Determining the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among commercial pig herds in South Africa

by

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Declaration

I, Shani van Lochem, hereby declare that the study presented in this dissertation, entitled 'Determining the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among commercial pig herds in South Africa' was conceived, planned and executed by myself, and apart from the normal guidance from my supervisors, I received no assistance.

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Abstract

In this study the prevalence of methicillin resistant Stahylococcus aureus (MRSA) among commercial piggeries in South Africa was determined. Twenty five commercial herds across South Africa participated. From each herd 18 finisher pigs' nasal contents were sampled at the abattoir between stunning and sticking. These samples were pooled into three pools with six samples per pool and selectively cultured to determine the presence of MRSA. A herd was classified as MRSA positive if one or more of the three pooled samples cultured positive for MRSA. In this study three out of the 25 herds tested positive for MRSA, equating to a 12% herd prevalence (95% CI: 2.5 - 31%) among South African commercial piggeries. In other countries nasal carrier status of MRSA has been described in pigs. Concerns exist over the zoonotic risk positive carriers pose to workers. In the current study the prevalence of nasal MRSA carriers amongst large commercial pig herds in South Africa was extremely low compared to what has been reported in other parts in the world. This study suggests a low zoonotic MRSA risk to workers in South African commercial piggeries and abattoirs.

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List of Abbreviations

CA-MRSA	community associated methicillin resistant *Staphylococcus aureus*
HA-MRSA	hospital-acquired/associated methicillin resistant *Staphylococcus aureus*
HIV	human immunodeficiency virus
LA-MRSA	livestock associated methicillin resistant *Staphylococcus aureus*
MLST	multi-locus sequence typing
MRSA	methicillin resistant *Staphylococcus aureus*
NT	non-typeable
PBP2a	penicillin-binding protein 2a
PFGE	pulse field gel electrophoresis
SAPPO	South African Pork Producers Organization
SCC	staphylococcal cassette chromosome
ST	sequence type

Chapter 1 Introduction and literature review

1.1 Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the leading nosocomial human pathogens causing hospital-associated infections worldwide. In 2008, MRSA accounted for 44% (171 200 cases) of hospital-associated infections in Europe of which 5 400 cases (3.15%) resulted in death (Köck et al. 2010). Between 2008 and 2011 it was estimated MRSA bacteraemia accounted for between 30 and 65% of hospital mortalities in Europe (de Kraker et al. 2011). In the United States there were 80 461 cases of invasive MRSA infections during 2011 (Dantes et al. 2013) of which the deaths due to MRSA surpassed deaths due to human immunodeficiency virus (HIV), tuberculosis and influenza combined in that year (Hoyert & Xu 2012). A prevalence study conducted from 1998 to 1999 documented a high prevalence of MRSA in hospitals of Asia-Pacific and South Africa, it was considered the most common cause of bloodstream infections, skin and soft tissue infections and pneumonia (Bell &Turnidge 2002). In South Africa, 94 *S. aureus* isolates were sampled from 213 institutions of which 41.5% were methicillin resistant (Bell & Turnidge 2002). Alarmingly, rifampin resistance was rife among South African strains studied (Bell & Turnidge 2002).

Livestock associated MRSA (LA-MRSA) carried by livestock is seen as a potential threat to humans working with livestock. The first human case of LA-MRSA from pigs was detected in a Dutch hospital in 2004 from a six month old baby hailing from a pig farming family (Voss et al. 2005). Several studies in different livestock sectors including poultry and veal calves have been conducted since.

In South Africa, the prevalence of LA-MRSA colonization of commercial pigs which might pose a risk to humans working with pigs, has never been determined. This study aimed to determine the prevalence of LA-MRSA among commercial pig herds in South Africa.

1.2. Literature Review

1.2.1. Aetiological agent

Staphylococcus aureus is a gram-positive coccus which can grow both aerobically and anaerobically and belongs to the *Micrococcaceae* family (Lowy 1998). It grows in grape-like clusters (*staphyle*) and forms golden colonies (*aureum*) on blood agar (Bannerman 2006). The genome is made up of a circular chromosome, prophages, plasmids and transposons (Lowy 1998). Genes responsible for antimicrobial resistance are carried on both the chromosome and extrachromosomal elements with the latter enabling antimicrobial resistance gene transfer between gram-positive species (Lowy 1998).

Staphylococcus aureus' ability to develop antimicrobial resistance is well documented in human medicine literature since 1942. One year after the discovery of the novel antibiotic penicillin, *Staphylococcus aureus* was found to be resistant via an inducible enzyme today known as beta-lactamase (Rammelkamp & Maxon 1942, Bondi & Dietz 1945). B-lactamase is a serine protease which inactivates penicillin by hydrolysis of the beta-lactam ring; today over 95% of *Staphylococcus aureus'* are resistant to penicillin (Lowy 1998).

In 1959 the first beta-lactamase resistant semi-synthetic penicillin was developed to overcome penicillin resistance, namely methicillin then known as celbenin (Jackson 1962). Only two years thereafter case reports surfaced describing failure of treatment due to a methicillin resistant *Staphylococcus aureus* (Jackson 1961), hence the name methicillin resistant *S. aureus*.

Staphylococcus aureus facilitates methicillin resistance via a transpeptidase penicillin-binding protein 2a (PBP2a) which reduces *Staphylococcus aureus'* affinity for beta-lactam antimicrobials (Hartman & Tomasz, 1981). The PBP2a is encoded for by the gene *mecA*, which is located in the transferable staphylococcal cassette chromosome (SCC) known as

SCC*mec* (Katayama, Ito & Hiramatsu 2000). The presence of SCC*mec* is essential for MRSA's antibiotic resistance. Methicillin resistant *S. aureus*' ability to acquire different mechanisms of antibiotic resistance through plasmids or chromosome cassettes (Lowy 2003) realises its potential to be a truly multidrug-resistant potential pathogen. MRSA isolates frequently carry resistance genes against other antimicrobial groups such as glycopeptides (e.g. vancomycin), quinolones, aminoglycosides (e.g. gentamycin), trimethoprim-sulfamethoxazole, tetracyclines, erythromycin, clindamycin, linezolid and/or daptomycin (Lowy 2003).

1.2.2. Diagnostic tests for detection of MRSA

1.2.2.1. Methicillin resistant *Staphylococcus aureus* screening methods

There are various older screening methods which all make use of a solid agar medium, an indicator substance and an inhibitory substance such as methicillin, oxacillin or more recently cefoxitin which enables the selection of MRSA (Brown et al. 2005). The older generation screening on solid agar used mannitol as a carbohydrate source, phenol red as a pH indicator and either methicillin or oxacillin as selective agents (Brown et al. 2005). The sensitivities for the mannitol salt agar method ranged between 46% and 90%, while the specificities ranged between 89% to 93% (Brown et al. 2005). Newer chromogenic agar plates using cefoxitin as the selective agent, for example the chromID™ MRSA agar plate (bioMérieux SA, Marcy-l'Etoile, France) outperformed the older methods. These screening plates used chromogenic enzyme substrates for key enzymes present in the pathogen being screened for (Van Hoecke et al. 2011). The chromID™ MRSA agar plate showed growth of green colonies if the sample is MRSA positive due to *S. aureus'* alpha glucosidase activity on the chromogenic substrate (Van Hoecke et al. 2011). Screening human nasal samples with chromID™ MRSA gave a sensitivity of 81.4% and 88.6% after 24 hours and 48 hours of incubation respectively, while the specificity was 97.9% and 95.8% after 24 hours and 48 hours of incubation respectively (Van Hoecke et al. 2011). Pre-enrichment of screening swabs can additionally increase sensitivity, although this increased costs and turnaround time (Brown et al. 2005).

Molecular methods utilising multi-plexed polymerase chain reactor (PCR) primers to detect the *mecA gene* such as the commercial kit IDI-MRSA assay (Infectio diagnostic, Ste-Foy, Quebec) amplifies part of the SCC mec gene which gives results within two hours (Brown et al. 2005). Superior results in especially sensitivity of 91.7% among 288 human patients' nasal contents were observed, however cost is still a factor not making this the routine method of choice (Brown et al. 2005).

1.2.2.2. Confirmation methods

Once a screening method does show a positive growth one has to confirm whether the colonies are truly *S. aureus*. The rapid slide agglutination test Staphaureux* (Remel Europe Ltd, Kent, UK) differentiates staphylococci which possess coagulase and/ or Protein A, in particular *S. aureus*, from other staphylococci which possess neither of these factors. 97% of *S.aureus* isolates possess both and 95% produce protein A independently. Staphaureux* reagent consists of polystyrene latex particles coated with fibrinogen and IgG. When this is mixed on a slide with a suspension of *S. aureus* the reaction of a clumping factor with the fibrinogen and/or of protein A with IgG will cause rapid, strong agglutination of the latex particles. A positive reaction would be clearly visible as clumping of the latex particles and indicate the presence of either coagulase or protein A, or both. The sensitivity of Staphaureux* is 99.8% and the specificity 99.5% (according to package insert).

1.2.2.3. Methicillin susceptibility testing

The antimicrobial disc diffusion method is still the most widely used method to determine whether the colonies isolated are susceptible or resistant to an antibiotic based on whether there is an inhibitory growth zone around the antibiotic disc or not respectively (Brown et al. 2005). Cefoxitin is these days mostly used to test for methicillin resistance due to the fact that methicillin is no longer on the market and oxacillin gave inconsistent results which required special mediums and incubation temperatures (Brown et al. 2005).

1.2.2.4. Methicillin resistant *Staphylococcus aureus* isolate identification

MRSA clones are named after a specific pulse field gel electrophoresis (PFGE) pattern done after digestion with the restriction endonuclease *Sma*I (Stryjewski & Corey 2014). The MRSA strains which are isolated in livestock are however non-typeable (NT) by the standard method of PFGE (Voss et al. 2005). This is due to the presence of a DNA methylation enzyme, protecting *S. aureus'* DNA from being digested by the restriction endonuclease *Sma*I (Bens, Voss & Klaassen 2006).Therefore different classification systems are used to identify livestock associated MRSA, such as multi-locus sequence typing (MLST) and *spa* typing (Stryjewski & Corey 2014).

Multi-locus sequence typing (MLST) is a nucleotide sequence-based method for bacterial typing which indexes allelic variation at multiple housekeeping loci directly by nucleotide sequencing of internal gene fragments (Urwin & Maiden 2003). Classification of MRSA via MLST involves sequencing DNA fragments of seven housekeeping loci of which the nucleotide sequences of these genes are compared to known alleles at each locus via the MLST website (Robinson & Enright 2004). Each isolate is described by a seven-integer allelic profile that defines a sequence type (ST) (Robinson & Enright 2004). Using MLST for MRSA identification is a systemic and objective method for assigning MRSA isolates to known clones or novel clones which provides unprecedented information about its genome diversity and epidemiology (Lindsay & Holden 2004). MLST's weakness as typing method is that it has a low throughput at a high cost (Stefani et al. 2012). The predominant isolate found in pigs and people working with pigs is ST398 (De Neeling et al. 2007, Khanna et al. 2008, Smith et al. 2009, Wulf et al. 2008)

Spa typing is an identification method where the *spa* gene's variable X region, which encodes for *S. aureus* protein A, sequence polymorphism is determined (Stefani et al. 2012). This is a

rapid, high throughput, dynamically evolving method of identification, but can lead to misclassification of a small number of lineages (Stefani et al. 2012).

1.2.3. Epidemiological reservoirs

There are currently three epidemiological reservoirs of MRSA recognised: hospital-acquired/associated (HA-MRSA), community associated (CA-MRSA) and livestock associated (LA-MRSA) (Köck et al. 2010).

Methicillin resistant *Staphylococcus aureus* is endemic in hospitals worldwide (DeLeo et al. 2010). The highest prevalence rates of over 50% are reported in Asia, North and South America and Malta with countries such a Sri-Lanka reporting an estimated HA-MRSA prevalence of 86.5% (Stefani et al. 2012).

Community associated MRSA is distinct from HA-MRSA as it does not originate from healthcare facilities (DeLeo et al. 2010). It is seen as an epidemic in some countries and is worsened by restricted treatment of infections which enhances virulence and transmission (DeLeo et al. 2010). Community associated MRSA occurs mostly in children, prisoners, poverty stricken adolescents, soldiers, athletes and people in day-care centres which are all populations exposed to a high risk of cross-infection (DeLeo et al. 2010).

Livestock associated MRSA originates from livestock and is again distinct from both HA-MRSA and CA-MRSA. It is seen as a relatively new epidemiological MRSA reservoir and has been found in pigs, veal calves (Graveland et al. 2010), poultry (Mulders et al. 2010), dairy cattle (Vanderhaeghen et al. 2010) and turkeys (Richter et al. 2012). In Germany 10% of MRSA infections in humans are due to LA-MRSA which were initially associated with livestock (Cuny et al. 2015).

1.2.4. Pathogenesis of MRSA

Staphylococcus aureus is a ubiquitous coloniser of human epithelia and a typical commensal bacteria living in the anterior nares of 30 % of the human population (Peacock et al. 2001, Wertheim et al. 2005). United States and Dutch data predicted that worldwide 2 – 53 million people carry MRSA (Stefani et al. 2012). Nasal carriage is one of the major risk factors for developing staphylococcal infection as it was demonstrated that rates of infection are higher in carriers compared to non-carriers, affected individuals are usually infected with their own isolate, and by temporarily eradicating carriage by the use of topical mupirocin reduced nosocomial infection in both patients undergoing dialysis or surgery (Peacock et al. 2001). The immunocompromised are more likely to be persistent nasal carriers as people infected with the human immuno-deficiency virus (HIV) have a far higher *S. aureus* nasal carriage rate than that of health care workers or patients with chronic diseases (Peacock et al. 2001). Nasal carriage rates are also higher in humans with insulin-dependent diabetes, patients undergoing repeated dialysis and intra-venous drug abusers (Peacock et al. 2001).

Staphylococcus aureus can infect any tissue in the human body once it enters through a break in either the skin or mucous membranes (Lindsay & Holden 2004). Patients in hospitals are at most risk to be infected with *S. aureus* due to frequent catheterisation and injection administration (Lindsay & Holden 2004). *Staphylococcus aureus* is able to cause disease in humans due to the evasion of the innate immune response, for e.g. resistance to phagocytic leucocytes, and by secreting cytotoxins which lyse host cells (DeLeo et al. 2010). Once *S. aureus* enters the body it can cause skin, soft-tissue, pleuro-pulmonary, bone, joint and endovascular infections of which the majority of these infections only occur in people which are immunocompromised (Lowy 1998). Life threatening infections with MRSA are due to bacteraemia, endocarditis, metastatic infections, sepsis and toxic shock-like syndromes (Lowy 1998).

Livestock associated MRSA ST398 have the same virulence potential as *S. aureus* infecting humans (Cuny et. Al 2015). This type of MRSA is responsible for 13% of MRSA associated severe skin and soft tissue infections in humans (Layer et al. 2012). However, the impact on quality of life from 44 persistent LA-MRSA human carriers in the Netherlands had recently been assessed and the impact on their health and health related quality of life was found to be limited (Van Cleef et al. 2016).

1.2.5. MRSA incidence

Invasive MRSA infections in humans have a documented mortality rate of 20% in the United States (Stefani et al. 2012). Globally the incidence varies between industrialised and non-industrialised countries as there is less information on the latter (Stafani et al. 2012). In 2008, MRSA accounted for 44% (171 200 cases) of hospital-associated infections in Europe of which 5 400 cases (3.15%) resulted in death (Köck et al. 2010). Between 2008 and 2011 it was estimated that hospital mortalities due to MRSA bacteraemia ranged from 30% to 65% in Europe (de Kraker et al. 2011). In the United States there were 80 461 cases of invasive MRSA infections during 2011 (Dantes et al. 2013). Deaths due to MRSA infections in the United States surpassed deaths due to human immunodeficiency virus (HIV), tuberculosis and influenza combined (Hoyert & Xu 2012). A prevalence study conducted from 1998 to 1999 documented a high prevalence of MRSA in hospitals of Asia-Pacific and South Africa, it was considered the most common cause of bloodstream infections, skin and soft tissue infections and pneumonia (Bell &Turnidge 2002). In South Africa, 94 *S.aureus* isolates were sampled from 213 institutions of which 41.5% were methicillin resistant (Bell & Turnidge 2002). Alarmingly, rifampin resistance was rife among South African strains studied (Bell & Turnidge 2002).

In eight European countries LA-MRSA ST398 accounted for less than 2% of the MRSA isolates in humans (Cuny et al. 2015). Higher proportions of these isolates were found in pig and veal calf dense areas combined with high density human populations (Cuny et al. 2015).

1.2.6. The significance of LA-MRSA in pigs

The significance of LA-MRSA in pigs is focussed on the potential risk of pigs carrying LA-MRSA transferring this status to humans which would result in humans becoming LA-MRSA carriers. There is however evidence of LA-MRSA in pigs causing severe skin infections which could be mistaken for greasy pig disease (Hall et al. 2015). In England 60 piglets from 11 litters presented with mutifocal skin lesions of 2 mm to 20 mm with a crusted, fibrinous exudate from which pure growth of MRSA was isolated; six of the piglets died (Hall et al. 2015).

Prevalence and association studies of LA-MRSA in both pigs and humans working with pigs have been conducted in several countries such as the Netherlands, Denmark, Canada and the United States over the past few years (Broens et al. 2011c, De Neeling et al. 2007, Khanna et al. 2008, Smith et al. 2009, Verhegghe et al. 2013, Voss et al. 2005 & Wulf et al. 2008). The first human LA-MRSA was isolated from a six-month old girl in a pre-screening room in a Dutch hospital during 2004 (Voss et al. 2005). Both her parents and the pigs on the farm carried identical MRSA strains, which demonstrated for the first time the possible transmission between animals and humans (Voss et al. 2005).

1.2.6.1. MRSA prevalence in pigs

In 2005 De Neeling et al. (2007) screened 540 pigs from nine slaughterhouses in the Netherlands. The MRSA prevalence amongst the 540 pigs tested was 39% of which all isolates belonged to one clonal group namely sequence type (ST) 398 (De Neeling et al. 2007), the same type isolated from the study of Voss et al. (2005). A study followed concerning herd prevalence in the Netherlands among 202 herds which were studied from 2007 to 2008 (Broens et al., 2011c). Of 171 breeding herds, 67.3% were MRSA positive, and of 31 finisher herds, 71% were MRSA positive (Broens et al., 2011c). The larger the unit in terms of sow units, the more likely a positive MRSA status would be found (Broens et al., 2011c). Pooled samples from this study showed that suckling and weaned pigs

were more likely to be positive than finishers and sows. MRSA prevalence of 53.4% in sucking piglets, 52.9% in weaned pigs, 38.7% in finishers and 38.3% in sows were observed (Broens et al., 2011c). A longitudinal study amongst four complete production chain herds, from breeding to slaughter, was followed to determine the age at which piglets become colonized with MRSA (Verhegghe et al., 2013). On the two farms with the highest MRSA prevalence, an MRSA prevalence of 90% to 100% was detected in piglets from birth to 70 days of age, thus persistent until the end of the weaner phase (Verhegghe et al., 2013). At 165 days of age in the finisher stage, just prior to slaughter, the prevalence decreased to 85% (Verhegghe et al., 2013). On the two farms with the lowest MRSA prevalence, an MRSA prevalence of 82% to 92% was detected in weaner pigs aged 45 to 70 days, while the finisher herd at 165 days, at slaughter age, had an MRSA prevalence of 75% (Verhegghe et al., 2013). This illustrates that MRSA prevalence in finisher pigs at the age of slaughter is less than that of weaner pigs, but is still very high.

However, the Australian pork industry detected only a 0.9% MRSA prevalence among 324 pigs of five commercial herds and one feral herd (Groves et al., 2014). The more recent study in the United States of America (USA) found no MRSA in 36 herds across 11 states (Sun et al., 2015). The latter study did however find *S. aureus* on 35 out of the 36 farms of which 100% of isolates were resistant to spectinomycin, 94% were resistant to tetracyclines and 75% were resistant to clindamycin (Sun et al. 2015). Out of 130 *S.aureus* isolates 89% were resistant to more than five antibiotics, but not to methicillin (Sun et al. 2015).

In Africa two studies have been conducted thus far. Locally the Eastern Cape of South Africa, Adegoke & Okoh (2014) sampled 64 pigs from different ages of which 15 pigs were MRSA positive giving a prevalence in these pigs of 23%. In Ilora, Nigeria, MRSA prevalence on-farm in 11 different herds was 9% (18/200) (Okunlola & Ayndale, 2015).

1.2.6.2. LA-MRSA human association studies

In Ontario, Canada, 9 out of 20 farms (45%) tested positive for MRSA of which 24.9% of the 285 pigs sampled were MRSA carriers; of the 25 pig farmers that participated, 20% were colonized with MRSA; from the isolates which were typed from both the pigs and humans, 59.2% were typed as ST398 (Khanna et al. 2008). Thereafter a study followed from two different production systems in Iowa and Illinois, United States. Only one of the two production systems tested positive for MRSA; the overall MRSA prevalence among 299 pigs sampled was 49%; the prevalence among 20 pig caretakers was 45% (Smith et al. 2009). Once again the predominant isolate was ST398 (Smith et al. 2009).

1.2.6.3. LA-MRSA prevalence in humans

Swine veterinarians from 38 countries were screened for MRSA and among the 272 participating veterinarians 12.5% of participants from nine countries (Belgium, Canada, Denmark, France, Germany, Italy, The Netherlands, Spain and Thailand) tested positive with the predominant isolate being LA-MRSA ST398 (Wulf et al. 2008). Italy had the highest percentage of carriers per country at 61% out of 21 participants which tested positive, followed by Germany with 33% out of 52 participants which tested positive (Wulf et al. 2008). An association factor which was of significance was that 94% of the carriers had frequent contact with pigs, i.e. daily and/or more than five hours per week, and the remaining 6% less than 5 hours per week pig contact with a minimum of once per month (Wulf et al. 2008). Protective clothing in the form of gowns, gloves and masks did not make any difference of LA-MRSA carriage compared to non-carriers, but it was speculated that breaches in adherence to biosecurity measures such as negligence in washing hands might have contributed to this (Wulf et al. 2008). In a Belgian study it was again proved that the strongest direct association between LA-MRSA carriage was working with live pigs with an odds ratio of 12.1 and a 95% confidence interval (Garcia-Graells et al. 2012).

A German study revealed that the dissemination of LA-MRSA ST398 into the community of non-exposed humans is infrequent and only spreads within family members of exposed individuals in contact with pigs (Cuny et al. 2009). The study furthermore revealed that of 113 humans working on pig farms, 86% were positive, while only 4.3% of their 116 family members were carriers. In this study 462 pupils of a school in a pig dense area were also tested and only three pupils tested positive for the presence of MRSA; all three pupils hailed from pig farms (Cuny et al. 2009). This highlights the tendency that only people with frequent pig exposure are likely to be carriers of LA-MRSA ST398.

A recent prospective cohort study traced nasal carriage of LA-MRSA in pig farmers for over a year, illustrating the persistence in the epidemiology of LA-MRSA in humans (Cleef et al., 2014). Of the 110 pig farmers from 49 pig farms an average LA-MRSA prevalence of 63% and a LA-MRSA persistence of 38% were found (Cleef et al., 2014).

MRSA ST398 was the predominant MRSA type in all of the above studies illustrating that MRSA ST398 is the predominant MRSA type carried by pigs and people working with pigs. The overall prevalence of LA-MRSA carriers among people working with livestock ranged from 12.5% to 86% (Khanna et al. 2008, Wulf et al. 2008, Smith et al. 2009). This is extremely high in contrast to HA-MRSA carriers if one considers that the national prevalence of any MRSA carriers in in-patients at US health care facilities in 2010 was only 6.6% (Jarvis, Jarvis & Chinn 2012). The high carrier rate of LA-MRSA underlines the importance of LA-MRSA ST398 as a potential nosocomial agent.

1.2.7. LA-MRSA as a veterinary public health concern

LA-MRSA is an important veterinary public health concern. This is due to the risk of introducing LA-MRSA into human hospitals through people working with livestock and being potential carriers of LA-MRSA. Identifying high risk human carriers of MRSA via a ‘Search and Destroy’ policy has been in use in the Netherlands and Scandinavian countries since 2001

(Van Rijen & Kluytmans 2009). High risk MRSA carriers are identified as people being treated in hospitals abroad as well as people who have been in contact with pigs and/ or veal calves (Van Rijen & Kluytmans 2009). These patients are first screened by collecting a nasal swab sample which is then tested for the presence of MRSA, if the sample tests positive the patients are first submitted to an isolation ward where targeted antibiotic therapy is given to eliminate the MRSA carrier status before the patient can be submitted to the rest of the hospital (Van Rijen & Kluytmans 2009). Over a time period of seven years, the 'Search and Destroy' policy has proved to be highly cost effective as it estimated to prevent 36 cases per year which resulted in an annual saving of €427 356 and ten lives per year (Van Rijen & Kluytmans 2009).

In South Africa the prevalence of LA-MRSA in pigs and people working with pigs has not yet been determined. Introducing LA-MRSA into human hospitals is potentially dangerous in a country with a high prevalence of HIV infection which is one of the risk factors associated with an increased chance of acquiring an MRSA infection (Shisana 2005). In future the identification of high risk carriers may become an important preventative strategy when facing multidrug-resistant MRSA. The majority of studies elsewhere in the world associating LA-MRSA in people have been demonstrated in pigs and their caretakers, therefore it would be appropriate to first screen South African pigs for MRSA.

1.2.8. Classifying herds as MRSA positive or negative

Different sampling methods have been evaluated to determine which samples are the most sensitive to detect MRSA presence in pigs. In a study comparing pooled nasal swabs, single environmental swabs and pooled environmental swabs amongst 147 herds, the apparent prevalence varied greatly, being 70.8%, 53.1% and 19.1% respectively (Broenset al. 2011a). This indicates that nasal sampling is the preferred method to determine presence of MRSA.

It is important to know at what stage of production MRSA would be most prevalent within a herd. This would enable sampling of the correct group of animals to determine MRSA

presence in a herd. Broens (2011c) eluded that suckling and weaning pigs were more likely to be positive than finishers and sows due to MRSA prevalence's found of 53.4% in suckling piglets, 52.9% in weaned piglets, 38.7% in finisher pigs and 38.3% in sows as referred to previously. Verhegghe's (2013) longitudinal study amongst four production chains found 90% to 100% suckling piglets and weaners till 70 days to be MRSA carriers which dropped to 85% at 165 days of age which is their finisher stage. Therefore, prevalence of MRSA amongst suckling and weaner pigs is the highest and this group would be the ideal group of pigs to sample on a farm to detect MRSA. However, the prevalence amongst finisher pigs are still high, ranging between 38.7% to 85%. In the current study it was decided to sample the latter due to logistical difficulties, but the reduced prevalence amongst finisher pigs were considered in determining a proper sample size to enable the classification of a herd as positive or negative based on sampling only finisher pigs.

In terms of herd size Broens (2011c) stated that larger sow units are more likely to have positive MRSA carriers. In South Africa larger sow units are classified as herds with more than 500 sow units according to the South African Pork Producers Organization (SAPPO).

1.3. Hypothesis

Livestock associated – methicillin resistant *Staphylococcus aureus* (LA-MRSA) is present in large South African commercial pig herds of over 500 sow-units.

1.4. Objective of the study

The main objective of this study was to determine the South African commercial pig herd's MRSA status as it was unknown at the time. In case the prevalence was of significance one can further investigate the type of MRSA found in South African pigs and whether humans working with positive pigs were carriers of the same type of MRSA present in the pigs. Studies to follow will be valuable for overall awareness if the risk indeed exists in South Africa which

may assist healthcare institutions to identify high risk patients and pre-screen for appropriate treatment if necessary.

Chapter 2 MATERIALS AND METHODS

2.1 Study design

A cross-sectional survey with two-stage sampling was used. A random sample of large commercial pig herds was selected. From each herd the nasal contents of randomly sampled finisher pigs were sampled at the abattoir in order to determine the herd's MRSA infection status. Prevalence among large commercial piggeries in South Africa was determined in terms of the proportion of large commercial pig herds which were positive for MRSA. Therefore, a herd was defined as positive for MRSA if one or more pooled MRSA positive cultures were obtained from the samples taken from the slaughter pigs. A farm was deemed negative for MRSA if no positive MRSA culture was obtained from the slaughter pigs.

2.1.1. Selection of herds

Finisher pigs in South Africa, originating from large commercial piggeries, are sent to centralized abattoirs weekly for slaughtering. These abattoirs are located mainly in Gauteng, Kwazulu-Natal and the Western-Cape. In order to determine the MRSA status of commercial piggeries it was decided to take samples at these centralised locations to enable the study to include herds from all over South Africa.

Samples were only taken from commercial piggeries with over 500 sow units as MRSA is more likely to be found in larger herds (Broens et al., 2011c) and these herds are also defined as large commercial enterprises by the South African Pork Producers Organisation (SAPPO). According to the South African Pork Producers Association at the time of sample size determination there were only 56 herds which had more than 500 sow units and produced finisher pigs. 84% of the national herds' pigs are in Gauteng, Limpopo, Mpumalanga, North-West, Kwazulu-Natal and Western Cape. These herds' finisher pigs are slaughtered in Gauteng, Kwazulu-Natal and the Western Cape.

Expected prevalence amongst herds was taken as 50% as it was unknown at the stage of drawing up the protocol whether herds' are MRSA positive or not. This was however in line with Fromm et al. (2014) where a meta-analysis of pooled data calculated a prevalence of 53.5% among 400 grower herds.

In order to calculate the number of herds needed to be tested to determine the national herd MRSA prevalence the following equation was applied:

$n = \frac{1.96^2 PQ}{L^2}$ (Thrusfield et al. 2001)

P is the expected prevalence. Expected prevalence amongst commercial herds in South Africa was unknown at that stage and was therefore taken as 50%.

Q is 1 minus P.

Q = 1 – P

L is the absolute allowable error which was taken as 15%

$n = \frac{1.96^2 * 0.5 * 0.5}{0.15^2}$

= 42.7 herds

The population (N) of herds with ≥ 500 sow units were only 56 herds. Therefore, the calculated sample size, n, was larger than 0.1N.

The new sample size n* was calculated as the reciprocal of $\frac{1}{n} + \frac{1}{N}$

$\frac{1}{n*} = \frac{1}{42.7} + \frac{1}{56}$

n* = 25 herds

Therefore 25 herds were to partake in this study.

Microsoft Excel® was used to select the 25 large commercial herds which slaughtered in the three main provinces: Gauteng, Kwazulu-Natal and the Western Cape. Five abattoirs were identified slaughtering the 25 herds; three abattoirs in Gauteng, one abattoir in Kwazulu-Natal

and one abattoir in the Western Cape. Abattoirs were contacted and written consent for sampling was obtained. Herds were identified using alphabetical letters ranging from A to Y. Herds' identities were and are to be kept strictly anonymous.

2.1.2. Selection of pigs to be tested within a herd

The smallest unit in the study was a 500 sow unit which would have approximately 5 000 pigs as part of their production chain at any given time. Per week a 500 sow unit would send a batch of approximately 250 pigs to the market. In both Broens et al. (2011) and Verhegghe et al. (2013) studies it was shown that the highest prevalence within a herd was in the weaned and grower pig groups followed by the finisher pigs with a 15% to 17% decrease in prevalence. This was taken into consideration in determining the minimum expected prevalence within the finisher herd.

In the Dutch prevalence study, a low minimum expected prevalence of 2 – 5% for MRSA detection in nasal passages was used (Broens et al., 2011c). The true prevalence in four herds tested by Verhegghe et al. (2013) calculated true prevalence to be at least 75% in finisher pigs. For this study the minimum expected prevalence was taken as 20% in finishing pigs taking into consideration the other studies conducted thus far on various sample sizes.

Six nasal samples were pooled into one sample for selective MRSA culture in order to reduce laboratory fees. Pooling of samples does however decrease the diagnostic sensitivity of culturing MRSA compared to that of culturing individual samples. According to Gremek-Kosnik et al. (2005) it would comparatively decrease sensitivity to 86%. The estimated sensitivity of MRSA detection was therefore reduced by 14%. Lancet Laboratories South Africa also claimed an estimated sensitivity of their culture method of 95%. Thus the calculated estimated sensitivity for this study, taking into consideration pooling of samples and laboratory culture methods, was 95% x 86% = 81.7%. The final estimated sensitivity used for this study was 80%.

The specificity for detection of MRSA on an MRSA selective plate was 99.8% according to Nsira et al. (2006). However, false positives were not to be considered as a possibility in this study due to confirmation of a positive status by selective laboratory procedures which were confirmed by a microbiologist. Specificity of laboratory procedures was therefore 100%.

In order to calculate the sample size needed to determine the presence of MRSA within the smallest batch of finisher pigs taken as 250 pigs, Free Calc2 software (www.ausvet.com.au) was used. An alpha value of 0.05 and a beta value of 0.05 was used. The minimum expected prevalence was taken as 20% with a test sensitivity of 80% and specificity of 100%. The sample size calculated to detect freedom of colonization among 250 pigs was 17 pigs. Using the same parameters and software, but changing the population to 2 500 pigs in order to determine the presence of MRSA in the largest batch of pigs from a possible 5 000 sow unit, a calculated sample number of 18 finisher pigs were needed to determine freedom of colonization. Thus from each chosen herd 18 finisher pigs were needed for sampling.

At the abattoir convenience sampling was used to select 18 finisher pigs from each chosen herd.

2.2 Experimental procedures

2.2.1. Nasal content collection

The nasal contents of 18 finisher pigs per participating herd was taken at the abattoir between stunning and sticking. A single sterile swab (Copan Transystem®, Copan Diagnostics Inc, Murrieta, USA) was used to collect the contents of both nares per pig and inserted into Amies medium. Each sample was marked with the farm's alphabetical letter allocated to it according to the enrolment list, followed by the number of pig sampled e.g. C3. The swabs were then transferred to a polystyrene cooler box with ice packets - the swabs were insulated from the iced gel packs to prevent direct contact between the swabs and the iced gel packs. Swabs were transported to Lancet laboratories with this method within 1 - 2 days after sampling. In-between sampling and transport, samples were refrigerated at between 4 - 8ºC.

2.2.2. Sample processing

Samples were processed at Lancet laboratories under the guidance of Dr B. Prinsloo and Mr M. Neve (012 483 0100, Lancet Laboratories South Africa).

Samples were directly processed upon arrival as the laboratory runs a 24-hour service. The 18 swabs from each herd were divided into three pools of six swabs per pool. Pooled samples were identified with the alphabetical identity followed by the swab numbers pooled together e.g. C 7 - 12.

2.2.2.1. MRSA screening

The pooled samples were directly swabbed onto chromID™ MRSA agar plates (bioMérieux SA, Marcy-l'Etoile, France) used to screen for MRSA. With each batch of chromID™ MRSA agar plates processed, one plate was inoculated with an authentic MRSA strain as a positive control. ChromID™ MRSA agar plates are selective plates with a medium favouring MRSA growth due to the selective media containing cefoxitin. These selective plates enabled direct detection of resistant bacterial colonies due the chromogenic substrate α-glucosidase activity which resulted in the visualisation of green colonies. These plates were incubated in aerobic conditions at 37ºC for 48 hours.

2.2.2.2. Rapid slide agglutination test

Positive plates with green colonies were then selected for a rapid slide agglutination procedure to confirm whether the colonies were truly a *S.aureus*. Staphaureux* (Remel Europe Ltd, Kent, UK) differentiates staphylococci which possess coagulase and / or Protein A, particularly *S. aureus*, from staphylococci which possess neither of these factors. 97% of *S.aureus* isolates possess both and 95% produce protein A independently. Staphaureux* reagent consists of polystyrene latex particles coated with fibrinogen and IgG.

When this is mixed on a slide with a suspension of *S. aureus* the reaction of a clumping factor with the fibrinogen and/or of protein A with IgG will cause rapid, strong agglutination of the latex particles. A positive reaction would be clearly visible as clumping of the latex particles and indicate the presence of either coagulase or protein A, or both. The sensitivity of Staphaureux* is 99.8% and the specificity 99.5%. An authentic MRSA strain was used as a positive control for each batch of suspected colonies tested with Staphaureux*.

2.2.2.3. Mass spectrometry

If a sample was both positive on the chromID™ MRSA agar plate and on the Staphaurex* slide, colonies were then sampled from the MRSA agar plate for further identification confirmation via mass spectrometry. Therefore, a pooled sample could only be MRSA positive with 100% specificity after it was positive on the chromID™ MRSA agar plate, positive in the slide agglutination test namely Staphaurex*and identified as a *S. aureus* on mass spectrometry.

2.2.2.4. Confirming antibiotic resistance

A random selection of positive samples resistance to cefoxitin was confirmed with an antibiogram with the antibiotic diffusion disc 'Fox'. If growth was inhibited with ≤ 21 mm this validated that the colonies were truly resistant and that the cefoxitin concentration used in the chromID™ MRSA agar plate batch was working as expected.

After the first 15 herds' samples were tested, six individual swabs from each of four herds were used to evaluate the MRSA screening method using three methods.

Firstly, the six swabs were individually inoculated directly onto the chromID™ MRSA agar plates. This was to determine whether pooling had substantially decreased sensitivity, resulting in failure to detect the organism in infected pigs.

Secondly the samples were individually enriched with thioglyccollate broth for 24 hours at 37ºC after which an inoculum was then inoculated onto the chromID™ MRSA agar plate which was read after 24 hours. This was to validate whether sample enrichment was necessary; if the screening plates were positive with this method, but negative against directly plating individual swabs onto screening plates then it meant that a pre-enrichment broth was necessary.

Thirdly the individual swabs were used to inoculate 5% sheep blood agar swabs with colistine for 24 hours at 37ºC where-after the colonies were then inoculated onto the chromID™ MRSA agar plates. This was to validate that Gram negative bacteria were not inhibiting the growth of the Gram positive bacteria. If these MRSA screening plates were positive and all the others were negative then one would conclude that the Gram negative bacteria were inhibiting the growth of MRSA.

2.3. Data analysis

A herd was classified MRSA positive if at least one pooled nasal sample tested positive for MRSA. MRSA herd prevalence was calculated with an exact binominal 95% confidence interval. Microsoft Excel was used to store all data.

2.4. Ethics statement

This study, project number V093-14, was approved by the Animal Ethics Committee of the University of Pretoria. Informed consent was given by SAPPO and the participating pig abattoirs. Refer to Appendix A for consent forms.

Chapter 3 RESULTS

The results of the study are summarized in Table 1 below. Out of the 25 participating herds identified from A to Y only seven pooled samples, from three herds, were positive on both the selective chromID™ agar plate and the Staphaureux* agglutination test. Herds T and Y both tested all three pools MRSA positive, whereas herd U had two pools testing MRSA negative, and one pool testing MRSA positive. The positive colonies identities were further confirmed with mass spectrometry. All positive colonies were identified to be *S. aureus* on mass spectrometry. The remainder of the pooled samples from herds A – S and V – X all produced negative chromID™ MRSA agar plates. The individual Lancet Laboratory reports for each sample are shown in Appendix 1.

The overall prevalence of MRSA-infected large commercial pig herds in South Africa was therefore estimated to be 12% (95% confidence interval: 2.5, 31.2%).

All positive colonies from herd T and one positive pool from herd U were further inoculated onto antibiogram plates containing cefoxitin discs confirming resistance and at the same time validating the MRSA selective plates. Herd T produced growth inhibition zones of 17 mm for a pooled sample consisting out of swabs 1 – 5, 19 mm for swab 6 tested in isolation, 16 mm for pooled sample two from swabs 7 – 12 and 17 mm for pooled sample three from swabs 13 – 18 (Table 2). Herd U's third pooled sample from swabs 13 – 18 produced a growth inhibition zone of 16 mm. Growth inhibition zones less than or equal to 21 mm indicate true resistance to cefoxitin. These colonies therefore proved to be truly cefoxitin resistant and thus confirming that the MRSA selective agar plates' cefoxitin was active. Therefore it was unnecessary to further process farm Y with an antibiogram.

Six extra swabs from four herds namely R, S, T and U were used to validate the tests due to the overall negative results found in herds A – O. The first validation test was to validate whether pooling swab samples were decreasing sensitivity and each swab was individually

inoculated directly onto the chromID™ MRSA agar plates. Herds R and S' swabs all tested negative while all six individual swabs from herd T and three individual swabs from herd U tested positive, thus corresponding to the pooled sample results and therefore suggesting that pooling of samples did not decrease sensitivity. The second validation test was to validate whether samples needed pre-enrichment and was tested by pre-enriching each sample individually before plating it onto the MRSA selective plates. All 12 swabs from herds R and S tested negative after pre-enrichment while all six swabs from herd T and three swabs from herd U tested positive after pre-enrichment. This corresponded with the direct processed samples therefore validating that pre-enrichment of samples was not necessary. The third validation was to test whether Gram negative bacteria were not inhibiting the growth of Gram positive MRSA colonies by first inoculating individual swabs onto 5% sheep blood agar plates with colistine and thereafter proceed with selective MRSA testing. Herds R and S all tested negative on the MRSA selective plates while all six swab samples from herd T and three swabs from herd U tested positive on the MRSA plates after pre-inoculation onto the Gram positive selective plates. The results once again corresponded to that of the direct MRSA testing and therefore validating that Gram negative bacteria did not inhibit possible MRSA colony growth.

Three herds were identified as MRSA positive namely T, U and Y. MRSA herd prevalence among large commercial piggeries in South Africa was calculated with an exact binominal 95%confidence interval to be 12%.

Table 1: MRSA results of both selective MRSA plates and Staphaureux* agglutination

Herd identity	**Pool 1 (1-6)**	**Pool 2 (7-12)**	**Pool 3 (13-18)**
A	Negative	Negative	Negative
B	Negative	Negative	Negative
C	Negative	Negative	Negative

D	Negative	Negative	Negative
E	Negative	Negative	Negative
F	Negative	Negative	Negative
G	Negative	Negative	Negative
H	Negative	Negative	Negative
I	Negative	Negative	Negative
J	Negative	Negative	Negative
K	Negative	Negative	Negative
L	Negative	Negative	Negative
M	Negative	Negative	Negative
N	Negative	Negative	Negative
O	Negative	Negative	Negative
P	Negative	Negative	Negative
Q	Negative	Negative	Negative
R	Negative	Negative	Negative
S	Negative	Negative	Negative
T	Positive	Positive	Positive
U	Negative	Negative	Positive
V	Negative	Negative	Negative
W	Negative	Negative	Negative
X	Negative	Negative	Negative
Y	Positive	Positive	Positive

Table 2: Antibiogram results of MRSA positive colonies

Sample Identity	Cefoxitin growth inhibition zone	Result
T 1-5	17mm	Resistant
T 6	19mm	Resistant
T 7 - 12	16mm	Resistant
T 13 - 18	17mm	Resistant
U 13 - 18	16mm	Resistant

Chapter 4 DISCUSSION

In this study 450 slaughter pigs' nasal contents were sampled from 25 large commercial pig herds to determine the national MRSA herd prevalence among large commercial piggeries in South Africa. MRSA was only detected in three of the 25 herds and therefore MRSA herd prevalence among large commercial piggeries in South Africa was estimated to be between 2.5 and 31%, with 95% confidence, with a point estimate of 12%. Despite the relatively large range of the estimate, we can conclude that substantially at most one third of piggeries appear to be infected with MRSA. This relatively low prevalence was unexpected compared to the study of Broens et al. (2011c) in the Netherlands where a herd prevalence of 67.3% among 171 breeding herds and a 71% herd prevalence among 31 finisher herds were observed. However, in both Nigeria and the USA low MRSA herd prevalence's were estimated. In Ilora, Nigeria, MRSA herd prevalence from 11 participating herds was 9% (Okunlola & Ayndale, 2015). This study correlates well with the 12% MRSA herd prevalence found in South Africa. A more recent study in the United States of America (USA) in fact found no MRSA in 36 herds across 11 states (Sun et al., 2015). It is therefore still possible to have a low herd prevalence of MRSA in pig herds despite the high prevalences found in other pig dense countries in the world. One may speculate that all three countries with low MRSA herd prevalence's have relatively healthy herds due to being far less pig dense in comparison with pig dense countries such as the Netherlands with a high MRSA herd prevalence. The increased distances between piggeries increases overall biosecurity resulting in less spread of disease between herds with consequently healthier herds and an overall reduction in the use of antibiotics to curtail disease. The influence of pig density on MRSA herd prevalence warrants further investigation.

The prevalence of MRSA in infected herds could have been underestimated if the herd-level sensitivity of the tests was low. In this study the specified minimum expected within-herd prevalence was 20% which was far less compared to true prevalence which Verhegghe et al.

(2013) determined to be at least 75% in finisher pigs. Therefore, a minimum expected within-herd prevalence of 20% used to determine the sample size of pigs per herd to be sampled among finisher pigs was correct. Locally in the Eastern Cape of South Africa, Adegoke & Okoh (2014) sampled 64 pigs from different ages of which 15 pigs were MRSA positive giving a within-herd prevalence of 23%, which correlates with the in-herd specified minimum expected prevalence of 20% used in this current study. It has to be borne in mind though, pigs from different ages (including piglets) were sampled in the Eastern Cape herd. Therefore, the true within-herd prevalence on a South African pig farm is still an area in which further research might be indicated. Further investigation to determine which group of pigs will have the highest MRSA prevalence in a South African production system might also be looked into.

On the other hand, the herd prevalence might have been over-estimated due to possible cross contamination between pigs from different herds or environmental contamination as the pigs did spend time in the lairage before sampling; some herds would overnight at the abattoir which would increase the risk of transfer of LA-MRSA significantly to them (Schmithausen et al., 2015), where others would have only rested for the period travelled which will be a minimum of one hour. Sampling was delayed to between stunning and sticking. Transmission of MRSA to pigs would be due nasal contact between carriers and non-carriers or from a contaminated environment. MRSA transmission via nose to nose contact was possible at one abattoir due to non-solid partitions between pig pens, whereas the other abattoirs all had solid partitions between pigs. Only one herd out of a total of six herds at the abattoir with the non-solid partitions tested positive which might indicate that this was not a significant risk factor as one would expect to find more positive herds from the said abattoir. Environmental contamination is suspected to be low due to lairages at all abattoirs being washed and disinfected between batches of pigs. Taking the above into consideration there might only be a slim chance of this being a factor for an over estimation of herd prevalence. Environmental swabs from the abattoir would have to be taken to estimate the risk of environmental contamination as this has not been done.

Herds were only sampled once-off and not repeatedly which might influence the results either to an over- or an underestimation of MRSA herd prevalence. The risk of herds acquiring MRSA is not only dependant on transmission between carriers and non-carriers, but is significantly increased via the administration of antibiotics to groups of pigs (Van Duijkeren et al. 2008) which might change from time to time within a herd depending on the health status and treatment programme followed on the farm prior to sampling, which may be seasonal if one considers an increase in severity of respiratory diseases during the fall and winter. In order to clarify this one would have to sample herds successively together with the on-farm treatment program.

The first 15 herds tested were all negative. It was then decided that an extra six samples of four herds were to be taken to validate the current sample processing procedure which was followed. These extra samples were tested as discussed under sample processing validation in parallel with the usual 18 samples which were pooled into three pools and processed as per the protocol. The results of the extra validation tests were in accordance with the results of the normal protocol. Two of the herds, T and U, were MRSA positive on all three processing methods while the other two herds, R and S, were negative on all three processing methods. This validation process answered three important questions. Firstly, pre-enrichment of the nasal swabs was not necessary to detect MRSA positive colonies. Secondly, pooling of samples did not appear to significantly decrease the sensitivity of MRSA detection on the selective MRSA agar plates. Thirdly, Gram negative bacteria did not inhibit the growth of *S.aureus* on the MRSA selective plate. For the other herds not evaluated with this method, authentic MRSA strains were used to validate both the chromogenic selective MRSA agar plates and the rapid slide agglutination Staphaureux* test. Furthermore, Normano et al. 2014 used the same plates for testing 215 slaughter pigs and got a prevalence of 37.6%. It can be concluded that the laboratory procedures were accurate and could be trusted.

Selection for MRSA in finisher pigs in South Africa is potentially decreased by the fact that there are no penicillin based or cephalosporin antibiotics registered for in-feed or in-water use in pigs, which would increase the risk of pigs becoming MRSA carriers (Van Duijkeren et al. 2008). These substances can however be used either off-label or compounded on special request by the herd veterinarian, but practices such as this in finisher pigs are mostly not done due to strict annual on-farm food safety audits on the majority of commercial piggeries. There are however injectable formulas available registered for pigs, but once again strict meat withdrawal periods have to be adhered to.

The importance of MRSA in pigs as a veterinary public health concern might have been over estimated in South Africa. Considering that herd prevalence among 25 large commercial herds tested was only 12% it does not raise significant concern that finisher pigs at the abattoir would be a severe risk to abattoir personnel. This statement does however leave a question as to what will be the LA-MRSA carrier status amongst abattoir personnel. Within-herd MRSA prevalence was not estimated and if this is found to be higher in a specific group of pigs such as the sows then further investigation on farm level personnel to determine their LA-MRSA carrier status would be indicated. Overall it is the author's opinion that LA-MRSA in pigs in South Africa is not of significance as a general public health threat, but further investigation to on-farm carrier status in both pigs and people working with pigs will be necessary before one can conclude that it is not a threat to the pig caretakers in South Africa.

Conclusion

The perceived prevalence of MRSA positive herds in South Africa is lower than expected. The within herd prevalence might be different as only slaughter pigs were sampled. None the less one can conclude that the risk of abattoir personnel being infected with MRSA from pigs will be lower than expected in South Africa, but this will have to be confirmed with sampling both abattoir personnel and slaughter pigs and typing all MRSA positive strains to prove transfer from pigs to humans. It would seem that the lack of registration of in-feed or in-water antibiotics containing beta-lactam and cephalosprins in South Africa, together with the strict monitoring of medication withdrawal periods dictated by food quality assurance schemes has reduced the overall MRSA prevalence among large commercial piggeries. The high health status of commercial piggeries in South Africa compared to that of more densely populated pig countries will also contribute to a lower antibiotic usage in South African piggeries overall which will reduce the overall MRSA prevalence.

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