

**EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE OF
STAPHYLOCOCCUS SPP. ISOLATED FROM CLINICAL CANINE CASES
PRESENTED AT ONDERSTEPSPOORT VETERINARY HOSPITAL**

**BOITHUTO BA MAFU LE BOEMO BA MANGANGA A THIBELO HO
DITHEHEFATSI KOKWANA-HLOKONG YA *STAPHYLOCOCCUS* SPP. HO
TSWA HO MAFU A DINTJA, E ILENG YA TEKWA SEPETLELENG SA
BONGAKA BA DIPHOOFULO SA ONDERSTEPSPOORT**

by

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Dedication

This thesis is dedicated to my wife Cher and our two beautiful kids; Hope and Doran, for their many sacrifices and support throughout this journey.

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Abstract

Canine *Staphylococcus* spp. have been isolated from several clinical conditions including pyoderma, otitis, wound and post-surgical infections. Studies have shown that transmission from dogs to humans occurs, and infected dogs play an important role in the epidemiology of human infections. Understanding the epidemiology of these infections and their antimicrobial resistance profiles is important in guiding control strategies. Therefore, the objective of this study was to investigate the epidemiology and antimicrobial resistance of *Staphylococcus* spp. isolated from canine clinical cases presented at the Onderstepoort veterinary hospital in South Africa between 2007 and 2012.

Records of 1,497 clinical canine samples submitted to the bacteriology laboratory at the veterinary academic hospital between 2007 and 2012 were included in this study. Crude and factor-specific proportions of *Staphylococcus* spp. infections and antimicrobial resistance and their 95% confidence intervals were estimated. Associations between proportions of *Staphylococcus* spp. and a number of suspected predictors were assessed using Chi-square or Fisher's exact tests as appropriate. Cochran–Armitage trend tests were used to assess temporal trends in the proportion of resistant isolates to each antimicrobial agent. Multinomial logistic models were used to investigate and identify predictors of the polytomous *Staphylococcus* spp. infections variable categorized as *S. pseudintermedius*, *S. aureus*

and *Staphylococcus* negative. However, the predictors of antimicrobial resistance (AMR) and multidrug resistance (MDR) were assessed using ordinary logistic models. In this study, AMR was defined as resistance to at least one class of antimicrobial agent while MDR was defined as resistance to at least three classes of antimicrobial agents. Spatial Empirical Bayesian (SEB) smoothing was used to investigate spatial patterns of *Staphylococcus* spp. infections, AMR and MDR, while spatial scan statistics were used to identify their geographic hotspots.

Twenty-seven percent of the samples were positive for *Staphylococcus* spp. There was a significant ($p=0.0027$) increasing temporal trend in the risk of *S. pseudintermedius*. Dogs ≤ 8 years of age compared to dogs >8 years old were significantly more likely to test positive for *S. aureus* or *S. pseudintermedius*. Ear canal and skin specimens compared to other specimens were more likely to test positive for *S. pseudintermedius* and *S. aureus* than test negative. The overall level of isolates that were AMR and MDR was 80.5% and 28.7% respectively. There were significant increases in the proportions of *S. pseudintermedius* isolates that were resistant to trimethoprim-sulphamethoxazole ($p=0.004$), clindamycin ($p=0.022$) and orbifloxacin ($p=0.042$) during the study period. A significant ($p=0.0052$) increase in the proportion of *S. aureus* isolates resistant to enrofloxacin was also observed. Both AMR and MDR isolates were more common among *S. aureus* (98.2%; 42.9%) than *S. pseudintermedius* (76.98; 25.9%) isolates. At the municipality spatial scale, hot-spots

of AMR were identified in the City of Johannesburg, Emfuleni, Westonaria, Midvaal, Randfontein and Ekurhuleni local municipalities. Significant hot-spots of AMR were also identified at the town spatial scale in several towns including Walkerville and Boksburg.

The observed increasing temporal trend in *Staphylococcus* spp. resistant to sulphonamides, lincosamides and fluoroquinolones is concerning and requires more detailed investigations using primary-base studies. Development of antimicrobial stewardship programs are warranted to address the increasing temporal trend in antimicrobial resistance. For instance, a requirement of an antibiogram before prescription of antimicrobials could be made mandatory to reduce treatment failures and slow down development of antimicrobial resistance. Finally, since *Staphylococcus* spp. infections and AMR tended to cluster in certain geographical areas, future studies need to investigate local socio-economic and environmental factors responsible for these hotspots to guide control efforts.

Ketapele

Mafu a dintja le kokwana-hloko ya *Staphylococcus* spp. haesale di arolwa mafung a mang a dintja ho kenyeletsa mafu a letlalo, a ditsebe, maqeba le ditshwaetso tse bang teng ha ho na le tshebetso e qetang ho etswa ka madimong. Diphuputso di bontsha hore tshwaetso ho tloha ho dintja ho ya ho batho e ya etsahala mme dintja tse nang le tshwaetso di hlaloswa e le tsona tse fetisang tshwaetso ya mafu ho batho. Ho bohlokwa ho utlwisisa tsela eo mafu a tshwaetsanang ka yona le ho tseba hore na manganga a thibelo e ba a jwang hore ho be le meolwane ya tataiso bakeng sa taolo ya mafu ana. Kahona, sepheo sa boithuto bona ke ho batlisisa sebopeho sa mafu le sekgahla seo ho bang le manganga a thibelo dithethefatsing tse itseng kgahlanong le kokwana-hloko ya *Staphylococcus* spp. mabapi le mafu a dintja e tlang ho tekwa mane sepetleleng sa bongaka ba diphoofolo sa Onderstepoort Afrika Borwa dipakeng tsa 2007 le 2012.

Direkote tse 1,497 tsa disampole tsa mafu a dintja di ile tsa fanwa laboratoring e sebetsanang le dibaketheria sepetleleng seo sa bongaka ba diphoofolo dipakeng tsa 2007 le 2012, mme direkote tseo e bile karolo ea boithuto bona. Ditekanyo tsa mantlha bakeng sa tshwaetso ya kokwana-hloko ya *Staphylococcus* spp. le sekgahla seo dikwokwana-hloko di bang le manganga a thibelo ho dithethefatsi kgahlanong le mafu mmoho le makgetlo a etsang dipresente tse 95 di ile tsa hakanywa. Kamano ya ditshiya tsena e hlahlobuwe ho

sebediswa dimetho tsa *Chi-square* le sa *Fisher*. Semetho sa *Cochran–Armitage* se ile tsa sebediswa ho hlahloba sekgahla sa manganga a thibelo ho dithethefatsi tse lwantshang mafu. Mekgwa e meng e fapaneng e sebedisitswe ho fuputsa le ho hlwaya matshwao a tshwaetso ya *polytomous Staphylococcus* spp. tse behwang mokgahlelong wa *S. pseudintermedius*, *S. aureus* le *Staphylococcus negative*. Leha ho le jwalo, matshwao a tshireletso ka sekgahla sa manganga a thibelo ho dithethefatsi tse lwantshang mafu (AMR) di ile tsa hlahlojwa ho sebediswa diteko tse tlwahelehileng. Diphuputsong tsena, AMR e hlaloswa e le sekgahla sa manganga a thibelo ho dithethefatsi tse phekolang mafu bonyane ka mokgahlelo o le mong. *Spatial Empirical Bayesian (SEB)* e sebedisitswe ho hlahloba boleng ba tshwaetso ya *Staphylococcus* spp. mme dipalopalo tsa AMR le MDR di sebedisitswe ho hlwaya moo di fumanehang teng ka bongata. Dipresente tse 27 tsa disampole di bontshitse di na le tshwaetso ya kokwana-hloko ya *Staphylococcus* spp. Ho bile le keketseho ya ho ba tlokotsing ya ho fumana tshwaetswo ya *S. pseudintermedius*. Dintja tse dilemo di ka tlaase ho 8 ha di bapiswa le tse ka hodimo ho tse dilemo di 8 di tlokotsing ya ho fumanwa di na le tshwaetso ena ya *S. aureus* kapa *S. pseudintermedius* ha di hlahlojwa. Bokahare ba tsebe le disampole tsa letlalo ha di bapiswa le disampole tse ding di monyetleng wa ho fumanwa di na le lefu lena la *S. aureus* kapa *S. pseudintermedius* ha di hlahlojwa. Kakaresto ea boemo ba AMR le MDR ke dipresente tse 80 le 28.7 ka karohano bobedi ba tsona. Ho bile le ho eketseha hwa

boleng ba *S. pseudintermedius* bakeng sa ho ba le manganga a thibelo ho dithethefatsi tsa *trimethoprim-sulphamethoxazole* ($p=0.004$), *clindamycin* ($p=0.022$) le *orbifloxacin* ($p=0.042$) nakong ya boithuto bona. Ho bile hape le ho eketseha ho hoholo hwa boleng ba sekgahla sa manganga a thibelo ho *S. aureus* mabapi le *enrofloxacin* ho ile hwa hlahella le hona. Bobedi disampole tsa AMR le MDR di ne di tshwana ho *S. aureus* (98.2%; 42.9%) ho feta disampole tsa *S. pseudintermedius* (76.98; 25.9%). Ka sekala sa Mmasepala, dibaka tseo AMR e leng teng di ile tsa hlwauwa toropong ya Johannesburg le bo-Mmasepaleng ba haufi ba Emfuleni, Westonaria, Midvaal, Randfontein le Ekurhuleni. Dibaka tse ding moo AMR e leng teng di ile tsa hlwauwa ka sekala sa toropo ho kenyeletsa ditoropo tsa Walkerville le Boksburg.

Temoho ya ho phahama hwa sekgahla sa manganga a thibelo ho *Staphylococcus* spp. kgahlanong le *sulphonamides*, *lincosamides* le *fluoroquinolones* e ya ngongorehisa mme e hloka diphuputso tse keneletseng ho ipapisitswe le dipatlisiso tsa mathomo. Ho hlahisa manane a manganga a thibelo ho dikokwanahloko ho a hlokahala. Mohlala, ho hlokahala sethethefatsi sa *antibiogram* pele ho ka fanwa ka meriana, mme sena se ka etswa e le ho fokotsa ho hloleha dintlheng tsa phekolo mmoho le ho thefula ho tswelapele hwa manganga a thibelo ho meriana e lwantshang dikokwana-hloko. Ha re phethela, kaha ditshwaetso tsa *Staphylococcus* spp. le AMR di atisa ho fumaneha nq'a e le nngoe, kamoso diphuputso di tla lokela ho

tsepamiswa le ho dintlha tsa bophelo tse shebaneng le moruo le dintlha tsa tikoloho
tse nang le seabo dibakeng tsena hore taolo e tle e be bobebe.

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List of abbreviations

RNA: Ribonucleic acid

rRNA: Ribosomal ribonucleic acid

CoPS: Coagulase Positive *Staphylococcus* species

CoNS: Coagulase Negative *Staphylococcus* species

UK: United Kingdom

PFGE: Pulsed field gel electrophoresis

AFLP: Amplified fragment length polymorphism

MLST: Multilocus sequence typing

tRNA: Transfer ribonucleic acid

DNase: Deoxyribonuclease

MRSA: Methicillin Resistant *Staphylococcus* species

MSSA: Methicillin Sensitive *Staphylococcus* species

PBP2: Penicillin-Binding Protein 2

HA-MRSA: Health care-associated Methicillin Resistant *Staphylococcus aureus*

CA-MRSA: Community acquired Methicillin Resistant *Staphylococcus aureus*

SCCmec: Staphylococcal cassette chromosome mec

LA-MRSA: Livestock-associated Methicillin-Resistant *Staphylococcus aureus*

MDR: Multi-drug resistance

VISA: Vancomycin intermediate resistant *S. aureus*

VRSA: Vancomycin-resistant *S. aureus*

SEB: Spatial empirical Bayesian

MEET: Tango's Maximized Excess Events Test

US: United States

RRR: Relative Risk Ratio

AKC: American Kennel Club

CI: Confidence interval

OR: Odds Ratio

SAS: Statistical Analysis System

CAT: Cochran-Armitage Trend

AMR: Antimicrobial resistance

UTI: Urinary tract infection

AVH: Academic veterinary hospital

RR: Relative Risk

GIS: Geographic information system

CHAPTER 1

1.1 Introduction

Staphylococcus spp. are known to colonize the nasal mucosa of animals and human beings. However, they are also known to be pathogenic to numerous species of animals including humans (Abulreesh, 2011; Adegoke & Okoh, 2014). *Staphylococcus* spp. are often associated with community and hospital acquired infections affecting the skin, soft tissue, surgical sights, bones and joints in humans. In some cases, *Staphylococcus* spp. infections have been associated with life-threatening post-surgical toxic shock syndrome with high rates of morbidity and mortality (Shittu & Lin, 2006; Marais et al., 2009). In dogs, *Staphylococcus* spp. have been isolated in clinical conditions including pyoderma, otitis, and post-surgical infections (Frank et al., 2003; May et al., 2005; Kramer, Schwebke & Kampf, 2006; Cohn & Middleton, 2010; Ishihara et al., 2010; Kawakami et al., 2010; Weese & van Duijkeren, 2010). The majority of studies on the prevalence of staphylococci in dogs show that skin remains the main body site associated with *Staphylococcus* infections Blunt et al (2013).

A number of studies have demonstrated that transmission of *Staphylococcus* species from dogs to humans do occur. Therefore, exposure to carrier or infected dogs is a risk factor for staphylococcal infections in humans (Bagcigil et al., 2007; Boost, O'Donoghue & Siu, 2007; Faires, Tater & Weese, 2009; Frank et al., 2009;

Pantosti, 2012). The zoonotic potential of *Staphylococcus* spp. is of great concern in developing countries where there is a high burden of communicable diseases and large proportions of immunocompromised individuals. Due to this risk, contact between infected animals and humans should be restricted, where possible, in order to mitigate the risk of infection and its associated public health concerns (Faires, Tater & Weese, 2009).

The presence of multi-drug resistant *Staphylococcus* spp. and methicillin resistant *Staphylococcus aureus* (MRSA) often worsens the prognosis of affected human and animal patients. Unfortunately, resistance profiles of *Staphylococcus* spp. from clinical cases of dogs in South Africa have not been investigated and yet this information is important for patient prognosis. The only published study in South Africa on antimicrobial susceptibility profiles of *Staphylococcus* spp. in dogs was done by Blunt et al (2013). In that study, Blunt and co-workers reported increased resistance to ampicillin, tetracycline and potentiated sulphonamides relative to other antimicrobials. Other studies elsewhere have reported an increased prevalence of resistance to enrofloxacin, gentamycin, erythromycin and clindamycin compared to other antimicrobials (Prescott et al., 2002; Hauschild & Wójcik, 2007). There is a consensus among researchers that indiscriminate use of antimicrobial agents is among the main drivers of resistance in veterinary medicine.

In veterinary epidemiology, spatial analytic tools have been used to investigate disease patterns (Carpenter, 2001; Pfeiffer et al., 2008). Identified spatial clusters or hotspots that can be used to identify populations at high risk and

potential risk factors that may be linked to the disease of interest in a particular region. This could, in turn, be used to inform the implementation of appropriate control strategies. Such methods need to be used to investigate the epidemiology of *Staphylococcus* in South Africa. Results of such studies may be used to identify communities that are at high risk of staphylococcal infections and also guide future investigations as well as infection control programs. The use of spatial scan statistics which has been implemented successfully in a number of epidemiological studies to detect and evaluate disease clusters offers opportunities to conduct such studies in South African (Kulldorff et al., 1998; Kulldorf, 1999; Pfeiffer et al., 2008).

With the above issues in mind, the objectives of this thesis were to:

- (1) investigate the burden and predictors of *Staphylococcus* spp. infections among dogs presented at an academic veterinary hospital in South Africa (2007-2012).
- (2) investigate patterns and predictors of antimicrobial resistance among *Staphylococcus* spp. from canine clinical cases presented at a veterinary academic hospital in South Africa.
- (3) describe geographic disparities of staphylococcal infections and antimicrobial resistance among selected *Staphylococcus* spp. from dogs presented at a veterinary academic hospital, South Africa.

This thesis is divided into five chapters. Chapter one is the literature review, chapters two to four are based on the three objectives outlined above, while chapter five is a general summary of the thesis, recommendations, and conclusions.

1.2 Literature Review

1.2.1 Staphylococcal organisms and infections

1.2.1.1 Aetiology, pathogenesis and clinical signs

More than sixty species and subspecies of the genus *Staphylococcus* have been described globally in humans and animals. Most *Staphylococcus* species are commensal and pose minimum to no risk to humans and animals (Burton et al., 2008; Chanchaithong et al., 2014). However, these organisms have the potential to cause clinical conditions in both humans and animals including dogs. *Staphylococcus* species are divided into coagulase positive *Staphylococcus* (CoPS) and coagulase negative *Staphylococcus* (CoNS). Of the two, the CoPS are the predominant group causing several clinical conditions in dogs (Cohn, Middleton 2010). Although infections associated with CoNS are rare in dogs, new evidence shows an increasing trend of CoNS nosocomial infections in other animals (Petzer et al., 2009; Abulreesh, 2011).

Staphylococcus species have a host preference, for example, human *Staphylococcus* clinical cases are often associated with *S. aureus*, while dog clinical cases are mainly due to *S. pseudintermedius* infections. Therefore, *S. pseudintermedius* and *S. aureus* remain the most prevalent *Staphylococcus* species compared to *S. schleiferi*, *S. epidermidis*, *S. xylosus* and *S. felis* (Boost, O'Donoghue & Siu, 2007; Hanselman et al., 2009; Chanchaithong et al., 2014). Infected and carrier dogs, as well

as contaminated environmental surfaces, remain potential sources of *Staphylococcus* infections to susceptible dogs.

The skin serves as a protective layer for *Staphylococcus* infections and a breach may result in dissemination of the infection to other organs. Hence, clinical signs associated with *Staphylococcus* infections often involve the skin and soft tissue around it. The dissemination of infection results in the formation of an abscess in the affected organ. Abscess formation is a host mechanism to contain and eliminate the pathogen and often resolves spontaneously (Kobayashi, Malachowa & Deleo, 2015).

Clinical conditions associated with *Staphylococcus* infections in dogs include pyoderma, otitis externa, wound infection, sepsis, nasal infection, pneumonia, nephritis, and post-surgical infections (Frank et al., 2003; May et al., 2005; Kramer, Schwebke & Kampf, 2006; Cohn & Middleton, 2010; Ishihara et al., 2010; Kawakami et al., 2010; Weese & van Duijkeren, 2010). However, pyoderma seems to be the most reported clinical condition in dogs (Hoekstra & Paulton, 2002; Kawakami et al., 2010; Gandolfi-Decristophoris et al., 2013; Schmidt et al., 2014). The severity of the clinical signs is often linked to the presence and the expression of the virulence genes. These genes enable the *Staphylococcus* organism to bind to the cell wall, form a biofilm, produce toxins and circumvent the immune system (Morris et al., 2017).

1.2.2 Diagnosis and treatment

1.2.2.1 Diagnosis

The diagnosis of *Staphylococcus* infection in dogs is based on initial clinical signs and the presence of characteristic lesions (Hillier et al., 2014). In addition, history of other underlying endocrine diseases may also be relevant. The most common lesions include skin pustules, hair folliculitis, hypopigmentation or hyperpigmentation of the skin (Hillier et al., 2014). Cytology of the site of the lesion can also be performed to identify cocci bacteria and assess co-infection. Additional tests that can be done include skin scrapings, Wood's lamp test, microscopic hair examination and histopathology to rule in and out *Staphylococcus* infections (Hillier et al., 2014).

Bacterial culture is recommended for suspected *Staphylococcus* infections especially where there is a suspicion of antimicrobial resistance or there is no evidence of patient recovery (Hillier et al., 2014). On culture, *Staphylococcus* species are identified on the basis of colony morphology and standard phenotypic tests. Colonies are medium sized, raised and unpigmented, and display large incomplete β - and small complete δ -haemolysis.

Differentiation between CoNS and CoPS is based on the coagulase test (Bannoehr & Guardabassi, 2012). The test uses the ability of *Staphylococcus* organism to convert fibrinogen to fibrin due to the presence of the coagulase enzyme (Cox et al., 1985; Cohn & Middleton, 2010). In situations where the organism exhibits both

coagulase positive and coagulase negative properties, further biochemical tests or molecular techniques are recommended (von Eiff, Peters & Heilmann, 2002). The CoNS species are further differentiated based on their susceptibility to novobiocin as novobiocin-resistant, and novobiocin-susceptible. Novobiocin-resistant species include *S. cohnii*, *S. saprophyticus*, *S. sciuri* and *S. xyloso*, while, novobiocin-susceptible species include *S. auricularis*, *S. capitis*, *S. caprae*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. pasteurii*, *S. saccharolyticus*, *S. schleiferi*, *S. simulans* and *S. warneri* (von Eiff, Peters & Heilmann, 2002).

Two methods of identifying *Staphylococcus* species are described in literature, namely, biochemical and molecular. The biochemical and molecular methods follow traditional culturing followed by identification based on colony characterization, biochemical tests including catalase, polymixin-B, D-mannitol, deoxyribonuclease (DNase) tests, and Gram-staining (Quinn et al., 1994; Cohn & Middleton, 2010). If a biochemical method is used, *S. pseudintermedius*, *S. intermedius*, and *S. delphini* are classified as *Staphylococcus intermedius* group (SIG) as the test is unable to distinguish these species from each other. Therefore, it is suggested that for correct species identification of the SIG, molecular methods are necessary (Bannoehr & Guardabassi, 2012).

Molecular techniques including pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST), and matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) have been used for *Staphylococcus* species

identification (Loeffler et al., 2007; Bannoehr et al., 2009; Black et al., 2009; S et al., 2010; Sasaki et al., 2010; Decristophoris et al., 2011; Chanchaithong et al., 2014). Amplified fragment length polymorphism (AFLP) and multilocus sequence typing (MLST) identify *Staphylococcus* species based on the 16S ribosomal ribonucleic acid (rRNA) gene with new studies showing the benefit of additional genes such as *rpoB* (β -subunit of RNA polymerase), *tu* (elongation factor Tu), and *dnaJ* (heat shock protein 40) in the characterization of *Staphylococcus* species (Lamers et al., 2012). The following clusters of *Staphylococcus* species and their subgroups have been described based on the 16s rRNA sequences: *S. auricularis* (*S. auricularis*); *S. aureus* (*S. simiae*, *S. aureus*); *S. epidermidis* (*S. capitis*, *S. saccharolyticus* *S. caprae*, *S. epidermidis*); *S. hyicus-intermedius* (*S. felis*, *S. schleiferi* *S. hyicus*, *S. pseudintermedius*, *S. intermedius*, *S. lutrae*, *S. muscae* *S. microti*, *S. rostri*, *S. delphini*, *S. chromogenes*); *S. warneri* (*S. pasteurii*, *S. warneri*); *S. lugdunensis* (*S. lugdunensis*); *S. simulans* (*S. simulans*); *S. sciuri* (*S. stepanovicii*, *S. fleurettii*, *S. lentus*, *S. vitulinus* *S. sciuri*); *S. saprophyticus* (*S. leei*, *S. cohnii*, *S. equorum*, *S. gallinarum*, *S. arlettae* *S. kloosii*, *S. xylosus* *S. nepalensis*, *S. saprophyticus*, *S. succinus*); *S. carnosus* (*S. simulans* *S. massiliensis* *S. piscifermentans*, *S. carnosus*, *S. condiment*) and *S. haemolyticus* (*S. hominis* *S. haemolyticus*, *S. devriesei*) (Takahashi, Satoh & Kikuchi, 1999; Lamers et al., 2012).

1.2.2.2 Treatment

Staphylococcus infections in dogs often resolve spontaneously, however, persistent infections may require medical intervention. The choice of treatment may

be influenced by the site of infection and the severity of the presenting clinical signs. For example, a patient presenting with light skin infections may only require topical antimicrobial cream, while systemic antimicrobial treatments may be required in patients with deep pyoderma. The commonly used topical cream contains chlorhexidine or benzoyl peroxide as an antimicrobial agent. While broad spectrum antimicrobial agents are used in deep pyoderma cases (Hillier et al., 2014).

Lilenbaum et al. (2000) observed that *Staphylococcus* infections in the dogs with otitis externa were highly susceptible to gentamicin and recommended the use of gentamicin for the treatment of staphylococcal infections in dogs. In a case of a young male dog with urinary tract infection due to methicillin-resistant *Staphylococcus pseudintermedius*, the use of doxycycline resulted in the resolution of clinical signs (Rubin & Gaunt, 2011). Dogs with *Staphylococcus* spp. septic arthritis at the Murdoch University were treated effectively with amoxicillin-clavulanate and cephalexin and the majority of them recovered (Marchevsky & Read, 1999).

As indicated above, broad spectrum antimicrobial agents are the treatment of choice for *Staphylococcus* infections in dogs (Hillier et al. 2014) and antimicrobial agents such as cefovecin, amoxicillin-clavulanate, clindamycin, trimethoprim sulphamethoxazole have been reported to have fair to good efficacy (Pellerin et al., 1998; Hauschild & Wójcik, 2007; Hillier et al., 2014). However, some antimicrobial agents are not effective against *Staphylococcus* species due to acquired or inherent resistance. Hence, veterinarians need to be made aware of the importance of requesting for an antibiogram before making treatment decisions (Hiller et al. 2014).

1.2.3 Epidemiology of *Staphylococcus* infections

1.2.3.1 Distribution of *Staphylococcus* species

Staphylococcus species have been isolated in various proportions in several studies in dogs. For example, Wedley et al. (2014) reported a 55.1% prevalence of *Staphylococcus* and 19% prevalence of CoNS among dogs visiting veterinary clinics in the mainland UK. In the same country, Schmidt and co-workers (2014) identified *Staphylococcus* spp. (99%) and CoNS (95%) among healthy Labrador retrievers. *Staphylococcus* species (68%), CoNS (61%) and CoPS (39%) have also been isolated from dogs with otitis externa in Brazil (Lilenbaum et al., 2000). In another study by Gandolfi-Decristophoris et al. (2013), CoNS (60%) were identified among healthy dogs in Switzerland. Of the two *Staphylococcus* species, CoPS compared to CoNS are more commonly associated with clinical conditions in dogs (Cohn & Middleton, 2010; Schmidt et al., 2014; Wedley et al., 2014).

Although *S. pseudintermedius* and *S. aureus* are frequently commensal, they can cause clinical conditions in dogs (Boost, O'Donoghue & Siu, 2007; Hanselman et al., 2009; Chanchaithong et al., 2014). A UK study by Schmidt et al. (2014) reported a 44% prevalence of *S. pseudintermedius* compared to 8% for *S. aureus* in healthy Labrador retrievers (Boost, O'Donoghue & Siu, 2007). Hanselman et al. (2009) in Ontario, Canada also reported more *S. pseudintermedius* (46%) than *S. aureus* (14%) among healthy dogs in their study. Hoekstra and Paulton (2002) in British Columbia, Canada also identified more *S. intermedius* (60.7%) isolates than *S. aureus* (39.3%)

from clinical cases of dogs. These studies are in agreement with studies that indicate that *S. pseudintermedius* is the common species isolated from canine clinical conditions (Wedley et al. 2014, Griffeth et al. 2008, Lilenbaum et al. 2000). *Staphylococcus pseudintermedius* has been reported in high proportions in dog clinical cases in Japan (89.5%) (Kawakami et al., 2010) as well as mong health animals in Switzerland (87%) (Gandolfi-Decristophoris et al., 2013) and Denmark (69%) (Paul et al., 2012). The high frequency of *S. pseudintermedius* in dogs has been attributed to its ability to successfully adhere to the corneocytes of canines with atopic dermatitis (Kawakami et al., 2010; Gandolfi-Decristophoris et al., 2013; Schmidt et al., 2014).

Grundmann et al. (2010) observed that infections associated with *Staphylococcus* organisms show variations in geographical distribution. For example, they identified clustering of *Staphylococcus* infections in The Czech Republic, Germany, Bulgaria and Romania as well as another cluster involving the UK, Ireland, Italy and Croatia based on the spa type. Globally, cases and sporadic outbreaks of methicillin resistant *S. pseudintermedius* (MRSP) shows clonal MRSP lineages. Moreover, Oliveira et al. (2002) identified five major clonal lineages among MRSA isolates, namely Iberian, Brazilian, Hungarian, New York/Japan, and paediatric pandemic MRSA clones. A study by Cain (2013) showed differences in MRSP clonal lineage between North America and Europe.

In Zambia, *S. aureus* (10.9%) and *S. pseudintermedius* (47.5%) have been reported from dogs presented at the veterinary teaching hospital (Youn et al., 2014). Schaumburg and co-workers (Schaumburg et al., in press) reported *S. aureus* from

three dogs in Côte d'Ivoire, while MRSA isolates were recovered from two dogs in Egypt (Abdel-moein, El-Hariri & Samir, 2012). Katakweba (2014) in Tanzania also reported *S. aureus* (11%) and *S. pseudintermedius* (4%) among healthy dogs. In South Africa, the only study that has reported *Staphylococcus* spp. infections in dogs is the one done by Blunt and co-workers in 2014. In that study, they observed *S. intermedius* isolates as the most common cause of canine pyoderma (Blunt, van Vuuren & Picard, 2013).

1.2.3.2 Risk factors for infection with *Staphylococcus* species

Several risk factors of *Staphylococcus* infections in dogs including sex, age, and site of the body have been investigated (Cohn & Middleton, 2010). Regarding body site, a study by Griffeth et al. (2008) in the USA, reported that *Staphylococcus* species were isolated in high numbers in the nose and the mouth of healthy dogs. Similarly, Wedley and co-workers isolated *Staphylococcus* organisms commonly from the nasal mucosal surfaces of dogs visiting veterinary clinics in the UK (Wedley et al., 2014). Staphylococci were also found to be more common on the nasal mucosae (16%) than the perineum (4%) in a small number of dogs in the UK. In the same study, the authors observed species differences in the site of infections with *S. epidermidis* being common (52%) in the nasal cavity, while *S. pseudintermedius* was equally distributed in the nasal mucosae and the perineum (Schmidt et al., 2014).

The risk of *Staphylococcus* infections is higher in dermatological conditions compared to other body sites (Hoekstra & Paulton, 2002; Kawakami et al., 2010;

Schmidt et al., 2014). For example, Kawakami et al. (2010) in Japan, identified *S. pseudintermedius* as a common (89.5%) cause of clinical pyoderma in dogs. While a higher risk of *Staphylococcus* species infection was reported in dogs presenting with otitis externa at a veterinary hospital in Brazil (Lilenbaum et al., 2000). Gandolfi-Decristophoris and co-workers also isolated high numbers of *Staphylococcus* species from the nostril (176) and the ear (108) of dogs in Switzerland (Gandolfi-Decristophoris et al., 2013).

Boost et al. (2008) observed a significantly higher occurrence of *S. aureus* infection in female dogs than male dogs, as well as younger dogs than older ones. In the same study, the odds of a *Staphylococcus* species infection was significantly higher in dogs from households with ≤ 3 people than >3 people and dogs of healthcare workers compared to other workers. The presence of a penetrating wound, a compromised immune system, underlying clinical conditions, and long stay at veterinary hospitals are known to increase the risk of *Staphylococcus* infections (Cohn & Middleton, 2010; Kawakami et al., 2010).

1.2.4 Antimicrobial resistance among staphylococci

1.2.4.1 Background

The ability of the bacterium to develop resistance is a biologic response for survival, and can be intrinsic or acquired. Intrinsic resistance has been observed in bacteria with inherent resistance to a certain antimicrobial agent. While acquired resistance is often due to genetic mutation and selection pressure. An example of

acquired resistance is that relating to the emergence of first generation penicillin resistant staphylococcal organism due to selection pressures associated with overuse of penicillin drugs. The other mechanism by which an organism can acquire resistance is by a genetic transfer of information that encodes resistance from a resistant organism to a susceptible organism (Tenover, 2006). The presence and ability of the *Staphylococcus* organism to acquire penicillin-binding protein (PBP) encoded by the *mecA* gene confers both intrinsic and acquired resistance to all beta-lactam antibiotics.

Of concern is the overuse of antimicrobial agents which has been linked to increased prevalence of resistance among pathogenic and non-pathogenic bacteria (Mathew, Cissell & Liamthong, 2007). Moreover, Werckenthin and co-workers suggest that the prevalence of antimicrobial drug resistance in staphylococcal isolates from dogs, cattle and pigs is on the rise (Werckenthin et al., 2001). Hauschild and Wójcik, as well as Pellerin and co-workers suggest that this increase in dogs might be attributed to the rapid introduction and the over prescription of new antimicrobial drugs in companion animals (Pellerin et al., 1998; Hauschild & Wójcik, 2007).

Resistant *Staphylococcus* organisms are often difficult to treat and result in increased morbidity, mortality, and financial burdens to the owners (Kim et al., 2006). Of greater concern is the presence of multiple drug resistant *Staphylococcus* organisms where the prognosis for patients is worse. In the event that an isolate is multidrug resistant (MDR), veterinary clinicians have fewer to no effective

antimicrobial agents to treat such infections. Hence, providing the clinician with the most likely antibiotics to work based on the susceptibility breakpoints helps improve clinical outcomes (Turnidge & Paterson, 2007).

Breakpoints are used to define susceptibility and resistance among *Staphylococcus* organism. A third breakpoint category, intermediate, was added between the resistant and susceptible to prevent error in interpretation (Turnidge & Paterson, 2007). Minimum inhibitory concentration (MIC) levels can also be reported where clinicians have knowledge of the pharmacodynamics (PD) in order to optimize treatment and patient prognosis.

A bacterium is said to be susceptible if its growth is inhibited by an antibacterial agent concentration in the range found in wild-type strains. While a bacterium is said to be resistant if its growth is inhibited by a concentration higher than the range seen for wild-type strains (Turnidge & Paterson, 2007). Magiorakos et al. (2012) define multidrug resistance (MDR) as non-susceptibility to at least one agent in three or more antimicrobial categories. Extensive drug resistance (XDR) is defined as resistance to all antimicrobial categories with the exception of antimicrobial agents from one or two antimicrobial categories. While pan-drug resistance (PDR) is non-susceptibility of an isolate to all agents in all antimicrobial categories.

Staphylococcus infections were initially treated with penicillin. However, overwhelming resistance to penicillin and other β -lactam antimicrobials has been

reported (Lim & Strynadka, 2002; Ball et al., 2008; Rubin & Gaunt, 2011; Rice, 2012). Authors have attributed this to the intrinsic resistance of *Staphylococcus* spp. and the ability of these organisms to acquire resistance by spontaneous mutation or acquisition of resistance genes from other organisms. Rapid introduction and the over prescription of new antimicrobial drugs have also been cited as the factors attributing to the increased prevalence of antimicrobial resistance among *Staphylococcus* organisms (Pellerin et al., 1998; Hauschild & Wójcik, 2007).

1.2.4.2 Distribution of antimicrobial resistance

Prevalence of resistance to antimicrobial agents among *Staphylococcus* species differs by antimicrobial agent, *Staphylococcus* species identified and region. Hauschild and Wójcik (2007) in Poland reported that 41% and 35% of *S. intermedius* isolates from dogs with dermatitis were resistant to erythromycin and clindamycin, respectively. Gandolfi-Decristophoris et al. (2013) reported 49% and 46% resistance to penicillin and ampicillin among *S. aureus* isolates from healthy dogs at various nursing homes in Switzerland. A study on antimicrobial resistance of staphylococci isolated from healthy dogs in the UK reported a 58% prevalence of resistance to oxacillin (Schmidt et al., 2014). Kawakami et al. (2010) in Japan, observed that *S. pseudintermedius* isolates from dogs with pyoderma had a high prevalence of resistance to cefalexin (58%), ofloxacin (62.9%), norfloxacin (61%), clindamycin (71%) and lincomycin (93%).

Temporal changes in antimicrobial resistance among *Staphylococcus* isolates have been reported. For example, Prescott et al. (2002) observed a significant decline in the proportion of *S. aureus* and *S. intermedius* isolates from clinical dog cases resistant to penicillin-G and ampicillin between 1984 and 1998. In the same study, a significant increase in the prevalence of resistance to enrofloxacin and gentamycin among *S. intermedius* isolates was observed. In the UK, between 1980 and 1996, Lloyd and co-workers reported an increased prevalence of resistance to penicillin from 69% to 89% among *Staphylococcus* species from canine clinical cases (Lloyd, Lamport & Feeney, 1996). In France, Pellerin and co-workers reported increased proportion of multidrug resistance in *S. intermedius* among canine pyoderma cases from 11% to 28% between 1986 and 1996 (Pellerin et al., 1998). Similarly, a study done in Switzerland observed an increase in the proportion of *S. intermedius* resistance to penicillin, neomycin, sulphonamides, co-trimoxazole and erythromycin isolated from clinical dog cases from May 1999 to February 2000 (Wissing, Nicolet & Boerlin, 2001). Detwiler and co-workers also reported a significant increase in overall resistance among *S. pseudintermedius* isolates from canine patients at a veterinary teaching hospital between 2006 and 2009 (Detwiler et al., 2013).

In a study done in Zambia, penicillin resistance was observed in 52.1% of *S. aureus*, while 10.4% *S. pseudintermedius* isolates were found to be multidrug resistant (Youn et al., 2014). In Côte d'Ivoire, *S. aureus* showed high levels of resistance to penicillin (90%) and tetracycline (71%), while those from Democratic Republic of Congo showed high levels of resistance to penicillin (68%). However, resistance to

penicillin among *S. aureus* from Gabon was uncommon (Schaumburg et al., in press). In Tanzania, all *S. pseudintermedius* from dogs were found to be resistant to ampicillin and penicillin (Katakweba & Selemani, 2014). A study done by Blunt et al. (2013) in South Africa reported high proportions of resistance to penicillin (66%), ampicillin (66%), and doxycycline (49%) among *S. intermedius* isolates from dogs presented with pyoderma.

1.2.4.3 Methicillin Resistant *Staphylococcus* species

Methicillin resistant *S. aureus* in the USA account for 94,000 hospital acquired human infections and 18,650 deaths per year (Klevens et al., 2014). In veterinary medicine, MRSA infections have been reported in cattle, poultry, horses, dogs, cats and exotic animals (Stastkova, Karpiskova & Karpiskova, 2009). The majority of *Staphylococcus* infection studies in veterinary medicine mention MRSA. These studies show that veterinary cases of MRSA infections have significantly increased in the last decade (Diekema et al., 2001).

Historically, MRSA infections were considered to be nosocomial. However, reports of MRSA infections in humans with no record of exposure to health care settings have emerged. As a result, MRSA was re-classified as healthcare-associated (HA-MRSA) and community acquired (CA-MRSA) (File, 2008). In addition to the origin of the patient, antimicrobial resistance patterns and molecular profile of the isolates are also used in the differentiation (Cohn & Middleton, 2010). A new category of MRSA called livestock-associated methicillin-resistant *Staphylococcus*

aureus (LA-MRSA) has also emerged in the last decade, the first reported human case was in the Netherlands (Voss et al., 2005).

Loeffler and co-workers (2011) reported a 2.1% prevalence of MRSA among dogs in the Greater London area. In another study by Hanselman et al. (2008), methicillin-resistant *S. pseudintermedius* (MRSP) was isolated from 2.1% of dogs entering a veterinary teaching hospital. Gronthal et al. (2015) reported 3% prevalence of MRSP in guide dogs in Finland. Methicillin-resistant *S. intermedius* have also been reported in three dogs in Slovenia (Vengust et al., 2006).

The presence of methicillin resistance has been attributed to the ability of *Staphylococcus* isolates to acquire the *mecA* gene encoding for the protein PBP2a needed to build the cell wall. The PBP2a has very low affinity for β -lactam antibiotics and confers resistance to methicillin and the other beta-lactams except for the newest generation of cephalosporins (Pantosti, Sanchini & Monaco, 2007). Methicillin-resistant *Staphylococcus aureus* isolates are largely multidrug resistant and present a clinical challenge as commonly used antimicrobial agents including aminoglycosides, fluoroquinolones, and lincosamides are not effective.

1.2.4.4 Vancomycin resistant *Staphylococcus* species

Vancomycin is currently the treatment of choice for serious infections caused by MRSA (Hiramatsu, 2001; Micek, 2007; Deresinski, 2009). It has a completely different mechanism of action compared to beta-lactam antibiotics. Thus, there has been an increase in the use of vancomycin for treatment of MRSA infections.

However, recently two types of vancomycin resistance have emerged in these bacteria, namely, vancomycin intermediate resistant *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) (Hiramatsu, 2001). Fortunately, vancomycin resistance in veterinary medicine is not common. Perreten et al. (2010) reported no vancomycin resistance among *S. pseudintermedius* in Europe and North America. Similarly, Kwon et al. (2006) found no vancomycin resistant isolates from hospitalized dogs investigated. In the UK, Baptiste and co-workers also reported no vancomycin resistance among methicillin-resistant staphylococci isolated from companion animals presented at a veterinary hospital (Baptiste et al., 2005)

1.2.4.5 Multidrug resistant *Staphylococcus* species

Studies show an association between methicillin resistance and multi-drug resistance (Kim et al., 2006; Cohn & Middleton, 2010). The increased risk of multi-drug resistance among methicillin resistant isolates has been attributed to a staphylococcal cassette chromosome mec (SCCmec) that allows for the incorporation of antimicrobial resistance markers to drug groups such as macrolides and fluoroquinolones (Cohn, Middleton 2010). High prevalence of multidrug resistance among *Staphylococcus* spp. in veterinary and human medicine is of public health concern as it complicates treatment (Werckenthin et al., 2001), and results in increased morbidity, mortality, and financial burden to animal owners and the public (Kim et al., 2006).

A study by Schmidt et al. (2014), focussing on antimicrobial resistance and characterization of *Staphylococcus* isolated from healthy Labrador retrievers in the United Kingdom, reported 34% MDR among *Staphylococcus* isolates. Another study done in Switzerland reported 17% prevalence of MDR among *Staphylococcus* isolates (Gandolfi-Decristophoris et al., 2013). Multiple drug resistance has been identified in 77.3% of MRS isolates from dogs with pyoderma in northern California, USA (Eckholm et al., 2013). A study in Michigan, USA, reported a 27.5% prevalence of MDR Staphylococci from canine patients visiting a veterinary teaching hospital (Detwiler et al., 2013). In 2014, Wedley and co-workers reported multidrug resistance among MRCoNS (87.5%) and CoPS (21.8%) from a dog population in mainland UK (Wedley et al., 2014).

Multidrug resistance among *Staphylococcus* isolates has also been reported in Africa. For example, a study done by Youn and co-workers (2014) in Zambia, reported multidrug resistance in 10.4% of *S. pseudintermedius* isolates tested and none among *S. aureus*. However, Blunt et al. (2013) in South Africa, observed no multidrug resistance among *S. intermedius* isolates from dogs presented with pyoderma.

1.2.4.6 Risk factors for antimicrobial resistance

Staphylococcus aureus isolated from ear samples have been shown to exhibit significantly higher levels of resistance to trimethoprim-sulphamethoxazole than isolates from other sites (Hoekstra & Paulton, 2002). In the same study, the isolates

from reproductive extremities also had a significantly higher prevalence of resistance to cephalothin, cloxacillin, enrofloxacin and lincomycin than isolated from other anatomical sites. In addition, significantly higher levels of resistance to enrofloxacin and susceptibility to cephalothin were observed in older compared to younger dogs.

In a study by Gandolfi-Decristophoris et al. (2013) in Switzerland, dogs that had stayed in a veterinary clinic in the last year had higher odds of colonization with MDR Staphylococci compared to those that had not stayed in a veterinary hospital (Gandolfi-Decristophoris et al., 2013). Faires and colleagues (2010) observed a significant association between previous exposure to antimicrobial agents and identification of MRSA. In the same study, dogs with intravenous catheters had higher odds of infection with MRSA compared to those that had not been catheterized. In another study by Loeffler et al. (2005), the odds of MRSA infection was higher among dogs with clinical conditions than among healthy dogs.

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CHAPTER 2

2.1 Burden and predictors of *Staphylococcus aureus* and *S. pseudintermedius* infections among dogs presented at an academic veterinary hospital in South Africa (2007-2012)

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2.2 Abstract

Staphylococci are commensals of the mucosal surface and skin of humans and animals, but have been implicated in infections such as otitis externa, pyoderma, urinary tract infections and post-surgical complications. Laboratory records provide useful information to help investigate these infections. Therefore, the objective of this study was to investigate the burdens of these infections and use multinomial regression to examine the associations between various *Staphylococcus* infections and demographic and temporal factors among dogs admitted to an academic veterinary hospital in South Africa. Records of 1,497 clinical canine samples submitted to the bacteriology laboratory at a veterinary academic hospital between 2007 and 2012 were included in this study. Proportions of staphylococcal positive samples were calculated, and a multinomial logistic regression model was used to identify predictors of staphylococcal infections. Twenty-seven percent of the samples tested positive for *Staphylococcus* spp. The species of *Staphylococcus* identified were *S. pseudintermedius* (19.0%), *S. aureus* (3.8%), *S. epidermidis* (0.7%) and *S. felis* (0.1%). The remaining 2.9% consisted of unspciated *Staphylococcus*. Distribution of the species by age of dog showed that *S. pseudintermedius* was the most common (25.6%) in dogs aged 2-4 years while *S. aureus* was more frequent (6.3%) in dogs aged 5-6 years. *Staphylococcus pseudintermedius* (34.1%) and *S. aureus* (35.1%) were the most frequently isolated species from skin samples. The results of the multinomial logistic

regression model identified specimen, year and age of the dog as significant predictors of the risk of infection with *Staphylococcus*. There was a significant temporal increase (RRR=1.17; 95% CI: 1.06-1.29) in the likelihood of a dog testing positive for *S. pseudintermedius* compared to testing negative. Dogs ≤ 8 years of age were significantly more likely to test positive for *S. aureus* than those >8 years of age. Similarly, dogs between 2-8 years of age were significantly more likely to test positive for *S. pseudintermedius* than those >8 years of age. In addition, dogs 2-4 years of age (RRR=1.83; 1.09-3.06) were significantly more likely to test positive for *S. pseudintermedius* compared to those <2 years of age. The risk of infection with *S. pseudintermedius* or *S. aureus* was significantly higher in ear canal and skin specimens compared to other specimens. The findings suggest that *S. pseudintermedius* and *S. aureus* were the most commonly isolated species from dogs presented at the study hospital. Age of the dog and the location of infection were significant predictors of infection with both *Staphylococcus* species investigated. Significant increasing annual temporal trend was observed in the proportion of *S. pseudintermedius* compared to negative samples. This was not the case for *S. aureus*. This information is useful for guiding clinical decisions as well as future research.

Tlaleho ya matshwao a tshwaetso ya *Staphylococcus aureus* le *S. pseudintermedius* dintjeng e ileng ya tekwa Sepetleleng sa Bongaka ba Diphoofolo Afrika Borwa ka selemo 2007-2012

2.3 Ketapele

Di-*Staphylococci* ke sebopelo sa letlalo diphoofolong le ho batho mme se ameha ditshwaetsong tsa mafu a ditsebe, mafu a letlalo, tshwaetso ya methapo ya mosese mmoho le mathata a hlahang kamora ho sebetswa madimong. Direkote tsa laboratoro di fana ka lesedi la bohlokwa ho etsa diphuputso ka ditshwaetso tsena. Kahona, sepheo sa mantlha sa diphuputso tsena ke ho batlisisa boleng ba ditshwaetso tsena le ho sebedisa meriana ho hlahloba kamano dipakeng tsa ditshwaetso tse ngata tsa *Staphylococcus* ho shebuwe maemo a palo ya dintja tse ileng tsa alafshwa sepetleleng sa bongaka ba diphoofolo Afrika Borwa. Direkote tse 1,497 tsa disampole tsa mafu a dintja tse ileng tsa fanwa laboratoring ya dibaketheria sepetleleng sa bongaka ba diphoofolo dipakeng tsa 2007 le 2012 di kenyeleditswe boithutong bona. Pokello ya disampole tsa *Staphylococcus* e ile ya hlahlojwa mme hwa sebediswa mekgwa e fapaneng ho hlwaya matshwao a ditshwaetso tsa *Staphylococcus*. Dipresente tse 27 di ile tsa fumanwa di na le tshwaetso ya *Staphylococcus* spp. Mefuta ya kokwana-hloko ya *Staphylococcus* e fumanweng ke ya *S. pseudintermedius* (19.0%), *S. aureus* (3.8%), *S. epidermidis* (0.7%) le *S. felis* (0.1%). Dipresente tse 2.9 tse setseng di kenyeletsa mefuta ya dikokwana-hloko tsa

Staphylococcus tse sa tsejweng. Papiso ya mefuta ya dintja ka dilemo tsa tsona e bontsha hore kokwana-hloko ya *S. pseudintermedius* e hlaha ka bongata ka dipresente tse 25.6 dintjeng tse dilemo di dipakeng tsa tse pedi le le tse nne ho kokwana-hloko ya *S. aureus* e ne e hlahella ka dipresente tse 6.3 dintjeng tse dilemo di dipakeng tsa tse hlano le tse tsheletseng. Kokwana-hloko ya *S. pseudintermedius* yona e hlahella ka dipresente tse 34.1 mme ya *S. aureus* e hlaha ka dipresente tse 35.1% ha ho hlahlojwa disampole tsa mofuta wa letlalo. Sephetho se ileng sa fumanwa ho ipapisitswe le mofuta le dilemo tsa ntja di ne di nkwa e le ditshiya tse behang dintja tlotlokotsing ya ho tshwaetswa ke kokwana-hloko ya *Staphylococcus*. Ho bile le keketseho ya boleng ba (RRR=1.17; 95% CI: 1.06-1.29) ntlheng ya dintja tseo ha di hlahlojwa di fumanwang di na le kokwana-hloko ya *S. pseudintermedius* papisong le dintja tse fumanwang di se na bolwetse bona. Dintja tse ka tlaase ho dilemo tse robedi di tlokotsing ya ho fumanwa di na le tshwaetso ya kokwana-hloko ya *S. aureus* ho feta dintja tse ka hodimo ho dilemo tse robedi. Ka ho tshwana, dintja tse dilemo di pakeng tsa dilemo tse robedi di tlokotsing ya ho fumanwa di na le kokwana-hloko ya *S. pseudintermedius* ho feta tse katlaase ho dilemo tse 8. Ho feta mona, dintja tse dipakeng tsa dilemo tse pedi ho isa ho tse nne (RRR=1.83; 1.09-3.06) di bonahetse di le tlokotsing ya ho fumanwa di na le tshwaetso ya kokwana-hloko ya *S. pseudintermedius* ha di bapiswa le tse dilemo di katlaase ho pedi. Ho bonahetse ho ba tlokotsing ya ho fumana tshwaetso ya kokwana-hloko ya *S. pseudintermedius*

kapa *S. aureus* e fumaneha haholo kahare ho ditsebe mmoho le letlalong la dintja ha ho bapiswa le disampole tse ding. Tshibollo ho hlaha diphuputsong tsena e supa hore kokwana-hloko ya *S. pseudintermedius* le *S. aureus* ke tsona tse fumanehang ka bongata mefuteng e mmalwa ya dintja. Dilemo tsa ntja le sebaka seo kokwana-hloko e fumanehang ho sona e ne e le matshwao a bohlokwa a tsela ya tshwaetso mefuteng e mmedi ya kokwana-hloko ya *Staphylococcus* tse ileng tsa fuputswa. Ho bonahetse keketseho e kgolo kahare ho boleng ba kokwana-hloko ya *S. pseudintermedius* ha ho bapiswa le disampole tse se nang tshwaetso. Empa ho ne ho se jwalo ho kokwana-hloko ya *S. aureus*. Lesedi lena le bohlokwa bakeng sa ho fana ka tataiso diqetong tsa bongaka mmoho le diphuputso tsa kamoso.

2.4 Introduction

Staphylococcus bacteria are commensal organisms of mucosal surfaces and skin of both humans and animals but are also associated with a variety of diseases. The organisms can survive for months on environmental surfaces and serve as sources of infection (Neely, Maley 2000, Coughenour, Stevens & Stetzenbach 2011). Transmission from dogs to humans occurs following exposure to carrier or infected dogs (Pantosti 2012, Faires, Tater & Weese 2009, Frank et al. 2010, Boost, O'Donoghue & Siu 2007). Due to this risk, some authors have recommended that, where possible, contact between animals and humans should be restricted to mitigate the risk of infection and its associated public health concerns (Faires, Tater & Weese 2009).

Staphylococcus spp. in dogs have been isolated in several clinical conditions including: pyoderma, otitis, wound infections, sepsis, nasal infections, pneumonia, nephritis, and post-surgical infections (Weese, van Duijkeren 2010, Kawakami et al. 2010, Cohn, Middleton 2010, Ishihara et al. 2010, Kramer, Schwebke & Kampf 2006, May et al. 2005, Frank et al. 2003). However, the majority of infections have been associated with canine dermatologic conditions.

Due to the high rates of colonization of dogs with *Staphylococcus pseudintermedius* and *Staphylococcus aureus*, these species make up the majority of

Staphylococcus related infections in dogs. For example, studies conducted in Canada, Hong Kong and Japan have reported the prevalence of *S. pseudintermedius* in dogs as ranging between 61% and 89.5% (Kawakami et al. 2010, Boost, O'Donoghue & Siu 2007) whereas the prevalence of *S. aureus* ranges from 9% to 40% in dogs (Hanselman et al. 2009, Boost, O'Donoghue & Siu 2007). Dogs also get infected with other species of *Staphylococcus* such as *S. schleiferi*, *S. epidermidis*, *S. xylosus* and *S. felis* (Chanchaithong et al. 2014).

There is paucity of information on the epidemiology of staphylococcal infections in dogs in South Africa. Specifically, there is no evidence of studies that have attempted to investigate temporal patterns of infections and the predictors of staphylococcal infections in dogs in South Africa. Furthermore, many of the investigations of predictors of infections described in the literature are based on the analysis of binary outcomes using logistic regression models, and there is no evidence of studies that have tried to ascertain if the predictors of the various *Staphylococcus* species differ using multinomial models. Therefore, the objective of this study was to investigate the burdens of these infections and use multinomial regression to examine the associations between infection with various *Staphylococcus* spp., and the demographic and temporal factors among dogs admitted to an academic veterinary hospital in South Africa.

2.5 Materials and Methods

2.5.1 Data collection and management

Data for this study were obtained from the bacteriology laboratory of the University of Pretoria academic veterinary hospital and included cases of *Staphylococcus* spp. infections isolated from all dog samples submitted to the laboratory for microbiological diagnosis between January 2007 and December 2012.

The data were assessed for duplicate entries. The dataset did not contain multiple tests from the same patient nor were there mixed infections in the samples analysed. *Staphylococcus* species were identified based on characteristics of the colony and chemical tests as described by Quinn et al. (1994). The laboratory records of all specimens from dogs submitted to the laboratory for diagnostic purposes were assessed. For the purpose of this study, only records of dogs from the Gauteng Province were included for analysis. This was to control for potential confounding by region as most of the samples were from Gauteng province and very few were from other provinces. Variables included in the dataset included age (in months), sex, breed, type of specimen submitted, address of the owner of the dog, and the date of specimen submission. The study excluded records of dogs with incomplete or inaccessible information (n=12). The breed classification used in the study was adapted from the American Kennel Club (AKC), and included the following

categories: working, sporting, herding, hound, toy, terrier, nonsporting and mixed breeds (American Kennel Club 2016).

2.5.2 Statistical analysis

2.5.2.1 Descriptive analysis

Age was categorised into five categories: <2 years, 2-4 years, 5-6 years, 7-8 years and >8 years. Crude and factor-specific proportions of *S. aureus* and *pseudintermedius* positive samples and their confidence intervals (95% CI) were computed. The factors (independent variables) included in these computations were breed, season, year, sex, age category and specimen type. In addition, annual changes in the proportion of samples that tested positive for *Staphylococcus* between 2007 and 2012 were displayed in temporal graphs.

2.5.2.2 Univariable and multivariable models

The first step in the investigation of the predictors of *Staphylococcus* infections in dogs was to fit univariable multinomial logistic regression models to assess the relationships between the potential predictors (sex, age, year, season, breed and specimen type) and the polytomous outcome variable, *Staphylococcus* status. This outcome variable represented the species of *Staphylococcus* (*S. pseudintermedius*, *S. aureus* and *Staphylococcus* negative that was used as the reference category). For this initial investigation, potential predictors at a p-value ≤ 0.20 were considered for

inclusion in the multivariable model to be fitted in the second step. The variable age was modelled as a categorical variable for ease of interpretation. Since the variable specimen type had too many categories to include in the model in its original form, it was re-coded into four categories (ear-canal, urine, skin and all others) for inclusion in the model with all others as the referent category. To be able to assess temporal trend, the predictor variable year was included in the model as a continuous variable. However, since it does not have a meaningful interpretation at the value 0, it was scaled by subtracting the lowest value in the data (2007) before inclusion in the model.

In the second step, a multivariable multinomial logistic regression model was fit using manual backwards selection with the polytomous *Staphylococcus* status variable as the outcome. At this step, statistical significance was assessed at $\alpha=0.05$. Confounding was assessed by comparing the change in model coefficients with and without the suspected confounders. If the removal of a suspected confounding variable resulted in a 20% or greater change in another model coefficient, the removed variable was considered a confounder and retained in the model regardless of its statistical significance. All two-way interaction terms among variables in the final main effects model were also assessed. Additionally, linearity assumption of the predictor variable, year was assessed by examining the significance of adding a

quadratic term to the model. The quadratic term was not statistically significant and hence it was removed from the model.

Relative risk ratios (RRRs) and their corresponding 95% confidence intervals were computed for all variables included in the final model. To directly assess if there were statistically significant differences between the predictors for *S. pseudintermedius* and *S. aureus*, the multinomial model was also fit with *S. pseudintermedius* as the reference category of the outcome (instead of the negative samples) and the predictors assessed for significance using an $\alpha=0.05$.

To assess the goodness-of-fit of the multinomial logistic regression models, ordinary logistic regression models were fit to each pairwise combination of the three potential outcome categories as proposed by Dohoo et al. (2009). Hosmer-Lemeshow goodness-of-fit test was then used to assess these models. This approach was used because currently there are no available multinomial model fit assessment tests in SAS, the only software package available to the authors.

2.5.2.3 Ethical statement

The study was approved (approval number: V051-14) by the Ethics Committee of the University of Pretoria.

2.6 Results

2.6.1 Characteristics of dogs tested

A total of 1,626 dogs were tested for *Staphylococcus* related infections between 2007 and 2012. Of these, 1,497 (92.1%) from Gauteng province were included in the study. The majority (86.4%) of dogs included in the study were from the City of Tshwane Municipality. Of these, 65.1% were from the Pretoria area (a suburb of Tshwane). The highest (24.7%) proportion of dogs was tested in 2009, and the lowest (7.8%) in 2012. Compared to other seasons, most samples (37.6%) were tested during summer months, while the lowest number was tested in spring (15.4%) (Table 2.1). The median age of the dogs in the study was 66 months (5 years) and the interquartile range was 27-101 months. Most dogs (29.9%) were >8 years old, followed by those <2 years (21.4%). The majority of samples tested were from female dogs (52.2%), with samples from males making up the remaining 47.8%.

A total of seventy-three breeds of dogs were identified, and of these 11.8% were Boerboel followed by German Shepherd (9.1%), Bullterrier (7.0%), Dachshund (6.7%), Labrador Retriever (5.0%), Jack Russel (4.8%), Crossbreed (4.7%), Rottweiler (3.3%), Spaniel (2.8%), Yorkshire Terrier (2.8%), Maltese (2.5%), English Bulldog (2.4%), Husky (2.2%), Border Collie (2.1%) and Great Dane (2.1%). For the purpose of this study, breeds that made up <2% of the study population were classified as “all

others”, and all together this group contributed 30.8% of the samples submitted and processed. When the breeds were further classified according to the American Kennel Club (AKC) dog classification system, the highest proportion of samples tested were from working breeds (25.9%) while samples from crossbreeds (4.8%) made up the lowest proportion of submissions (Table 2.1).

A total of 48 different types of specimens were submitted and classified into four main groups: urine (31.0%), ear canal swabs (17.0%), skin (11.0%) and other specimen types classified as ‘all others’ (40.9%).

2.6.2 Isolated *Staphylococcus* species

Of the 1,497 samples tested, 26.5% (396/1497) were positive for *Staphylococcus* spp. From the submitted diagnostic samples, *S. pseudintermedius* was isolated most often (19.0%, 284/1497), followed by *S. aureus* (3.8%, 57/1497), unspciated *Staphylococcus* (2.9%, 43/1497), *S. epidermidis* (0.7%, 11/1497) and one *S. felis* (0.1%, 1/1497). The majority (96.7%, 341/353) of *Staphylococcus* spp. identified were coagulase-positive (*S. pseudintermedius* and *S. aureus*), and only 3.3% (12/353) were coagulase negative (*S. epidermidis* and *S. felis*).

Table 2. 1: Temporal and host-factor distribution of *Staphylococcus* infections among dogs presented at the academic veterinary hospital, 2007-2012.

Variable	*All Samples Tested		<i>S. pseudintermedius</i>		<i>S. aureus</i>	
	Proportion	² 95% CI	¹ Proportion	² 95% CI	¹ Proportion	² 95% CI
Year						
2007	18.6 (279/1497)	16.8-20.7	14.3 (40/279)	10.7-18.9	2.9 (8/279)	1.5-5.6
2008	20.4 (305/1497)	18.4-22.5	19.3 (59/305)	15.3-24.2	4.9 (15/305)	3.0-8.0
2009	24.7 (369/1497)	22.5-26.9	16.5 (61/369)	13.1-20.7	3.0 (11/369)	1.7-5.2
2010	15.6 (234/1497)	13.9-17.6	19.2 (45/234)	14.7-24.8	4.7 (11/234)	2.7-8.2
2011	12.9 (193/1497)	11.3-14.7	21.8 (42/193)	16.5-28.1	3.1 (6/193)	1.4-6.6
2012	7.8 (117/1497)	6.7-9.3	31.6 (37/117)	23.9-40.5	5.1 (6/117)	2.4-10.7
Season						
Autumn	20.0 (299/1497)	18.0-22.1	21.1 (63/299)	16.8-26.0	2 (6/299)	0.9-4.3
Spring	15.4 (230/1497)	13.6-17.2	21.3 (49/230)	16.5-27.0	4.3 (10/230)	2.4-7.8
Summer	37.6 (563/1497)	35.2-40.0	19.7 (111/563)	16.6-23.2	4.8 (27/563)	3.3-6.9
Winter	27.1 (405/1497)	24.9-29.4	15.1 (61/405)	11.9-18.9	3.5 (14/405)	2.1-5.7
Age Category						
<2 years	21.4 (320/1497)	19.4-23.5	17.2 (55/320)	13.5-21.7	3.8 (12/320)	2.2-6.4
2-4 years	13.8 (207/1497)	12.2-15.7	25.6 (53/207)	21.0-33.0	5.3 (11/207)	3.0-9.3
5-6 years	16.8 (252/1497)	15.0-18.8	22.2 (56/252)	17.5-27.8	6.3 (16/252)	4.0-10.1
7-8 years	18.1 (271/1497)	16.2-20.1	23.6 (64/271)	19.0-29.0	4.4 (12/271)	2.6-7.6
>8 years	29.9 (447/1497)	27.6-32.2	12.5 (56/447)	9.8-15.9	1.3 (6/447)	0.6-2.9
Sex						
Female	52.2 (767/1468)	49.7-54.8	17.2 (132/767)	12.5-19.7	4.3 (33/767)	3.1-6.0
Male	47.8 (701/1468)	45.2-50.3	21.0 (147/701)	18.1-24.1	3.4 (24/767)	2.3-5.0
Breed						
Working	26.3 (387/1474)	24.01-28.6	16 (61/387)	12.5-19.7	3.9 (15/387)	2.4-6.3
Sporting	18.0 (265/1474)	16.1-20.0	22.3 (59/265)	17.7-27.7	3.4 (9/265)	1.8-6.3
Herding	12.0 (177/1474)	10.5-13.8	14.1 (25/177)	9.7-20.0	5.1 (9/177)	2.7-9.4
Hound	11.1 (163/1474)	9.6-12.8	14.1 (23/163)	9.6-20.3	4.3 (7/163)	2.1-8.6
Toy	11.1 (163/1474)	9.6-12.8	16.6 (27/163)	11.6-23.0	3.1 (5/163)	1.3-7.0
Terrier	10.7 (158/1474)	9.2-12.4	26.6 (42/158)	20.3-34.0	3.8 (6/158)	1.8-8.0
Nonsporting	6.5 (95/1474)	5.3-7.8	29.5 (28/95)	21.3-39.3	2.1 (2/95)	0.6-7.4
Crossbreed	4.8 (71/1474)	3.8-6.0	18.3 (13/71)	11.0-28.9	5.6 (4/71)	2.2-13.6
Specimen						
Urine	31.0 (463/1493)	28.7-33.4	4.5 (21/463)	3.0-6.8	1.7 (8/165)	2.5-9.3
Ear-canal	17.0 (254/1493)	15.2-19.0	34.7 (88/254)	29.1-40.7	5.1 (13/254)	3.0-8.6
Skin	11.1 (165/1493)	9.6-12.7	58.8 (97/165)	51.2-66.0	12.1 (20/165)	8.0-18.0
All Others	40.9 (611/1493)	38.5-43.4	12.8 (78/611)	10.4-15.7	2.6 (16/611)	1.6-4.2

*All samples tested included those tested for all species of *Staphylococcus* (not just *S. pseudintermedius* and *S. aureus*)

¹Proportion of positive samples under each category

²95% confidence interval

While there was an overall increasing temporal trend in both the annual proportion of samples that tested positive for *Staphylococcus* spp. or *S. pseudintermedius* during the study period, the proportion of *S. aureus* positive samples remained stable (Figure 2.1).

Based on graphing of the crude proportions of samples that tested positive for *Staphylococcus* spp., *S. aureus* and *S. pseudintermedius*, no seasonal patterns in the distribution of the three variables were apparent (Figure 2.2). The very high proportion of *Staphylococcus* positive samples (100%) and *S. pseudintermedius* (69.2%) seen in spring of 2010 could be attributed to the small numbers of samples submitted during that season.

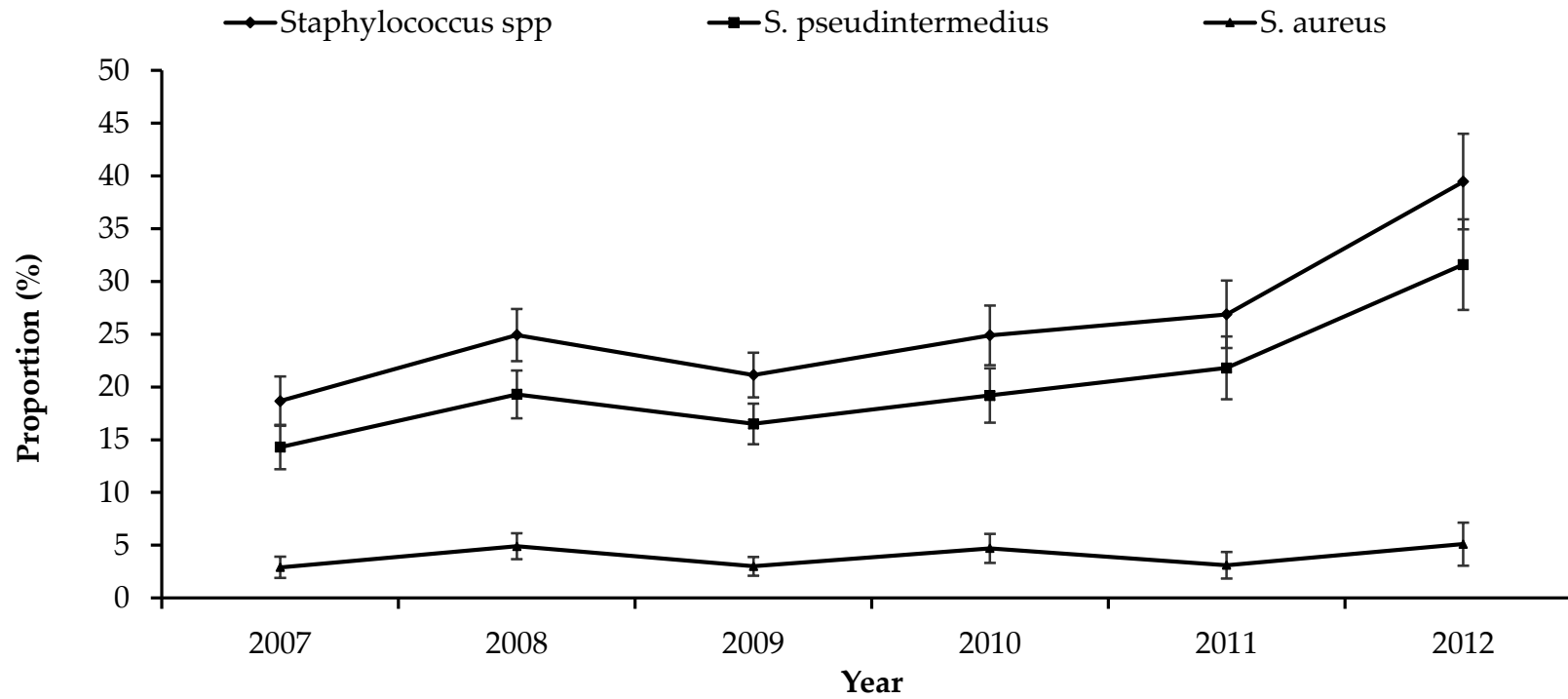


Figure 2. 1: Annual patterns of the proportions and 95% confidence intervals of *Staphylococcus* infections among canine samples tested at the academic veterinary hospital, 2007-2012.

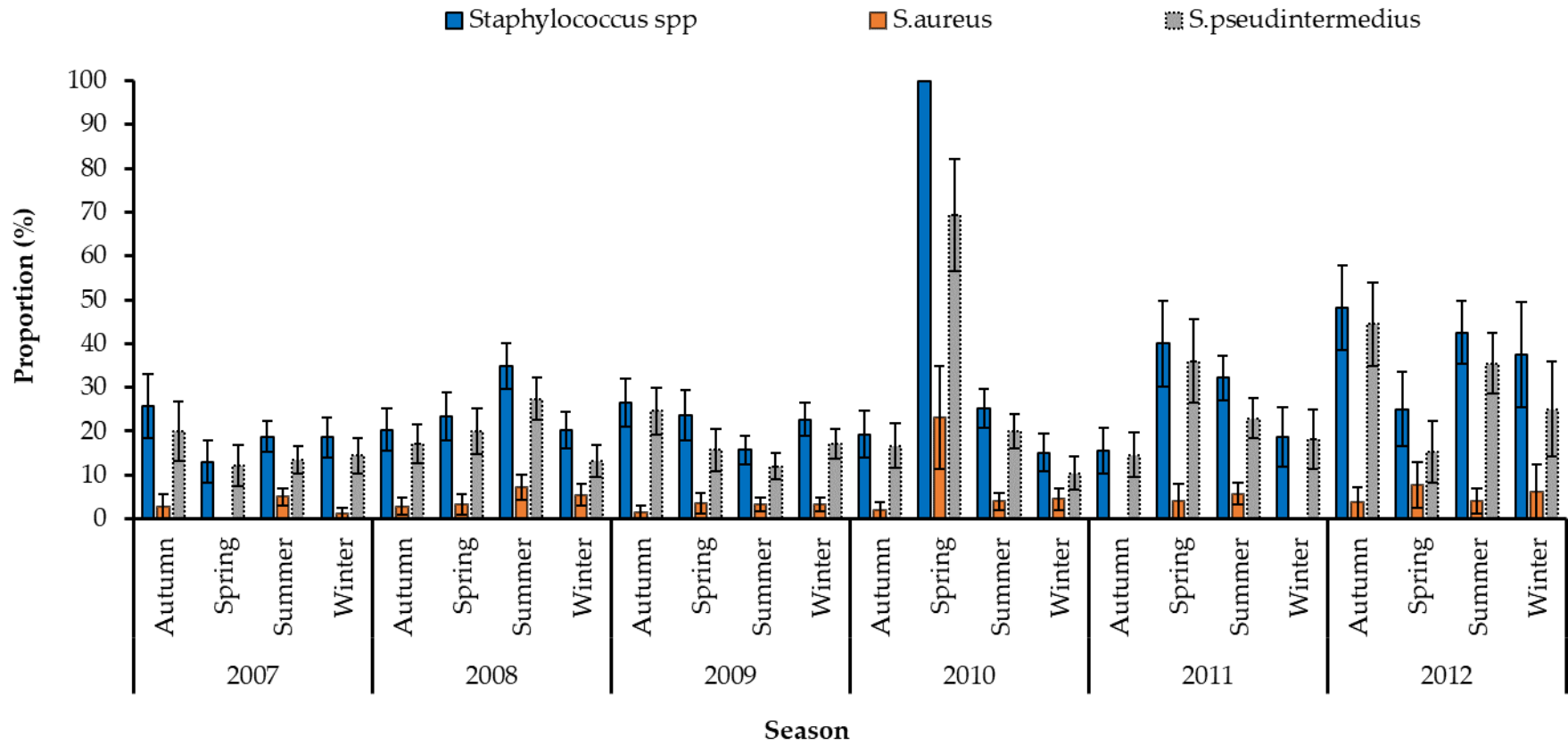


Figure 2. 2: Seasonal patterns in the proportions and 95% confidence intervals of *Staphylococcus* infections among canine samples tested at the academic veterinary hospital, 2007-2012.

Overall, 2012 had the highest proportion of samples that were positive for *S. pseudintermedius* (31.6%) and *S. aureus* (5.1%). The highest proportion of *S. pseudintermedius* positive samples were in Autumn (21.1%) and Spring (21.3%), while the highest proportion of *S. aureus* positive samples were in Summer (4.8%) (Table 2.1).

Staphylococcus pseudintermedius was most common in 2-4 year old dogs (25.6%) followed by dogs in the 7-8 year age category (23.6%). On the other hand, *S. aureus* was most common in dogs 5-6 years old (6.3%) followed by dogs 2-4 years old (5.3%). Male dogs had the highest proportion of samples that tested positive for *S. pseudintermedius* (21.0%), while females had the highest proportion of samples that tested positive for *S. aureus* (4.3%) (Table 2.1).

Overall, non-sporting breeds had the highest proportion (29.5%) of samples positive for *S. pseudintermedius* followed by terriers (26.6%). By contrast, crossbreeds had the highest proportion (5.6%) of samples that tested positive for *S. aureus* followed by herding breeds (5.1%) (Table 2.1).

S. pseudintermedius and *S. aureus* were commonly isolated from both skin and ear samples, while abscesses and bone samples did not yield any *S. aureus* (Figure 2.3).

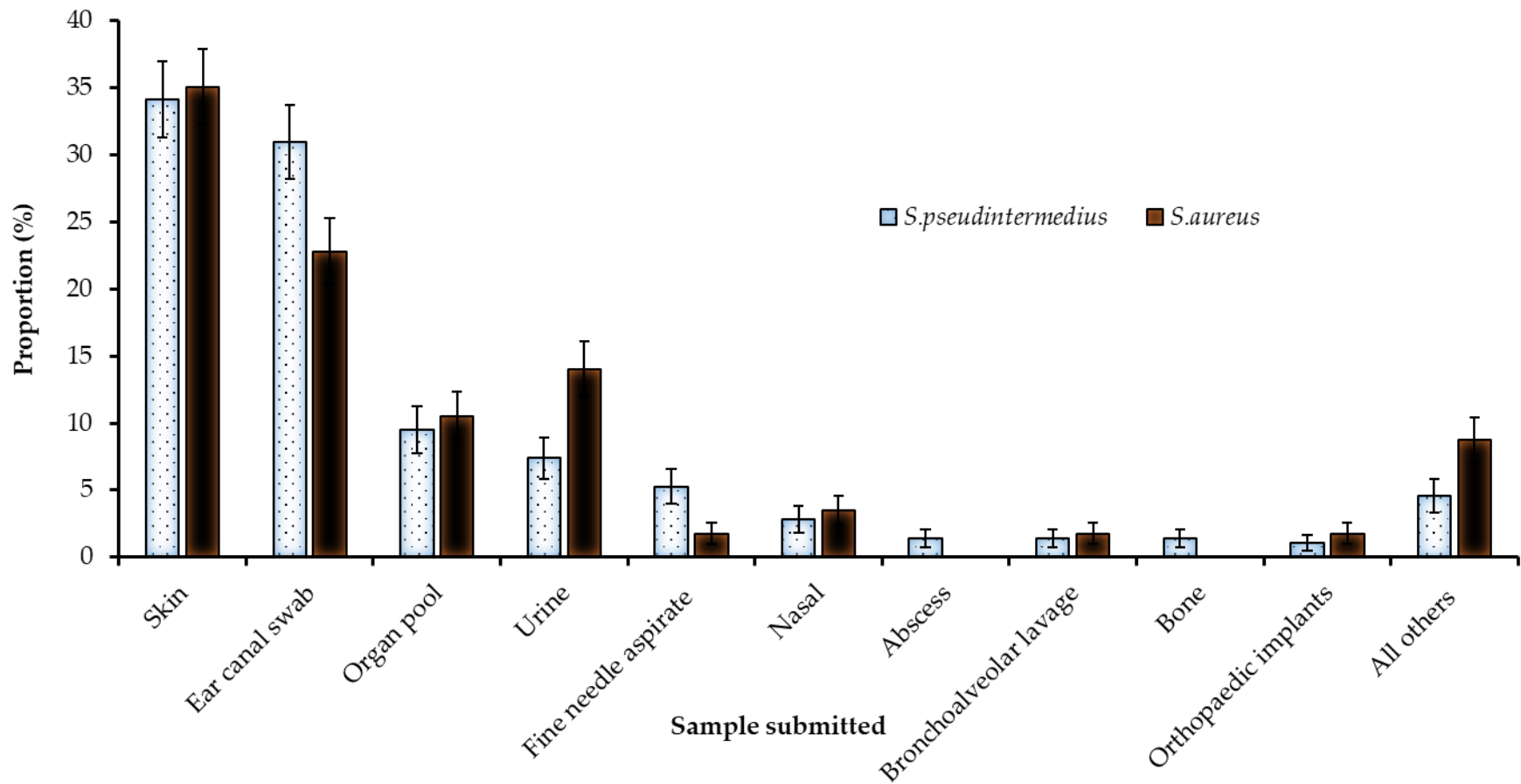


Figure 2. 3: Distribution of the proportions and 95% confidence intervals of *S. pseudintermedius* and *S. aureus* infections among canine samples tested at the academic veterinary hospital, 2007-2012

2.6.3 Predictors of *Staphylococcus* infections based on the multinomial logistic regression

Following assessments using univariable models, age ($p=0.0003$), season ($p=0.1435$), breed ($p=0.0588$), year ($p=0.0004$), specimen type ($p < 0.0001$) and sex ($p=0.0728$) were considered for inclusion in the multivariable model based on a liberal p -value of 0.20 (Table 2.2). However, in the multivariable multinomial model only age, specimen-type and year were significantly associated with infection classification (Table 2.3).

Compared to dogs >8 years of age, the following age classes were significantly more likely to have dogs that tested positive for *S. aureus* compared to testing negative for staphylococci: <2 years, 2-4 years and 5-6 years (Table 2.3). Similarly, 2-4 and 5-6 year old dogs compared to >8 year old dogs, were significantly more likely to test positive for *S. pseudintermedius* than test negative (Table 2.3). In addition, dogs 2-4 years of age compared to dogs <2 years of age were significantly more likely to test positive for *S. pseudintermedius* than testing negative (RRR=1.83, 95% CI: 1.09-3.06, $p=0.0221$; Table 2.4).

The type of specimen tested was also significantly associated with both *S. aureus* and *S. pseudintermedius*. Ear canal and skin specimens were more likely to test positive (as opposed to testing negative) for *S. pseudintermedius* and *S. aureus* compared to specimen categorized as 'all others'. However, urine samples were less

likely to test positive (as opposed to testing negative) for *S. pseudintermedius* compared to specimen classified as 'all others'. In addition, there was a significant temporal increase (RRR=1.17, 95% CI:1.06-1.29, p=0.0027) in the likelihood of a dog testing positive to *S. pseudintermedius* compared to testing negative.

Compared to skin and ear canal samples, the other specimen types were significantly less likely to be infected with either *Staphylococcus* species (Table 2.4), and skin specimens were significantly more likely to be infected with either bacterial species than ear-canal samples (Table 2.4).

To directly assess if there were statistically significant differences between the predictors of *S. pseudintermedius* and *S. aureus*, a multinomial model was fit with *S. pseudintermedius* positive as the reference group (instead of the negative samples). The results of this model showed no significant differences between the predictors of *S. pseudintermedius* and those of *S. aureus* (Table 2.5). Effects of changing the reference categories of the categorical predictors of the model presented in Table 2.5 is shown in Table 2.6.

Table 2. 2: Results of univariable multinomial logistic models investigating potential predictors of canine *Staphylococcus* infections in samples tested at the academic veterinary hospital, 2007-2012.

Variables	<i>S. aureus</i>				<i>S. pseudintermedius</i>			
	¹ RRR	² 95% CI		p-values	¹ RRR	² 95% CI		p-values
Season								
Autumn	0.60	0.23	1.58	0.2975	1.44	0.97	2.13	0.0696
Spring	1.37	0.60	3.16	0.4582	1.54	1.01	2.35	0.0441
Summer	1.46	0.75	2.83	0.2652	1.38	0.97	1.94	0.0710
Winter	Ref
Breed								
Crossbreed	1.50	0.48	4.70	0.4862	1.20	0.61	2.34	0.5943
Herding	1.28	0.54	2.99	0.5753	0.87	0.52	1.45	0.5953
Hound	1.04	0.41	2.61	0.9363	0.84	0.50	1.42	0.5099
Nonsporting	0.60	0.13	2.68	0.5016	2.06	1.22	3.47	0.0068
Sporting	0.92	0.40	2.15	0.8486	1.48	0.99	2.22	0.0544
Terrier	1.08	0.41	2.86	0.8723	1.86	1.19	2.93	0.0069
Toy	0.76	0.27	2.13	0.6015	1.01	0.61	1.66	0.9744
Working	Ref
Sex								
Female	1.20	0.70	2.06	0.5021	0.78	0.60	1.02	0.0728
Male	Ref
Age								
<2 years	2.62	0.97	7.07	0.0577	1.29	0.86	1.93	0.2252
2-4 years	4.46	1.62	12.29	0.0039	2.30	1.50	3.53	0.0001
5-6 years	5.09	1.96	13.25	0.0009	1.91	1.26	2.89	0.0022
7-8 years	2.73	1.01	7.38	0.0477	1.56	1.05	2.32	0.0276
>8 years	Ref
Year								
2007-2012	1.08	0.907	1.285	0.3876	1.167	1.071	1.272	0.0004
Specimen								
Ear-canal	2.83	1.33	6.02	0.01	3.93	2.75	5.61	<0.0001
Urine	0.59	0.25	1.39	0.22	0.32	0.19	0.52	<0.0001
Skin	14.70	7.09	30.48	<0.0001	14.63	9.48	22.57	<0.0001
All Others

¹RRR= Relative Risk Ratio

²95% CI: 95% Confidence Intervals

Table 2. 3: Results of the final multinomial logistic model showing predictors of *Staphylococcus* species infections in dogs presented at the academic veterinary hospital, 2007 and 2012.

Variables	<i>S. aureus</i>			<i>S. pseudintermedius</i>		
	¹ RRR	² 95% CI	p-value	¹ RRR	² 95% CI	p-value
Age						
<2 years	2.93	1.04 8.22	0.0416	1.26	0.78 2.05	0.3406
2-4 years	4.27	1.49 12.27	0.007	2.31	1.39 3.84	0.0012
5-6 years	5.10	1.89 13.78	0.0013	2.06	1.27 3.36	0.0037
7-8 years	2.49	0.89 6.96	0.083	1.41	0.89 2.25	0.1479
>8 years	Ref
Specimen						
Ear-canal	3.13	1.44 6.82	0.0041	3.99	2.72 5.84	<0.0001
Urine	0.56	0.23 1.34	0.1901	0.32	0.19 0.53	<0.0001
Skin	15.42	7.21 33.00	<0.0001	14.26	9.02 22.55	<0.0001
All Others	Ref
Time						
Year (scaled)	1.09	0.91 1.30	0.3597	1.17	1.06 1.29	0.0027

¹RRR= Relative Risk Ratio

²95% CI: 95% Confidence Intervals

Table 2. 4: Results of changing reference categories of categorical variables included in the final model presented in Table 2.3.

Variables	<i>S. aureus</i>				<i>S. pseudintermedius</i>			
	¹ RRR	² 95% CI	p-value	¹ RRR	² 95% CI	p-value		
Age								
<2 years	1.18	0.5	2.78	0.7094	0.9	0.56	1.45	0.654
2-4 years	1.72	0.71	4.19	0.2334	1.64	0.99	2.71	0.0548
5-6 years	2.05	0.91	4.65	0.0852	1.46	0.9	2.37	0.1225
>8 years	0.4	0.14	1.13	0.083	0.71	0.45	1.13	0.1479
7-8 years	Ref
<2 years	0.57	0.25	1.31	0.1852	0.61	0.37	1.01	0.0538
2-4 years	0.84	0.36	1.96	0.6823	1.12	0.67	1.88	0.6658
7-8 years	0.49	0.22	1.11	0.0852	0.68	0.42	1.11	0.1225
>8 years	0.2	0.07	0.53	0.0013	0.49	0.3	0.79	0.0037
5-6 years	Ref
<2 years	0.69	0.28	1.67	0.4049	0.55	0.33	0.92	0.0221
5-6 years	1.19	0.51	2.79	0.6823	0.89	0.53	1.5	0.6658
7-8 years	0.58	0.24	1.42	0.2334	0.61	0.37	1.01	0.0548
>8 years	0.23	0.08	0.67	0.007	0.43	0.26	0.72	0.0012
2-4 years	Ref
2-4 years	1.46	0.6	3.55	0.4049	1.83	1.09	3.06	0.0221
5-6 years	1.74	0.77	3.96	0.1852	1.63	0.99	2.68	0.0538
7-8 years	0.85	0.36	2.01	0.7094	1.12	0.69	1.8	0.654
>8 years	0.34	0.12	0.96	0.0416	0.79	0.49	1.28	0.3406
<2 years	Ref
Specimen								
All Others	0.32	0.15	0.7	0.0041	0.25	0.17	0.37	<0.0001
Urine	0.18	0.07	0.45	0.0003	0.08	0.05	0.14	<0.0001
Skin	4.93	2.18	11.12	0.0001	3.58	2.22	5.76	<0.0001
Ear-canal	Ref
All Others	0.07	0.03	0.14	<0.0001	0.07	0.04	0.11	<0.0001
Urine	0.04	0.01	0.09	<0.0001	0.02	0.01	0.04	<0.0001
Ear-canal	0.2	0.09	0.46	<0.0001	0.28	0.17	0.45	<0.0001
Skin	Ref

Table 2. 5: Results of the final multinomial logistic model showing predictors of

***Staphylococcus* spp. infection with *S. pseudintermedius* as the reference category, 2007-2012.**

	<i>Staphylococcus</i> Negative				<i>S. aureus</i>			
	¹ RRR	² 95% CI		p-value	¹ RRR	² 95% CI		p-value
Age								
<2 years	0.79	0.49	1.28	0.3406	2.31	0.79	6.79	0.1262
2-4 years	0.43	0.26	0.72	0.0012	1.85	0.63	5.47	0.2667
5-6 years	0.49	0.30	0.79	0.0037	2.47	0.88	6.94	0.0851
7-8 years	0.71	0.45	1.13	0.1479	1.76	0.61	5.11	0.2968
8> years								
Specimen								
Ear-canal	0.25	0.17	0.37	<0.0001	0.79	0.35	1.78	0.5613
Urine	3.14	1.88	5.22	<0.0001	1.75	0.65	4.70	0.2695
Skin	0.07	0.04	0.11	<0.0001	1.08	0.51	2.29	0.838
All Others								
Time								
Year	0.86	0.78	0.95	0.0027	0.93	0.77	1.12	0.4608

¹RRR= Relative Risk Ratio

²95% CI: 95% Confidence Intervals

Table 2. 6: Results of changing reference categories of categorical variables included in the final model presented in Table 2.5.

	<i>Staphylococcus</i> Negative			<i>S. aureus</i>				
	¹ RRR	² 95% CI	p-value	¹ RRR	² 95% CI	p-value		
Age								
2-4 years	0.55	0.33	0.92	0.0221	0.8	0.32	2.01	0.6335
5-6 years	0.61	0.37	1.01	0.0538	1.07	0.45	2.54	0.8796
7-8 years	0.9	0.56	1.45	0.654	0.76	0.31	1.87	0.5522
8> years	1.26	0.78	2.05	0.3406	0.43	0.15	1.27	0.1262
<2 years								
<2 years	1.83	1.09	3.06	0.0221	1.25	0.5	3.15	0.6335
5-6 years	1.12	0.67	1.88	0.6658	1.34	0.56	3.21	0.5144
7-8 years	1.64	0.99	2.71	0.0548	0.95	0.38	2.38	0.918
8> years	2.31	1.39	3.84	0.0012	0.54	0.18	1.6	0.2667
2-4 years								
<2 years	1.63	0.99	2.68	0.0538	0.94	0.39	2.22	0.8796
2-4 years	0.89	0.53	1.5	0.6658	0.75	0.31	1.79	0.5144
7-8 years	1.46	0.9	2.37	0.1225	0.71	0.3	1.67	0.4347
8> years	2.06	1.27	3.36	0.0037	0.4	0.14	1.13	0.0851
5-6 years								
<2 years	1.12	0.69	1.8	0.654	1.313	0.535	3.225	0.5522
2-4 years	0.61	0.37	1.01	0.0548	1.049	0.42	2.619	0.918
5-6 years	0.68	0.42	1.11	0.1225	1.404	0.599	3.289	0.4347
8> years	1.41	0.89	2.25	0.1479	0.567	0.196	1.645	0.2968
7-8 years								
Specimen								
Urine	12.51	7.37	21.22	<0.0001	2.23	0.8	6.2	0.1257
Skin	0.28	0.17	0.45	<0.0001	1.38	0.62	3.04	0.4279
All Others	3.99	2.72	5.84	<0.0001	1.27	0.56	2.89	0.56
Ear-canal								
Ear-canal	0.08	0.05	0.14	<0.0001	0.45	0.16	1.25	0.1257
Skin	0.02	0.01	0.04	<0.0001	0.62	0.23	1.64	0.3355
All Others	0.32	0.19	0.53	<0.0001	0.57	0.21	1.54	0.2695
Urine								
Ear-canal	3.58	2.22	5.76	<0.0001	0.73	0.33	1.6	0.4279
Urine	44.72	24.81	80.62	<0.0001	1.62	0.61	4.29	0.3355
All Others	14.26	9.02	22.55	<0.0001	0.93	0.44	1.96	0.838
Skin								

2.7 Discussion

This study was designed to assess the burdens and predictors of staphylococcal infections in dogs presented at an academic veterinary hospital in South Africa between 2007 and 2012. We used multinomial logistic regression models to investigate predictors of staphylococcal infections to assess if there were significant differences in predictors across species or if the strength of association differed. Therefore, the results of this study contribute significantly to improving our understanding of the epidemiology of staphylococcal bacterial infections in dogs presented at an academic veterinary hospital in South Africa.

Our results show that *Staphylococcus* bacteria were isolated from 26.5% of samples submitted to the academic veterinary hospital. This is lower than the 55.1% reported by Wedley et al. (2014) in mainland United Kingdom (UK) and 67.7% by Lilenbaum et al. (2000) in Brazil. The low proportion of *Staphylococcus* positive samples identified in this study compared to the above mentioned studies may be due to differences in target populations. In this study, we assessed staphylococcal infections among clinical cases of hospitalized dogs, whereas, Wedley et al. (2014) assessed infections in outpatient dogs and Lilenbaum et al. (2000) specifically investigated infection in dogs with otitis.

The significant increasing annual trend in the proportion of *Staphylococcus pseudintermedius* infections has not been previously reported/investigated in other dog populations. However, this could be attributed to increased access to veterinary services in South Africa since the dawn of democratic South Africa. This would especially be true if there is systematic access to and utilization of veterinary services such that dogs that potentially have high *Staphylococcus* infection risks are more likely to be presented for diagnostic services at the academic veterinary hospital compared to those with lower staphylococcal infection risks. It is also possible that the increase in the proportions of staphylococcal related infections in this study may be due to progressive poorer health and wellbeing of dogs living in and around the area which makes them susceptible to staphylococcal infections. Furthermore, changes in referral practices by surrounding veterinary clinics, changes in owner attitudes towards seeking treatment for chronic skin conditions, and/or changes in lab procedures could have resulted in the increased identification of these infections.

Evidence from the current study showed no seasonal pattern in the risk of canine *S. pseudintermedius* or *S. aureus* infection. This contrasts with reports of seasonal atopic dermatitis with secondary staphylococcal infections that have been published by other authors (Simou et al. 2005).

Our study found that Coagulase-Positive *Staphylococcus* (CoPS) organism were more common than Coagulase-Negative *Staphylococcus* (CoNS). This is

consistent with the findings by Wedley et al. (2014) who reported higher proportions of CoPS (38%) in dogs compared to CoNS (19%) in the UK. In contrast, Schmidt et al. (2014), in a UK study and Lilenbaum et al. (2000) in a Brazilian study reported a higher frequency of CoNS (95% and 61%) than CoPS (41% and 39%) in dogs. However, it should be noted that in contrast to our study, Schmidt et al. (2014) tested samples collected from healthy Labrador Retrievers. These could explain the differences in the proportions of dogs with CoPS and CoNP infections in the two studies.

Staphylococcus pseudintermedius was more commonly isolated (19.0%) than *S. aureus* in this study. However, the observed proportion was lower than 87% reported in Switzerland (Gandolfi-Decristophoris et al. 2013) and 69% reported in Denmark (Paul et al. 2012). Although the prevalence of *S. pseudintermedius* was higher than that of *S. aureus* there was no significant difference between the two species in terms of their affinity for different sample types. Nonetheless, a higher proportion of *S. pseudintermedius* infections was observed from skin (34.1%) and ear (31.0%) samples compared to other sites/specimens. This is not unexpected because previous studies have reported higher frequency of *S. pseudintermedius* in dogs with pyoderma or otitis (Wedley et al. 2014, Griffeth et al. 2008, Lilenbaum et al. 2000). These findings can be explained by the fact that *S. pseudintermedius* is known to be more adapted to colonise the skin surface of dogs than *S. aureus* (Gandolfi-Decristophoris et al. 2013,

Schmidt et al. 2014, Kawakami et al. 2010). Moreover, increased adherence by *S. pseudintermedius* to corneocytes of canines with atopic dermatitis has been previously reported (McEwan, Mellor & Kalna 2006) suggesting that dogs are more likely to be infected with *S. pseudintermedius* compared to other species. Additionally, the results of the study show that ear canal and skin sites compared to other sites are more likely to test positive for *S. pseudintermedius* or *S. aureus* than test negative. This further supports research that reports that skin and ear canal compared to other body sites are more likely to be infected with *Staphylococcus* organisms than other bacterial organisms (Gandolfi-Decristophoris et al. 2013, Schmidt et al. 2014, Kawakami et al. 2010).

The high frequency of *S. pseudintermedius* in dogs visiting the academic veterinary hospital is of public health significance because of association between *S. pseudintermedius* colonization in dogs and infection in humans with a history of exposure to carrier dogs (Hanselman et al. 2009). Since *S. pseudintermedius* is not a commensal in the nasal passages of healthy humans, dogs are thought to be an important reservoir for human infections with this pathogen (Tanner, Everett & Youvan 2000).

Although the results of the descriptive analysis indicated that female dogs carried more *S. aureus*, the results from the multinomial logistic regression indicated that sex of the dog was not a significant predictor of staphylococcal infections in

dogs presented at the academic veterinary hospital in South Africa. This is consistent with the findings by Hanselman et al. (2009) who reported no significant association between sex and staphylococcal infections in dogs in Canada. However, our findings are in contrast to findings by Boost et al. (2008), who reported that female dogs in Japan were more likely to be infected with *S. aureus* compared to their male counterparts.

The significant association between age and the risk of *Staphylococcus* infection in dogs observed in the present study was anticipated because age has previously been described as a predictor for *Staphylococcus* infection in dogs (Boost, O'Donoghue & James 2008). However, unlike the study by Boost et al. (2008) that reported more colonization in older dogs compared to puppies and middle aged dogs, in this study dogs ≤ 6 years of age and 2-6 years of age compared to dogs > 8 years of age had a significantly higher risk of *S. aureus* and *S. pseudintermedius* infection, respectively, compared to testing negative. It was also noted that the risk of *S. pseudintermedius* infection was significantly higher in 2-4 year old dogs compared to dogs < 2 years of age.

The external validity of this study should be interpreted with caution since it is based on submitted laboratory samples. Moreover, the history of previous use of antibiotics or anti-inflammatory agents was not included in the analysis. It is possible that this could have affected the recovery rates of *Staphylococcus* species.

Since the population under study did not include outpatient cases, the study population should not be regarded as representative of the dog population in Gauteng or visiting the teaching hospital. Nonetheless, the results provide a useful preliminary indication of the burden and predictors of staphylococcal infections in dogs presented to the academic veterinary hospital.

2.8 Conclusions

This study confirmed that skin, ear, and urinary tract infections in dogs presented at the academic veterinary hospital are mainly due to *S. pseudintermedius* followed by *S. aureus*. Furthermore, the risk of infection with *S. aureus* or *S. pseudintermedius* in dogs presented at the academic veterinary hospital differed by age, with dogs ≤ 8 years of age often being at higher risk of infection compared to those > 8 years old. This is useful information to guide clinical decisions and future studies.

2.9 Acknowledgement

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CHAPTER 3

3.1 Patterns and predictors of antimicrobial resistance among *Staphylococcus* spp. from canine clinical cases presented at a veterinary academic hospital in South Africa.

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3.2 Abstract

Antimicrobial resistance in staphylococci, often associated with treatment failure, is increasingly reported in veterinary medicine. The aim of this study was to investigate patterns and predictors of antimicrobial resistance among *Staphylococcus* spp. isolates from canine samples submitted to the bacteriology laboratory at the University of Pretoria academic veterinary hospital between 2007 and 2012. Retrospective data of 334 *Staphylococcus* isolates were used to calculate the proportion of samples resistant to 15 antimicrobial agents. The Cochran-Armitage trend test was used to investigate temporal trends and logistic regression models were used to investigate predictors of antimicrobial resistance in *Staphylococcus aureus* and *Staphylococcus pseudintermedius*. Results show that 98.2% (55/56) of the *S. aureus* isolates were resistant to at least one drug while 42.9% were multidrug resistant. Seventy-seven percent (214/278) of the *S. pseudintermedius* isolates were resistant to at least one drug and 25.9% (72/278) were multidrug resistant. Resistance to lincospectin was more common among *S. aureus* (64.3%) than *S. pseudintermedius* (38.9 %). Similarly, resistance to clindamycin was higher in *S. aureus* (51.8%) than *S. pseudintermedius* (31.7%) isolates. There was a significant ($p=0.005$) increase in *S. aureus* resistance to enrofloxacin over the study period. Similarly, *S. pseudintermedius* exhibited significant increasing temporal trend in resistance to trimethoprim-sulphamethoxazole ($p=0.004$), clindamycin ($p=0.022$) and orbifloxacin ($p=0.042$).

However, there was a significant decreasing temporal trend in the proportion of *S. pseudintermedius* isolates resistant to doxycycline (p=0.041), tylosin (p=0.008), kanamycin (p=0.017) and amoxicillin/clavulanic acid (p=0.032). High levels of multidrug resistance and the increasing levels of resistance to sulphonamides, lincosamides and fluoroquinolones among *Staphylococcus* spp. isolates in this study are concerning. Future studies will need to investigate local drivers of antimicrobial resistance to better guide control efforts to address the problem.

Sebopeho le matshwao a meriana e lwantshang kokwana-hloko ya *Staphylococcus* spp. ho tswa ho ditlaleho tse fanweng tsa mafu a dintja tse ileng tsa tekwa Sepetleleng sa Bongaka ba Diphoofole Afrika

3.3 Ketapele

Twantsho ya kokwana-hloko ya *staphylococci*, eo hangata e amahangwang le ho hloleha ha meriana, e tlalehwa e ntse e ja setsi bongakeng ba diphoofole. Sepheo sa boithuto bona ke ho batlisisa sebopeho le matshwao a twantshong ya kokwana-hloko ya *Staphylococcus* spp. ho hlaha disampoleng tsa mafu a dintja tse ileng tsa fanwa laboratoring ya dibaketheria tsa lefapha la bongaka ba diphoofole Yunifeshing ya Tshwane dipakeng tsa 2007 le 2012. Tlathlobisiso ya disampole tse 334 tsa kokwana-hloko ya *Staphylococcus* di ile tsa sebediswa ho batla sekgahla sa disampole tse 15 tsa meriana e lwantshang bolwetse bona. Semetho sa *Cochran-Armitage* se ile sa sebediswa ho fumana sebopeho sa mefuta ya kokwana-hloko ena e le ho fumana boitshwaro ba meriana e lwantshang kokwana-hloko ya *Staphylococcus aureus* le ya *Staphylococcus pseudintermedius*. Sepheo se bontsha hore dipresente tse 98.2 tsa kokwana-hloko ya *S. aureus* di bonahala di lwantsha kapa ho ba manganga a thibelo ho bonyane sethethefatsi se le seng empa dipresente tse 42.9 di bontshitse di le manganga a thibelo ho dithethefatsi tse mmalwa. Dipresente tse 70 (214/278) tsa kokwana-hloko ya *S. pseudintermedius* di bontshitse di le manganga a thibelo bonyane ho sethethefatsi se le seng mme dipresente tse 25.9 (72/278) di ne di le

manganga a thibelo ho dithethefatsi tse mmalwa. Manganga a thibelo ho sethethefatsi sa *lincospectin* e bonahetse boholo ho kokwana-hloko ya *S. aureus* ka dipresente tse 64.3 ho feta ya *S. pseudintermedius* ka dipresente tse 38.9. Ka tsela e tshwanang, ho ba manganga a thibelo ho sethethefatsi sa *clindamycin* ho na le ho phahama ka dipresente tse 51.8 kokwana-hloko ya *S. aureus* ho feta ya *S. pseudintermedius* ka dipresente tse 31.7. Ho bile le keketseho ya ($p=0.005$) ho kokwana-hloko ya *S. aureus* e bileng le bokgoni ba ho ba manganga a thibelo ho sethethefatsi sa *enrofloxacin*, nakong ya boithuto bona. Ka mokgwa o tshwanang, kokwana-hloko ya *S. pseudintermedius* e bontshitse keketseho ntlheng ya ho ba manganga a thibelo ho dithethefatsi tse latelang: *trimethoprim-sulphamethoxazole* ($p=0.004$), *clindamycin* ($p=0.022$) le *orbifloxacin* ($p=0.042$). Leha ho le jwalo, ho bile le ho theoha ho ho holo hwa sekgahla sa manganga a thibelo metswakong e bileng manganga a thibelo ho dithethefatsi tsa *doxycycline* ($p=0.041$), *tylosin* ($p=0.008$), *kanamycin* ($p=0.017$) le *amoxicillin/clavulanic acid* ($p=0.032$). Ho na le ho phahama ho ngongorehisang diphuputsong tsena hwa manganga a thibelo a bonahetseng dithethefatsing tsa *sulphonamides*, *lincosamides* le *fluoroquinolones* malebana le kokwana-hloko ya *Staphylococcus* spp. Diphuputso tse tla etswa kamoso di lokela ho kenyeletsa dintlha tse susumetsang manganga a thibelo merianeng mabapi le dikokwana-hloko e le ho fana ka tataiso ya hore na bothata bona bo ka fengwa jwang.

3.4 Introduction

Resistance of *Staphylococcus* spp. organisms to antimicrobial agents used in dogs may complicate treatments and result in increased morbidity, mortality and financial burdens to the owners (Kim et al., 2006). According to Werckenthin et al. (2001), the prevalence of antimicrobial drug resistance in staphylococcal infections in dogs, cattle and pigs is on the rise. Prescott et al. (2002), in their study conducted in Canada, observed that the majority of *S. intermedius* isolates from dogs were resistant to enrofloxacin and gentamycin. In another study by Hauschild and Wójcik (2007) carried out in Poland, it was reported that 41% and 35% of *S. intermedius* isolates from dogs tested were resistant to erythromycin and clindamycin, respectively. In both studies, the authors attributed the high levels of resistance to changes in the pattern of antimicrobial use for treatment of staphylococcal infections. Moreover, Hauschild and Wójcik (2007) as well as Pellerin et al. (1998) suggest that rapid introduction and the over prescription of new antimicrobial drugs in companion animals may also be contributing to the increased antimicrobial resistance seen in staphylococcal organisms. Hoekstra and Paulton (2002) also reported that the type of organism, site of isolation, sex and age of the dogs is associated with the risk of resistance.

In South Africa, 269 794 kg of parenteral antimicrobials were sold between 2002 and 2004, with penicillins being the most commonly (60%) sold antimicrobial followed by tetracyclines (32%) (Eagar, Swan & Vuuren, 2012). A study by Kudakwashe (2014) on antimicrobial usage patterns among companion animal veterinarians in South Africa reported that the most commonly used antimicrobials by the respondents were cephalosporins (100%) , followed by penicillins (98%), quinolones (95%), and lincosamides (52%). In addition, 28% of the respondents indicated that they did not undertake routine antibiograms in cases of therapeutic failures. This is surprising considering 81% of the respondents indicated that patients returned to the clinic due to treatment failures following use of the prescribed antimicrobials (Kudakwashe, 2014).

Although several studies have reported increased antimicrobial resistance among *S. intermedius* isolates to ampicillin, penicillin and tetracycline (Pellerin et al., 1998; Moodley, Damborg & Nielsen, 2014), not much information is available on antimicrobial resistance trends in *Staphylococcus* spp. in South Africa. Moreover, no work has been done to investigate the predictors of resistance among *Staphylococcus* spp. isolates from clinical cases. Therefore, the aim of this study was to investigate patterns and predictors of antimicrobial resistance among *Staphylococcus* spp. isolates from canine samples submitted to the bacteriology laboratory at the University of Pretoria academic veterinary hospital between 2007 and 2012.

3.5 Materials and Methods

3.5.1 Data collection and preparation

This was a retrospective study of *Staphylococcus* spp. isolates from canine clinical samples submitted to the University of Pretoria bacteriology laboratory as part of the routine diagnostic evaluation of cases presented to the university veterinary academic hospital between January 2007 and December 2012. All samples originated from cases treated at the university hospital. The bacteriology laboratory does not process samples from other sites/clinics/hospitals implying that samples included in the study are all from animals that were treated at the academic hospital. The data were assessed for duplicate entries, mixed infections (i.e., more than one isolate per sample), and if any animals were sampled multiple times during the study period. No duplicates were identified. Moreover, there were no samples with mixed infections (i.e., no samples with more than one isolate). Additionally, the dataset did not contain multiple tests from the same patient. The analyses were performed at isolate-level. However, since each sample had only one isolate, it implies that the results of isolate-level analysis are the same as sample level analysis (i.e., no problem of clustering arises).

A total of 334 confirmed *Staphylococcus* isolates consisting of *S. aureus* or *S. pseudintermedius* isolates were included in this study. Each case included in the analysis had data on the following variables: site of collection, breed, sex, age, date

of submission and the antimicrobial agents tested. The American Kennel Club (AKC) breed classification was modified and used to classify breeds of dogs into the following categories: working, sporting, herding, hound, toy, terrier, nonsporting and mix-breed (American Kennel Club, 2017). *Staphylococcus* species were identified based on their phenotypic characteristics including colony characteristics, catalase, D-mannitol, deoxyribonuclease (DNase) tests, and Gram-staining as described by Quinn et al. (1994).

3.5.2 Antimicrobial susceptibility testing

Staphylococcus isolates were subjected to antimicrobial susceptibility testing against a panel of 15 drugs using the disc diffusion method. The following antimicrobials were included in the panel: 30µg amikacin (AK), 30µg doxycycline (DOX30), 5µg enrofloxacin (ENR), 10µg gentamicin (CN), 10µg ampicillin (AM), 10µg penicillin G (P), 25µg trimethoprim-sulphamethoxazole (co-trimoxazole) (SXT), 30µg chloramphenicol (C), 30µg cephalothin (KF), 30µg kanamycin (K), 2µg clindamycin (MY), 100µg lincospectin (LS100), 5µg orbifloxacin (OBX5), 20/10µg amoxicillin/clavulanic acid (AMC20/10) and 15µg tylosin (TY). Unfortunately, since the laboratory does not routinely assess for methicillin susceptibility, the panel did not include this drug and therefore this study was not able to investigate the resistance of *Staphylococcus* spp. to methicillin. The laboratory from where the data were obtained, follows the Clinical and Laboratory Standards Institute (CLSI)

procedures (Clinical and Laboratory Standards Institute, 2007, 2015, Clinical and Laboratory Standards Institute & Standards, 2010, 2011; Clinical and Laboratory Standards Institute & Standards, 2012; Clinical and Laboratory Standards Institute & Institute, 2013) for isolation, testing and classification to determine the susceptibility profile (Susceptible, Intermediate or Resistant) of *Staphylococcus* isolates. The original raw data with exact diameter measurements of the inhibition zones were not available for this retrospective study. Thus, only interpretations of the susceptibility test results (i.e., Susceptible, Intermediate or Resistant) were available. Therefore, although a newer version of the CLSI document (Clinical and Laboratory Standards Institute, 2015) is currently available, it was not possible to interpret the susceptibility profile of isolates using this newer version of the document. For the purposes of the study, intermediate susceptibility was considered as susceptible and therefore re-coded as such for all subsequent analyses.

3.5.3 Data analysis

All the statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) statistical package. The data were assessed for missing data and inconsistencies such as improbable values. Age was categorised into <2 years, 2-4 years, 4-6 years, 6-8 years and >8 years. The frequencies and proportions of all categorical variables together with their 95% confidence intervals were calculated. Associations between categorical variables were assessed using Chi-square or

Fishers Exact tests where appropriate. The Cochran–Armitage trend tests were used to assess temporal trends in the proportion of samples resistant to each antimicrobial agent between 2007 and 2012. Statistical significance was assessed at 5% level of significance for all the above tests.

Investigation of the predictors of antimicrobial resistance (resistance to at least one antimicrobial) was performed in two steps. In the first step, univariable logistic regression models were fitted with antimicrobial resistance status (yes/no) as the outcome and each of the suspected predictors available in the dataset (age, sex, breed) as the explanatory variables. For each of the simple binary univariable regression models, the predictor variables with p-values less than 0.20 were considered for inclusion in the multivariable logistic regression model fit in the second step of the modeling effort. In this second step, a multivariable logistic regression model using manual backwards selection was fitted containing all variables that had potential univariable associations ($p < 0.2$) with the outcome. Confounding was assessed by comparing the change in parameter estimate of the variables in the model with and without the suspected confounding variable. If there was a 20% change in the estimate, the variable of interest was considered to be a significant confounder and was retained in the final model. The odds ratios and their corresponding 95% confidence intervals were computed for all variables included in the final model. The predictor variables with p-values < 0.05 were considered to be

statistically significant based on the Wald Chi-Squared Test. Steps 1 and 2 above were repeated with multi-drug resistance compared to no multidrug resistance (resistance to three or more antimicrobial classes) as the outcome variable. Goodness of fit of the final models was assessed using the Hosmer-Lemeshow goodness of fit test.

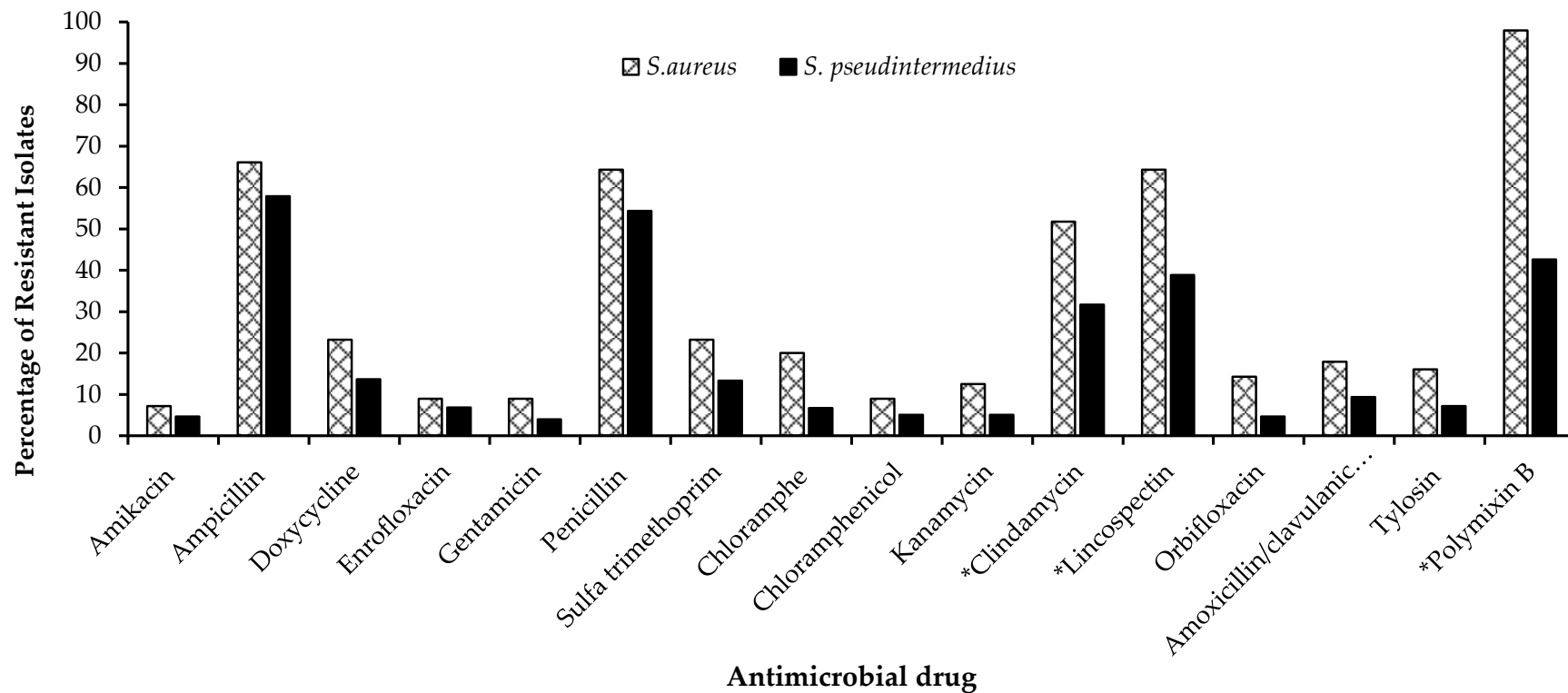
3.6 Results

3.6.1 Antimicrobial resistance patterns

Of the 1,497 samples tested, 26.5% (396/1497) were *Staphylococcus* positive and included *S. aureus* (n=57), *S. epidermidis* (n=11), *S. pseudintermedius* (n=284), *Staphylococcus. spp.* (n=43) and *S. felis* (n=1). Seven isolates [*S. pseudintermedius* (n=6) and *S. aureus* (n=1)] were not included in subsequent analyses due to missing information. Therefore, this study focuses only on the 334 samples that were positive for *S. pseudintermedius* (n=278) or *S. aureus* (n=56) and that did not have missing information. Twenty-five different types of specimens tested positive for *Staphylococcus* spp. with the skin contributing the most samples (34.4%, 115/334), followed by ear canal (29.9%, 100/334) while the rest of the 23 different specimen types making up the remaining 35.6% (119/334).

Over 50% of the *S. aureus* isolates were resistant to ampicillin (66.1%), penicillin (64.3%), lincospectin (64.3%), and clindamycin (51.8%). Similarly, over 50% of the *S. pseudintermedius* isolates were resistance to ampicillin (57.9%) and penicillin (54.3%) (Figure 3.1). In addition, significant differences were observed in the proportion of *S. pseudintermedius* and *S. aureus* resistance to lincospectin ($p=0.0006$) and clindamycin ($p=0.0055$) (Figure 3.1). In both instances, the proportion of *S. aureus* isolates were significantly higher than those of *S. pseudintermedius* isolates. These

results show that the level of resistance among *Staphylococcus* isolates from dogs presented at the teaching hospital was very high, and also showed that *S. aureus* tended to exhibit higher resistance levels to certain antimicrobials compared to *S. pseudintermedius*.



*=Significant ($p < 0.05$) differences in the percentage of resistant isolates between *S. aureus* and *S. pseudintermedius*

Figure 3. 1: Proportions of *S. aureus* and *S. pseudintermedius* isolates resistant to each of the fifteen antimicrobials tested at University of Pretoria bacteriology laboratory, 2007 and 2012.

3.6.2 Temporal patterns in resistance patterns of *S. aureus* and *S. pseudintermedius*

The proportions of *S. aureus* isolates resistant to the different antimicrobials is shown in Table 3.1. Cochran-Armitage trend test showed that the proportion of *S. aureus* isolates that were resistant to doxycycline ($p=0.0412$) or tylosin ($p=0.0083$) significantly decreased from 37.5% in 2007 to 0.0% in 2012, while the proportion resistant to kanamycin significantly ($p=0.0167$) decreased from 26.7% in 2008 to 0.0% in 2012. Similarly, a significant ($p=0.0317$) decrease (37.5% to 0%) in the proportion of *S. aureus* isolates resistant to amoxicillin/clavulanic acid was observed between 2007 and 2011. However, increasing levels of resistance among *S. aureus* isolates to amoxicillin/clavulanic acid emerged by the end of 2012. A significant ($p=0.0052$) increase in the level of resistance to enrofloxacin among *S. aureus* isolates was also observed between 2007 and 2012 (Figure 3.2).

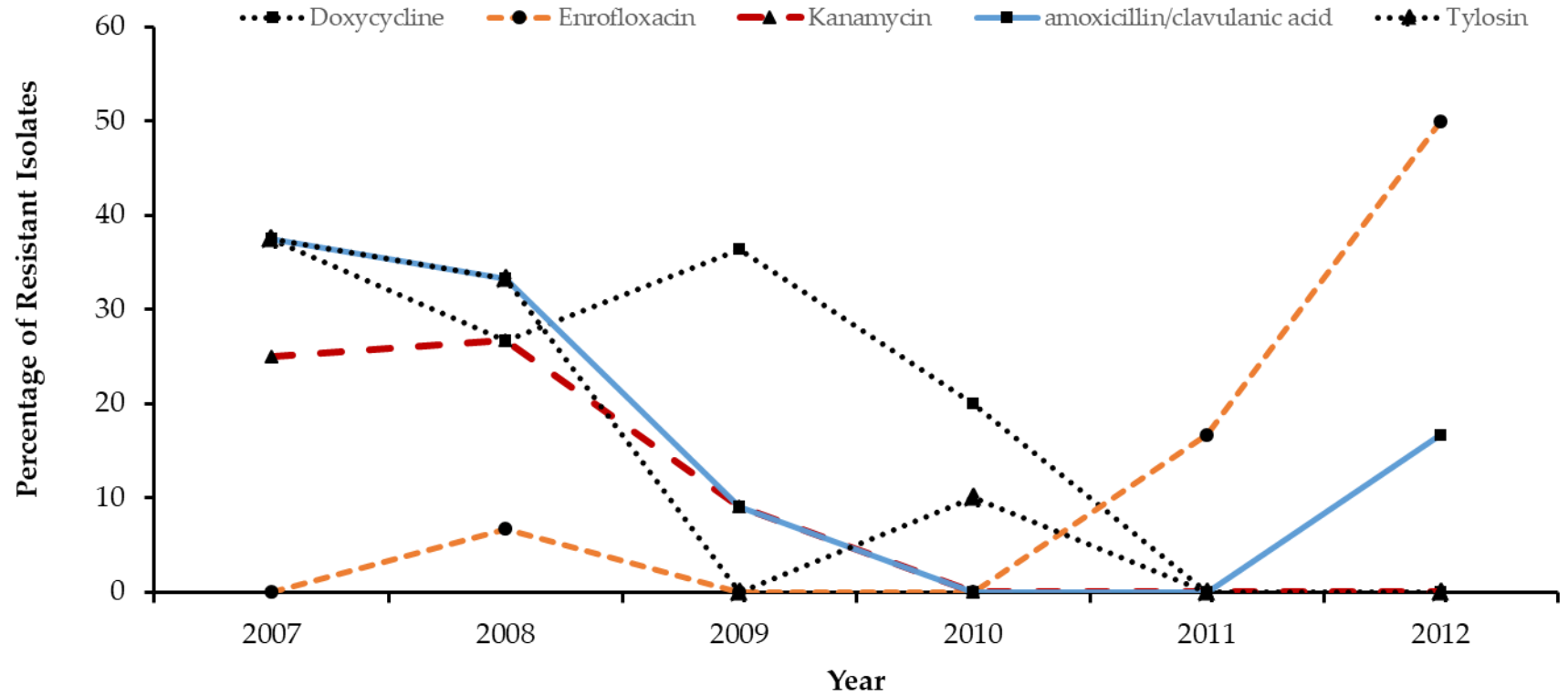


Figure 3. 2: Antimicrobial agents showing significant temporal trends in resistance based on the Cochran-Armitage tests among the *S. aureus* isolates from canine samples submitted to the bacteriology laboratory between 2007-2012.

Table 3. 1: Trends in antimicrobial resistance of *S. aureus* from samples tested at the University of Pretoria bacteriology laboratory, 2007-2012.

Group	Antimicrobials	Resistance to antimicrobial agents by year						¹ P-values of CAT Test
		2007	2008	2009	2010	2011	2012	
β-lactams	Penicillin	75.0 (6/8)	80.0 (12/15)	63.6 (7/11)	50.0 (5/10)	50.0 (3/6)	50.0 (3/6)	0.089
	Ampicillin	75.0 (6/8)	80.0 (12/15)	63.6 (7/11)	60.0 (6/10)	50.0 (3/6)	50.0 (3/6)	0.124
	Cephalothin	25.0 (2/8)	6.7 (1/15)	9.1 (1/11)	10.0 (1/10)	0.0 (0/6)	0.0 (0/6)	0.181
Aminoglycosides	Amikacin	0.0 (0/8)	20.0 (3/15)	9.1 (1/11)	0.0 (0/10)	0.0 (0/6)	0.0 (0/6)	0.258
	Gentamicin	12.5 (1/8)	20.0 (3/15)	9.1 (1/11)	0.0 (0/10)	0.0 (0/6)	0.0 (0/6)	0.098
	Kanamycin	25.0 (2/8)	26.7 (4/15)	9.1 (1/11)	0.0 (0/10)	0.0 (0/6)	0.0 (0/6)	0.017
Tetracyclines	Doxycycline	37.5 (3/8)	26.7 (4/15)	36.4 (4/11)	20.0 (2/10)	0.0 (0/6)	0.0 (0/6)	0.041
Fluoroquinolones	Enrofloxacin	0.0 (0/8)	6.7 (1/15)	0.0 (0/11)	0.0 (0/10)	16.7 (1/6)	50.0 (3/6)	0.005
	Orbifloxacin	12.5 (1/8)	20.0 (3/15)	9.1 (1/11)	0.0 (0/10)	16.7 (1/6)	33.3 (2/6)	0.718
Potentiated-sulfas	Co-trimoxazole ²	37.5 (3/8)	33.3 (5/15)	27.3 (3/11)	10.0 (1/10)	0.0 (0/6)	16.7 (1/6)	0.067
Amphenicols	Chloramphenicol	33.3 (1/3)	20.0 (3/15)	9.1 (1/11)	10.0 (1/10)	40.0 (2/5)	33.3 (2/6)	0.552
Macrolides	Tylosin	37.5 (3/8)	33.3 (5/15)	0.0 (0/11)	10.0 (1/10)	0.0 (0/6)	0.0 (0/6)	0.008
Aminoglycoside-lincosamides	Lincospectin ³	37.5 (3/8)	80.0 (12/15)	54.6 (6/11)	70.0 (7/10)	50.0 (3/6)	83.3 (5/6)	0.478
Lincosamides	Clindamycin	50.0 (4/8)	60.0 (9/15)	45.5 (5/11)	60.0 (6/10)	50.0 (3/6)	33.3 (2/6)	0.552
Others	Amoxicillin/clavulanic acid	37.5 (3/8)	33.3 (5/15)	9.1 (1/11)	0.0 (0/10)	0.0 (0/6)	16.7 (1/6)	0.032

¹ P-values of CAT Test= Cochran-Armitage trend test

² Co-trimoxazole = Trimethoprim-sulphamethoxazole

³ Lincospectin = Espectinomycine-lincomycine

The distributions of resistance among *S. pseudintermedius* isolates to the different antimicrobials is shown in Table 3.2. Significant increases in the proportions of resistant isolates to trimethoprim-sulphamethoxazole ($p=0.0040$), clindamycin ($p=0.0221$) and orbifloxacin ($p=0.0418$) were observed among *S. pseudintermedius* isolates between 2007 and 2012 (Figure 3.3, Table 3.2). Among *S. pseudintermedius* isolates, the proportion that were resistant to clindamycin increased until 2011 (59%) but subsequently decreased to 28% in 2012, while, the proportion of isolates that were resistant to orbifloxacin increased from 0% to 11% by 2012. Similarly, we observed an increase in the proportion of isolates resistant to trimethoprim-sulphamethoxazole from 2.5% in 2007 to 22% in 2012 among *S. pseudintermedius* isolates.

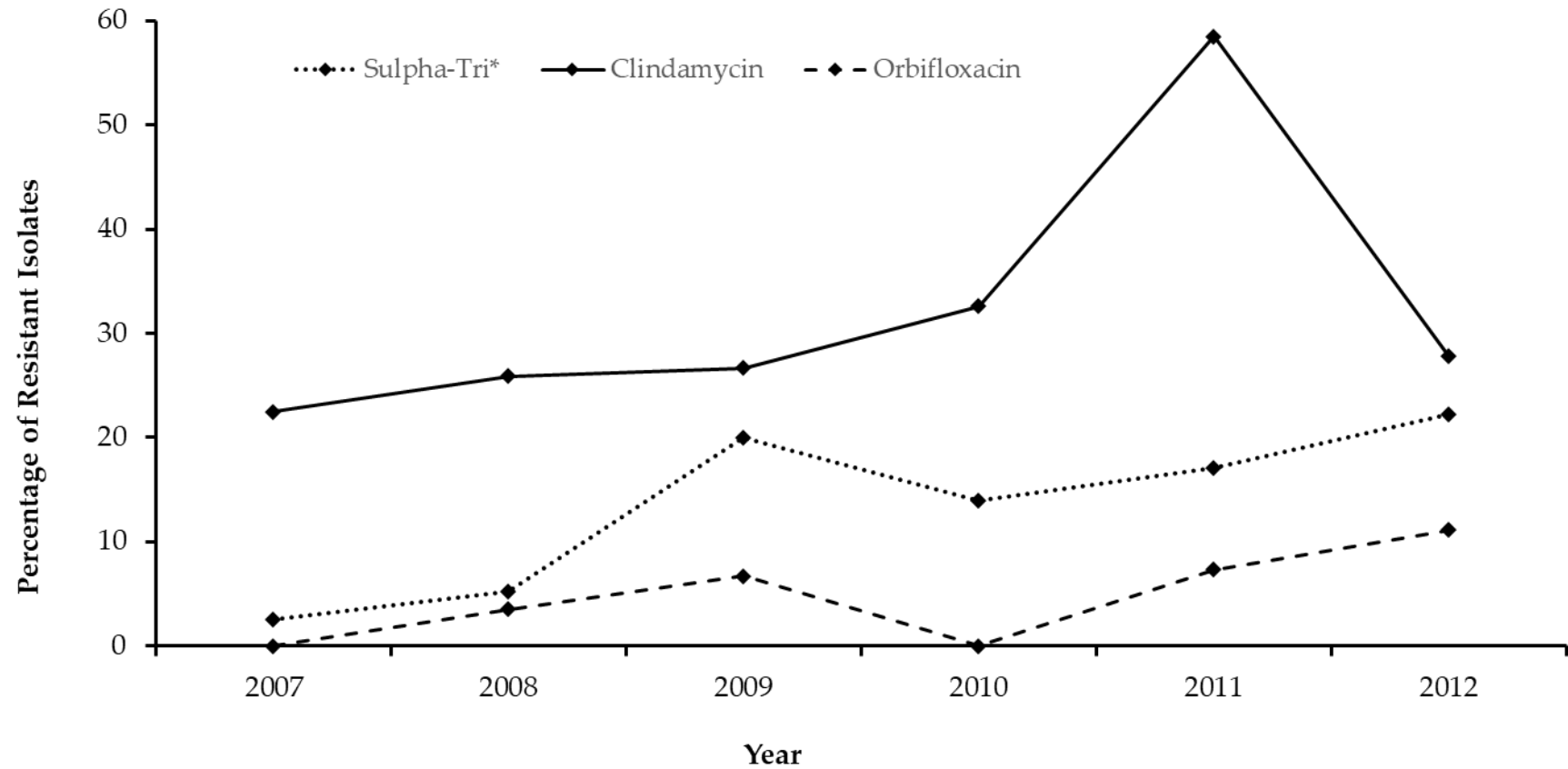


Figure 3. 3: Antimicrobial agents showing significant temporal trends in resistance based on the Cochran Armitage tests among the *S. pseudintermedius* isolates from canine samples submitted to the bacteriology laboratory between 2007-2012.

Table 3. 2: Trends in antimicrobial susceptibility of *S. pseudintermedius* to antimicrobial agents from samples tested at the University of Pretoria academic veterinary laboratory, 2007-2012.

Group	Antimicrobial	Resistance to antimicrobial agents by year						¹ P-value of CAT Test
		2007	2008	2009	2010	2011	2012	
β-lactams	Penicillin	37.5 (15/40)	58.6 (34/58)	50.0 (30/60)	51.2 (22/43)	73.2 (30/41)	55.6 (16/36)	0.052
	Ampicillin	50.0 (20/40)	56.9 (33/58)	53.3 (32/60)	55.8 (24/43)	73.2 (30/41)	61.1 (22/36)	0.104
	Cephalothin	7.5 (3/40)	5.2 (3/58)	5.0 (3/60)	2.3 (1/43)	7.3 (3/41)	2.8 (1/36)	0.555
Aminoglycosides	Amikacin	0.0 (0/40)	13.8 (8/58)	3.3 (2/60)	0.0 (0/43)	4.9 (2/41)	2.8 (1/36)	0.382
	Gentamicin	0.0 (0/40)	10.3 (6/53)	3.3 (2/60)	0.0 (0/43)	2.4 (1/41)	5.6 (2/36)	0.777
	Kanamycin	2.5 (1/40)	3.5 (2/58)	8.3 (5/60)	4.7 (2/43)	4.9 (2/41)	5.6 (2/36)	0.612
Tetracyclines	Doxycycline	12.5 (5/40)	22.4 (13/58)	15.0 (9/60)	2.3 (1/43)	17.1 (7/41)	8.3 (3/36)	0.213
Fluoroquinolones	Enrofloxacin	5.0 (2/40)	5.2 (3/58)	6.7 (4/60)	4.7 (2/43)	4.9 (2/41)	16.7 (6/36)	0.122
	Orbifloxacin	0.0 (0/40)	3.5 (2/58)	6.7 (4/60)	0.0 (0/43)	7.3 (3/41)	11.1 (4/36)	0.042
Potentiated sulfas	Co-trimoxazole ²	2.5 (1/40)	5.2 (3/58)	20.0 (3/60)	14.0 (2/41)	17.1 (7/41)	22.2 (8/36)	0.004
Amphenicols	Chloramphenicol	4.8 (1/21)	7.0 (4/57)	5.0 (3/60)	4.9 (2/41)	12.8 (5/36)	5.6 (2/36)	0.626
Macrolides	Tylosin	7.5 (3/40)	6.9 (4/58)	6.7 (4/60)	7.0 (3/43)	9.8 (4/41)	5.6 (2/36)	1
Aminoglycoside-lincosamides	Lincospectin ³	22.5 (9/40)	48.3 (28/58)	26.7 (16/60)	39.5 (17/43)	58.5 (24/41)	38.9 (14/36)	0.066
Lincosamides	Clindamycin	22.5 (9/40)	25.9 (15/58)	26.7 (16/60)	32.6 (14/43)	58.5 (24/41)	27.8 (10/36)	0.022
Others	Amoxicillin/clavulanic acid	22.5 (9/40)	6.9 (4/58)	5.0 (3/60)	2.3 (1/43)	17.1 (7/41)	5.6 (2/36)	0.225

¹P-value of CAT Test= Cochran-Armitage trend test

² Co-trimoxazole = Trimethoprim-sulphamethoxazole

³ Lincospectin= Espectinomycine-lincomycine

3.6.3 Proportions of AMR or MDR among isolates of *S. aureus* and *S. pseudintermedius*.

Of the 334 isolates, 80.5% (269/334) exhibited antimicrobial resistance (AMR) to at least one drug, while 28.7% (96/334) were multidrug resistant (MDR). AMR was significantly ($p=0.0003$) more common among *S. aureus* (98.2%, 55/56) than *S. pseudintermedius* (76.98%, 214/278). Similarly, MDR was also significantly ($p=0.0105$) more frequent among *S. aureus* (42.9%, 24/56) than *S. pseudintermedius* (25.9%, 72/278).

No significant temporal trends were observed in the proportion of AMR *S. aureus* ($p=0.1580$) or *S. pseudintermedius* ($p=0.7312$) between 2007 and 2012 (Figure 3.4). However, the proportion of AMR samples was significantly ($p=0.0003$) higher among *S. aureus* than *S. pseudintermedius*. For example, between 2007 and 2012 the proportion of AMR *S. aureus* ranged from 87.5% to 100% compared to 68% to 84% among *S. pseudintermedius* isolates.

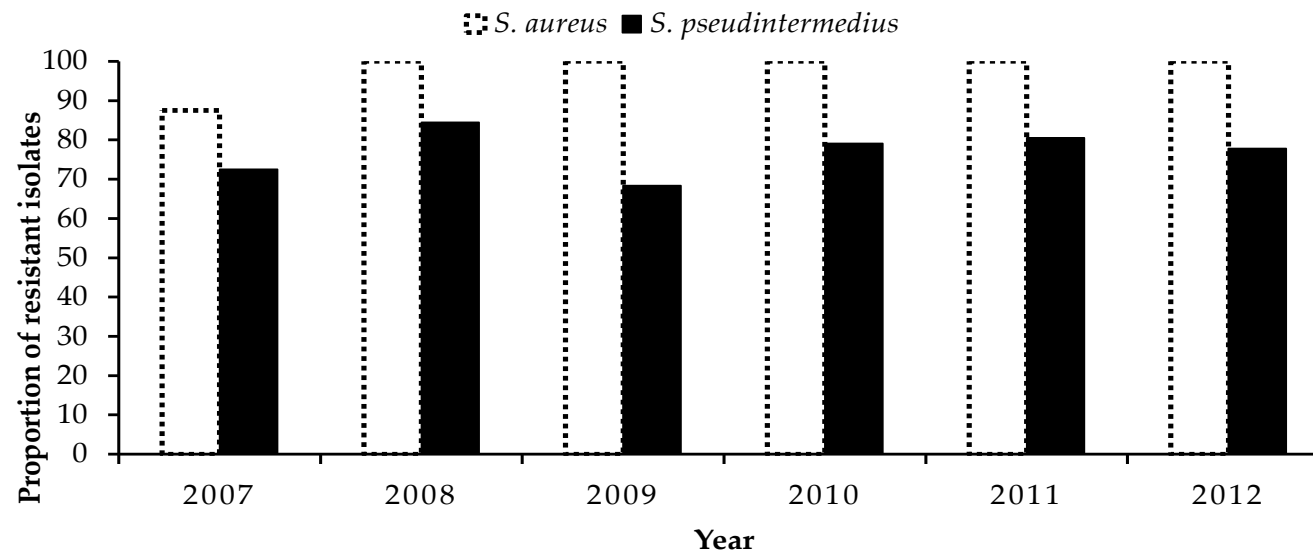


Figure 3. 4: Proportion of *S. aureus* and *S. pseudintermedius* isolates resistant to at least one antibiotic from samples tested at the University of Pretoria bacteriology laboratory, 2007-2012.

Multidrug resistance *S. aureus* were more common than MDR *S. pseudintermedius* during the period 2007 to 2012 with the exception of 2011. In 2011, 39% of *S. pseudintermedius* were MDR compared to 33% of *S. aureus* isolates. While, between 2007-2010 and 2012 the proportion of MDR *S. aureus* ranged from 53% to 30% compared to 16% to 39% among *S. pseudintermedius* isolates. No significant temporal trends were observed in the proportions of MDR *S. aureus* ($p= 0.8212$) or MDR *S. pseudintermedius* ($p= 0.0932$) (Figure 3.5).

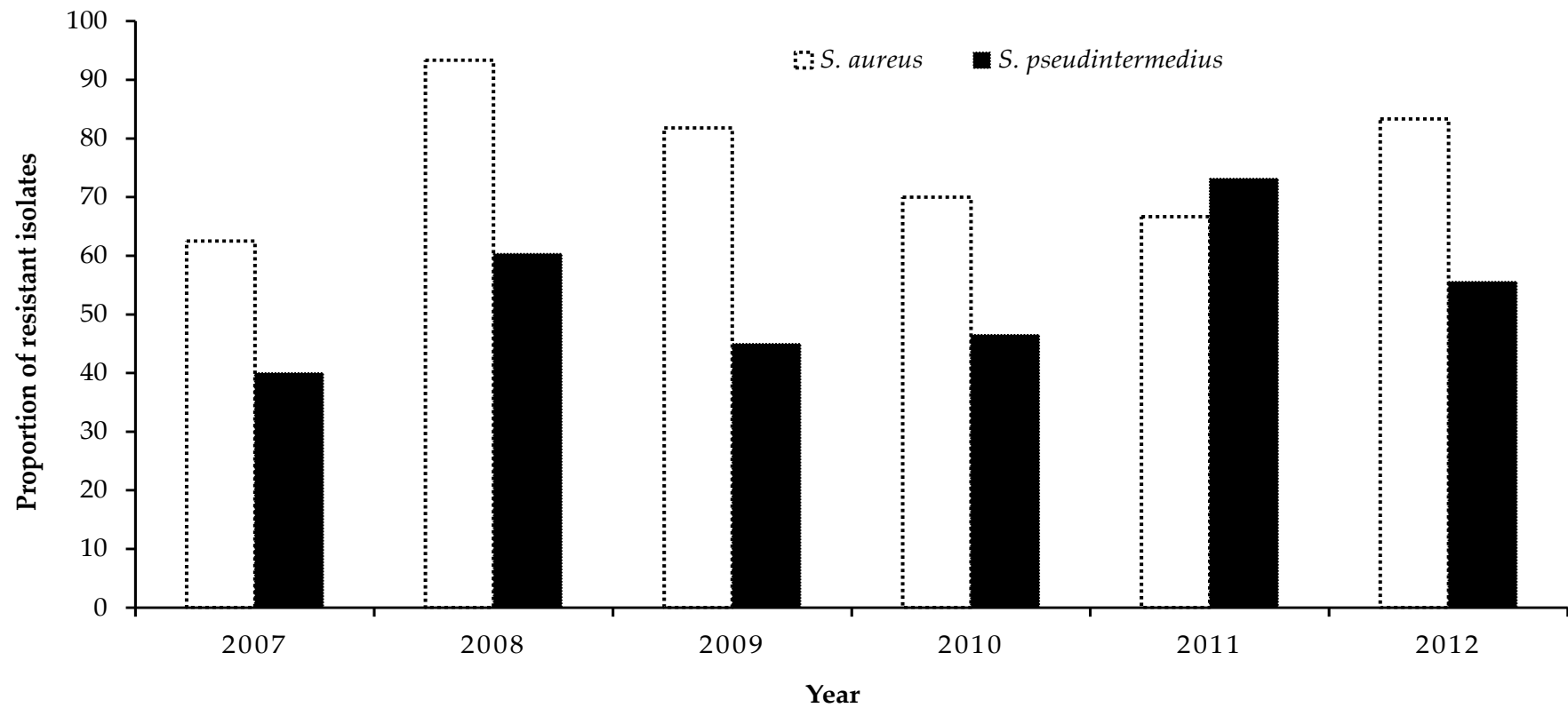


Figure 3. 5: Proportion of multidrug resistant *S. aureus* and *S. pseudintermedius* isolates from samples tested at the University of Pretoria veterinary bacteriology laboratory between 2007 and 2012.

3.6.4 Predictors of AMR or MDR *Staphylococcus* spp.

None of the variables investigated in this study had significant associations with the log odds of either AMR or MDR.

3.7 Discussion

Antimicrobial resistance in veterinary medicine and especially in companion animals is of increasing public health concern (Normand et al., 2000; Werckenthin et al., 2001). This problem is exacerbated by the fact that the relationship between companion animals and their owners have been implicated in the cross-transmission and spread of antimicrobial resistant organisms (van den Bogaard et al., 2000; Guardabassi, Schwarz & Lloyd, 2004; Lloyd, 2007). Since South Africa lacks continuous assessment and reporting of veterinary related staphylococcal infections and antimicrobial resistance, the results of this study contribute significantly to improving our understanding of the trends in antimicrobial resistance among *S. aureus* and *S. pseudintermedius* clinical cases of dogs.

3.7.1 Antimicrobial resistance patterns

In the present study, we observed high proportions of *S. aureus* and *S. pseudintermedius* isolates resistant to β -lactam antibiotics (ampicillin and penicillin). This is consistent with previous studies that have reported significantly higher levels of resistance of *Staphylococcus* species to β -lactam antibiotics compared to other classes of antimicrobials (Malik, Peng & Barton, 2005; Boost, O'Donoghue & James, 2008). This may be associated with the expression of intrinsic low-affinity to penicillin-binding proteins (PBPs) among *Staphylococcus* species (Rice, 2012) or the

excessive use of β -lactam antibiotics in the treatment of *S. aureus* and *S. pseudintermedius* infections (Prescott et al., 2002). High levels of resistance to β -lactam antibiotics observed in this study will undoubtedly affect treatment and management of *Staphylococcus* spp. infections.

A significantly higher proportion of *S. aureus* than *S. pseudintermedius* isolates exhibited resistance to lincosamides (lincospectin and clindamycin). The reasons for this difference are unclear but these findings are consistent with reports of high levels of lincosamide resistance among *S. aureus* and *S. pseudintermedius* in the United States (Gold & Lawhon, 2013). These findings suggest that lincosamides should not be used as alternative drugs in the treatment of staphylococcal infections in this population of dogs (Guardabassi et al., 2008). Of concern is the fact that lincosamides, such as clindamycin, have been generally known to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug resistant staphylococci (Frank & Loeffler, 2012). However, we observed that up to 59% of the *S. pseudintermedius* positive samples were resistant to lincosamides. This seems to suggest that lincosamides should not be considered for treatment of *S. pseudintermedius* infections in this population of dogs without first performing antibiograms.

3.7.2 Temporal patterns

Over the study period, *S. aureus* resistance to doxycycline, kanamycin, amoxicillin/clavulanic acid and tylosin decreased significantly while resistance to fluoroquinolones among *S. aureus* and *S. pseudintermedius* positive samples significantly increased. A study conducted over a 14-year period in Canada by Prescott et al. (2002) reported no significant temporal changes in *S. aureus* resistance to fluoroquinolones. Similar to findings by Pellerin et al. (1998) in France among *S. pseudintermedius* isolates tested between 1987 and 1996, we observed an increasing trend in resistance to trimethoprim-sulphamethoxazole among *S. pseudintermedius*. The observed decrease or increase in antimicrobial resistance in the current study may be due to changes in usage patterns as suggested by other authors (Prescott et al., 2002; Gold & Lawhon, 2013). Moreover, a causal link between trimethoprim-sulphamethoxazole usage and trimethoprim-sulphamethoxazole resistance in patients with urinary tract infections (UTI) has been established (Metlay, Strom & Asch, 2003). It has also been suggested that rapid introduction of fluoroquinolone and trimethoprim-sulphamethoxazole drugs into companion veterinary medicine practice may also be a contributing factor to increased prevalence of resistance (Walker & Thornsberry, 1998). The authors of the present study think that this might be the case in this study. However, further investigations of the antimicrobial

prescription practices among clinicians at the veterinary hospital is needed to better understand this.

Hauschild and Wójcik (2007) reported 88% resistance to at least one antimicrobial drug among canine *Staphylococcus* isolates in Poland, whereas, Lilenbaum et al. (Lilenbaum et al., 2000) in Brazil, reported 90.9% resistance among *Staphylococcus* spp. In our study, 80.5% of the *Staphylococcus* spp. isolates exhibited resistance to at least one antimicrobial drug. With regards to multi-drug resistance, the percentage MDR isolates in the current study was higher (28.7%) than the 24.5% reported by Gandolfi-Decristophoris et al. (2013) in Switzerland and lower than the 34% as reported by Schmidt et al. (Schmidt et al., 2014) in the UK. The reasons for the high MDR among *Staphylococcus* spp isolates in this study is unclear. However, we hypothesise that this may be an early indication of changes in the usage patterns among veterinarians caring for animals whose samples were included in the study. In view of this, more research needs to be done to provide a much clearer picture of the situation.

3.7.3 Distribution and predictors of AMR or MDR

Almost 100% of the *S. aureus* positive samples were resistant to at least one antimicrobial agent compared to 77% of *S. pseudintermedius* positive samples. Although resistance to at least one antimicrobial agent was lower among *S.*

pseudintermedius, it was much higher than 5.2% reported as by Vanni et al. (2009) in Italy and 2-40% as reported by Blunt et al. (2013) in South Africa. Our findings are consistent with reports of higher levels of MDR among *S. aureus* (ranging from 51 to 67%) compared to *S. pseudintermedius* (ranging from 5% to 14%) in the UK (Wedley et al., 2014) and Canada (Hoekstra & Paulton, 2002) respectively. In contrast, Jung-Ho Youn et al. (2014) reported no MDR in *S. aureus* compared to 10.4% MDR among *S. pseudintermedius* in Zambia. The results of this study suggest that MDR is more common in *S. aureus* isolates than *S. pseudintermedius* from dogs presented at the veterinary academic hospital in South Africa.

3.7.4 Study limitations

Hoekstra and Paulton (2002) reported that the site of isolation is a risk factor of resistance. Unfortunately, we could not assess this in the current study due to the large number of categories (site types) and the small sample sizes associated with each category (site type). Some of the categories in some of the sub-analyses performed had low sample sizes and high variances hence lower precision. Previous exposure to antimicrobial agents has been associated with increased risk of development of antimicrobial resistance (van den Bogaard et al., 2000). Unfortunately, due to the retrospective nature of the current study, the history of antibiotic use was not available. Therefore, we could not investigate the association between previous antibiotic use and antimicrobial resistance patterns. Furthermore,

the study focused on canine clinical cases that were submitted to the bacteriology laboratory for diagnosis. Often such cases would include animals that have not responded well to previous treatments. Therefore, it is possible that a large population of dogs that responded to empirical treatments were not included in this study. Some sub-analyses were not possible due to relatively low sample sizes associated with some categories of some variables such as specimen type. Additionally, the time when culture and sensitivity test was done in relation to the time after hospital admission of the dogs was not available making it impossible to distinguish nosocomial from community acquired infections. Moreover, the geographic area covered by the study was limited to Gauteng Province which is not representative of South Africa as a whole nor is it representative of other veterinary hospitals in South Africa. These limitations, notwithstanding, the findings from this study provide useful information to guide future studies to better understand antimicrobial resistance in dogs.

3.8 Conclusion

Antibiotic resistance among *Staphylococcus* spp. from dogs presented to the veterinary academic hospital was high and continued to increase for enrofloxacin, trimethoprim-sulphamethoxazole, clindamycin and orbifloxacin during the 7-year study period. Of concern are the increasing levels of resistance to fluoroquinolones

and sulphonamides among *S. pseudintermedius*. This calls for urgent action to address the problem. The actions may include development of an antimicrobial stewardship program for veterinary and para-veterinary personnel to be offered by the university as part of continuing education. Furthermore, training of veterinary students should have a strong emphasis on antimicrobial stewardship. Lastly, the need for *Staphylococcus* species characterization and request for antibiogram as part of the protocol for diagnosis and treatment of *Staphylococcus* spp. infections should be emphasized and encouraged.

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CHAPTER 4

4.1 Geographic distribution of *Staphylococcus* spp. infections and antimicrobial resistance among dogs presented at a veterinary teaching hospital in South Africa.

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My contributions to this paper included data preparation, analysis, interpretation of results gathering and reviewing of literature, formulation of discussion topics, as well as drafting and editing the manuscript.

4.2 Abstract

The objective of this study was to investigate spatial patterns of staphylococcal infections and antimicrobial drug resistance patterns of clinical isolates from dogs tested at a veterinary teaching hospital (VTH) in South Africa. Data from records of 1,497 dog clinical samples submitted to the VTH between 2007 and 2012 were used in this study. Spatial empirical Bayesian smoothed risk maps were used to investigate spatial patterns of staphylococcal infections, antimicrobial resistance (AMR), and multidrug resistance (MDR). Moran's I and spatial scan statistics were used to investigate spatial clusters at municipal and town spatial scales. Significant clusters of staphylococcal infections were identified at both the municipal (Relative Risk [RR]=1.71, $p=0.003$) and town (RR=1.65, $p=0.039$) scales. However, significant clusters of AMR ($p=0.003$) and MDR ($p=0.007$) were observed only at the town scale. Future larger studies will need to investigate local determinants of geographical distribution of the clusters so as to guide targeted control efforts.

Maemo a dibaka tseo tshwaetso ya kokwana-hloko ya *Staphylococcus* spp. e susumetsang boemo ba manganga a thibelo ho meriana ho dintja, e leng tokomane e ileng ya tekwa setsing sa boithuto sa sepetlele sa bongaka Afrika Borwa.

4.3 Ketapele

Sepheo sa boithuto bona ke ho fuputsa boleng ba tshwaetso ya kokwana-hloko ya boemo ba *Staphylococcus* mmoho le boleng ba manganga a thibelo ho meriana ho shebanwe le dintja tse ileng tsa hlahlojwa setsing sa boithuto sa sepetlele sa bongaka (VTH) Afrika Borwa. Lesedi ho hlaha direkteng tse 1,497 tsa disampole tsa mafu a dintja tse fanweng ho VTH dipakeng tsa 2007 le 2012 di sebedisitswe diphuputsong tsena.

Sekala sa *Spatial Empirical Bayesian* se ile sa sebediswa ho hlahloba dikarolo tsa tshwaetso kokwana-hlokong ya *Staphylococcus*, manganga a thibelo ho dithethefatsi ka bomong (AMR) le manganga a thibelo ho dithethefatsi tse mmalwa (MDR). Semetho sa *Moran's I* le sekenara sa dipalopalo se ile sa sebediswa ho fuputsa dibaka boemong ba ditlelasetara tsa bommasepala le ditoropong. Boholo ba ditlelasetara tsa tshwaetso ya *Staphylococcus* di ile tsa hlwauwa sebakeng sa mmasepala (*Relative Risk [RR]=1.71, p=0.003*) le ditoropong (*RR=1.65, p=0.039*). Leha ho le jwalo, boholo ba ditlelasetara tsa AMR ($p=0.003$) le tsa MDR ($p=0.007$) di ile tsa bonwa boemong ba ditoropo. Diphuputso tse kgolo tsa kamoso di tla lokela ho shebisisa ditshiya tsa lehae tse amanang le dibaka tsa ditlelasetara tsa dibaka e le tataiso ntlheng ya taolo.

4.4 Introduction

Although *Staphylococcus* spp. are commensals on the skin and mucosal surface of dogs, association between colonization and the risk of infection with these organism has been reported (Biberstein, Jang & Hirsh, 1984; Beça et al., 2015). *Staphylococcus* spp. are the leading causes of pyoderma, otitis media, and wound infections in companion animals such as dogs (Ganiere, Medaille & Mangion, 2005; Weese et al., 2006; Kroemer et al., 2014). However, in South Africa in particular, the burden of staphylococcal infections among dogs presented at veterinary hospitals has not been investigated. Prevalence of *Staphylococcus* spp. infections among healthy and clinical dog cases have been shown to vary greatly (Duquette & Nuttall, 2004; Ganiere, Medaille & Mangion, 2005; Gandolfi-Decristophoris et al., 2013). Moreover, in South Africa, the incidence of *Staphylococcus* spp. among dogs is reported to be on the rise (Qekwana et al., 2017a). These findings are of public health significance because transmission of infections from dogs to humans have been reported following exposure to carrier or infected dogs (Guardabassi, Schwarz & Lloyd, 2004; Boost, O'Donoghue & Siu, 2007; Faires, Tater & Weese, 2009; Frank et al., 2010; Pantosti, 2012; Pompilio et al., 2015).

Of concern is the significantly higher proportion of *S. aureus* and *S. pseudintermedius* isolates resistant to lincosamides, fluoroquinolones and trimethoprim-sulphamethoxazole among dogs in South Africa (Qekwana et al., 2017b). Another South

African study by Blunt et al. (2013) also reported high proportions *S. pseudintermedius* isolates resistant to ampicillin and doxycycline among pyoderma cases in dogs. This is not surprising as resistance to various antimicrobial agents among *S. aureus* and *S. pseudintermedius* in dogs has also been reported in other studies (Werckenthin et al., 2001; Hoekstra & Paulton, 2002; Prescott et al., 2002; Guardabassi, Schwarz & Lloyd, 2004).

Although reports by Qekwana et al. (2017a,b) suggest that variations in risks of *Staphylococcus* infections and antimicrobial resistance among the *Staphylococcus* isolates are due, in part, to host risk factors, it is quite possible that local environmental factors might also play a role. Therefore, studies investigating the spatial epidemiology of *Staphylococcus* spp. infections, antimicrobial resistance (AMR) and multi-drug resistance (MDR) among *Staphylococcus* spp. isolates are needed to help identify the geographic distribution of these infections. This information is useful to help better predict the risk of *Staphylococcus* spp. infection and resistance in both humans and companion animals to guide control efforts (Pfeiffer et al., 2008).

After disease clusters are identified the information gathered can be used to assess potential factors associated with disease occurrence in identified regions and help develop mitigation strategies. Kulldorff's spatial scan statistics, implemented in SaTScan™ (Kulldorff et al., 1998), has been successfully used in a number of

epidemiological studies to detect and evaluate disease clusters (Kulldorff et al., 1998; Kulldorf, 1999; Haddow, Bixler & Odoi, 2011; Saman, Walsh, et al., 2012). Grundmann and co-workers used spatial scan statistics to investigate clustering of methicillin resistance *Staphylococcus aureus* (MRSA) in Europe and reported existence of regional clusters of MRSA isolates in their study region (Grundmann et al., 2010). It is possible that risks of *Staphylococcus* infections, AMR and MDR among *Staphylococcus* spp. isolates in South Africa also exhibit a spatial pattern that if identified would guide control efforts. Therefore, the objective of this study was to investigate spatial patterns of risks of *Staphylococcus* spp. infections, AMR and MDR among *Staphylococcus* isolates from dogs presented at a veterinary teaching hospital in South Africa.

Materials and Methods

4.4.1 Study Area

This study was conducted in Gauteng province, located in the Highveld region of South Africa. The province is estimated to be approximately 18,178 km² in size with a population of 12.3 million people. It is surrounded by four provinces: Free State province to the South, North-West province to the west, Limpopo to the north, and Mpumalanga to the east. Gauteng has nine administrative municipalities: Ekurhuleni, Emfuleni, Midvaal, Mogale City, Randfontein, Westonaria, Johannesburg, Tshwane and Lesedi (Figure 4.1). The province has a subtropical climate with an annual summer rainfall of approximately 700 mm. It has four seasons: summer (November-March), autumn (April-May), winter (June-August) and spring (September-October).

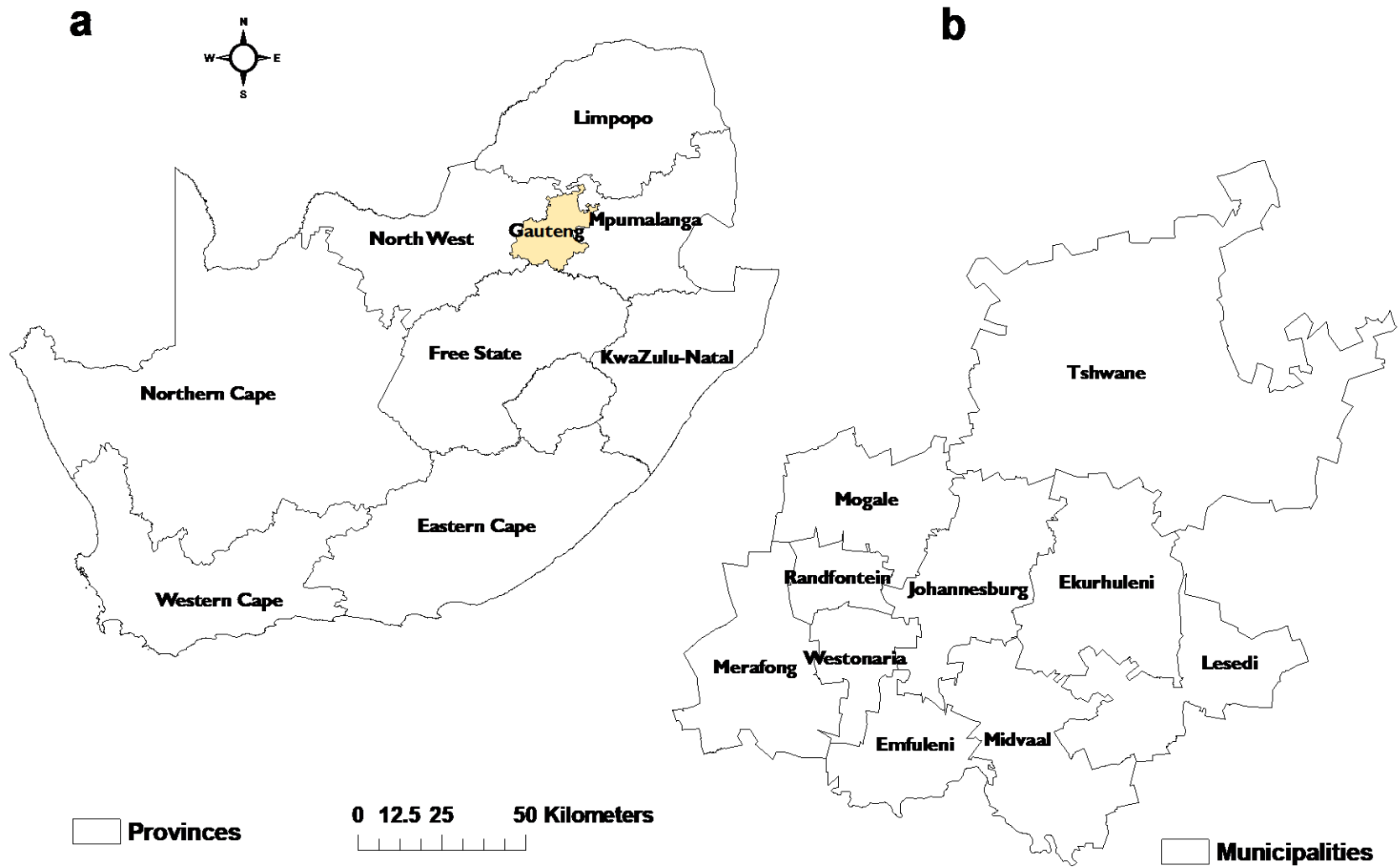


Figure 4. 1:Map of South Africa showing the location of the study area: (a) Gauteng Province and (b) local municipalities of Gauteng Province.

4.4.2 Data Source

Laboratory records of clinical samples from dogs presented at the University of Pretoria VTH for microbiological diagnosis between January 2007 and December 2012 were included in this study. Of the 1,479 samples from Gauteng province, 382 were *Staphylococcus* positive and were included in subsequent analyses. The following fields were extracted from each record: residential address, submitted specimen-type, *Staphylococcus* species isolated and antimicrobial susceptibility test results. The data were inspected for inconsistencies such as missing and incorrect addresses, assessed for duplicate entries and if any animals were sampled multiple times during the study period. No duplicates were identified and the dataset did not contain multiple tests from the same patient. There were also no results of mixed infections. All cases were geocoded using GIS software, ArcView GIS10.1 (ESRI Inc., Redlands, California, USA) and linked to the municipal and town-level polygon shape files.

4.4.3 Data Analysis

The proportions of *Staphylococcus* positive samples and antimicrobial resistant *Staphylococcus* isolates were calculated at local municipal and town spatial scales using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) statistical package.

The proportions of *Staphylococcus* positive samples and antimicrobial resistance among *Staphylococcus* isolates at the town spatial resolution were smoothed using

spatial empirical Bayesian (SEB) smoothing in GeoDa (Anselin, Syabri & Kho, 2006) using first-order queen contiguity spatial weights. In small area mapping, spatial empirical Bayesian smoothing adjusts for spatial autocorrelation and non-homogeneity of variance associated with differences in sample sizes across geographic units (in this case, towns) under study (Cuzick & Edwards, 1990; Bernardinelli & Montomoli, 1992; Pfeiffer et al., 2008; Haddow, Bixler & Odoi, 2011; Pedigo, Aldrich & Odoi, 2011; Saman, Cole, et al., 2012).

4.4.4 Detection of Spatial Clusters

Global Moran's I was used to assess spatial clustering of unsmoothed proportions of *Staphylococcus* infections and AMR among *Staphylococcus* isolates at both local municipal and town spatial scales. Significance of Moran's I was assessed using 9999 permutations (Anselin, Syabri & Kho, 2006).

Spatial scan statistics software, developed by Kulldorff (Kulldorff & Nagarwalla, 1995), was used to identify local clusters of *Staphylococcus* spp. infections and AMR at town spatial scale. In this study, retrospective purely spatial analysis using Poisson models were used to identify significant purely spatial high risk clusters of *Staphylococcus* infections and AMR. No geographic overlap of the clusters were allowed. The maximum cluster size was left at a 50% of the total population at risk and the significance of the clusters was assessed at $\alpha=0.05$ using 9999 Monte Carlo replications.

ArcView GIS (version10.1) was used to display the results of identified clusters. The following information was reported for the identified clusters: location, number of towns, numbers of observed and expected cases, relative risk (RR) and p-value.

4.5 Results

At the local municipal level, Johannesburg had the highest (43.9%) unsmoothed proportion of *Staphylococcus* positive samples followed by the East Rand local municipalities (31.2%). Similarly, the results of the SEB smoothing showed higher proportions of *Staphylococcus* isolates in western and south-central parts of the province (Table 4.1, Figure 4.2a). Although similar patterns were observed at the town spatial scale with the risk ranging from 47% to 56%, this spatial scale provided more detail in spatial heterogeneity than the municipal spatial scale (Figure 4.2b).

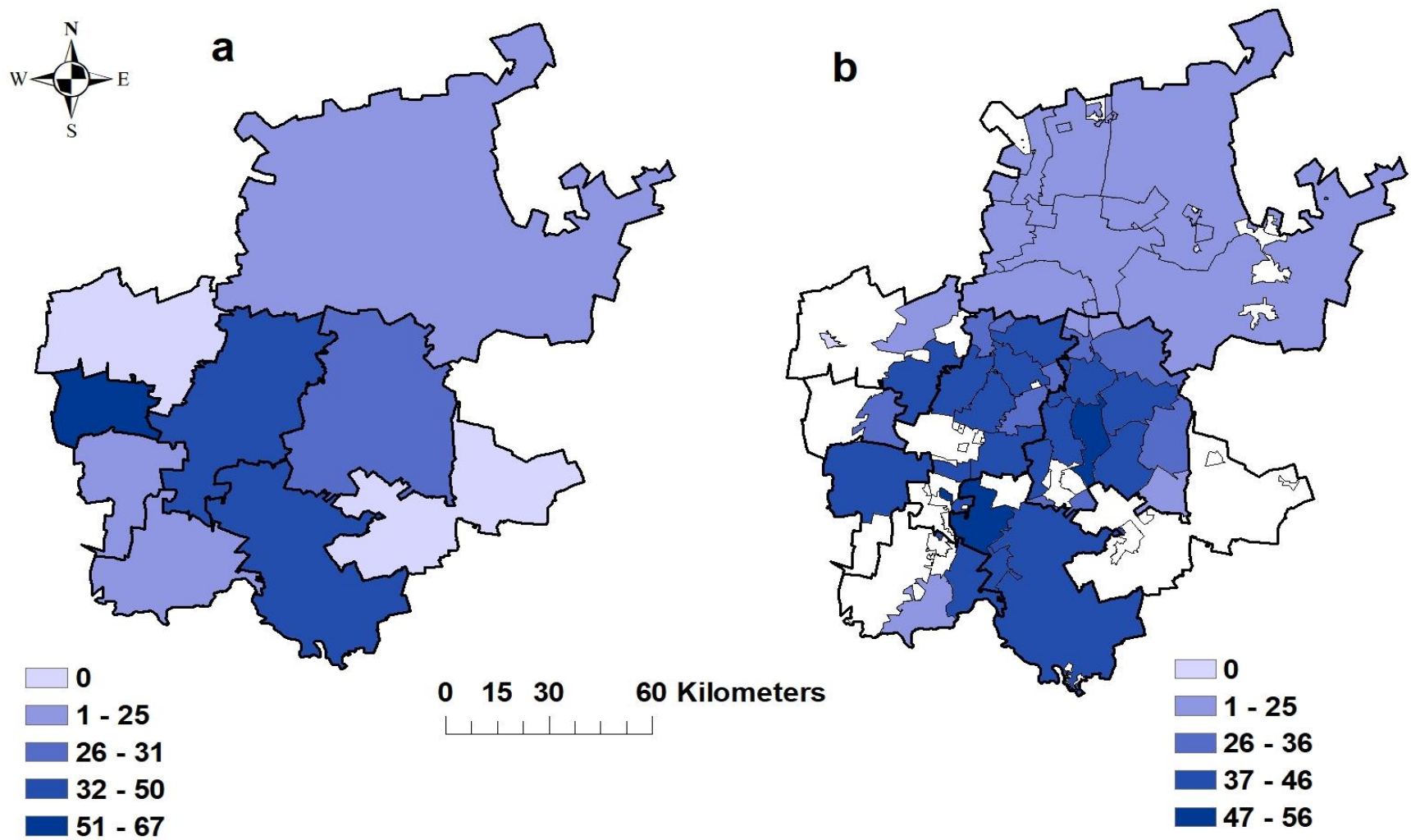


Figure 4. 2: Geographic distribution of the percentage of *Staphylococcus* positive isolates at (a) municipality spatial scale and (b) town spatial scale.

Table 4. 1: Distribution of *Staphylococcus* infections based on clinical samples tested at the bacteriology laboratory of a veterinary teaching hospital, 2007 and 2012.

	Samples processed		<i>Staphylococcus</i> positive samples	
	Number	Percent (95% CI)	Number	Percent
Metropolitan municipality				
City of Tshwane	1294	86.4 (84.61-88.08)	321	24.8 (22.53-27.23)
City of Johannesburg	98	6.6 (5.40-7.91)	43	43.9 (34.47-53.75)
Ekurhuleni	77	5.1 (4.14-6.38)	24	31.2 (21.93-42.2)
West Rand	15	1.0 (0.61-1.65)	3	20.0 (7.05-45.18)
Sedibeng	13	0.9 (0.51-1.48)	5	38.5 (17.71-64.48)
Local municipality				
Pretoria	1242	83.0 (80.98-4.79)	308	24.8 (22.48-27.28)
Johannesburg	98	6.6 (5.40-7.91)	43	43.9 (34.47-53.75)
East Rand	77	5.1 (4.14-6.38)	24	31.2 (21.93-42.2)
Cullinan	32	2.1 (1.52-3.00)	7	21.9 (11.02-38.75)
Bronkhorstspuit	20	1.3 (0.87-2.06)	6	30.0 (14.55-51.9)
All Others	28	1.9 (1.30-2.69)	8	28.6 (15.25-47.06)
Towns				
Pretoria	974	65.1 (62.61-7.44)	247	25.4 (22.73-28.18)
Akasia	129	8.6 (7.3-10.15)	33	25.6 (18.83-33.74)
Centurion	58	3.9 (3.0-5.0)	11	19.0 (10.93-30.85)
City of Tshwane Rural	43	2.9 (2.14-3.85)	10	23.3 (13.15-37.74)
Randburg	23	1.5 (1.03-2.30)	10	43.5 (25.64-63.19)
Kungwini Rural	20	1.3 (0.9-2.06)	6	30.0 (14.55-51.9)
Roodepoort	20	1.3 (0.9-2.06)	10	50.0 (29.93-70.07)
Kempton Park	17	1.1 (0.71-1.81)	7	41.2 (21.61-63.99)
Roodeplaat	17	1.1 (0.71-1.81)	5	29.4 (13.28-53.13)
Germiston	15	1.0 (0.61-1.65)	8	53.3 (30.12-75.19)
All other Towns	181	12.1 (10.54-13.84)	49	27.1 (21.13-33.97)

Resistance to at least one antimicrobial category among *Staphylococcus* spp. isolates at local municipality level was highest in western and south-central areas of the province (Table 4.2, Figure 4.3). Again, although a similar general spatial pattern is evident at the town spatial scale, a lot more detail and heterogeneity is revealed. Multiple drug resistant (MDR) among *Staphylococcus* isolates at the local municipal spatial scale was highest in the southern region of Gauteng province. Similar patterns of MDR among *Staphylococcus* isolate was observed at town spatial scale although some additional towns with quite high risks are revealed to the north of the study area; these could not be shown by the higher level municipal mapping (Figure 4.4)

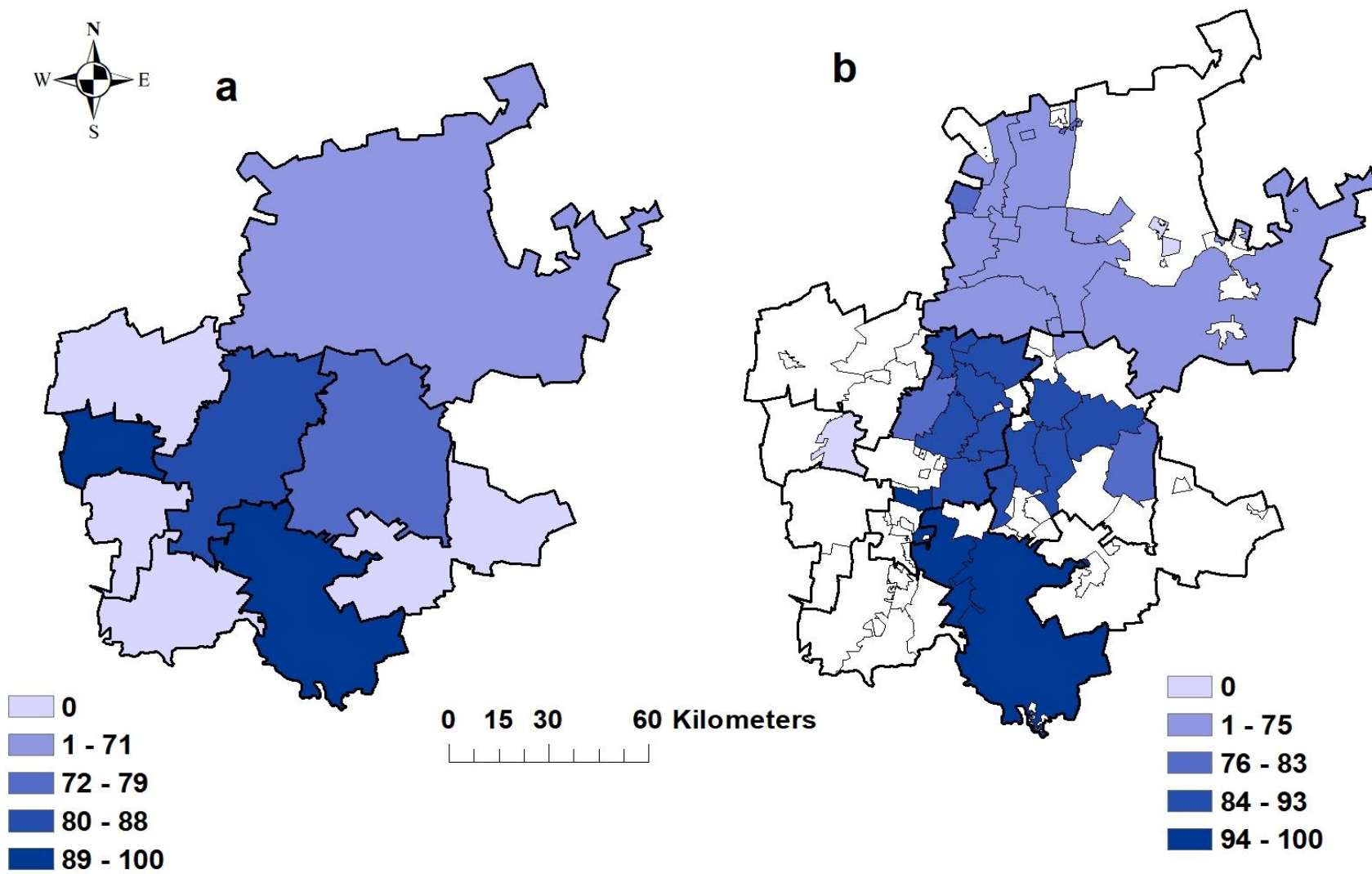


Figure 4. 3: Geographic distribution of resistant (AMR) *Staphylococcus* at (a) municipal spatial scale and (b) town spatial scale

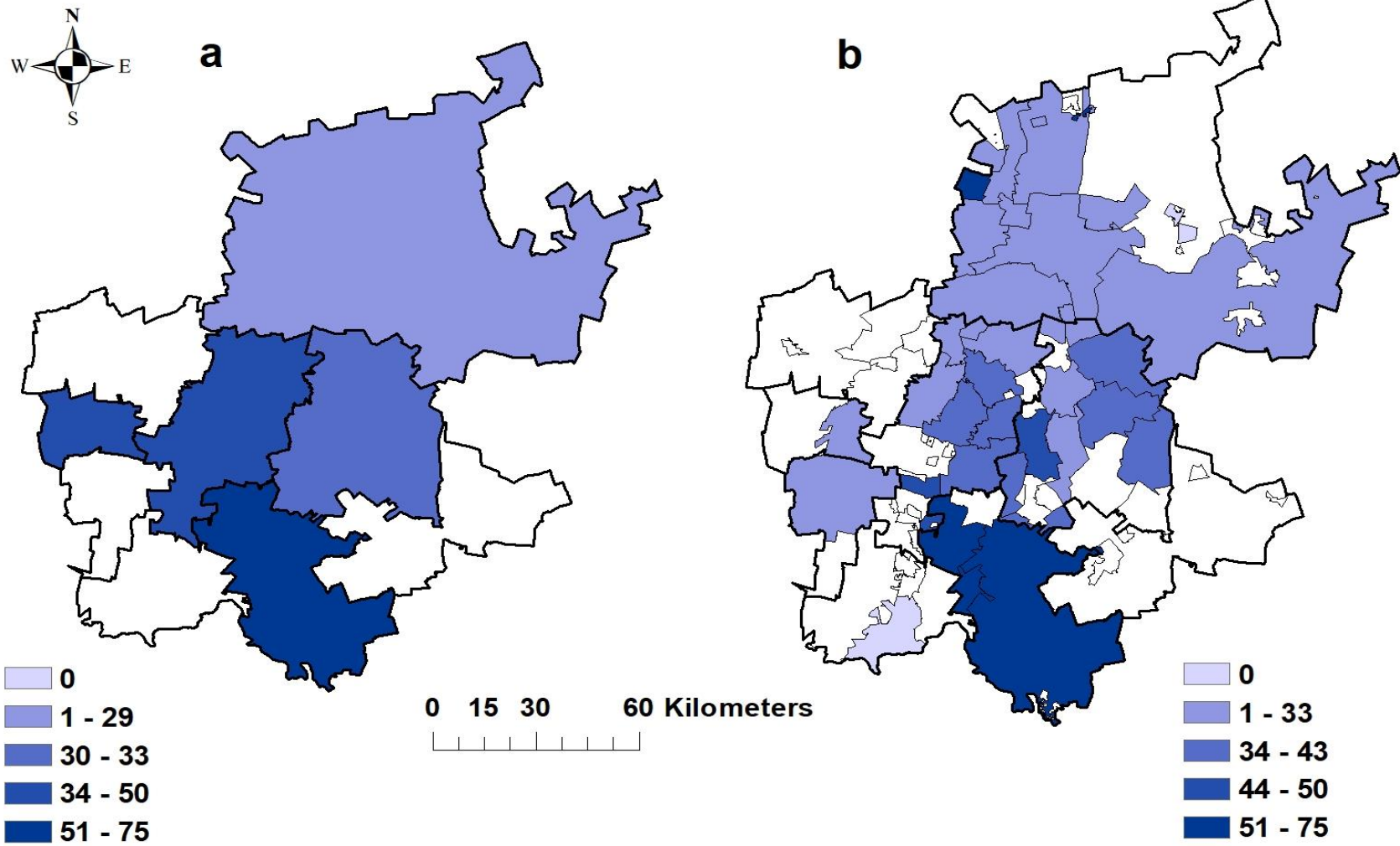


Figure 4. 4: Geographic distribution of multi-drug resistant (MDR) at (a) municipal spatial scale and (b) town spatial scale

Table 4. 2: Geographical distribution of antimicrobial resistance patterns among *Staphylococcus* spp. isolated from canine clinical samples tested at the bacteriology laboratory of a veterinary teaching hospital, 2007-2012.

	<i>Staphylococcus</i> spp.		Antimicrobial Resistance		Multidrug Resistance	
	Number	Percent (95% CI)	Number	Percent (95 % CI)	Number	Percent
Metropolitan						
City of Tshwane	309	80.9 (76.65-84.52)	243	78.6 (73.73-82.84)	169	54.7 (63.49-74.99)
City of Johannesburg	43	11.3 (8.47-14.82)	40	93.0 (81.39-97.6)	34	79.1 (70.93-92.94)
Ekurhuleni	22	5.8 (3.83-8.57)	19	86.4 (66.67-95.25)	14	63.6 (51.21-88.19)
Sedibeng	5	1.3 (0.56-3.03)	4	80.0 (37.56-96.38)	4	80.0 (51.01-100)
West Rand	3	0.8 (0.27-2.28)	2	66.7 (20.77-93.85)	2	66.7 (34.24-100)
Local municipality						
Pretoria	296	77.5 (73.04-1.39)	232	78.4 (73.34-82.69)	161	54.4 (63.19-74.97)
Johannesburg	43	11.3 (8.47-14.82)	40	93.0 (81.39-97.6)	34	79.1 (70.93-92.94)
East Rand	22	5.8 (3.83-8.57)	19	86.4 (66.67-95.25)	14	63.6 (51.21-88.19)
Cullinan	7	1.8 (0.90-3.73)	6	85.7 (48.69-97.43)	5	71.4 (43.65-96.99)
Bronkhorstspuit	6	1.6 (0.72-3.38)	5	83.3 (43.65-96.99)	3	50.0 (23.07-88.24)
Meyerton	4	1.1 (0.41-2.66)	4	100.0 (51.01-100)	4	100.0 (51.01-100)
Randfontein	2	0.5 (0.14-1.89)	2	100.0 (51.01-100)	2	100.0 (51.01-100)
Vereeniging	1	0.3 (0.05-1.47)	0	0.0	0	0.0
Westonaria	1	0.3 (0.05-1.47)	0	0.0	0	0.0
Towns						
Pretoria	237	62.0 (57.08-66.77)	182	76.8 (71.02-81.71)	131	55.3 (48.91-61.47)
Akasia	31	8.1 (5.78-11.29)	25	80.7 (63.72-90.81)	15	48.4 (31.97-65.16)
Centurion	11	2.9 (1.62-5.08)	10	90.9 (62.27-98.38)	6	54.5 (28.01-78.73)
Tshwane Rural	10	2.6 (1.43-4.75)	10	100.0 (72.25-100)	7	70.0 (39.68-89.22)
Randburg	10	2.6 (1.43-4.75)	7	70.0 (39.68-89.22)	6	60.0 (31.27-83.18)
Roodepoort	10	2.6 (1.43-4.75)	10	100.0 (72.25-100)	7	70.0 (39.68-89.22)
Midrand	9	2.4 (1.24-4.42)	9	100.0 (70.09-100)	9	100.0 (70.09-100)
Germiston	8	2.1 (1.07-4.08)	8	100.0 (67.56-100)	5	62.5 (30.58-86.31)
All Others	56	14.7 (11.46-18.56)	47	83.9 (72.19-91.31)	37	66.1 (65.1-88.01)

There was evidence of global spatial clustering of *Staphylococcus* infections at both the local municipal spatial scale (Moran's $I = 0.342$, $p = 0.006$) and the town spatial scale (Moran's $I = 0.398$, $p = 0.001$). However, AMR and MDR did not show evidence of significant global spatial clustering at the municipal scale (Table 4.3). In contrast, both AMR and MDR showed evidence of significant global spatial clustering at the town spatial scale (Table 4.3).

Table 4. 3: The results of the global Moran's I spatial autocorrelation tests on proportion of *Staphylococcus* infections and antimicrobial resistance and multidrug resistance in Gauteng Province, South Africa, 2007-2012.

	Local municipalities		Towns	
	Morans'I	P-value	Morans'I	P-value
<i>Staphylococcus</i> infection	0.342	0.006	0.398	0.001
Antimicrobial resistance	0.209	0.094	0.356	0.003
Multidrug resistance	0.232	0.055	0.303	0.007

Table 4. 4: Significant spatial clusters *Staphylococcus* spp. isolated from canine clinical samples tested at the bacteriology laboratory of a veterinary teaching hospital, 2007-2012

	# Areas	Observed	Expected	RR	P-Value
Local municipalities					
<i>Staphylococcus</i>	5	51	31.48	1.71	0.003
Towns					
<i>Staphylococcus</i>	17	64	41.53	1.65	0.045

Significant local spatial clusters of *Staphylococcus* infections were detected at both the local municipal ($p=0.003$) and town ($p=0.045$) spatial scales in the central and southwestern regions of the province (Table 4.4 and Figure 4.5). The risk of infection in the municipality local cluster was 1.7 (RR=1.71) higher than in municipalities outside the cluster. Similarly, the risk within the local cluster identified at the town spatial scale was also approximately 1.7 (RR=1.65) times higher than in towns outside the cluster (Table 4.4 and Figure 4.5).

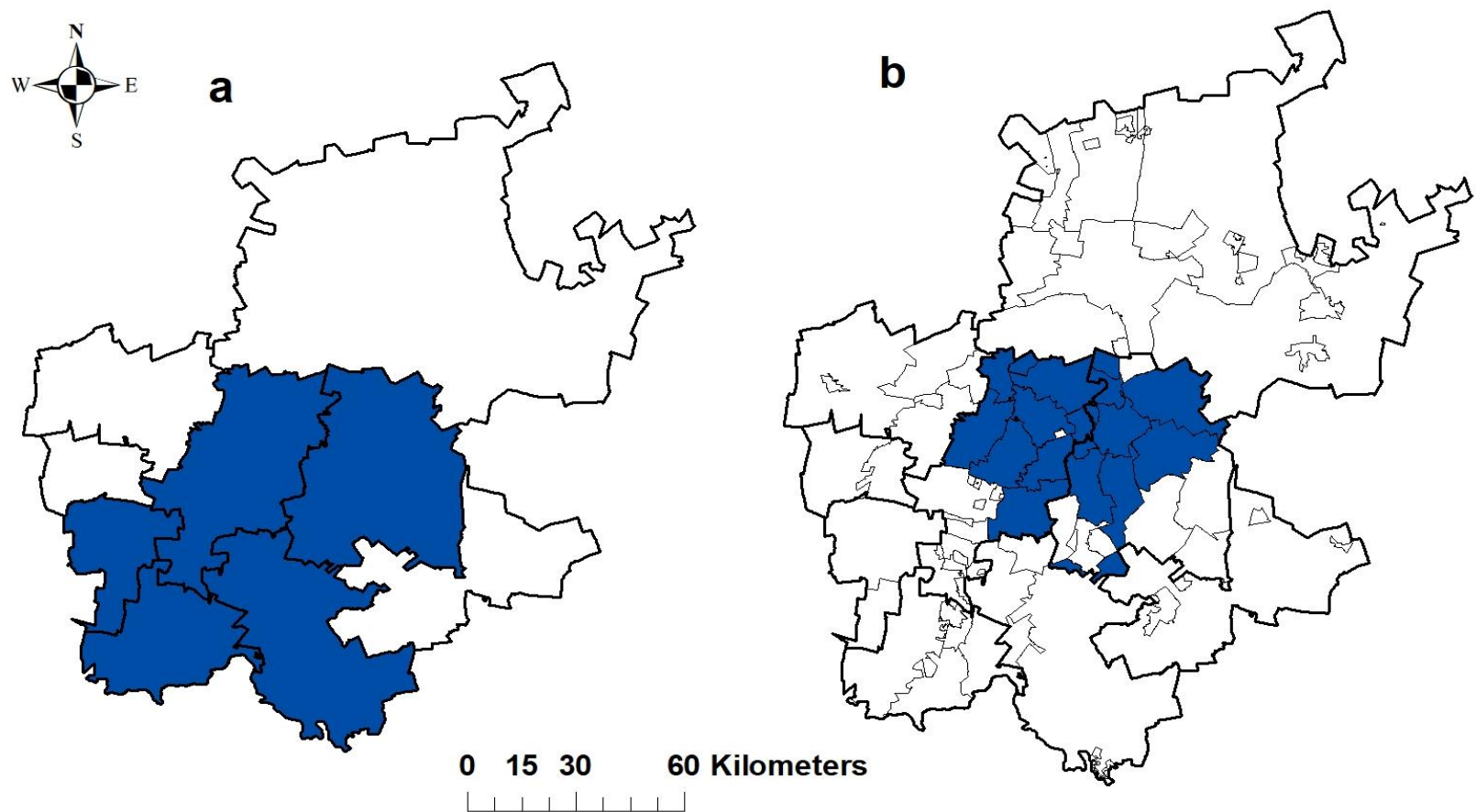


Figure 4. 5: Spatial clusters of *Staphylococcus* isolates in Gauteng province (a) municipal and (b) town spatial scale, 2007-2012.

4.6 Discussions

This study was designed to identify spatial patterns of canine *Staphylococcus* spp. infections as well as their AMR and MDR patterns with a view to identifying geographic hotspots. We used three spatial analytical techniques to address the objectives. Study findings show evidence of clustering of *Staphylococcus* isolates in the Western, Central and Southern regions of Gauteng province. The reasons for these clusters are unclear at this time since the sample size in our dataset did not allow for further detailed investigations of determinants of the identified hotspots. However, we hypothesize that local environmental factors may be responsible for the observed patterns. Suffice it to say that studies in Europe and US on the molecular epidemiology of MRSA have reported regional clustering of *Staphylococcus* cases. In Europe, MRSA compared to MSSA infections clustered in regions in close proximity to hospitals (Grundmann et al., 2010). While in the USA, MRSA infections were more common in the western states compared to other states (Carrel, Perencevich & David, 2015). Unfortunately, no similar studies have been done in South Africa and hence our study provides an initial clue regarding the spatial epidemiology of these infections in this region. However, it is important to note that similar to our findings, the authors of the two studies above could not provide the reasons for clustering of MRSA infections. We hypothesise that clustering of *Staphylococcus* isolates in the current study could be due

to socio-economic and environmental factors (Onozuka & Hagihara, 2007; Li et al., 2013), differences in population distribution based on breed and age, differences in dog population management strategies including fencing of household dogs, vaccinations, and animal health could be at play (Qekwana et al., 2017a). However, more research needs to be done to investigate factors responsible for the observed patterns.

The results of the spatial scan statistics show no evidence of clustering of resistant isolates at local municipal and town spatial level. This may suggest that there is spatial homogeneity in antimicrobial resistance among *Staphylococcus* isolates in this study (Kulldorff, 1998; Pfeiffer et al., 2008) probably due to similarity in prescription practices among veterinarians in the areas under study. However, since this is the first study that investigated spatial patterns of antimicrobial resistance among *Staphylococcus* isolates in veterinary medicine in this region, more studies will need to be done using information from other veterinary clinics in these regions to gain better understanding of the spatial epidemiology of antimicrobial resistance.

This study is not without limitations. The samples used in this study were from a veterinary teaching hospital which is not the only veterinary hospital or clinic in Gauteng. Therefore, some cases of infections that were seen in other clinics were not included in our study. In addition, the population under study did not include outpatient cases. The use of antibiotics could have resulted in lower recovery rates of

Staphylococcus species. Unfortunately, this information was not available for inclusion in the study. Notwithstanding these limitations, this study provides useful base line information on the spatial distribution of *Staphylococcus* infections, AMR and MDR among dogs presented at the veterinary teaching hospital.

4.7 Conclusions

Staphylococcus infections among dogs presented at the veterinary teaching hospital in South Africa clusters in certain local municipalities and towns. Further research must be undertaken to investigate determinants of these spatial patterns so as to inform control efforts.

4.8 References

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CHAPTER 5

5.1 Summary, discussions and conclusions

This chapter reviews the objectives of the study and summarizes the key findings. It also provides conclusions and recommendations for future research. The main aim of this study was to describe the epidemiology and antimicrobial resistance of *Staphylococcus* species isolated from canine clinical cases presented at Onderstepoort Veterinary Hospital. The objectives of this study were to: (1) investigate the burden and predictors of *Staphylococcus* spp. infections among dogs presented at an academic veterinary hospital in South Africa (2007-2012); (2) investigate patterns and predictors of antimicrobial resistance among *Staphylococcus* spp. from canine clinical cases presented at a veterinary academic hospital in South Africa.; and (3) describe geographic disparities of staphylococcal infections and antimicrobial resistance among selected *Staphylococcus* spp. from dogs presented at a veterinary academic hospital, South Africa.

The proportion of *Staphylococcus* positive samples in this study was lower than previously reported in the United Kingdom and in Brazil (Lilenbaum et al., 2000; Schmidt et al., 2014; Wedley et al., 2014). We hypothesise that this may be due to the fact that we assessed the burden of staphylococcal infections among all hospitalized dogs, whereas, the other studies assessed the burden of infections in

outpatient dogs and dogs with specific clinical signs. In addition, hospitalized dogs may have been exposed to antimicrobial drugs which could have resulted in lower proportions of infections. Nonetheless, the results of this study suggest that infections associated with *Staphylococcus* isolates are common among dogs presented to the veterinary teaching hospital in South Africa. Therefore, future studies should investigate the role that may be played by dogs in the transmission of *Staphylococcus* to their owners.

Staphylococcus pseudintermedius infections among dogs presented at the veterinary academic hospital in South Africa have been on the rise and remain the most predominate Coagulase-Positive *Staphylococcus* (CoPS) species causing infections. The observed increase could be attributed to increased access to veterinary services where dogs that potentially have high *Staphylococcus* infection risk are more likely to be presented for diagnostic services. It may also be due to the progressive poor health of dogs living in and around the area making them susceptible to staphylococcal infections. Although there are reports of seasonal atopic dermatitis with secondary *Staphylococcus* infections, in this case, there was no evidence of seasonality in the risk of *S. pseudintermedius* or *S. aureus* infection among dogs presented at the veterinary academic hospital. The results of this study suggest that the most likely species causing the infection will be *S. pseudintermedius*. In addition, the veterinary clinicians must also be aware that clinical cases associated

with *S. pseudintermedius* may present throughout the year, therefore, laboratory diagnosis is always recommended.

The ear canal and skin sites were more likely to test positive for *S. pseudintermedius* or *S. aureus*, compared to other sites. Therefore, our findings support previous reports of *Staphylococcus* species as a common cause of dermatological conditions in dogs (Hoekstra & Paulton, 2002; Kawakami et al., 2010; Gandolfi-Decristophoris et al., 2013; Schmidt et al., 2014). The risk of *Staphylococcus* infections in dogs presented at the veterinary academic hospital differed by age group. In the current study, the risk of either *S. aureus* and *S. pseudintermedius* infection was higher in dogs ≤ 6 years of age compared to dogs > 8 years of age. This was contrary to the findings of other studies which reported a higher proportion of *Staphylococcus* infection in older dogs compared to younger dogs.

We observed high proportions of *S. aureus* and *S. pseudintermedius* isolates resistant to β -lactam antibiotics. This is not surprising as *Staphylococcus* species have an intrinsic resistance to β -lactam antimicrobials (Hartman & Tomasz, 1984). In addition, the high resistance to lincosamides among *S. aureus* and *S. pseudintermedius* isolates confirms that they should not be used as alternative antimicrobial drugs in the treatment of staphylococcal infections in dogs presented at the veterinary academic hospital in South Africa.

Over the study period, there was a significant increase in the risk of resistance to fluoroquinolones among *S. aureus* and *S. pseudintermedius* isolates. In addition, increasing resistance to trimethoprim-sulphamethoxazole among *S. pseudintermedius* isolates was also observed. However, contrary to findings from other studies (Gandolfi-Decristophoris et al., 2013; Schmidt et al., 2014) we observed lower proportions of MDR among *Staphylococcus* isolates. It was not surprising to see higher MDR among *S. aureus* than *S. pseudintermedius* as similar observations have been made in other studies (Hoekstra & Paulton, 2002; Wedley et al., 2014; Youn et al., 2014). The presence of MDR among *Staphylococcus* isolates in this study suggests that clinicians should always request an antibiogram to help improve treatment and patient prognosis. In situations where previous antimicrobial drug use has failed and an antibiogram is not feasible, empirical treatment using antimicrobial drugs known to be effective against MDR *Staphylococcus* isolates is recommended. The observed increasing resistance to antimicrobial agents and the presence of MDR in this study require further investigation in order to assess its relationship to the prescription practice among veterinary clinicians in South Africa.

Spatial analytic tools have been used in veterinary epidemiology to investigate disease patterns and identify populations at risk of disease. In this study, spatial empirical Bayesian (SEB) was used to describe proportions of *Staphylococcus* positive and antimicrobial resistant isolates processed at the veterinary academic

hospital. We identified higher proportions of *Staphylococcus* isolates in the City of Johannesburg. In addition, within Johannesburg local municipality, Roodepoort town had the highest proportions of *Staphylococcus* positive samples.

Significant clustering of *Staphylococcus* spp. infections at local municipal and town spatial levels were identified. At town spatial scale, a high proportion of infection was observed in two areas, Walkerville and Boksburg. The findings of this study suggest that there may be local factors at local municipal and town levels that may be responsible for the higher risks in some locations. Therefore, clinicians need to be aware of the origin of the patient as it may play an important role in the diagnosis of *Staphylococcus* infections. Therefore, patient history is important in ascertaining whether a patient comes from a *Staphylococcus* geographic hotspot. Future studies will need to investigate factors associated with infection based on larger primary base studies.

Staphylococcus pseudintermedius and *S. aureus* were the most common *Staphylococcus* species causing skin and ear infections among dogs at the veterinary academic hospital. Younger dogs were at a higher risk of *Staphylococcus* infections than older dogs. In addition, there was clustering of *Staphylococcus* infection at local municipal and town spatial scales. Therefore, veterinary clinicians should be aware that the site of infection, origin of the dog, age of the dog and *Staphylococcus* species isolated are important in clinical diagnosis of *Staphylococcus* infection

We observed a significant increase in resistance to enrofloxacin, trimethoprim-sulphamethoxazole, clindamycin and orbifloxacin among the *Staphylococcus* isolate. More importantly, *S. aureus* showed a higher prevalence of antimicrobial resistance than *S. pseudintermedius*. This highlights the importance of *Staphylococcus* speciation and antibiogram profiling as part of the protocol for diagnosis and treatment of *Staphylococcus* spp. infections. Therefore, continuing education programs on antimicrobial usage must be developed and offered to veterinary and para-veterinary personnel and undergraduate veterinary science students to mitigate the development of antimicrobial resistance.

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