

**Prevalence and antimicrobial susceptibility of coagulase positive *Staphylococcus*  
species from milk samples submitted to the Faculty of Veterinary Science  
Onderstepoort**

by

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## ABSTRACT

**Background:** Coagulase positive *Staphylococcus* (CoPS) species are commensal of the skin and the mucosal surface of animals, including dairy cattle. However, they have also been implicated in *sub-clinical* and clinical mastitis in dairy cattle. In South Africa, information on the prevalence and antimicrobial drug resistance of CoPS from clinical mastitis cases of dairy cattle is limited. Therefore, the aim of this study was to investigate the prevalence and antimicrobial resistance patterns of CoPS isolated from dairy cow milk samples submitted to the Onderstepoort milk laboratory for routine clinical diagnosis.

**Methodology:** A multistage sampling technique was used to obtain a representative sample of 2862 randomly selected cow milk samples. Coagulase-positive *Staphylococcus* species were identified based on morphology and biochemical tests and confirmation was done using the Analytical Profile Index (API). Antimicrobial susceptibility to twelve antimicrobial agents was determined using the disk diffusion method and interpreted according to the Clinical Laboratory Standard International guidelines.

**Results:** Twenty-three percent (23.2%; 665/2862) of samples tested positive for *Staphylococcus* species. Of these, 21.1% (605/2862) were coagulase negative *Staphylococcus* species (CoNS) and 2.0% (58/2862) CoPS. Among CoPS, *Staphylococcus aureus* (*S. aureus*, 86.2%, 50/58) was more prevalent than *Staphylococcus hyicus* (*S. hyicus*, 13.8%; 8/58). All (100%) *S. aureus* isolates exhibited antimicrobial resistance (AMR) to at least one category of antimicrobials, while 92.0% (46/50) were multidrug resistant (MDR). The highest proportion of *S. aureus* isolates were resistant to streptomycin (92.0%; 42/50) followed by erythromycin (62.0%; 31/50) and ampicillin (62.0%; 31/50). Almost half of *S. aureus* isolates were resistant to oxacillin (46.0%; 23/50) and only 8.0% (4/50) resistant to

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cefoxitin. *Staphylococcus hyicus* exhibited a high prevalence of resistance to streptomycin (100%; n=8) followed by erythromycin (75.0%; n=6) and ampicillin (75.0%; n=6).

**Conclusion:** *Staphylococcus aureus* is the predominant CoPS species associated with infectious mastitis among dairy herds in this study. *Staphylococcus aureus* and *Staphylococcus hyicus* exhibited high prevalence of resistance to aminoglycosides, macrolides and penicillins. In addition, several *S. aureus* isolates from milk samples submitted to the Onderstepoort milk laboratory exhibited multidrug and methicillin resistance.

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## LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance/ resistance to at least one antimicrobial
BTA	Blood tryptose agar
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase negative <i>Staphylococcus</i>
CoPS	Coagulase positive <i>Staphylococcus</i>
Co-trimoxazole	Sulfamethoxazole + trimethoprim
DAFF	Department of Agricultural Forestry and Fisheries
DNA	Deoxyribose nucleic acid
IMI	Intramammary infection
KZN	Kwa-Zulu Natal
MDR	Multidrug resistant/ resistant to at least three antimicrobials
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSA	Mannitol salt agar
NNIS	National Nosocomial Infections Surveillance
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
SANAS	South African National Accreditation System
SCC	Somatic cell count
SIG	<i>Staphylococcus intermedius</i> group
Spp.	Species
VRSA	Vancomycin Resistant <i>Staphylococcus aureus</i>

## CHAPTER 1

### 1.1 INTRODUCTION

Coagulase positive *Staphylococcus* (CoPS) species are commensal of the skin and mucosal surface of animals and humans (Heikens et al. 2005; Madigan et al. 2009). However, clinical conditions associated with CoPS have been reported in both humans and animals. In humans, clinical conditions associated with CoPS include boils, food poisoning, impetigo, toxic shock syndrome and dermatitis (Coton et al. 2010; Kebede and Hagos 2015; Ryan and Ray 2004; Trinidad, Nickerson, and Alley 1990). In dairy cattle, CoPS have been associated with clinical and *sub-clinical* mastitis, with *Staphylococcus aureus* (*S. aureus*) being the most predominant species (Capurro 2009; Fikru 2014; Halasa et al. 2007; R. Mohammed 2015; Roberson et al. 1996). Herds with high prevalence of *S. aureus*, experience low levels of milk production, increased levels of somatic cell count (SCC), increased veterinary and treatment costs, premature culling and reduced milk quality (Halasa et al. 2007).

*Staphylococcus aureus* infections as a cause of mastitis in dairy has been reported in Asia (Ali et al. 2008; Kheirabadi, Ebrahimi, and Barati 2008; Krithiga et al. 2011; Nam et al. 2010; Rahman et al. 2010; Yang et al. 2011), Europe (Capurro 2009; Unnerstad et al. 2009), America (Riekerink et al. 2008; Roberson et al. 1996), Eastern and Northern Africa (Abdel-Rady and Sayed 2009; Abera et al. 2012; Hakem et al. 2012; Iraguha, Hamudikuwanda, and Mushonga 2015; Kasozi, Tingiira, and Vudriko 2014; J. Mohammed 2015; R. Mohammed 2015; Mureithi and Njuguna 2016). Studies done in South Africa have also reported *S. aureus* infections in clinical mastitis cases in dairy cattle (Ateba et al. 2010; Fosgate, Petzer, and Karzis 2013; Petzer et al. 2009, 2013; Schmidt, Kock, and Ehlers 2015).

Antimicrobial drugs including  $\beta$ -lactams, tetracyclines, and aminoglycosides are a treatment of choice for *S. aureus* mastitis. In addition, glycopeptides, fluoroquinolones and lincosamides have been reported to be effective against MRSA and multidrug resistant

*Staphylococcus* species (Fikru 2014; Frank and Loeffler 2012; Kowalski, Karenchak, and Romanowski 2003). Resistance to penicillins among *S. aureus* have been reported in Europe (Nunes et al. 2007) and United states (Erskine et al. 2002). Similarly, in South Africa, resistance to  $\beta$ -lactams, aminoglycosides and macrolides among *S. aureus* have also been reported (Ateba et al. 2010; Schmidt 2011; Schmidt et al. 2015).

Studies in South Africa on prevalence of staphylococci in mastitis clinical cases have focused largely on infections associated with *S. aureus*. Therefore, the burden and antimicrobial susceptibility profile of coagulase positive *Staphylococcus* species in dairy herds in South Africa has not been extensively studied. In view of this it possible that infection associated with coagulase positive *Staphylococcus* species in dairy cattle in South Africa is common and that CoPS display a certain resistant pattern.

## **1.2 AIM AND OBJECTIVES**

### **1.2.1 Aim of the study**

The aim of this study was to investigate the prevalence and antimicrobial susceptibility profile of coagulase positive *Staphylococcus* species from cow milk samples submitted to the Onderstepoort milk laboratory for routine clinical diagnosis.

### **1.2.2 Objectives**

This study was based on two objectives:

1. To investigate prevalence of coagulase positive *Staphylococcus* species in cow milk samples of dairy cattle submitted to the Onderstepoort milk laboratory.
2. To determine the antimicrobial susceptibility profiles of the coagulase positive *Staphylococcus* species isolates from cow milk samples of dairy cattle submitted to Onderstepoort milk laboratory.

### **1.3 BENEFITS OF THE STUDY**

The study will contribute to the body of knowledge on the burden of coagulase positive *Staphylococcus* species in dairy herds in South Africa. In addition, information generated from this study will contribute to better understanding of the antimicrobial resistance patterns of coagulase positive *Staphylococcus* species among dairy herds in South Africa. This information may further be used to advise farmers on prudent use of antimicrobial agents in treatment of clinical mastitis cases.

### **1.4 ETHICAL CONSIDERATIONS**

Ethical clearance for this study was obtained from the University of Pretoria, Animal Ethics Committee (Ref V121-16) (Appendix B) and the farms from which the samples were obtained were kept anonymous.

### **1.5 COMPONENTS OF THIS RESEARCH**

This dissertation is divided into six chapters, namely: Chapter 1: Introduction, Chapter 2: Literature review, Chapter 3: Materials and methods, Chapter 4: Results, Chapter 5: Discussions, Chapter 6: Conclusions and recommendations, Chapter 7: Appendices.

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## CHAPTER 2

### 2.1 LITERATURE REVIEW

#### 2.1.1 Infectious mastitis

Mastitis is defined as the inflammation of the mammary gland and can be characterized into sterile or infectious mastitis (Schroeder 2012). The aetiology of infectious mastitis is complex and multi factorial. Over 135 infectious mastitis causing pathogens have been identified including *Staphylococcus* species (Evenson et al. 1988; Martinko and Madigan 2005; Pyörälä 2003; Watts 1988). *Staphylococcus* species have been reported in both chronic and *sub-clinical* mastitis cases in dairy cattle (Kebede and Hagos 2015).

*Staphylococcus* belong to the family *Micrococcaceae* (Baird-Parker 1974) and over fifty species of the genus *Staphylococcus* have been identified (Götz, Bannerman, and Schleifer 2006). These species differ from other genera of the *Micrococcaceae* family due to their ability to produce heat-stable endonuclease and their cell wall composition (Baird-Parker 1974).

Coagulase positive staphylococci (CoPS), compared to coagulase negative staphylococci (CoNS), remain the predominate *Staphylococcus* species associated with *sub-clinical* and clinical mastitis cases in dairy cattle with *S. aureus* being the most predominate among CoPS associated with *sub-clinical* mastitis. Other CoPS reported in mastitis cases include *S. delphini*, *S. hyicus*, *S. intermedius* and *S. schleiferi* subspecies *coagulans* (Roberson et al. 1998).

Age, sex, days in milk, breed, and presence of an underlying clinical condition are considered risk factors for occurrence of staphylococcal infectious mastitis. In addition, a contaminated environment increases the risk of staphylococcal infectious mastitis among dairy cows (Weinrauch and Zychlinsky 1999). Martinko and Madigan (2005) and Evenson et al. (1988) suggest that a high proportion of carrier and infected cattle also increase the risk of infection and spread of staphylococcal mastitis on the farm.

In general, mastitis in cattle results in poor milk quality, decreased milk production, increased somatic cell counts (SCC), premature culling of infected cows, and increased veterinary and labour costs (Hogeveen, Huijps, and Lam 2011; Radostits et al. 2000). Losses of up to \$1 billion per year associated with mastitis in dairy cattle has been reported in the U.S (Pursley and Martins 2011). In South Africa, mastitis has been associated with losses of up to R92.2 million (+/- \$6.9 million) a year (Lactodata 2017; Milk Producers' Organisation 2017).

### **2.1.1.1 Pathogenesis of *Staphylococcus* infection**

Successful infection with *Staphylococcus* spp. depends on the initial bacterial load, access to the target tissue, the type of strain, and host immunity (Projan et al. 1997). In the mammary tissue, *Staphylococcus* induces an inflammatory response characterized by an initial intense polymorphonuclear leukocyte response and the subsequent infiltration of the infected area by macrophages and fibroblasts (Gyles et al. 2011). In addition, staphylococci produce toxins which destroy cell membranes resulting in damage and scarring of the mammary tissue. In cases of *Staphylococcus aureus* infection, they can get established in the deep-seated pockets of alveoli resulting in abscessation. These abscesses can later reopen, further spreading the pathogens within the mammary tissue. In case of abscessation, the prognosis is often poor due to inability of antibiotics to penetrate the area (Pettersson-Wolfe, Mullarky, and Jones 2010).

### **2.1.2 Clinical signs associated with *Staphylococcus* infection**

The majority of staphylococcal related mastitis cases in dairy cattle are *sub-clinical*. However, in the acute phase, clinical signs such as swelling, warm, and gangrenous udder can be observed (Benić, Habrun, and Kompes 2012). In addition, a slight elevation in somatic cells count has been reported in staphylococcal clinical mastitis cases (DeGraves and Fetrow 1993; Harmon 1994; Heeschen 2010; Petzer et al. 2017).

### **2.1.3 Laboratory diagnosis of *Staphylococcus* species**

During laboratory diagnosis, *Staphylococcus* species are characterised based on colony morphology, coagulase and phosphatase reactions, acetoin production, and sugar fermentation (Baird-Parker 1974; Buchanan and Gibbons 1974). Coagulase positive staphylococci can be differentiated from coagulase negative staphylococci based on their ability to convert fibrinogen to fibrin. In addition, catalase, DNase, lipase and phosphatase tests are also used to characterize *Staphylococcus* species (Götz et al. 2006). Selective media such as mannitol salt agar have also been used to further characterise *Staphylococcus* spp. due to their ability to withstand high salt concentrations. This growth medium is used to characterize *S. aureus* among CoPS (Götz et al. 2006).

Biochemical tests such as Analytical Profile Index (API) (BioMérieux, France) are frequently used to identify and characterize *Staphylococcus* species (bioMérieux 2013). However, rapid molecular techniques such as real-time polymerase chain reaction (RT-PCR) are increasingly being used to characterize *Staphylococcus* species and identify new species (Götz et al. 2006). These molecular techniques have been recommended for correct species identification including that of the *Staphylococcus intermedius* group (SIG) due to the weakness of biochemical tests to characterize these species (Bannoehr and Guardabassi 2012).

### **2.1.4 *Staphylococcus* treatment and control**

#### **2.1.4.1 Treatment of staphylococcal infections**

Antimicrobial drugs such as  $\beta$ -lactams have successfully been used for the treatment of staphylococcal mastitis in dairy cattle (Kowalski, Karenchak, and Romanowski 2003). Drugs like ciprofloxacin, tetracyclines and trimethoprim-sulfamethoxazole are also known to be effective against *Staphylococcus* species (Lowy 1998). On the other hand, vancomycin is the drug of choice for the treatment of methicillin resistant *Staphylococcus aureus* (MRSA)

infections in human medicine. However, this drug is prohibited for use in veterinary medicine (Michel and Gutmann 1997).

#### **2.1.4.2 Control of staphylococcal infections**

Antimicrobial drugs alone are ineffective in the management of *Staphylococcus* spp. mastitis cases in dairy cattle. This is partially attributed to the high prevalence of antimicrobial resistance among the coagulase positive staphylococci, in particular, *S. aureus* (Fikru 2014; Magiorakos et al. 2011; Shorr 2007; Shorr and Lodise 2006). In addition, Smith et al. Middleton (1998) in Washington indicated that even in well-managed dairy herds, *S. aureus* remains a challenge.

*Staphylococcus aureus* can persist for long periods of time in the mammary gland of infected animals and may continue to be a source of infection to susceptible animals (Anderson and Lyman 2006; Larsen et al. 2000). Therefore, prevention of new infections and culling of infected cows is critical in reducing the prevalence of CoPS infection in dairy cattle (Petersson-Wolfe et al. 2010). Vaccination of susceptible dairy cattle has also been recommended as a potential control strategy. However, the success rate of vaccination is limited (Hoe and Ruegg 2005).

Talbot and Lacasse (2005) discovered ways to prevent new infections of mastitis to include: isolation or removal of fomites, evaluation of teat skin condition, and backflush. While Neave et al. (1969) and Haveri (2008) described a “five-point plan” for the control of contagious mastitis involving staphylococci. This program includes post-milking teat disinfection, total dry cow therapy, prudent use of antimicrobial agents, proper maintenance of the milking machines, and culling of chronically infected cows. In view of this, a multidisciplinary approach remains the most effective method of controlling and managing staphylococcal infections in dairy farms (Hoe and Ruegg 2005).

## 2.1.5 Epidemiology of *Staphylococcus* infections

### 2.1.5.1 Distribution of *Staphylococcus* species

*Staphylococcus* species have been isolated from 16.0% from dairy cattle with clinical mastitis in England (Bradley et al. 2007) and 15.7% in Australia (Shum et al. 2009). In Uruguay 45.0% of clinical mastitis from different dairy farms were caused by *Staphylococcus* species (Giannechini et al. 2002).

A number of studies in Africa reported *Staphylococcus* spp. involvement in clinical mastitis in dairy cattle. Mohammed (2015) reported that 24.0% of dairy cattle in Sudan had staphylococcal clinical mastitis. In Uganda, Byarugaba et al. (2008) reported a 42.4% and Abrahmsén (2014) a 55.6% prevalence of *Staphylococcus* infection in dairy cattle. In Kenya, 60.9% of dairy cattle with *sub-clinical* mastitis were reported to be infected with *Staphylococcus* species (Mureithi and Njuguna 2016).

Studies done in South Africa show a disparity in the occurrence of *Staphylococcus* infection at herd and cow level (Blignaut 2013; Petzer et al. 2009; Schmidt, Kock, and Ehlers 2015). Blignaut (2013) reported that 23.5% in 2008 and 16.2% in 2013 of dairy cattle were positive for *Staphylococcus* species. Petzer and co-workers (2009) reported that between 1996 and 2007, 54.3% of dairy cattle had mastitis caused by *Staphylococcus* species. In another study conducted by Schmidt et al. (2015), 7.3% of dairy cattle in KwaZulu Natal had staphylococcal mastitis.

### 2.1.5.2 Distribution of coagulase positive *Staphylococcus*

Mastitis caused by coagulase positive *Staphylococcus* has a worldwide distribution. *Staphylococcus hyicus* (17.7%), *S. intermedius* (0.2%) and *S. aureus* (82.1%) have been reported in cattle with mastitis in Virginia, USA (Roberson et al. 1996). Capurro (2009) in Sweden reported infection caused by *S. aureus* (97.0%), *S. intermedius* (2.0%) and *S. hyicus*

(1.0%) in mastitis cases of dairy cattle. In a study conducted by Mohammed (2015) in Sudan, *S. aureus* (16.0%) and *S. hyicus* (8.0%) were commonly isolated from clinical mastitis cases in dairy cattle. *Staphylococcus aureus* (17.2%), *S. hyicus* (8.2%) and *S. intermedius* (7.4%) have also been reported among dairy cattle in Ethiopia (Fikru 2014). Studies suggest that among CoPS in dairy cattle, *S. aureus* is the predominant species associated with intramammary infections in dairy cattle (Fikru 2014; R. Mohammed 2015; Rabie 2015; Roberson et al. 1996).

### **2.1.5.3 Distribution of *S. aureus* infections**

*Staphylococcus aureus* has been reported in 21.3% of clinical mastitis cases of dairy cattle in Sweden (Unnerstad et al. 2009). In Canada, *S. aureus* was isolated in 21.7% of dairy cattle with mastitis (Riekerink et al. 2008). Yang et al. (2011) in Asia, reported that 29.5% of clinical mastitis cases of dairy cattle were caused by *S. aureus*.

In Africa, *S. aureus* has also been isolated in mastitis in dairy cattle. Abera and co-workers (2012) in Ethiopia, reported that 48.6% of mastitis cases among dairy cattle were caused by *S. aureus*. Mureithi and Njuguna (2016) in Kenya, reported that 35.5% of *sub-clinical* mastitis in cattle was associated with *S. aureus*. In a study done in Uganda by Kasozi, et al. (2014), it was observed that *S. aureus* infection was responsible for 21.8% of mastitis cases in dairy cattle. Iraguha et al. (2015) in Rwanda observed that 6.25% *sub-clinical* mastitis in dairy cattle were due to *S. aureus*.

In Niger, Issa et al. (2016) reported that 55.7% of *sub-clinical* mastitis cases in dairy cattle were due to *S. aureus*. In Egypt, *S. aureus* were isolated from 52.5% of mastitis cases in dairy cattle (Abdel-Rady and Sayed 2009). In Zimbabwe, Katsande et al. (2013) reported 16.3% proportion of *S. aureus* in *sub-clinical* and clinical mastitis from smallholder dairy cattle. In South Africa, Petzer et al. (2009) reported a prevalence of 25.1% and Petzer et al. (2013) reported a 0.9% of *S. aureus* in mastitis cases in dairy cattle. In 2013, Fosgate et al.

(2013) reported *S. aureus* prevalence of 2.7% in mastitis cases submitted for clinical diagnosis. In 2015, Schmidt and co-workers (2015) reported a higher proportion of 4.3% among cattle with mastitis in KwaZulu Natal.

## 2.1.6 Antimicrobial resistance

Pathogenic and non-pathogenic organisms are increasingly becoming resistant to commonly used antimicrobial drugs both in human and veterinary medicine (Shitandi and Sternesjö 2004; Talbot et al. 2006; There and Wadhai 2013). This has been attributed to a number of factors including the inappropriate and sub-therapeutic use of these antimicrobial agents (Levy and Bonnie 2004). Resistance to commonly used antimicrobial drugs among the coagulase positive staphylococcal isolates from mastitis cases is on the rise (Brouillette and Malouin 2005; Sears and McCarthy 2003). This is of great concern since resistant coagulase positive staphylococci are able to persist in the environment for a longer period and may be a source of infection to susceptible cows (Deresinski 2005; Haveri 2008; Levy and Bonnie 2004). Studies show that resistance to  $\beta$ -lactams, quinolones and aminoglycosides among coagulase positive staphylococci from dairy cows with mastitis already exists (Alalem 2008; Bjorland, Sunde, and Waage 2001; Rice 2006; Schmidt 2011).

### 2.1.6.1 Mechanisms of action and resistance

Antimicrobial agents work in variety of ways (**Table 1**). For example, penicillin G inhibit bacterial cell wall synthesis. While fluoroquinolones and sulphonamides inhibit DNA synthesis. In view of this, resistance can occur through a number of mechanisms including genetic transfer of antimicrobial resistant genes which may be carried on the bacterial chromosome, plasmids, and transposons (Alemayehu 2015; Levy and Bonnie 2004; Rychlik, Gregorova, and Hradecka 2006). On the other hand, penicillin resistance can occur due to the expression of inducible  $\beta$ -lactamase encoded by the *blaZ* gene (Abraham and Chain 1940) or through the acquisition of modified penicillin-binding proteins (PBP) in a case of *S. aureus* (Monecke et al. 2007).

**Table 1: Classification of different antimicrobial drugs based on mechanisms of action and mode of action**

Antibiotic class*	Example	Mechanism of action	Mode of action
Anti staphylococcal $\beta$ -lactams	Oxacillin, Ampicillin	Block cross linking via competitive inhibition of the transpeptidase enzyme	Bactericidal
Anti-MRSA Cephalosporins	Cefoxitin	Inhibits bacterial cell wall synthesis via competitive inhibition of the transpeptidase enzyme	Bactericidal
Glycopeptides	Vancomycin	Disrupt peptidoglycan cross-linkage of the cell-wall	Bactericidal
Aminoglycosides	Streptomycin	Inhibit protein synthesis (bind to 30s subunit)	Bactericidal
Macrolides	Erythromycin	Inhibit protein synthesis (bind to 50S subunit)	Bacteriostatic
Fluoroquinolones	Ciprofloxacin	Inhibit DNA gyrase enzyme, inhibiting DNA synthesis	Bactericidal
Ansamycins	Rifampicin	Inhibits RNA transcription by inhibiting RNA polymerase	Bactericidal
Oxazolidinones	Linezolid	Inhibit protein synthesis by binding at the P site at the ribosomal 50S subunit	Bacteriostatic
Folate pathway inhibitors	Co-trimoxazole	Inhibit folic acid synthesis	Bacteriostatic
Phenicol	Chloramphenicol	Inhibit the 50s subunit to inhibit protein synthesis	Bacteriostatic

\*Adopted from Magiorakos et al. (2011).

## 2.1.6.2 Antimicrobial resistance among coagulase positive staphylococci

### 2.1.6.2.1 General antimicrobial resistance

High prevalence of resistance to penicillins among coagulase positive staphylococci (CoPS) have been reported in clinical dairy mastitis cases, for example, 69.0% and 61.0% penicillin resistance among *S. aureus* isolated from *sub-clinical* mastitis in dairy cattle have been reported in Europe (Nunes et al. 2007) and United State (Erskine et al. 2002) respectively. Resistance of *S. aureus* to penicillins has also been reported in Argentina (40.0%) (Gentilini et al. 2000) and Italy (22.0%) (Normanno et al. 2007). In South Africa, Schmidt (2011) reported 47.8% penicillin and 65.6% ampicillin prevalence of resistance among *S. aureus* from mastitis cases.

In Sudan, 82.5% of CoPS from cows with mastitis were reported to be resistant to  $\beta$ -lactams (El-Zubeir, Kutzer, and EL Owni 2006). In Kenya, Gitau et al. (2014) found 57.0% and in Uganda, Byarugaba et al. (2008) found 62.8% resistance to  $\beta$ -lactams among CoPS isolated from cows with *sub-clinical* mastitis. In Egypt, Haftu et al. (2012) reported 82.5% prevalence of resistance to  $\beta$ -lactams among CoPS isolated from cow with mastitis.

Coagulase positive *Staphylococcus* species have also been reported to show resistance against macrolides. To give an example, in Egypt (Haftu et al. 2012) and Sudan (El-Zubeir et al. 2006), 58.8% and 23.8% macrolide resistance among *S. aureus* isolates from cows with mastitis were respectively observed. Schmidt (2011) reported 2.2% resistance to macrolides among *S. aureus* from mastitis cases in South Africa.

Resistance to fluoroquinolones has been reported in 48.6% of *S. aureus* isolated from cows with mastitis in Sudan (El-Zubeir et al. 2006). In another study, 48.6% of CoPS from cows with mastitis in Sudan were reported to be resistant to fluoroquinolones (Gitau et al.

2014). In Ethiopia, 5.9% *S. aureus* from clinical mastitis cases in cattle were resistant to fluoroquinolones (Haftu et al. 2012).

#### **2.1.6.2.2 Methicillin resistant *Staphylococcus aureus* (MRSA)**

When *Staphylococcus aureus* strains exhibit resistance to methicillin or oxacillin, they are classified as methicillin resistant *Staphylococcus aureus* (MRSA) (Deurenberg and Stobberingh 2008). Methicillin resistant *S. aureus* belong to the clonal complex 398 and are classified as zoonotic (Hata et al. 2010; Paterson et al. 2012). The majority of MRSA generally resistant to other antibiotics such as aminoglycosides, macrolides, chloramphenicol, and fluoroquinolones are considered multidrug resistant (MDR) MRSA (Lee 2003; Schwarz et al. 2010).

Coelho et al. (2009) in Brazil reported that 25.0% of *Staphylococcus aureus* from cows with *sub-clinical* mastitis were methicillin resistant. In Europe, Vanderhaeghen et al. (2010) reported 9.3% MRSA in clinical bovine mastitis. Feßler et al. (2012) in Netherlands observed 4.0% MRSA in cows with clinical mastitis. In Canada, Erskine et al. (2002) and Saini et al. (2012) reported 1.0% and 0.1% MRSA in cattle with mastitis, respectively. In Ethiopia, Daka et al. (2012) reported 60.0% MRSA in cattle with clinical mastitis. Suleiman et al. (2013) in Nigeria reported 35.6% MRSA in cattle with clinical mastitis. Schmidt (2011) in South Africa reported 1.1% MRSA in cows with mastitis in KwaZulu Natal.

#### **2.1.6.2.3 Vancomycin resistant *Staphylococcus aureus* (VRSA)**

Vancomycin is the drug of choice for the treatment of MDR staphylococci as well as MRSA infections in human medicine and is prohibited in veterinary medicine (Michel and Gutmann 1997; There and Wadhai 2013). However, there are reports of vancomycin resistant coagulase positive staphylococci in dairy cattle. It is possible that vancomycin resistance among *Staphylococcus* species occur via genetic transfer of from humans to animals (There

and Wadhai 2013). In addition, the introduction of vancomycin for therapeutic treatment of clinical mastitis in dairy in the past could also have contributed to the emergence of VRSA in the dairy industry (Levy and Bonnie 2004; Myllys et al. 1998).

In Ethiopia, 38.5% and 52.4% VRSA have been reported in bovine mastitis cases by Daka et al. (2012) and Alemayehu (2015) respectively. In Tanzania, a lower prevalence of VRSA (2.17%) were reported from bovine mastitis cases (J. Mohammed 2015). In South Africa, Ateba et al. (2010) observed 4.7% vancomycin resistance among *S. aureus* from dairy farms in North West Province.

#### **2.1.6.2.4 Multi drug resistance**

Multidrug resistant *S. aureus* are not uncommon in *sub-clinical* and clinical mastitis cases in dairy cattle. Schmidt et al. (2015) reported 1.4% MDR *S. aureus* among dairy cows in KwaZulu Natal Province, South Africa. In India, 70.0% of *S. aureus* isolated from clinical mastitis cases of cattle were multidrug resistant (Sharma, Sharma, and Malik 2011). In Ethiopia, Mekuria et al. (2013) reported that 47.6% *S. aureus* isolates from cows with mastitis were MDR. In the same country, Fikru (2014) reported that 92.4% *S. aureus* in bovine mastitis were MDR. In Tanzania, Mohammed (2015) reported 26.1% of MDR *S. aureus*.

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## CHAPTER 3

### 3.1 METHODOLOGY

#### 3.1.1 Study area and study population

The Onderstepoort Milk Laboratory, at the Faculty of Veterinary Science, University of Pretoria receives both composite and quarter milk samples from dairy farms across South Africa for routine diagnosis of mastitis cases. A study sample was drawn from all the milk samples submitted to the Onderstepoort Milk Laboratory from lactating cows of all ages during July 2016 to January 2017.

#### 3.1.2 Sample type and collection method

For the purpose of this study, composite cow milk samples were used. Composite milk samples are approximate equal volumes of milk from the four quarters of the udder in one vial. At farm level, milk samples were collected aseptically from all individual cows in a herd by trained personnel. The samples were identified, packaged and transported on ice packs to the Onderstepoort Milk Laboratory and the analysis was performed immediately upon arrival.

#### 3.1.3 Sampling strategy

A multistage sampling technique was adopted for this study. The first stage involved sampling herds. The number of herds to be sampled was calculated based on an estimated 6.0% prevalence ( $P_1$ ) (according to information received from the laboratory). The second stage entailed calculation of the total number of cattle to be sampled in each herd. This was determined based on a prevalence ( $P_2$ ) of 25.1% (Petzer, Karzis, Watermeyer et al., 2009).

The required sample size was calculated using the formula described by Thrusfield (2013):  $n = \frac{Za^2 \times P(1-P)}{e^2}$  where ( $Za$ ) =95% confidence interval and ( $e$ ) is the allowable error of 5%.

### 3.1.3.1 Calculating the number of herds to be included in the study

$$n = \frac{1.96^2 \times 0.06(1 - 0.06)}{0.05^2}$$

The number of herds sampled was calculated to be  $n = 87$ . When herds received in the laboratory were less or greater than 87, the herds number was adjusted using the formulae  $n' = \frac{1}{\frac{1}{N} + \frac{1}{n}}$  where  $N =$  herd size;  $n =$  estimated herds ( $n = 87$ ) (Appendix A). In the laboratory, the total number of herds sampled per year was estimated to be 90. Over a 6 months period, we estimated the herds submitting milk to the laboratory to be 45. Therefore, based on the calculation, the total number of herds to be sampled during the study period was estimated to be 30.

### 3.1.3.2 Calculating the number of cows from each herd to be included in the study

$$n = \frac{1.96^2 \times 0.251(1 - 0.251)}{0.05^2} \quad n = 289$$

The number of cows to be sampled was adjusted based on the formulae by Dohoo et al. (2009)

$$n' = \frac{1}{\frac{1}{N} + \frac{1}{n}} \text{ where } N = \text{herd size; } n = \text{estimated sample size (=289). Herds were randomly}$$

selected during routine laboratory diagnosis. Each herd was only sampled once.

## 3.2 LABORATORY ANALYSIS

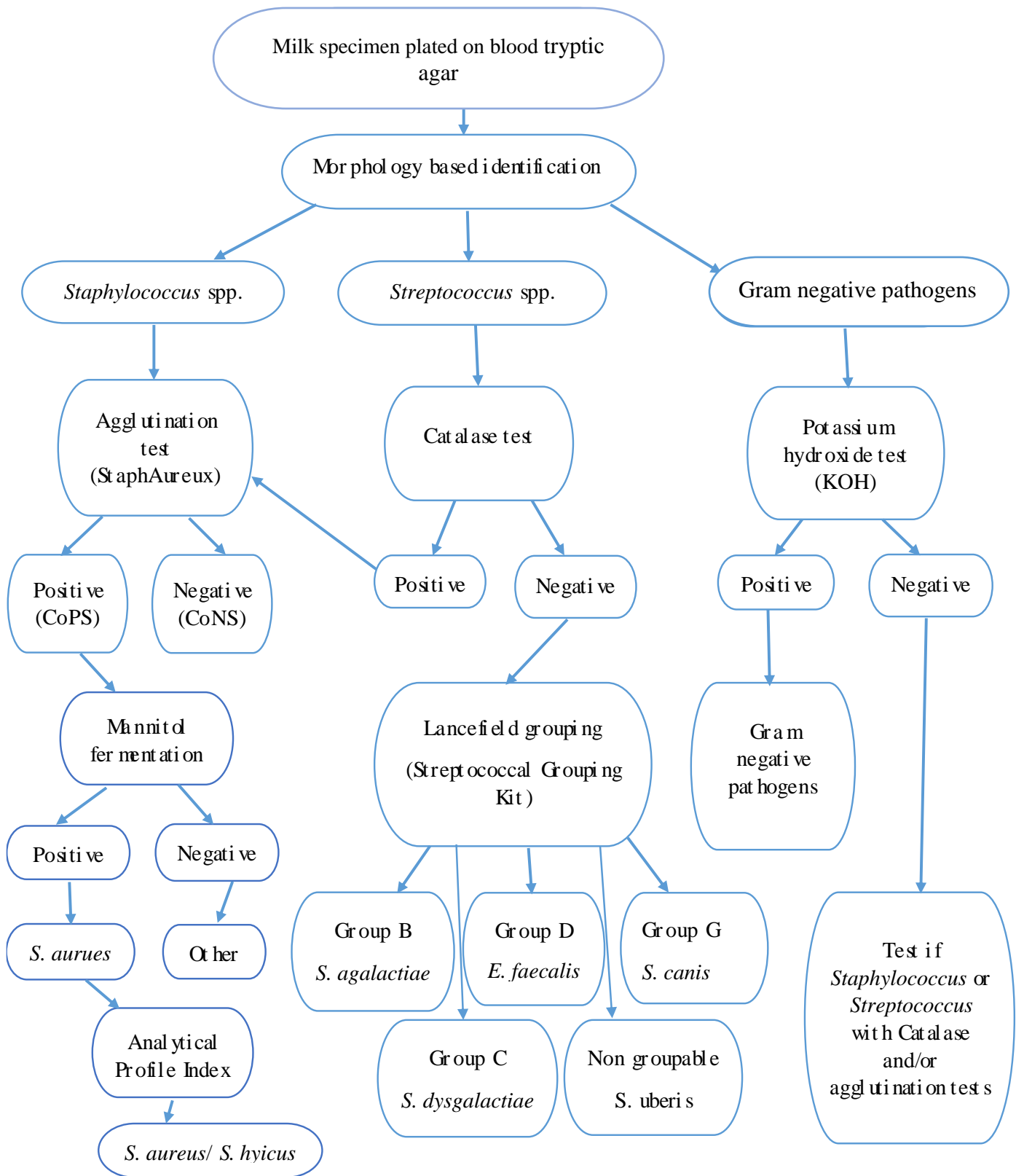
### 3.2.1 Bacterial culture

Blood Tryptic Agar (BTA) media (Oxoid, ThermoFisher Scientific, USA) with 5% bovine blood, Mannitol Salt Agar (MSA) (Oxoid, ThermoFisher Scientific, USA) and Mueller Hinton agar (MHA) (Oxoid, ThermoFisher Scientific, USA) were used in this study (United Kingdom Standards for Microbiology Investigations, 2014a). Using a 10 microliter plastic sterile loop (Quality Biological, USA), milk samples were plated on BTA and incubated at

37°C for 16-24 hrs. Presumptive colonies were divided into *Staphylococcus* spp., *Streptococcus* spp. and gram negative spp. on their phenotypic expression.

### 3.2.1.1 Identification of *Streptococcus* spp.

Haemolysis produced by colonies on blood agar and Lancefield serological grouping are crucial factors in the presumptive identification of *Streptococcus* pathogens. On blood tryptic agar, *Streptococcus* spp. appear as round colonies with smooth edges. Some strains produce mucoid colonies and various degrees of haemolysis. In addition, *Streptococcus* species are catalase negative (United Kingdom Standards for Microbiology Investigations, 2014b) and thus presumptively identified based on this factor. Catalase positive species were suspected to be of *Staphylococcus* species and subsequently tested with the agglutination test. This process is illustrated in the flow diagram in **Figure 1**.



**Figure 1: The process of the identification of mastitis causing pathogens from milk samples submitted to Onderstepoort Milk Laboratory for routine diagnosis**

The presumptive *Streptococcus* isolates were confirmed and grouped based on the Lancefield serological grouping using the Oxoid Streptococcal Grouping Kit (Oxoid, Thermo Fisher Scientific Inc, USA) (Table 2).

**Table 2: Lancefield serological grouping of common *Streptococcus* species at the Onderstepoort milk laboratory.**

Group	<i>Streptococcus</i> species
A	<i>Streptococcus pyogenes</i>
B	<i>Streptococcus agalactiae</i>
C	<i>Streptococcus dysgalactiae</i>
D	<i>Enterococcus faecalis</i> (previously known as <i>Streptococcus faecalis</i> )
F	<i>Streptococcus anginosus</i>
G	<i>Streptococcus canis</i>
Non groupable	<i>Streptococcus uberis</i>

(Medical Laboratories, 2013)

### 3.2.1.2 Identification of gram negative pathogens.

The Gram-negative pathogens were preliminarily identified based on the potassium hydroxide test after growth on BTA at 35-37°C for 18-24hrs. When Gram negative species react with potassium hydroxide, a white mucoid string forms (Chandra & Mani, 2011). Potassium hydroxide negative isolates were suspected to be either *Streptococcus* or *Staphylococcus* spp. These isolates were further subjected to the catalase and agglutination test (**Figure 1**). Species differentiation was not performed for this group of organisms because it was out of the scope of this study.

### 3.2.1.3 Identification of *Staphylococcus* spp.

Preliminary identification was based on phenotypic morphology on BTA, which includes round, convex, 1-4 mm diameter colonies with a sharp border, some frequently surrounded by zones of clear beta-haemolysis, golden-white and sometimes grey in colour.

Presumptive *Staphylococcus* colonies from BTA were tested for the presence of coagulase using the slide agglutination test kit (StaphAureux® kit, Oxoid, ThermoFisher Scientific, USA). Coagulase positive isolates were streaked on mannitol salt agar (MSA) (Oxoid, ThermoFisher Scientific, USA) and incubated for 16-24hrs at 37°C (United Kingdom Standards for Microbiology Investigations, 2014a). Resultant colonies were used to identify *Staphylococcus aureus*, first by inoculating pure colonies on MSA after purifying them on BTA (**Figure 1**). Mannitol salt agar is regarded as a valuable medium for the isolation of *S. aureus* from water, milk, skin and respiratory tract secretions (Kampf, Lecke, Cimbali et al., 1998). It contains a high concentration (~7.5%-10%) of salt (NaCl), which makes it selective for gram positive bacteria such as staphylococci (as well as other species from the *Micrococcaceae* family), since this level of NaCl is inhibitory to most other organisms. In this study MSA was used to streak and isolate the coagulase positive isolates (**Table 3**).

Differentiation of *Staphylococcus* species which were positive on the MSA were conducted using the API® Staph™ kit (Analytical Profile Index Staph test kit, Biomerieux, South Africa) following the manufacturer's manual. Isolates which were mannitol positive were *S. aureus*. The mannitol positive isolates were preserved in tryptic soy agar with 10% glycerol solution at -80°C (**Figure 1**)

**Table 3: Biochemical tests for characterization of *Staphylococcus* species into coagulase positive and coagulase negative *Staphylococcus* species.**

Species	Haemolysis present on blood agar	Coagulase	Mannitol
<i>S. aureus</i>	+	+	+
<i>S. hyicus</i>	-	+	-
<i>S. pseudintermedius</i>	+	+	-
<i>S. schleiferi subsp. coagulans</i>	+	+	±
<i>S. epidermidis</i>	±	-	-
<i>S. lugdunensis</i>	+	-	-

+: Positive, -: Negative ±: Positive or Negative; (Phillips & Kloos, 1981; Roberson, Fox, Hancock et al., 1992; Cunha, Sinzato & Silveira, 2004; VetBact, 2015)

### 3.2.2 Antimicrobial susceptibility

Only the API confirmed *S. aureus* (n= 50) and *S. hyicus* (n= 8) were subjected to antimicrobial susceptibility testing using disc diffusion method on Mueller Hinton agar (MHA) (Oxoid, ThermoFisher Scientific, USA) using the method described by CLSI (CLSI, 2013). The isolates were streaked on MHA, pressed with antibiotic discs and incubated at 37°C for 16-24 hrs. *Staphylococcus aureus* ATCC 25923 was used as a control.

The panel of antibiotics tested included ampicillin (AMP, 10ug), erythromycin (E, 15ug), chloramphenicol (C, 30ug), linezolid (LZD, 30ug), ciprofloxacin (CIP, 5ug), vancomycin (VA, 30ug), rifampicin (RD, 5ug), co-trimoxazole (SXT, 25ug), oxacillin (OX, 1ug), polymyxin B (PB, 300 units) and streptomycin (S, 10ug).

The methicillin resistance mediated through the *mecA* gene was done using the antibiotic cefoxitin (FOX, 30ug) on the Mueller Hinton agar.

The results of the antimicrobial tests were interpreted as prescribed by the CLSI (2013) guidelines for disc diffusion shown in **Table 4**. However, for purposes of this study, the intermediate resistant isolates were reclassified as resistant.

**Table 4: Antimicrobial susceptibility characterization of *S. aureus* based on the inhibition zones**

Antibiotics	Inhibition diameters (mm)		
	Susceptible	Intermediate	Resistant
Ampicillin	≥29	-	≤28
Erythromycin	≥23	-	≤13
Chloramphenicol	≥12	10-11	≤9
Linezolid	≥20	-	≤21
Ciprofloxacin	≥21	16-20	≤15
Vancomycin	≥18	13-17	≤12
Rifampicin	≥20	17-19	≤16
Cefoxitin	≥22	-	≤21
Co-trimoxazole	≥16	11-15	≤10
Oxacillin	≥22	-	≤21
Polymyxin B*	≥12	9-11	≤8
Streptomycin	≥22	17-19	≤11

Interpretation by CLSI (2013). \*Interpretation by Oranim (2017)

### **3.3 DATA MANAGEMENT AND DATA ANALYSIS**

#### **3.3.1 Data management**

Laboratory results were entered into a Microsoft Excel spread sheet (Microsoft Corporation, USA). The antibiotic names were abbreviated for ease of capturing data.

The data was first checked for improbable entries and missing data before analysis of the data commenced. Data was analyzed using both SPSS v24.00 (IBM SPSS Statistics) and Microsoft Excel (Microsoft Corporation, USA).

#### **3.3.2 Data analysis**

Analysis was performed using the Statistical Package for Social Science (SPSS version 24) and Microsoft Excel (Microsoft Corporation, USA). Descriptive statistics such as percentages and frequency distributions of the antimicrobial susceptibility profile of bacteria were computed and presented as tables and figures. Isolates that were resistant to at least one antimicrobial were classified as AMR, while those that showed resistance to 3 or more categories of antimicrobials were classified MDR (Magiorakos, Srinivasan, Carey et al., 2011).

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## CHAPTER 4

### 4.1 RESULTS

#### 4.1.1 The number of farms and samples tested

A total of 2862 cow milk composite samples from 43 farms with an average of 67 (CI: 61.0-72.2) cows per farm were tested for mastitis causing pathogens.

#### 4.1.2 Proportions of mastitis-causing organisms isolated from milk samples

Out of the 2862 composite cow milk samples that were tested, the majority (69.5%; n= 1987) were negative for bacterial growth. Of the positive samples (30.8%; n= 875), the majority of the organisms isolated were positive for *Staphylococcus* spp. (23.2%; n= 665) followed by 4.3% (n= 124) that were positive for *Streptococcus* spp. (Table 5).

**Table 5: Proportions of organisms isolated from milk samples submitted to the Onderstepoort milk laboratory.**

Organisms	Pathogens isolated			
	Frequency	Percent (%)	Confidence Interval	
<i>Staphylococcus</i> species	665	23.2	21.4	24.4
<i>Streptococcus</i> species	124	4.3	3.9	5.1
Gram negative pathogens	43	1.5	1.1	2.0
Other	43	1.5	1.1	2.0

Among the *Streptococcus* species (n=124) that were isolated, *S. uberis* (62.9%; n= 78) was the most commonly isolated followed by *S. agalactiae* (16.1%; n= 20) and *S. dysgalactiae* (12.1%; n= 15). The least common species was *S. canis* (3.2%; n= 4) (Table 6).

**Table 6: Distribution of *Streptococcus* species (n = 124) isolated from milk samples submitted to the Onderstepoort quick diagnosis” milk laboratory.**

Organisms	<i>Streptococcus</i> isolated			
	Frequency	Percent (%)	Confidence interval	
<i>Streptococcus uberis</i>	78	62.9	54.4	71.4
<i>Streptococcus agalactiae</i>	20	16.1	9.7	22.6
<i>Streptococcus dygalactiae</i>	15	12.1	6.7	17.8
<i>Enterococcus faecalis</i>	7	5.7	1.6	9.7
<i>Streptococcus canis</i>	4	3.2	0.1	6.3
<b>Total</b>	<b>124</b>	<b>100</b>	<b>100</b>	<b>100</b>

Coagulase negative *Staphylococcus* (CoNS) species (91.3%; n= 607), were more common than coagulase positive *Staphylococcus* (CoPS) which constituted only 8.7% (n=58) of the total number of *Staphylococcus* species (n= 665) (Table 7).

**Table 7: Distribution of *Staphylococcus* species isolated from bovine milk samples submitted to the Onderstepoort milk laboratory.**

Organisms (n= 665)	<i>Staphylococcus</i> spp. isolated			
	Frequency	Percent (%)	Confidence interval	
Coagulase negative <i>Staphylococcus</i>	607	91.3	89.1	93.4
Coagulase positive <i>Staphylococcus</i>	58	8.7	6.6	10.9

*Staphylococcus aureus* (86.2%; n= 50) was more prevalent than *S. hyicus* (13.8%; n= 8) among the isolated CoPS (Table 8).

**Table 8: Distribution of coagulase positive *Staphylococcus* species from bovine milk samples submitted to the Onderstepoort milk laboratory.**

Organisms (n= 665)	<i>Staphylococcus</i> spp. isolated		
	Frequency	Percent (%)	Confidence interval
<i>Staphylococcus aureus</i>	50	86.2	77.33 – 95.08
<i>Staphylococcus hyicus</i>	8	13.8	4.92 – 22.67

### 4.1.3 Antimicrobial susceptibility profiles of *Staphylococcus*

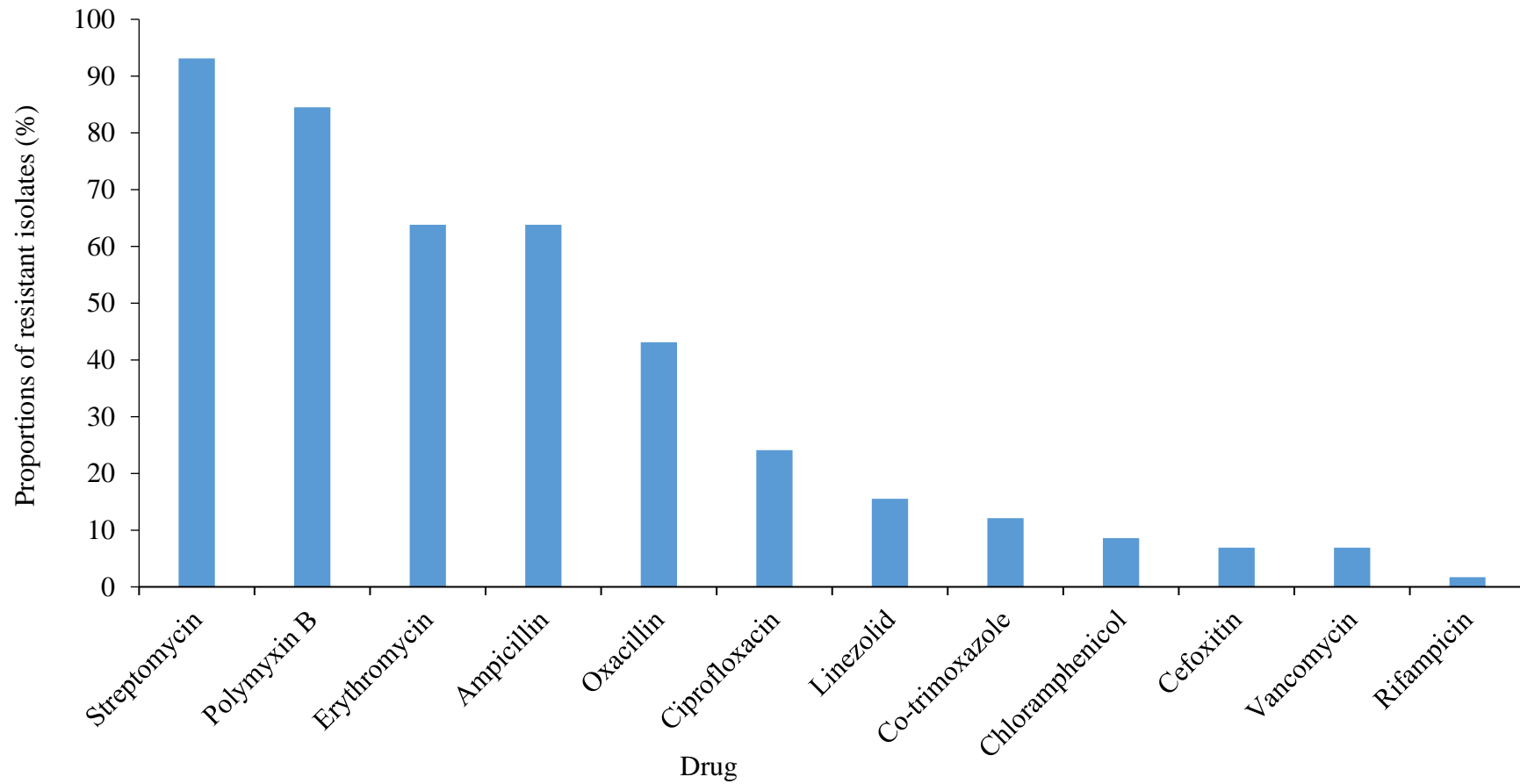
#### 4.1.3.1 Antimicrobial resistance profile of coagulase positive *Staphylococcus* (CoPS)

The majority of CoPS isolates were resistant to streptomycin (93.1%), polymyxin B (84.5%), ampicillin (63.8%) and erythromycin (63.8%). While 43.1% and 6.9% were resistant to oxacillin, cefoxitin and vancomycin respectively. The lowest level of resistance (1.7%) among CoPS, was observed against rifampicin (**Figure 2**).

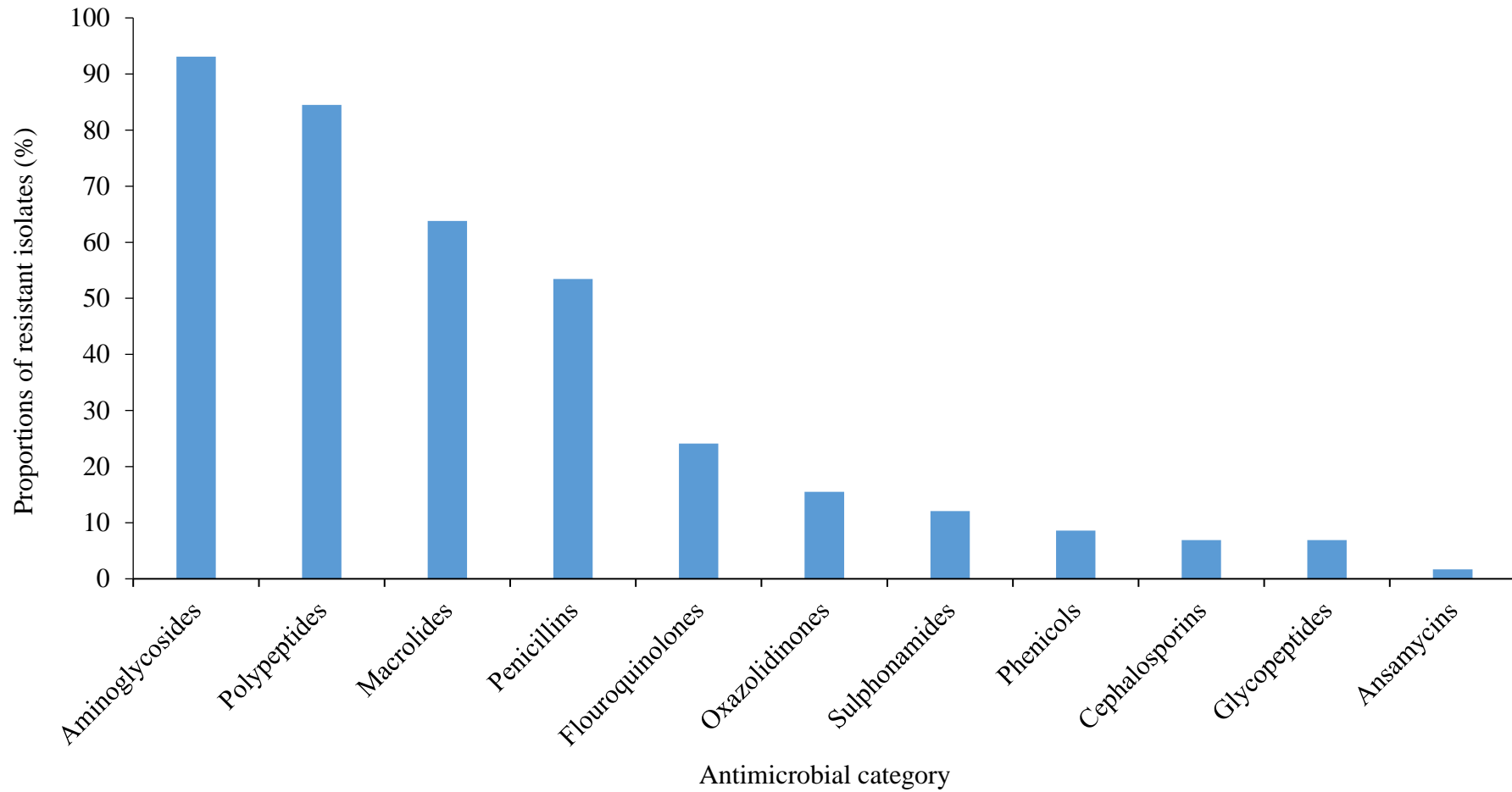
Based on class of antimicrobials, the CoPS were more frequently resistant to aminoglycosides (93.1%), macrolides (63.8%) and penicillins (53.5%). Lower resistance levels among CoPS isolates were observed against ansamycins (1.7%) (**Figure 3**).

It was observed that all (100%) of the CoPS spp. were resistant to at least one category of antimicrobials (AMR), while 91.4% (n= 53) were resistant to three or more classes of antimicrobials (MDR) (**Figure 4**).

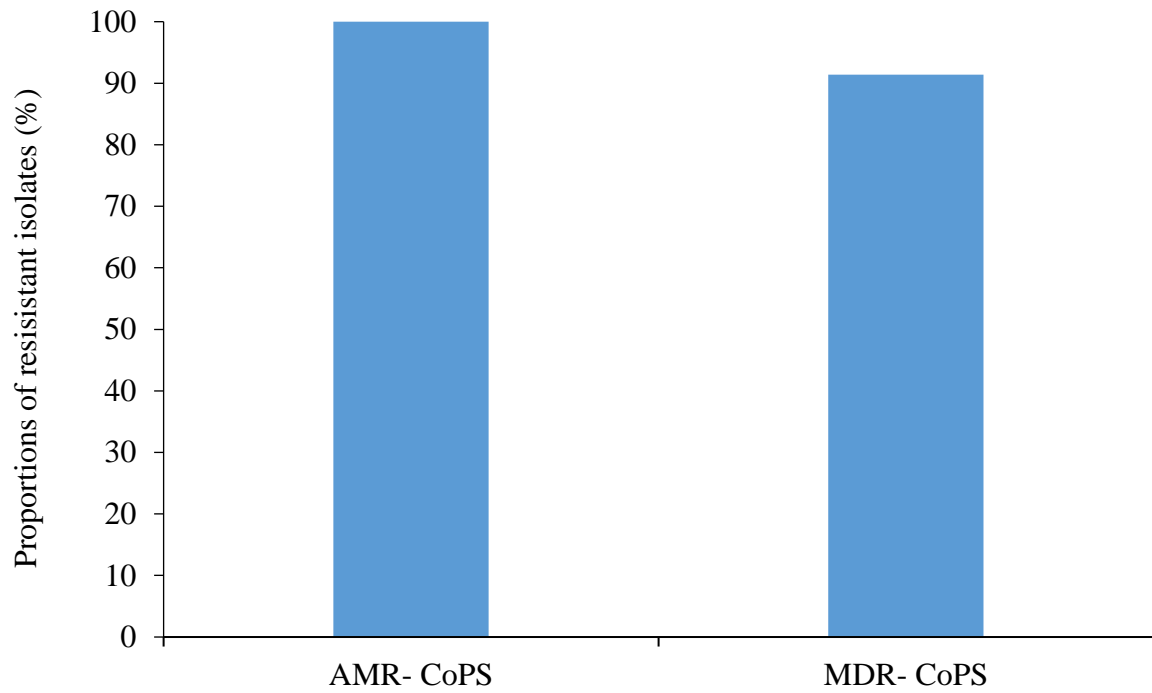
Most of the CoPS isolates were resistant to the drug combinations E S PB (10.3%; n= 6), followed by E AMP OX S PB (8.6%; n= 5), AMP S PB (6.9%, n= 4), S PB, E CIP AMP S PB and AMP OX S PB with 5.2% (n= 3) each. Isolates that exhibited the combination involving the E AMP S PB combination was the least observed (3.4%; n= 2) (**Table 9**).



**Figure 2: Proportions of resistant Coagulase Positive *Staphylococcus* species to each antimicrobial drug tested at the Onderstepoort milk laboratory.**



**Figure 3: Proportions of resistant Coagulase Positive *Staphylococcus* species isolates from milk samples submitted to Onderstepoort milk laboratory based on antimicrobial category.**



**Figure 4: Proportions of AMR and MDR Coagulase Positive *Staphylococcus* species isolated from dairy milk samples tested at the Onderstepoort milk laboratory**

**Table 9: Antimicrobial drug resistant combinations among coagulase positive *Staphylococcus* species isolates from milk samples submitted to Onderstepoort milk laboratory.**

Drug combinations	Proportions of isolates resistant			
	Frequency	Percent (%)	Confidence interval	
E S PB	6	10.3	2.51	18.18
E AMP OX S PB	5	8.6	1.40	15.84
AMP S PB	4	6.9	0.38	13.42
S PB	3	5.2	0.0	10.87
E CIP AMP S PB	3	5.2	0.0	10.87
AMP OX S PB	3	5.2	0.0	10.87
E AMP S PB	2	3.4	0.0	8.144
SXT S PB	1	1.7	0.0	5.074
LZD S PB	1	1.7	0.0	5.074
LZD AMP S PB	1	1.7	0.0	5.074
LZD AMP S	1	1.7	0.0	5.074
LZD AMP OX S PB	1	1.7	0.0	5.074
E S PB C	1	1.7	0.0	5.074
E S FOX	1	1.7	0.0	5.074
E OX SXT PB	1	1.7	0.0	5.074
E OX S PB C	1	1.7	0.0	5.074
E LZD S	1	1.7	0.0	5.074
E LZD AMP S RD PB C	1	1.7	0.0	5.074
E LZD AMP OX SXT S	1	1.7	0.0	5.074
E CIP S PB	1	1.7	0.0	5.074
E CIP S	1	1.7	0.0	5.074
E CIP OX S PB	1	1.7	0.0	5.074
E CIP LZD AMP OX	1	1.7	0.0	5.074
E CIP AMP SXT S PB	1	1.7	0.0	5.074
E CIP AMP OX SXT S PB	1	1.7	0.0	5.074
E CIP AMP OX S PB FOX	1	1.7	0.0	5.074
E CIP AMP OX S PB	1	1.7	0.0	5.074
E AMP S	1	1.7	0.0	5.074
E AMP OX SXT S PB FOX	1	1.7	0.0	5.074
E AMP OX S VA	1	1.7	0.0	5.074
E AMP OX S PB VA C	1	1.7	0.0	5.074
E AMP OX S PB VA	1	1.7	0.0	5.074
E AMP OX PB	1	1.7	0.0	5.074
CIP S PB	1	1.7	0.0	5.074
CIP OX SXT S PB	1	1.7	0.0	5.074
CIP AMP OX S PB VA	1	1.7	0.0	5.074
AMP S PB VA	1	1.7	0.0	5.074
AMP S	1	1.7	0.0	5.074
AMP OX FOX	1	1.7	0.0	5.074

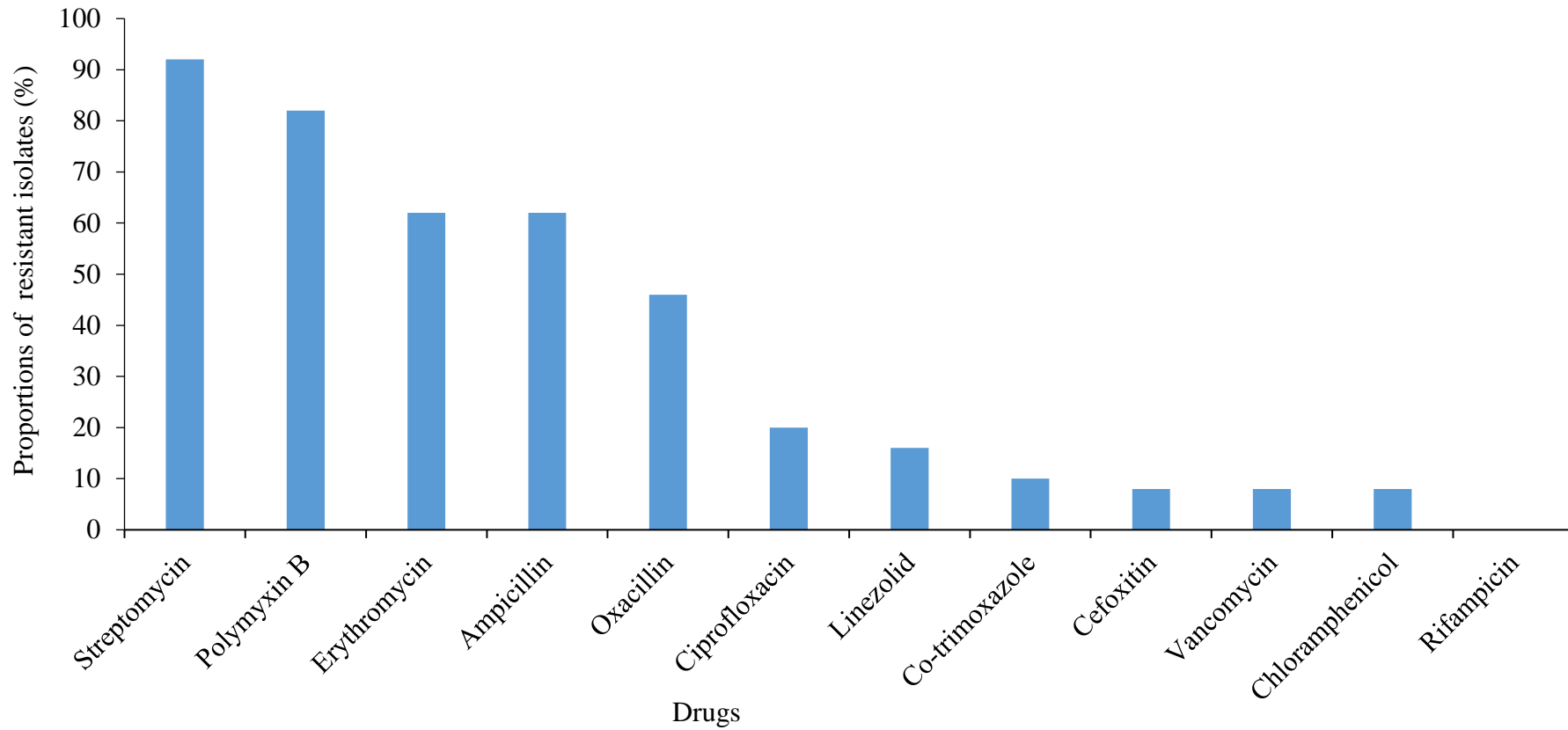
AMP: Ampicillin; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; FOX: Cefoxitin; LZD: Linezolid; OX: Oxacillin; PB: Polymyxin B; RD: Rifampicin; S: Streptomycin; SXT: Co-trimoxazole; VA: Vancomycin

#### 4.1.3.2 Antimicrobial resistance profiles of *Staphylococcus aureus*

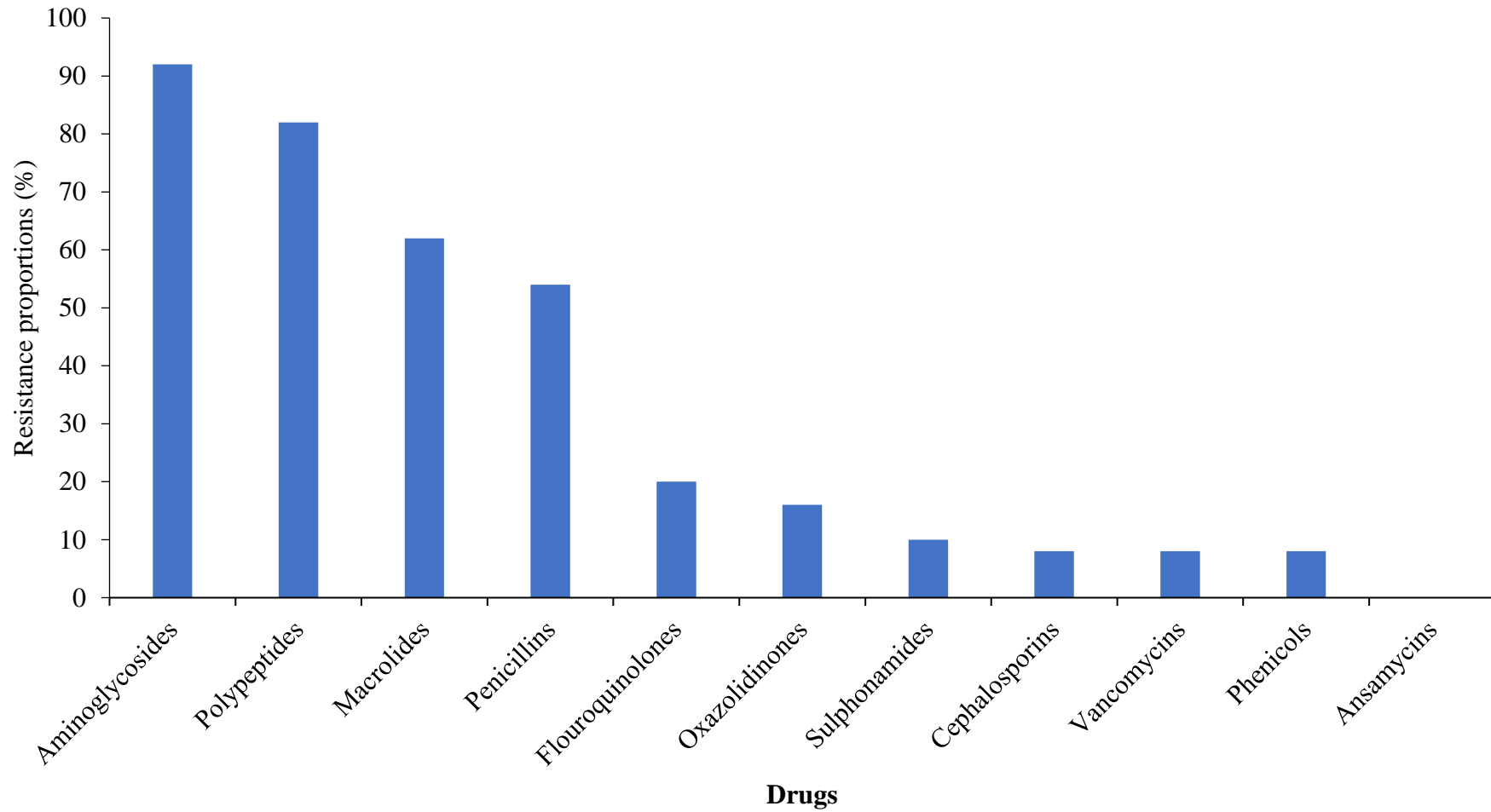
*Staphylococcus aureus* exhibited high resistance to streptomycin (92.0%, n= 45), polymyxin B (82.0%, n= 41) and erythromycin (62.0%; n= 31) and ampicillin (62.0%; n= 31). Resistance against ceftiofuran and vancomycin was the lowest, 10% respectively (**Figure 5**). Based on antimicrobial class, *S. aureus* exhibited high prevalence of resistant to aminoglycosides (92.0%, n= 45), macrolides (62.0%, n= 31) and penicillins (54.0%, n= 54). While lower prevalence of resistance was observed for cephalosporins (8.0%), glycopeptides (8.0%) and phenicols (8.0%) (**Figure 6**).

All (100%) *S. aureus* isolates were resistant to at least one antimicrobial drug category, while 92.0% (n= 46) exhibited multidrug resistance (**Figure 7**). The most common combination of drugs to which *S. aureus* isolates were resistant included: E S PB (12.0%, n= 6), E AMP OX S PB (8.0%, n= 4), AMP S PB (6.0%; n= 3) and AMP OX S PB (6.0%; n= 3) (**Table 10**).

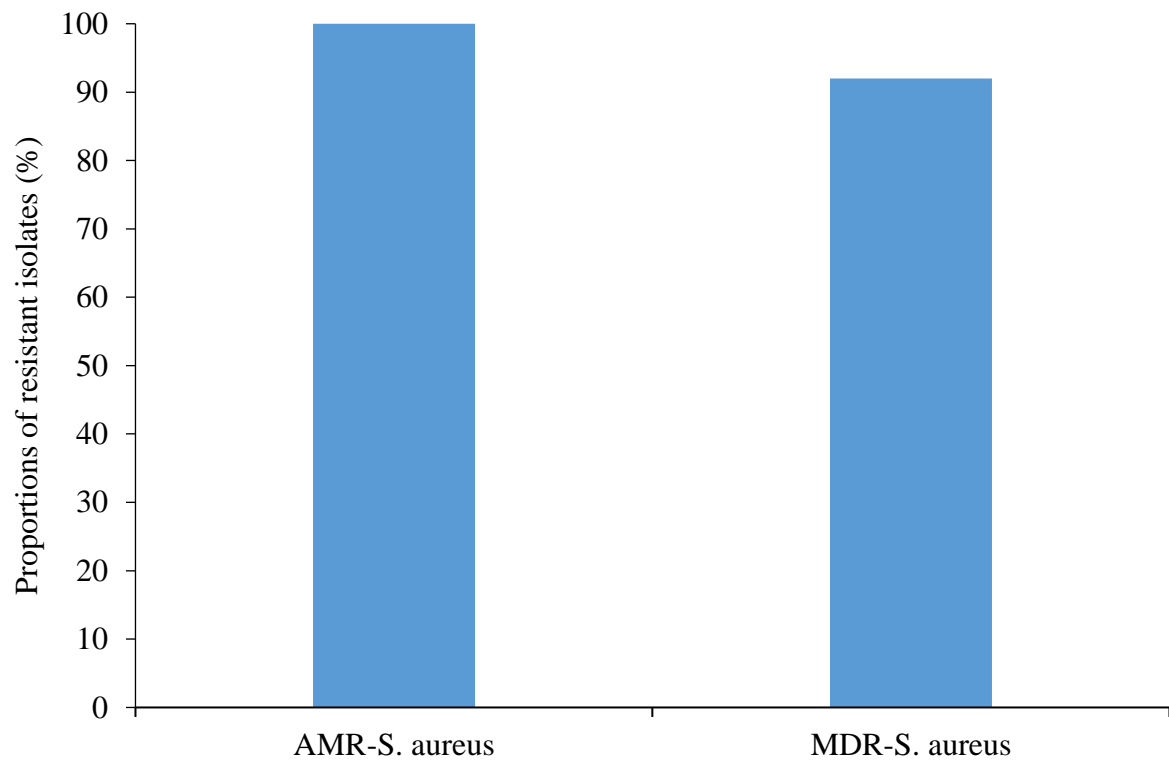
Among MDR *S. aureus* isolates, the antimicrobial classes most commonly involved in resistance combinations were aminoglycosides (94.3%), polypeptides (86.8%), macrolides (69.8%) and penicillins (73.6%). The antimicrobial drugs least commonly involved in MDR combinations were ansamycins (1.9%), cephalosporins (5.7%) and glycopeptides (7.5%) (**Figure 8**).



**Figure 5: Proportions of resistant *S. aureus* isolated to each antimicrobial drug tested from milk samples submitted to the Onderstepoort milk laboratory**



**Figure 6: Proportions of resistant *S. aureus* isolates from milk samples submitted to Onderstepoort milk laboratory based on antimicrobial categories**

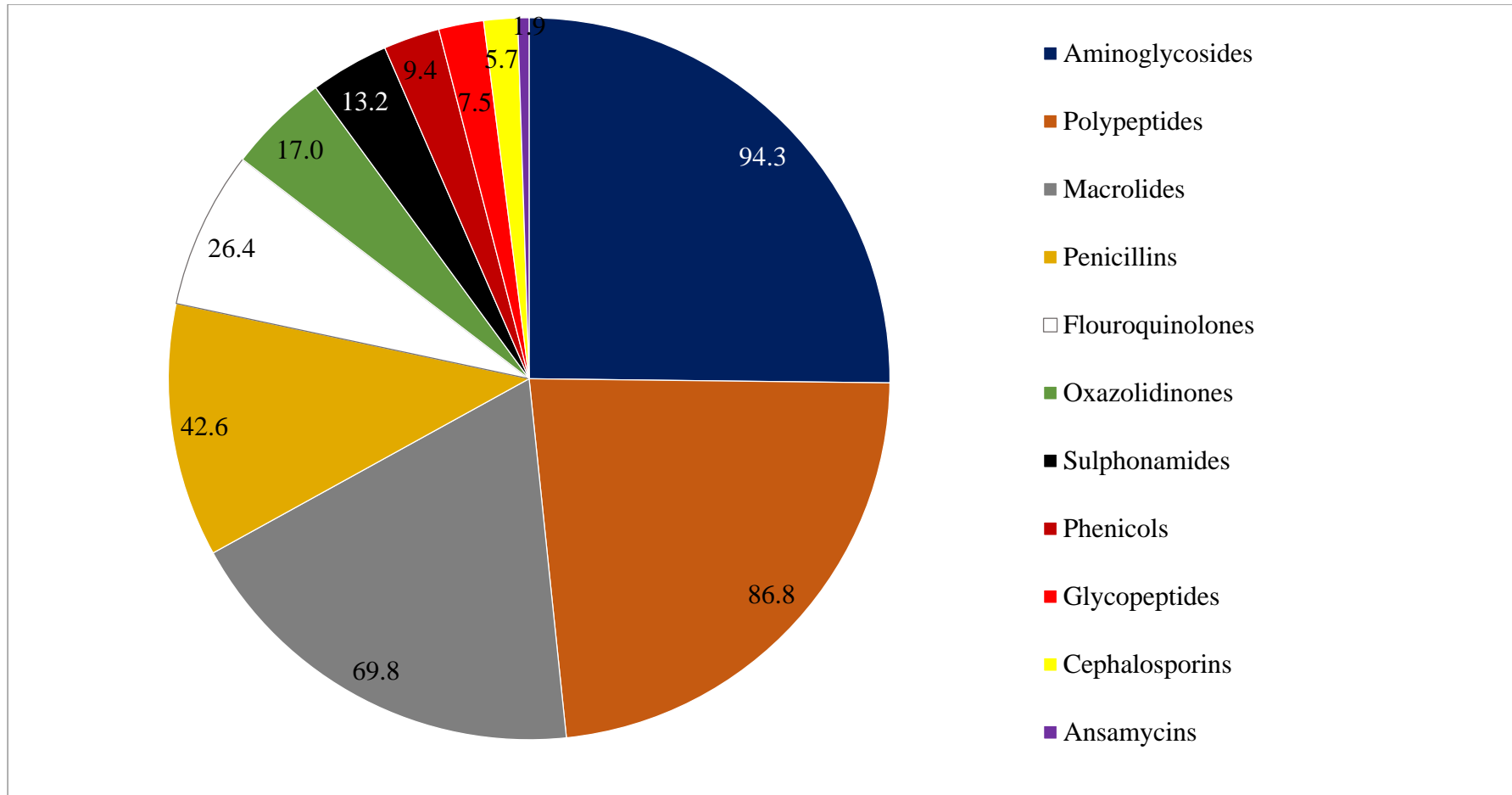


**Figure 7: Proportions of antimicrobial resistant and multidrug resistant *S. aureus* isolated from samples submitted to the Onderstepoort milk laboratory**

**Table 10: Antimicrobial drug resistant combinations among *S. aureus* isolated from milk samples submitted to the Onderstepoort milk laboratory.**

Drug combinations	Proportions of isolates resistant			
	Frequency	Percent (%)	Confidence interval	
E S PB	6	12	2.993	21.01
E AMP OX S PB	4	8	0.4807	15.52
AMP OX S PB	3	6	0.0	12.58
AMP S PB	3	6	0.0	12.58
E AMP S PB	2	4	0.0	9.431
E CIP AMP S PB	2	4	0.0	9.431
S PB	2	4	0.0	9.431
AMP OX FOX	1	2	0.0	5.88
AMP S	1	2	0.0	5.88
AMP S PB VA	1	2	0.0	5.88
CIP AMP OX S PB VA	1	2	0.0	5.88
CIP OX SXT S PB	1	2	0.0	5.88
CIP S PB	1	2	0.0	5.88
E AMP OX PB	1	2	0.0	5.88
E AMP OX S PB VA	1	2	0.0	5.88
E AMP OX S PB VA C	1	2	0.0	5.88
E AMP OX S VA	1	2	0.0	5.88
E AMP OX SXT S PB FOX	1	2	0.0	5.88
E AMP S	1	2	0.0	5.88
E CIP AMP OX S PB	1	2	0.0	5.88
E CIP AMP OX S PB FOX	1	2	0.0	5.88
E CIP LZD AMP OX	1	2	0.0	5.88
E CIP OX S PB	1	2	0.0	5.88
E CIP S	1	2	0.0	5.88
E LZD AMP OX SXT S	1	2	0.0	5.88
E LZD S	1	2	0.0	5.88
E OX S PB C	1	2	0.0	5.88
E OX SXT PB	1	2	0.0	5.88
E S FOX	1	2	0.0	5.88
E S PB C	1	2	0.0	5.88
LZD AMP OX S PB	1	2	0.0	5.88
LZD AMP S	1	2	0.0	5.88
LZD AMP S PB	1	2	0.0	5.88
LZD S PB	1	2	0.0	5.88
SXT S PB	1	2	0.0	5.88
<b>Total</b>	<b>50</b>	<b>100</b>	<b>100</b>	<b>100</b>

AMP: Ampicillin; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; FOX: Cefoxitin; LZD: Linezolid; OX: Oxacillin; PB: Polymyxin B; RD: Rifampicin; S: Streptomycin; SXT: Co-trimoxazole; VA: Vancomycin

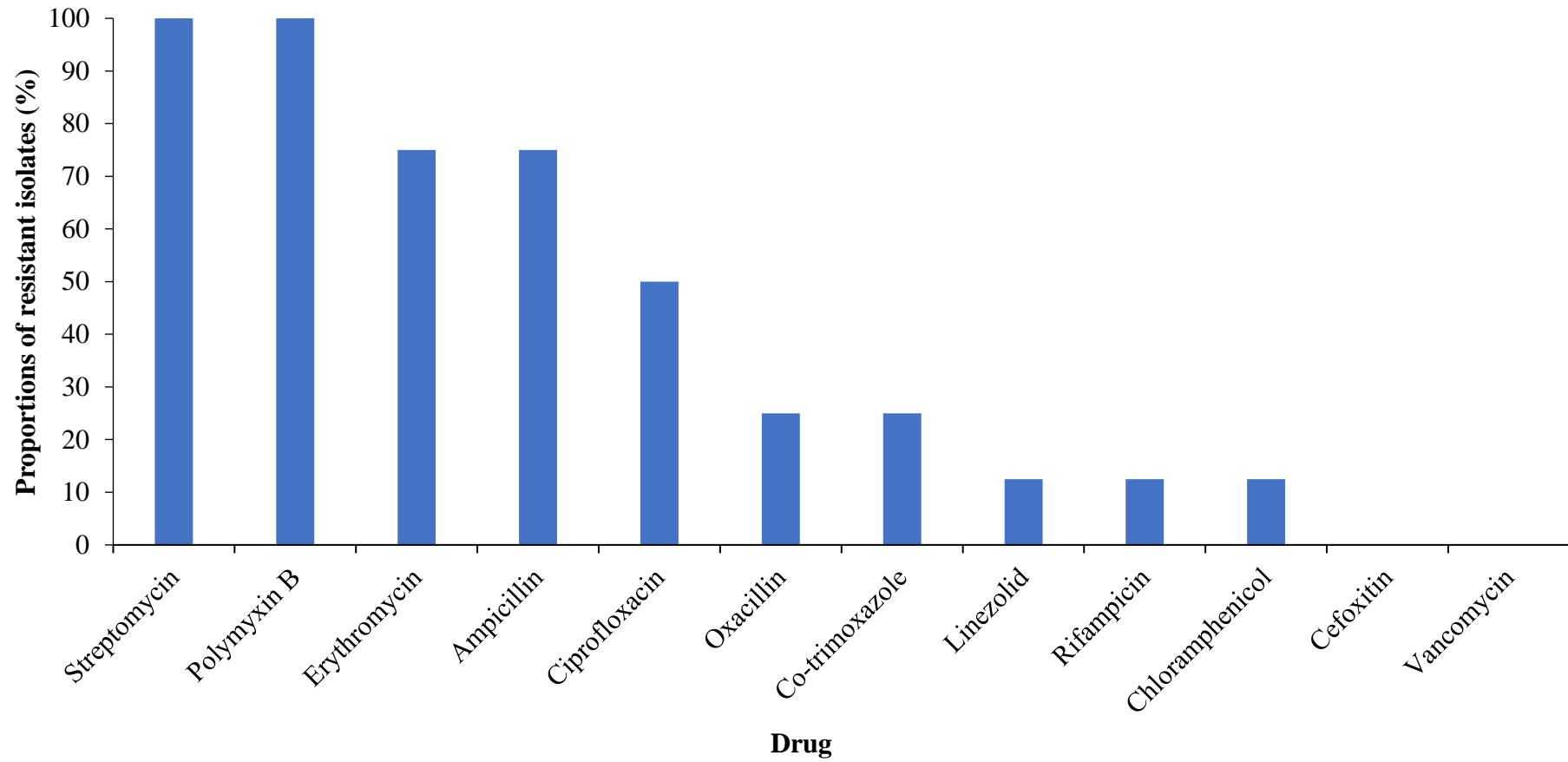


**Figure 8: Proportion (%) of resistant *S. aureus* isolated based on antimicrobial categories from milk samples submitted to Onderstepoort milk laboratory.**

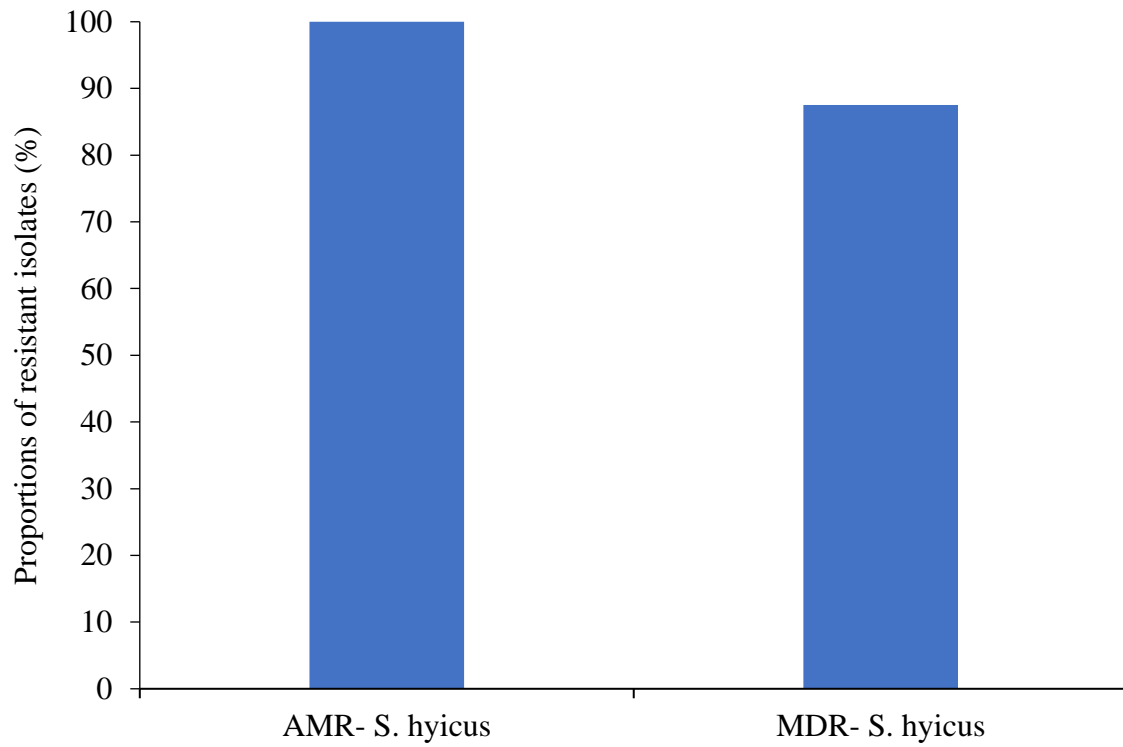
#### 4.1.3.3 Antimicrobial resistance profile of *Staphylococcus hyicus*

All (100%) *S. hyicus* were resistant to both streptomycin and polymyxin B. While 75.0% were resistant to both erythromycin and ampicillin respectively. No resistance to cefoxitin and vancomycin was observed (**Figure 9**). In addition, all (100%) *S. hyicus* isolated were resistant to at least one category of antibiotics, while 87.5% were MDR (**Figure 10**).

Each MDR *S. hyicus* isolate had a unique combination of drug, giving a total of eight (n=8) MDR combinations (**Table 11**).



**Figure 9: Proportions of resistant *S. hyicus* to each antimicrobial drug tested from milk samples submitted to the Onderstepoort milk laboratory**



**Figure 10: Proportions of resistant and multidrug resistant *S. hyicus* isolated from milk samples submitted to the Onderstepoort milk laboratory**

**Table 11: Antimicrobial drug combinations among multidrug *S. hyicus* isolated from milk samples submitted to the Onderstepoort milk laboratory.**

Drug combinations	Proportions of isolates resistant			
	Frequency	Percent (%)	Confidence interval	
AMP S PB	1	12.5	0.0	35.42
E AMP OX S PB	1	12.5	0.0	35.42
E CIP AMP OX SXT S PB	1	12.5	0.0	35.42
E CIP AMP S PB	1	12.5	0.0	35.42
E CIP AMP SXT S PB	1	12.5	0.0	35.42
E CIP S PB	1	12.5	0.0	35.42
E LZD AMP S RD PB C	1	12.5	0.0	35.42
S PB	1	12.5	0.0	35.42
<b>Total</b>	<b>8</b>	<b>100</b>	<b>100</b>	<b>100</b>

AMP: Ampicillin; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; OX: Oxacillin; PB: Polymyxin B; RD: Rifampicin; S: Streptomycin; SXT: Co-trimoxazole

## CHAPTER 5

### 5.1 DISCUSSION

#### 5.1.1 *Staphylococcus* infection

Coagulase positive *Staphylococcus* (CoPS) species as a cause of *sub-clinical* and clinical mastitis in dairy herds have been widely reported (Blignaut 2013; Bradley et al. 2007; Byarugaba et al. 2008; Giannechini et al. 2002; D. K. Mureithi and Njuguna 2016). In South Africa, information on the burden and antimicrobial drug resistance patterns of CoPS from mastitis clinical cases in dairy cattle is limited (Blignaut 2013; Petzer et al. 2009). Therefore, this study investigated the prevalence and antimicrobial drug resistance patterns of CoPS from mastitic milk samples submitted to the Onderstepoort Milk Laboratory, South Africa.

In this study, *Staphylococcus* species were isolated in 23.2% of milk samples tested. This is higher than the 16.0% *Staphylococcus* spp. infection reported by Bradley et al. (2007) from dairy milk samples in England. Similarly, findings reported here were higher than the 15.7% *Staphylococcus* spp. infection reported among dairy cattle in Australia (Shum et al. 2009). However, a previous study by Blignaut (2013) in South Africa compared to this study reported lower (14.4%) proportion of *Staphylococcus* spp. infection among dairy cattle which were on total mix ration and pasture-based feeding system in the years 2008 and 2013.

Mureithi et al. (2016) in a study conducted in Kenya, have observed a higher (60.9%) *Staphylococcus* spp. infection among dairy cattle with clinical mastitis. Byarugaba et al. (2008) in Uganda, also observed a higher (42.4%) proportion of *Staphylococcus* spp. infection in dairy cattle. A higher proportion (45.0%) of *Staphylococcus* spp. infection was also reported among dairy cattle in Uruguay (Giannechini et al. 2002). The difference in the proportions of *Staphylococcus* spp. isolated in this study compared to other studies could be attributed to differences in herd sizes, management practices, hygiene status and the breed composition (Abinet 2015; Kivaria, Noordhuizen, and Msami 2007; Tafa et al. 2015). This is confirmed by

a study by Kivaria et al. (2007) who indicated that management practices such as poor hygiene results in an increased exposure of mastitis pathogens, such as *Staphylococcus* species.

Byarugaba et al. (2008) has revealed that high treatment costs and lack of veterinary services are the major constraints to the control of mastitis causing pathogens while Kivaria, et al. (2007) indicated that management practices such as poor hygiene results in an increased exposure of mastitic pathogens, such as *Staphylococcus* species. However, in South Africa, the major constraints of mastitis or *Staphylococcus* spp. control have not been studied.

Nonetheless, the results of this study suggest that *Staphylococcus* spp. are a major cause of mastitis among dairy herds in South Africa. Therefore, more studies on the causes and control constraints of mastitis and *Staphylococcus* spp. should be conducted in order to implement effective treatment and control strategies.

### **5.1.2 Coagulase negative and coagulase positive *Staphylococcus***

In the current study, coagulase negative *Staphylococcus* spp. (CoNS) were more common than CoPS. This is not surprising given that a previous study by Petzer, et al. (2009) had reported more CoNS (61.0%) than CoPS (25.1%) among dairy cattle in South Africa. In contrast, a study by Fikru (2014) in Ethiopia reported more CoPS (37.7%) than CoNS (9.1%) from cow milk samples from dairy cattle. The reason why the proportions of CoPS were lower than CoNS in the Ethiopian study is not clear. A study done by Tafa et al. (2015) in Ethiopia, observed breed predisposition to CoPS infection with the odds of infection being higher in cross bred cows than local breeds. In South Africa, it has not been reported whether breed influences herd proportions of *Staphylococcus* spp. infections.

Compared to CoNS, CoPS are known to cause severe morbidity in dairy cattle with clinical mastitis (Weinrauch and Zychlinsky 1999). This is because the two species differ in terms of how they infect and survive in the host. Coagulase positive *Staphylococcus* spp. are often associated with chronic infection while CoNS are often involved in acute outbreaks (Kebede

and Hagos 2015; Weinrauch and Zychlinsky 1999). Therefore, the fact that in this study more CoNS (21.2%; n= 607) compared to CoPS (2.0%; n= 58) (Table 7), were observed is a positive result for the dairy farmers with respect to the *Staphylococcus* spp. observed, because CoNS are less virulent and less prone to antimicrobial resistance than CoPS (Shimels 2014). It is possible that differences in geographical distribution could have played a role in the differences between the rates at which the *Staphylococcus* spp. infections were observed among studies.

Among CoPS species isolated in this study, *S. aureus* was more common than *S. hyicus*. These findings agree with those reported by Capurro (2009) who reported more *S. aureus* (97.0%) compared to *S. intermedius* (2.0%), and *S. hyicus* (1.0%) in a study done in Sweden among dairy milk samples. The current results also agree with those by Mohammed (2015) in Sudan and Roberson et al. (1996) in Virginia, USA. The results of this study support reports that indicate that among CoPS, *S. aureus* is the most common cause mastitis in dairy cattle. Therefore, further studies on the control of CoPS must be conducted as it is known that implementation of effective control measures reduce the incidence of *S. aureus* mastitis in herds (Petersson-Wolfe, Mullarky, and Jones 2010).

### **5.1.3 *Staphylococcus aureus* infection**

*Staphylococcus aureus* infection observed in this study was slightly higher than the 0.9% prevalence reported by Petzer et al. (2013) in cow milk samples from dairy cattle in South Africa. However, proportion of samples positive for *S. aureus* reported in this study was lower than 2.3% and 25.1% reported among dairy cows in South Africa by Fosgate et al. (2013) and Petzer et al. (2009) respectively. Ateba et al. (2010) also reported a higher proportion (100%) of *S. aureus* among dairy farms in North-West Province, South Africa. *Staphylococcus aureus* infection in this study was also lower compared to the results reported in Sweden (21.3%) (Unnerstad et al. 2009), Canada (21.7%) (Riekerink et al. 2008) and China (29.5%) (Yang et al. 2011).

Higher proportions of *S. aureus* infection in dairy herds have also been reported in many African countries compared to what was observed in this study. For example, a study by Issa et al. (2016) in Niger reported that 55.7% of the samples from dairy cattle were positive for *S. aureus*. In Egypt, Abdel-Rady and Sayed (2009) also reported a higher (52.5%) proportion of *S. aureus* infection in dairy cattle with *sub-clinical* mastitis. While, Abera et al. (2012) in a study conducted in Ethiopia reported that 48.6% *S. aureus* milk samples were positive for *S. aureus*.

In Kenya, Mureithi and Njuguna (2016) reported a higher proportion (35.5%) of cow milk samples from animals with *sub-clinical* mastitis were infected with *S. aureus*. Likewise a study by Knight-Jones et al. (2016) also reported a higher proportion (22.1%) of *S. aureus* in milk samples of dairy cattle in Zambia. In Uganda, 21.8% of the milk samples from dairy cattle were positive for *S. aureus* (Kasozi, Tingiira, and Vudriko 2014). Laba and Udonsek (2013) in Nigeria, Iraguha et al. (2015) in Rwanda and Katsande et al. (2013) in Zimbabwe also reported that 13.0%, 6.25% and 3.4% of the milk samples respectively were positive for *S. aureus*.

The difference in the proportions of *S. aureus* in this study compared to the other studies is attributed to geographical difference, sampling methods and study population. For example, Laba and Udonsek (2013) analysed milk samples from milking bowls, while Katsande et al. (2013) used convenience sampling. In addition, the differences may also be due to different management practices, treatment and control strategies (Kebede and Hagos 2015). Nonetheless, the lower *S. aureus* proportions in this study suggests that the prevalence of staphylococcal mastitis in dairy herds in South Africa is lower compared to other African countries. It is therefore important that good farm management practices be maintained and improved in order to achieve even lower prevalence of infection. This is because *S. aureus* is a known major mastitis causing pathogen even in well-managed dairy herds (Smith, Fox, and Middleton 1998).

#### **5.1.4 *Staphylococcus hyicus* infection**

The proportions of *S. hyicus* positive samples observed in this study was lower than the 3.9% reported by Schmidt et al. (2015) among dairy herds in South Africa. Likewise, higher proportions of *S. hyicus* positive samples have been reported in other countries as compared to what was observed in this study. For example, Giannechini et al. (2002) observed 0.6% of milk samples from dairy cattle with sub-clinical mastitis were positive for *S. hyicus* in Southern America. Similarly, Capurro (2009) also reported a high proportion (1.0%) of *S. hyicus* in dairy milk samples from clinical dairy mastitis cases in Sweden. Furthermore, a higher proportion of *S. hyicus* infection (8.2%) was observed in milk samples of dairy cattle in a study by Fikru (2014) in Ethiopia. Studies done in the US have also reported higher *S. hyicus* proportions (17.7%) (Roberson et al. 1996) and 26.0% (Gillespie et al. 2009) in milk samples from dairy cattle. In Egypt, El-Ashker et al. (2015) reported a slightly higher (1.5%) proportion of *S. hyicus* from milk samples of dairy cattle. While in Sudan, Mohammed (2015) reported that 8.0% of the milk samples from dairy cows were positive for *S. hyicus*.

It is possible that the lower prevalence of *S. hyicus* observed in this study may be due to differences in laboratory identification methods. For example, it is common for *S. hyicus* to be classified as a coagulase negative *Staphylococcus* pathogen (Devriese et al. 1978, 2005; Gillespie et al. 2009; Hajek 1976; Schmidt et al. 2015; Taponen et al. 2006). Nonetheless, the results of this study suggest that *S. hyicus* is not a common cause of mastitis infection (Roberson et al. 1996).

#### **5.1.5 Antimicrobial resistance (AMR)**

Antimicrobial resistance among *Staphylococcus* spp. is of clinical and public health concern (Deresinski 2005). In dairy herds, antimicrobial resistance in *Staphylococcus* spp.

leads to decreased productivity and increased culling rates (Kebede and Hagos 2015). However, based on the South African literature obtained from various search engines, research on antimicrobial resistance among *Staphylococcus* spp. from dairy cattle in South Africa is limited (Ateba et al. 2010; Petzer et al. 2007, 2009; Schmidt et al. 2015). Therefore, to close this knowledge gap, this section provides recent antimicrobial resistance status of dairy herds in South Africa.

#### **5.1.5.1 Antimicrobial resistance (AMR) in coagulase positive *Staphylococcus* spp.**

Tafa et al. (2015) in Ethiopia reported a higher proportion of AMR (98.8%) and multi drug resistance (MDR) (87.6%) among and CoPS isolates from dairy cows with *sub-clinical* mastitis as compared to AMR (100%) and MDR (91.4%) observed in this study. The majority of MDR CoPS (91.4%; n=53) isolates in this study were resistant to aminoglycosides (93.1%), macrolides (63.8%) and penicillins (53.4%). This compares to the results from a study by Tafa et al. (2015), who observed a high prevalence of resistance to  $\beta$ -lactams like ampicillin (90.1%) and penicillin (67.9%), and aminoglycosides (streptomycin) (35.8%) among MDR CoPS mastitis cases.

The high prevalence of AMR (100%; n= 58) and MDR (91.4%; n= 53) CoPS in this study is concerning. This is because MDR-CoPS have been linked to high rates of treatment failure and reduced productivity. Therefore, action is needed to reduce antimicrobial resistance among CoPS. This can be done by implementing antimicrobial stewardship, improving farm management practices, and biosecurity in order to reduce incidences of new infections on the farm (Iraguha et al. 2015; Kebede and Hagos 2015; Sañé Schepisi et al. 2013; Yohannis and Molla 2013).

### 5.1.5.2 Antimicrobial resistance among *S. aureus*

Similar to studies in Ethiopia by Mekuria et al. (2013) and Fikru (2014), all (100%) *S. aureus* isolates in this study were resistant to at least one antimicrobial agent. Schmidt (2011) also observed a very high prevalence (91.1%) of resistance against at least one antimicrobial among samples from dairy cows with clinical mastitis in South Africa. Similarly, a Tanzanian researcher (J. Mohammed 2015), also reported high proportion (80.4%) of AMR *S. aureus* from dairy milk samples. The high prevalence of resistance among *S. aureus* proportions observed in this study may be due to inappropriate use among farmers (Levy and Bonnie 2004; Werckenthin et al. 2001). However, further research must be done to investigate the role of farmers in antimicrobial resistance in dairy farms.

#### 5.1.5.2.1 Streptomycin resistance in *S. aureus*

Resistance to streptomycin among *S. aureus* isolates in this study (**Figure 5**) was higher than the 61.5% reported by Ateba et al. (2010) from cow milk samples in North-West Province area. Resistance to streptomycin observed in this study was also higher compared to 8.2% reported by Schmidt et al. (2015) among *S. aureus* isolated from milk samples of cows with mastitis.

Lower proportion of *S. aureus* isolates resistant to streptomycin have also been reported in Ethiopia compared to what was observed in this study. Tigabu, Kassa & Asrat (2015) reported that 8.3% *S. aureus* in bovine milk were resistant to streptomycin while Shimels (2014) reported 19.7%. In Uganda, Kasozi et al. (2014) reported that 42.6% of *S. aureus* isolates from bovine milk samples were resistant to streptomycin. Likewise, in Kenya, fewer (6.0%) *S. aureus* isolates from bovine milk samples were resistant to streptomycin (Gitau et al. 2014). Bamaiyi & Aniesona (2013) in Nigeria also reported lower (3.7%) streptomycin resistant *S. aureus* from milk samples of dairy cows.

The high proportions of *S. aureus* resistance to streptomycin in the current study is of great concern because of the zoonotic potential of the organism. Moreover, streptomycin is common for the treatment of human tuberculosis cases, especially among HIV positive patient (Hospira 2017). Therefore, more research needs to be done to investigate the zoonotic potential associated with streptomycin resistance *S. aureus* to humans.

#### **5.1.5.2.2 Erythromycin resistance in *S. aureus***

Resistance against erythromycin among *S. aureus* isolates reported here (62.0%, n=31) was higher than 24.6% isolated from cow milk samples in North-West Province (Ateba et al. 2010). Several authors have also reported lower prevalence of erythromycin resistance among *S. aureus* in dairy cows in Ethiopia (Beyene 2016; Haftu et al. 2012; Mekuria et al. 2013; Shimels 2014; Tafa et al. 2015; Tigabu et al. 2015). For example, 4.6% (Tigabu et al. 2015), 22.0% (Beyene 2016) and 23.8% (Mekuria et al. 2013) of *S. aureus* isolates from bovine milk samples were reported to be resistant to erythromycin.

In contrast, 69.2% *S. aureus* from cow milk samples in Uganda (Byarugaba et al. 2008) and 58.8% in Ethiopia (Haftu et al. 2012) were resistant erythromycin. The reasons for the lower prevalence of *S. aureus* resistant to erythromycin observed in the present study compared to Uganda and Ethiopia is not clearly explained in literature.

#### **5.1.5.2.3 Ampicillin resistance in *S. aureus***

Compared to the current findings, Schmidt (2011) reported a lower proportion (28.8%) of *S. aureus* isolates resistant to ampicillin from dairy milk samples in KwaZulu Natal. Similarly, Tafa et al. (2015) and Tigabu et al. (2015) also reported lower proportion *S. aureus* resistant to ampicillin from bovine milk samples in Ethiopia, 30.1% and 31.2% respectively. Contrary to the findings of this study, higher proportions of *S. aureus* resistant to ampicillin were observed in Ethiopia by Haftu et al. (2012) and Beyene (2016) from cow milk samples. Previous studies

in South Africa, such as Ateba et al. (2010) and Schmidt et al. (2015) also reported higher proportions of *S. aureus* resistant to ampicillin.

#### **5.1.5.2.4 Methicillin resistance in *S. aureus***

##### **5.1.5.2.4.1 Oxacillin**

Oxacillin test is used as an indicator for methicillin-resistant *S. aureus* (MRSA) (CLSI 2013). Based on the results of the current study (**Figure 5**), the proportion of MRSA was higher than the 1.1% reported by Schmidt (2011) from cow milk samples in South Africa. Similarly, findings reported here were higher than the 29.7% reported by Byarugaba et al. (2008) in Uganda and 33.3% by Mekuria et al. (2013) in Ethiopia from milk samples of cows with *sub-clinical* mastitis.

On the contrary, findings reported here were lower than 80.8% MRSA reported in India from milk samples of dairy cows with clinical mastitis (Chandrasekaran et al. 2014). Unlike what was observed here, Ateba et al. (2010) reported a higher proportion of MRSA (81.2 and 93.2%) in two communal dairy herds in North-West Province.

##### **5.1.5.2.4.2 Cefoxitin**

The *mecA* gene is the gold standard for detecting MRSA (Brown 2001) and cefoxitin is used to test for the presence of *mecA* gene in methicillin resistant *S. aureus* strains (CLSI 2013). *S. aureus* isolates resistant to cefoxitin are regarded as being resistant to  $\beta$ -lactam antimicrobials with the exception of anti-MRSA drugs such as cephalosporins,  $\beta$ -lactamase inhibitors, and carbapenems (Fernandes, Fernandes, and Collignon 2005; Magiorakos et al. 2011).

Although a study by Schmidt et al. (2015) in South Africa did not report any *S. aureus* isolates from dairy cow milk samples exhibiting cefoxitin resistance, the present study

observed that 8.0% of the isolates tested exhibited cefoxitin resistance. In another study by Mohammed (2015) lower proportion (4.4%) of cefoxitin resistance *S. aureus* from cow milk samples in Tanzanian was reported. The high proportion of cefoxitin resistant *S. aureus* in this study compared to that reported by Schmidt et al. (2015) could be due to differences in geographical areas sampled. It is also possible that the differences in antibiotic use patterns among farmers could have also played a role (Zanichelli et al. 2018). In addition, South African dairy farmers are technologically advanced and have access to high quality veterinary services compared to those in countries such as Tanzania (Farmer's Weekly 2018; Misaki, Apiola, and Gaiani 2016).

Higher proportion of cefoxitin resistant *S. aureus* compared to this study have been reported by Abinet (2015) (32.4%), Shimels (2014) (67.2%), and Alemayehu (2015) (75.7%) in cow milk samples in Ethiopia. Joshi et al. (2014) also reported a higher proportion (62.2%) of cefoxitin resistant *S. aureus* from dairy milk samples in Nepal.

The presence of cefoxitin resistance in this study is suggestive of the presence of the *mecA* gene among *S. aureus* which is an indicator of MRSA strains. This is concerning as MRSA strains are difficult to treat patient prognosis is often poor (Seguin et al. 1999). In addition, there are concerns of transmission between infected cows and susceptible humans (Juhász-Kaszanyitzky et al. 2007; There and Wadhai 2013). Therefore, continuous monitoring of MRSA mastitis infection is needed in order to identify carries animals (Oliver, Murinda, and Jayarao 2011; Seguin et al. 1999).

#### **5.1.5.2.5 Vancomycin resistance in *S. aureus* (VRSA)**

Vancomycin is the drug of choice for the treatment of MRSA and MDR *S. aureus* infections (Levy and Bonnie 2004; Myllys et al. 1998). The proportion of VRSA (8.0%) in this study was higher than the 4.7% observed in milk samples in Mafikeng by Ateba et al. (2010). Similarly,

the current findings were higher than 2.2% and 2.4% proportion of VRSA from cow milk samples in Tanzania (J. Mohammed 2015) and Ethiopia (Mekuria et al. 2013) respectively. In contrast, Belayneh et al. (2013), Daka and Yihdego (2012), and Alemayehu (2015) reported higher proportions of VRSA from cow milk samples in Ethiopia, 16.0%, 52.4%, and 38.5% respectively.

Although vancomycin use is prohibited in veterinary medicine in South Africa, the presence of VRSA in the herds studied provides justification for the need to monitor and measure the occurrence of VRSA in dairy herds. Moreover, the animal handlers should be regularly checked for vancomycin resistance as cross contamination between animals and humans is possible (Abinet 2015).

#### **5.1.5.2.6 Multidrug resistance in *S. aureus* isolates**

Multidrug resistant *S. aureus* strains in dairy herds are not uncommon (Ateba et al. 2010; Fikru 2014; Normanno et al. 2007; Stefani and Goglio 2010; Wulf and Voss 2008). It was therefore not surprising that a high proportion of *S. aureus* in this study were MDR and it was higher than 1.4% reported in dairy cows in Kwa-Zulu Natal (Schmidt et al. 2015). It is worth noting that the proportions of MDR-*S. aureus* observed in the present study was lower than the 47.6% reported among milk samples from dairy cows in Ethiopia (Mekuria et al. 2013).

On the other hand, other authors such as Mohammed (J. Mohammed 2015) have reported lower proportion (26.1%) of MDR-*S. aureus* in dairy herds in Tanzania compared to what has been observed in this study. Similarly, Normanno et al. (2007) in Italy reported a lower proportion (22.2%) of MDR-*S. aureus* from many types of samples including cow milk.

The high proportions of MDR *S. aureus* isolates in this study suggests that multidrug resistance among dairy herds in South Africa is more common than previously reported (Ateba

et al. 2010; Schmidt et al. 2015). This is indicative of lack of prudent use of antimicrobials among dairy farmers.

In this study, streptomycin and erythromycin were commonly involved in MDR combinations involving *S. aureus*. This further infers the intense use of these antibiotics in treatment which could have led to the development of MDR *S. aureus* strains among herds (Levy and Bonnie 2004; There and Wadhai 2013). Contrary to the findings in this study, Ateba et al. (2010) in a study done in South Africa observed that ampicillin-sulphamethoxazole-nitrofurantoin were more commonly involved in MDR combinations among *S. aureus* isolates from dairy herd samples. In Ethiopia, Fikru (2014) reported that the most frequent antibiotic involved in MDR combinations among *S. aureus* isolates was penicillin. While in Ethiopia, Mekuria et al. (2013) reported penicillin and tetracycline resistance as the most frequent antimicrobial drugs among MDR *S. aureus* from cow milk samples. In India, Sharma, Sharma, & Malik (2011) reported fluoroquinolones, penicillins, macrolides, aminoglycosides, and glycopeptides as common antimicrobial agents among MDR *S. aureus* isolates from cow milk samples.

### **5.1.5.3 Antimicrobial resistance in *S. hyicus***

*S. hyicus* isolates in this study were commonly resistant to aminoglycosides (92.0%), macrolides (62.0%), penicillins (54.0%), and fluoroquinolones (20.0%). This is contrary to findings by Schmidt et al. (2015), whom instead, observed no resistance among *S. hyicus* isolates from cow milk samples in KwaZulu-Natal. There is a paucity of research on prevalence and antimicrobial susceptibility of *S. hyicus* and other CoPS in dairy cattle in general. In view of this, further research needs to be done on antimicrobial resistance patterns of *S. hyicus* in dairy cattle.

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## CHAPTER 6

### 6.1 CONCLUSION

The observed prevalence of 23.2% CoPS isolated from bovine mastitis cases in South African dairy herds is concerning and is an indication that a lot needs to be done to improve the management and control of mastitis caused by CoPS. *Staphylococcus aureus* is the predominant CoPS species associated with infectious mastitis among dairy herds in South Africa. Since both *S. aureus* and *S. hyicus* show high levels of resistance to streptomycin, erythromycin and ampicillin these drugs may not be effective in the treatment of CoPS mastitis in dairy herds. The presence of MDR and possible methicillin resistance is of public health concern and calls for improved antimicrobial stewardship to limit further development of resistance.

Vancomycin resistance observed among herds in this study means that they can act a source for VRSA in humans. However, the risk is low because of the low prevalence.

### 6.2 RECOMMENDATIONS

In view of the high prevalence of CoPS infection and high resistance patterns observed, there is a need for studies to investigate the factors driving both CoPS infection and antimicrobial resistance among dairy cows. This may include visiting the farms and assessing the hygiene of the milking parlours, handlers and water, and antimicrobial use among the herds. Furthermore, this calls for emphasis on the proper prevention and control strategies of mastitis to be established. In light of the high resistance to streptomycin, erythromycin, and ampicillin, the author recommends that measures including training in prudent use of antimicrobial must be implemented to help reduce high levels of resistance to these drugs. In addition, presence of rifampicin and vancomycin resistance is of great concern and study must be done to monitor changes in resistance overtime. There is also a need for studies on molecular epidemiology of resistance genes among CoPS spp. since phenotypic tests have been known to have high rates

both false negative and false positive results (Vanderhaeghen, Cerpentier, Adriaensen et al., 2010).

This study highlighted the occurrence of VRSA in dairy herds. However, given the limited scope of this study, more studies are needed to assess the occurrence of VRSA in dairy herds in South Africa. In addition, given the limited literature on *S. hyicus*, and yet high levels of resistance were observed in this study, further studies must be done to investigate the prevalence and antimicrobial resistance profile of these organism in South Africa.

### 6.3 REFERENCES

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## APPENDICES

### 6.1 APPENDIX A

The number of cows per farms tested for Coagulase Positive *Staphylococcus* species at the Onderstepoort milk laboratory.

Number of herds	Cows Tested	
	Cow number in each herd	Total cows
2	82	164
3	80	240
2	79	158
5	78	390
1	77	77
2	76	152
2	75	150
2	74	148
2	73	146
4	72	288
4	71	284
4	70	280
1	68	68
1	65	65
1	60	60
1	34	34
2	32	64
1	31	31
1	28	28
1	19	19
1	16	16