UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolates from clinical cases of canine pyoderma in South Africa

---

CATHERINE ANN BLUNT

A research report submitted in partial fulfillment of the requirements for the degree of

Magister Scientiae (Veterinary Tropical Diseases)

Department of Veterinary Tropical Diseases

Faculty of Veterinary Science

University of Pretoria

South Africa

Supervisor: Prof. M. van Vuuren

2011

PREFACE

Declaration by Student

I, Catherine Ann Blunt declare that this dissertation is my own work, carried out originally under the supervision of Dr. Jackie Picard of the University of Pretoria and is in accordance with the requirements of the University for the degree Magister Scientiae (Veterinary Tropical Diseases). Prof. M. van Vuuren served as supervisor during the final stage of the project.

_____ _____

Date Signature

ACKNOWLEDGEMENTS

My sincerest gratitude must be conveyed to the following persons:

- Dr Rick Last and Dr Johan Vorster of Vetdiagnostix Veterinary Pathology Services, for affording me the opportunity of pursuing my lifelong dream of attaining my MSc degree.
- My supervisor, Dr Jackie Picard, for her patience, enthusiasm, motivation, advice and supervision in the planning and execution of this study.
- Prof Moritz van Vuuren for his assistance during the final stages of the project.
- My parents, for enabling me to pursue this degree and for their unconditional belief in and support of me.
- My husband, for his continual love and patience and, for always having faith in my abilities.
- The rest of my family, friends and colleagues for their constant assistance and encouragement.

TABLE OF CONTENTS

PREFACE ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES vi

LIST OF FIGURES vii

ABSTRACT 1

LIST OF ABBREVIATIONS 3

CHAPTER 1 5

1. INTRODUCTION 5

1.1 Motivation for the Research Project 5

1.2 Aims and Objectives 5

CHAPTER 2 6

2. LITERATURE REVIEW 6

2.1 Background 6

2.2 Antimicrobial Resistance 7

2.4 Conclusion 9

2.5 Null (H_O) and Alternative (H_A) Hypotheses 10

CHAPTER 3 11

3. METHODOLOGY 11

3.1 Sampling 11

3.2 Identification of *Staphylococcus intermedius* 11

3.3 Antimicrobial Susceptibility Tests 12

3.4 Additional Data 16

3.5 Statistical Analyses 16

CHAPTER 4 17
4. RESULTS 17
4.1 Kirby-Bauer and Signalment Data between 2007 and 2010 17
4.2 Kirby-Bauer and MIC Data between 2007 and 2010 27
CHAPTER 5 30
5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS 30
5.1 Age 30
5.2 Gender 31
5.3 Breed 31
5.4 Temporal Distribution 31
5.5 Geographical Distribution 31
5.6 Susceptibility Testing 32
5.7 Kirby-Bauer and MIC Data 32
5.8 Conclusion and Recommendations 33
APPENDIX A 35
REFERENCES 36

LIST OF TABLES

Table 1: Zone diameter interpretive standards for the various antibiotics tested (CLSI, 2008)..... 13

Table 2: Antimicrobial dilution ranges used on the COMPAN1F sensititre plates..... 15

Table 3: Percentage age distribution of dogs from which skin samples yielded Staphylococcus intermedius isolates between 2007 and 2010 17

Table 4: Gender distribution of dogs sampled between 2007 and 2010. 19

Table 5: Proportion of dog breeds sampled between 2007 and 2010...... 20

Table 6: Monthly distribution of Staphylococcus intermedius isolates between 2007 and 2009..... 21

Table 7: Percentage susceptibility of Staphylococcus intermedius isolates from canine skin samples between 2007 and 2010..... 26

Table 8: Comparison of percentage susceptibility of Kirby-Bauer and broth dilution MIC data between 2007 and 2010...... 27

Table 9: Percentage MIC distribution of Staphylococcus intermedius strains isolated between June 2009 and June 2010 29

LIST OF FIGURES

Figure 1: Phagocytosed coccal-shaped staphylococci in a Diff-Quik® stained smear prepared from a canine skin sample (100X) (Picture: C. Blunt) 6

Figure 2: Colony characteristics of Staphylococcus intermedius when grown on 5% sheep blood agar (Picture: C. Blunt)..... 11

Figure 3: Positive DNase reactions of staphylococci grown on DNase agar following incubation and the addition of 1% hydrochloric acid (Picture C. Blunt) 12

Figure 4: Age distribution of dogs from which skin samples yielded Staphylococcus intermedius isolates between 2007 and 2010...... 18

Figure 5: Gender distribution of dogs sampled between 2007 and 2010...... 19

Figure 6: Proportion of breeds sampled between 2007 and 2010. Large breed shorthaired included Boerboel, Ridgeback, Bull Mastiff, Great Dane, Doberman, Rottweiler, Pointer and Dalmatian; Bull terrier type included English Bull Terrier, Staffordshire Bull Terrier and American Pitt Bull Terrier; large thick coat included Labrador, Golden Retriever, Schnauzer and Siberian Husky; medium to miniature shorthaired pure breed included the Fox Terrier, Jack Russell Terrier, Dachshund, Bulldog. Shepherd type included the German Shepherd Dog and Belgian Shepherd Dog...... 20

Figure 7: Temporal distribution of Staphylococcus intermedius isolates between 2007 and 2010...... 21

Figure 8: Overview of the geographical distribution of Staphylococcus intermedius strains isolated in 2007..... 22

Figure 9: Geographical distribution of Staphylococcus intermedius strains isolated in 2008..... 23

Figure 10: Geographical distribution of Staphylococcus intermedius strains isolated in 2009..... 24

Figure 11: Geographical distribution of Staphylococcus intermedius strains isolated in 2010..... 25

Figure 12: Percentage susceptibility of Staphylococcus intermedius isolates from canine skin samples between 2007 and 2010. Breakpoint zone of inhibition diameters derived from CLSI, 2008..... 27

Figure 13: Comparison of percentage susceptibility of Kirby-Bauer and broth dilution MIC data between 2007 and 2010..... 28

ABSTRACT

Successful treatment of canine pyoderma has become increasingly difficult due to the development of antimicrobial resistance and recurrence of infection. The development and spread of antimicrobial resistance has major implications because treatment failures have been associated with increased mortality, morbidity and costs related to disease. All canine skin samples submitted to Vetdiagnostix Veterinary Pathology Services for microbiological culture and sensitivity between January 2007 and June 2010, from which *Staphylococcus intermedius* was isolated, were selected for this investigation. A total of 319 samples from dogs of various ages, sexes and breeds from various locations throughout South Africa were obtained. In addition to the antimicrobial susceptibility data, data relating to dog signalment (age, gender, breed), case history and any other aspects of the history provided e.g. diagnosis, sample type and geographical location of the patient were collected. The number of skin samples yielding *Staphylococcus intermedius* was high in dogs up to the age of 6 years and, then decreased with only a few cases in dogs aged 11 years or older. The distribution of samples collected in 2010 was unusual in that there were two peaks, one in dogs two years old or less and one in 6 to 9-year-old dogs. With the exception of skin samples taken in 2008, a high percentage of dogs were under the age of one year old. 2008 had a lower percentage of affected dogs less than one year of age compared to the other years. Staphylococcal pyoderma is more common in dogs below the age of five years. Dogs above this age are less likely to contract this condition. The genders tended to be equally distributed throughout the years, with almost equal proportions of affected males and females present. The Bull Terrier types and Shepherd types were grouped separately as they were over-represented and are known to be prone to pyoderma. Large short haired dogs were consistently worse affected throughout the years sampled, followed by the Bull Terrier types. In 2010, small short haired breeds were worse affected compared to the numbers between 2007 and 2009. The monthly distribution, with the exception of 2009, tended to be consistent throughout the years. The unusual temporal distribution in 2009 could be associated with a general distribution in the number of samples compared to the other years sampled. Samples tended to be mainly from practices located in the KwaZulu-Natal province of South Africa. This is most likely due to the fact that the laboratory is located in this province, with a courier network that arranges collection from these practices. Practices in other regions send their samples to the laboratory via private courier companies and the South African Post Office. Antimicrobial resistance of *S. intermedius* was greatest to ampicillin followed by tetracycline and then potentiated sulphonamides. The results also showed that, in general, antimicrobial resistance was low. Very few methicillin resistant isolates were detected. Temporal trends were not noted, with the exception of ampicillin where isolates became more susceptible and potentiated sulphonamides (co-trimoxazole) where isolates were becoming more resistant. *Staphylococcus intermedius* is significantly less resistant to erythromycin, clindamycin, cephalexin, oxacillin, amoxicillin-clavulanic acid, enrofloxacin, marbofloxacin and gentamicin, with most strains being susceptible to these drugs. Resistance to penicillin and tetracycline is frequently found in *Staphylococcus intermedius* and is on the increase. Resistance to most other antimicrobials, particularly newer generation antimicrobial agents such as the fluoroquinolones, is still comparatively low. In general, both the Kirby-Bauer and broth dilution MIC tests yielded similar results for the antimicrobial agents tested. The main difference between the two tests was evident

in the over-estimation of resistance by the Kirby-Bauer test in the cases of ampicillin, co-trimoxazole, penicillin and doxycycline. This could be related to the instability of these particular drugs *in vitro*. Inoculum densities may also have played a role, with denser inocula producing smaller zone sizes for the drugs tested. Using the MIC method, all of the isolates tested were found to be completely sensitive to ticarcillin, oxacillin, amoxicillin-clavulanic acid, imipenem, ceftiofur, chloramphenicol, doxycycline, gentamicin, amikacin and co-trimoxazole. Of the isolates tested using the MIC method, between 2-40% showed some level of resistance to the following antimicrobials: erythromycin, penicillin, ampicillin, enrofloxacin, clindamycin and marbofloxacin. The highest level of resistance observed was shown to erythromycin. The increase in resistance to the lincosamides, lincomycin, clindamycin and erythromycin may be attributed to the increased use of these drugs in the last decade. Knowledge of trends in bacterial resistance is important for veterinarians when determining treatment for canine skin infections. The information obtained from the analysis of the antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolated from canine pyoderma cases will provide veterinarians with valuable information on choosing the most appropriate drug to treat *S. intermedius* skin infections as well as re-enforcing the need for the prudent use of antimicrobial drugs in companion animals.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
µg	Micrograms
µl	Microlitres
AK	Amikacin
AMC	Amoxicillin-clavulanic acid
AMP	Ampicillin
ATCC	American type culture collection
C	Chloramphenicol
CLSI	Clinical Laboratory Standards Institute
CN	Gentamicin
DA	Clindamycin
DNA	Deoxyribose nucleic acid
DO	Doxycycline
E	Erythromycin
EFT	Ceftiofur
ENR	Enrofloxacin
ermB	Gene conferring erythromycin resistance
IPM	Imipenem
KF	Cephalothin
MAR	Marbofloxacin
mecA	Gene conferring methicillin resistance
MIC	Minimum inhibitory concentration
MIC_{50}	Median MIC value
MIC_{90}	MIC required to inhibit the growth of 90% of organisms
ml	Millilitres
mm	Millimetres
N	Neomycin
OX	Oxacillin
PEN	Penicillin

Abbreviation	Meaning
PCR	Polymerase chain reaction
RD	Rifampicin
SXT	Trimethoprim-sulphamethoxazole
TE	Tetracycline
TIC	Ticarcillin

CHAPTER 1

1. INTRODUCTION

1.1 Motivation for the Research Project

Successful treatment of canine pyoderma has become increasingly difficult due to the development of antibiotic resistance and recurrence of infection. The development and spread of antimicrobial resistance has major implications because treatment failures have been associated with increased mortality, morbidity and costs related to disease. In light of this, the present study has been undertaken to investigate the antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolates from clinical cases of canine pyoderma in South Africa. The results from this study will be specific to South Africa and, since resistance is often based on usage patterns, which are largely regional in nature, these results cannot necessarily be extrapolated to other countries.

Knowledge of trends in bacterial resistance is important for veterinarians when determining treatment for canine skin infection. The information obtained from the analysis of the antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolated from canine pyoderma cases will provide veterinarians with valuable information on choosing the most appropriate drug to treat *S. intermedius* skin infections as well as allowing for the prudent use of antimicrobial drugs in companion animals.

1.2 Aims and Objectives

Aims

- To investigate the antimicrobial susceptibility profiles of *Staphylococcus intermedius* isolates from clinical cases of canine pyoderma in South Africa.
- To compare the Kirby-Bauer and broth microdilution methods of antimicrobial susceptibility testing.

Objectives

- To determine if there is a correlation between the antimicrobial profiles obtained, the age, sex, breed of dog as well as the geographical distribution of the isolates.
- To determine if there is any correlation between the results from the Kirby-Bauer and broth microdilution methods of antimicrobial susceptibility testing with particular reference to methicillin resistance testing.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Background

The staphylococci are facultatively anaerobic, Gram-positive, coccal-shaped bacteria (Figure 1) which belong to the family *Micrococcaceae* (Rich 2005). They are mostly harmless commensals of the skin and mucous membranes but are potentially pathogenic to man and many other animal species (Vanni *et al.* 2009).

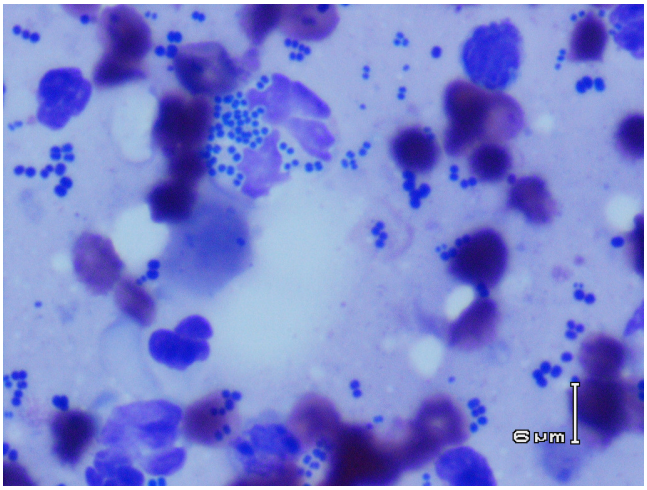

Figure 1: Phagocytosed coccal-shaped staphylococci in a Diff-Quik® stained smear prepared from a canine skin sample (100X) (Picture: C. Blunt)

Nearly all cases of pyoderma in dogs are caused by *Staphylococcus intermedius* (DeBoer 2006). The name *Staphylococcus intermedius* was proposed for those isolates which differed from *Staphylococcus aureus* in various biochemical reactions and in cell wall composition (Hajek 1976). The name *Staphylococcus pseudintermedius* is now given to the canine-specific strain of *Staphylococcus intermedius*. *Staphylococcus intermedius* is also an important cause of wound infections, otitis externa, cystitis, ocular and respiratory disease. Comparable to *Staphylococcus aureus* colonisation of humans, healthy dogs frequently carry *S. intermedius* as part of their normal microflora. It is a transient inhabitant of the skin and hair coat. Reservoir sites include the oral and nasal cavities as well as the perineum and anus (Hartmann *et al.* 2005). Although *Staphylococcus intermedius* is not usually isolated from humans as it shows a host-specificity for canine corneocytes, transmission between humans and their pets has been shown to occur (Fitzgerald 2009).

Dogs affected by pyoderma usually have histories of multiple episodes of pyogenic and pruritic skin infections, usually associated with poor response to corticosteroids and partial response to antimicrobial drugs. Lesions

may involve the superficial epidermis, dermis and hair follicles and may manifest as papules or pustules. Deeper infections are far more severe, with resulting destruction of the hair follicle and deep dermis and subcutaneous tissue invasion. The characteristics of deep pyoderma are cellulitis, furunculosis, fistulous tracts and skin ulceration (Morales *et al.* 1994). This skin condition typically results from an underlying skin disorder such as ectoparasitism, atopy, hormonal imbalances or immune-mediated dermatitis (Vanni *et al.* 2009).

2.2 Antimicrobial Resistance

Since the introduction of antimicrobials, the staphylococci have shown a rapid development and spread of resistance, particularly in nosocomial infections (Werckenthin *et al.* 2001). Antimicrobial resistance is of steadily increasing concern, in both veterinary and human medicine. Its development and spread has lead to treatment failures and hence increased morbidity, mortality and treatment costs (Pellerin *et al.* 1998). Increased attention has been devoted to small animal welfare which has resulted in increased expenditure on veterinary care. Consequently, antimicrobial agents are frequently utilised in pet animals, including preparations licensed for human use and the treatment of human infections (Guardabassi 2004b). The treatment of deep pyoderma associated with *S. intermedius* is possibly the most common reason for antimicrobial treatment in dogs (Guardabassi *et al.* 2004a). This therapy is generally of an empirical nature. The following guidelines are widely accepted by small animal practitioners (DeBoer 2006):

- The use of penicillin, ampicillin, amoxicillin and tetracycline is not indicated as resistance to these drugs is widespread;
- Potentiated sulphonamides, erythromycin, lincomycin or clindamycin are reasonable choices as many isolates are still susceptible to these drugs;
- Cephalosporins, amoxicillin-clavulanic acid and penicillinase-resistant penicillins such as oxacillin are good choices as nearly all strains are susceptible;
- Aminoglycosides and fluoroquinolones should be reserved for more serious infections where other options are not available.

Hoekstra and Paulton (2002) reported that resistance in *S. intermedius* was commonly observed for penicillin G, lincomycin, tetracycline and trimethoprim-sulphamethoxazole. They also found that adult male dogs were worse affected than juvenile female dogs. This could be due to the fact that they carry greater numbers of resistant *S. intermedius* strains. Similarly, Hartmann *et al* (2005) recognised that resistance to penicillin and tetracycline was most common while most isolates tested were sensitive to erythromycin, clarithromycin and clindamycin. Pellerin *et al* (1998) established that more than 95% of the *S. intermedius* strains tested were susceptible to oxacillin, amoxicillin-clavulanic acid, cephalexin, gentamicin, fucidic acid, enrofloxacin and marbofloxacin. Vanni *et al* (2009) reported that resistance was observed mainly against macrolides, chloramphenicol and lincosamides.

The method of prescribing drugs without the use of microbiological culturing and sensitivity testing is most likely a major contributor to the emergence of resistant strains of staphylococci (Lilenbaum *et al.* 2000). Antimicrobial resistance is, however, a very complex problem involving various bacterial species, resistance mechanisms, transfer mechanisms and reservoirs (Guardabassi *et al.* 2004b).

Antimicrobial resistance occurs when an organism is resistant to one or more antimicrobials. It can also be acquired by spontaneous mutation or the attainment of resistance genes from another organism via conjugation, transduction or transformation (Cohn & Middleton 2010). The most well-known mechanism of resistance in staphylococci is through the production of various beta-lactamase enzymes. These are in turn classified as penicillinases and cephalosporinases. These enzymes may be encoded by chromosomes or plasmids and may be constitutive or inducible (Pellerin *et al.* 1998). One of the most problematic resistance mechanisms of recent times is methicillin resistance. Methicillin resistance refers to strains that are resistant to all beta-lactam antimicrobial agents, and can cause a spectrum of infection and disease, from subclinical carriage to life-threatening focal infections and bacteraemia, It is mediated by vectors which are called staphylococcal cassette chromosome (SCC) elements containing the *mecA* gene which in turn encodes a penicillin-binding protein which has a broad affinity for beta-lactam antibiotics. These SCCs can be spread horizontally between staphylococci leading to a rapid spread of resistance (Epstein *et al.* 2009).

Awareness and monitoring of antimicrobial resistance in veterinary staphylococcal isolates is of great importance as the development of resistance in animal pathogens can result in treatment failure in individual patients which may pose a zoonotic risk to their owners. Moreover, increases in resistance to antimicrobial classes that are important in human medicine may result in the restriction of precious antibacterial agents from veterinary use (Loeffler *et al.* 2007).

2.3 Antimicrobial Susceptibility Testing

The *in vitro* susceptibility of a pathogen to an antimicrobial agent can be performed by disk diffusion or by the measurement of the minimum inhibitory concentration (MIC), the lowest drug concentration capable of inhibiting the growth of the bacterium under investigation. Susceptibility testing is controlled for incubation in or on suitable media, atmosphere, temperature and incubation duration (Blondeau 2009).

The Kirby-Bauer disc diffusion method is a flexible and relatively inexpensive technique, which is commonly used in diagnostic laboratories. The standard procedure is used mainly to test rapidly growing aerobic bacteria. Filter paper discs containing specified amounts of antimicrobial agents are placed on agar uniformly seeded with the test bacterium. The diameter of each zone of inhibition is measured in millimetres and the results compared with standards for interpretation of the zone size. Susceptibility to an antimicrobial drug indicates that the infection caused by the bacterium may respond to treatment if the drug reaches therapeutic levels in the affected tissues (Quinn et al. 2002). The disk diffusion technique has been standardised primarily for the testing of rapidly growing bacteria. The test has, however, been modified to allow testing of certain fastidious bacteria. The

diameter of the zone of inhibition is influenced by the rate of diffusion of the antimicrobial agent through the agar, which may vary among different drugs. The zone size is however inversely proportional to the MIC. Criteria currently recommended for interpreting zone diameters and MIC results for commonly used antimicrobial agents are published by the Clinical and Laboratory Standards Institute (Murray et al. 1999, CLSI 2008).

In the broth microdilution test, drugs are added to a growth medium in a 96-well microtitre plate and serially diluted to the lowest drug concentration to be tested. After the addition of the test organism, the plate is incubated for 18-24 hours. The lowest drug concentration visibly preventing growth is recorded as the MIC. The antimicrobial susceptibility is subsequently determined by comparing the MIC value to established breakpoints that take the following into account: the drug's *in vitro* activity, achievable and sustainable drug concentrations within the host, distribution and elimination data and drug toxicity (Blondeau 2009). Dilution methods offer flexibility in the sense that the standard medium used to test frequently encountered organisms can be supplemented or replaced with another medium to allow for accurate testing of various fastidious bacteria not reliably tested by disk diffusion. Dilution methods are also adaptable to automated methods. The flexibility of dilution testing is also evident in the reporting formats that may be used. Quantitative results (in micrograms per millilitre) or category results (susceptible, intermediate and resistant) or both can be used (Murray *et al.* 1999).

Due to its greater stability, oxacillin has been widely used for the *in vitro* detection of staphylococcal resistance to the class of penicillinase-resistant penicillins (Bemis *et al.* 2009). Oxacillin susceptibility tests can be applied to other penicillinase-stable penicillins e.g. cloxacillin, dicloxacillin, flucloxacillin, methicillin and nafcillin. Oxacillin is more resistant to degradation and is therefore more likely to detect heteroresistant staphylococcal strains compared to methicillin or nafcillin (CLSI 2008). Most veterinary laboratories use the disk diffusion or broth microdilution methods for oxacillin susceptibility testing. Commercial microdilution test panels do, however, not always contain a sufficient range of oxacillin concentrations to detect low levels of oxacillin resistance (Bemis *et al.* 2009). Because of the heterogeneous nature of methicillin resistance, no single best method for susceptibility testing exists. The methods used in the clinical laboratory to detect methicillin resistant staphylococci are empirically derived and may be several steps removed from the detection of the genetic or biochemical determinants associated with methicillin resistant strains. This undoubtedly accounts for some of the confusion encountered in determining which strains are resistant and which are not (Hackbarth & Chambers 1989).

Tests for *mecA* e.g. PCR or the protein encoded by *mecA* (the penicillin-binding protein 2a) are the most accurate methods for prediction of resistance to oxacillin/methicillin. These tests could be used to confirm results of staphylococcal isolates from serious cases of infection (CLSI 2008).

2.4 Conclusion

In order to detect early changes in *Staphylococcus intermedius* sensitivity patterns before a high prevalence of resistance is selected or developed, regular monitoring of antimicrobial resistance in companion animals is

necessary. Prudent use of antimicrobial drugs to treat *Staphylococcus intermedius* infections should be employed in conjunction with laboratory culture and susceptibility testing, especially if a dog has received prior antimicrobial therapy.

2.5 Null (H_O) and Alternative (H_A) Hypotheses

- H_O: There is no correlation between the antimicrobial profiles obtained, the age, sex, breed of dog as well as the geographical distribution of the isolates.
- H_A: There is a correlation between the antimicrobial profiles obtained, the age, sex, breed of dog as well as the geographical distribution of the isolates.
- H_O: There is no correlation between the results from the Kirby-Bauer and broth microdilution methods of antimicrobial susceptibility testing with particular reference to methicillin resistance testing.
- H_A: There is a correlation between the results from the Kirby-Bauer and broth microdilution methods of antimicrobial susceptibility testing with particular reference to methicillin resistance testing.

CHAPTER 3

3. METHODOLOGY

3.1 Sampling

All canine skin samples submitted to Vetdiagnostix Veterinary Pathology Services for microbiological culture and sensitivity testing between January 2007 and June 2010, and from which *Staphylococcus intermedius* was isolated, were selected for this investigation. A total of 319 samples from dogs of various ages, sexes and breeds from various locations throughout South Africa yielded *Staphylococcus intermedius* and were therefore included in this study. Duplicates were not excluded.

These samples included skin swabs, skin biopsies, skin abscess and pustule swabs and fine needle aspirates.

3.2 Identification of *Staphylococcus intermedius*

Following growth on 5% sheep blood agar (Appendix A) after 24 hours incubation at 37˚C, *Staphylococcus intermedius* was identified on the basis of colony characteristics (Figure 2), catalase production, Gram's stain, lack of pigment production, delayed acid production from D-mannitol, slow or weak maltose production and positive DNase reaction (Figure 3) on DNase agar (Appendix A) as outlined in Clinical Veterinary Microbiology (Quinn, Carter, Markey & Carter 1994). All media used was quality controlled using *Staphylococcus aureus* ATCC 25923. The name *Staphylococcus pseudintermedius* is now given to the canine-specific strain of *Staphylococcus intermedius*. This can only be determined accurately by DNA sequencing. For the purposes of this study, the isolates will be referred to as *Staphylococcus intermedius*, as phenotypic typing cannot reliably distinguish the canine-specific species from others.

Figure 2: Colony characteristics of Staphylococcus intermedius when grown on 5% sheep blood agar (Picture: C. Blunt)

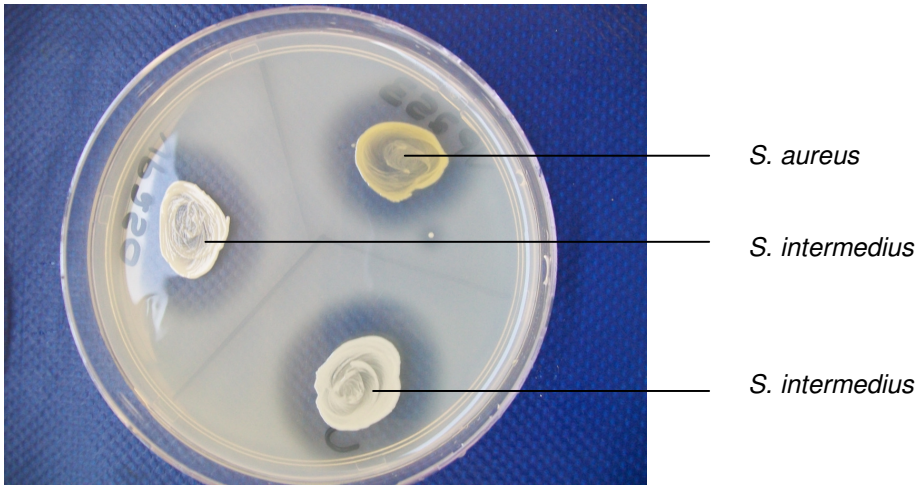

Figure 3: Positive DNase reactions of staphylococci grown on DNase agar following incubation and the addition of 1% hydrochloric acid (Picture: C. Blunt)

3.3 Antimicrobial Susceptibility Tests

The bacteria identified as *Staphylococcus intermedius* were tested for antimicrobial susceptibility by the disk diffusion method on Mueller Hinton agar (Appendix A). A 0.5 McFarland suspension of the test organism in sterile saline was evenly spread onto the Mueller Hinton agar plates, after which the plates were disked and incubated at 37˚C for 18-24 hours (CLSI, 2008). All media was quality controlled using *Staphylococcus aureus* ATCC 25923.

The following antimicrobial agents were tested for all isolates: Ampicillin (AMP, 10 µg), cephalothin (KF, 30µg), chloramphenicol (C, 30µg), amoxicillin-clavulanic acid (AMC, 30 µg), enrofloxacin (ENR, 5 µg), gentamicin (CN, 10 µg), neomycin (N, 30 µg), oxacillin (OX, 1IU), tetracycline (TE, 30 µg) and trimethoprim-sulphamethoxazole (SXT, 25 µg) (Appendix A).

The isolates collected from June 2009 to June 2010 were tested against the following additional antimicrobial agents: amikacin (AK, 30 µg), ceftiofur (EFT, 30 µg), clindamycin (DA, 2 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), imipenem (IPM, 10 µg), marbofloxacin (MAR, 5 µg), penicillin (PEN, 10 IU), rifampicin (RD, 5 µg) and ticarcillin (TIC, 75 µg) (Appendix A). After measuring the zones of inhibition, the strains were classified as sensitive or resistant to the drugs tested according to the Clinical and Laboratory Standards Institute standards and criteria (Table 1) (CLSI 2008). The intermediate strains were interpreted as sensitive or resistant depending on whether the reading obtained fell closer to the sensitive or resistant cut-off.

Table 1: *Zone diameter interpretive standards for the various antibiotics tested (CLSI, 2008).*

Antimicrobial drug	Zone Diameter (mm)		
	Sensitive	**Intermediate**	**Resistant**
Amikacin	>17	15-16	<14
Gentamicin	>15	13-14	<12
Rifampicin	>20	17-19	<16
Amoxicillin-clavulanic acid	>20	-	<19
Ampicillin	>29	-	<28
Oxacillin	>18	-	<17
Penicillin	>29	-	<28
Ticarcillin	>20	15-19	<14
Imipenem	>16	14-15	<13
Cephalothin	>18	15-17	<14
Ceftiofur	>21	18-20	<17
Enrofloxacin	>23	17-22	<16
Marbofloxacin	>20	15-19	<14
Trimethoprim-sulphamethoxazole	>16	11-15	<10
Clindamycin	>21	15-20	<14
Erythromycin	>23	14-22	<13
Neomycin	>17	13-16	<12
Chloramphenicol	>18	13-17	<12
Tetracycline	>19	15-18	<14
Doxycycline	>19	15-18	<14

The MIC results for the above-mentioned antimicrobial drugs were determined for the *Staphylococcus intermedius* isolates collected from June 2009 to June 2010. The laboratory does not routinely make use of the MIC method and therefore retrospective data was not available. The broth microdilution method was utilised and commercial COMPAN1F Sensititre MIC plates were purchased for this purpose (Trek Diagnostics). Table 2 shows the dilution ranges used. A 0.5 McFarland suspension of the test organism was prepared in sterile saline and 10 µl of this suspension was added to 990 µl of cation-adjusted Mueller Hinton broth (Appendix A). 0.1ml of chilled calcium and magnesium ion stock solutions were added per litre for each desired increment of 1mg/l in the final concentration in the adjusted Mueller-Hinton broth (CLSI 2008).100µl of this inoculated broth suspension was then pipetted into each of the 96 wells on the commercial microtitre plate and the plate incubated at 37˚C for 18-24 hours.

Table 2: *Antimicrobial dilution ranges used on the COMPAN1F sensititre plates*

Antibiotic	Dilution Range (µg/ml)
Ampicillin	0.25-16
Amoxicillin-Clavulanic Acid	4/2-32/16
Ticarcillin	8-64
Trimethoprim-Sulphamethoxazole	0.5/9.5-2/38
Gentamicin	1-8
Penicillin	0.06-8
Ceftiofur	0.25-4
Enrofloxacin	0.25-2
Amikacin	4-32
Imipenem	1-8
Erythromycin	0.5-4
Marbofloxacin	0.25-2
Oxacillin	0.25-4
Clindamycin	0.5-4
Doxycycline	2-8
Chloramphenicol	4-16
Rifampicin	1-2

3.4 Additional Data

In addition to the antimicrobial susceptibility data, data relating to dog signalment (age, gender, breed), case history and any other aspects of the history provided e.g. diagnosis, sample type and geographical location of the patient were also collected.

3.5 Statistical Analyses

Microsoft Excel was used to calculate, compare and graph the different parameters measured e.g. percentage susceptibility, age, breed, sex and geographical locations of the practices. The statistics used are descriptive using numerator/ denominator data.

CHAPTER 4

4. RESULTS

4.1 Kirby-Bauer and Signalment Data between 2007 and 2010

4.1.1 Age

Table 3 and Figure 4 show that the number of skin samples yielding *Staphylococcus intermedius* were high in dogs up to the age of 6 years and, then decreased with only a few cases in dogs aged 11 years or older. Of the 319 samples that yielded *Staphylococcus* intermedius, data related to age of the patients was only available for 289 of the cases. The distribution of samples collected in 2010 is unusual in that there are two peaks, one in dogs two years old or less and one in 6 to 9 year-old dogs. With the exception of skin samples taken in 2008, a high percentage of dogs were under the age of one year old. 2008 had a lower percentage of affected dogs less than one year of age compared to the other years.

Table 3: *Percentage age distribution of dogs from which skin samples yielded Staphylococcus intermedius isolates between 2007 and 2010*

Age (Years)	2007 (n=98)	2008 (n=97)	2009 (n=76)	2010 (n=18)
<1	14.29	5.15	11.84	16.67
1	3.06	10.31	5.26	16.67
2	12.24	10.31	9.21	11.11
3	8.16	12.37	6.58	5.56
4	10.20	14.43	13.16	5.56
5	15.31	10.31	11.84	5.56
6	10.20	8.25	10.53	11.11
7	4.08	6.19	5.26	11.11
8	5.10	7.22	6.58	0.00
9	5.10	6.19	7.89	11.11
10	8.16	5.15	3.95	0.00
11	1.02	2.06	2.63	0.00
12	1.02	0.00	0.00	5.56
13	1.02	0.00	2.63	0.00
14	0.00	0.00	1.32	0.00
15	0.00	1.03	1.32	0.00
16	1.02	1.03	0.00	0.00

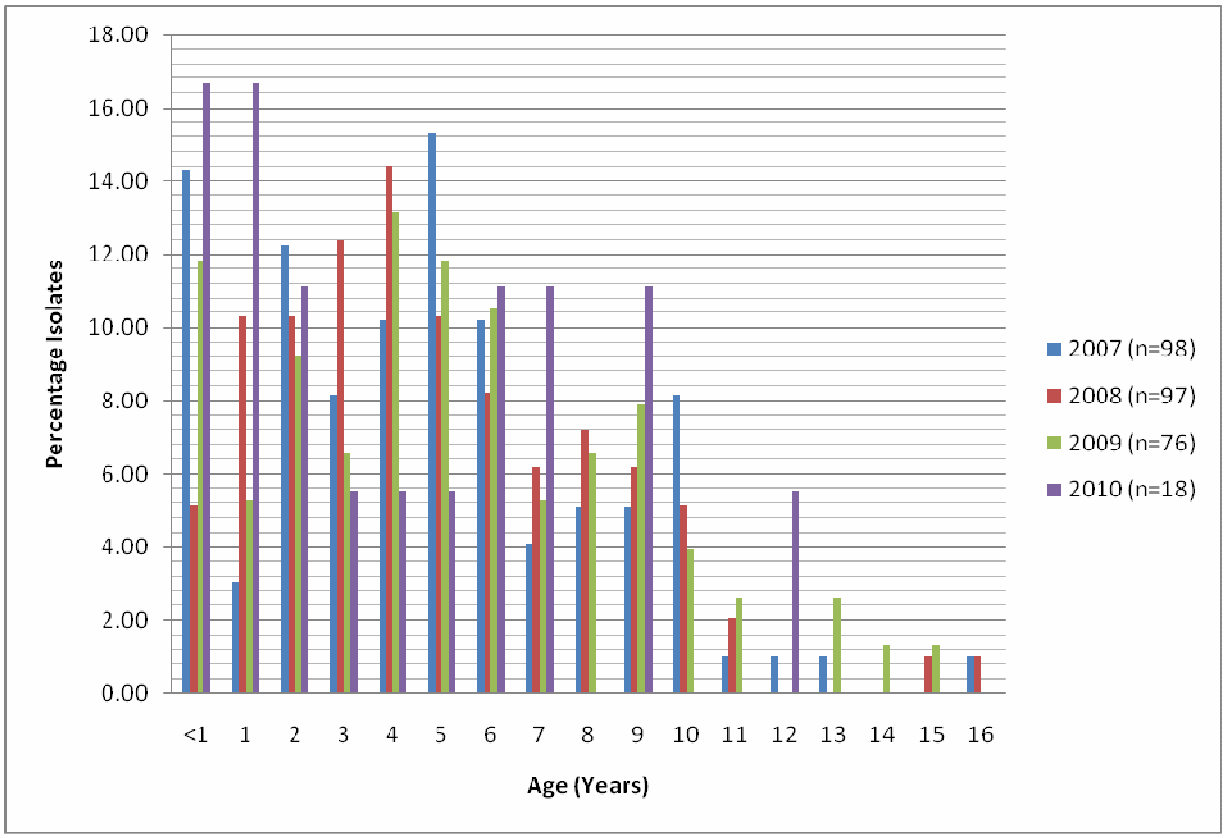

Figure 4: *Age distribution of dogs from which skin samples yielded Staphylococcus intermedius isolates between 2007 and 2010.*

4.1.2 Gender

The genders tended to be equally distributed throughout the years, with almost equal proportions of affected males and females present (Table 4 and Figure 5).

Table 4: *Gender distribution of dogs sampled between 2007 and 2010.*

	Male	Female
2007 (n=113)	58.56	41.44
2008 (n=105)	47.47	52.53
2009 (n=80)	50.63	49.37
2010 (n=21)	45.00	55.00

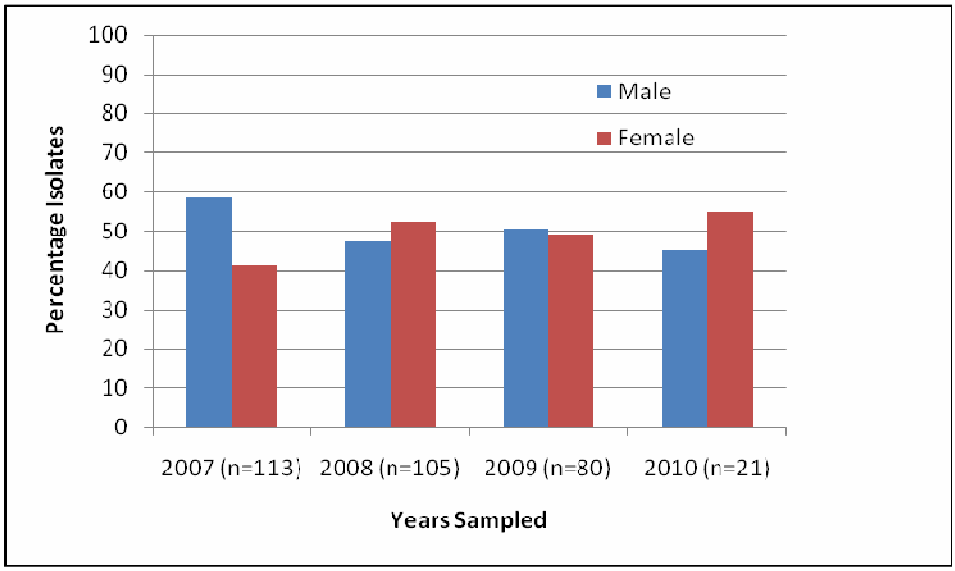

Figure 5: *Gender distribution of dogs sampled between 2007 and 2010.*

4.1.3 Breed

The Bull Terrier and Shepherd type dogs were grouped separately as they were over-represented and are known to be prone to pyoderma. As shown in Table 5 and Figure 6, the large short haired group was consistently worse affected throughout the years sampled, followed by Bull Terrier types. In 2010, small short haired breeds were worse affected compared to the numbers between 2007 and 2009.

Table 5: *Proportion of dog breeds sampled between 2007 and 2010.*

Breed Group	2007 (n=104)	2008 (n=98)	2009 (n=76)	2010 (n=19)
Bull Terrier Type	19.00	15.00	20.00	11.00
Large Short Hair	36.00	32.00	36.00	21.00
Large Thick Coat	13.00	13.00	8.00	11.00
Shepherd Type	11.00	11.00	13.00	5.00
Small Short Hair	13.00	14.00	12.00	32.00
Small Long Hair	7.00	11.00	9.00	16.00
Cross-Breed	2.00	3.00	3.00	5.00

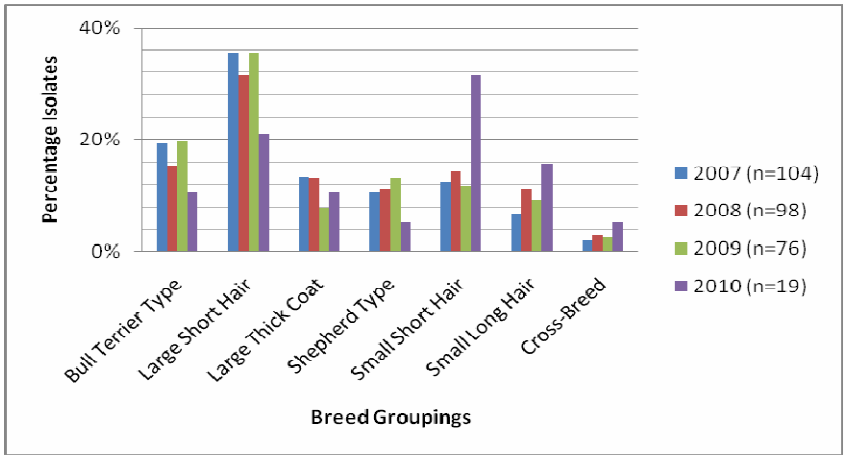

Figure 6: *Proportion of breeds sampled between 2007 and 2010. Large breed shorthaired included Boerboel, Ridgeback, Bull Mastiff, Great Dane, Doberman, Rottweiler, Pointer and Dalmatian; Bull terrier type included English Bull Terrier, Staffordshire Bull Terrier and American Pitt Bull Terrier; large thick coat included Labrador, Golden Retriever, Schnauzer and Siberian Husky; medium to miniature shorthaired pure breed included the Fox Terrier, Jack Russell Terrier, Dachshund, Bulldog. Shepherd type included the German Shepherd Dog and Belgian Shepherd Dog.*

4.1.4 Temporal Distribution

The monthly distribution, with the exception of 2009, tended to be consistent throughout the years (Table 6 and Figure 7).

Table 6: *Monthly distribution of Staphylococcus intermedius isolates between 2007 and 2009.*

Month	2007 (n=113)	2008 (n=105)	2009 (n=80)
January	11.50	9.52	7.50
February	11.50	7.62	20.00
March	6.19	7.62	12.50
April	7.96	8.57	21.25
May	7.08	10.48	13.75
June	7.08	9.52	3.75
July	9.73	9.52	3.75
August	9.73	5.71	1.25
September	4.42	15.24	5.00
October	6.19	5.71	5.00
November	10.62	6.67	3.75
December	7.96	3.81	2.50

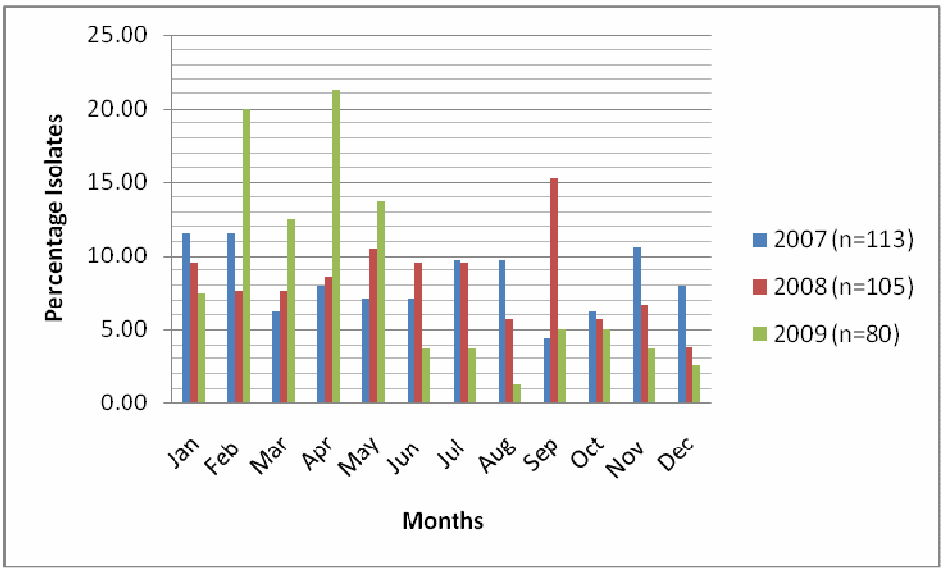

Figure 7: *Temporal distribution of Staphylococcus intermedius isolates between 2007 and 2010.*

4.1.5 Geographical Distribution

The samples tended to be mainly from practices located in the KwaZulu-Natal province of South Africa (Figures 8-11).

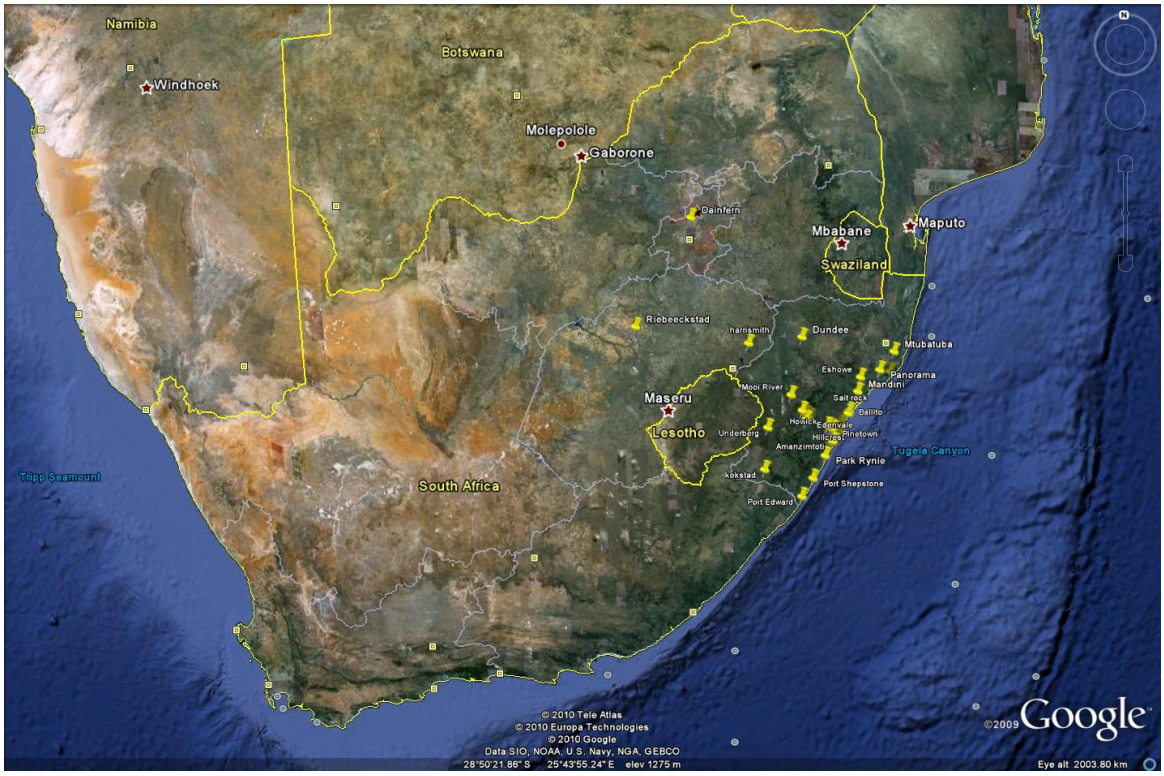

Figure 8: *Overview of the geographical distribution of Staphylococcus intermedius strains isolated in 2007.*

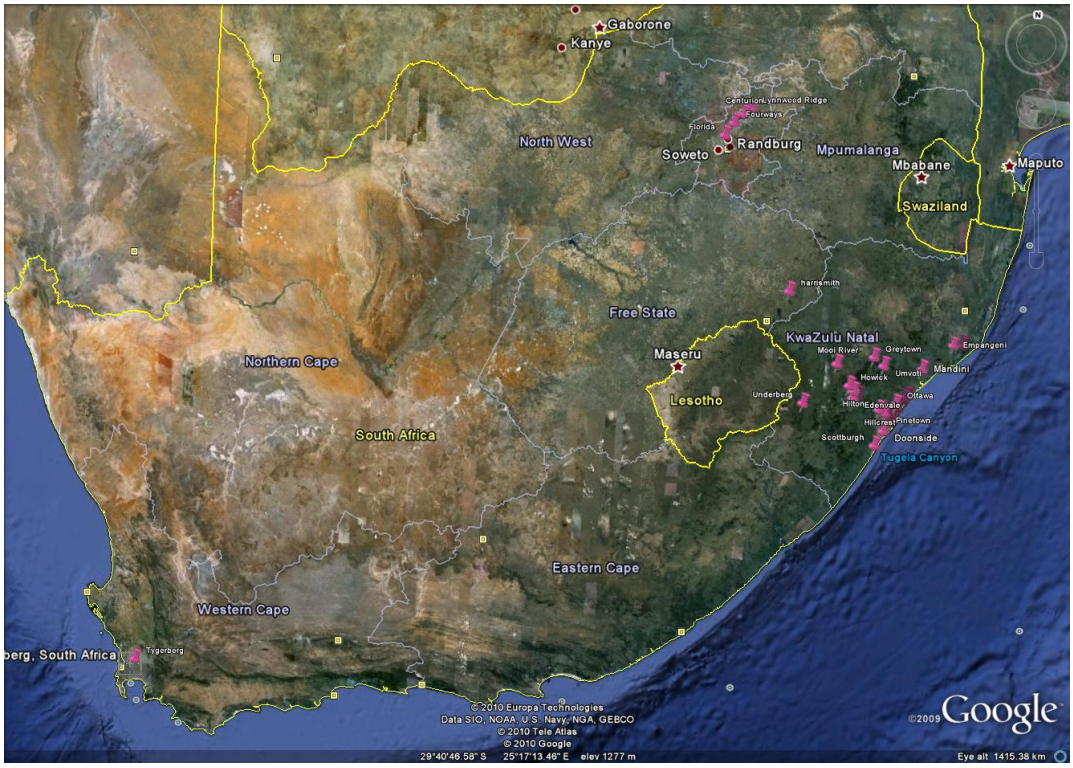

Figure 9: *Geographical distribution of Staphylococcus intermedius strains isolated in 2008*

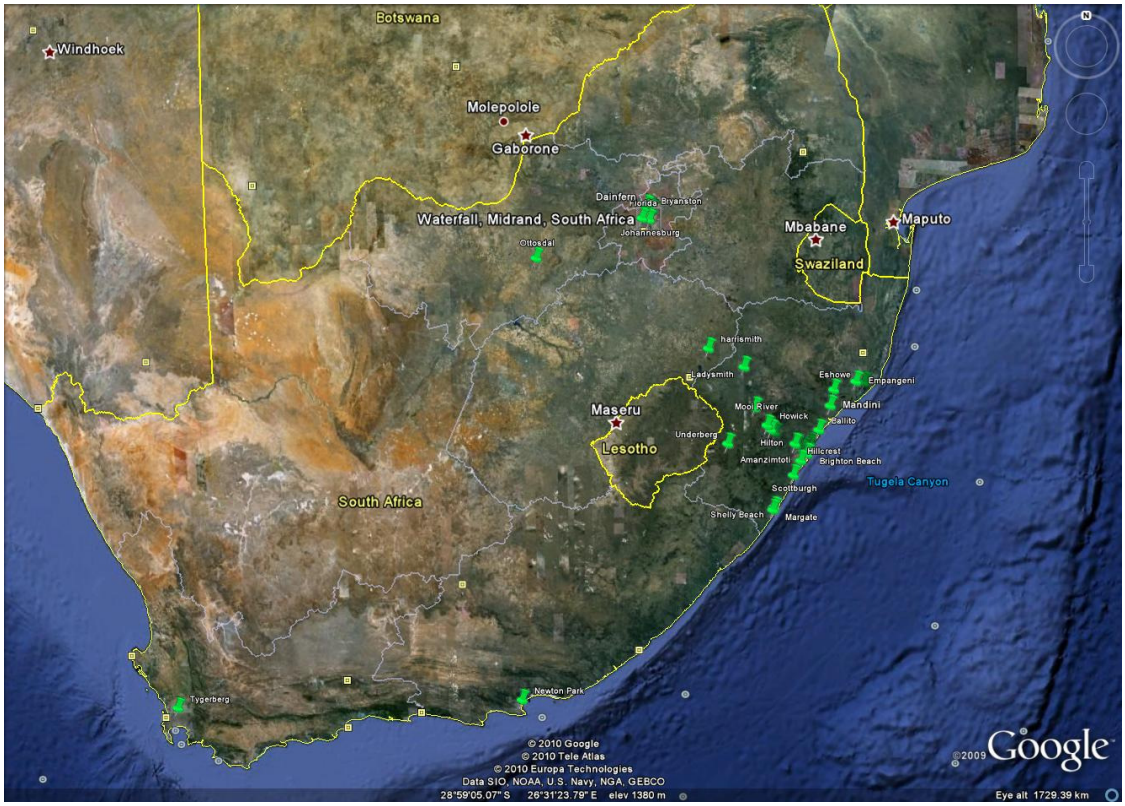

Figure 10: *Geographical distribution of Staphylococcus intermedius strains isolated in 2009.*

Figure 11: *Geographical distribution of Staphylococcus intermedius strains isolated in 2010.*

4.1.6 Susceptibility Testing

As noted in Table 8 and Figure 12, antimicrobial resistance of *S. intermedius* was greatest to ampicillin followed by tetracycline and then potentiated sulphonamides. The results also showed that, in general, antimicrobial resistance was low. Very few methicillin resistant isolates were detected. Temporal trends were not noted, with the exception of ampicillin where isolates became more susceptible and potentiated sulphonamides (co-trimoxazole) where isolates were becoming more resistant.

Table 7: *Percentage susceptibility of Staphylococcus intermedius isolates from canine skin samples between 2007 and 2010*

Antibiotic	2007 (n=113)	2008 (n=105)	2009 (n=80)	2010 (n=21)
Ampicillin	23.89	37.14	40.00	42.86
Cephalothin	99.12	99.05	100.00	95.24
Chloramphenicol	100.00	100.00	100.00	100.00
Co-Trimoxazole	95.58	98.10	88.75	71.43
Enrofloxacin	100.00	99.05	97.50	95.24
Gentamicin	98.23	100.00	98.75	95.24
Neomycin	96.46	96.19	96.25	85.71
Oxacillin	99.12	99.05	97.50	100.00
Amoxicillin-clavulanic acid	100.00	92.38	98.75	90.48
Tetracycline	56.64	55.24	46.25	61.90

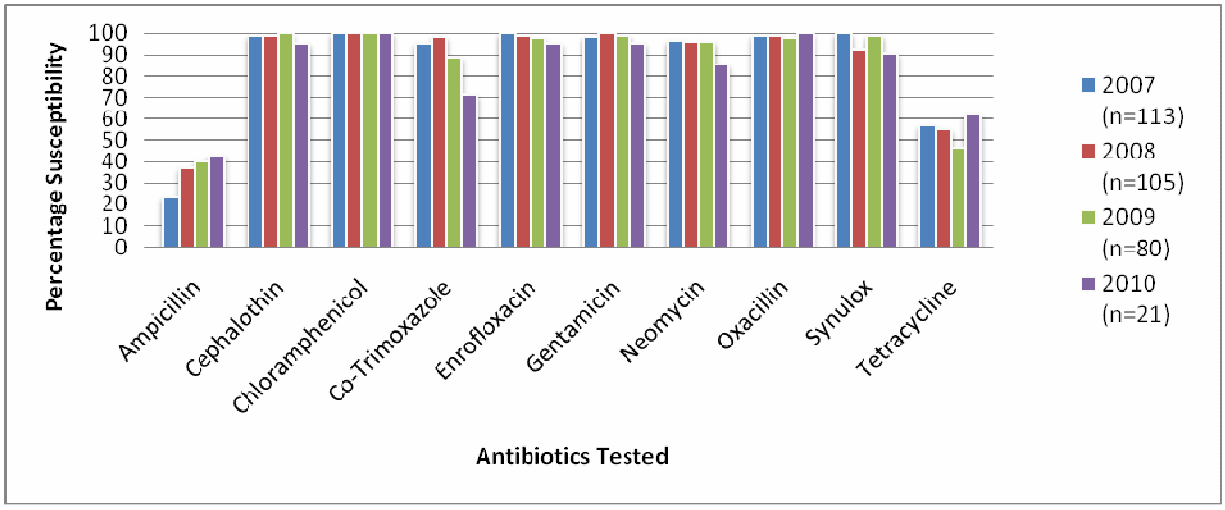

Figure 12: *Percentage susceptibility of Staphylococcus intermedius isolates from canine skin samples between 2007 and 2010. Breakpoint zone of inhibition diameters derived from CLSI, 2008.*

4.2 Kirby-Bauer and MIC Data between 2007 and 2010

In general, both the Kirby-Bauer and broth dilution MIC tests yielded similar results for the antimicrobial agents tested (Table 8 and Figure 13).

Table 8: *Comparison of percentage susceptibility of Kirby-Bauer and broth dilution MIC data between 2007 and 2010.*

Antibiotic	Kirby-Bauer	Broth Dilution MIC
Amikacin	82.92	100.00
Ampicillin	34.14	87.80
Amoxicillin-clavulanic acid	92.68	100.00
Clindamycin	97.56	97.56
Co-Trimoxazole	75.61	95.12
Enrofloxacin	97.56	100.00
Erythromycin	95.12	100.00
Gentamicin	97.56	100.00
Imipenem	100.00	100.00
Penicillin	34.14	68.29
Rifampicin	100.00	100.00
Chloramphenicol	100.00	100.00
Marbofloxacin	97.56	100.00
Doxycycline	51.21	100.00
Ticarcillin	97.56	100.00
Ceftiofur	97.56	100.00
Oxacillin	100.00	100.00

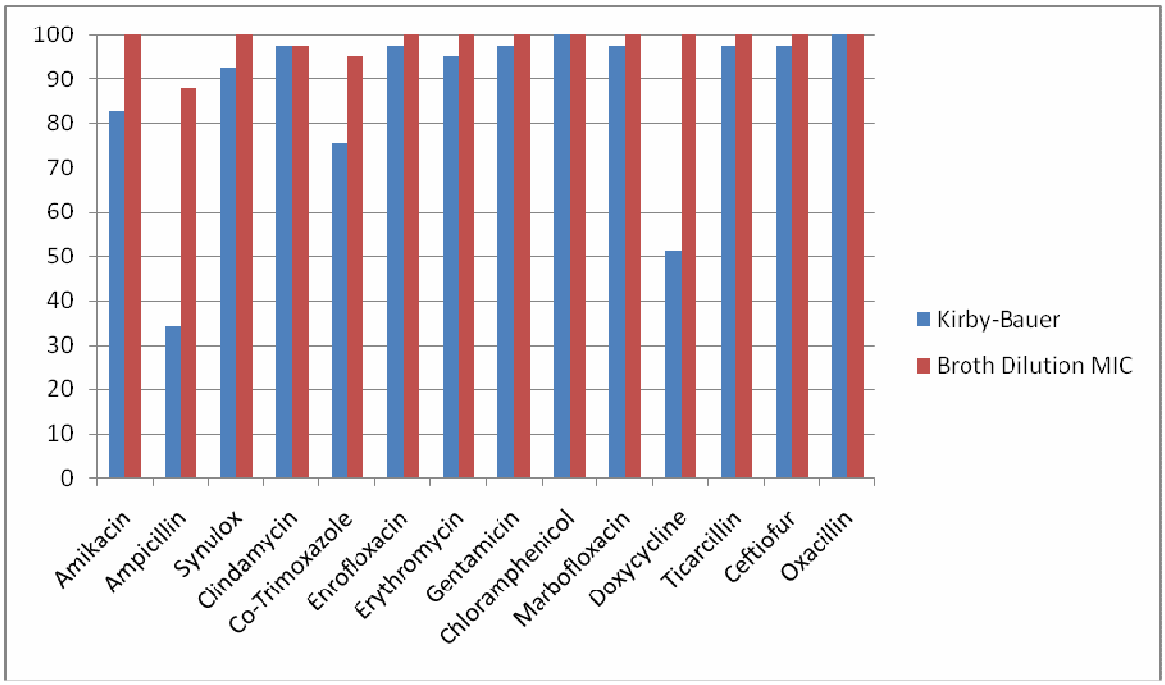

Figure 13: *Comparison of percentage susceptibility of Kirby-Bauer and broth dilution MIC data between 2007 and 2010*

Table 9 depicts the percentage MIC distribution of the isolates for each dilution as well as the MIC_{50} (median value) and MIC_{90}. The percentage resistance represented in the table was based on published breakpoints (CLSI, 2008). Using the MIC method, all of the isolates tested were found to be completely sensitive to ticarcillin, oxacillin, amoxicillin-clavulanic acid, imipenem, ceftiofur, chloramphenicol, doxycycline, gentamicin, amikacin and co-trimoxazole. Of the isolates tested, between 2-40% showed some level of resistance to the following antimicrobials: erythromycin, penicillin, ampicillin, enrofloxacin, clindamycin and marbofloxacin. The highest level of resistance was shown to erythromycin.

Table 9: *Percentage MIC distribution of Staphylococcus intermedius strains isolated between June 2009 and June 2010*

Antimicrobial	% Susceptible	% with MIC less than tested range	Concentration of antimicrobial tested (µg/ml) 0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	MIC_{50}	MIC_{90}
Penicillin	68.29	43.90	9.76	14.63	12.20	7.32	7.32	0.00	2.44	2.44				0.06	0.5
Ampicillin	87.80	80.49			7.32	4.88	2.44	2.44	2.44	0.00	0.00			<0.125	0.5
Ticarcillin	100.00	100.00								0.00	0.00	0.00	0.00	<8	<8
Oxacillin	100.00	100.00			0.00	0.00	0.00	0.00	0.00					<0.25	<0.25
Amoxicillin-clavulanic acid	100.00	100.00							0.00	0.00	0.00	0.00		<4/2	<4/2
Imipenem	100.00	100.00					0.00	0.00	0.00	0.00				<1	<1
Ceftiofur	100.00	63.41			34.15	2.44	0.00	0.00	0.00					<0.25	0.25
Erythromycin	53.66	39.02				14.63	7.32	17.07	21.95					0.5	4
Clindamycin	97.56	97.56				0.00	0.00	0.00	2.44					<0.5	<0.5
Chloramphenicol	100.00	14.63							78.05	7.32	0.00			4	4
Doxycycline	100.00	48.78						17.07	34.15	0.00				<2	4
Enrofloxacin	92.68	68.29			17.07	7.32	4.88	2.44						<0.25	0.5
Marbofloxacin	97.56	9.76			65.85	19.51	2.44	2.44						0.25	0.5
Gentamicin	100.00	90.24					7.32	2.44	0.00	0.00				<1	<1
Amikacin	100.00	87.80							7.32	4.88	0.00	0.00		<4	4
Co-Trimoxazole	100.00	82.93				12.20	0.00	4.88	0.0					<0.5/9.5	0.5/9.5

The shaded areas indicate the concentration range tested for each antimicrobial used. Blue shading indicates that the MIC was within the tested range while red shading indicates resistance to the particular antimicrobial, based on the various breakpoints used (CLSI, 2008). The concentration range for Amoxicillin-clavulanic acid was 4/2 – 32/16(µg/ml). The concentration range for Co-Trimoxazole was 0.5/9.5 – 2/38(µg/ml).

CHAPTER 5

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The antimicrobial resistance predicament in human medicine has brought to light various aspects of the use of these substances in animals. There is, however, little useful data on antimicrobial resistance and usage in companion animals (Prescott *et al*., 2002). The purpose of this study was to investigate the antimicrobial resistance patterns in *Staphylococcus intermedius* isolates from canine pyoderma cases in South Africa. *Staphylococcus intermedius* is a significant cause of juvenile and adult canine pyoderma. Chronic and recurrent pyoderma is a complex syndrome involving cell-mediated hypersensitivity, endocrine disorders and genetic predisposition. It responds poorly to antibiotic therapy alone. Staphylococcal pustular dermatitis occurs in neonates and adults under conditions of poor hygiene. It responds readily to antimicrobial therapy (Quinn *et al.,* 1994). Pyoderma is one of the commonest causes of canine skin disease worldwide. The canine *stratum corneum* is thinner and more compact with intercellular spaces that are permeated with less protective emulsion when compared to other species. Canine hair follicle infundibula are also relatively unprotected (Siugzdaite *et al*., 2008).

Various aspects of the cases studied were considered such as age, gender, breed, temporal distribution, geographical distribution and the susceptibility profiles of the isolates. The Kirby-Bauer and broth microdilution methods were also compared.

5.1 Age

The number of skin samples yielding *Staphylococcus intermedius* was high in dogs up to the age of 6 years and, then decreased with only a few cases in dogs aged 11 years or older (Table 3 and Figure 4). The distribution of samples collected in 2010 was unusual in that there were two peaks, one in dogs two years old or less and one in 6 to 9 year-old dogs. This may just be related to the numbers of cases sampled in 2010 compared to the other years. With the exception of skin samples taken in 2008, a high percentage of dogs were under the age of one year old. 2008 had a lower percentage of affected dogs less than one year of age compared to the other years. In the literature, many authors have indicated that staphylococcal pyoderma is more common in dogs below the age of five years. Dogs above this age are less likely to contract this condition (Siugzdaite *et al*., 2008). This is largely in agreement with the results found in this study.

5.2 Gender

The genders tended to be equally distributed throughout the years, with almost equal proportions of affected males and females present (Table 4 and Figure 5). According to Holm *et al.* (2002), pyoderma is more common in males. Most authors, however, reported no statistically significant difference between the number of affected males and females (Siugzdaite *et al.*, 2008).

5.3 Breed

The Bull Terriers and Shepherds were grouped separately as they were over-represented and are known to be prone to pyoderma. The large short haired group was consistently worst affected throughout the years sampled, followed by the Bull Terrier types. In 2010, small short haired breeds were worse affected compared to the numbers between 2007 and 2009 (Table 5 and Figure 6). This may just be related to the numbers of cases sampled in 2010 compared to the other years. Firm conclusions cannot, therefore, be made for this reason, The literature revealed some controversy with regards to breed disposition to this disease. Some authors stated that pyoderma is more common in long-haired breeds while others maintain that short-haired breeds were worse affected. The opinion of yet others was that hair length does not affect the occurrence of skin diseases (Siugzdaite *et al.*, 2008).

5.4 Temporal Distribution

The monthly distribution, with the exception of 2009, tended to be consistent throughout the years (Table 6 and Figure 7). The unusual distribution in 2009 could be associated with a general distribution in the number of samples compared to the other years sampled. The literature presented limited data about the manner in which bacterial disease is affected by seasonal changes. Holm *et al.* (2002) reported that seasonal changes do not have any significant influence which is in line with the findings of this study.

5.5 Geographical Distribution

The samples tended to be mainly from practices located in the KwaZulu-Natal province of South Africa (Figures 8-11). This is most likely due to the fact that the laboratory is located in this province, with a courier network that arranges collection from these practices. Practices in other regions send their samples to the laboratory via private courier companies and the South African Post Office. Therefore, no conclusions can be drawn as to whether dogs in certain areas of South Africa are more prone to pyoderma than those in other areas.

5.6 Susceptibility Testing

Antimicrobial resistance of *S. intermedius* was greatest to ampicillin followed by tetracycline and then potentiated sulphonamides. The results also showed that, in general, antimicrobial resistance was low. Very few methicillin resistant isolates were detected. Temporal trends were not noted, with the exception of ampicillin where isolates became more susceptible and potentiated sulphonamides (co-trimoxazole) where isolates were becoming more resistant (Table 8 and Figure 12). Pellerin *et al.* (1998) and Hartmann *et al.* (2005) similarly showed that resistance was most commonly observed to penicillin, tetracycline and sulphamethoxazole-trimethoprim (co-trimoxazole). These drugs should not be used without prior susceptibility testing (Aarestrup, 2006). *Staphylococcus intermedius* is less resistant to erythromycin, clindamycin, cephalexin, oxacillin, amoxicillin-clavulanic acid, enrofloxacin, marbofloxacin and gentamicin, with most strains being susceptible to these drugs (Pellerin *et al.*, 1998). Werckenthin *et al.* (2001) found that resistance to penicillin and tetracycline is frequently found in *Staphylococcus intermedius* and is on the increase. Resistance to most other antibiotics, particularly newer generation antimicrobial agents such as the fluoroquinolones, is still comparatively low.

5.7 Kirby-Bauer and MIC Data

In general, both the Kirby-Bauer and broth dilution MIC tests yielded similar results for the antimicrobial agents tested (Table 8 and Figure 13). The main difference between the two tests was evident in the over-estimation of resistance by the Kirby-Bauer test in the cases of ampicillin, co-trimoxazole, penicillin and doxycycline. This could be related to the instability of these particular drugs *in vitro*. Inoculum densities may also have played a role, with denser inocula producing smaller zone sizes for the drugs tested.

The Kirby-Bauer method remains as a convenient, low-cost means of conducting antimicrobial susceptibility tests and is widely used in veterinary laboratories. The test provides qualitative results that categorise isolates as susceptible, intermediate or resistant. Almost all veterinary-specific agents are available in the antimicrobial-impregnated disks. However, low-volume veterinary-specific agents may only be available from the pharmaceutical manufacturer. Smaller veterinary laboratories may have difficulties standardising the inoculum used in this method, however, commercial systems are available for this purpose (Aarestrup, 2006).

Table 9 depicts the percentage MIC distribution of the isolates for each dilution as well as the MIC_{50} (median value) and MIC_{90}. The percentage resistance represented in the table was based on published breakpoints (CLSI, 2008). Using the MIC method, all of the isolates tested were found to be completely sensitive to ticarcillin, oxacillin, amoxicillin-clavulanic acid, imipenem, ceftiofur, chloramphenicol, doxycycline, gentamicin, amikacin and co-trimoxazole. Of the isolates tested, between 2-40% showed some level of resistance to the following

antimicrobials: erythromycin, penicillin, ampicillin, enrofloxacin, clindamycin and marbofloxacin. The highest level of resistance was shown to erythromycin. The genes which confer erythromycin resistance in canine staphylococci are almost exclusively *ermB* genes. The increase in resistance to the lincosamides, lincomycin, clindamycin and erythromycin may be attributed to the increased use of these drugs in the last decade (Pellerin *et al.*, 1998).

The MIC method may be performed in a variety of ways. This method provides a quantitative value as well as a categorisation of the organism as susceptible or resistant. Standardised methods for testing more fastidious organisms such as anaerobes and *Campylobacter* species have been developed. The MIC method is preferred for use in surveillance or epidemiological investigations as it allows for calculation of summary statistics. Of the various MIC formats used, the broth microdilution method is most widely used and is available in a variety of commercial systems as either dry or frozen panels. It permits testing of a wide range of antimicrobials on a small scale. The disadvantages of these MIC panels are that these panels are inflexible unless the laboratory is willing to bear the cost of custom panels. Not all veterinary-specific agents are available on all panels. Laboratories involved in surveillance programmes or epidemiological studies usually prefer to test a smaller number of antimicrobial agents for an extended number of dilutions. Many diagnostic laboratories choose to use a breakpoint panel. Breakpoint panels allow the laboratory to test a larger number of compounds with dilution ranges spanning the interpretive criteria or breakpoints for each agent (Aarestrup, 2006).

5.8 Conclusion and Recommendations

Antimicrobial resistance patterns largely reflect changing fashions in the use of antimicrobial drugs. Reports of resistance from diagnostic laboratories often represent treatment failures rather than treatment successes as animals that have been previously treated will be more likely to yield resistant bacteria than those that have not. The problem of antimicrobial resistance in bacterial pathogens in dogs should be much less serious than in those isolated from humans. This is because dogs are less likely to be exposed to antimicrobials, other than for short, sporadic periods. They are also less likely to be hospitalised. Chronically ill dogs may be euthanized and immune-compromised animals are not normally treated with broad-spectrum and very potent drugs, like imipenem (Prescott *et al.*, 2002). *Staphylococcus intermedius* isolates from dogs have increased in resistance to some drugs while decreasing in resistance to others. These changes reflect the changing patterns in the use of individual drugs over time. Antimicrobial resistance is not yet at a crisis stage in canine medicine but there have been warning signs. More information is needed on antimicrobial resistance and its molecular basis in canine medicine.

Bacteria that exhibit antimicrobial resistance will always be a part of our lives. The resistance dilemma in human medicine has brought to light how community-acquired resistance can arise in a relatively short time period. Data pertaining to antimicrobial resistance and the way in which drugs are used in companion animal practice is still

relatively hard to come by. There needs to be a continual process of improving prudent use guidelines in companion animal practice so that resistance problems can be avoided. It would be extremely beneficial if an international agreement could be reached amongst veterinarians on a simple but effective approach to prudent companion animal drug use. There should be a concerted effort by veterinary hospitals and companion animal practices to develop formal guidelines for the usage of antimicrobials. Active and effective infection control programmes need to be implemented in veterinary hospitals in order to minimise the spread of resistant organisms or their resistance genes. Veterinary clinical microbiologists also need to agree on standards for monitoring and reporting resistance in companion animal bacteria. This data needs to be used in conjunction with data on antimicrobial drug usage. The limitations of the available data also need to be understood and improved on.

APPENDIX A

Media

1. Agar Technical (Oxoid L13)
2. Columbia Blood Agar Base (Oxoid CM331)
3. DNase Agar (Oxoid CM321)
4. Mueller Hinton Agar (Oxoid CM337)
5. Mueller Hinton Broth (Oxoid CM405)
6. Maltose (Associated Chemical Enterprises M1417NN00100)
7. Mannitol (Associated Chemical Enterprises M1425NN00250)
8. Phenol Red Broth Base (Difco 0092)

Antibiotic Sensitivity Discs

1. Ampicillin (Oxoid CT0003B)
2. Cephalothin (Oxoid CT0010B)
3. Chloramphenicol (Oxoid CT0013B)
4. Amoxicillin-Clavulanic Acid (Oxoid CT0223B)
5. Enrofloxacin (Oxoid CT0639B)
6. Gentamicin (Oxoid CT0024B)
7. Neomycin (Oxoid CT0033B)
8. Oxacillin (Oxoid CT0159B)
9. Tetracycline (Oxoid CT0054B)
10. Trimethoprim-Sulphamethoxazole (Oxoid CT0052B)
11. Amikacin (Oxoid CT0107B)
12. Ceftiofur (Oxoid CT1751B)
13. Clindamycin (Oxoid CT0064B)
14. Doxycycline (Oxoid CT0018B)
15. Erythromycin (Oxoid CT0020B)
16. Imipenem (Oxoid CT0455B)
17. Marbofloxacin (Becton Dickinson 232170)
18. Penicillin (Oxoid CT0043B)
19. Rifampicin (Oxoid CT0207B)
20. Ticarcillin (Oxoid CT0167B)

REFERENCES

AARESTRUP, F.M. 2006. *Antimicrobial Resistance in Bacteria of Animal Origin*. Washington D.C.: ASM Press.

BEMIS, D.A., JONES, R.D., FRANK, L.A. & KANIA, S.A. 2009. Evaluation of susceptibility test breakpoints used to predict *mecA*-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs. *Journal of Veterinary Diagnostic Investigation*, 21: 53-58.

BLONDEAU, J.M. 2009. New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. *Veterinary Dermatology,* 20: 383-396.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). 2008. *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard – third edition*. CLSI document M31-A3. Philadelphia: CLSI.

COHN, L.A. & MIDDLETON, J.R. 2010. A veterinary perspective on methicillin resistant staphylococci. *Journal of Veterinary Emergency and Critical Care,* 20(1): 31-45.

DEBOER, D.J. 2006. Canine staphylococcal pyoderma. US Companion Animal Health. 26-28 www.touchbriefings.com/pdf/2397/deboer.pdf (Accessed on 28/04/2009)

EPSTEIN, C.R., YAM, W.C., PEIRIS, J.S.M. & EPSTEIN, R.J. 2009. Methicillin-resistant commensal staphylococci in healthy dogs as a potential zoonotic reservoir for community-acquired antibiotic resistance. *Infection, Genetics and Evolution,* 9: 283-285.

FITZGERALD, J.R. 2009. The *Staphylococcus intermedius* group of bacterial pathogens: species re-classification, pathogenesis and the emergence of methicillin resistance. *Veterinary Dermatology,* 20: 490-495.

GUARDABASSI, L., LOEBER, M.E. & JACOBSON, A. 2004a. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Veterinary Microbiology,* 98: 23-27.

GUARDABASSI, L., SCHWARTZ, S. & LLOYD, D.H. 2004b. Pet animals as reservoirs of antimicrobial-resistant bacteria. *Journal of Antimicrobial Chemotherapy,* 54: 321-332.

HACKBARTH, C.J. & CHAMBERS, H.F. 1989. Methicillin-resistant staphylococci: genetics and mechanisms of resistance. *Antimicrobial Agents and Chemotherapy,* 33(7): 991-994.

HAJEK, V. 1976. *Staphylococcus intermedius*, a new species isolated from animals. *International Journal of Systematic Bacteriology,* 26(4): 401-408.

HARTMANN, F.A., WHITE, D.G., WEST, S.E.H., WALKER, R.D. & DEBOER, D.J. 2005. Molecular characterisation of *Staphylococcus intermedius* carriage by healthy dogs and comparison of antimicrobial susceptibility patterns to isolates from dogs with pyoderma. *Veterinary Microbiology,* 108: 119-131.

HOEKSTRA, K.A. & PAULTON, R.J.L. 2002. Clinical prevalence and antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus intermedius* in dogs. *Journal of Applied Microbiology,* 93: 406-413.

HOLM, B.R., PETERSSON, U., MORNER, A., BERGSTROM, K., FRANKLIN, A. & GREKO, C. 2002. Antimicrobial resistance in staphylococci from canine pyoderma: a prospective study of first-time and recurrent cases in Sweden. *The Veterinary Record,* 151: 600-605.

LILENBAUM, W., VERAS, M., BLUM, E. & SOUZA, G.N. 2000. Antimicrobial susceptibility of staphylococci isolated from otitis externa in dogs. *Letters in Applied Microbiology,* 31:42-45.

LOEFFLER, A., LINEK, M., MOODLEY, A., GUARDABASSI, L., SUNG, J.M.L., WINKLER, M., WEISS, R. & LLOYD, D.H. 2007. First report of multiresistant *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. *Veterinary Dermatology*, 18:412-419.

MORALES, C.A., SCHULTZ, K.T. & DEBOER, D.J. 1994. Antistaphylococcal antibodies in dogs with recurrent staphylococcal pyoderma. *Veterinary Immunology and Immunopathology,* 42: 137-147.

MURRAY, P.R., BARON E.J., PFALLER, M.A., TENOVER, F.C. & YOLKEN, R.H. 1999. *Manual of Clinical Microbiology*. Washington: ASM Press. 115: 1469-1472.

PELLERIN, J.L., BOURDEAU, P., SEBBAG, H. & PERSON, J.M. 1998. Epidemiosurveillance of antimicrobial compound resistance of *Staphylococcus intermedius* clinical isolates from canine pyodermas. *Comparative Immunology, Microbiology and Infectious Diseases,* 21:115-133.

PRESCOTT, J.F., HANNA, W.J.B., REID-SMITH, R. & DRAST, K. 2002. Antimicrobial drug use and resistance in dogs. *Canine Veterinary Journal*, 43: 107-116.

QUINN, P.J., CARTER, M.E., MARKEY, B. & CARTER, G.R. 1994. *Clinical Veterinary Microbiology.* London: Wolfe Publishing.

QUINN, P.J., MARKEY, B.K., CARTER, M.E., DONNELLY, W.J. & LEONARD, F.C. 2002. *Veterinary Microbiology and Microbial Disease.* Oxford: Blackwell Publishing.

RICH, M. 2005. Staphylococci in animals: prevalence, identification and antimicrobial susceptibility, with an emphasis on methicillin-resistant *Staphylococcus aureus*. *British Journal of Biomedical Science,* 62(2): 98-104

SIUGZDAITE, J., ZAMOKAS, G., GRIGONIS, A., MACIJAUSKAS, V. & LASYS, V. 2008. Antimicrobial susceptibility of *Staphylococcus* spp. Isolated from dogs with pyoderma. *Medycyna Wet.,* 64: 991-994.

VANNI, M., TOGNETTI, R., PRETTI, C., CREMA, F., SOLDANI, G., MEUCCI, V. & INTORRE, L. 2009. Antimicrobial susceptibility of *Staphylococcus intermedius* and *Staphylococcus schleiferi* isolated from dogs. *Research in Veterinary Science,* 87: 192-195.

WERCKENTHIN, C., CARDOSO, M., MARTEL, J.L. & SCHWARTZ, S. 2001. Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus,* porcine *Staphylococcus hyicus* and canine *Staphylococcus intermedius*. *Veterinary Research,* 32: 341-362.