of good husbandry practices such as biosecurity, hygiene, better nutrition, good genetic resistance to diseases, and proper stocking rate.

6.4.3 Farm characteristics and participants’ perception

Although this study established a non-significant association for MRSA carriage in pigs with biosecurity (i.e open system, failing to quarantine replacement pigs) and hygiene practices, a lower proportion of MRSA was detected in piggeries reporting better biosecurity and hygiene practices in place compared to those with no adequate measures such as open farming systems, continuous practice, and not disinfecting the pig sheds every time stock is removed. However, other studies have demonstrated that better biosecurity could markedly reduce the risk of MRSA occurrence in pigs. For example, Alt et al. (2011) found that limiting the number of sources for replacement pigs can reduce the risk of introducing and disseminating MRSA in pigs. Similarly, Dorado-Garcia et al. (2015) revealed that having a closed farming system is a protective factor against MRSA in pigs compared with a continuous farming system. Moreover, studies have reported MRSA contaminated environments in association with MRSA carriage in pigs (EFSA, 2009d; Espinosa-Gongora et al., 2012) highlighting the importance of shed cleaning and hygiene. The non-statistical association observed in this study might be due to other confounders that have not been accounted for or the
insufficient sample size. Future studies with a larger sample size will be useful to identify the most important biosecurity and hygiene-related factors associated MRSA carriage in pigs in Australia.

The overall perception of participants indicates that the majority would like to take action in relation to the issue of antibiotic resistance and that they are keen to explore alternative options to antibiotics. Some participants in the current study indicated that they would reduce antibiotics if the change would not compromise on farm productivity. Studies in other parts of the world have investigated alternative options to antibiotics with positive impact on pig health and overall farm profitability. In line with this, a study conducted in Belgium on 61 commercial pig herds demonstrated no loss in production after reducing antimicrobial use by more than 50% coupled with improved biosecurity and vaccination (Postma et al., 2017). In addition, the study revealed improvement in piglets’ survival rate and weight gain. There is a need to develop a strategy of communicating such beneficial research to the producers that could help in promoting good husbandry practices with less antibiotic usage. In addition, further research is required to explore options within Australian domestic farming practices to improve pig health with the aim of reducing antibiotic usage. Developing economic models for the cost-benefit analysis can help to evaluate alternative farm management strategies including antibiotics substitutes, reducing stocking density, investing extra in improving farm biosecurity and hygiene. This may further promote reduced antibiotic use within the pig industry.

Overall, the perceptions of the respondents indicate a moderate degree of risk awareness and willingness to reduce antibiotic usage and tackle the resistance issue. However, this response is likely influenced by the fact that these herds were selected based on willingness to participate representing the population with interest and concern in this area. The producers’ perception and attitude towards antibiotic use and emerging resistance could further be promoted through communicating and raising farmers’ awareness about the risk of extensive antibiotic use. Studies have found an inverse relationship for antibiotic usage on farms with farmers’ knowledge and perceived risk where producers with better knowledge and correct risk perception towards bacterial resistance tend to use fewer antibiotics on their farms (Kramer et al., 2017, Visschers et al., 2016). Veterinarians can play an important role in raising awareness and promoting good practice amongst pig producers as they represent one of the most common sources among pig producers for seeking information regarding antibiotics and their risks, and on alternative strategies (Visschers et al., 2015).

6.5 Conclusion

This study found that MRSA lineage ST398 is widespread among commercial piggeries participating in the study and that ST93 was not present in these farms. These results are similar to what is reported in many parts of the world. However, the proportion of antibiotic resistance to most of the tested non-
betalactam antibiotics were observed to be lower in comparison to previous studies abroad. This study also found that the biosecurity level and hygienic conditions of the studied farms could be further improved. Examples of improvements include breeding own replacement pigs, ensuring replacement pigs are sourced from MRSA free suppliers (which is likely to be difficult in the current system) and implementation of all-in-all-out systems coupled with effective cleaning and disinfection of sheds.

To further reduce the resistance level and overall MRSA prevalence in the pig industry, it is recommended that antibiotic stewardship practices are strengthened across the industry. A systematic effort is required to quantitatively measure the use of antibiotics in pigs at a farm level as well as at a national scale. Establishment of a nationally coordinated antibiotic resistance surveillance program could help in monitoring trends in antibiotic resistance prevalence. Such a program would enable the industry to develop strategic plans for the management of antibiotic resistance issue.
Table 6.1 Multi-drug resistance (MDR) pattern amongst MRSA ST398 isolates collected in 26 piggeries to investigate the MRSA prevalence in national herds in Australia.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla, tet</td>
<td>32</td>
<td>5.25</td>
</tr>
<tr>
<td>bla, cli, tet</td>
<td>29</td>
<td>4.76</td>
</tr>
<tr>
<td>bla, chl, cli, tet</td>
<td>22</td>
<td>3.61</td>
</tr>
<tr>
<td>bla, cip, cli, tet</td>
<td>26</td>
<td>4.27</td>
</tr>
<tr>
<td>bla, cli, ery, tet</td>
<td>288</td>
<td>47.29</td>
</tr>
<tr>
<td>bla, cli, neo, tet</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>bla, chl, cip, tet</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>bla, cli, ery, qd, tet</td>
<td>5</td>
<td>0.82</td>
</tr>
<tr>
<td>bla, chl, cli, sxt, tet</td>
<td>7</td>
<td>1.15</td>
</tr>
<tr>
<td>bla, cli, ery, sxt, tet</td>
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<td>0.16</td>
</tr>
<tr>
<td>bla, chl, cip, cli, tet</td>
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</tr>
<tr>
<td>bla, cip, cli, ery, tet</td>
<td>9</td>
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</tr>
<tr>
<td>bla, chl, cli, ery, tet</td>
<td>59</td>
<td>9.69</td>
</tr>
<tr>
<td>bla, cip, cli, neo, tet</td>
<td>4</td>
<td>0.66</td>
</tr>
<tr>
<td>bla, chl, cli, ery, qd, tet</td>
<td>6</td>
<td>0.99</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>bla, chl, cip, cli, neo, tet</td>
<td>3</td>
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</tr>
<tr>
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<td>bla, chl, cli, ery, qd, sxt, tet</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>bla, cip, cli, ery, neo, qd, tet</td>
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<tr>
<td>bla, chl, cip, cli, ery, qd, tet</td>
<td>39</td>
<td>6.40</td>
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<tr>
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<td>1.81</td>
</tr>
<tr>
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<td>0.16</td>
</tr>
<tr>
<td>bla, chl, cip, cli, ery, gen, neo, qd, tet</td>
<td>11</td>
<td>1.81</td>
</tr>
</tbody>
</table>
Figure 6.1 The prevalence of MRSA carriage among pig samples collected in 26 piggeries to investigate the MRSA prevalence in national herds in Australia. MRSA was detected on 54% (n = 14/26) of the study piggeries.

Figure 6.2 Antibiotics resistance phenotype pattern in MRSA ST398 isolates (n=609) collected from pigs and environment in 26 piggeries to investigate the MRSA prevalence in national herds in Australia.

*amc (amoxicillin-clavulanate), fox (cefoxitin), chl (chloramphenicol), cip (ciprofloxacin), clin (clindamycin), ery (erythromycin), gen (gentamicin), neo (neomycin), qd (quinupristin-dalfopristin), tet (tetracycline), sxt (sulfamethoxazole-trimethoprim)
Figure 6.3 Antibiotic resistance phenotype pattern in MRSA ST398 isolates originated from pigs (n = 564) and environment (n = 45) collected in 26 piggeries to investigate the MRSA prevalence in national herds in Australia.

*amc (amoxicillin-clavulanate), fox (cefoxitin), chl (chloramphenicol), cip (ciprofloxacin), clin (clindamycin), ery (erythromycin), gen (gentamicin), neo (neomycin), qd (quinupristin-dalfopristin), tet (tetracycline), sxt (sulfamethoxazole-trimethoprim)

Figure 6.4 Proportion of pig herds reported as having used particular antibiotics and heavy metals over the 12 months prior to survey among 26 pig herds participating in the study.

*amc (amoxycillin), apra (apramycin), avo (avoparvin), cer (ceftiofur), ctc (chlortetracycline), cu (copper), flor (florfenicol), kita (kitasamycin), lin (lincomycin), neo (neomycin), olanq (olaquindox), pen (peniceline), salino (salinomycin), spect (spectinomycin), sxt (sulfamethoxazole-trimethoprim), tet (oxytetracycline), tiam (tiamulin), tilm (tilmicosin), tul (tulathromycin), tylo (tylosin), virg (virginiamycin ), zn (zinc)
Figure 6.5 Proportion of pig herds reporting the frequency of injectable antibiotic being used in the past 12 months. (Negative percentages represent 'never used' responses and positive responses represent certain levels of usage (rarely, sometimes, often, frequently)).

Figure 6.6 Proportion of pig herds reporting the frequency of oral antibiotic and heavy metals being used in the past 12 months. (Negative percentages represent 'never used’ responses and positive responses represent certain levels of usage (rarely, sometimes, often, frequently)).
Figure 6.7 Proportion of pig herds reporting the frequency of disinfectants being used in the past 12 months. (Negative percentages represent ‘never used’ responses and positive responses represent certain levels of usage (rarely, sometimes, often, frequently)).

*QAC = Quaternary ammonium compound

Figure 6.8 Box plot for the distribution of MRSA+ isolates found among 26 pig herds reported as having types of farming system in place.
Figure 6.9 Box plot for the distribution of MRSA+ isolates found among 26 pig herds reported as having quarantine system in place for replacement pigs.

Figure 6.10 Box plot for the distribution of MRSA+ isolates found among 26 pig herds reported as having practice disinfecting the weaner shed after removal of stock.
Chapter 7.

Assessment of the potential economic impact of MRSA on the Australian pig industry
Abstract

Introduction

Livestock-associated (LA) MRSA ST398 has been described as an occupational health issue in many countries with intensive pig production (EFSA, 2012). Recently, a high prevalence of MRSA carriage has been identified on a pig enterprise recognised for recurrent MRSA outbreaks among workers (Sahibzada et al., 2017) and reported, for the first time, a community-associated (CA) MRSA ST93 as an occupational health risk (Chapter 5). A subsequent investigation among Australian commercial piggeries identified MRSA ST398 carriage among pigs in 54% (CI 35.5-71.2) of participating piggeries (Chapter 6). Although ST398 is reported to represent an additional burden and cost to MRSA infections in some countries (EFSA, 2009c), the impact of the newly emerged CA-MRSA strains, such as ST93 in piggery workers is uncertain.

Aims and methods

The current study aims to estimate the potential economic impact of MRSA infections among piggery workers in the Australian pig industry. To achieve this aim, a risk assessment approach based on scenario trees and a Monte Carlo simulation model has been used. Three different situational scenarios were considered depending on strain carriage in piggery workers, these being: Scenario 1) ST93 MRSA carriage only; Scenario 2) ST398 MRSA carriage only; and Scenario 3) A combination of ST398 and ST93 carriage (4:21 ratio). The key input parameters considered in the models were the probability of positive MRSA carriage in pigs in piggeries, the probability of MRSA carriage and infection in piggery workers, the probability of required hospitalisations among infected piggery workers and the associated costs due to infection and hospitalisation.

Results

Scenario 1 resulted in the highest number of infections, with a median of 830 (5%-95%, 477-1316), and hospitalisations (median = 262, 106-539), costing the industry a median of AU$4m (1.74-7.92) per year. Scenario 2 resulted in the lowest rate of infections (68, 18-153) with a median of only two people requiring hospitalisation and a median cost of $15,206 (2,250-5,657). In Scenario 3, a median of 710 (409-1119) people are estimated to be infected per year, resulting in a median of 220 (89-453) hospitalisations and costing the industry a median of $3.36m (1.47-6.66). The majority of infections (median = 697, 401-1105) in Scenario-3 were associated with ST93.

Conclusion

This study indicates that MRSA carriage and infection in piggery workers could potentially cause a significant economic impact to the Australian pig industry, and the emergence of new MRSA strains like ST93 amongst piggery workers could pose a major economic challenge.
7.1 Introduction

MRSA represents a large challenge for infection control in human hospitals and certain sections of the community. Some domestic animals are efficient hosts of MRSA, with pigs being the most frequently reported livestock species with asymptomatic carriage. The MRSA lineage carried by livestock species is known as livestock-associated (LA-MRSA). The strain type ST398 is one of the most frequently reported LA-MRSA in pigs and people in contact with pigs (Goerge et al., 2017, Sahibzada et al., 2017). ST398 is believed to be less pathogenic in humans in comparison to hospital-associated (HA) and community-associated (CA) strains of MRSA (Kock et al., 2011, Mutters et al., 2016). HA-MRSA usually causes infections in hospital patients; in contrast, CA-MRSA strains, the most common in Australia being ST93, are known for enhanced virulence and fitness level, resulting in severe infections in otherwise healthy individuals (Coombs et al., 2017, Otto, 2013).

It is evident from a number of studies that MRSA represents an additional burden of staphylococcal infections, causing increased morbidity and mortality (in comparison to non-MRSA infections), and resulting in an additional economic burden (Filice et al., 2010, Uematsu et al., 2016). In Europe, MRSA was estimated to have caused 5,400 attributable extra deaths, and an additional hospital cost of €380 million in 2007 (ECDC, 2009). In the USA, MRSA causes over 80,000 invasive infections annually which lead to 11,000 deaths (Dantes et al., 2013). In Australia, each year there are approximately 6,900 reported cases of *S. aureus* infections, with 34% reported as being MRSA, which have been estimated to cost approximately AU$150 million (Nimmo et al., 2011, Collignon et al., 2005). In a hospital setup, the MRSA cost for each patient upto AU$60,000 (Cosgrove et al., 2005), with an average of approximately AU$10,000 (Uematsu et al., 2016, Wernitz et al., 2005). The high cost of MRSA infections in hospitals is mainly due to the long hospital stay, which is reported to be on average 20 days, but ranging from 10 to 90 days (Wernitz et al., 2005, Papia et al., 2015, Tejiram et al., 2017).

Studies have reported up to 85% of MRSA prevalence among people working in piggeries affected by MRSA in pigs (Cuny et al., 2009, Reynaga et al., 2016). The carriage within the at-risk group is linked to the MRSA status and prevalence in pigs, as MRSA carriage in people working on MRSA negative farms has rarely been reported (van Duijkeren et al., 2008). Despite ST398 being reported as an occupational risk for nasal MRSA carriage, subsequent infections have rarely been documented for this strain. One study reported 6% skin and soft tissue infections in piggery workers, but none of these were caused by ST398 (Nadimpalli et al., 2016). Although ST398 is usually reported to caused mild skin infections (Becker et al., 2017), some severe infections involving ST398 have also been reported (Becker et al., 2017). ST398 is reported to represent an additional burden to MRSA carriage.
and infections in hospital setups for some European countries (Camozz et al., 2013, van Alen et al., 2017).

The Australian pork industry plays a vital role in employing people and providing quality pork to the nation, contributing approximately $1.4 billion to the gross domestic product (GDP) of the Australian economy (ABARES, 2017). The pork industry GDP represents 6% of the total gross value of the livestock industries and 2% of the total Australian farm production income. In 2015–16, around five million pigs were slaughtered, which produced 378,000 tonnes of pig meat. Australia’s pork sector mainly raises pigs for the domestic market; however, the industry also contributes to the export economy. In 2015-16, Australia exported 29,000 tonnes of pig meat valued at $129 million (ABARES, 2017). According to Australian Bureau of Statistics (ABS), there are approximately 1,800 pig enterprises, with a total of 271,000 sows (ABS, 2017a); however, many of them operate on a small scale. According to Australian Pork Limited (APL), the Australian pork industry research and development organisation, there are only 272 commercial piggeries with more than 150 breeding sows, which produce approximately 90% of the total national pig produce (L.K. Van Breda, personal communication, December 21, 2017). The exact number of people working in the pig industry is unknown, but previous reports estimated the total number to be between 17,000 and 20,000 people (ABARES, 2017, Kerr, 2015).

While the majority of studies are looking at the economic burden of MRSA related to HA-MRSA, the economic impact due to human infections with MRSA within the commercial pig industry has not been investigated. Recently, a high prevalence of carriage of MRSA in people and pigs was identified on an Australian pig enterprise with a recurrent MRSA outbreak in workers (Sahibzada et al., 2017). A subsequent study investigated the prevalence and distribution of MRSA amongst pigs in Australian commercial piggeries on a national level (Chapter 6). This study confirmed that MRSA carriage in pigs occurred in over half of participating piggeries (54%). These findings imply that an outbreak of MRSA in piggery workers represents a challenge for the pig industry and may result in marked economic losses for pig producers, through medical expenses, workers’ compensation and loss of productive working days. In addition, there may be other associated costs, such as workers’ welfare losses, anxiety among carriers and putative loss of lives among those infected. The current study was designed to estimate the potential economic losses to the pig enterprises and the overall Australian pig industry due to the carriage of different MRSA strains among pigs and piggery workers using a risk assessment approach.
7.2 Methods

7.2.1 Development of the simulation model

The first step in this study was to develop a scenario tree (Martin et al., 2007), to describe the pathway of MRSA infection and hospitalisation among piggery workers in a commercial piggery and estimate its corresponding probability (Figure 7.1). The nodes included in the scenario tree were: 1) probability of the piggery being affected with MRSA (in pigs); 2) probability of MRSA carriage in piggery workers; 3) probability of MRSA infection among piggery workers carrying MRSA; and, 4) probability of severe MRSA infections requiring hospitalisation. In this study, ST93 MRSA was used as a representative of a range of CA-MRSA that exist and might possibly colonise piggery workers as a result of their occupational exposure.

Three different situational scenarios were considered depending on the MRSA strain carriage in piggery workers, given the severity of the infection, and as such, the economic impact, differs according to strain: 1) ST93 MRSA carriage only; 2) ST398 MRSA carriage only; and 3) A combination of ST93 and ST398 carriage. In the last scenario, the piggery workers would be carrying ST398 and ST93 with a ratio of 4:21 based on the reported MRSA carriage in an Australian piggery affected by a recurrent MRSA outbreak in workers (Sahibzada et al., 2017). A conceptual model of the three situational scenarios is shown in Figure 7.2.

The scenario tree was developed using Microsoft Excel (PC/Windows 7, 2010) and probabilities estimated using Monte Carlo stochastic simulation modelling in @Risk 7.5 (Palisade Corporation, 2016) with input parameters incorporated into the model as probability distributions. Each simulation consisted of 100,000 iterations sampled using the Monte Carlo sampling method with a fixed random seed.

Using the outcomes of each of the three situational scenarios, the financial loss arising from MRSA cases in piggery workers was derived as an industry-wide (national) figure. This cost was comprised of the following components: sick leave, medical costs and hospitalisation, and replacement of the sick workers, which was aggregated to the national level by considering, the total number of piggery workers in commercial piggeries. Simulations determined the unit cost of an MRSA infection in human from a distribution and multiplied by the number of hospitalisations in piggery workers. Similarly, the unit cost of sick leave was estimated by multiplying the number of people diagnosed with MRSA infections and the number of sick leaves per infection.
7.2.2 Data sources

The study uses available data from several sources to estimate the input probabilities, with most of the data sourced from our recent investigation examining the risk for MRSA carriage in piggery workers on a pig farm with a recurrent MRSA outbreaks in people (Chapter 3 and 5). In this investigation, information on health history, previous MRSA diagnosis, hospitalisation, the number of days stayed in a hospital, and the number of days taken off from work due to MRSA diagnosis were collected from a total of 52 piggery workers. The study found 60% (31/52) MRSA carriage in piggery workers, with 16% of the MRSA detected typed as ST398 and 84% as ST93. A total of 42% of those who were positive for MRSA had been diagnosed with MRSA infection on the farm and 31% of these required hospitalisation due to severe infection. Additional information about the total number of infections over the outbreak period and the total cost per infection was collected from the farm records. Scientific literature was used to gather additional data required to estimate input parameters for the models. A detailed description of data used for each of the model input parameters is described in the following section and Table 7.1.

7.2.3 Scenario tree probabilities

7.2.3.1 Probabilities of the piggery being infected with MRSA (in pigs) and of MRSA carriage and infection in piggery workers

Recently, a national MRSA prevalence study was conducted on a representative sample of commercial piggeries in Australia and reported 54% \((n = 14/26)\) of the participating farms, had pigs that carried MRSA (Chapter 6). This proportion has been used to estimate the probability of a piggery being MRSA positive \((P (farm \ affected \ with \ MRSA \ in \ pigs))\) and incorporated into the model using a beta distribution to account for the uncertainty around this estimate \((\text{beta}(15, 13))\).

Since working on MRSA affected farms has been recognised as the most important factor for MRSA carriage in piggery workers, the current study only considers MRSA carriage in workers of those piggeries that are MRSA positive. The carriage is a state when a worker carries MRSA in the nose without expressing symptoms of infection or disease due to this pathogen. According to Sahibzada et al. (2017), 60% \((n = 31/52)\) of piggery workers on a pig farm was found to be carrying MRSA. The current model has used a beta distribution around this proportion, to estimate the probability of MRSA carriage in people \((P (\text{nasal MRSA carriage in workers}))\). This distribution was used both, for ST398 and ST93 carriage. As stated earlier MRSA ST398 is commonly isolated from piggery workers where over 80% carriage has been reported in Europe (Cuny et al., 2009, van Cleef et al., 2010b); however, non-ST398, for example, ST93, has rarely been reported. Given ST93 has only been found on a single
occasion in piggery workers, MRSA carriage due to ST93 is difficult to be estimated. As such, the same MRSA carriage value was used for both, ST398 and ST93, based on results reported by Sahibzada et al. (2017).

Nasal MRSA carriage can subsequently develop into an infection in people, depending on the MRSA strain involved. As the ability of both strains (ST93 and ST398) in developing infection is different, separate probability distributions were used to estimate the probability of MRSA infection in those positive for carriage with either of the strains \(P(\text{infection} \mid \text{positive for nasal MRSA carriage})\). For ST93 carriage, the infection rate was estimated from our recent study (Chapter 5), where 42\% \(n = 13/31\) of participants carrying nasal MRSA had previously been diagnosed with MRSA infections. This study assumes that all those infections were caused by ST93 and a beta distribution around this proportion was used in the model. Another study has also reported a high infection (45\%) subsequent to the nasal carriage of CA-MRSA (Rahimian et al., 2007). For ST398 carriage in piggery workers, no prior epidemiological studies had explored the risk of subsequent infections. However, observational studies investigating clinical MRSA isolates have reported MRSA ST398 related infections with a larger variation from 0 to 8\% (Grundmann et al., 2010, van Alen et al., 2017). Since there is a higher level of uncertainty related to ST398 infections, a betapert distribution also referred as RiskPert (Vose, 1996, Palisade Corporation, 2016) was used in this model to estimate the conditional infection rate for those carrying ST398, with a most likely value of 3\%, and a minimum and maximum of 0 and 10\%, respectively (Table 7.1).

### 7.2.3.2 Probability of severe MRSA infections requiring hospitalisation

Hospitalisation rate is an important cost-driver in our model owing to the level of healthcare required for affected individuals. For each occupationally-derived MRSA infection, the employer must meet hospitalisation and sick leave cost. The probability of hospitalisation given a person is diagnosed with MRSA infection \(P(\text{hospitalisation} \mid \text{positive for infection})\) is different for ST93 and ST398. For estimating the number of people hospitalised, given they were diagnosed with ST93 infection, a beta distribution \(\text{beta}(5, 10)\) derived from information collected in Chapter 5 was used. In this study, 13 piggery workers reported MRSA infections, and of these, four required hospitalisations.

MRSA infections may become systemic and result in bacteraemia that requires hospitalisation. As the likelihood of systemic infection due to ST398 is very low, the probability of hospitalisation for these cases is expected to be lower (Becker et al., 2017) than that of cases arising from ST93 infections (Nimmo et al., 2013). A study investigating 2,890 Staphylococcus isolates collected from different countries in Europe, found no ST398 among MRSA collected from clinical specimens (Grundmann et al., 2010); whereas, another study in Germany that investigated 6,555 MRSA isolated from humans
in pig-dense regions found 8% \((n = 124)\) of the clinical isolates belonged to ST398; however, only seven isolates were associated with systemic infection or bacteraemia (van Alen et al., 2017). Similarly, a study in the Netherlands using a deterministic approach, estimated a very low probability (0.09%) of bacteremia subsequent to ST398 nasal carriage among farmers, when the carriage was assumed to be 38% in veal calf farmers and 68% in pig farmers (van Cleef et al., 2013). To model the likelihood of severe infections due to ST398 requiring hospitalisation, a betapert\((0, 0.02, 0.08)\) distribution was used in the current study.

7.2.4 Estimating the cost associated with MRSA infections in piggeries in Australia under different situational scenarios

7.2.4.1 Number of piggeries and piggery workers at risk in Australia

Previous studies have found an increased risk of MRSA carriage in pigs on large commercial pig farms compared to smallholdings. Similarly, the risk of subsequent transmission of MRSA to people in direct pig contact has been reported to be higher in larger herds (Fang et al., 2014, Fromm et al., 2014). As such, the current study only considers commercial piggeries when estimating the potential costs of MRSA, and piggeries with more than 150 breeding sows have been included. According to data collected from APL, the number of piggeries with more than 150 breeding sows is estimated to be 272 (LK. Van Breda, personal communication, December 21, 2017).

Given there is a lack of data on the number of staff working on large commercial piggeries, the total number of piggery staff working on commercial piggeries with different herd sizes was calculated in this study. The distribution of herd sizes was obtained from APL (LK. Van Breda, personal communication, December 21, 2017), while the number of workers required in these farms was estimated from an earlier industry survey (APL, 2016). A total of 6,365 workers were estimated to work in commercial piggeries with over 150 sows (shown in Table 7.2). Using a uniform probability distribution, 20% variability was considered in either direction of the total estimated workers.

7.2.4.2 Calculation of the number of MRSA infections and hospitalisations among piggery workers of commercial piggeries in Australia

In this step of the modelling, the probability estimates used in the scenario tree were combined with the number of piggeries and piggery workers at risk in Australia, to estimate the number of MRSA infections and hospitalisations, under the three different situational scenarios: 1) ST93 MRSA carriage only; 2) ST398 MRSA carriage only; and 3) A combination of ST398 and ST93 carriage at a ratio of 4:21.
A stochastic process, using 100,000 iterations was used to obtain the output probabilities results in relation to the following equations. Firstly, the number of MRSA carriage (W) and infections (Y) amongst piggery workers for each strain was calculated. Secondly, the estimated number of hospitalisations (Z) and non-hospitalisations (X) for each strain was estimated. Lastly, the output probabilities obtained in these equations were used to estimate the associated costs of infections as described in the following sections.

**Number of farm workers carrying MRSA (W) =**

\[ W = P(\text{farm affected with MRSA in pigs}) \times P(\text{nasal MRSA carriage in workers}) \times \text{Total workers in commercial piggeries} \]

**Number of farm workers diagnosed with MRSA infection (Y) =**

\[ Y = W \times P(\text{infection | positive for nasal MRSA carriage}) \]

Where ‘W’ is the number of total workers positive for nasal MRSA carriage given they are working on MRSA affected farms, and ‘Y’ is the total number of workers diagnosed for MRSA infection given they were positive for nasal MRSA carriage.

**Number of infected farm workers hospitalised (Z) =**

\[ Z = Y \times P(\text{hospitalisation | positive for infection}) \]

Where Z is the number of workers that were diagnosed with severe infection requiring hospitalisation given they were working on MRSA affected farm and given they were positive for nasal MRSA carriage.

**Number of infected farm workers not-hospitalised (X) =**

\[ X = Y - Z \]

### 7.2.4.3 Number of days of sick leave among MRSA infected piggery workers

The number of days required off work depends on the severity of the infection. In the current study, information obtained from the infected piggery with MRSA infection in workers and the information collected in Chapter 5 shows a higher mean number of days off work among people affected with MRSA infections requiring hospitalisation (18 days) compared to those not requiring hospitalisation (5 days). Therefore, considering the severity of infection, the proportion of days off work was estimated separately for hospitalised and non-hospitalised workers.
For hospitalised workers, a betapert distribution with a most likely value of 18 days (range 7-56) sick leave was used. Although the probability of hospitalisation due to ST398 is very low compared to ST93, this study assumed the length of days off work to be similar for both strains in case of severe infections. For those who were diagnosed with MRSA infections but not hospitalised, different assumptions were used to estimate the number of days off work for each strain. For ST93 infections, a betapert distribution with a most likely value of 5 days (range 0-28 days) was used to estimate the number of days of sick leave. In contrast, for ST398, when infection does not require hospitalisation, the model assumed no sick leave, as it is evident from the literature mentioned earlier that ST398 normally causes mild superficial skin infections.

7.2.4.4 The cost of MRSA infections among piggery workers

There are two types of costs that the model considers when calculating the total losses due to MRSA infections, including the cost incurred by sick leaves and hospitalisation.

Sick leave cost:
Sick leave cost was computed from the attributable cost of a single day off work due to MRSA infection and to replace the person taking sick leaves by additional workers. On average, a farm worker earns $751 weekly (ABS, 2017b), which equates to $150 per day. According to Fair Work Ombudsman Australia (FWO, 2017), a piggery attendant is paid $673 to $808 weekly, depending on their employment level; with a minimum hourly rate for casual employees $22 to $27 for weekday work (not for weekends or public holidays). In the current study, a flat rate of $150 per day irrespective of the type of employment (casual or permanent) has been used. The total cost of sick leave was calculated considering the unit (day) sick leave cost ($150), the number of days of sick leave and the number of infected farm workers obtained from previously described distributions, and the sick leave replacement costs as described below:

\[
\text{Total sick leave cost} = (Z \times \text{number of days off work} \times \$150) + (X \times \text{number of days off work} \times \$150) + \text{(Sick leaves replacement cost)}
\]

Hospitalisation cost:
In this study, an average annual cost of $3,000 (ranging from 1000-9000) per person due to hospitalisation was used based on data obtained from the affected piggery. The farm data comprised of the details relating to the total cost incurred to the employer by each individual infection over a three years period of MRSA outbreak among workers. It is also reported that MRSA infection in Australian hospital setup causes a longer hospital stay (average 29 days) that leads to an average of
AU$3,200 per patient (The Expert Working Group, 2001). Therefore, an average estimate of $3000 was used as the most likely value of a pert distribution, with a minimum of $1,000 and a maximum of $9,000. The total hospitalisation costs were calculated using the following formula:

\[
\text{Total hospitalisation cost} = Z \times \text{unit hospital cost}
\]

### 7.3 Results

The results are shown as the median of the output probability distribution and the 5th and 95th percentile of the distribution is given in brackets. The simulation results estimated that 1,991 (1324 - 2781) piggery workers have nasal carriage of MRSA, independently of the strain. The infection, hospitalisation rate and total cost are estimated to be different for each situational scenario investigated in this study, and results are detailed in the following paragraphs. The results for each situational scenario, presented as output probability distributions, are shown in Figure 7.3, 7.4 and 7.5.

#### 7.3.1 Scenario 1: ST93 MRSA carriage only

The first scenario, where MRSA carriage in pig workers is only due to ST93, results in the highest number of infections and hospitalisations when compared with Scenario 2 and 3. The median number of infections per year in this scenario is 830 (477-1316), with 262 (106-539) requiring hospitalisations. The pig enterprises are short of 10,076 (4026-20805) days of labour. In this scenario, the industry faces the highest economic loss, with AU$4 million (1.74-7.92) per year, with an individual median annual cost for the infected piggery of $27,000.

#### 7.3.2 Scenario 2: ST398 MRSA carriage only

The second scenario, where all MRSA carriage is due to ST398, results in the lowest infection rate among all the scenarios (68; 18-153), with a median of only two (0-5) severe infections requiring hospitalisations. The labour shortage was estimated to be a 32 (4-127) days. This scenario is estimated to result in the lowest cost to the industry, with a median cost of $15,206 (2,245-56,570).

#### 7.3.3 Scenario 3: A combination of ST398 and ST93 carriage at a ratio of 4:21

Scenario 3, where MRSA carriage in piggery workers is caused by both, ST398 and ST93 in a 4:21 ratio, the median total number of infections is estimated to be 710 (409-1119), with only 11 (3-24) of these being caused by ST398, and the rest by ST93. This scenario is causing the industry 8,472 (3,385-17,481) days of labour shortage, where ST398 contribution is minimal, with only causing 5 (1-20) days of a labour shortage. The model estimates 220 (89-453) cases requiring hospitalisation due to severe infections, with all these hospital cases being due to ST93. The estimated median cost to the
industry is AU$3.36 million (1.47-6.66), which equates to $23,000 for each individually infected piggery. ST398 is only contributing $2,000 (359-9,000) to the overall costs of this scenario.

7.4 Discussion

This study shows that there are likely to be vastly different occupational health issues and economic consequences based on differing strains entering and becoming endemic within individual piggeries and in the national herd. Recently, a study reported an unexpectedly high prevalence of highly pathogenic CA-MRSA ST93 carriage in humans and pigs on a piggery in Australia (Sahibzada et al., 2017). These findings were quite different from other studies conducted elsewhere in the world, where LA-MRSA in pigs and people with pig exposure have been mainly reported (Liu et al., 2015, Crombé et al., 2013). The current study simulated the impact of newly emerging pathogenic MRSA strains, like ST93, along with ST398, on the Australian pig industry and results indicate that non-ST398 poses a greater economic risk for the pig industry than ST398. Moreover, it is evident from this study (scenarios 1 and 3) that a situation where non-ST398 was to become endemic amongst piggeries, would have a severe impact on the industry, leading towards a substantial amount of loss and resulting in occupational infections in piggery workers on a large scale. The endemic presence of non-ST398 in the industry is estimated to generate a direct economic cost of up to eight million dollars (95th percentile) representing almost 1% of the total Australian GDP of pig production. The results show that the marked impact under the Scenario 3 is largely driven by ST93 rather than by ST398, which is also obvious from the outputs of Scenario 2 (carriage and infection due to ST398 only) having little impact on the industry.

There is evidence that livestock, including pigs, represent a reservoir for the emergence of new human-pathogenic S. aureus clones with the capacity for pandemic spread (Spoor et al., 2013). And it has been shown in an experimental study that non-ST398, like CA-MRSA ST8, are not only capable of colonising pigs but also outcompete ST398 (Moodley et al., 2012). Other human-adapted strains, for example ST5, have also emerged in pigs (Narvaez-Bravo et al., 2016, Frana et al., 2013, Khanna et al., 2008). It is quite possible that in the future, new strains of highly pathogenic community-associated MRSA could successfully evolve in pigs in the presence of selection pressure, which would further enhance its ability to colonise both hosts and might become a more challenging zoonotic agent. ST93, which is the predominant CA-MRSA in Australia (Coombs et al., 2016), exhibits marked virulence factors compared to healthcare and other community-associated MRSA strains (Chua et al., 2011b). Although ST93 has only been reported in a single piggery in Australia at this stage, further spread among piggeries in the future cannot be ruled out.
This study identified ST398 to be less impactful compared to non-ST398, with respect to the number of infections and the overall economic loss. ST398 is the predominant strain in piggery workers in Europe, where a higher carriage and infection due to this pathogen has been reported compared to the rest of the world (Liu et al., 2015).

In this study, the likelihood of ST398 carriage and infection in piggery workers were assumed to be similar to that reported in Europe (i.e. a maximum estimate for the Australian situation) and still estimated fewer infections for Australia compared to other countries in Europe which is evident from Scenario 2. Despite using double the proportion (2%) of the reported severe infections for ST398 in Scenario 2, the model predicted only a median of 68 infections in workers. In addition, only a median of two of the total infected workers were predicted to require hospitalisation under this scenario, indicating that the majority are likely to be diagnosed with mild infections. However, our estimated number of infections in Scenario 2 is higher than that estimated by Cox and Popken (2014), who assessed the risk of MRSA infection amongst pig farmers in the USA and predicted only one infection per year out of 41,777 ST398 carriages; however, the previous study assumed no infections due to ST398 for the at-risk group in the model. While some studies have associated ST398 with severe cases of infections, the overall risk amongst farmers is apparently negligible (Cuny et al., 2015b, Nadimpalli et al., 2016). Therefore, the true risk of infection due to ST398 is likely to be considerably smaller than our estimates, and the risk of severe infection could possibly be null. Although the current study estimated a low number of severe infections due to ST398 in piggery workers, the nasal carriage and infection rate was high, therefore, the ST398 impact cannot be overlooked. In contrast to our finding, another study in Sweden has estimated a considerable cost incurred by the endemic spread of ST398 in the pig industry (Höjgård et al., 2015). The study predicted a cost of €1,233,510 to Swedish healthcare arising from endemic ST398 in a risk group of 6,000 people, comprised of people working in direct pig contact and their family members. At the time of assessment, the study considered Sweden free of LA-MRSA and assumed a similar prevalence for the at-risk group than the one previously reported in the Netherlands (6-28%).

Although no human MRSA infections due to ST398 have been reported in Australia to date, a proactive approach to avoid them in the future is advised. Since the first discovery of ST398 in 2005, the initial epidemiological studies in Europe recognised this pathogen as only being associated with asymptomatic carriage in piggery workers; however, a gradual increase in reported infections has been observed in the last few years (Goerge et al., 2017, Becker et al., 2017). There is evidence that this strain is becoming more human-adapted over the passage of time (Bosch et al., 2016). In Denmark, a 156 times increase in the number of infections due to ST398 has been noted over a five year period since 2009 (DANMAP, 2014, DANMAP, 2016, DANMAP, 2010). It is therefore not out
of the question that this strain might undergo further genetic changes and become more virulent and pathogenic to cause infections in piggery workers on a larger scale.

The estimates from this study only include the direct medical cost to pig producers incurred by an infected worker in a hospital and paid sick leave days. The cost could increase if additional medical costs, including diagnostic and therapeutic expenses incurred by workers diagnosed with MRSA but not hospitalised, were included. Other non-health service costs, such as informal care costs, patient travel costs and out-of-pocket expenses were not included in this study either. A previous study has found an increase of 100 times the estimated economic losses due to MRSA infections, when societal costs are included (Lee et al., 2013). In addition to the economic impact, the widespread nature of MRSA and presence of human infections in the pig industry could attract unfavourable publicity amongst consumers that may result in losing trust and increasing levels of public concern for worker welfare. Moreover, the increase in the number of infections amongst piggery workers could also result in a high rate of employee turnover, and attracting new skills and labour might become a challenge. Thereby, the pig industry, which is already facing a labour shortage (Kerr, 2015), may be challenged by further staff turnover which may also impact farm productivity.

### 7.5 Conclusion

There is substantial potential for adverse economic impact due to zoonotic transmission of MRSA in the Australian pig industry. The bulk of the potential loss is attributable to CA-MRSA with the capacity to colonise or be carried by pigs. In particular, ST93 has been shown to have this capacity and potentially other CA-MRSA present in humans may well demonstrate a similar capacity. This may result in marked economic losses if an endemic situation among pigs occurs.

Further investigation is required to identify cost-effective interventions to prevent the transfer of MRSA from humans to pigs (like ST93), resulting in the emergence of this pathogen in pigs and consequential spread amongst piggery workers. The considerable economic losses due to MRSA infections in piggery workers may justify further investment in prevention and control. This study suggests the need for active surveillance that will help in early diagnosis of new emerging MRSA strains and assist in limiting further spread of existing strains including ST93.
Table 7.1 Description of the input parameters used in a simulation model estimating the economic impact due to MRSA carriage and infection in piggery workers in commercial pig enterprises (>150 sows) in Australia

<table>
<thead>
<tr>
<th>Input variable</th>
<th>Distribution</th>
<th>Distribution parameters</th>
<th>Data sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P(farm \text{ affected with MRSA in pigs})$</td>
<td>beta ($s+1$, $n−s+1$)</td>
<td>$s= 14$ $n= 26$</td>
<td>Chapter 6</td>
</tr>
<tr>
<td>$P(nasal MRSA carriage in workers)$</td>
<td>beta ($s+1$, $n−s+1$)</td>
<td>$s= 31$ $n= 52$</td>
<td>Chapter 3</td>
</tr>
<tr>
<td>$P(MRSA infection due to ST93</td>
<td>+nasal MRSA carriage)$</td>
<td>beta ($s+1$, $n−s+1$)</td>
<td>$s= 13$ $n= 31$</td>
</tr>
<tr>
<td>$P(MRSA infection due to ST398</td>
<td>+nasal MRSA carriage)$</td>
<td>betapert (min, most, max)</td>
<td>min= 0 most= 0.03 max= 0.1</td>
</tr>
<tr>
<td>$P(Hospitalisation due to ST93</td>
<td>+MRSA infection)$</td>
<td>beta ($s+1$, $n−s+1$)</td>
<td>$s= 4$ $n= 13$</td>
</tr>
<tr>
<td>$P(Hospitalisation due to ST398</td>
<td>+MRSA infection)$</td>
<td>betapert (min, most, max)</td>
<td>min= 0 most= 0.2 max= 0.08</td>
</tr>
<tr>
<td>Number of days of sick leave due to ST93 infection</td>
<td>betapert (min, most, max)</td>
<td>min= 0 most= 5 max= 28</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Number of days of sick leave due to ST398 infection</td>
<td>0</td>
<td>-</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Number of days of sick leave due to ST93 hospitalisation</td>
<td>betapert (min, most, max)</td>
<td>min= 7 most= 18 max= 56</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Number of sick leaves due to ST398 hospitalisation</td>
<td>betapert (min, most, max)</td>
<td>min= 7 most= 18 max= 56</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Unit cost of hospitalisation</td>
<td>betapert (min, most, max)</td>
<td>min= 1000 most= 3000 max= 9000</td>
<td>This study</td>
</tr>
<tr>
<td>Number of piggery workers</td>
<td>betapert (min, most, max)</td>
<td>min= 5092 most= 6365 max= 7638</td>
<td>This study</td>
</tr>
</tbody>
</table>
Table 7.2 An estimation of the number of required staff in piggeries in Australia according to the total number of piggeries and the herd size.

<table>
<thead>
<tr>
<th>Herd size (Breeding sows)</th>
<th>No of businesses</th>
<th>Number of sows</th>
<th>% of total sows</th>
<th>staff required</th>
<th>total staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>469</td>
<td>9,340</td>
<td>3.45</td>
<td>3.1</td>
<td>1,454</td>
</tr>
<tr>
<td>51-150</td>
<td>168</td>
<td>16,591</td>
<td>6.13</td>
<td>2.7</td>
<td>454</td>
</tr>
<tr>
<td>151+</td>
<td>272</td>
<td>244,534</td>
<td>90.41</td>
<td>23.4</td>
<td>6,365</td>
</tr>
</tbody>
</table>

Figure 7.1 Generic scenario tree for the assessment of the probability of MRSA infections in piggery staff working on MRSA affected commercial piggeries (node 1) subsequently leads to the probability of MRSA carriage (node 2), infection (node 3), and severe infections that requires hospitalisation (node 4).
Figure 7.2 A conceptual model of three situational scenarios used to estimate the economic impact of MRSA carriage among piggery workers in the Australia pig industry, according to the MRSA strain (ST93, ST398).

Figure 7.3 The outcome probability distributions of annual MRSA infection cases among piggery workers in commercial piggeries in Australia, under three different situational scenarios according to the strain.
Figure 7.4 The outcome probability distributions of annual MRSA hospitalisation cases among piggery workers in commercial piggeries in Australia, under three different situational scenarios according to the strain carriage.

Figure 7.5 The outcome probability distributions of the total cost due to MRSA carriage among piggery workers in commercial piggeries in Australia, under three different situational scenarios according to the strain carriage.
Chapter 8.

General Discussion
8.1 Background to the thesis

The overarching aims of this thesis were to investigate the potential contribution of pigs to an outbreak of MRSA among piggery workers on a single pig enterprise in Australia, to identify the potential risk factors for MRSA carriage associated with the occupational activity on the outbreak piggery and to investigate the presence, prevalence and distribution of MRSA, with a particular focus on likely molecular type, in commercial piggeries in Australia. This will allow comparison of our domestic pig industry with other parts of the world where MRSA is considered a challenge in pigs and people with pig exposure (Wardyn et al., 2015, Dorado-Garcia et al., 2015, van Cleef et al., 2014).

This investigation commenced within a single pig enterprise that had been the focus of an ongoing outbreak of MRSA in piggery workers. In this piggery, high prevalence carriage of MRSA in pigs as well as the workers was found. Prior to the current study, this particular piggery was recognised by the State Department of Health as having a marked occurrence of MRSA infections in people, some of which required hospitalisation. Additional information provided by the farm showed that each MRSA infection in humans costs the enterprise on average $3000 and 18 days of sick leave (Chapter 7). The common question posed by both the Department of Health and the management of the pig enterprise was whether there was any involvement of pigs in the recurrent MRSA outbreak.

This enterprise had two sites that were affected (site-A and site-B), with a higher number of infections in workers reported from site-A. The investigation commenced at site-A, with samples collected from pigs, pig environment (sheds, ponds), all workers, and the environment shared by workers (showers, toilets, kitchen, sitting areas, and offices). The initial focus of the study was on the detection rather prevalence of MRSA in pigs as the aim was to identify whether pigs were carrying MRSA in the first instance, given that there was only one prior report of MRSA in pigs in Australia (Groves et al., 2014).

The research reported in Chapter 3 revealed that pigs acted as the key host in the MRSA outbreak among piggery workers; however, anthropozoonotic transmission was also identified.

The results of this chapter indicated a possible human-to-pig host transmission and identified pigs as a likely reservoir for CA-MRSA with the capacity for zoonotic transmission. It is evident from WGS that ST93 was likely introduced into the pig population in this piggery by humans, and that it subsequently evolved in pigs and acquired further antibiotic resistance determinants against drugs that are commonly used in pigs. Although pigs were found to be the most likely reservoir for ST93 on this piggery, human to human transmission is also possible and indeed likely. ST93 is one of the dominant and most successful CA-MRSA in Australia and is frequently isolated from people in the community and hospitals (Coombs et al., 2015, Harch et al., 2017). However, the overall prevalence
of CA-MRSA ST93 found amongst piggery staff in this study is greater than previously reported in other cohorts (veterinarians, hospital staff, or community groups) in Australia (Verwer et al., 2012, Brennan et al., 2013, Munckhof et al., 2009, Vlack et al., 2006, Jordan et al., 2011). A large proportion of ST93 found in this study did not carry PVL and IEC genes, in contrast to usual findings, suggesting loss of these genes and adaptation of this strain to the pig environment. It is possible that ST93 could further acquire (or re-acquire) genetic traits in the presence of selection pressure that would enhance its ability to colonise both hosts and might become a more formidable zoonotic agent.

The second MRSA strain found on this farm, ST398, did not harbour PVL and IEC, indicating this strain is a pig specific clade. Although this study did not identify the presence of PVL and IEC which enhance the virulence and adherence ability of ST398 in humans, it is not guaranteed that this strain will not undergo further genetic change. Early reports in Europe recognised asymptomatic carriage of ST398 in at-risk groups with the absence of human virulence and enterotoxin genes, but these genes have become more prevalent in recent years (Diene et al., 2017). In addition, this strain is reportedly emerging as a potentially pathogenic strain in humans in some European countries, resulting in an additional clinical burden to existing MRSA infections (Becker et al., 2017). A survey conducted in 26 European countries that investigated 2,890 invasive Staph isolates collected from humans between 2006 and 2007 identified no LA-MRSA (Grundmann et al., 2010). However, another study conducted in 2014 in 27 European countries reported 3.9% (n = 533) as LA-MRSA ST398 among 13,756 MRSA isolates collected through screening and clinical sampling in patients, with a majority (n = 401) isolated from clinical samples (Kinross et al., 2017). Confirming this trend towards pathogenicity, a study investigating the molecular characteristics of over 9,000 LA-MRSA isolates collected through the Dutch public health surveillance from 2003 to 2014 proposed that ST398 had become more human-adapted over time and could transfer among humans, independent of pig contact (Bosch et al., 2016). The whole phylogenetic analysis performed in the current study revealed that this lineage is likely to have been introduced into Australia from Europe or North America on multiple occasions. Since there is no live pig importation in Australia, the implication is that this strain has been introduced by humans (likely veterinarians or farmers) exposed to this strain overseas.

In conclusion, this study found that pigs can act as a reservoir for LA- as well as CA-MRSA, both of which may spread to humans; similarly, humans can be a source of transmission of new strains of MRSA to pigs. These findings have major public health implications and highlight the importance of a One Health approach to disease investigation.

In Chapter 4, the prevalence of carriage of different MRSA strains was investigated on a pig level at both sites of the aforementioned farm. Given a high prevalence of carriage of ST93 in humans was
found in the farm under investigation (Chapter 3), it was hypothesised that a high prevalence of carriage of the same strain was to occur in pigs. In addition, in Chapter 3 it was found that ST93 was the only MRSA present in nasal swabs from staff working on site-A, whereas both ST93 and ST398 were detected on site-B. Therefore, it was hypothesised that prevalence of MRSA strain carriage in pigs would be different for both sites, reflecting the differences in human carriage. To test this hypothesis, all pig samples collected on site-B were genotyped, as reported in Chapter 3, while a new set of swabs were processed for site-A to provide individual animal, rather than pooled sample, as explained in Chapter 4. To gain an insight into the epidemiological details of the collected MRSA isolates, this work used different primers to detect the MLST types and PVL genes in each isolate; and a subset of MRSA isolates were characterised for the *spa* typing.

Three quarters (75.2%) of pigs were found to be carrying MRSA, with the majority identified as ST93 (71%). The results show a significantly ($P < 0.01$) higher proportion of ST93 (80%) amongst MRSA found on site-A compared to site-B (67%) which is in line with the high proportion of carriage of ST93 in humans on site-A. Although ST398 was identified in pigs on site-A, the proportion of carriage was lower compared to ST93. However, ST93 was not detected in workers on site-A. This supports the proposition that ST93 outcompetes ST398 in workers likely due to the high affinity for human colonisation (Hewagama et al., 2016, Chua et al., 2011b). The results in this chapter indicate the importance of MRSA strain prevalence in pigs, a high carriage of a certain strain in pigs could reflect the carriage of in-contact humans. Moreover, one-third of the ST93 (31%) isolates collected in pigs did not have PVL encoding genes confirming the finding in Chapter 3 where three out of twelve pig isolates (a selected subset) belonging to ST93 were lacking PVL, suggested pig adaptation of this strain. Overall, the antibiotic susceptibility profiles of ST93 isolates demonstrated resistance to a much greater number of antibiotics than previously reported for ST93 and has likely resulted through antibiotic selective pressures encountered within pig farming.

In addition, in this study, the odds of CA-MRSA ST93 detection were higher in weaners compared to other stages which is hypothesised to be likely due to selection pressure, since weaners are recognised for a high antibiotic usage compared to others stages of pig production (Postma et al., 2016, van Rennings et al., 2015, DANMAP, 2016).

Chapter 5 investigated the risk factors for MRSA carriage in piggery workers on the outbreak enterprise. A direct association between MRSA carriage and pig contact was found, which was strongly associated with the intensity of contact and each hour increase in occupational contact per day increased the risk of MRSA carriage by 1.44 times (95% CI 1.14-1.96). These associations were found for LA-MRSA ST398 as well as CA-MRSA ST93, and therefore this study reports ST93 as a potential occupational-health-issue in piggery workers for the first time. In addition to the generalised
increase in likelihood of carriage with pig exposure, those working with farrowing sows were found to have increased odds of MRSA carriage compared with those who did not which is possibly due to the intense nature of the pig contact required when working in this shed.

In Chapter 6, MRSA prevalence in commercial piggeries in Australia was investigated. The high prevalence of MRSA in pigs and people found on the outbreak piggery was expected, as the piggery had already been identified for the recurrent MRSA infections in humans. Given that ST398 and ST9 are the predominant LA-MRSA in other parts of the world and ST93 had never been reported as an occupational health issue in piggeries, it was hypothesised that the molecular epidemiology of MRSA in the Australian pig industry might be different compared to the rest of the world. To test the hypothesis, a study was designed to investigate MRSA carriage in pigs and the environment among commercial piggeries (with >300 sows) in Australia. The study did not aim to investigate MRSA carriage in humans; however, the study gathered information on MRSA infections in people working on these farms, via a questionnaire. Only commercial piggeries were targeted for this study, as larger herds have been previously identified to be at higher risk of MRSA carriage in pigs and people with pig exposure, compared to smaller farms (van Den Broek et al., 2009, Reynaga et al., 2016). According to APL estimates, there are 200 enterprises with over 300 breeding sows (LK. Van Breda, personal communication, December 21, 2017). All of these enterprises were invited to participate, and 26 agreed to participate in the study. The sensitive nature of the topic under investigation is likely to be the reason for the low participation rate.

Prior to this study it was unknown whether MRSA was established in the Australian pig industry. Previously, MRSA was considered likely to be less prevalent in pigs in Australia compared to other parts of the world as a single study had previously found only three MRSA (ST398) positive samples among 324 samples collected in pigs from five different commercial pig farms and one feral herd (Groves et al., 2014). In the study conducted in this thesis, MRSA was found on 54% (CI 35.5-71.2) of the study farms with pig prevalence ranging from 1.6% to 100% within infected farms, indicating that Australia is not very different from other pig producing countries, where a high MRSA prevalence has been reported (Cuny et al., 2009, Dorado-Garcia et al., 2015, Broens et al., 2011b).

Lack of detection of ST93 and no reported infections in humans on any of the farms sampled suggests the pathogenic MRSA strain ST93 is unlikely to be widespread amongst commercial piggeries in Australia. However, it is also possible that MRSA infections are present in workers, but not brought to the attention of management, given that MRSA is not thought of as an occupational issue within the medical sector in Australia. Also, potential spread to other piggeries in the future cannot be ruled out as it has been shown that ST93 has the potential to adapt and circulate within the pig environment in Chapter 3. However, it is unclear whether it is possible or how long it could take for ST93 to
become widely established in the pig industry in Australia. Moreover, the well-established ST398 in pigs in Australia could also potentially emerge as a source of human infections. Although this strain has mainly been associated with carriage rather than infection since its discovery in 2005, Denmark has reported a significant increase (156 times) in the infection rate due to ST398 over a five year period since 2009 (DANMAP, 2014, DANMAP, 2016, DANMAP, 2010).

The survey in the current study collected information on antibiotic usage and indicated a high usage of betalactams (amoxicillin, penicillins, and ceftiofur) followed by macrolides, tetracyclines, sulphonamides, aminoglycosides and lincosamides. These antibiotics have been associated with MRSA prevalence in pigs due to selection pressure (Dorado-Garcia et al., 2015, Pyörälä et al., 2014, Smith et al., 2013, Broens et al., 2012a). Although the findings reported in this thesis identify large amount of resistance against antibiotics that are commonly used in pigs (Chapter 3, Chapter 4, and Chapter 6), the proportion of antibiotic resistance to most of the tested non-betalactam antibiotics was observed to be lower in comparison to previous studies abroad (EFSA, 2014). Australia has very strict control and regulation regarding antibiotic usage in food animals and such regulation might have resulted in lower resistance to non-betalactams amongst MRSA isolates identified in this study. Only a restricted number of antibiotics are permitted to be used in pigs in Australia. It is important that the therapeutic use of these antibiotics remain available for pigs’ health and welfare under an efficient regulatory and monitoring system; however, antibiotic use as growth promotion or prophylactic use need to be reconsidered, and responsible therapeutic antibiotic use needs to be ensured.

Overall, this study provides an insight into the epidemiology of MRSA as well as the antibiotic usage and the biosecurity and hygiene practices among participating pig farms. However, caution should be taken when extrapolating results of this study to the Australian pig industry, due to the low number of participating farms and the fact that only weaner pigs were sampled in each farm. Nevertheless, within the constraints of resources and time, this study confirms that LA-MRSA ST398 is established among Australian pigs and ST93 was not present on any of the sampled properties.

Chapter 7 aimed to estimate the potential economic impact due to MRSA carriage and infection among piggery workers in the Australian pig industry, using a risk assessment approach. The predicted outputs of this study show that emerging new CA-MRSA strains within the pig industry, using ST93 as an example, have the potential to cause substantial disruption to pork production, with a high number of infections in workers and resultant marked economic loss. Although the current study estimated a low number of severe infections due to ST398 in piggery workers, the nasal carriage and infection rate was high. The study shows that the spread of ST93 to other pig farms could change the epidemiology of infections in piggery workers, with the potential to increase the MRSA burden in communities and hospitals further. An experimental study investigating the adherence of different
S. aureus strains to pig corneocytes to determine their colonisation potential reported that certain CA-MRSA, such as ST8 have an extended-host spectrum that makes them able to adapt to pigs (Moodley et al., 2012). Also, this previous study claimed that ST8 could outcompete ST398 for colonisation in pigs. Although ST8 is endemic in the USA and has a capacity for pig colonisation, this strain has not yet emerged in pigs on a large scale. However, another human epidemic MRSA strain, ST5, has been reported to successfully adapt to pigs in the USA and Canada (Narvaez-Bravo et al., 2016, Frana et al., 2013, Khanna et al., 2008).

The host jump and the reasons why some clones are more successful than others have not been determined. It is possible that in the future new strains or other CA-MRSA strains could emerge in pigs and acquire genetic traits in the presence of selection pressure that would further enhance their ability to colonise both hosts and become challenging zoonotic agents. It is also evident from Chapter 3 that pigs can potentially act as a reservoir for ST93, and further spread infections.

8.2 Future studies

Although pigs are suggested to be the most likely reservoir for MRSA in this study (Chapter 3), it is unclear whether ST93 carriage found in humans in this single piggery are derived from pigs or ancestral human strains. If required, a detailed molecular investigation to examine core variable genes encoding predicted surface proteins might explain the actual source (either human or pig) of the outbreak.

This study investigated nasal swabs from piggery workers at one point in time (Chapter 3 and 5); thereby, carriage might be due to transient contamination of the nasal passages or true colonisation. A longitudinal cohort study involving repeated measures of MRSA carriage in piggery workers would further improve understanding of the potential risk for persistent MRSA carriage in people.

The current study (Chapter 6) only collected qualitative information on the use of antibiotics among targeted commercial piggeries with no information on the quantity of antibiotic usage. Therefore, the statistical association between qualitative use in this study was limited by the constraints of the limited number of herds investigated. Although antibiotic use has been linked with resistance determinants in pigs (Dorado-Garcia et al., 2015; Smith et al., 2013), it is a very complicated phenomenon and depends on various factors, such as the quantity, frequency, route of administration (oral, injectable), dosage level (therapeutic or subtherapeutic), purpose of drug use (prophylaxis or growth promotion) and class of antibiotic used. Collecting epidemiological data on the quantitative use of different antibiotics in a larger number of farms would help to identify the antibiotics that contribute the most to the emergence of resistance. In addition to data on antibiotic usage, collecting other farm-specific information, such as stocking rate, nutritional management, and vaccination (that were not investigated in the current
study) could also help to identify the important risk factors for MRSA emergence in pigs (Alt et al., 2011, Fang et al., 2014, Postma et al., 2017, Slifierz et al., 2015a, Amachawadi et al., 2015). This may provide important information that can help in developing strategies to reduce antibiotic usage in pigs without compromising pig health and welfare.

8.3 Recommendations from the research

This thesis shows that MRSA in pigs and piggery workers represents a challenge for the pig industry in Australia. ST398 is already established in commercial piggeries (Chapter 6), and it is possible that emerging strains may have a marked impact on the health and welfare of pigs and/or farmers, thus, affecting overall farm productivity. Thereby, this issue requires full recognition from the industry and a proactive approach. The complexity of MRSA in pigs suggests that there is no single solution to reduce MRSA load on farms, and a holistic approach is required. Strategies like reducing antibiotic usage and improving biosecurity and hygiene measures can play a vital role in limiting MRSA emergence and spread. However, other farm-specific management practices could also play an important role within a control strategy such as adequate housing, optimum stocking rate, increased weaning age, improvement of nutritional management, and vaccination. Some of the strategies to control MRSA in piggeries in relation to the findings presented in this thesis are explained in the following sections.

8.3.1 Minimising MRSA transmission through human vectors

The results in Chapter 3 suggest that humans are important sources of introduction and transmission of new strains in pig herds and this is also supported by other studies (Grøntvedt et al., 2016). Humans can transfer MRSA through mechanical means (clothing, footwear, dirty clothes) or biological means such as nasal carriage in workers. However, biological transmission has not gained much attention, therefore, no specific measures of prevention have been investigated in the literature. One could argue for a targeted screening and decolonisation of personnel before working in pig herds as a pre-emptive control strategy; however, the efficacy of decolonisation treatment is controversial (Sai et al., 2015), and decolonisation may not be successful due to recolonisation from the source (Lozano et al., 2011, Davis et al., 2017). Nevertheless, adapting standard occupational hygiene practices and use of regular PPE can limit this transmission. In recommending the uptake of such measures, the results in Chapter 5, which suggest that efforts are needed to improve knowledge and perception of farm owners and piggery workers regarding biosafety measures, must be considered. Overall, targeted training that focuses on the following strategy could help to minimise the chances of introducing MRSA onto a farm and spreading the pathogen among piggery workers:
The workers should be encouraged to wear company-specific clothing (i.e. overalls) and boots when working with animals and to change between each activity, regular use of clean disposable gloves and mask, taking regular showers before and after work and always using a clean towel. The change room with shower-in facility needs to be hygienically clean with clear distinction of dirty and clean areas, where external objects (i.e. clothing and boots) do not touch the clean area or farm-specific objects.

All workers should practice good personal hygiene with regular hand washing using soap and water or an alcohol-based hand rub before and after work activity.

Workers should avoid touching body parts (i.e. face or nose) and scratching their skin with uncleaned hands or gloves.

Skin or soft tissue infections, whether acquired off-farm or on-farm, may become a potent source of CA-MRSA moving from humans to pigs and for LA-MRSA moving from pigs to humans. Therefore, it is important to ensure all wounds are kept covered with an occlusive dressing while at work and medical attention is sought if infections develop.

MRSA can also be spread by presenting contaminated animal handling equipment, containers and other objects in human use such as mobile phone, camera, laptop that are used on another farm. In order to reduce this, workers and visitors should be discouraged from bringing external objects into the farms, or make sure it is hygienically clean and does not come into contact with other animals.

**8.3.2 Improving farm biosecurity and hygiene**

The overall biosecurity and hygiene measures reported on the study farms in Chapter 6 indicate that there is potential for improvement. Biosecurity measures are required to limit the emergence and transmission of MRSA including newly emergent strains like ST93 (Chapter 3) within and between the piggeries. External biosecurity is the most important factor for preventing the introduction of MRSA and establishment of this pathogen in the recipient farms (Alt et al., 2011, Espinosa-Gongora et al., 2012a). In case a pathogen is already present on a farm, tightened biosecurity can prevent the introduction of other virulent strains that potentially could have a marked impact on a piggery in terms of infections in workers (Chapter 5). Over one half (58%) of the study farms, reported in Chapter 6, had open farming systems, and the majority of farms that were outsourcing their replacement pigs did not quarantine the incoming pigs. To limit the risk of introducing MRSA in farms, it is recommended that pigs are purchased from a minimum number of origins (Alt et al., 2011), MRSA free sources (Espinosa-Gongora et al., 2012a) or from piggeries with higher biosafety and health status if MRSA status is not known (unlikely to be possible in Australia at this time).
Similar to external biosecurity, internal biosecurity is also important to control MRSA spread within the herd. Since this thesis (Chapter 4) found MRSA carriage associated with the age group of pigs, it is therefore suggested to avoid staff moving from working on different age groups of pigs within a single shift, where possible, to reduce the chances of MRSA spread between different ages. However, when working in multiple sheds is required, changing clothing and use of shed specific boots is recommended where possible. Equipment should be dedicated for use in a specific shed and should not be used in another shed (across pig age groups) and in cases where equipment must be shared, equipment should be cleaned and disinfected before use in other sheds.

In the current study, one-quarter of the farms reported that they never disinfect the weaners’ sheds after stock removal (Chapter 6). Several studies have reported MRSA contaminated environments in association with MRSA carriage in pigs (Espinosa-Gongora et al., 2012b, EFSA, 2009d). Therefore, adoption of good shed hygiene (cleaning and disinfecting shed) measures are the prime determinants to minimise the dissemination of MRSA (Moodley et al., 2012).

8.3.3 Raising awareness regarding antibiotic resistance

About one-quarter (23%) of the respondents in the national survey (Chapter 6) study disagreed with the statement that antibiotic resistance was an issue in the industry and some participants in this study did not show a willingness to reduce antibiotic usage. This implies a need to raise awareness amongst producers as well as veterinarians about the risk of resistance and the consequences of imprudent antibiotic usage which can help to reduce the emergence and spread of resistant pathogens including MRSA. Studies have found an inverse relationship between antibiotic usage on farms and farmers’ knowledge and perceived risk, where producers with better knowledge and correct risk perception towards bacterial resistance tend to use less antibiotics on the study farms (Kramer et al., 2017, Visschers et al., 2016). It is evident from the literature that counselling farmers towards less antibiotic use could be achieved without compromising production by informing them about feasible alternatives to antibiotics (Postma et al., 2017).

8.3.4 Monitoring MRSA

This thesis provides a base for sampling and analysis in the target population if further surveys are required to monitor the presence and distribution of MRSA in pigs and piggery workers over time. Regular monitoring may help in the early identification of high-risk emergent clones that are particularly able to adapt and spread in the pig environment as found in Chapter 3. The early recognition of emerging MRSA strains will benefit the industry in developing strategic plans and decision making on the control and prevention of emerging resistance at the human-livestock.
interface without jeopardising pig health and welfare. The considerable economic losses estimated in this study (Chapter 7) due to MRSA infections in piggery workers may justify further investment in monitoring, prevention and control strategies that would ultimately deliver both social and economic benefits for people working in the pig industry.

Currently, there is no monitoring or surveillance system for tracking antibiotic resistance in pigs in Australia. Where industry sees a benefit, a biannual or annual sampling across the large commercial farms is suggested to track the changing molecular epidemiology of MRSA over time. In case of limited logistics, environmental samples of pig farms (as described in Chapter 6) may also be used for a rudimentary assessment of MRSA presence on the farms; however, the low sensitivity of testing MRSA need to be considered. A flow diagram suggested for detection of MRSA and microbiological analysis to be performed on the samples that are collected in pigs, human, or environment for detection of MRSA is attached as Annex-5 in this thesis.

8.4 Conclusion from the research

The findings of this thesis can be explained in a form of a hypothesis that describes the events unfolding from an MRSA outbreak on a pig farm in rural Australia and leads to the presence of MRSA in pigs and piggery workers. The investigation on a single pig enterprise indicates that humans are a reservoir of CA-MRSA and have transferred the CA-MRSA strain ST93 to pigs. In pigs, ST93 acquired further antibiotic resistance according to selection pressure and adapted this new host, and subsequently zoonotic transmission may have occurred. However, in the subsequent survey investigating the prevalence and molecular epidemiology of MRSA in the Australian national pig herd found no ST93. This suggests that the high prevalence of CA-MRSA ST93 found on the outbreak piggery is unique. For all this to occur, humans must be predisposed to infection. A high degree of predisposition, leading to a high rate of human MRSA disease, may be the fundamental cause of the outbreak given that CA-MRSA are present in the Australian community. In addition, the national pig herd survey confirmed that individual-animal prevalence in Australia is similar to other pig producing countries where ST398 is the predominant LA-MRSA in pigs. However, the thesis also suggests that evolution in MRSA strains in adapting to new hosts is possible in pigs and people working in direct pig contact.

Due to a direct association between MRSA carriage in pigs and humans identified in this study, it is suggested that any intervention strategy implemented in pig farms on the animal level will have direct impacts on piggery workers’ health and welfare. The findings and recommendations outlined in this thesis could be used as a reference point to advocate for and develop relevant strategies and interventions to limit the MRSA spread among pigs and piggery workers.
Annex-1

Participants information sheet for the MRSA outbreak investigation in piggery workers
Participant Information Sheet

Project title: Epidemiology and molecular characterisation of methicillin resistant *Staphylococcus aureus* in the Australian pig industry.

Principal Investigators:

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Thank you for taking the time to consider participating in this research project. This survey invites people who are working with pigs at the current piggery. If you are over the age of 18 and interested in participating in this study, you will be asked to self-collect a nasal swab. Please note that you will be ineligible to participate if you have any existing nasal problems. We would also appreciate if you could complete the attached questionnaire. The questionnaire takes approximately 20 minutes to complete and it is completely voluntary.

Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish.

What is the purpose of this study?

The main focus of this study is to conduct an investigation of the currently MRSA cases identified among employees of this piggery. This study will help to determine the potential factors posing a risk
for MRSA carriage among piggery staff members. Your participation will help not only to investigate the current identified MRSA cases but also to better understand if any farm practices could be associated with MRSA carriage.

**What is MRSA?**

*Staphylococcus aureus (Staph)* is a bacteria that commonly colonises the skin and nose. Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be resistant to multiple antibiotics including penicillins and cephalosporins that commonly used to treat ordinary staph infections. According to the European Centre for Disease Prevention and Control (ECDC), about 30% of people carry *Staphylococcus aureus* and up to 10% carry MRSA on their skin and nostrils. Normally this colonisation goes unnoticed. Normally, colonisation does not lead to disease symptoms, unless favourable conditions are meet like immunosuppression.

**Why have I been invited to participate in this study?**

For this study we are seeking the participation of people working at this piggery, where cases of MRSA have been observed.

**What does this study involve?**

If you agree to participate you will be asked to complete a questionnaire and swab a sterile nasal swab in your nostrils. You will be responsible for rotating the nasal swab in your external nares before placing it in a covered sterile transport tube. A PhD scholar and senior researchers will supervise you with explicit instructions during the sample collection and questionnaire completion. The questionnaire takes approximately 15-20 minutes to complete and it is completely voluntary. Information will be collected regarding your gender, qualification, any known infections, current or previous antimicrobial treatment, attitude and behaviour toward antimicrobial uses.

Participation in the research is completely voluntary; you can withdraw participation anytime from the research.

**Are there any risks and benefits to me in taking part in this study?**

Participating in this research, you have the option to be notified about the MRSA testing results. You will be sent a standard letter and if your results are positive further advice and guidance will be provided.
How is this study being paid for?

The project is being undertaken by researchers from Charles Sturt University and is being funded by research funds from within the organisation.

Will I be charge for any laboratory tests?

You will not be charged for any laboratory tests performed for this research. All the required laboratory tests will be performed on the expenses of the research funds.

What if I don’t want to take part in this study?

Participation in this research is entirely your choice. Only those people who give their informed consent will be included in the project. Whether or not you decide to participate is your decision and will not disadvantage you.

What if I participate and want to withdraw later?

Participation in this survey is completely voluntary and you can withdraw from the study at any time without giving a reason during or after the survey is conducted. All your information will be deleted that could identify you.

How will my confidentiality be protected?

Responses to this survey will be given unique codes to ensure that anonymity and confidentiality is maintained. The code could be used to re-identify those who showed consent to be notified if they were MRSA positive. However, they could be identified by the assigned researchers only.

In addition, any information collected by the researchers which might identify you will be stored securely and only accessed by the researchers unless you consent otherwise, except as required by law. The information which might identify you will not be disclosed without prior consent. Data will be retained for at least 5 years at Charles Sturt University, School of Animal and Veterinary Sciences, Wagga Wagga.

What will happen to the information that I give you?

It is the intention of the researchers to publish the outcomes of the study in scientific journals and as PhD thesis without mentioning names and addresses of the participants. Individuals will not be identified in any reports arising from the research.
What should I do if I want to discuss this study further before I decide to participate or not?

If you would like further information please contact:

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mhernandez-jover@csu.edu.au

Who should I contact if I have concerns about the conduct of this study?

Charles Sturt University’s Human Research Ethics Committee has approved this study. I understand that if I have any complaints or concerns about this research, I can contact.

Executive Officer  
Human Research Ethics Committee  
Office of Academic Governance  
Charles Sturt University  
Panorama Avenue  
Bathurs NSW 2795  
Tel: +61 2 6338 4628  
Email: ethics@csu.edu.au

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

Thanks for taking the time to consider participating in this research.

**This information sheet is for you to keep.**
Questionnaire for the MRSA outbreak investigation in piggery workers
MRSA outbreak investigation

Section 1: Work related

Note: Please ✓ the most appropriate check box

1. How many years in total have you worked with pigs? ____________years

2. Do you own any livestock and/or pets?
   □ Yes   □ No

3. If yes what type?
   □ Pigs   □ Horses   □ Cattle   □ Sheep   □ Alpacas
   □ Dogs   □ Cats   □ Other ____________________________.

4. Do you have any other family members or housemates working on a pig farm?
   □ Yes   □ No

5. If yes, where do they work?
   □ This farm   □ Somewhere else

6. Do any of your household members work in healthcare?
   □ Yes   □ No

7. If yes, where do they work?
   □ Hospital   □ Nursing home   □ Medical Centre   □ Other ____________

8. How long have you been working at the current farm? (Please mention years/months) ____________

9. At which site do you currently work most of your time?
   □ Site-A   □ Site-B

10. Do you have to move and work at the other site apart from the one you mentioned above?
    □ Yes   □ No

11. If yes on average, how many times in last month did you have to work at the other sites?
12. How many days per week do you work at the farm? _______________ days

13. On average how many hours per day do you work at this farm? _______ hrs

14. What is your main working role at the farm?
   - ☐ Working with pigs (Go to Q17)
   - ☐ Piggery maintenance (Go to Q17)
   - ☐ Pastoral
   - ☐ Agricultural & cropping
   - ☐ Feed mill
   - ☐ Transporting pigs
   - ☐ Administrative

15. Do you ever come into contact with pigs?
   - ☐ Yes
   - ☐ No

16. If yes, how frequently do you contact pigs in a week?
   - ☐ Less than once
   - ☐ Once
   - ☐ Twice
   - ☐ More than twice

Note: Please continue if your main role is “working with pigs” and/or “Piggery maintenance” as mentioned in Q14 otherwise go to section 2

17. Which unit do you work most of the time at site-B?
   - ☐ Unit 1/3
   - ☐ Unit 2
   - ☐ the new Unit

18. How often do you have to work in the other unit apart from the one you have mentioned above?
   - ☐ Once a day
   - ☐ Once a week
   - ☐ Twice a week
   - ☐ Once a month

19. In which shed do you work most of the time?
   - ☐ Dry sow
   - ☐ BB Shed
   - ☐ Farrowing
   - ☐ Lactation
   - ☐ Weaner
   - ☐ Grower
   - ☐ Finisher

20. How often do you have to work in the other shed apart from the one you have mentioned above in Q 17?
   - ☐ Once a day
   - ☐ Once a week
   - ☐ Twice a week
   - ☐ Once a month
21. In the last week, approximately how many hours have you spent on each site and shed conducting the following activities?

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dry sows sheds</th>
<th>BB Shed</th>
<th>Farrowing sheds</th>
<th>Weaner sheds</th>
<th>Growing sheds</th>
<th>Finishing shed</th>
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<tbody>
<tr>
<td>Cleaning/Washing sheds</td>
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<td>Vaccinating pigs</td>
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<td>Moving pigs</td>
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</table>

**Section 2 – Health History**

22. Have you been diagnosed with MRSA infection?

☐ Yes       ☐ No (Go to Q69)

23. How many times have you been diagnosed with MRSA? ________________

24. Do you currently have clinical signs of an MRSA infection?

☐ Yes       ☐ No

1st time diagnosis with MRSA

158
25. When were you diagnosed with MRSA? (Approximate date, month and year)  
__________________________

26. Were you working at the current farm at the time of diagnosis?  
☐ Yes ☐ No (Go to Q29)

27. Which site were you working at?  
☐ Site-A ☐ Site-B

28. In the last 4 weeks before the diagnosis was made, what types of animals were you working with most of the time?  
☐ Dry Sows ☐ Farrowing ☐ Piglets ☐ Weaners  
☐ Finishers ☐ Boars ☐ Unsure

29. If not working at the current farm where were you working?  
__________________________

30. Were any of your family members diagnosed with MRSA at around the same time as you?  
☐ Yes ☐ No

31. How many family members were diagnosed with MRSA? _____________________

32. Did you take time off work as a result of your MRSA infection?  
☐ Yes, please indicate the approximate number of days _____or weeks _____taken off work.  
☐ No

33. Were you hospitalized during the MRSA infection?  
☐ Yes, please indicate the number of days _____or weeks _____that you were hospitalized.  
☐ No

34. Had you visited/worked at a pig farm or had any direct contact with pigs in a different country up to 6 months prior to your MRSA diagnosis?  
☐ Yes ☐ No

35. If yes please indicate in which country _________________________________

2nd time diagnosis with MRSA
36. When were you diagnosed with MRSA? (Approximate date, month and year)  
____________________________

37. Were you working at the current farm at the time of diagnosis?  
☐ Yes  ☐ No (Go to Q40)

38. Which site were you working at?  
☐ Site-A  ☐ Site-B

39. In the last 4 weeks before the diagnosis was made, what types of animals were you working with most of the time?  
☐ Dry Sows  ☐ Farrowing  ☐ Piglets  ☐ Weaners  
☐ Finishers  ☐ Boars  ☐ Unsure

40. If not working at the current farm where were you working?  
____________________________

41. Were any of your family members diagnosed with MRSA at around the same time as you?  
☐ Yes  ☐ No

42. How many family members were diagnosed with MRSA? _________________

43. Did you take time off work as a result of your MRSA infection?  
☐ Yes, please indicate the approximate number of days _____or weeks _____taken off work.  
☐ No

44. Were you hospitalized during the MRSA infection?  
☐ Yes, please indicate the number of days _____or weeks _____that you were hospitalized.  
☐ No

45. Had you visited/worked at a pig farm or had any direct contact with pigs in a different country up to 6 months prior to your MRSA diagnosis?  
☐ Yes  ☐ No

46. If yes please indicate in which country _________________________________

3rd time diagnosis with MRSA
47. When were you diagnosed with MRSA? (Approximate date, month and year)
__________________________

48. Were you working at the current farm at the time of diagnosis?

☐ Yes  ☐ No (Go to Q51)

49. Which site were you working at?

☐ Site-A  ☐ Site-B

50. In the last 4 weeks before the diagnosis was made, what types of animals were you working with most of the time?

☐ Dry Sows  ☐ Farrowing  ☐ Piglets  ☐ Weaners

☐ Finishers  ☐ Boars  ☐ Unsure

51. If not working at the current farm where were you working?
__________________________

52. Were any of your family members diagnosed with MRSA at around the same time as you?

☐ Yes  ☐ No

53. How many family members were diagnosed with MRSA? _____________________

54. Did you take time off work as a result of your MRSA infection?

☐ Yes, please indicate the approximate number of days _____or weeks ____taken off work.

☐ No

55. Were you hospitalized during the MRSA infection?

☐ Yes, please indicate the number of days _____or weeks _____that you were hospitalized.

☐ No

56. Had you visited/worked at a pig farm or had any direct contact with pigs in a different country up to 6 months prior to your MRSA diagnosis?

☐ Yes  ☐ No

57. If yes please indicate in which country _________________________________
4th time diagnosis with MRSA

58. When were you diagnosed with MRSA? (Approximate date, month and year)
______________________________

59. Were you working at the current farm at the time of diagnosis?
☐ Yes ☐ No (Go to Q62)

60. Which site were you working at?
☐ Site-A ☐ Site-B

61. In the last 4 weeks before the diagnosis was made, what types of animals were you working with most of the time?
☐ Dry Sows ☐ Farrowing ☐ Piglets ☐ Weaners
☐ Finishers ☐ Boars ☐ Unsure

62. If not working at the current farm where were you working?
______________________________

63. Were any of your family members diagnosed with MRSA at around the same time as you?
☐ Yes ☐ No

64. How many family members were diagnosed with MRSA? _____________________

65. Did you take time off work as a result of your MRSA infection?
☐ Yes, please indicate the approximate number of days _____or weeks ____taken off work.
☐ No

66. Were you hospitalized during the MRSA infection?
☐ Yes, please indicate the number of days _____or weeks ____that you were hospitalized.
☐ No

67. Had you visited/worked at a pig farm or had any direct contact with pigs in a different country up to 6 months prior to your MRSA diagnosis?
☐ Yes ☐ No

68. If yes please indicate in which country ______________________

69. Has any family member ever been diagnosed with MRSA?
70. Please indicate how many family members? ______________

71. When were they diagnosed with MRSA? ______________

72. Have you been hospitalized at all in the last 12 months? [Yes] [No] (Go to Q72)
   a. When was the last time? Date: ______________
   b. What was the reason? ____________________

73. Have you had any of the following infections in the last 12 months that have required antibiotic treatment?
   [ ] Respiratory [ ] Urinary [ ] Skin [ ] Other

74. Do you remember what antibiotics were you treated with? [Yes] [No]

75. If yes please indicates the names?
   ___________________________________________ ___________________________ _______________________
   ___________________________________________ ___________________________ _______________________

76. Do you consider yourself to have a weakened immune system (i.e. having a disease such as diabetes, psoriasis, cancer, or AIDS etc)? [Yes] [No]

77. Have you been diagnosed with any chronic disease (i.e. arthritis, chronic hepatitis, asthma, heart diseases)? [Yes] [No]

78. Do you smoke? [Yes] [No] (Go to Q79)

79. Have you ever smoked? [Yes] [No]

80. When did you quit smoking? (Approximate months/years) ______________

81. Do you drink alcohol? [Yes] [No]
82. On average, how many standard drinks do you have in a week?
__________________.

Section 3- Knowledge

83. As you are working with pigs, how concerned are you that …….?

<table>
<thead>
<tr>
<th>Not concerned</th>
<th>Slightly Concerned</th>
<th>Moderately Concerned</th>
<th>Very concerned</th>
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</thead>
<tbody>
<tr>
<td>…you could get a disease from them</td>
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<tr>
<td>…you could transfer a disease to them</td>
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<tr>
<td>…your co-workers could get a disease from them</td>
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<tr>
<td>…your family could get a disease from them through you</td>
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</table>

84. How best do you think we can prevent disease transmission from animals to humans?

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Slightly disagree</th>
<th>Slightly agree</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Don't know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing hands after touching an animal</td>
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<tr>
<td>Washing hands before eating</td>
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<tr>
<td>Taking a shower after working with animals</td>
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<tr>
<td>Using a disposable mask all the time while working with animals</td>
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<tr>
<td>Using a clean overall when working with animals and change it between each activity</td>
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<tr>
<td>Using clean disposable gloves</td>
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</table>
85. How likely do you think it would be that the following activities could increase the level of risk of exposure to MRSA?

Please use a scale of 0 to 5 where 0 is no risk and 5 is maximum possible risk

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<tr>
<th>Activity</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Working on a pig farm</td>
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<tr>
<td>Working on a dairy farm</td>
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<tr>
<td>Working on a beef farm</td>
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<tr>
<td>Working on a sheep farm</td>
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<tr>
<td>Working in a veterinary clinic</td>
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<tr>
<td>Working in a public hospital</td>
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<tr>
<td>Working in an aged care facility</td>
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</table>

86. Do you think the following activities are likely to increase the occurrence of resistant bacteria (like MRSA)?

Yes | No | I don’t know
---|----|-------------------
Using antibiotics as growth promotants        |   |   |   |
Poor quality of protein concentrates          |   |   |   |
Treating healthy animals with antibiotics to prevent disease occurrence |   |   |   |
Treating diseased animals with antibiotics    |   |   |   |
Cleaning shed floors with antibacterial substances |   |   |   |
Washing shed floors and walls with soapy water |   |   |   |
Washing animals with water                     |   |   |   |

Section 4 – Occupational hygiene

87. How confident are you about your personal hygiene and protection when working at the farm?

- [ ] Not confident
- [ ] Slightly confident
- [ ] Somehow confident
- [ ] Very confident
88. How would you rate your current level of knowledge of occupational hygiene practices to avoid acquiring and spreading of MRSA infection?

Please use a scale of 0 to 5 where is 0 is very low (minimum) level and 5 is very high (maximum) level of knowledge.

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<tr>
<td></td>
<td>Low risk</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>High risk</td>
</tr>
</tbody>
</table>

89. How confident are you about your co-workers’ personal hygiene and protection when working at the farm?

- [ ] Not confident
- [ ] Slightly confident
- [ ] Somehow confident
- [ ] Very confident

90. Do you dry your hands after washing using the same towel which is used by others?

- [ ] Yes
- [ ] No, I use a different towel
- [ ] I use paper towel
- [ ] I don’t dry my hands

91. Have your personal hygiene practices at the farm improved after the MRSA outbreak?

- [ ] Yes
- [ ] No

92. Have you notice any improvement in your co-workers’ personal hygiene practices at the farm after the MRSA outbreak?

- [ ] Yes
- [ ] No

Section 5 Your Background

93. What is your age group?

- [ ] 18-21
- [ ] 22-29
- [ ] 30-39
- [ ] 40-59
- [ ] 60+

94. What is your gender?

- [ ] Male
- [ ] Female

95. What is the highest level of education you have completed?

- [ ] Primary School
- [ ] High School
- [ ] TAFE
- [ ] University
- [ ] Other ____________________
96. What is your ethnic background (please ✓ one or more than one boxes if it applies to you)?

- [ ] Indigenous Australian
- [ ] North-West European
- [ ] Southern and Eastern European
- [ ] People of the Americas
- [ ] North African and Middle Eastern
- [ ] Sub-Saharan African
- [ ] South-East Asian
- [ ] North-East Asian
- [ ] Southern and Central Asian

THANK YOU SO MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE. YOUR PARTICIPATION IS HIGHLY APPRECIATED.

Please provide the following information if you want to be notified of positive sample results.

Name: _______________________________________

Phone: ___________________________ Mobile: ___________________

Email: _____________________________________________________

Postal Address:

Unit ________ Street No _______ Street Name ______________________________
suburb ____________________ State ___________________ Post Code __________.

How would you like us to contact you? Please indicate your preference

- [ ] Mobile
- [ ] Telephone
- [ ] Email
- [ ] Post
Annex-3

Participants information sheet for the MRSA prevalence investigation in national herds
Participant Information Sheet

Project title: Epidemiology and molecular characterisation of methicillin resistant \textit{Staphylococcus aureus} in the Australian pig industry.

Thank you for taking the time to consider participating in this research project. This survey invites producers who keep at least 300 sows on their property, registered with Australian Pork Limited (APL) and are located in New South Wales or Victoria. Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve.

What is the purpose of this study?

The main focus of this study is to identify the presence of \textit{Methicillin-resistant S. aureus} (MRSA) in commercial pig enterprises in New South Wales and Victoria. This study will help to determine virulence and resistance pattern, and the diversity amongst prevalent MRSA strains on those farms.

What is MRSA and why is it important for pig producer to monitor it on their farms?

MRSA is well known for a wide range of diseases that are difficult to treat, like skin and soft tissue infections and invasive systemic diseases, particularly in humans. Although carriage as high as 70% has been reported in pigs in parts of Europe, it does not affect the wellbeing of pigs. However, a number of studies have demonstrated that working directly with pigs carrying MRSA is an occupational risk, as MRSA can potentially transfer to workers and may cause various infections.

An outbreak of MRSA in piggery workers represents a challenge for the pig industry and may result in marked economic losses for individual pig producers, in relation to medical expenses, workers compensation, and loss of productive working days. In one Australian piggery, where an ongoing MRSA outbreak among piggery workers have been reported, the direct cost to the producer was estimated to be approximate $150,000 and over 200 days of sick leave over three years duration.
**What does this study involve?**

If you agree to participate one or two researchers will visit your farm on a day that is convenient to you. They will collect nasal swabs from sixty randomly selected weaners. Five environmental samples will also be collected from inside the shed.

**Will I be charged for any laboratory tests?**

You will not be charged for any laboratory tests performed for this research. All the required laboratory tests will be paid for by research funds.

**What if I don’t want to take part in this study?**

Participation in the research is completely voluntary; you can withdraw your participation in the research project at any time.

**How will my confidentiality be protected?**

Responses to this survey will be given unique codes to ensure that anonymity and confidentiality is maintained. In addition, any information collected by the researchers which might identify you or your farm will be stored securely and only accessed by the researchers unless you consent otherwise, except as required by law. The information which might identify you or your farm will not be disclosed to anyone without prior consent.

**What will happen to the information that I give you?**

It is the intention of the researchers to publish the outcomes of the study in scientific journals and as a PhD thesis without mentioning names and addresses of the farm or any other information that could lead to identification of those farms. Individual farm will not be identified in any reports arising from the research.

**What should I do if I want to discuss this study further before I decide to participate or not?**

If you would like further information please contact the following research investigators:

**Mr Shafi ullah Sahibzada**
School of Animal and Veterinary Sciences  
Charles Sturt University  
PhD Student  
Mobile: 0411375186  
Email: sshafullah@csu.edu.au

**Dr. Jane Heller**
Associated Professor  
School of Animal and Veterinary Sciences  
Charles Sturt University  
02 69332839  
jheller@csu.edu.au
Who should I contact if I have concerns about the conduct of this study?

Charles Sturt University’s Human Research Ethics Committee has approved this study. I understand that if I have any complaints or concerns about this research, I can contact:

Executive Officer
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue
Bathurs NSW 2795
Tel: +61 2 6338 4628
Email: ethics@csu.edu.au

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

What do I do next?

If you are interested in participating, please click here on “MRSA prevalence in Australia” or directly contact the researcher (details given below).

Dr. Marta Hernandez-Jover
Associate Professor
School of Animal and Veterinary Sciences
Charles Sturt University
02 69332086
mhernandez-jover@csu.edu.au

Mr. Shafi
Ph.D. Scholar
Mobile: 0411375186
Email: sshafiullah@csu.edu.au
School of Animal and Veterinary Sciences
Charles Sturt University, Wagga Wagga.

Thanks for taking the time to consider participating in this research.
Annex-4

Questionnaire for the MRSA prevalence investigation in national herds
NATIONAL HERD MRSA PREVALENCE IN WEANERS

Section 1 Hygiene and Biosecurity

1. Is the farm site that we swabbed pigs on today a closed system?
   □ Yes (go to question 5)    □ No (go to question 2)

   Comments ____________________________________________________________

2. What type of production system do you have?
   □ All in all out  □ Continuous  □ Other, please state _____________

3. Where do you get replacement pigs from?
   □ Breeding company  □ Sale yard  □ Breed our own on other sites
   □ Other, please state _____________

4. If you receive replacement pigs from other sites of the same establishment, how often do you isolate incoming pigs from those sites upon arrival?
   Never          Sometime          Always
   □              □                   □

5. How often do you isolate incoming pigs (from other farms) upon arrival?
   Never          Sometime          Always
   □              □                   □

6. Please identify the approximate number of pigs on the farm site where the swabs were collected?
   Sows ____________,  Farrowing ____________,  Weaners ____________,
   Growers and Finishers ________________.

7. Please identify the approximate number of weaners that are housed in the shed from where the swabs were collected?
   Total number of weaners in the shed ________________.

8. Please identify the approximate area in square meters (m2) of the shed from where the swabs were collected?
   Shed area in m2 ________________.
9. How often do you disinfect the weaner shed?

- [ ] Once a year only
- [ ] Twice a year
- [ ] Every time after removing the weaner stock
- [ ] Never
- [ ] Other, please specify ________________

10. Please list the chemical that you have used to disinfect/sanitize a weaner shed in the last 12 months?

Examples of generic/trade names have been given in brackets

<table>
<thead>
<tr>
<th>Chemical</th>
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<tr>
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<tr>
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<td>VIRKON</td>
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<td>Chloride</td>
<td></td>
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<td>(Viralfx, Viraban, Farmcare)</td>
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<td>CHLORAMINE</td>
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<td>Phenol</td>
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<td>(Bacrasan, Nufarm, Polyphen)</td>
<td></td>
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</tr>
</tbody>
</table>
Section 2 – Health History

11. Please list the injectable antibiotics that have been used in the current weaning lot where swabs were collected?

_______________________________________________________________________________
_______________________________________________________________________________

12. Please list the oral (mixed in feed or water) antibiotics that have been used in the current weaning lot where swabs were collected?

_______________________________________________________________________________

13. How often have you used the following antibiotics in injectable form on your farm for any production stage pigs in the last 12months?

Examples of generic/trade names have been given in brackets

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<tr>
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<td><strong>Oxytetracycline</strong></td>
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<td><em>(Oxytet, Terramycin, Alamycin, Tetravet, OTC)</em></td>
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<td><em>(Excede, Cefomax, Norocef, Calefur, Excenel)</em></td>
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<tr>
<td><em>(Bomox, Bimoxyl, Amoxyvet, Betamox)</em></td>
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<td><em>(Linopharm, Linco-Sol, Lincomix, Abbeylinc)</em></td>
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<td><em>(Neomycin Penicillin, Neoject, Jurox)</em></td>
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<td><strong>Avoparcin</strong></td>
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<th>1 Rarely used</th>
<th>2 Sometimes used</th>
<th>3 Often used</th>
<th>4 Frequently used</th>
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<td>Tulathromycin (Draxxin)</td>
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<td>Amoxicillin (Amoxyvet, Moxylan, Betamox, Solumox, Abbeymox)</td>
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<td>Antibiotic</td>
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<td>Salinomycin</td>
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<tr>
<td>(Coxistac, Salindo)</td>
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<tr>
<td>Sulpha/Trimethoprim</td>
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</table>

15. Have you had any MRSA infections that you know of in humans on your farm?

☐ Yes       ☐ No

16. If yes, in how many workers has MRSA been diagnosed in the last 12 months?

__________________________________________________________________________

17. Do you think antibiotic resistance is an issue for the pig industry?

☐ Yes       ☐ No       ☐ don’t know

Comments: ________________________________________________

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18. Would you be interested in reducing antibiotic usage on your farm?

☐ Yes    ☐ No    ☐ Maybe, if productivity isn’t affected

Comments: _______________________________

19. How often do you seek the following information from a veterinarian regarding antibiotic prescription?

<table>
<thead>
<tr>
<th>Information</th>
<th>Never ask</th>
<th>Sometimes</th>
<th>Always ask</th>
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<tr>
<td>The risks of antibiotic use</td>
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<td>☐</td>
<td>☐</td>
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<tr>
<td>How antibiotics work</td>
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<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Any alternative strategies</td>
<td>☐</td>
<td>☐</td>
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</tr>
<tr>
<td>Other ____________________________</td>
<td>☐</td>
<td>☐</td>
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</tr>
</tbody>
</table>

20. Would you like to know about your farm results?

☐ Yes    ☐ No

21. If yes, please enter the following details

Farm name: ____________________________________________

Phone: __________________________ Mobile: __________________________

Email: _____________________________________________________

Postal Address:

Unit _______ Street No _______ Street Name __________________________

suburb ________________ State ________________ Post Code ________________

THANK YOU SO MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE. YOUR PARTICIPATION IS GREATLY APPRECIATED.

If you have any questions, please do not hesitate to contact Shafi via email at sshafiullah@csu.edu.au mobile: 0411 375 186
Annex-5

Flow diagram for detection of MRSA
Collect samples from the farms using *Flocked Nylon Swabs (Copan ESwab™)* having Liquid Stuart’s transport medium and send to laboratory for analysis as soon as possible. In humans and pigs rotate a single swabs in both external nares. From the pig environment collect at least five swabs from shed. The sample should be collected from approximately 500 cm² dorsal surfaces including partition walls walk way, water trough, feeder, fence, and pen floors. After collection, label each sample and place the swabs in the transported medium without contamination and send it to the laboratory as soon as possible otherwise refrigerate until posting.

1. In the laboratory, open carefully the tubes containing the swab samples in transport medium (in a laminar air flow bench and using protective gloves) and inoculate each swab into a tube containing 10 ml of Mueller-Hinton broth (MHB) (BD™ Difco™) supplemented with 6.5 % sodium chloride. Mix thoroughly and incubate at 37 °C for 24 hours.

2. Take one millilitre of this first pre-enrichment culture and then inoculate into 9 ml Tryptone Soya Broth (TSB) (CM0129, Oxoid™) containing 3.5 mg/L cefoxitin and 75 mg/L aztreonam. Mix thoroughly, and incubate for 24 hours at 37 °C.

3. Following incubation, a loopful (10µl loop) of the selective enriched cultured and streak onto a Chromogenic MRSA agar plate (CHROMagar™ MRSA) and incubate for at least 24 hours at 37 °C.

4. Observe the colony morphology (size and coloration) on the Chromogenic Agar plate. As per the manufacturer’s instructions, rose to mauve coloured round colonies were preliminarily identified as MRSA.

5. Choose one colony from the Chromagar plat, if not sure than up to five pink colonies indicative for being MRSA and isolate them onto a new 5% sheep blood agar and incubated for 24 hours at 37°C.

6. Check for purity and re-isolate if necessary.

7. Subsequently to identified as *S. aureus* perform Gram staining, catalase production and the *S. aureus* Protein-A latex agglutination test (staphylase, Oxoid™). Use *S. aureus* ATCC 29213 as the control strain.


9. Presumptive isolates of methicillin resistant *Staphylococcus aureus* should at this stage either be stored under appropriate conditions (–80°C) for later identification and characterisation or processed immediately.
Flow Diagram for MRSA isolation in a laboratory

Day 1  
**Pre-Enrichment: in Mülle-Hinton Broth** (37°C, 18-24 h.)  
(6.5 % NaCl)  
Each sample in 10ml broth

Day 2  
**Selective Enrichment: in TSB Broth** (37°C, 18-24 h.)  
(Transfer 1ml MHB in 9ml TSB with 3.5 mg/L cefoxitin and 75 mg/L aztreonam)

Day 3  
**Isolation: on Chromogenic agar** (37°C, 22-26 h.)  
streak with a 10 μl inoculation loop: CHROMagar™ (37°C, 24 –48 h.) (colony: mauve pink colour)

Day 4  
**Cultivation: on sheep blood agar** (37°C, 24 h.)  
(Colony: golden and usually surrounded by a zone of haemolysis)

Day 5  
**Identification: Biochemical tests**  
Latex agglutination test (preferably using **Staphytec plus, Oxoid**)

**Antibiogram**
1. Select one colony to make turbidity equal to 0.5McFarland solution  
2. Take this solution with cotton swab and inoculate on MHA.  
3. Insert antibiotic discs and incubate at (37°C, 24 h.)  
4. Measure diameter of the zones

**Storage**

**Samples storage:** 750μL aliquot into 1.5 mL cryovials containing 750μL of MH broth with 20% glycerol.  
**Isolate storage:** Store in -80°C freezer
<table>
<thead>
<tr>
<th>Class</th>
<th>Antimicrobial agent</th>
<th>Abbreviation (Oxoid&lt;sup&gt;TM&lt;/sup&gt;)</th>
<th>Disk content (μg)</th>
<th>Zone diameter</th>
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<td>Ceftiofur</td>
<td>EFT</td>
<td>30</td>
<td>17 I 18-20 21</td>
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<td>14 I 15-18 19</td>
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