PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS FROM SURGICAL PATIENTS AND NORMAL DOGS

CECILIA NJOROGE (BVM)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY SURGERY (MVET SURGERY) OF THE UNIVERSITY OF NAIROBI

FACULTY OF VETERINARY MEDICINE

OCTOBER 2016

DECLARATION

This is my original work and I declare that it has not been submitted for award of a degree in any other University.

DR. CECILIA W. NJOROGE, BVM (UNIVERSITY OF NAIROBI)

SIGNATURE:_____ **DATE:**_____

This thesis has been submitted for examination with our approval as University Supervisors:

PROF. JOHN DEMESI MANDE (BVM, MSc, PhD)

SIGNATURE:_____ **DATE:**_____

PROF. SIMON ERIC MITEMA (BVM, MSc, PhD)

SIGNATURE:_____ **DATE:**_____

DR. JAFRED M.A. KITAA (BVM, MSc, PhD)

SIGNATURE:_____ **DATE:**_____

DEDICATION

To my parents, Dr. B.Ngaruiya and Mrs. Pauline Njoroge.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my supervisors Prof. J.D. Mande, Prof. S.E. Mitema and Dr. J.M.A. Kitaa, their expertise; vast knowledge and guidance during all levels of this project were invaluable. Special thanks to Prof. Mande, who gave me the foundation to start my postgraduate degree by encouraging me to enrol for graduate study. His mentorship, persistence and technical assistance during the course of my study truly made a difference in my life. I also want to acknowledge Dr. Aboge for taking time out of his busy schedule to help me navigate the world that is Molecular Biology. His vast expertise in this field and willingness to guide me through it was remarkable and I am eternally grateful. I am grateful to Prof. Mitema and NACOSTI (National Commission for Science, Technology and Innovation), for the financial support towards the research project.

I would like to thank the staff at the Department of Public Health and Pharmacology and Toxicology, especially Mr. Nduhiu Gitahi, Mr. Alfred Mainga and James Macharia for the technical assistance, support and encouragement they offered me during the course of my project. Appreciation also to Beatrice, Carol, Masinde and Lucy.

I acknowledge the staff at the University of Nairobi Small Animal Hospital and Andy's Veterinary clinic for facilitating access to clinical cases in particular, Dr. W. Mwangi, Dr. L. Mathai and Dr. D. Muasya of the UoN Small Animal Clinic and Dr. A. Matole and Dr. A. Gitari of the Andy's Veterinary Clinic.

I would also like to express my deepest gratitude to my friends Maryanne Kagai, Robert Ndung'u, Anne Olang'o, Paul Kimweli and David Ochieng'. Their support through what has been a very challenging journey have truly made a lasting impact in my life and taught me how to face challenges head on. My gratitude also goes to my postgraduate classmates Dr. Hassan Mohammed and Dr. Jamleck Muriuki for their friendship, company, useful insights and exchange of ideas throughout my study.

I would also like to thank my family for the support they provided me through my entire life and in particular, I must acknowledge my parents for their unwavering support, guidance and love. Their dedication to education and inspires me to keep aiming higher.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Staphylococcus spp. are globally recognized as colonisers of the skin and important causes of infection in the skin of animals and humans. The increasing prevalence of antimicrobial resistance, and in particular multi-drug resistant methicillin resistant *Staphylococcus aureus* (MRSA) and the emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs has made treatment more challenging. The objectives of this study were to determine bacterial ecology and their antimicrobial susceptibilities from wound and ear swabs with emphasis on *Staphylococcus aureus* and to determine the prevalence of MRSA/MRSP in normal dogs and surgical patients using phenotypic and genotypic assays. The study also undertook Basic Local Alignment Search Tool (BLAST) analysis of sequenced polymerase chain reaction (PCR) amplicons of the resistance determinant.

The study was divided into two parts, retrospective and prospective components. The retrospective component of the study was designed to determine the bacterial ecology and antimicrobial susceptibility from samples taken from surgical patients. Records were retrieved from clinical laboratory of 291 bacteriological samples collected from 200 dogs submitted to the University of Nairobi Small Animal Clinic over a 10 year period between January 2004 and December 2013. Information collected included the location from where the sample was collected (wound or ear swab) as well as age, sex of the animal, microbial isolates and antimicrobial susceptibility profile. In addition, for samples obtained from wounds, records were further reviewed to determine the type, nature, location and causes of the wounds.

In the prospective component of the study, investigations were done on 191 samples obtained from dogs presented at the University of Nairobi Small Animal Clinic and a community veterinary clinic. Identification of coagulase positive *Staphylococcus* spp. (COPS) was undertaken using mannitol salt agar as a selective medium and coagulase testing using

reconstituted rabbit plasma. Final confirmation of COPS was done by PCR using primers specific to the 16S rRNA gene of *Staphylococcus* Genus and primers specific to the *nuc* (thermonuclease) gene of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* spp. Antimicrobial susceptibility testing (AMST) was performed using Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. *Staphylococcus aureus* ATCC 25923 was used as the reference organism. Oxacillin was used as a surrogate for methicillin. For each isolate, susceptibility testing was done twice and the mean zone diameter of inhibition calculated. The mean diameter was then compared to the CLSI interpretive standard break points for *Staphylococcus aureus* and *Staphylococcus pseudintermedius* for oxacillin and the number of resistant isolates noted.

DNA of the phenotypically resistant COPS isolates were extracted and thereafter specific PCR assays were used to detect the resistance determinant among the resistant isolates. The PCR amplicons were electrophoresed on 1.5 % agarose gel in Tris-acetate-EDTA buffer supplemented with 0.5µg/ml of ethidium bromide and calibrated using 100 bp DNA ladder. The gels were visually inspected by Ultra Violet (UV)-transilluminator. The amplicons obtained were purified and sequenced using the ABI PRISM 3770 genetic analyzer. BLAST analysis was done to confirm the identities of the sequenced amplicons, their location on chromosomal DNA, the geographical distribution and diversity of hosts from which genes' homologues had previously been isolated. The sequenced resistance gene was submitted to the National Center for Biotechnology Information genetic sequence database (NCBI GenBank) for assignment of accession numbers.

The retrospective study findings revealed that the most prevalent microbial isolates recovered from dogs diagnosed with wounds, surgical site infections and otitis externa, were *Staphylococcus aureus* 50 % (133/267) and *Proteus spp.* 14 % (38/267) respectively. Other frequently recovered isolates included *Pseudomonas spp.* 10 % (28/267), other *Staphylococcus spp.* 8.2 % (22/267), *Streptococcus spp.* 6.7 % (18/267) and *E. coli* 5.6 % (15/267) respectively.

Resistance to antimicrobial drugs was observed in the majority of the isolates in the retrospective study, with 97% (262/267) of the isolates demonstrating antimicrobial resistance to at least one drug. Resistance to sulphonamides (96%), potentiated sulphonamides (89%), ampicillin (68%), amoxicillin (62%) and tetracycline (56%) was relatively high for all bacterial species examined.

Staphylococcus aureus isolates showed 95% resistance to sulfamethoxazole, 55% to ampicillin, 52% to tetracycline and 52% to amoxicillin/clavulanic acid respectively. *Pseudomonas* spp. showed the highest multidrug resistance with all (100%) isolates showing resistance to amoxicillin, amoxicillin/clavulanic acid and sulfamethoxazole, the isolates also showed high resistance to cotrimoxazole (93%), ampicillin (93%) and tetracyclines (80%) respectively. Low resistance to gentamicin (9%), norfloxacin (24%) and chloramphenicol (33%) was observed in all bacterial isolates.

The cause of 33% (18/54) wounds was not specified in the records. Common source of wound swabs included, surgical site infections (SSI) 23.9% (11/46) followed by bite wounds 21.7% (10/46), and traumatic injuries 15.2% (7/46). Majority of the wounds 67% (31/46) were recorded on the limbs of affected animals with hindlimbs 32.6% (15/46) being more affected than hindlimbs 28.3% (13/46).

Data from the prospective study revealed that presumptive *Staphylococcus spp.* were isolated from 34% (65/191) of the samples. Coagulase positive *Staphylococcus* spp. (COPS) accounted for 43% (28/65) of the *Staphylococcus* spp. isolated. Phenotypic resistance to oxacillin was detected in 53.6% (15/28) of COPS. The PCR assay detected *mec*A gene as a 286 bp gene fragment amplicon in 2 of the 15 (7%) oxacillin resistant phenotypes. BLAST analysis of the sequenced PCR products revealed that one of the resistance genes had 99 % nucleotide identity to sequences in the NCBI GenBank database, while the other sample had a 95 - 97% identity. Further analysis of the resistant determinants by BLAST revealed that all the resistant *Staphylococcus* strains were *Staphylococcus aureus* strains.

This study confirms *Staphylococcus aureus* as the most prevalent bacterial isolate from wounds, surgical site infections and otitis externa. *Proteus* spp., *Pseudomonas* spp., other *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli* in descending order, were also frequently isolated. Gentamicin, norfloxacin and chloramphenicol in that order were the most effective antimicrobial agents in management of wounds, surgical site infections and otitis externa in the retrospective study. The study reports the first case of MRSA strains in dogs in Kenya which were associated with mobile genetic elements (*SCCmec*) and have the potential to be transferred from dogs to humans. The MRSA resistant determinants observed are similar to some human like isolates reported in several countries.

CHAPTER ONE

1.0. INTRODUCTION

Staphylococcal species are commensal bacteria and leading causes of community and hospital-associated disease in humans and animals worldwide (Vengust *et al.*, 2006). The most clinically relevant staphylococci in veterinary medicine are the coagulase positive *Staphylococcus aureus* and members of the *Staphylococcus intermedius* group, particularly *Staphylococcus pseudintermedius* (Weese and Duijkeren, 2009).

Although *S. aureus* can colonize and infect companion animal species, the most common commensal staphylococci of canines is *Staphylococcus pseudintermedius* (formerly *S. intermedius)* with isolation rates of between 46- 92% in healthy dogs compared to 10% *S. aureus* (Hanselman *et al.*, 2009; Rubin and Chirino-trejo, 2011; Paul *et al.*, 2011). *Staphylococcus pseudintermedius* can be isolated from the nares, mouth, pharynx, forehead, groin and anus of healthy dogs and cats. It is an opportunistic pathogen and a leading cause of skin and ear infections, infections of other body tissues and cavities, and post-operative wound infections in dogs and cats (Guardabassi et al., 2004; van Duijkeren et al., 2011).

Staphylococcal infections are frequently treated with antibiotics and, consequently, antibiotic resistance and⁄or acquired resistance have developed (Normand *et al*., 2000). The increasing prevalence of antimicrobial resistance has made staphylococcal infections become more dangerous and costly to treat. Of considerable concern is Methicillin-resistant *S. aureus* (MRSA) and emergence of Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs and cats. These resistant strains of bacteria pose a new threat to animal health due to the limitations in their management (EMA, 2011).

MRSA was first identified in the United Kingdom, and was then recognized as a nosocomial pathogen worldwide (HA-MRSA) (Petinaki and Spiliopoulou, 2012). Subsequently, there have been reports of MRSA infections occuring in people with no exposure to a healthcare setting; these have been designated community acquired (CA-MRSA). There are differences in epidemiology of HA-MRSA and CA-MRSA including resistance determinants, *SCCmec* types and clonal complexes. HA-MRSA has also been found to be resistant to more antimicrobials than CA-MRSA, and to be responsible for more invasive infections (Cohn and Middleton, 2010). In animals, methicillin resistance was first documented in the early 1970's after isolation of MRSA from a dairy cow with mastitis. The emergence of MRSA in livestock and in people in contact with livestock have introduced a new epidemiological dimension to MRSA infections. These strains are designated LA-MRSA and are phenotypically and genotypically distinct from the HA-MRSA and CA-MRSA genotypes (Petinaki and Spiliopoulou, 2012). Although pet animals, especially dogs and cats may become contaminated, colonized, or infected with *S. aureus*, including MRSA, these species are not believed to be natural reservoir hosts for *S. aureus*. The MRSA strains found in companion animals are frequently identical to human epidemic strains of MRSA, making it more likely that MRSA originates from a person than a pet (Cohn and Middleton, 2010). Majority of MRSA infections in dogs and cats appear to be in high-risk patients and are acquired by direct contact with human carriers (Duquette and Nutall, 2004).

The MRSA isolates in dogs have been associated with clinical samples from surgical site infections, wound infections (Baptiste *et al*., 2005; Vincze *et al*., 2014), catheter site infections, urinary tract infections, pneumonia, and skin infections (Vengust *et al*., 2006).

These observations demonstrate the clinical importance and therapeutic challenge of MRSA in the management of conditions of dogs and cats.

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged in small animals worldwide and represents a major challenge for small animal practitioners due to its characteristic multidrug resistance phenotype (Paul *et al*., 2011) and its characteristics of a nosocomial pathogen (Frank and Loeffler, 2012). It has been isolated from various conditions including wound infections, otitis externa and canine pyoderma (Beck *et al*., 2012). An important aspect of MRSA and MRSP control is identification of potential sources of exposure.

There are limited reports of MRSA in humans in Kenya; Maina *et al*. (2013) found MRSA prevalence of 84.1% amongst *Staphylococcus aureus* isolated from patients with skin and soft tissue conditions. A different study by Aiken *et al*. (2014) reported low carriage rate of *Staphylococcus aureus* (85/950) in hospitalized patients, with only 7.0% of these isolates being MRSA. There is limited data in literature on the prevalence as well as phenotypic and molecular characteristics of microbial isolates from normal and surgical conditions in dogs in Kenya. Mande and Kitaa (2005) found *Staphylococcus aureus* as the most common isolate from ear swabs of dogs suffering from otitis externa and also reported multidrug resistance among bacterial isolates. Available records in the UoN Small Animal Clinic laboratory indicated frequent isolation of bacteria from dogs and cats. However, no systematic data or meta-analysis was available describing the full extent of the phenotypic and molecular characteristics of the different types of microbial isolates in dogs in Kenya.

This study was therefore designed with the aim of addressing the identified gap in the knowledge and skills in order to improve the therapeutic and clinical management of dogs undergoing surgical or medical procedures in Kenya.

1.1. Justification

Increasing isolation of methicillin resistant staphylococci in dogs has serious implications not just on canines but also for in contact humans due to the potential of zoonotic transmission. MRS are primarily transmitted via contact with contaminated objects/ environment, persons or animals.

The relationship between many pets and their owners has dramatically changed. Most dogs no longer live in kennels outside the home, people keep pets who live in the household almost as family members and thus there is frequent contact between the pets and family members. The intimate contact between pets (namely, cats and dogs) and their owners creates favourable conditions for MRSA/MRSP transmission.

Preliminary review of facilities at the University of Nairobi's Small Animal Clinic revealed that surgical patients, clinical cases and healthy dogs share the same environment. These facilities include:- common reception area, consultation rooms, corridors, surgical theatres) and in some cases are housed in the same kennel. Although veterinary patients are often attended to by the same clinicians, there was no documentary evidence of existence of Standard Operating Procedures for decontamination of facilities and staff between cases. Animals colonised with MRSA/MRSP may serve as sources of these pathogens in hospital environments. Contamination of contact surfaces may be a risk factor for acquisition of these resistant pathogens by surgical cases, with subsequent infection.

Bacteria of the genus *Staphylococcus* and in particular *Staphylococcus aureus* are the most commonly isolated microbes from samples collected from cases at the small animal clinic. These microbes are on average resistant to 3 or more antibiotics suggesting existence of multidrug resistance phenotypes. These microbes pose a danger due to the possibility of transfer of these resistant genes amongst other staphylococci and some clinically important pathogens.

1.2. General objective

To determine the prevalence of *Staphylococcus aureus* and other microbial isolates in surgical patients and normal dogs with emphasis on MRSA/MRSP at the University of Nairobi small animal clinic, upper Kabete and a community veterinary clinic.

1.3. Specific objectives

The specific objectives of the study were;

(i) To determine retrospectively the prevalence of common bacterial flora in isolates from wounds surgical site infections and otitis externa with emphasis on *Staphylococcus aureus*.

(ii) To determine the antimicrobial suscpetibility profiles of the various bacterial isolates to antibiotics used in the antimicrobial susceptibility tests.

(iii) To determine the prevalence of MRSA/MRSP in normal dogs and surgical patients using phenotypic and genotypic tools.

(iv) To sequence resistant PCR amplicons and thereafter validate the sequences.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Structure and function of the canine skin

The skin (integument) is composed of two major layers; the outer stratified epithelium known as epidermis and an underlying dermis (Figure 1). The integument provides a primary barrier against infectious agents, thus serving as the body's first line of defence against microorganisms. The epidermis consists three principal layers; stratum basale, stratum spinosum and stratum corneum (Pavletic, 2003).

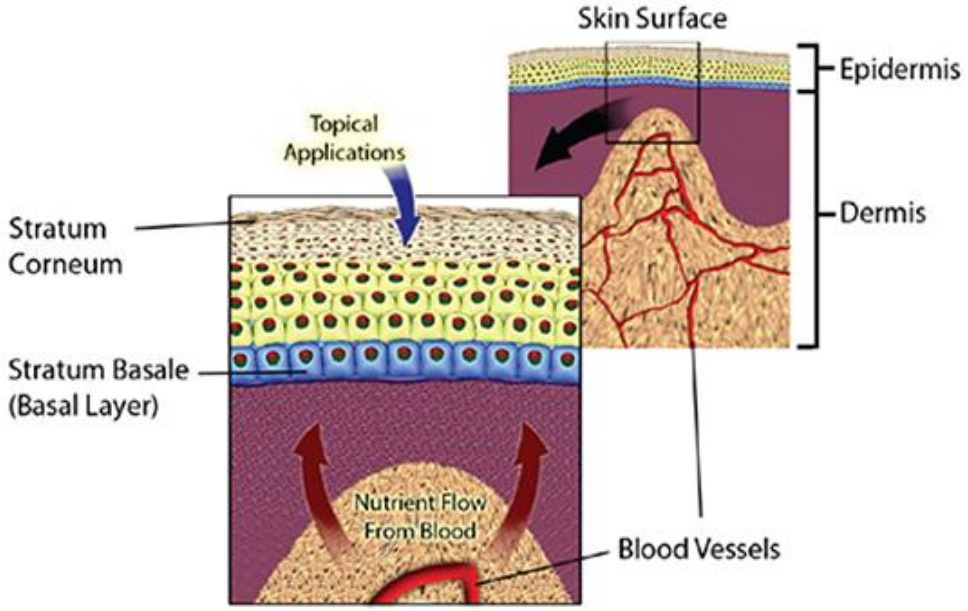


Figure 1: Skin Structure

Adapted from **http://lpi.oregonstate.edu/mic/micronutrients-health/skin-health**

The skin is a primary source for harboring microorganisms that present as being a potential cause of cross contamination (AST Standards, 2008). There is an intimate relationship between this population of micro-organisms, particularly the bacterial component, and the host. This relationship has a critical role in both protection and development of disease (Weese, 2012). The principal types of skin flora include resident and transient flora. The resident flora consist permanent inhabitants of the skin. Resident bacteria become established on the skin where they multiply and are able to persist on a long-term basis (Kampf and Kramer, 2004). Resident skin flora are mainly found under the superficial cells of stratum corneum (Verwilghen *et al.* 2011).

Transient skin flora consist of micro-organisms including bacteria and fungi that are found passively on the skin. They do not replicate on the skin, but they survive and take advantage of changing conditions. If the conditions allow, they multiply and may cause clinical infection (Saijonmaa- Koulumies and Lloyd, 1996; Mason *et al*., 1996). Transient flora only colonise the superficial layers of intact skin. They are acquired by contact with other people, animals, or contaminated environmental surfaces (Verwilghen *et al*., 2011). Upon wounding, there is damage to the epidermis, local vasculature, possibly the dermis and underlying tissue, depending on the extent (Daunton *et al*., 2012). Exposure of subcutaneous tissue provides a favourable substratum for a wide variety of microbes to contaminate and colonise (Padhy *et al*., 2014).

Isolation of low numbers of coagulase positive Staphylococci from the canine skin surface indicates transient status. However, these organisms are readily isolated from the muco-cutaneous junction. This suggests a resident status in mucosal surfaces which then act as reservoir for transmission to the skin and hair through grooming (Mason *et al*., 1996).

2.2. Concepts of surgical asepsis, surgical site infection and infection control in small animal practice

Surgical hand antisepsis aims to reduce the number of transient microorganisms as much as possible as well as to depress resident microflora of the hands and forearms (Slatter, 2003). However, traditional methods of hand antisepsis such as scrubbing, have been implicated as one of the factors leading to skin damage. In one study, the hands of surgical staff were found to have higher bacterial counts and more pathogenic organisms than hands of others. Prolonged or repeated washing leads to damaged barrier function of the stratum corneum and strips the skin of protective agents like amino acids and antimicrobial factors present in the water–lipid layers of the superficial skin (Verwilghen *et al.,* 2011).

The hands of healthcare workers are often contaminated with opportunistic pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks in veterinary patients have been associated with colonized surgeons and staff (McLean and Ness, 2008). This is due to frequent and close contact with patients and the hospital environment. Healthcare workers have been implicated as critical sources of hospital acquired infections (Wang *et al*., 2001; Weber *et al*., 2002). Hand carriage of *Staphylococcus aureus* and other multi-drug resistant (MDR) bacteria on the hands of medical professionals, including veterinary surgeons, makes prevention of transmission of skin bacteria to the surgical wound particularly important. *Staphylococcus aureus* survives on hands for at least 150 minutes and for an even longer time on surfaces, with MRSA being isolated for upto seven months on inanimate surfaces (Neely *et al*., 2000). However, compliance rates with hand hygiene practices have been reported to be low, with an overall average of 40% (Kampf and Kramer, 2004). Lack of routine hand washing after handling household pets has been found to be significantly associated with *S.*

pseudintermedius colonization in humans (Hanselmann *et al*., 2009)**.** Contaminated surfaces, including hands of healthcare workers may therefore act as sources of transient colonisation for in-contact humans and animals.

The normal skin flora is a major cause of postoperative infections in animals. The most commonly isolated genus from surgical site infections is *Staphylococcus* (Turk, 2013; Padhy *et al.,* 2014). Prevention of exposure to this flora is most important at the time of surgery and is achieved through pre-operative preparation of the patients including clipping of hair and scrubbing of the surgical site (Slatter, 1993). However, a patient's skin cannot be completely sterile and all surgical wounds become contaminated with bacteria; increasing the risk of infection. For this reason, use of prophylactic antibiotics in surgical patients is common place in veterinary clinics (Turk, 2013).

2.3. Methicillin resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus pseudintermedius* in dogs

Methicillin resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus pseudintermedius* have emerged as important pathogens in companion animals. MRSA is an important pathogen that has been implicated as a leading cause of hospital acquired infections in people (Singh *et al.,* 2013). Methicillin belongs to a class of semi-synthetic β-lactamase resistant penicillins introduced to treat infections caused by β-lactamase-producing *Staphylococcus* strains. Within a year of its introduction to clinical use, the first reports of methicillin (oxacillin) resistant *Staphylococcus aureus* emerged.

Historically, the vast majority of MRSA infections were nosocomial and were isolated from patients associated with hospitals (Cohn and Middleton, 2010). Such strains were designated

hospital associated MRSA (HA-MRSA) clones. Subsequently, these resistant *Staphylococcus aureus* organisms established themselves in hospitals and communities, spreading throughout the world (Hafeez *et al*., 2004). MRSA infections due to HA-MRSA were associated with serious illness and even death (Kuehnert *et al*., 2005). In recent years, there has been a shift in the epidemiology of MRSA infections with an increase in the proportion of MRSA infections occurring in humans with no exposure to healthcare settings. These MRSA infections have been designated community- acquired (CA-MRSA) lineages and these can be carried for long periods by healthy people (Harris *et al*., 2013; Cohn and Middleton, 2010). Methicillin resistance in staphylococci in samples from animals has been documented since the early 1970s with the isolation of MRSA from a dairy cow**.** Indeed, MRSA have been isolated from wound infections (Vincze *et al*., 2014), canine pyoderma (Beck *et al*., 2012), otitis externa, bovine mastitis, equine wound infections (Vengust *et al*., 2006), porcine exudative epidermitis and soft tissue infections of cats (Weese, 2010).

Methicillin-resistant *Staphylococcus aureus* infections in people occur in high-risk environments such as intensive care units, or are associated with infections acquired during or after orthopaedic surgery (Manian, 2003). The reservoir of infection is usually other colonised or infected patients or hospital staff, and the organism is frequently transmitted via the transiently colonised hands of healthcare workers (Baptiste *et al*., 2005; Leonard *et al*., 2006). Dogs and cats are not considered resevoir hosts of *Staphylococcus aureus*. However, they may become contaminated, colonized, or infected with *S. aureus*, including MRSA (Cohn and Middleton, 2010).

In dogs, *S. pseudintermedius* is the predominant *Staphylococcus spp.* with reported isolation frequencies between 20% and 90% from healthy canine skin and mucosal sites (Griffeth *et*

al., 2008; Hanselman *et al.*, 2009; Rubin and Chirino-trejo, 2011). *Staphylococcus pseudintermedius* can be isolated from the nares, mouth, anus, groin and forehead of healthy dogs and cats as well as from dogs and cats with inflammatory skin disease (Abraham *et al*., 2007; Griffeth *et al*., 2008). The perineum and the mouth are the most frequently colonized body sites. The combination of the samples from the two body sites, allowed detection of 90% (75/82) of dog carriers in one study (Paul *et al.*, 2012). Rubin and Chirino-Trejo (2011) recommended screening of at least the pharynx and rectum, which together accounted for 99.3% of the carriers in their study. MRSP has been isolated from dermatologic conditions, especially canine pyoderma, otitis externa and wound infections (Beck *et al*., 2012). Simultaneous sampling of the pharynx, perineum, the corner of the mouth and wounds (if present) is recommended for MRSP screening (Windahl *et al.*, 2012). However, a negative culture from a non-purulent wound should not be used as a criterion for a dog being MRSP negative. In a study by Windahl *et al.* (2012), almost 20% of the wound samples were negative, despite the bacteria being found in cultures from other sites that were sampled simultaneously. *Staphylococcus aureus* is a common isolate from the skin and can persist in the nares, and up to 60% of humans are thought to be carriers of *S. aureus*. Nasal carriage is indicative of exposure and is associated with an increased risk of clinical infection in hospitalized patients (Davis *et al*., 2004; Stevens *et al*., 2010). There has been an increase in reports of MRSA infections in animals; MRSA has been reported in almost all domesticated species, including dogs, cats, horses, cattle and sheep (Hartmann *et al*., 1997; Tomlin *et al*., 1999; Goñi *et al*., 2004; Rich and Roberts, 2004).

Prevalence of MRSA is variable, with documented studies reporting prevalence ranging between 0-4% in healthy animals (Loeffler *et al*., 2005; Abraham *et al*., 2007; Griffeth *et al*.,

2008). Various studies on MRSA colonization or infection among pets have shown that both human-to-animal and animal-to-human transmission can occur, and that environmental sources in veterinary clinics, veterinary staff and other hospitalized animals play a crucial role (Petinaki and Spiliopoulou, 2012). Loefller *et al*. (2005) in their study isolated MRSA from staff, dogs and environmental sites. Eighty two (82%) percent of the isolates were indistinguishable from EMRSA-15, an epidemic strain dominant in UK hospitals. The high prevalence of MRSA in people and pets in known infected households as well as the identification of indistinguishable strains in humans and domestic animals suggested that there was interspecies transmission of MRSA (Faires *et al*., 2009) though the direction of transmission remains unclear. This demonstrates the zoonotic importance of MRSA/MRSP in veterinary practice as well as pet owning households. Transmission of MRSA between veterinary personnel and their patients is a concern in veterinary facilities (Baptiste *et al.*, 2005; Bergstrom *et al.*, 2012) with both animal health and zoonotic implications. Leonard *et al*. (2006) isolated MRSA from five dogs with wound discharges after surgical procedures at a veterinary practice in Ireland. In the same study, MRSA with similar molecular and phenotypic characteristics was isolated from the nares of one veterinary surgeon**.** While the direction of transmission is not known, it suggests that veterinary hospitals and colonised staff may play a role in the dissemination of MRSA and thus emphasises the zoonotic potential of MRSA and the need for infection control in hospitals to prevent outbreaks of nosocomial MRSA infections (van Duijkeren *et al*., 2011).

Risk factors include repeated courses of antibiotics, hospitalizations, intravenous catheterization and surgery. Nienhoff *et al*. (2011) reported an association between antimicrobial treatment and MRSP carriage. This finding was also reported by Bergstrom *et*

al. (2012). In their study, all dogs that tested positive for MRSP had been treated with antimicrobials and although healthy dogs were included in their study, none of them were MRSP positive, despite sharing a common environment with the sick dogs (Bergstrom *et al*., 2012). Thus, antimicrobial treatment should be considered as one potential factor contributing to MRSP isolation from patients.

2.4. Mechanism of MRSA resistance

Methicillin-resistance in MRSP is mediated by acquisition of *mecA*, which is carried on a mobile genetic element identified as the Staphylococcal Cassette Chromosome mec (SCCmec) encoding the penicillin binding protein 2_a ($PBP2_a$). β-lactam antibiotics bind to PBP of *S. pseudintermedius* to prevent cell wall construction by the bacterium. The modified PBP of MRSP has a low affinity for β -lactams and therefore cell wall construction is not prevented by these antimicrobials (van Duijkeren *et al*., 2011). Thus, cell wall construction in these MRS strains continues even in the presence of otherwise inhibitory concentrations of β -lactam antibiotics (Paterson *et al*., 2014). According to the Clinical Laboratory Standards Institute (CLSI) standards, MRS should be considered resistant to all β -lactams agents i.e penicillins, β-lactam/ β-lactamase inhibitor combinations, both oral and parenteral cephems including cephalosporins and carbepenems regardless of the results obtained from susceptibility testing. This is since most cases of documented MRS infections have responded poorly to therapy with β -lactam agents (CLSI, 2013).

Some MRSA strains encode a novel *mecA* homologue termed *mecC* originally designated as $mecA_{LGA251.}$ This homologue has 70% nucleotide identity with *mecA* and encodes a PBP that is 63% identical at the amino acid level to the PBP2α encoded by *mecA* (Paterson *et al*., 2014). The *mecC* wielding MRSA isolates have been isolated from bovine milk (Paterson *et*

al., 2014), domestic dog (Paterson *et al*., 2014) and from a cat suffering from chronic conjunctivitis (Medhus *et al*., 2012). Prevalence of *mecC* from animal species is low, as screening of bovine milk samples for MRSA yielded a prevalence of 2% in Britain (Paterson *et al*., 2014). A study on samples from Dutch cattle did not isolate any *mecC* MRSA (van Duijkeren *et al*., 2014). The *mecC* MRSA pose a potential diagnostic loophole since the *mecC* gene is not detected by the PCR method established for the detection of *mecA* and consequently *mecC* strains will be potentially misidentified as methicillin susceptible *Staphylococcus aureus* (Paterson *et al*., 2014; Gomez *et al*., 2014).

There are two methicillin resistance phenotypes namely, homogenous and heterogenous. While homogenous strains express a uniformly high level resistance, heterogenous strains have a small proportion of a highly resistant subpopulation in a largely susceptible population. Due to the selective pressure of antibiotics, the resistant minority predominates providing clinical resistance (Niemeyer *et al*., 1996). Detection of resistance is made difficult by additional genes, which are also found in susceptible isolates. These genes can affect the expression of methicillin resistance in *S. aureus*, resulting in heterogenecity of strains (Brown *et al*., 2005).

Additional genetic determinants frequently confer concurrent resistance to other clinically relevant antibiotics (Bond and Loeffler, 2012). High resistance rates in MRS isolates have been observed in isolates from different regions. In Germany, majority of the isolates were resistant to fluoroquinolones, aminoglycosides and macrolides (Ruscher *et al*., 2009). In North America, a study of 103 isolates found that aside from β-lactam resistance, 90 % isolates were also resistant to ciprofloxacin, clindamycin, erythromycin, kanamycin, streptomycin and trimethoprim; resistance to gentamicin and tetracycline was observed in

70 % and to chloramphenicol in 57 % (Perreten *et al*., 2010). The multidrug resistance profile of MRSP in Europe and North America includes resistance to all oral antimicrobials routinely used for the treatment of infections in pets, and the drugs to which they remain susceptible are not authorized for use in animals (Perreten *et al*., 2010).

2.5. Detection of methicillin resistance

Various methods have been described for identification of MRS from clinical samples (Chambers, 1997; Brown, 2001; Brown *et al*., 2005). These include genotypic and phenotypic methods. Phenotypic tests rely on standard culture media together with conventional laboratory tests and AST for the identification of MRSA. Conventional methods require isolation of *S. aureus* first before antimicrobial susceptibility testing (Velasco *et al*., 2014). Clinical Laboratory Standards Institute recommends the use of broth microdilution and disk diffusion for detection of methicillin resistance in coagulase positive staphylococci. Interpretive criteria of ⩽21mm for disk diffusion and >4µg/mL for broth microdilution is used for methicillin resistance (CLSI, 2013).

Most clinical laboratories use either oxacillin or cefoxitin as a surrogate for methicillin (Loeffler *et al*., 2007; Bemis *et al*., 2009). However, studies have indicated that cefoxitin testing is more superior and reliable than oxacillin (Table 1) for detection of MRSA strains (Rostami *et al*., 2013). Oxacillin disk testing has been proven to be unreliable for MRSA detection, since it suffers from lower specificity relative to cefoxitin (Chambers, 1997).

Table 1: Comparison of efficiency of methods used for susceptibility testing

Method	Species	Sensitivity	Specificity	Reference
Oxacillin (DD)	MRSA	100	92.8	Rostami *et al*., 2013
Oxacillin (DD)	MRSA	95.83	58.33	Jain *et al*., 2008
Cefoxitin	MRSA	100	100	Rostami *et al*., 2013
Cefoxitin	MRSA	94.44	100	Jain *et al*., 2008

Interpretive criteria specific for veterinary staphylococci, including *Staphylococcus pseudintermedius*, remain to be established. Cefoxitin disc diffusion testing using the interpretative criteria for *Staphylococcus aureus* leads to an unacceptably high percentage of false-negative results and is an inappropriate screening test for MRSP isolated from dogs (Schissler *et al*., 2009; Bemis *et al*., 2009). An oxacillin MIC of $\geqslant$0.5 mg/L (agar and broth dilution) and a zone diameter of $\leqslant$17 mm around a 1 mg oxacillin disc (disc diffusion) used for coagulase-negative staphylococci (CNS) are highly correlated with the detection of *mecA* in *S. pseudintermedius* (van Duijkeren *et al*., 2011) and are therefore the recommended screening tests for phenotypic detection of MRSP.

Phenotypic methods for AST are time consuming and laborius; in addition, several culture conditions can also influence methicillin resistance such as the temperature, pH and concentration of sodium chloride (NaCl) in the medium (Brown *et al*., 2005). These factors impair the process of detection and may cause misidentification of some strains as methicillin susceptible *Staphylococcus aureus* (MSSA) when in fact they are MRSA.

Genotypic methods are more accurate in detecting methicillin resistant staphylococci as compared to conventional susceptibility methods and detection of the *mecA* gene by PCR is considered the gold standard for identification of MRS (Schissler *et al*., 2009; Cohn *et al*., 2010). PCR can produce results within 24 hours as compared to the conventional methods which require at least 48 hours. This quick turnaround time ensures that MRS infections are quickly diagnosed and appropriate therapy started (Sajith Khan *et al*., 2012). However, few laboratories perform PCR for *mecA* in routine diagnostics, since it has greater technical demands, uses expensive reagents and requires specialised laboratory equipment (Han *et al*., 2007; Schissler *et al*., 2009).

Detection of the altered gene product of *mecA*, i.e. Penicillin Binding Protein ($PBP2_a$), in MRSA can also be used to diagnose MRSA (Hanselmann *et al*., 2006; Griffeth *et al*., 2008; Julian *et al*., 2012). This test reliably differentiates between MRSA and MSSA. However, PBP2a latex agglutination testing developed for MRSA can result in false-positive reactions when applied to *S. pseudintermedius* isolates, and is therefore not recommended as the sole test for confirmation of methicillin resistant *Staphylococcus pseudintermedius* (van Duijkeren *et al*., 2011). In the study by Griffeth *et al*. (2008), it was found that the latex agglutination test failed to identify 2 out of 13 MR isolates. Both the isolates were methicillin resistant *Staphylococcus pseudintermedius* isolates. This finding could be due to the fact that the test

has not been validated for *Staphylococcus pseudintermedius* as it has for *Staphylococcus aureus*.

Several chromogenic media have been approved for the detection of MRSA in pure cultures. These media have been shown to reliably identify MRSA with sufficient sensitivity and specificity for routine use (Han *et al.*, 2007; Riedel *et al.*, 2010). In the study by Han *et al.* (2007), CHROMagar *S. aureus* (CSA) recovered 89.7 % and 94.9 % MRSA at 24 and 48 hours, respectively while CHROMagar MRSA (CSA-MRSA) recovered 87.2 % and 94.9 % of the MRSA isolates at 24 and 48 hours. There was no significant difference between the two agars in detection of MRSA. MRSA *Select* agar demonstrated a sensitivity and specificity of 99 % and 98 % respectively in detecting MRSA from blood cultures. However, the specificity of the tests can be greatly improved by combining with either the tube coagulase test or a commercial biochemical typing system to presumptively identify staphylococci. Once presumptive MRSA are identified, molecular detection of *mecA* or latex agglutination test for PB2a is recommended.

Selective media for detection of MRSP have not been identified. A recent study compared the use of conventional MRSA selective media for isolation of MRSP. Five different screening media were used in the study :- mannitol salt agar with oxacillin, CHROMagar MRSA, chromID MRSA agar, oxacillin resistance screening agar base (ORSAB) and Brilliance MRSA agar. The study found ORSAB and Brillance MRSA agar to be the most reliable in detection and isolation of MRSP from clinical material (Horstmann *et al*., 2012).

2.6. Contamination, colonisation and infection

Colonization is the presence, growth and multiplication of MRS in one or more body sites without observable clinical signs or immune reaction. Colonization by methicillin resistant Staphylococci (MRS) of any species poses a risk for plasmid encoded transfer of antimicrobial resistance determinants between staphylococci and other bacterial organisms. Colonisation in humans has been associated with a four-fold risk of infection compared to non- colonised patients (Safdar and Bradley, 2008). Colonization is incriminated as a risk factor for *S. pseudintermedius* infection, since most dogs are infected with strains residing on their body (Pinchbeck *et al.*, 2006; Sasaki *et al.*, 2007; Fazakerley *et al.*, 2010). In a study on dogs presented to a private dermatology clinic, Beck *et al*. (2012) demonstrated persistence of MRSP after resolution of MRSP pyoderma. Of the dogs that initially had an MRSP pyoderma, 26 of 42 (61.9 %) were colonized at one or more sites at follow-up, even though the pyoderma had resolved.

Contamination on the other hand means that the bacteria can be easily washed off and often only one culture is MRSP positive, while subsequent cultures are negative. Most studies done on MRSA/MRSP are cross-sectional, making it difficult to determine if individuals with MRSP positive cultures are merely contaminated or carriers. A longitudinal study carried out by Paul *et al.* (2012) demonstrated that dogs were either persistent, transient or sporadic carriers of *S. pseudintermedius*. Dogs positive for *S. pseudintermedius* at all sampling times were classified as persistent carriers. Intermittent carriers were distinguished between transient carriers that tested positive in at least three consecutive samples and sporadic carriers that were positive at only one or two of the nine sampling times. Non-carriers were defined as dogs testing negative at all sampling times (Paul *et al.*, 2012).

Methicillin resistant *Staphylococcus pseudintermedius* has been reported as a contaminant in cages for large dogs, the top surface of X-ray stand and the intensive care unit (Ishihara *et al*., 2010). Another study found hospital clothing to have a high prevalence of methicillin resistant Staphylococci (17.5 %); of these 3.5 % were MRSA and 14.0 % were MRSP (Singh *et al*., 2013). In this study, technicians were 9.5 times more likely than students to have clothing contaminated with MRSA. Julian *et al*. (2012) isolated MRS from 3/123 (2.4 %) cellular phones (CPs) belonging to personnel in a veterinary teaching hospital; MRSP was isolated from two (1.6 %) CPs, while MRSA was isolated from one (0.8 %) CP. Cellular phones and hospital clothing may serve as formites for pathogenic bacteria with transmission to patients or personnel through subsequent contamination of the hands.

Infections with methicillin resistant staphylococci in small animals, especially dogs, have been reported. Baptiste *et al.* (2005) isolated MRSA from 3 dogs with clinical infections; joint infection, pleuro-pneumonia and wound infection respectively. The dog with joint infection also tested positive for nasal and faecal carriage of MRSA. Two months after the initial isolation, a similar MRSA strain was associated with clinical disease in two other dogs. These dogs had no history of contact with the other dogs, suggesting hospital acquired transmission could also occur in veterinary centres. Beck *et al*. (2012) collected skin, nasal and rectal swabs of dogs that were presented to a dermatology referral service with pyoderma and healthy control dogs. Skin cultures yielded MRSP in 70 (40.5 %) dogs, methicillin-resistant *Staphylococcus aureus* (MRSA) in three (1.7 %) and methicillin-resistant *Staphylococcus schleiferi* ssp. coagulans (MRSScoag) in five (2.9 %).

Contact with other MRSP colonized dogs or humans might also serve as a source of reinfection, as well as contaminated objects in the household (Windahl *et al*., 2012). Isolation

of MRSP remains uncommon in humans, screening of veterinarians and veterinary personnel via nasal culture for MRSP carriage, revealed a carriage rate of 3.9–5.3 % (Ishihara *et al*., 2010)**.** Pet owners of animals with MRSP were screened and a nasal carriage rate of 4–13 % was observed. The genetic identity of some isolates from owner–pet pairs supported interspecies transmission (Frank and Loeffler, 2012). Carriage rate has been reported to be higher in veterinarians attending to known MRSA/MRSP cases. Loeffler *et al*. (2010) in their case-control study on colonisation rate in veterinarians and owners of small animals with known MRSA infection reported carriage rates of 12.3 % and 7.5 % respectively. The rates in the control group i.e animals with MSSA (methicillin Susceptible *Staphylococcus aureus*) were significantly lower at 4.8 % and 0 % respectively for veterinarians and owners. The findings of this study indicated an occupational risk for MRSA carriage in small animal general practitioners, veterinary staff and owners of MRSA-infected pets.

Although methicillin resistant staphylococci are not necessarily more virulent than methicillin-susceptible staphylococci, treatment options are often severely limited by multi-drug resistance (Cain, 2013). MRS infections are more resistant to some treatments than methicillin-sensitive *Staphylococcus* (MSS). There are concerns regarding the role of pets in MRSA transmission with various authors reporting concurrent colonisation of humans and their pets with indistinguishable MRSA strains. Many companion animals if not all, have come into contact with humans and other animals of the same species, creating the potential for transmission of organisms such as MRS (Vengust *et al*., 2006). Some reports have noted that infection of human subjects with MRSA persisted until the pet and any other colonized or infected cohabitants was treated with antimicrobials to which the bacteria were susceptible (Manian 2003, van Duijkeren *et al*., 2004, Sing *et al*., 2008).

There are speculations that epidemic MRSA in humans drives the parallel epidemic in companion animals. Despite the growing importance of these pathogens in veterinary medicine, especially for surgical patients, no studies have been reported on the prevalence of these pathogens in dogs in small animal practices in Kenya. In a preliminary study, *Staphylococcus* species was identified as the most common isolate from wound swabs from the University of Nairobi Small Animal Clinic. A high percentage of these isolates were resistant to ampicillin and other B-lactam antibiotics such as amoxycillin and amoxycillin-clavulanic (Njoroge *et al*., 2016). These preliminary findings led to a suspicion of the existence of Methicillin resistant *Staphylococcus* spp.. in dogs in Kenya and prompted further research to substantiate these claims.

CHAPTER THREE

3.0. MATERIAL AND METHODS

3.1. Study site

The study was undertaken at the University of Nairobi Small Animal Clinic, Upper Kabete. This facility receives patients mostly from the suburbs of Nairobi region and its environs. It also serves as a referral center for cases from other small animal clinics in Kenya. The Andy's community clinics whose patients are drawn from the Nairobi region and surrounding areas.

3.2. Study design

This study involved a retrospective and prospective component. The retrospective study component involved review of microbial isolates and antibiogram data from the bacteriology laboratory of samples submitted from surgical patients and dogs with otitis externa at the University of Nairobi Small Animal Clinic. The prospective component was a cross-sectional study that involved sampling of surgical patients and normal dogs presented at the Univeristy of Nairobi Small Animal Clinic and at a Community veterinary clinic located in Nairobi County.

3.3. Retrospective study: Survey of common bacterial isolates from wounds and otitis externa and their respective antimicrobial susceptibility profiles.

3.3.1. Animal patient biodata

The bacteriology laboratory records of clinical samples submitted between January 2004 and December 2013 were investigated. All the samples were from animals presented to the University of Nairobi's Small Animal Clinic during the study period. The records were

examined to retrieve data on culture samples of dogs and cats presented with otitis externa and wounds. Animal biodata retrieved from these records included: date of submission, sex and site where the sample was collected from (wound or ear swab).

3.3.2. Bacterial profile

For each clinical sample submitted, the number of microbial isolates and microorganisms isolated from either wounds or ear swab were recorded. The total number of various bacterial flora isolated were calculated and expressed as percentages. Bacteria of the Genus *Staphylococcus* were recorded as *Staphylococcus aureus* or broadly classified as other *Staphylococcus* spp. (for those that did not fit the characteristics of *S. aureus* in biochemical tests).

3.3.3. Antimicrobial susceptibility testing (AST)

Routine disk diffusion procedures were employed in AST by the laboratory. The bacterial isolates were tested against a panel of 8 antimicrobial agents namely, ampicillin (2µg), gentamicin (10µg), cotrimoxazole (25µg), chloramphenicol (10µg), tetracycline (10µg), potentiated amoxicillin (amoxycillin-clavulanic acid) (30µg), norfloxacin and sulfamethoxazole (25µg). Various bacteria in the AST were scored by the laboratory as either being susceptible or resistant to the respective antibiotic. If the zone of inhibition around the disk was found to be $\leqslant$14mm, the organism was scored as being resistant to that drug.

3.3.4. Wound characteristics

Patient case records from which wound and abscess swabs were collected were retrieved for further review. Information recorded for analysis included the cause and location (body region) of the wound or abscess swab.

3.4. Data analysis

All data was entered into a spreadsheet (Microsost Excel 2010) and a pivot table generated. The frequency of the various parameters (species, breed, sex) over the study period was calculated and expressed as percentages. The total number of bacterial flora isolated was calculated and expressed as percentages. Antimicrobial susceptibility was expressed as either susceptible or resistant. Overall resistance for each antimicrobial agent was calculated. Percentage resistance for each bacteria was calculated for each antimicrobial agent.

3.5. Prospective study: Prevalence of MRSA/MRSP in dogs

3.5.1. Study population

The following formula was used to calculate an appropriate sample size for the study

$$n = \frac{1.96^2\, p(1-p)}{d^2}$$

Where (p) = Estimate of the expected proportion (15%)

(d) = Desired level of absolute precision (0.05)

An estimated MRSA prevalence of 15% (Bond and Loeffler, 2012) in the population was used at 95% confidence interval. From the formula, we estimated our sample size to be 196 samples.

A total of 191 dogs were enrolled in this cross-sectional study, which entailed convenience sampling at the UoN Small Animal Clinic and a Community Owned Clinic. Criteria for inclusion entailed: - dogs of any age, sex, breed and obtaining written consent from owner or attending veterinarian to collect samples; preference was given to dogs presented for surgery, those with wounds and/or otitis externa. A brief questionnaire was filled by the owner or

attending veterinarian in order to obtain information on the patient including biodata like breed, sex, age, presenting complaint, history of the condition (first time/recurrent) and prior treatment administered (antibiotic use) in the past three months preceding the study.

3.5.2. Sample collection

Sampling was carried out between March 2014 and June 2015. Samples were collected from four sites on the affected surgical patients and normal dogs, specifically, anterior nares, buccal mucosa, perianal area, a wound swab if the patient presented with a wound and an ear swab in patients presenting with otitis externa. A sterile cotton tipped swab moistened with sterile normal saline was used to collect samples by swabbing the aforementioned sites. A separate swab was used for each anatomic location and swabs from each dog were pooled in a bijou bottle containing 3 ml of transport medium (Stuart's medium) and transported to the laboratory where they were stored in a refrigerator at 4^{o}C awaiting processing.

3.5.3. Bacteriological examination

3.5.3.1. Recovery of isolates

Samples were removed from the refrigerator and kept at room temperature for 4 hours before being cultured onto nutritive medium, tryptone soya broth supplemented with 6.5 % NaCl for selective enrichment of *Staphylococcus.* After incubation at 37^{o}C for 24 hrs, a loopful of broth was taken and cultured to Mannitol Salt Agar (MSA), a selective medium and incubated at 35^{o}C for 24- 48 hrs. Growth of yellow colonies on this medium and colour change of the media to yellow was taken as positive fermentation of mannitol and presumptive *Staphylococcus aureus* (Kateete *et al.,* 2010). Pink colonies on mannitol salt agar were also sub cultured and designated as presumptive *Staphylococcus pseudintermedius*.

The presumptive *Staphylococcus aureus* or *Staphylococcus pseudintermedius* colonies were subcultured on 5 % sheep blood agar (SBA) and incubated at 37^{o}C for 24 hours to isolate a pure culture. Those SBA plates that did not show any growth after 24 hours were incubated for a further 24 hours. Final identification of the presumptive coagulase positive *Staphylococcus* spp. characteristic colonies was on basis of colonial morphology, gram stain reaction, and positive catalase and coagulase tests. The presumed staphylococcus colonies were subjected to a Gram stain and the slide examined under a light microscope to check for gram reaction, size and shape of the colonies. Gram positive cocci that appeared as grapelike clusters in pairs and singles were presumed to be *Staphylococcus* spp.

3.5.4. Biochemical tests for confirmation

3.5.4.1. Catalase test

A sterile loop was used to pick organisms from the plate and place them on a slide. A drop of 3 % Hydrogen peroxide was added to the slide and mixed with the organisms. Visualization of bubbles was regarded as a positive reaction.

3.5.4.2. Tube coagulase test

This test was performed by transferring a single colony of inoculum to 1 ml of reconstituted rabbit plasma. The two were mixed by gently rotating the tubes. The tubes were then incubated at 37^{o}C and evaluated after 24 hrs. Formation of a clot in the tube was taken as a positive reaction. Presumptive coagulase positive Staphylococcus colonies were sub-cultured on Tryptic soy agar, awaiting susceptibility testing.

3.5.6. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed according to the Kirby-Bauer disc diffusion method. A sterile loop was used to pick organisms from the tryptone soy agar plate.

The organisms were added to a tube containing 4.5 ml of sterile physiological saline. The mixture was vortexed to create a smooth suspension. The turbidity of the suspension was adjusted to 0.5 McFarland standard. A sterile swab was dipped into the inoculum suspension. The Mueller Hinton (MH) plate was then inoculated by streaking across the agar surface ensuring that the entire plate was covered. The lid of the plate was left slightly open for 3-5 minutes for the agar surface to dry up.

Oxacillin was used as the surrogate antibiotic to methicillin (CLSI, 2008). Oxacillin (1 µg) discs (HiMedia Laboratories Pvt. Ltd, Mumbai, India) were peeled from the cartridge using forceps. The lid of the MH agar was lifted to allow placement of the discs on the agar surface. Once the disc was placed, it was gently pressed with forceps to ensure total contact with the agar surface. Plates were incubated at 35-37^{o}C for 24 hrs. The zone diameters of complete inhibition, including that of the disks, were measured to the nearest whole millimetre using a ruler. To measure the zones of inhibition, the ruler was held on the back of an inverted petri dish while holding it a few inches from a black non-reflecting background illuminated with reflected light.

For each isolate, antimicrobial susceptibility testing was done in duplicate and the mean zone diameter of inhibition calculated. The resistance zone diameter of <17mm around a 1 µg oxacillin disc was used as an indicator for methicillin resistance as recommended by Bemis *et al*. (2009), and approved by the Clinical and Laboratory Standards Institute (CLSI) subcommittee on Veterinary Antimicrobial Susceptibility Testing (CLSI, 2013).

3.6. Molecular identification and PCR detection of *mecA*

Isolates found to be resistant were amplified by polymerase chain reaction (PCR). *Staphylococcus aureus* ATCC 25923 served as the reference quality control strain. Primer pairs, sequences and amplicon size of primers used in the PCR reactions are shown in Table 2.

3.6.1. DNA extraction

Extraction of DNA was performed as described by Diaz-Campos (2012). Two or three colonies were obtained from 18 – 48 hours cultures inoculated on tryptic soy agar (4.1 %) and suspended in 400 µl of sterile distilled water. The bacterial suspension was boiled at 95°C for 7 minutes and then centrifuged at 15,000 *g* for 1 min and the supernatant collected. The DNA supernatant extracts were stored at -20°C until used as a template for the PCR reactions.

3.6.2. Validation of isolates

Amplification of 16S rRNA gene of all strains were performed at first to confirm that they were *Staphylococcus* strains. This was performed in a protocol adapted from Kondo *et al*. (2007). PCR reaction was done in a total volume of 20 µl containing 5 µl of DNA template and 0.25 µl of primers Staph-F and Staph-R. Thermal cycling reactions consisted of initial denaturation at 94°C for 10 min; followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 15 s, extension at 72°C for 1 min; and a final elongation at 72°C for 5 min. Amplification products were analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Gels were visualised under U.V light. Amplification of the 416bp PCR product indicated the strain to belong to the genus *Staphylococcus.*

3.6.3. Identification of coagulase positive staphylococci

Primers for species identication were designed to amplify a portion of the *nuc* gene. The procedure used was adapted from Asfour and Darwish (2014). The reaction was established in 25 µl reaction volume containing 10 µl of DNA as template. The amplification cycles were carried out in a thermocycler. Reaction conditions were optimized to be 94°C for 5 min, as initial denaturation, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55° C for 30 seconds and extension at 72°C for 60 seconds. A final extension step at 72°C for 10 min was followed. DNA isolated from *Staphylococcus aureus* ATCC 25923 was used as positive control. Amplification of 295 bp and 381 bp indicated the isolate to be *Staphylococcus aureus* and *Staphylococcus pseudintermedius* respectively.

3.6.4. Detection of *mec*A

Detection of the *mec*A gene was performed as previously described by Kondo *et al*. (2007). PCR reaction was performed in a final reaction volume of 25 µl containing 5 µl of DNA template. Amplification was done in a MJ minicycler (MJ Research Inc., USA) under the following conditions: initial denaturation at 94^{o}C for 2 minutes, followed by 30 cycles of 94^{o}C for 2 minutes, annealing temperature at 57^{o}C for 1 minute, extension temperature at 72^{o}C for 2 minutes, and a final extension step of 72^{o}C for 2 minutes. A 1.5 % agarose gel was used for electrophoresis after staining with ethidium bromide. Gels were visualized under ultraviolet illumination. A 100 bp DNA ladder was run simultaneously as a DNA marker. Amplification of the 286 bp band indicated the strains to harbour the *mecA* gene.

3.7. Sequencing of resistant genes

The PCR products obtained using gene-specific primers for resistance were purified and submitted for sequencing. The PCR products were purified with QIAquick PCR Purification

Kit (Qiagen, USA). This was done to remove excess primers, salts and Taq polymerase which interfere with the sequencing reaction. The purified products together with the forward and reverse primers initially used for the PCR detection of resistance were submitted to International Livestock Research Institute (ILRI), Segolip laboratory for sequencing which was done using the ABI PRISM 3770 genetic analyser (Applied Biosystems, US).

Table 2: Primer pairs and sequences used in the PCR reactions for identification of the Genus *Staphylococcus*, species identification of *Staphylococcus aureus* and detection of *mec*A medicated resistance.

Primer Name	Sequence 5'- 3'	Gene	Amplicon Size
***MecA*$_1$**	F- TGC TAT CCA CCC TCA AAC AGG	*mec*A	286bp
	R- AAC GTT GTA ACC ACC CCA AGA		
***MecA*$_2$**	F- AGA AAT GAC TGA ACG TCC GAT TT	*mec*A	887bp
	R- CAC CTG TTT GAG GGT GGA TAG		
Sau	F- CGA AAG GGC AAT ACG CAA AG	*Nuc*	295bp
	R- GGA TGC TTT GTT TCA GGT GTA TC		
Staph	F- GTA GGT GGC AAG CGTTAT CC	16S rRNA	416bp
	R- CGC ACA TCA GCG TCA G		

Key: F- Forward Primer R- Reverse Primer Sau- *Staphylococcus aureus*

3.8. Basic Local Alignment Sequence Tool (BLAST) analysis

The BLASTn tool of the NCBI Genbank database (http:/blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze the sequenced DNAs. The nucleotides were first read using GeneRunner software for further analysis. Analysis of the BLAST output was used to determine the *Staphylococcus spp.* harbouring the assayed resistance genes, their geographical distribution and hosts from which these homologues had been previously isolated. The homologues to the sequences including their nucleotide and amino-acid identity were identified using the BLASTn output.

3.9. Submission to NCBI GenBank

The sequenced resistance gene that was longer than 200 bp was submitted to the NCBI GenBank database for validation and assignment of an accession number.

3.10. Ethical issues

Ethical approval for the study was granted by the University of Nairobi's Faculty of Veterinary Medicine's Biosecurity, Animal Use and Ethics Committee (Ref No. 15/10). Informed consent was sought from the owners prior to sample collection. Consent to collect samples from the University Small Animal Clinic was requested and granted by the Chairman of the Department of Clinical Studies. The dogs used were the sole responsibility of the owner. Hospitalised dogs were housed in kennels located at the clinic.

CHAPTER FOUR

4.0. RESULTS

4.1. Retrospective study: Survey of common bacterial isolates from wounds and otitis externa of dogs and their antimicrobial susceptibility patterns

During the period between January 2004 and December 2013, a total of 291 samples were recorded from 191 individual dogs. The swab samples were obtained from wounds 27% (n=80) and ear infections 73% (n=211) respectively. Of these samples, growth was observed in 267 (92%) of the samples with 24 (8%) of the samples showing no growth after culture.

4.1.1. Animal Patient Biodata

The samples (n=291) were submitted from 200 dogs of which of which 145 were sampled once, 34 sampled twice, 15 sampled thrice, 3 sampled four times, 2 sampled 5 times and one animal sampled 12 times over the study period. Adult animals accounted for 89% (178/200) compared to 6% (12/200) young animals. Males accounted for 68% (136/200) compared to 27% (53/200) females while the sex of 11 animals was not indicated. Of the 200 samples from dogs, 119 (59.5 %) were German shepherd dogs, 29 (14.5 %) dogs were cross breeds, 8 (4%) Japanese spitz, 7 (3.5%) rottweilers dogs. The rest were breeds with 4 or less dogs in each breed.

4.1.2. Microbial isolates

The predominant isolates were *Staphylococcus aureus* 50% (133/267) and *Proteus* spp. 14% *(38/267)*. Other frequently isolated bacteria included *Pseudomonas* spp. 10% (28/267), *Staphylococcus* spp. 8.2% (22/267), *Streptococcus* spp. 6.7% (18/267), *E. coli* 5.6% (15/267). The frequency and source of isolation of the different spp. is represented in Table 3**.**

Staphylococcus aureus remained the most common isolate, regardless of the source of the sample. *Proteus* spp. were more frequently isolated in ear swabs (16.5%), than from wounds (3.7%). *Pseudomonas* spp. were also recorded as important pathogens in ear infections (12.3%) but were found to be minor pathogens in wound infections with isolation rate of 2.5%. *E. coli* was a common cause of contamination in wounds (16.3%) but did not seem to be an important cause of ear infections (0.95%).

Table 3: Prevalence of bacterial isolates from clinical samples of wounds and ear swabs in dogs

Isolate	Ear (Percent)	Wound (Percent)	Total	Percent
Staphylococcus aureus	103 (48.8%)	30 (37.5%)	133	49.8
Proteus spp.	35 (16.5%)	3 (3.75%)	38	14.2
Pseudomonas spp.	26 (12.3%)	2 (2.5%)	28	10
Staphylococcus spp.	19 (9%)	3 (3.75%)	22	8.2
Streptococcus spp.	10 (4.7%)	8 (10%)	18	6.7
Escherichia coli	3 (1%)	12 (16.3%)	15	5.6
Corynebacterium spp.	3 (1.4%)	2 (2.5%)	5	1.9
Actinomyces pyogenes	1 (0.5%)	1 (1.25%)	2	0.75
Diphtheroids	1 (0.5%)	-	1	0.4
Klebsiella spp.	-	1 (1.25%)	1	0.4
Norcardia spp.	-	1 (1.25%)	1	0.4
Pasteurella spp.	-	1 (1.25%)	1	0.4
Total	201	66	267	100

4.1.3. Antibiogram profile

Resistance to antimicrobial drugs was observed in the majority of the isolates in the study, with 97% (262/267) of the isolates demonstrating antimicrobial resistance to at least one drug. 4 isolates were not resistant to any drug and one of the isolates was a fungal, thus antimicrobial susceptibility was not done. Resistance to sulphonamides (96%), potentiated sulphonamides (89%), ampicillin (68%), amoxicillin (62%) and tetracycline (56%) was relatively high for all bacterial species examined (Table 4).

Staphylococcus aureus isolates displayed high multidrug resistance to sulfamethoxazole (95%), cotrimoxazole (87%), ampicillin (55%) and amoxicillin/clavulanic acid (51%). Resistance to sulfamethoxazole was a common finding, with more than (90%) of the isolates being resistant to this drug. *Proteus* spp. isolates were 100% resistant to amoxicillin and sulfamethoxazole and showed high level resistance to ampicillin (94%), cotrimoxazole (97%) and tetracyclines (69%). All *Pseudomonas* spp. isolates (100%) were resistant to sulfamethoxazole and amoxycillin. High level resistances to ampicillin (93%), amoxicillin/clavulanic acid (87%) tetracyclines (79%) and chloramphenicol (64%) were also observed among the *Pseudomonas* spp. isolates (Table 4).

Low resistance to gentamicin (9%), norfloxacin (22%) was observed in all bacterial isolates. The results of antimicrobial susceptibility testing are presented in Table 4. Multidrug resistance was also observed with majority of the isolates displaying resistance to 2 or more drugs (Table 5).

Table 4: Resistance (%) of six bacterial isolates from dogs to various antimicrobial agents (n=262).

Antimicrobial agent	*S. aureus* n=139	Proteus n=40	Pseud n=28	*E. coli* n=15	Staph n=24	Strep n=19	Total n=262
Amoxycillin	69%	100%	100%	N/A	67%	N/A	62%
Amoxicillin/Clavulanic	51%	58%	87%	100%	75%	46%	58%
Ampicillin	55%	94%	93%	79%	68%	65%	68%
Chloramphenicol	24%	45%	64%	33%	33%	7%	32%
Gentamicin	12%	0%	4%	8%	0%	29%	9%
Norfloxacin	28%	14%	15%	33%	21%	8%	22%
Tetracycline	50%	69%	79%	36%	50%	71%	56%
Cotrimoxazole	87%	97%	93%	93%	89%	67%	89%
Sulfamethoxazole	95%	100%	100%	91%	92%	100%	96%

KEY: *S. aureus*- *Staphylococcus aureus*; Proteus-*Proteus* spp.; Pseud-*Pseudomonas* spp.; Staph; Other *Staphylococcus* spp; Strep- *Streptococcus* spp.

Table 5: Phenotypic multidrug resistance profiles displayed by the bacterial isolates from dogs to various antimicrobial agents.

Resistance profile	Number of isolates resistant
COT, SXT	7
AMP**, COT**	5
AMP, COT, TET	7
AMP, COT, AMC, SXT	6
AMP, COT, TET, SXT	5
AMP, TET, AMC, SXT	5
AMP, COT, TET, AMC, SXT	7
AMP, CEF, COT, TET, AMC	6
AMP, COT, CHP, TET, AMC, SXT	6

KEY: AMP-Ampicillin; AMC-Amoxicillin/Clavulanic Acid; COT-Cotrimoxazole; TET-Tetracycline; CEF-Cefaclor; CHP-Chloramphenicol; SXT; Sulfamethoxazole.

4.1.4. Wound characteristics

Of the 80 samples collected from wounds in the retrospective study, only 58% (46/80) of records were retrievable from the medical records. Wounds commonly involved the limbs of the affected animals, with hindlimbs (32.6%) more affected than forelimbs (27.8%). The head region was also frequently presented with wounds 8 out of 46 (17.3%), Table 6.

Surgical site infections were a more frequent source of wound swabs than other causes, representing 23.9% of the sources. Bite wounds and traumatic wounds were also frequently sampled for culture and susceptibility testing (Table 7). The cause of 18 wounds sampled (33%) was not specified.

Table 6: Number of dogs presented with injury to different regions of the body

Region	**Number of dogs**	**%**
Abdomen	4	8.7
Cervical Region	4	8.7
Forelimbs	13	28.3
Head	8	17.3
Hindlimb	15	32.6
Pelvic Region	1	2.2
Thorax+Abdomen	1	2.2
Total	46	100

Table 7: Causes of wounds sampled for culture and sensitivity in dogs presented to the clinic.

Cause	**Number of dogs**	**%**
Bite Wound	10	21.7
Cellulitis	1	2.2
Fracture	1	2.2
Pododemodicosis	1	2.2
Surgical Site Infection	11	23.9
Tra**umatic**	7	15.2
Unknown	15	32.6
Total	46	100

4.2. Prevalence of MRSA/MRSP from normal dogs and surgical patients

4.2.1. Clinical history and animal biodata

Samples from the Community veterinary clinic accounted for 103 (54%) of the samples, while 88 samples (46%), were collected from the University of Nairobi Small Animal Clinic. Seventy two (37.7%) of the 191 dogs sampled presented with wound(s) on their body. Males were the predominant dogs sampled accounting for 56% (n=107) of the samples with 44 % (n=84) being females. Majority of the animals (60 %) had received antimicrobial treatment in the past three months prior to sampling.

4.2.2. Prevalence of staphylococci.

All the 191 samples successfully formed colonies in the enrichment media (Tryptone soya broth). The selective media, MSA, detected 65 (34 %) presumptive staphylococci species, the other samples yielded gram –ve bacteria which were not considered for further screening. The *Staphylococcus* spp. were subjected to a tube coagulase test and only 28 (14.7%) of the isolates tested positive and thus designated coagulase positive staphylococci.

4.2.3. Phenotypic characterisation of resistance

Antimicrobial susceptibility testing was done on the 28 coagulase positive isolates of which, 13 isolates (46.4%) were susceptible to oxacillin. Phenotypic resistance to oxacillin was observed in 15 isolates (53.6%).

4.2.4. Validation of isolates

The control PCR was performed to exclude any false positive results. It was done using a control primer pair targeting 416 bp fragment of 16S rRNA gene of genus Staphylococcus. Eleven out of the 15 presumptive coagulase positive staphylococci, were confirmed to be staphylococci **(**Figure 2**)**

4.2.5. Identification of coagulase positive staphylococci (COPS)

This PCR assay was done to differentiate the COPS by amplification of the 295 bp and 381bp specific PCR product for *Staphylococcus aureus* and *Staphylococcus pseudintermedius* respectively. Out of the 11 confirmed *Staphylococcus* species, this assay identified 7 (63.6%) *Staphylococcus aureus* strains. No *Staphylococcus pseudintermedius* strains were detected in this study.

4.2.6. MecA gene

Two *mecA*-positive MRSA strains were isolated from two dogs (Figure 3). One of the strains was from the wound of a dog with a post-operative infection that resulted after inguinal herniorrhaphy while the other was from a normal healthy puppy presented for vaccination.

4.2.7. BLAST analysis

4.2.7.1. Identification of DNA sequences

Analysis of the sequenced resistant determinants from the two samples revealed the genes were harboured by *Staphylococcus* spp. strains. The nucleotide sequence of isolate 1 (Lab ID: CS 100), was 97% identical to GenBank accession number AB547235.1, which is a *Staphylococcus sciuri mecA* gene and 96% identical to GenBank accession number KF058902.1 which is a *Staphylococcus aureus mecA* gene.

The nucleotide sequence of isolate 2 (Lab ID: CS 148) revealed 99% nucleotide identity to sequences in the NCBI databases belonging to different *Staphylococcus* spp. This isolate was 99% identical to GenBank accession numbers KR187111.1, KP265312.1 and HE984157.2 which were *mecA* genes from *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus pseudintermedius* respectively (Table 8).

4.2.7.2. Geographical distribution and host diversity of the homologue genes

The homologues containing the *mecA* gene showed varied global distribution with isolates from Brazil, Japan, China, Madagascar, Israel and Ireland. These strains were isolated from diverse sources including human, dogs, rodents and primates and with different conditions (Table 9).

4.2.7.3. Accesion numbers

The sequenced resistance gene submitted to the GenBank database was validated and subsequently assigned the accession number KX689749.

Figure 2: Agarose gel electrophoresis of PCR products of control PCR done for identification of *Staphylococcus* targeting 16S rRNA gene. Lane 1: 100bp ladder DNA marker, Lane 2: Negative Control; Lanes 3-11 Representative *Staphylococcus* (PCR Product 416 bp); Lane 13: *Staphylococcus aureus* ATCC 25923

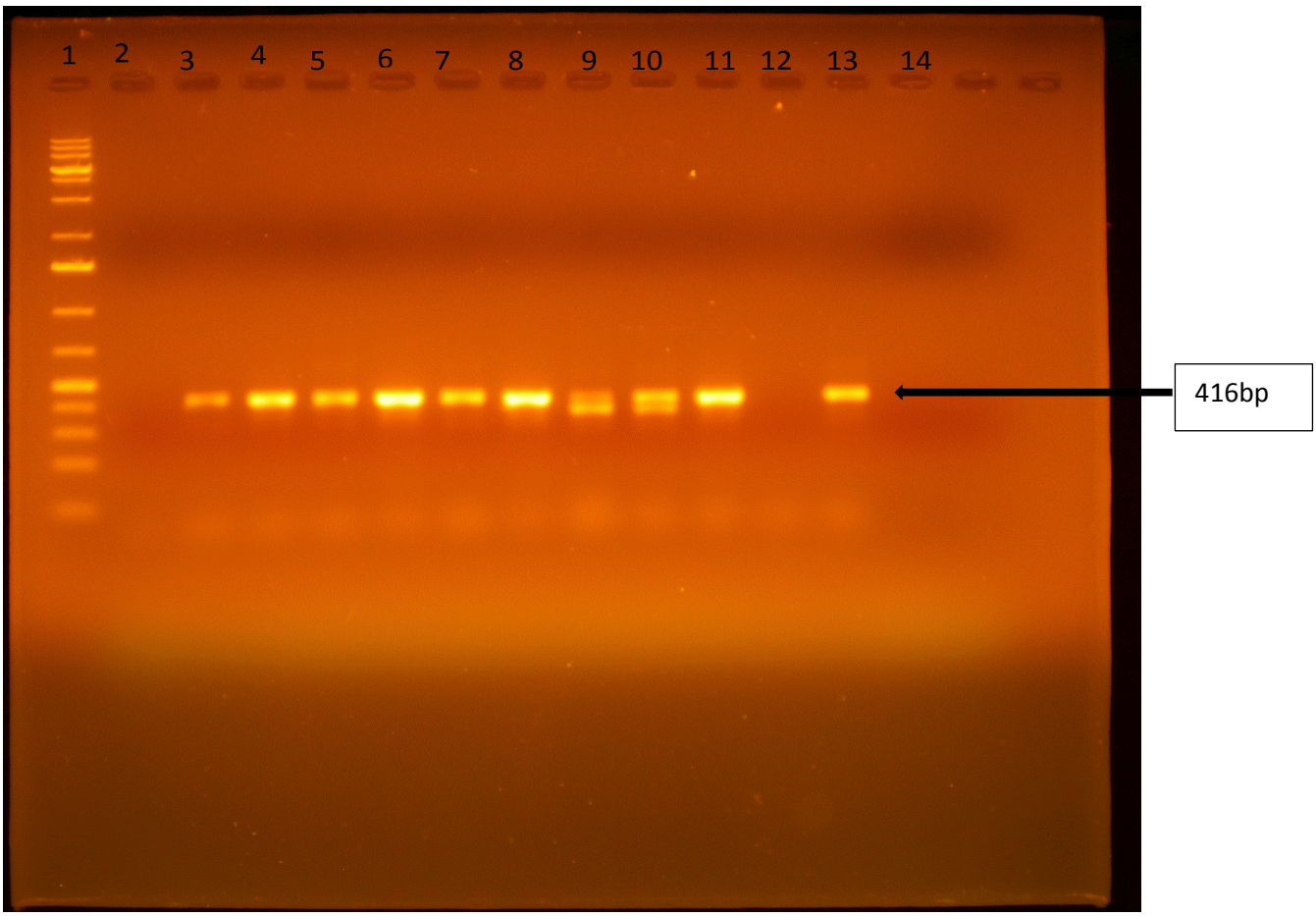


Figure 3: Agarose gel electrophoresis of PCR products of mecA positive strains. Lane 1: 1KB ladder DNA marker, Lane 3-6 mecA positive isolates (Positive PCR product 286 bp), Lane 7 Positive control.

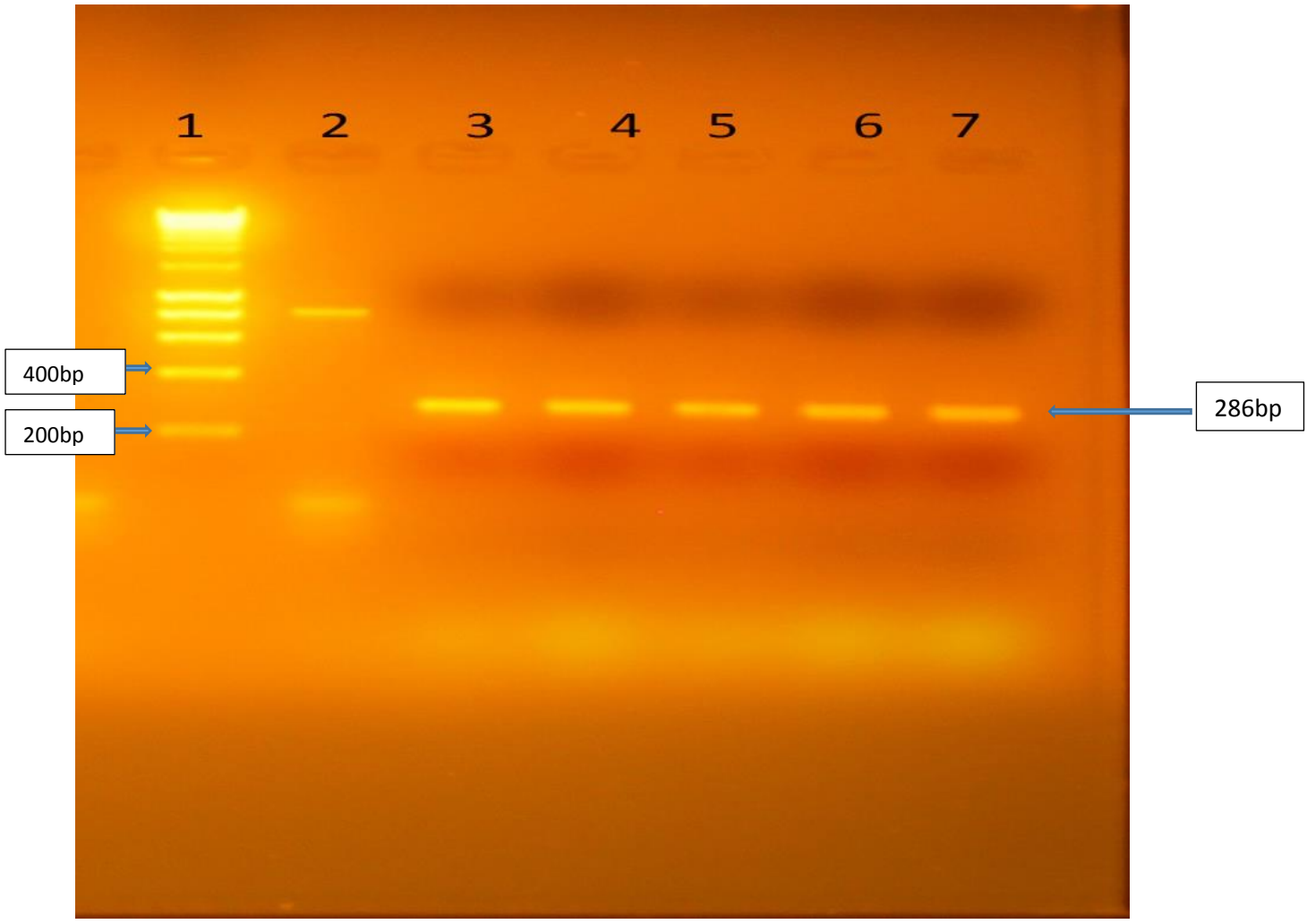


Table 8: Resistant gene nucleotide homologues and their identities in expressed in percentages

I.D	Homologue	%Identity	Accession Number
CS 100	*Staphylococcus sciuri mecA* gene	97%	AB547235
	Staphylococcus sciuri mecA gene	96%	JX094435.1
	Staphylococcus aureus mecA gene	96%	KF058902.1
CS 148	*Staphylococcus aureus mecA* gene	99%	KR187111.1
	Staphylococcus epidermidis mecA gene	99%	KP265312.1
	Staphylococcus aureus mecA gene	99%	KF058908.1
	Staphylococcus pseudintermedius mecA gene	99%	HE984157

Table 9: Diversity of hosts and geographical distribution of resistant gene homologues

Isolate I.D	Accession number	Host	Country
CS 100	AB547235	Rat	Japan
	JX094435.1	Primate (Sifaka)	Madagascar
	KF058902.1	Bovine (Mastitic milk)	Brazil
CS 148	KR187111.1	Bovine (Mastitic milk)	China
	KP265312.1	Canine (Fracture site)	Ireland
	KF058908.1	Human	Brazil
	HE984157	Canine (Rhinitis)	Israel

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

In this study, *Staphylococcus* spp. were the most common isolates from samples submitted in the laboratory. The findings of the retrospective study confirmed the etiological and clinical importance of *Staphylococcus* organisms as colonisers of skin and important causes of infection in the skin of animals. The high percentage of staphylococci (59.1%) was expected since Staphylococcal species are present on or in clinically normal individuals as commensals (Weese, 2010). However, they are opportunistic pathogens including *S. pseudintermedius* as well as *S. aureus* as leading cause of surgical site infections in animals (Vengust *et al.*, 2006; Turk, 2015). The observation in this study that *Staphylococcus aureus* was the most prevalent isolate from wounds is similar to reports from Bangladesh (Rahman *et al*., 2003) In contrast, Vincze *et al.* (2014) recorded a low prevalence of *Staphylococcus aureus* from wounds with isolation rates of 5.8% and 12.2% for dogs and cats respectively in Germany. *Staphylococcus aureus* has been recognized as an important wound pathogen and a major cause of delayed wound healing and infection. The prevalence of *Staphylococcus aureus* (37.5%) isolated from wounds of dogs in Kenya has previously not been reported.

The high prevalence of *Staphylococcus aureus* in this study was surprising. Other authors (Meyers *et al*., 2007; Urumova *et al*., 2012; Padhy *et al*., 2014) have reported *Staphylococcus intermedius* to be the major isolate from wounds in dogs. While dogs and cats may become colonised, contaminated and infected with *Staphylococcus aureus*, they are not considered reservoir hosts of this organism (Cohn and Middleton, 2010). The predominant *Staphylococcus spp.* in dogs has been reported to be *Staphylococcus pseudintermedius*

(Griffeth *et al*., 2008; Hanselmann *et al*., 2009). This finding may be due to the fact that at the laboratory, all coagulase positive Staphylococci were designated as *Staphylococcus aureus*.

In the present study, *E. coli*, *Streptococcus spp.*, other *Staphylococcus spp.* and *Proteus spp.*, were other microorganisms isolated from the wound swabs. This finding is similar to the study by Rahman *et al*. (2003) in Bangladesh, who isolated *E. coli*, *Klebsiella spp.* and *Proteus spp.* in wound swabs. The results of the present study are also in agreement with Urumova *et al*. (2012) who also found a high incidence of enterobactericiae in particlular *E.coli* in wounds. In this study, the polymicrobial growth was demonstrated, with 24 % of the swabs yielding more than one organism was consistent with other reports of similar nature conducted elsewhere (Meyers, 2007; Padhy *et al*., 2014). Colonisation in wounds is mostly polymicrobial involving different potentially pathogenic microorganisms (Bowler *et al*., 2001). The number and diversity of microorganisms in any wound is influenced by several factors among them are wound type, depth, location, and quality, the level of tissue perfusion, and the antimicrobial efficacy as well as the host immune response.

In vitro antimicrobial agent susceptibility of the isolates showed a high frequency of resistant strains, with 97% of the isolates showing resistance to at least one drug.. These observations are the cause for concern as they are an indication of existence of multidrug resistant isolates among dogs that might pose a clinical as well as therapeutical challenges.

In the retrospective study, 58% of bacteria isolated from ear swabs belonged to the Genus *Staphylococcus*, this is comparable to other studies (Lilenbaum *et al*., 2000; Lyskova *et al*., 2007; Petrov *et al*., 2013). Malayeri *et al*. (2010) reported a high prevalence of 73.8% of *Staphylococcus spp.* in Iran. Other bacteria isolated in the present study included *Proteus spp.* 16.5%, *Pseudomonas spp.* 12.3% and *Streptococcus spp.* 4.7% were comparable to a previous study by Mande and Kitaa (2005) where *Staphylococcus aureus* was found to be the most

prevalent isolate (51.2%), and *Streptococcus spp.* (14%), *Pseudomonas spp.* (14%) and *Proteus spp.* (10%) also commonly isolated (Mande and Kitaa, 2005). This study demonstrated an increase in staphylococcal isolation from otitis externa to 58% vs 51.2% compared to a previous Kenyan study. This study also shows that *Proteus spp.* is increasingly becoming an important pathogen with a prevalence of 16.5% up from 10% in the study by Mande and Kitaa (2005).

Previous studies in dogs, reported the pathogens isolated from wounds to be most sensitive to potentiated sulphonamides and amoxicillin/clavulanic acid preparations (Meyers *et al*., 2007; Urumova *et al*., 2012). This observation is not in agreement with findings in this study where comparatively higher resistance rates were observed to potentiated sulphonamides (89%) and amoxycillin/clavulanic (58%). Interestingly, Pedersen *et al*. (2007) found no resistance to amoxicillin/clavulanic acid in their study which involved bacterial isolates from clinical submissions in Denmark. Earlier reports by Authier *et al*. (2006) suggested amoxicillin/clavulanic acid to be an appropriate antimicrobial for treatment of skin infections by *Staphylococcus spp.* However, based on the results of this study, the use of these antimicrobials as the first line of treatment for empirical therapy might result in treatment failure, if the observation made represnts the general population in Kenya. The findings of this study demonstrated that gentamicin and norfloxacin were the most effective antimicrobial agents against majority of the isolates. Gentamicin has been indicated for the treatment of Staphylococcal infections (Lilenbaum *et al*., 2000). However, Authier *et al*. (2006) suggests that its use should be limited to cases where initial treatment has failed.

In the present study, surgical site infections were found to be a frequent cause for wound swabbing representing 24% of the wound swabs. Seventy five percent of surgical site infections sampled resulted from fracture fixation using an implant. Turk *et al*. (2015) reported the use of implants increases the risk for surgical site infections. Gallagher *et al*.

(2012) and Turk *et al*. (2015) further points out that, implants frequently become colonized with bacteria and may also act as substrates for bacterial biofilm formation. Majority of the wounds sampled were located on the extremeties with the hindlimbs being more affected than the forelimbs. These results are comparable with the report by Shamir *et al*. (2002) where the extremeties and the head were reported as the most frequent sites of bite wounds in dogs. Similarly, Meyers *et al*. (2007) also observed majority of wounds to involve the cranial half of the body, especially the head and thoracic limbs in dogs.

With respect to the susceptibility of coagulase positive *Staphylococcus spp.* to various antimicrobials the present study found 97% of the *Staphylococcus* isolates to be resistant to at least one drug.This finding is in agreement with a previous report which is in agreement with a report by Lilenbaum *et al*. (2000) who reported Staphylococcus isolates to display a high level of resistance in Brazil. They found 90.9 % of the isolates in their study to show resistance to at least one drug. However, the findings of this study were in contrast with thefindings reported by Junco and Barrasa (2002) who reported only 64.8% of COPS displaying resistance.

In the present study, the least effective antimicrobials against *Staphylococcus aureus* were sulphonamides (sulfamethoxazole), potentiated sulphonamides (cotrimoxazole), ampicillin and tetracycline. The highest level of resistance noted was for potentiated sulphonamides with a resistance rate of 95 %. Lilenbaum *et al*. (2000) also found majority of staphylococcal isolates in Brazil resistant to this drug though at a lower rate 72.7 %. On the other hand, a study in Denmark by Pedersen *et al*. (2007) described very low resistance of *S.intermedius* ear isolates to this drug combination. The most effective agent against Staphylococci was gentamicin, chloramphenicol and norfloxacin. Gentamicin susceptibility rate was 88% which is similar to the one reported by Lilenbaum *et al*. (2000). Most of the isolates were also found

to be susceptible to amoxicillin (62%), suggesting that this drug can be used as a first line of treatment prior to results of antimicrobial susceptibility testing.

Pseudomonas and *Proteus* isolates observed in this study displayed the highest resistance to most antimicrobial agents. *Pseudomonas* spp. are mostly isolated in chronic cases of canine otitis externa (Scott *et al*., 2001; Greene, 2006). This organism has been reputed for its high level of resistance to most antimicrobials. The multidrug resistance was observed to be the case in this study, with 92% of *Pseudomonas* spp. isolates showing resistance to 4 or more drugs. Highest resistance was recorded to amoxicillin, and sulfamethoxazole, with all the isolates tested against these drugs showing 100% resistance. These isolates also showed high resistance to ampicillin (93%) and amoxicillin/clavulanic acid (87%). Significant resistance to chloramphenicol (64%) and tetracycline (79%) was also observed in the *Pseudomonas* spp. isolates in this study. Pedersen *et al*. (2007) in their study found that all the *Pseudomonas* spp. isolates were resistant to ampicillin, amoxicillin/clavulanic acid and erythromicin. Malayeri *et al*. (2010) also concurred with these observations with all *Pseudomonas* spp. isolates in their study showing 100% resistance to ampicillin, amoxicillin/clavulanic, erythromicin, rifampin and penicillin G. In another study, Hariharan *et al*. (2006) found that Pseudomonas isolates to be highly resistant to chloramphenicol (99%) and doxycycline (98%). The least antimicrobial resistance in this study was observed against gentamicin (4%) and norfloxacin (15%). This observation is in agreement with Petersen *et al*. (2002) who reported most *Pseudomonas aeruginosa* isolates to be 100% susceptible to the two drugs.

In the present study, *Proteus* spp. was the second most frequently isolated microorganism after *Staphylococcus aureus*, accounting for 14.2% of all isolates; a finding that was similar to reports by other researchers (Pedersen *et al*., 2007; Lyskova *et al*., 2007; Petrov *et al*., 2013). The present study found all isolates to be resistant to amoxicillin and sulphonamides, but susceptible to gentamicin. High resistance was observed against ampicillin (94%),

cotrimoxazole (97%) and moderate resistance to tetracyclines (69%), amoxicillin/clavulanic (58%) and chloramphenicol (45%). Similar results have previously been reported by Petrov *et al*. (2013), who found all isolates to be susceptible to gentamicin. In addition, they observed resistance to tetracycline (81%) and chloramphenicol (74%) though at higher rates, and the isolates in their study were resistant to ampicillin. In contrast to this study, Pedersen *et al*. (2007), found all *Proteus* spp. isolates in their study to be resistant to tetracyclines and majority of the isolates susceptible to gentamicin and ciprofloxacin, which is in agreement to the findings in this study.

Prospective screening of dogs in this study showed a carriage rate of 34% (65/191) of *Staphylococcus spp.* This may be due to the prolonged storage time of some samples (up to 8 months for a few samples). In other studies, samples were cultured within 12 hrs (Gingrich *et al*., 2011) and 24 - 36 hours of collection (Bergstrom *et al*., 2012; Walther *et al*., 2012). The recent use of antimicrobial agents in most of the study animals prior to sampling may have led to suppression of the number of commensal bacteria, especially those resident on the skin.

Detection of the *mecA* gene by PCR revealed 2 out of the 15 (13%) phenotypically resistant isolates to be genotypically resistant to methicillin (oxacillin). These two isolates contained the *mecA* gene that encodes for resistance to β-lactam antibiotics. Ozturk *et al*. (2010) reported similar results in their study where all 5 *Staphylococcus* spp. isolates that were phenotypically resistant to oxacillin were *mecA* negative on PCR. This discrepancy between phenotypic and genotypic resistance in the isolates has been reported by Schmidt *et al*., 2014 and Elhassan *et al*., 2015. This discrepancy could be due to existence of the so-called borderline (low-level resistant) strains. These *mecA* negative strains are thought to result from overproduction of β-lactamase (Chambers, 1997). Other mechanisms associated with borderline resistance include acquisition of modified PBPs (Elhassan *et al*., 2015). The

existence of these borderline strains emphasise the need to screen *mecA* negative strains for other resistance mechanisms.

Two genes are known to encode for methicillin resistance in *Staphylococcus* spp. namely *mecA* and *mecC*. However in this study only *mecA* was investigated for genotypic characterization of methicillin resistance, since reports of *mecC* positive MRSA isolates are low with prevalence of 0-3% reported in European countries (Paterson *et al*., 2014). Previous studies have also shown *mecA* to be the most common gene encoding for methicillin resistance in *Staphylococcus spp*. (Weese, 2010; van Duijkeren *et al*., 2011).

No MRSPs were observed in this study, which is similar to reports by Garbacz *et al*. (2011), their study involved 39 *Staphylococcus pseudintermedius* isolates from clinical submissions and found all the isolates to be susceptible to oxacillin. Several studies by different authors have also failed to isolate any MRSP isolates (Murphy *et al*., 2009; Rubin and Chirino-Trejo, 2011; Schmidt *et al*., 2014). In most of these studies, the investigators collected samples from healthy animals. In the present study, samples were collected both from normal and clinically sick animals, some of which had received antibiotic treatment prior to sampling. This study found a prevalence rate of 7% (2/28) of MRSA among coagulase positive *Staphylococcus* spp. and an overall prevalence of 1% (2/191). In a Swedish animal hospital, no MRSA was isolated from surgical patients and healthy animals, although the prevalence of MRSA in the environment was found to be 5.3% (Bergstrom *et al*., 2012). The low prevalence of MRSA in this study is also similar to reports by Couto *et al*. (2011) who reported MRSA prevalence of 1% from 287 dogs and cats presented to a veterinary teaching hospital in Portugal, and is consistent with findings of Quitoco *et al*. (2013). A higher prevalence of MRSA of 15.8% was observed in a study on surgical site infections (Turk *et al*., 2015). A recent report by Aiken *et al*. (2014) found a similar prevalence (7%) of MRSA among *Staphylococcus aureus* strains isolated from patients admitted to a hospital in Kiambu County, Kenya. One of the MRSA

isolates in this study was recovered from a patient with a surgical site infection. The patient had been hospitalised for a week prior to the infection and was under treatment with an antimicrobial agent. This finding is similar to that by Middleton *et al*. (2005) whose sole postoperative MRSA isolate was from a canine patient with an orthopaedic pin tract infection. MRSA has emerged as an important pathogen in post-operative infections in previous studies (Tomlin *et al*., 1999; Turk *et al*., 2015). Antimicrobial drug therapy, hospitalization and surgery have been cited as factors predisposing to MRSA infection (Loeffler *et al*., 2005; Magalhaes *et al*., 2010; Faires *et al*., 2010; Davis *et al*., 2013). Multidrug resistant Staphylococci isolated from dogs with post-operative infections and wounds should raise suspicion of MRSA infection and appropriate care taken in handling such patients.

Analysis of the sequenced resistant determinants showed that the resistant genes were harboured by *Staphylococcus* spp. strains and that the resistant determinant is geographically widespread across various regions of the globe having previously been isolated from countries such as Ireland (McManus *et al*., 2015), China (Wu *et al*., 2015), Brazil (Melo *et al*., 2013), Israel (Perreten *et al*., 2013). The strains have been isolated from different *Staphylococcus* spp. isolated from bovine (milk), canine (orthopaedic implant and rhinitis) and human clinical submissions. The *mecA* genes contained in *SCCmec* have been reported to be almost identical regardless of the *Staphylococcus* species carrying it (Tsubakishita *et al*., 2010). This observation alludes to the fact that the *mecA* gene is transferable from different *Staphylococcus* spp. The *mecA* gene encodes for penicillin binding (*PBP2*) protein. PBP 2a, is a low-affinity penicillin-binding protein (PBP) that mediates methicillin resistance in *Staphylococcus* spp. (Weese, 2010). This modified PBP2 has a low affinity for β -lactams and therefore cell wall construction is not prevented by these antimicrobials (van Duijkeren *et al*., 2011).

Haphazard use of antimicrobials prior to testing can lead to selection of multidrug resistant strains. This may have been the case for one of the *Staphylococcus aureus* strains isolated that showed resistance to the complete panel of the 8 drugs tested against. This strain was isolated from a dog suffering from recurrent otitis externa. Selective pressure exerted by previous antimicrobial treatment in recurrent cases may lead to emergence of resistant strains (Guardabassi *et al*., 2004). Occurrence of antimicrobial resistance in companion animals is of significance to human health (Hawkey, 2008). The close contact between household pets and humans offers favourable conditions for the transmission of bacteria by direct contact or indirectly through contamination of the environment. Transmission of mobile resistance determinants between companion animals and humans may also occur (Guardabassi *et al*., 2004).

5.2. Conclusions

1. The present study confirms that the most prevalent microorganisms associated with wounds and otitis externa were *Staphylococcus aureus* (50.5%), *Proteus spp.* (14.04%), *Pseudomonas spp.* (9.82%), Other *Staphylococcus spp.* (8.42%), *Streptococcus spp.* (7.67%) and *E. coli* (5.62%).

2. From the results of our study, gentamicin, an aminoglycoside, is the most effective antimicrobial agent against all the isolates from wounds, surgical site infections and otitis externa in dogs. Norfloxacin, a fluoroquinolone, is relatively effective against Gram negative isolates (*Proteus spp*. and *Pseudomonas spp.*) and *Streptococcus spp*.

3. *Pseudomonas spp.* and *E. coli* from otitis externa and wounds respectively, are the most challenging organisms to treat in dogs.

4. The study findings report the first two cases of MRSA isolated from a normal healthy dog and a dog with a surgical site infection in Kenya. The study observed MRSA prevalence of 7% among coagulase positive *Staphylococcus* spp.

5. The resistant determinant *mecA* in this study was similar to some MRSA strains from human patients in other parts of the world and therefore demonstrates the zoonotic importance of these resistant strains.

5.3. Recommendations

1. Judicial use of antimicrobial agents is recommended especially the newer antibiotics to prevent development of resistance.
2. Development of antimicrobial treatment guidelines in companion animals or adoption of existing ones for empiric therapy.

3. Establishment of veterinary practice infection control policies to prevent spread of resistant isolates between patients and in- contact staff.
4. Further investigation is necessary to determine the extent of MRSA carriage by companion animals and livestock.
5. Further research is necessary to evaluate the factors leading to acquisition of these resistant isolates

CHAPTER SIX

6.0. REFERENCES AND APPENDICES

6.1. References

Abraham, J., Morris D., Griffeth, G., Shofer, F., Rankin, S. (2007). Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* ssp. *schleiferi*. *Veterinary Dermatology* **18**: 252-259.

Aiken, A.M., Mutuku, I.M., Sabat, A.J., Akkerboom, V., Mwangi, J., Scott, J.A.G, Morpeth, S.C., Friedrich, A.W. and Grundmann, H. (2014). Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. *Antimicrobial resistance and infection control* **3**(1): 22.

Asfour, H.A.E. and Darwish, S.F. (2014). Evaluation of Phenotypic Methods versus Molecular Methods for Differentiation of Coagulase Positive Staphylococci causing Bovine Mastitis with a Special Reference to atypical *Staphylococcus aureus*. *International Journal of Current Microbiology and Applied Science* **3(5)**: 543-558.

Association of Surgical Technologists (AST) standards. **(2008)**. Standards of practice for surgical attire, surgical scrub, hand hygiene and hand washing. Retrieved from: http://www.ast.org/uploadedFiles/Main_Site/Content/About_Us/Standard_Surgical_Attire_Surgical_Scrub.pdf.

Authier, S., Paquette, D., Labrecque, O. and Messier, S. **(2006)**. Comparison of susceptibility to antimicrobials of bacterial isolates from companion animals in a veterinary diagnostic laboratory in Canada between 2 time points 10 yrs apart. *Canadian Veterinary Journal* **47**: 774-778.

Baptiste, K., Williams, K., Williams, N., Wattret, A., Clegg, P., Dawson, S., Corkill, J., O'Neill, T. and Hart C. (2005). Methicillin-resistant staphylococci in companion animals. *Emerging Infectious Diseases* **11**: 1942–1944.

Beck, K. M., Waisglass, S. E., Dick, H. L. and Weese, J. S. (2012). Prevalence of methicillin resistant *Staphylococcus pseudintermedius* (MRSP) from skin and carriage sites of dogs after treatment of their methicillin-resistant or methicillin-sensitive staphylococcal pyoderma. *Veterinary Dermatology* **23**:369-75.

Bemis, D. A., Jones, R. D., Frank, L. A. and 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.

Bergström, A., Gustafsson, C., Leander, M., Fredriksson, M., Grönlund, U., and Trowald-Wigh, G. **(2012)**. Occurrence of methicillin-resistant Staphylococci in surgically treated dogs and the environment in a Swedish animal hospital. *Journal of Small Animal Practice* **53**(7): 404-410.

Bond, R. and Loeffler, A. (2012). What's happened to Staphylococcus intermedius? Taxonomic revision and emergence of multi-drug resistance. *Journal of Small Animal Practice* **53**: 147–154.

Bowler, P.G., Duerden, B.I. and Armstrong, D.G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews* **14**: 244-269.

Brown, D.F. **(2001)**. Detection of methicillin/oxacillin resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* **48**(1): 65–70.

Brown, J., Edwards, I. and Hawkey, M. **(2005)**. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant Staphylococcus aureus (MRSA). *Journal of Antimicrobial Chemotherapy* **56**(6): 1000-1018.

Cain, C.L. (2013). Methicillin resistant *Staphylococcal* infections. *Today's Veterinary Practice*, **3** (3):26-32.

Chambers, H.F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clinical Microbiology Review* **10**: 781–791.

CLSI (Ed) (2013) ***Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals;*** Approved Standard, 4th edition. CLSI document VET01-A. Wayne, PA: Clinical and Laboratory Standards Institute

CLSI (2008). ***Performance Standards for Antimicrobial Disk and Dilution Susceptibility Test for Bacteria Isolated from Animals***; Approved Standard, 3rd edition. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute.

Cohn, L.A. and Middleton, J.R. **(2010)**. A Veterinary Perspective on Methicillin Resistant Staphylococci. *Journal of Veterinary Emergency and Critical Care* **20**: 31-45.

Couto, N., Pomba, C., Moodley, A. and Guardabassi, L. (2011). Prevalence of methicillin-resistant staphylococci among dogs and cats at a veterinary teaching hospital in Portugal. *Veterinary Record* **162**: 72.

Daunton, C., Kothari, S., Smith, L. and Steele, D.A. (2012). A History Of Materials And Practices For Wound Management**.** *Wound Practice and Research* **20**(4)**:** 174-186.

Davis, K.A., Stewart, J.J., Crouch, H.K., Florez, C.E. and Hospenthal, D.R. (2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. *Clinical Infectious Disease* **39**:776–82.

Davis, M.F., Peterson, A.E., Julian, K.G., Greene, W.H., Price, L.B., Nelson, K., Whitener, C.J., Silbergeld, E.K. (2013). Household risk factors for colonization with multidrug-resistant *Staphylococcus aureus* isolates 8.doi:10.1371/journal.pone.0054733

Diaz- Campos, D.V. (2012). Molecular epidemiology and genetic analysis of *Staphylococcus* species in companion animal medicine (Doctorate dissertation). Retrieved from https://etd.auburn.edu/bitstream/handle/10415/3066/Dissertation,%20D%20Diaz,%204%2030%202012.pdf?sequence=2

Duquette, R.A. and Nuttall, T.J. (2004). Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *Journal of Small Animal Practice* **45:** 591-597.

Elhassan, M.M., Hani, A.O., Hassan, A.H., Miskelyemen A.E., and Leila, M.A. (2015). Absence of the mec A Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Shendi City , Sudan. *BioMed Research International*. doi: 10.1155/2015/895860

European Medicines Agency (EMA). **(2010)**. Reflection paper on Methicillin-resistant *Staphylococcus pseudintermedius.*

Faires, M., Tater, K. and Weese, J.S. (2009). An investigation of methicillin-resistant *Staphylococcus aureus* colonization in people and pets in the same household with an infected person or infected pet. *Journal of the American Veterinary Medical Association* **235**(5): 540-543.

Faires, M.C., Traverse, M., Tater, K.C., Pearl, D.L., Weese, J.S. (2010). Methicillin-resistant and -susceptible Staphylococcus aureus infections in dogs. *Emerging Infectious Diseases*. **16**:69–75. doi:10.3201/eid1601.081758

Fazakerley, J., Williams, N., Carter, S., McEwan, N. and Nuttall, T. (2010). Heterogeneity of *Staphylococcus pseudointermedius* isolates from atopic and healthy dogs. *Veterinary Dermatology* **21:** 578–585.

Frank, L.A. and Loeffler, A. (2012). Methicillin resistant *Staphylococcus pseudointermedius*; Clinical challenge and treatment options. *Veterinary Dermatology* **23**: 283-291

Gallagher, A.D., and Mertens, W.D. (2012). Implant removal rate from infection after tibial plateau leveling osteotomy in dogs. Veterinary *Surgery* **41**: 705- 711.

Garbacz, K., Piechowicz, L., Żarnowska, S., Haras, K. and Dąbrowska-Szponar, M. (2011). Heterogeneity of methicillin-sensitive *Staphylococcus pseudintermedius* strains isolated from diseased dogs. *Polish Journal of Veterinary Sciences* **14**(2): 283-284.

Gingrich, E.N., Kurt, T., Hyatt, D.R., Lappin, M.R. and Ruch-Gallie, R. **(2011).** Prevalence of methicillin-resistant staphylococci in northern Colorado shelter animals. *Journal of Veterinary Diagnostic Investigation* **23**: 947-950.

Gomez, P., Gonzalez-Barrio, D., Benito, D., Garcıa, J.T., Vinuela, J., Zarazaga, M., Ruiz-Fons, F. and Torres, C. (2014). Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in wild small mammals in Spain. *Journal of Antimicrobial Chemotherapy* **69**(8): 2061-2064.

Goni, P., Vergara, Y., Ruiz, J., Albizu, I., Vila, J. and Gomez-Lus, R. (2004). Antibiotic resistance and epidemiological typing of *Staphylococcus aureus* strains from ovine and rabbit mastitis. *International Journal of Antimicrobial Agents* **23**(3): 268-272

Greene, C.E. (2006). Otitis Externa. In: Greene CE (ed) Infectious Diseases of the Dog and Cat, 3rd edn. Saunders, Missouri: 815–823.

Griffeth, G.C., Morris, D.O., Abraham, J.L., Shofer, F.S. and Rankin, S.C. (2008). Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. *Veterinary Dermatology* **19**: 142–149.

Guardabassi, L., Loeber, M.E. and Jacobson, A. (2004). Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Veterinary Microbiology* **98**: 23-27.

Hafeez, R., Chughtai, A.S. and Aslam, M. (2004). Prevalence and antimicrobial susceptibility of methicillin resistant *S. aureus* (MRSA). *International Journal of Pathology* **2**(1): 10–15.

Han, Z., Lautenbach, E., Fishman, N. and Nachamkin, I. **(2007)**. Evaluation of mannitol salt agar, CHROMagar Staph aureus and CHROMagar MRSA for detection of meticillin-resistant *Staphylococcus aureus* from nasal swab specimens. *Journal of Medical Microbiology* **56**(1):43-46.

Hanselman, B.A., Kruth, S.A., Rousseau, J. and Weese, J.S. (2009). Coagulase positive staphylococcal colonization of humans and their household pets. *Canadian Veterinary Journal* **50:** 954-958.

Hanselman, B.A., Kruth, S.A., Rousseau, J., Low, D.E., Willey, B.M. (2006). Methicillin resistant *Staphylococcus aureus* colonization in veterinary personnel. *Emerging Infectious Diseases* **12**(12): 1933–1938.

Hariharan, H., Coles, M., Poole, D., Lund, L. and Page, R. **(2006)**. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Canadian Veterinary Journal* **47**:253–255.

Harris, S.R., Cartwright, E.J., Török, M.E., Holden, M.T., Brown, N.M., Ogilvy Stuart, A.L., Ellington, M.J., Quail, M.A., Bentley, S.D., Parkhill, J. and Peacock, S.J.

(2013). Whole-genome sequencing for analysis of an outbreak of methicillin resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infectious Diseases* **13**: 130–136.

Hartmann, F.A., Trostle, S.S. and Klohnen, A.A. (1997). Isolation of methicillin-resistant Staphylococcus aureus from a postoperative wound infection in a horse. *Journal of the American Veterinary Medical Association* **211**:590-592.

Hawkey, P.M. (2008). The growing burden of antimicrobial resistance. *Journal of Antimicrobial Chemotherapy* **62** *Suppl.* 1*: i1-i9.*

Horstmann, C., Mueller, R.S., Straubinger, R.K. and Werckenthin, C. (2012). Detection of methicillin resistant *Staphylococcus pseudintermedius* with commercially available selective media. *Letters in Applied Microbiology* **54:** 26-31.

Ishihara, K., Shimokubo, N., Sakagami, A., Ueno, H., Muramatsu, Y., Kadosawa, T., Yanagisawa, C., Hanaki, H., Nakajima, C., Suzuki, Y. and Tamura, Y. (2010). Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic Veterinary Hospital. *Applied Environmental Microbiology* **76:** 5165-5174.

Jain, A., Agarwal, A., Verma, R.K. (2008). Cefoxitin disc diffusion test for detection of Methicillin resistant Staphylococci. *Journal of Medical Microbiology* **57**: 957-961.

Julian, T., Singh, A., Rousseau, J. and Weese, J.S. (2012). Methicillin resistant staphylococcal contamination of cellular phones of personell in a veterinary teaching hospital. *BMC Research Notes* **5**: 193-197.

Junco, T.M. T. and Barrasa Martin J.L. (2002). Identification and antimicrobial susceptibility of coagulase positive Staphylococci isolated from healthy dogs and dogs suffering from otitis externa. *Journal of Veterinary Medicine B* **49**: 419–423.

Kampf, G. and Kramer, A. (2004). Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clinical Microbiology Review* **17**:863–893.

Kateete, D.P., Kimani, C.N., Katabazi, F.A., Okeng, A., Okee, M.S., Nanteza, A., Joloba, M.L. and Najjuka, F.L. (2010). Identification of Staphylococcus aureus: Dnase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals of Clinical Microbiology and Antimicrobials* **9:**23-30.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J. and Hiramatsu, K. (2007). Combination of Multiplex PCRs for Staphylococcal Cassette Chromosome *mec* Type Assignment: Rapid Identification System for*mec*, *ccr*, and Major Differences in Junkyard Regions. *Antimicrobial Agents and Chemotherapy* ***51***(1):264–274.

Kuehnert, M.J., Hill, H.A., Kupronis, B.A., Tokars, J.I., Solomon, S.L., and Jernigan, D.B. **(2005)**. Methicillin-resistant *Staphylococcus aureus* hospitalizations, United States. *Emerging Infectious Diseases* **11**:868–72.

Leonard, F., Abbott, Y., Rossney, A., Quinn, P., O'Mahony, R. and Markey, B. (2006). Methicillin-resistant *Staphylococcus aureus* isolated from a veterinary surgeon and five dogs in one practice. *Veterinary Record* **158**: 155–159.

Lilenbaum, W., Veras, M., Blum, E. and Souza, G.N. (2000). Antimicrobial susceptibility of Staphylococci isolated from otitis externa in dogs. *Letters in Applied Microbiology* **31**:42–45.

Loeffler, A., Boag, A.K., Sung, J., Lindsay, J.A. and Guardabassi, L., Dalsgaard, A., Smith, H., Stevens, K. B. and Lloyd, D. H. (2005). Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *Journal of Antimicrobial Chemotherapy* **56**(4): 692–697.

Loeffler, A., Linek, M., Moodley, A., Guardabassi, L., Sung, J.M., Winkler, M., Weiss, R. and 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**:412-421.

Loeffler, A., Pfeiffer, D.U., Lloyd, D.H., Smith, H., Soares-Magalhaes, R. and Lindsay, J.A. (2010). Methicillin resistant *Staphylococcus aureus* carriage in U.K veterinary staff and owners of infected pets: new risk groups. *Journal of Hospital Infections* **74**(3): 282-288.

Lyskova, P., Vydrzalova, M. and Mazurova, J. **(2007)**. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. *Journal of Veterinary Medicine A* **54**: 559-563.

Magalhaes, S.R.J., Loeffler, A., Lindsay, J., Rich, M., Roberts, L., Smith, H., Lloyd, D. H. and Pfeiffer, D. U. (2010). Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dogs and cats: a case-control study. *Veterinary Research* **41**:55.

Maina, E. K., Kiiyukia, C., Wamae, C. N., Waiyaki, P. G. and Kariuki, S. (2013). Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. *International Journal of Infectious Diseases* **17**(2): e115-119.

Malayeri, H.Z., Jamshidi, S. and Salehi, T.Z. (2010). Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs *Veterinary Research Communications* **34**:435–444.

Mande, J.D and Kitaa, J.M.A. **(2005)**. Microbial profile and antimicrobial susceptibility of isolates from dogs with otitis in Kenya. *The Kenya Veterinarian* **29**: 40-44.

Manian, F.A. (2003). Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clinical Infection and Disease* **36:** e26–e28.

Mason, I.S., Mason, K.V. and Llov, D.H. (1996). A review of the biology of canine skin with respect to the commensals *Staphylococcus intermedius*, *Demodex canis* and *Malassezia pachydermatis*. *Veterinary Dermatology* **7**: 119-132.

McLean, C.L and Ness, M.G. (2008). Methicillin-resistant *Staphylococcus aureus* in a veterinary orthopaedic referral hospital: staff nasal colonisation and incidence of clinical cases. *Journal of Small Animal Practice* **49**(4):170–177

McManus, B.A., Coleman, D.C., Deasy, E.C., Brennan, G.I., O' Connell, B., Monecke, S., Ralf Ehricht, R., Leggett, B., Leonard, N. and Shore, A.C. (2015). Comparative Genotypes, Staphylococcal Cassette Chromosome mec (SCCmec) Genes and Antimicrobial Resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Isolates from Infections in Humans and Companion Animals. *PLoS ONE* 10(9): e0138079.

Medhus A., Slettemea J.S., Marstein L, Larssen K.W. and Sunde M. (2012). Methicillin-resistant *Staphylococcus aureus* with the novel *mecC* gene variant isolated from a cat suffering from chronic conjunctivitis. *Journal of Antimicrobial Chemotherapy***, 0:** 1–2.

Melo, D.A., Coelho, I.S., Motta, C.C., Rojas, A.C., Dubenczuk, F.C., Coelho, S.M.O. and Souza, M.M.S. (2013). Implications of the use of human parameters for the mecA gene detection to predict beta-lactam resistance in bovine *Staphylococcus spp.* isolated from mastitis. Staphylococcus aureus strain 28C penicillin-binding protein (mecA) gen - Nucleotide - NCBI.htm (Unpublished).

Meyers, B., Schoeman, J.P., Goddard, A. and Picard, J. **(2007)**. The bacteriology and antimicrobial susceptibility of infected and non-infected dog bite wounds: fifty cases. *Veterinary Microbiology* **127**(3-4): 360–368.

Middleton, J., Fales, W., Luby, C., Oaks, J., Sanchez, S., Kinyon, J., Wu, C., Maddox, C., Welsh, R. and Hartmann, F. (2005). Surveillance of Staphylococcus aureus in veterinary teaching hospitals. *Journal of Clinical Microbiology* **43**:2916-2919.

Murphy, C., Reid-Smith, R. J., Prescott, J. F., Bonnett, B. N., Poppe, C., Boerlin, P., Weese, J. S., Janecko, N. and Mcewen, S. A. (2009). Occurrence of antimicrobial resistant bacteria in healthy dogs and cats presented to private veterinary hospitals in southern Ontario: preliminary study. *Canadian Veterinary Journal* **50**: 1047-53

Neely, A. N. and Maley, M. P. (2000). Survival of Enterococci and Staphylococci on hospital fabrics and plastic. *Journal of Clinical Microbiology* **38**: 724-726.

Nelson, L.L. (2011). Surgical site infections in small animal practice. *The Veterinary Clinics of North America Small Animal Practice* **41**: 1041-1056.

Niemeyer, D.M., Pucci, M.J., Thanassi, J.A., Sharma, V.K. and Archer, G.L. (1996). Role of *mecA* transcriptional regulation in the phenotypic expression of methicillin resistance in *Staphylococcus aureus*. *Journal of Bacteriology* **178** (18): 5464–5471.

Nienhoff, U., Kadlec, K., Chaberny, I.F., Verspohl, J., Gerlach, G.F., Kreienbrock, L., Schwarz, S., Simon, D. and Nolte, I. **(2011)**. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. *Veterinary Microbiology* **150**:191-197.

Njoroge, C.W., Mande, J.D., Mitema, E.S. and Kitaa, J.M.A. (2016). Multidrug resistance of common bacterial patogens from wounds and otitis externa in small animals during a 10 year period in Kenya. *International Journal of Veterinary Science* 5(4): 262-267

Normand, E.H., Gibson, N.R., Reid, S.W., Carmichael, S. and Taylor, D.J. (2000). Antimicrobial-resistance trends in bacterial isolates from companion animal community practice in the UK. *Preventative Veterinary Medicine* **46**: 267–278.

Öztürk, D., Avki, S., Türütoğlu, H., Yiğitarslan, K. and Sağnak, S. **(2010)**. Methicillin resistance among Coagulase-positive Staphylococci isolated from dogs with Otitis Externa, Skin Wounds and Pyoderma. *Kafkas Universitesi Veteriner Fakultesi Dergisi* **16**(4): 651-656.

Padhy, A., Mishra, R., Behera, S.S., Sahu, A.R. and Sahoo, S. (2014). Microbial profile of canine persistent wound infections. *Veterinary World* **7**(4): 244-247.

Paterson, G.K., Harrison, E.M. and Holmes, M.A. **(2014)**. The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiology* **22**: 42–47.

Paterson, G.K., Morgan, F.J.E., Harrison, E.M., Peacock, S.J., Parkhill, J., Zadoks, R.N. and Holme, M.A. (2014). Prevalence and properties of *mecC* methicillin resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. *Journal of Antimicrobial Chemotherapy* **69**: 598–602.

Paul, N.C., Bargman, S.C., Moodley, A., Nielsen, S.S. and Guardabassi, L. (2012). *Staphylococcus pseudintermedius* colonization patterns and strain diversity in healthy dogs: a cross-sectional and longitudinal study. *Veterinary Microbiology* **160:** 420–427.

Paul, N.C., Moodley, A., Ghibaudo, G. and Guardabassi, L. (2011). Carriage of Methicillin-Resistant *Staphylococcus pseudointermedius* in small animal veterinarians; Indirect Evidence of Zoonotic transmission. *Zoonoses and Public Health* **8**: 533-539.

Pedersen, K., Jensen, H., Finster, K., Jensen, V.F. and Heuer, O.E. (2007). Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *Journal of Antimicrobial Chemotherapy* **60**:775 – 781.

Perreten, V., Kadlec K., Schwarz S., Grönlund Andersson U., Finn M., Greko C., Moodley A., Kania S.A., Frank L.A., Bemis D.A., Franco A., Iurescia M., Battisti A., Duim B., Wagenaar J.A., van Duijkeren E., Weese J.S., Fitzgerald J.R., Rossano A. and Guardabassi L. (2010). Clonal spread of methicillin- resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *Journal of Antimicrobial Chemotherapy* **65**:1145 – 1154.

Perreten,V., Chanchaithong, P., Nuvee Prapasarakul, N., Rossano, A., Shlomo, E., Blum, S.E., Elad, D. and Schwendener S. (2013). Novel Pseudo-Staphylococcal Cassette Chromosome *mec* Element (SCC*mec*57395) in Methicillin-Resistant *Staphylococcus pseudintermedius* CC45. *Antimicrobial Agents and Chemotherapy* **57**(11): 5509–5515.

Petersen, A. D., Walker, R. D., Bowman, M. M., Schott, H. C. and Rosser Jr., E. J. (2002). Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius and Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992– 1997). *Journal of American Animal Hospitals Association* **38**: 407–13

Petinaki, E. and Spiliopoulou, I. (2012). Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals; Impact of human contact. *Clinical Microbiology and Infection* **18**: 626-634.

Petrov, V., Mihaylov, G., Tsachev, I., Zhelev G., Marutsov, P. and Koe, K. (2013). Otitis externa in dogs: microbiology and antimicrobial susceptibility. *Revue de Médecine Vétérinaire* **164 (**1): 18-22.

Pinchbeck, L.R., Cole, L.K., Hillier, A., Kowalski, J.J., Rajala-Schultz, P.J., Bannerman, T.L. and York, S. (2006). Genotypic relatedness of staphylococcal strains isolated from pustules and carriage sites in dogs with superficial bacterial folliculitis. *American Journal of Veterinary Research* **67:** 1337–1346.

Quitoco, I.M., Raimundo, M.S., Silva-Carvalho, M.C., Souza, R.R., Beltrame, C.O., Oliveira, T.F., Araújo, R., del Peloso, P.F., Coelho, L.R. and Figueiredo, A.M. (2013). First report in South America of companion animal colonization by the USA1100 clone of community-acquired methicillin-resistant *Staphylococcus aureus* (ST30) and by the European clone of methicillin-resistant *Staphylococcus pseudintermedius* (ST71) 336. *BMC Research Notes* **6**:336. doi: 10.1186/1756-0500-6-336.

Rahman, M.M., Biswas, D.B., Islam, M.M. and Islam, M.A. (2003). Cultural sensitivity of septic wounds in animals. *Pakistan Journal of Biological Sciences* **6**: 741-744.

Rich, M. and Roberts, L. (2004). Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. *Veterinary Record* **159**: 535-536.

Riedel, S., Dam, L., Stamper, P.D., Shah, S.A.R. and Carro, K.C. (2010). Evaluation of Bio-Rad MRSA*Select* Agar for detection of methicillin-resistant *Staphylococcus aureus* directly from blood cultures. *Journal of Clinical Microbiology* **48**(6): 2285–2288.

Rostami, S., Moosavian, M., Shoja, S., Torabipour, M. and Farshadzadeh, Z. (2013). Comparison of *mecA* gene based PCR with CLSI cefoxitin and oxacillin disc diffusion methods for detecting methicillin resistance in *Staphylococcus aureus* clinical isolates. *African Journal of Microbiology Research* **7**(21): 2438- 2441.

Rubin, J.E. and Chirino-Trejo, M. (2011). Prevalence, sites of colonization, and antimicrobial resistance among *Staphylococcus pseudintermedius* isolated from

healthy dogs in Saskatoon, Canada. *Journal of Veterinary Diagnostic Investigation* **23**: 351–354.

Ruscher, C., Lübke-Becker, A., Wleklinski, C.G., Soba, A., Wieler, L.H. and Walther, B. (2009). Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. *Veterinary Microbiology* **136**:197-201.

Safdar, N. and Bradley, E.A. (2008). The risk of infection after nasal colonization with *Staphylococcus aureus*. *American Journal of Medicine* **121**: 310–315.

Saijonmaa-Koulumies, L.E. and Lloyd, D.H. (1996). Colonization of the canine skin with bacteria. *Veterinary Dermatology*, **7**:153-162.

Sajith- Khan, A.K., Preetha, J.S., Lakshi, S.Y., Anandi, C. And Ramesh, R. (2012). Detection of mecA genes of methicillin resistant *Staphylococcus aureus* by PCR. *International Journal of Health and Rehabilitation Science* **1**(2): 64-68.

Sasaki, T., Kikuchi, K., Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K. (2007). Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. *Journal of Clinical Microbiology* **45**:1118–1125.

Schissler, J.R., Hillier, A., Daniels, J.B., Cole, L.K. and Gebreyes, W.A. (2009). Evaluation of Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs. *Journal of Veterinary Diagnostic Investigation* **21**: 684-688.

Schmidt, V.M., Williams, N.J., Pinchbeck, G., Corless, C.E., Shaw, S., McEwan, N., Dawson, S. and Nuttall, T. (2014). Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. *BMC Veterinary Research* **10:** 17.

Scott, D.W., Miller, W.H. and Griffin, C.E. **(2001)**. Muller and Kirk's small animal dermatology. 6th ed. W.B. Saunders; Philadelphia:pp 913.

Shamir, M.H., Leisner, S., Klement, E., Gonen, E. and Johnston, De. (2002). Dog bite wounds in dogs and cats: a retrospective study of 196 cases. *Journal of Veterinary Science A* **49**: 107-112.

Sing, A., Tuschak, C. and Hörmansdorfer, S. (2008). Methicillin-resistant Staphylococcus aureus in a family and its pet cat. *New England Journal of Medicine* **358**:1200–1.

Singh, A., Walker, M., Rousseau, J., Monteith, G.J. and Weese, J.S. (2013). Methicillin-resistant Staphylococcal contamination of clothing worn by personell in a veterinary teaching hospital. *Veterinary Surgery* **42**: 643-648.

Slatter, D. (2003). Textbook of small animal surgery, 3rd Edition. (ed. Slatter, D). W.B Saunders Company, Philadelphia: pp 163-178.

Stevens, A.M., Hennesy, T., Bagett, H.C., Bruden, D., Parks, D. and Klejka, J. (2010). Methicillin resistant *Staphylococcus aureus* carriage and risk factorsfor skin infections, Southwestern Alaska, USA. *Emerging Infectious Diseases* **16**: 797-803.

Pavletic, M.M. (2003). The Integument. *In* Slatter D.J. (Ed): Textbook of Small Animal Surgery, 3rd edn. W.B. Saunders Company, Philadelphia.pp 250-259.

Petersen, A. D., Walker, R.D., Bowman, M. M., Schott, H. C., Rosser, E. J. JR. (2002). Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992-1997). *Journal of American Animal Hospitals Assocciation*. **38**: 407-413.

Perreten, V., Kadlec, K., Schwarz, S., Gronlund A.U., Finn, M., Greko, C., Moodley, A., Kania, S. A., Frank, L. A., Bemis, D. A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J. A., Van Duijkeren, E., Weese, J. S., Fitzgerald, J.R.,

Rossano, A. and Guardabassi, L. (2010). Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *Journal of Antimicrobial Chemotherapy*. **65**:1145-54.

Tomlin, J., Pead, M.J., Lloyd, D.H., Howell, S., Hartmann, F., Jackson, H.A. and Muir, P. **(1999)**. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. *Veterinary Record* **144**: 60–64.

Tsubakishita, S., Kuwahara-Arai, K., Sasaki, T., and Hiramatsu, K. (2010). Origin and Molecular Evolution of the Determinant of Methicillin Resistance in Staphylococci. *Antimicrobial Agents and Chemotherapy* **54**(10): 4352–4359.

Turk, R. (2013). Prospective evaluation of epidemiology and microbiology of surgical site infections (Masters Thesis). Retrieved from https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/7431/Turk_Ryen_20130Msc.pdf?sequence=1.

Turk, R., Singh, A. and Weese, J.S. (2015). Prospective Surgical Site Infection Surveillance in Dogs. *Veterinary Surgery* **44**(1): 2-8.

Urumova, V., Chaprazov, T.S., Lyutskanov, M. and Borisov, I. (2012). Microbiological analyses of canine infected wounds. *Revue Médecin Veterinaire* **163**(4): 201-205.

Van Duijkeren, E., Catry, B., Greko, C., Moreno, M.A., Pomba, M.C., Pyörälä, S., Ruzauskas, M., Sanders, P., Threlfall, E.J., Torren-Edo, J. and Törneke, K. (2011). Review on methicillin-resistant *Staphylococcus pseudintermedius*. *Journal of Antimicrobial Chemotherapy* **66** (12): 2705-2714.

V**an Duijkeren, E., Jansen, M.D., Flemming, S.C., de Neeling, H., Wagenaar, J.A., Schoormans, A.H., van Nes, A. and Fluit, A.C. (2007)**. Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. *Emerging Infectious Diseases* **13**:1408-1410.

Van Duijkeren, E., Box, A.T., Heck, M.E., Wannet, W.J. and Fluit, A.C. (2004). Methicillin resistant staphylococci isolated from animals. *Veterinary Microbiology* **103**:91–97.

Van Duijkeren, E., Hengeveld, P.D., Albers, M., Pluister, G., Jacobs, P. and Heres, L. (2014). Prevalence of methicillin resistance *Staphylococcus aureus* carrying mecA or mecC in dairy cattle. *Veterinary Microbiology*. **171**(3-4): 364-367.

Velasco V., Sherwood J.S., Rojas-Garcı P.P. and Logue, C.M. (2014). Multiplex Real-Time PCR for Detection of *Staphylococcus aureus*, *mecA* and Panton Valentine Leukocidin (PVL) Genes from Selective Enrichments from Animals and Retail Meat. *PLoS One* **9**(5): e97617.

Vengust, M., Anderson, M.E.C., Rousseau, J. and Weese, J.S. (2006). Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. *Letters in Applied Microbiology* **43**:602-606.

Verwilghen, D. and Kampf, G. (2011). Presurgical Hand Antisepsis: Concepts and Current Habits of Veterinary Surgeons. *Veterinary Surgery* **40**:515–521.

Vincze, S., Stamm, I., Kopp, P. A., Hermes, J., Adlhoch, C., Semmler, T., Wieler, L. H., Lubke-Becker, A. and Walther, B. (2014). Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010-2012. *PLoS One* **9**(1): e85656.

Walther, B., Hermes, J., Cuny, C., Wieler, L.H., Vincze, S., Abou E.Y., Stamm, I., Kopp, P.A., Kohn, B., Witte, W., Jansen, A., Conraths, F.J., Semmler, T., Eckmanns, T. and Lübke-Becker, A. (2012). Sharing more than friendship--nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. *PloS One* **7**(4): e35197.

Wang, J.T., Chang S.C., Ko, W.J., Chang, Y.Y., Chen, M.L., Pan, H.J. and Luh, K.T. (2001). A hospital-acquired outbreak of methicillin-resistant *Staphylococcus aureus* infection initiated by a surgeon carrier. *Journal of Hospital Infection* **47**:104–109.

Weber, S., Herwaldt, L. A., Mcnutt, L.A., Rhomberg, P.,Vaudaux, P., Pfaller, M.A. and Perl. T.M. (2002). An outbreak of Staphylococcus aureus in a pediatric cardiothoracic surgery unit. *Infection Control and Hospital Epidemiology* **23**:77–81.

Weese, J.S. and Duijkeren, V.E. (2009). Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Veterinary. Microbiology* **140**:418-429.

Weese, J.S. (2010). Methicillin resistant *Staphylococcus aureus* in animals. *Institute for Laboratory Animal Research Journal* **51**:233–244.

Weese, J. S. (2012). Staphylococcal control in the veterinary hospital. *Veterinary Dermatology* **23**: 292-298, e57-8.

Windahl, U., Reimega˚, R.D., Holst, B.S., Egenvall, A., Fernstro¨, M.L., Fre-Driksson, M., Trowald-Wigh, G. and Andersson, U.G. (2012). Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs—a longitudinal study. *BMC Veterinary Research* **8**: 34- 42.

Wu, Z., Li, F., Liu, D., Xue, H. and Zhao, X. (2015). Novel type XII of Staphylococcal cassette chromosome mec harboring a new cassette chromosome recombinase, $CcrC_2$ allele. *Antimicrobial Agents and Chemotherapy* **59**(12):7597-7601.

http://lpi.oregonstate.edu/mic/micronutrients-health/skin-health.

6.2. Appendices

Appendix 1: Microbial isolates from wounds and ear swabs of dogs from the UoN Small Animal Clinic (2004-2013).

No.	Year	Species	Sex	Age	Breed	Source	*IRT*
1	2005	Canine	M	A	GSD	Abscess	*Actinomyces pyogenes*
2	2006	Canine	M	A	GSD	Ear swab	*Actinomyces pyogenes*
3	2009	Canine	F		Cross	wound	*Coliforms*
4	2007	Canine	M	A	GSD	Ear swab	*Corynebacterium*
5	2007	Canine	M	A	GSD	Ear swab	*Corynebacterium*
6	2007	Canine	F	A	Cross	Ear swab	*Corynebacterium*
7	2012	Canine	M	A	J.Spitz	wound	*Corynebacterium*
8	2013	Canine	M	A	GSD	wound	*Corynebacterium*
9	2007	Canine	M	*	GSD	Ear swab	*Diphtheroids*
10	2004	Canine	F	A	J.Spitz	wound	*E.coli*
11	2004	Canine	F	A	Cross	wound	*E.coli*
12	2007	Canine	M	A	GSD	Ear swab	*E.coli*
13	2008	Canine	M	7yrs+	Cross	wound	*E.coli*
14	2008	Canine	M	A	Dachschund	wound	*E.coli*
15	2009	Canine	F	6mnths	J.Spitz	wound	*E.coli*
16	2009	Canine	F	4mnt/yr	J.Spitz	wound	*E.coli*
17	2009	Canine	F	A	J.Spitz	wound	*E.coli*
18	2009	Canine	F	A	J.Spitz	wound	*E.coli*
19	2009	Canine	M	A	Cross	wound	*E.coli*
20	2009	Canine	F	11mnths	GSD	wound	*E.coli*
21	2010	Canine	M	A	GSD	Abscess	*E.coli*
22	2013	Canine	F	A	GSD	wound	*E.coli*
23	2010	Canine	M	*	GSD	Ear Swab	*E.coli*
24	2010	Canine	M	A	GSD	wound	*E.coli*
25	2006	Canine	M	A	Terrier	Ear swab	*Fungi*
26	2009	Canine	F	A	GSD	wound	*Klebsiella*
27	2007	Canine	F	*	GSD	Abscess	*Nocardia*
28	2008	Canine	M	A	Cross	wound	*Pasteurella*
29	2004	Canine	M	A	Cross	Ear Swab	*Proteus* spp.
30	2004	Canine	M	A	Cross	Ear swab	*Proteus* spp.
31	2004	Canine	M	A	Cross	Ear Swab	*Proteus* spp.
32	2004	Canine	F	A	*	Ear Swab	*Proteus* spp.
33	2005	Canine	M	A	GSD	Ear swab	*Proteus* spp.
34	2005	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
35	2005	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
36	2005	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
37	**2005**	Canine	M	A	GSD	Ear swab	*Proteus* spp.
38	2005	Canine	*	*	*	Ear swab	*Proteus* spp.

39	2005	Canine	M	A	GSD	Ear swab	*Proteus* spp.
40	2005	Canine	M	A	Husky Cross	Ear swab	*Proteus* spp.
41	2005	Canine	M	A	GSD	Ear swab	*Proteus* spp.
42	2005	Canine	M	A	GSD	Ear swab	*Proteus* spp.
43	2006	Canine	M	A	*	Ear swab	*Proteus* spp.
44	2007	Canine	M	A	GSD	Ear swab	*Proteus* spp.
45	2007	Canine	F	A	GSD	Ear swab	*Proteus* spp.
46	2007	Canine	M	A	Jack Rusell	Ear swab	*Proteus* spp.
47	2007	Canine	M	A	Jack Rusell	Ear swab	*Proteus* spp.
48	2007	Canine	M	A	GSD	Ear swab	*Proteus* spp.
49	2007	Canine	M	A	GSD	Ear swab	*Proteus* spp.
50	2007	Canine	M	A	*	Ear swab	*Proteus* spp.
51	2007	Canine	M	A	GSD	Ear swab	*Proteus* spp.
52	2008	Canine	M	A	Cross	Ear Swab	*Proteus* spp.
53	2008	Canine	F	A	GSD	Ear Swab	*Proteus* spp.
54	2009	Canine	M	Puppy	GSD	Ear Swab	*Proteus* spp.
55	2009	Canine	M	A	*	wound	*Proteus* spp.
56	2011	Canine	M	A	Cross	Ear Swab	*Proteus* spp.
57	2011	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
58	2012	Canine	F	A	GSD	Ear Swab	*Proteus* spp.
59	2012	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
60	2012	Canine	M	3yrs	Rottweiler	wound	*Proteus* spp.
61	2012	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
62	2013	Canine	M	A	J.Spitz	wound	*Proteus* spp.
63	2013	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
64	2013	Canine	M	A	Ridgeback	Ear Swab	*Proteus* spp.
65	2010	Canine	M	A	GSD	Ear Swab	*Proteus* spp.
66	2010	Canine	F	A	GSD	Ear Swab	*Proteus* spp.
67	2004	Canine	F	A	Beagle	Ear Swab	*Pseudomonas* spp.
68	2004	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
69	2005	Canine	M	A	GSD	Ear swab	*Pseudomonas* spp.
70	2005	Canine	M	A	GSD	Ear swab	*Pseudomonas* spp.
71	2005	Canine	*	A	GSD	Ear Swab	*Pseudomonas* spp.
72	2005	Canine	F	Puppy	Poodle	Ear Swab	*Pseudomonas* spp.
73	2005	Canine	M	Puppy	Poodle	Ear Swab	*Pseudomonas* spp.
74	2006	Canine	M	A	GSD	Ear swab	*Pseudomonas* spp.
75	2006	Canine	*	A	*	Ear swab	*Pseudomonas* spp.
76	2006	Canine	M	A	GSD X	Ear swab	*Pseudomonas* spp.
77	2006	Canine	M	A	GSD	Ear swab	*Pseudomonas* spp.
78	2008	Canine	F	A	GSD	Ear Swab	*Pseudomonas* spp.
79	2008	Canine	F	A	Boerboel	Ear Swab	*Pseudomonas* spp.
80	2009	Canine	M	A	Ridgeback	Ear Swab	*Pseudomonas* spp.
81	2009	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
82	2009	Canine	F	A	GSD	Ear Swab	*Pseudomonas* spp.

83	2010	Canine	M	A	J. spitz	wound	*Pseudomonas* spp.
84	2011	Canine	F	A	GSD	Ear Swab	*Pseudomonas* spp.
85	2011	Canine	F	A	GSD	Ear Swab	*Pseudomonas* spp.
86	2011	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
87	2012	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
88	2012	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
89	2010	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
90	2010	Canine	F	A	GSD	Ear Swab	*Pseudomonas* spp.
91	2010	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
92	2010	Canine	M	A	GSD	Ear Swab	*Pseudomonas* spp.
93	2010	Canine	M	A	*	Ear Swab	*Pseudomonas* spp.
94	2010	Canine	M	A	J.Spitz	wound	*Pseudomonas* spp.
95	2004	Canine	F	A	GSD	Ear swab	*S.aureus*
96	2004	Canine	M	A	GSD	Ear swab	*S.aureus*
97	2004	Canine	F	Puppy	GSD	Ear swab	*S.aureus*
98	2004	Canine	M	A	GSD	Ear swab	*S.aureus*
99	2004	Canine	F	A	GSD X	Ear swab	*S.aureus*
100	2004	Canine	F	A	GSD	Ear Swab	*S.aureus*
101	2004	Canine	M	A	GSD/Rott	Ear Swab	*S.aureus*
102	2004	Canine	M	A	GSD	Ear swab	*S.aureus*
103	2004	Canine	F	A	GSD	Ear Swab	*S.aureus*
104	2004	Canine	F	A	GSD	Ear Swab	*S.aureus*
105	2004	Canine	F	A	Cross	Ear Swab	*S.aureus*
106	2004	Canine	F	A	GSD	Ear Swab	*S.aureus*
107	2004	Canine	M	A	GSD/Rott	Ear Swab	*S.aureus*
108	2004	Canine	M	A	Cross	Ear Swab	*S.aureus*
109	2004	Canine	M	A	Husky Cross	Ear Swab	*S.aureus*
110	2004	Canine	M	A	GSD	Ear Swab	*S.aureus*
111	2004	Canine	F	A	Cross	Ear Swab	*S.aureus*
112	2005	Canine	M	A	GSD	Ear swab	*S.aureus*
113	2005	Canine	M	A	GSD	Ear Swab	*S.aureus*
114	2005	Canine	M	A	GSD	Ear swab	*S.aureus*
115	2005	Canine	F	A	GSD X	Ear Swab	*S.aureus*
116	2005	Canine	M	A	GSD	Ear Swab	*S.aureus*
117	2005	Canine	M	A	GSD	Ear swab	*S.aureus*
118	2005	Canine	M	A	GSD	Ear swab	*S.aureus*
119	2005	Canine	M	A	Rottweiler	Ear swab	*S.aureus*
120	2005	Canine	M	A	GSD	Ear swab	*S.aureus*
121	2006	Canine	F	*	GSD	Ear swab	*S.aureus*
122	2006	Canine	F	A	GSD	Ear swab	*S.aureus*
123	2006	Canine	M	A	GSD	Ear swab	*S.aureus*
124	2006	Canine	M	A	Ridgeback	Ear swab	*S.aureus*
125	2006	Canine	M	A	GSD	Ear swab	*S.aureus*
126	2006	Canine	M	A	GSD	Ear swab	*S.aureus*

127	2006	Canine	F	A	GSD	Ear swab	*S.aureus*
128	2006	Canine	M	A	Doberman	Ear swab	*S.aureus*
129	2006	Canine	M	Puppy	Chihuahua X	Ear swab	*S.aureus*
130	2006	Canine	M	A	GSD	Ear swab	*S.aureus*
131	2006	Canine	M	A	GSD	Ear swab	*S.aureus*
132	2006	Canine	F	A	GSD	Ear swab	*S.aureus*
133	2006	Canine	M	A	GSD	Ear swab	*S.aureus*
134	2007	Canine	M	A	Cross	Ear swab	*S.aureus*
135	2007	Canine	F	A	GSD	Ear swab	*S.aureus*
136	2007	Canine	F	A	GSD	Ear swab	*S.aureus*
137	2007	Canine	F	A	*	Ear swab	*S.aureus*
138	2007	Canine	M	A	GSD	Ear swab	*S.aureus*
139	2007	Canine	M	A	*	Ear swab	*S.aureus*
140	2007	Canine	F	A	GSD X	Ear swab	*S.aureus*
141	2007	Canine	M	A	GSD	Ear swab	*S.aureus*
142	2007	Canine	*	*	GSD	Ear swab	*S.aureus*
143	2007	Canine	M	A	GSD	Ear swab	*S.aureus*
144	2007	Canine	M	A	GSD	Ear swab	*S.aureus*
145	2007	Canine	M	A	GSD X	Ear swab	*S.aureus*
146	2007	Canine	F	A	GSD	Ear swab	*S.aureus*
147	2007	Canine	M	A	GSD	Ear swab	*S.aureus*
148	2007	Canine	F	A	GSD	Ear swab	*S.aureus*
149	2007	Canine	M	A	J.Spitz	Ear swab	*S.aureus*
150	2007	Canine	M	A	Terrier	Ear swab	*S.aureus*
151	2007	Canine	M	Puppy	Rott X GSD	wound	*S.aureus*
152	2008	Canine	M	A	Rottweiler	Ear Swab	*S.aureus*
153	2008	Canine	M	A	GSD	Ear Swab	*S.aureus*
154	2008	Canine	M	A	GSD	Ear Swab	*S.aureus*
155	2008	Canine	M	A	GSD	Ear Swab	*S.aureus*
156	2008	Canine	M	A	Ridgeback X	Ear Swab	*S.aureus*
157	2008	Canine	M	A	Local	wound	*S.aureus*
158	2008	Canine	M	A	GSD	wound	*S.aureus*
159	2008	Canine	F	A	J. Spitz	Ear Swab	*S.aureus*
160	2009	Canine	M	A	Daschund	Abscess	*S.aureus*
161	2009	Canine	M	3yrs	GSD	Abscess	*S.aureus*
162	2009	Canine	M	A	GSD	Ear Swab	*S.aureus*
163	2009	Canine	M	Puppy	Cross	Ear Swab	*S.aureus*
164	2009	Canine	M	A	GSD	Ear Swab	*S.aureus*
165	2009	Canine	*	*	*	Ear Swab	*S.aureus*
166	2009	Canine	M	A	Cross	Ear Swab	*S.aureus*
167	2009	Canine	M	A	G.dane	Eye swab	*S.aureus*
168	2009	Canine	M	A	Doberman	wound	*S.aureus*
169	2009	Canine	M	A	GSD	wound	*S.aureus*

170	2009	Canine	M	A	Cross	wound	*S.aureus*
171	2009	Canine	M	A	Cross	wound	*S.aureus*
172	2009	Canine	M	A	Doberman	wound	*S.aureus*
173	2009	Canine	M	4yrs	G.dane	Abscess	*S.aureus*
174	2009	Canine	F	A	GSD	Ear Swab	*S.aureus*
175	2010	Canine	*	8yrs	*	Abscess	*S.aureus*
176	2010	Canine	F	A	Cross	wound	*S.aureus*
177	2010	Canine	M	A	GSD	wound	*S.aureus*
178	2011	Canine	F	A	Terrier	Abscess	*S.aureus*
179	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
180	2011	Canine	F	A	GSD	Ear Swab	*S.aureus*
181	2011	Canine	M	A	Ridgeback	Ear Swab	*S.aureus*
182	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
183	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
184	2011	Canine	F	A	Cross	Ear Swab	*S.aureus*
185	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
186	2011	Canine	F	A	GSD	Ear Swab	*S.aureus*
187	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
188	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
189	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
190	2011	Canine	M	6mnths	*	wound	*S.aureus*
191	2011	Canine	M	*	Labrador	wound	*S.aureus*
192	2011	Canine	F	2.5yrs	Rottweiler	wound	*S.aureus*
193	2012	Canine	F	A	GSD	Ear Swab	*S.aureus*
194	2012	Canine	F	A	French Bullmastiff	Ear Swab	*S.aureus*
195	2012	Canine	F	A	Cross	Ear Swab	*S.aureus*
196	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
197	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
198	2012	Canine	F	A	GSD	Ear Swab	*S.aureus*
199	2012	Canine	F	A	GSD	Ear Swab	*S.aureus*
200	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
201	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
202	2012	Canine	M	A	Local	Ear Swab	*S.aureus*
203	2012	Canine	M	A	GSD	wound	*S.aureus*
204	2012	Canine	F	A	Rottweiler	wound	*S.aureus*
205	2012	Canine	M	A	Cross	wound	*S.aureus*
206	2012	Canine	M	1yr	GSD	wound	*S.aureus*
207	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
208	2012	Canine	F	A	GSD	wound	*S.aureus*
209	2012	Canine	M	A	GSD	Ear Swab	*S.aureus*
210	2013	Canine	M	A	GSD	Ear Swab	*S.aureus*
211	2013	Canine	F	A	Cross	wound	*S.aureus*
212	2013	Canine	M	A	Rottweiler	wound	*S.aureus*
213	2013	Canine	*	*	Rottweiler	wound	*S.aureus*

214	2010	Canine	M	A	Cross	Ear Swab	*S.aureus*
215	2010	Canine	F	A	GSD	Ear Swab	*S.aureus*
216	2010	Canine	F	A	GSD	Ear Swab	*S.aureus*
217	2010	Canine	*	*	*	Ear Swab	*S.aureus*
218	2010	Canine	*	*	*	Ear Swab	*S.aureus*
219	2010	Canine	M	*	GSD	Ear Swab	*S.aureus*
220	2010	Canine	F	A	GSD	Ear Swab	*S.aureus*
221	2010	Canine	M	A	GSD	Ear Swab	*S.aureus*
222	2010	Canine	M	A	Cross	wound	*S.aureus*
223	2010	Canine	M	Puppy	GSD	wound	*S.aureus*
224	2010	Canine	F	A	Cross	wound	*S.aureus*
225	2010	Canine	M	A	GSD	Ear Swab	*S.aureus*
226	2011	Canine	M	A	GSD	Ear Swab	*S.aureus*
227	2012	Canine	M	A	Cross	Ear Swab	*S.aureus*
228	2006	Canine	M	A	Ridgeback	Ear swab	*Staphylococcus* spp.
229	2007	Canine	M	A	GSD	wound	*Staphylococcuss spp.*
230	2007	Canine	M	A	GSD	Ear swab	*Staphylococcus* spp.
231	2010	Canine	M	7mnths	GSD	wound	*Staphylococcus* spp.
232	2011	Canine	F	A	Lhasa Apso	Ear Swab	*Staphylococcus* spp.
233	2011	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
234	2011	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
235	2011	Canine	M	*	GSD	Ear Swab	*Staphylococcus* spp.
236	2011	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
237	2011	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
238	2013	Canine	M	A	Belgian	Ear Swab	*Staphylococcus* spp.
239	2013	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
240	2013	Canine	F	A	GSD	Ear Swab	*Staphylococcus* spp.
241	2013	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
242	2013	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
243	2013	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
244	2010	Canine	F	A	*	wound	*Staphylococcus* spp.
245	2010	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
246	2010	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
247	2010	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
248	2010	Canine	M	A	Ridgeback	Ear Swab	*Staphylococcus* spp.
249	2010	Canine	M	A	GSD	Ear Swab	*Staphylococcus* spp.
250	2004	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
251	2004	Canine	M	A	Cross	Ear Swab	*Streptococcus* spp.
252	2004	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
253	2005	Canine	M	A	GSD	Abscess	*Streptococcus* spp.
254	2005	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
255	2007	Canine	M	A	GSD	Ear swab	*Streptococcus* spp.
256	2007	Canine	F	A	GSD	Ear swab	*Streptococcus* spp.
257	2008	Canine	M	A	Cross	Abscess	*Streptococcus* spp.
258	2008	Canine	*	*	Ridgeback	Abscess	*Streptococcus* spp.

259	2009	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
260	2009	Canine	M	A	*	Ear Swab	*Streptococcus* spp.
261	2010	Canine	M	A	Cross	wound	*Streptococcus* spp.
262	2010	Canine	F	A	Jack Russell	wound	*Streptococcus* spp.
263	2012	Canine	F	2yrs	G. dane	Abscess	*Streptococcus* spp.
264	2013	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
265	2010	Canine	M	A	Rottweiler	Abscess	*Streptococcus* spp.
266	2010	Canine	M	A	GSD	Ear Swab	*Streptococcus* spp.
267	2010	Canine	M	A	Cross	wound	*Streptococcus* spp.

KEY: *S. aureus*: *Staphylococcus aureus*, Staphylococcus: Other *Staphylococcus* spp., *E. coli*: *Escherichia coli*; AMST- Antimicrobial susceptibility testing. M: Male, F: Female, A: Adult, GSD: German Shepherd *: Information missing from record.

Appendix 2: Antimicrobial susceptibility pattern of bacterial isolates (2004-2013)

No.	*IRT*	Pen	Amp	Ery	Apc	Cef	Gen	Cot	Chl	Tet	Kan	Str	Nor	Acv	Axy	Sul
1	*Actinomyces pyogenes*	*	R	*	*	R	R	R	S	*	R	R	S	*	*	*
2	*Actinomyces pyogenes*	*	S	*	*	R	S	*	S	S	*	R	S	S	*	*
3	*Coliforms*	*	R	*	*	*	S	R	*	R	*	*	S	R	*	R
4	*Corynebacterium*	*	S	R	*	S	S	R	R	S	*	*	*	R	*	*
5	*Corynebacterium*	*	R	*	*	S	S	R	R	S	*	*	R	S	*	*
6	*Corynebacterium*	*	R	*	*	S	S	R	R	R	*	*	R	S	*	*
7	*Corynebacterium*	*	*	R	R	*	*	*	*	S	*	R	*	S	R	R
8	*Corynebacterium*	*	R	*	*	*	S	R	S	*	R	R	*	R	R	R
9	*Diphtheroids*	*	R	R	S	*	*	R	S	S	*	S	R	*	*	*
10	*E.coli*	*	R	*	R	R	S	R	R	S	S	*	R	*	*	R
11	*E.coli*	*	R	*	*	R	R	R	R	S	*	*	S	R	*	*
12	*E.coli*	*	R	*	R	*	*	R	*	R	*	*	*	*	*	*
13	*E.coli*	*	S	*	S	*	S	R	S	*	*	*	S	*	*	R
14	*E.coli*	*	R	*	*	*	S	R	S	R	*	*	S	R	*	R
15	*E.coli*	*	R	*	*	R	S	R	R	S	*	*	R	*	*	R
16	*E.coli*	*	*	R	S	R	*	*	*	S	*	S	R	R	*	*
17	*E.coli*	*	R	*	R	*	S	R	*	R	S	R	S	*	*	*
18	*E.coli*	*	R	*	R	R	*	R	R	S	*	*	R	*	*	R
19	*E.coli*	*	S	*	S	*	S	R	S	S	*	*	S	*	*	R
20	*E.coli*	*	R	*	*	R	S	R	S	R	*	*	S	*	*	R
21	*E.coli*	*	R	*	*	R	S	R	S	S	*	*	*	R	*	R
22	*E.coli*	*	S	*	*	*	S	S	S	S	*	*	S	R	*	S
23	*E.coli*	R	R	*	*	*	S	R	S	R	*	*	S	*	R	R
24	*E.coli*	*	R	*	*	R	S	R	S	S	*	*	*	R	*	R
25	*Klebsiella*	*	R	*	*	*	S	R	S	R	*	*	S	R	*	R
26	*Nocardia*	*	*	S	*	S	*	S	R	*	*	*	R	S	*	R
27	*Pasteurella*	S	S	*	*	S	*	R	S	*	*	*	S	S	*	*
28	*Proteus*	*	R	*	*	R	S	R	S	S	S	*	S	R	*	*
29	*Proteus*	*	R	*	*	*	*	R	R	*	R	R	*	*	*	R
30	*Proteus*	*	R	*	*	*	S	R	R	R	R	R	*	*	*	R
31	*Proteus*	*	R	*	*	*	S	R	R	R	R	R	*	*	*	R
32	*Proteus*	*	R	*	*	R	S	R	S	S	*	*	S	S	*	*
33	*Proteus*	*	R	*	R	*	S	R	S	R	*	*	S	R	*	*
34	*Proteus*	*	R	*	R	*	S	R	S	R	*	R	S	*	*	*
35	*Proteus*	*	R	*	R	*	S	R	*	S	R	*	*	R	*	*
36	*Proteus*	*	R	R	*	R	S	R	S	S	*	*	*	R	*	*
37	*Proteus*	*	*	R	S	R	*	R	S	*	*	*	R	*	*	R
38	*Proteus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*

39	*Proteus*	*	*	*	*	R	S	R	S	S	*	R	S	*	*	*
40	*Proteus*	*	R	*	*	R	S	R	S	R	*	*	*	R	*	*
41	*Proteus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*
42	*Proteus*	*	R	*	*	S	S	R	R	R	*	*	S	R	*	*
43	*Proteus*	*	R	*	*	R	S	R	S	R	*	*	S	R	*	R
44	*Proteus*	*	R	*	*	R	S	R	S	R	R	*	S	R	*	*
45	*Proteus*	*	R	*	*	R	S	R	R	R	*	*	R	S	*	*
46	*Proteus*	*	R	*	*	R	S	R	R	R	*	*	S	R	*	*
47	*Proteus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*
48	*Proteus*	*	R	R	*	S	S	R	S	R	S	*	R	*	*	*
49	*Proteus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*
50	*Proteus*	*	R	*	*	R	S	R	S	R	*	R	S	*	*	*
51	*Proteus*	*	R	R	*	*	S	R	R	S	*	*	S	R	*	*
52	*Proteus*	*	S	*	*	*	S	R	R	R	*	*	S	S	*	R
53	*Proteus*	*	R	*	*	*	S	R	R	R	*	*	S	*	R	*
54	*Proteus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	R
55	*Proteus*	*	R	*	*	*	S	R	S	S	*	*	S	*	R	R
56	*Proteus*	*	R	*	*	*	S	R	*	S	R	*	S	*	R	R
57	*Proteus*	*	*	*	R	*	S	*	R	*	*	S	S	S	R	R
58	*Proteus*	*	R	*	*	*	S	R	R	S	*	*	S	R	*	R
59	*Proteus*	*	R	*	*	*	S	R	*	S	*	*	S	R	*	R
60	*Proteus*	*	S	*	*	*	S	S	S	R	*	*	*	R	R	R
61	*Proteus*	*	R	*	*	R	S	R	R	S	*	*	*	S	*	R
62	*Proteus*	*	R	*	*	*	S	R	*	R	*	*	R	S	*	R
63	*Proteus*	*	R	*	R	R	S	R	*	R	S	S	*	*	*	*
64	*Proteus*	*	R	*	*	R	S	R	R	R	*	*	S	*	*	R
65	*Proteus*	*	R	*	*	R	S	R	R	R	*	*	S	*	*	R
66	*Pseudomonas*	*	R	*	*	R	S	R	R	S	*	*	R	R	*	*
67	*Pseudomonas*	*	R	*	*	*	S	R	R	R	R	R	*	*	*	R
68	*Pseudomonas*	*	R	*	*	*	S	R	R	R	*	*	*	R	*	R
69	*Pseudomonas*	*	R	*	*	R	S	R	R	S	*	*	*	R	*	*
70	*Pseudomonas*	*	R	S	S	R	*	R	*	*	*	*	*	S	*	*
71	*Pseudomonas*	*	R	R	R	*	S	R	R	R	*	*	*	R	*	*
72	*Pseudomonas*	*	R	R	R	*	S	R	R	R	*	*	*	R	*	*
73	*Pseudomonas*	*	R	*	*	R	S	R	S	R	*	*	*	R	*	*
74	*Pseudomonas*	*	S	*	*	R	S	S	S	S	*	S	S	*	*	*
75	*Pseudomonas*	*	R	*	*	R	S	R	S	R	*	*	S	R	*	*
76	*Pseudomonas*	*	R	*	*	R	S	R	S	R	*	*	S	R	*	*
77	*Pseudomonas*	*	R	*	*	R	S	R	S	R	*	*	S	*	*	*
78	*Pseudomonas*	*	R	*	*	*	S	R	R	*	*	*	S	*	R	*
79	*Pseudomonas*	*	R	*	*	*	S	R	R	R	*	*	S	*	R	R
80	*Pseudomonas*	*	R	*	*	R	S	R	*	R	*	*	*	*	R	R

81	*Pseudomonas*	*	R	*	*	R	S	S	*	R	*	S	S	*	*	*
82	*Pseudomonas*	*	R	*	*	R	S	R	R	*	*	*	*	R	*	R
83	*Pseudomonas*	R	R	*	*	R	S	R	S	R	*	R	*	*	R	*
84	*Pseudomonas*	*	S	*	R	*	S	*	S	R	*	*	*	*	R	R
85	*Pseudomonas*	R	R	*	*	R	S	R	R	R	*	*	*	*	R	*
86	*Pseudomonas*	*	R	*	*	*	S	R	R	*	R	*	R	S	*	R
87	*Pseudomonas*	*	R	*	R	*	S	R	S	S	*	*	*	R	*	R
88	*Pseudomonas*	R	R	*	*	*	S	R	R	R	*	*	S	*	R	R
89	*Pseudomonas*	*	R	*	*	*	S	R	R	R	*	R	S	R	*	*
90	*Pseudomonas*	*	R	*	*	R	R	R	R	R	*	*	*	R	*	*
91	*Pseudomonas*	*	R	*	*	*	S	R	R	S	S	*	S	*	*	R
92	*Pseudomonas*	*	R	*	*	*	S	R	S	R	*	*	S	R	*	R
93	*Pseudomonas*	*	R	*	*	R	S	R	R	R	*	*	*	*	*	R
94	*S.aureus*	*	R	*	*	*	S	R	S	S	S	S	*	*	*	*
95	*S.aureus*	*	R	*	*	S	S	R	S	R	S	S	*	*	S	R
96	*S.aureus*	*	S	*	*	*	S	R	S	R	R	R	*	*	*	R
97	*S.aureus*	*	S	*	*	S	S	R	S	S	R	*	*	*	*	R
98	*S.aureus*	*	R	*	*	*	S	R	S	R	*	*	S	*	S	R
99	*S.aureus*	*	R	*	*	*	S	R	S	S	S	*	S	S	*	*
100	*S.aureus*	*	R	*	S	S	R	S	R	R	R	R	S	R	*	R
101	*S.aureus*	*	R	*	*	S	S	R	R	S	*	*	R	R	*	*
102	*S.aureus*	*	S	*	*	S	S	*	S	S	S	S	S	*	*	*
103	*S.aureus*	*	S	*	*	*	S	R	S	S	R	R	*	*	*	R
104	*S.aureus*	*	S	*	*	*	S	R	*	R	S	S	*	*	*	R
105	*S.aureus*	*	R	*	*	S	S	*	S	R	R	*	R	S	*	*
106	*S.aureus*	*	R	*	*	R	S	*	R	S	S	*	S	S	*	*
107	*S.aureus*	*	R	*	*	R	S	R	R	R	*	*	R	S	*	*
108	*S.aureus*	*	R	*	S	S	R	S	R	R	R	R	S	R	*	R
109	*S.aureus*	*	R	*	*	*	S	S	S	R	R	S	*	*	*	R
110	*S.aureus*	*	S	*	*	R	S	R	S	S	*	*	*	S	*	*
111	*S.aureus*	*	S	*	S	S	S	R	S	S	*	*	*	*	*	*
112	*S.aureus*	*	S	*	*	S	S	R	S	*	*	*	S	S	*	*
113	*S.aureus*	*	R	*	*	S	S	R	*	S	S	S	*	*	*	*
114	*S.aureus*	*	R	*	*	R	S	R	S	S	*	R	*	R	*	*
115	*S.aureus*	*	S	*	*	S	S	*	S	S	*	*	S	S	*	R
116	*S.aureus*	*	S	*	*	R	S	R	S	S	*	*	*	S	*	*
117	*S.aureus*	*	R	*	*	*	S	R	S	R	S	*	S	*	*	*
118	*S.aureus*	*	R	*	*	R	R	*	S	R	*	*	S	S	*	R
119	*S.aureus*	*	R	R	*	S	S	*	*	R	R	S	*	S	*	*
120	*S.aureus*	*	S	*	*	S	S	R	S	S	*	*	R	S	*	*
121	*S.aureus*	*	R	*	R	*	S	R	S	R	*	*	S	S	*	*
122	*S.aureus*	*	R	*	*	R	R	R	S	S	*	*	R	R	*	*

123	*S.aureus*	*	S	*	*	S	S	R	S	S	S	*	S	*	*	*
124	*S.aureus*	*	R	*	*	S	S	R	S	S	*	*	S	S	*	*
125	*S.aureus*	*	R	*	R	S	S	R	S	R	*	*	S	*	*	*
126	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*
127	*S.aureus*	*	S	*	*	S	S	S	S	S	*	*	S	S	*	*
128	*S.aureus*	*	R	*	*	S	S	R	R	R	*	*	R	S	*	*
129	*S.aureus*	*	R	*	*	R	S	R	S	R	*	*	S	S	*	*
130	*S.aureus*	*	R	*	*	S	R	R	S	S	*	*	S	S	*	*
131	*S.aureus*	*	R	*	*	R	R	R	S	R	*	S	S	*	*	*
132	*S.aureus*	*	S	*	*	S	S	R	S	R	*	*	R	S	*	*
133	*S.aureus*	*	R	*	*	S	S	S	R	R	*	*	S	S	*	*
134	*S.aureus*	*	S	*	*	S	S	R	S	R	*	*	S	S	*	*
135	*S.aureus*	*	R	*	*	S	R	R	S	R	*	*	S	R	*	*
136	*S.aureus*	*	R	*	*	R	S	R	R	R	*	*	S	R	*	*
137	*S.aureus*	*	R	*	*	S	S	R	S	S	*	*	R	R	*	*
138	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	S	R	*	*
139	*S.aureus*	*	R	*	*	S	R	R	R	R	*	*	S	R	*	*
140	*S.aureus*	*	R	*	*	S	S	R	*	S	R	*	S	R	*	*
141	*S.aureus*	*	R	*	*	S	S	R	R	S	*	*	S	R	*	*
142	*S.aureus*	*	S	*	S	S	S	R	S	S	*	*	S	*	*	*
143	*S.aureus*	*	R	*	*	R	*	R	*	R	*	*	*	R	*	*
144	*S.aureus*	*	S	*	S	S	S	R	S	S	*	R	*	*	*	*
145	*S.aureus*	*	S	*	*	S	R	R	S	S	*	*	R	S	*	*
146	*S.aureus*	*	R	*	*	S	S	S	S	R	*	*	S	R	*	*
147	*S.aureus*	*	S	*	*	S	R	R	S	R	*	*	S	S	*	*
148	*S.aureus*	*	R	*	*	S	S	R	S	R	*	S	R	*	*	*
149	*S.aureus*	*	R	*	*	*	*	R	*	*	*	*	*	*	*	*
150	*S.aureus*	*	R	S	*	*	S	R	S	S	*	*	R	S	*	*
151	*S.aureus*	*	R	R	*	*	R	R	S	R	*	*	R	R	*	*
152	*S.aureus*	*	S	*	*	S	S	R	R	S	*	*	S	S	*	*
153	*S.aureus*	R	S	*	*	S	S	R	S	*	*	*	S	S	*	*
154	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	S	S	*	*
155	*S.aureus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	*
156	*S.aureus*	*	R	*	*	*	S	R	S	S	*	*	S	R	*	R
157	*S.aureus*	S	S	*	*	S	R	R	S	*	*	*	S	R	*	*
158	*S.aureus*	*	R	*	*	R	S	*	S	*	S	R	*	R	*	*
159	*S.aureus*	*	R	*	*	*	S	R	S	R	*	*	R	R	*	S
160	*S.aureus*	*	R	*	*	*	R	R	R	R	*	*	S	*	*	R
161	*S.aureus*	*	S	*	*	*	S	R	R	S	*	*	S	*	*	R
162	*S.aureus*	*	R	*	*	S	S	R	R	R	*	*	*	*	S	R
163	*S.aureus*	*	S	*	*	S	S	R	S	R	*	*	*	*	S	R
164	*S.aureus*	*	S	*	R	S	S	R	R	*	R	*	*	S	*	R

165	*S.aureus*	*	R	*	*	R	S	*	R	R	*	*	S	*	*	R
166	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	S	R	*	R
167	*S.aureus*	*	R	*	*	S	S	R	R	R	*	*	*	S	*	R
168	*S.aureus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	R
169	*S.aureus*	*	R	*	*	*	S	R	R	R	*	*	S	*	R	R
170	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	*	R	*	*
171	*S.aureus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	R
172	*S.aureus*	*	S	*	S	*	S	R	S	S	*	*	R	S	*	*
173	*S.aureus*	*	S	*	*	*	S	R	S	S	*	S	S	R	*	R
174	*S.aureus*	*	R	*	*	S	S	R	S	S	*	*	*	R	*	R
175	*S.aureus*	*	S	*	*	*	S	R	S	S	*	*	R	S	*	R
176	*S.aureus*	S	S	*	*	R	S	R	*	R	*	R	*	*	S	*
177	*S.aureus*	R	S	*	*	R	S	*	S	*	S	*	*	*	S	S
178	*S.aureus*	S	S	*	*	*	S	S	S	S	S	R	*	*	S	*
179	*S.aureus*	R	S	*	*	*	S	R	S	S	*	*	*	*	S	*
180	*S.aureus*	*	S	*	*	*	S	S	S	S	*	S	*	*	S	R
181	*S.aureus*	*	S	*	*	*	S	R	S	S	*	*	S	*	R	R
182	*S.aureus*	*	S	*	*	*	S	S	S	S	*	*	S	*	*	R
183	*S.aureus*	*	S	*	*	*	S	S	S	S	*	*	*	S	S	R
184	*S.aureus*	*	S	*	*	*	S	S	S	S	*	*	*	S	S	R
185	*S.aureus*	*	S	*	*	*	S	R	S	S	*	*	*	S	S	R
186	*S.aureus*	*	S	*	R	*	S	R	S	S	*	*	*	*	R	R
187	*S.aureus*	R	S	*	*	*	S	R	S	S	*	*	*	*	*	R
188	*S.aureus*	S	*	S	*	S	*	*	S	S	*	S	*	R	*	S
189	*S.aureus*	*	R	*	*	*	S	R	S	R	*	R	*	R	*	R
190	*S.aureus*	*	R	*	*	*	S	R	S	S	*	*	*	R	S	R
191	*S.aureus*	*	R	*	*	*	S	R	S	S	*	*	*	*	R	R
192	*S.aureus*	*	*	*	S	*	S	R	S	R	*	*	R	S	*	R
193	*S.aureus*	*	R	*	*	*	S	S	S	R	*	*	S	R	*	R
194	*S.aureus*	*	S	*	*	*	S	R	S	S	*	*	S	S	*	R
195	*S.aureus*	*	S	*	*	*	R	R	R	S	*	*	R	S	*	R
196	*S.aureus*	*	R	*	*	*	S	S	S	R	*	*	S	R	*	R
197	*S.aureus*	*	S	*	*	*	S	R	S	R	*	S	*	R	*	*
198	*S.aureus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	R
199	*S.aureus*	*	R	*	*	*	S	R	S	S	*	*	*	R	R	R
200	*S.aureus*	*	S	*	R	*	S	R	S	S	*	R	*	R	R	*
201	*S.aureus*	*	R	*	*	*	S	R	S	R	*	*	*	R	R	R
202	*S.aureus*	*	S	*	*	*	R	R	R	S	*	*	R	S	*	R
203	*S.aureus*	*	R	*	*	*	S	R	S	S	*	*	*	R	R	R
204	*S.aureus*	*	S	*	*	*	S	R	R	S	*	*	*	R	R	R
205	*S.aureus*	*	R	*	*	*	S	R	R	*	*	R	S	R	*	R
206	*S.aureus*	*	S	*	*	S	S	R	S	S	*	*	*	*	S	*

207	*S.aureus*	*	S	*	*	*	S	R	*	S	*	*	*	S	R	*
208	*S.aureus*	*	R	*	*	S	S	*	R	S	R	*	R	*	*	*
209	*S.aureus*	*	S	*	*	S	S	S	S	*	*	*	R	S	*	R
210	*S.aureus*	*	S	*	*	S	S	R	S	S	*	*	S	*	*	R
211	*S.aureus*	*	R	*	*	*	S	S	S	R	*	R	S	R	*	*
212	*S.aureus*	*	S	*	R	S	S	R	S	R	*	*	*	*	*	R
213	*S.aureus*	*	S	*	*	R	S	R	S	R	*	R	*	R	*	*
214	*S.aureus*	*	S	*	*	R	S	R	S	R	*	*	*	S	*	R
215	*S.aureus*	*	S	*	*	*	S	R	R	S	*	*	S	R	*	R
216	*S.aureus*	*	R	*	*	*	S	R	S	R	*	R	S	*	S	*
217	*S.aureus*	R	S	*	*	*	S	R	S	R	*	*	S	*	S	*
218	*S.aureus*	*	R	*	*	*	S	R	S	R	*	*	S	*	*	R
219	*S.aureus*	*	S	*	*	*	S	R	R	S	*	*	R	S	*	R
220	*S.aureus*	*	S	*	*	S	S	R	*	*	*	*	S	R	*	R
221	*S.aureus*	*	R	*	*	S	S	R	S	R	*	*	*	R	*	R
222	*S.aureus*	*	S	*	*	*	S	R	S	S	*	R	*	*	R	*
223	*S.aureus*	*	R	*	*	*	S	R	S	R	*	*	R	S	*	R
224	*Staphylococcus*	*	R	*	S	S	S	*	*	S	S	S	S	*	*	*
225	*Staphylococcus*	*	S	*	*	S	S	R	S	R	*	*	S	S	*	*
226	*Staphylococcus*	*	R	*	*	*	S	R	R	R	*	*	S	R	*	*
227	*Staphylococcus*	*	S	*	*	*	S	R	R	S	*	*	R	S	*	R
228	*Staphylococcus*	*	R	*	*	*	S	R	S	S	*	*	S	*	R	R
229	*Staphylococcus*	R	R	*	*	*	S	R	*	R	*	*	S	*	R	*
230	*Staphylococcus*	*	S	*	R	*	S	*	S	R	*	S	*	*	R	R
231	*Staphylococcus*	*	S	*	*	*	S	R	S	R	*	R	R	*	R	*
232	*Staphylococcus*	*	R	*	*	*	S	R	R	S	*	*	R	*	R	R
233	*Staphylococcus*	*	R	*	R	*	S	R	S	S	*	*	*	R	*	R
234	*Staphylococcus*	*	S	*	*	*	S	R	R	R	*	*	S	*	S	R
235	*Staphylococcus*	*	R	*	*	S	S	*	*	R	*	*	S	*	S	R
236	*Staphylococcus*	*	S	*	*	*	S	S	*	R	*	R	S	*	*	*
237	*Staphylococcus*	*	R	*	*	S	S	R	*	S	*	R	*	R	*	*
238	*Staphylococcus*	*	R	*	*	*	S	R	S	S	*	*	*	R	*	R
239	*Staphylococcus*	*	S	*	*	*	S	R	S	S	*	*	*	*	*	R
240	*Staphylococcus*	*	R	*	*	S	S	R	S	S	*	*	*	R	*	R
241	*Staphylococcus*	*	R	*	R	*	S	R	*	R	R	*	*	*	*	R
242	*Staphylococcus*	*	R	*	*	R	S	S	S	R	*	*	*	R	*	S
243	*Staphylococcus*	*	R	*	*	*	S	R	*	R	S	R	S	*	*	*
244	*Staphylococcus*	R	R	*	*	*	S	R	R	S	*	*	S	*	S	R
245	*Staphylococcus*	R	R	*	*	*	S	R	S	S	*	R	S	*	R	*
246	*Streptococcus*	*	R	*	*	*	S	R	*	R	R	R	*	*	*	R
247	*Streptococcus*	*	R	*	*	S	R	R	S	S	R	*	S	S	*	*
248	*Streptococcus*	*	R	*	*	S	S	*	S	R	*	*	S	R	*	R

249	*Streptococcus*	*	S	S	*	S	R	S	S	S	*	*	*	S	*	*
250	*Streptococcus*	*	S	R	*	S	S	R	S	R	*	*	S	S	*	*
251	*Streptococcus*	*	R	*	*	S	R	R	S	R	*	*	S	R	*	*
252	*Streptococcus*	*	R	*	*	R	S	R	S	S	*	S	R	*	*	*
253	*Streptococcus*	*	S	R	*	*	R	S	S	R	*	*	S	S	*	*
254	*Streptococcus*	*	S	*	*	*	R	S	S	S	*	*	S	*	*	*
255	*Streptococcus*	*	R	*	*	*	S	R	*	R	S	S	*	R	*	R
256	*Streptococcus*	*	R	*	*	*	S	*	S	R	*	*	S	R	*	R
257	*Streptococcus*	*	R	*	*	*	S	S	S	S	*	*	S	R	*	R
258	*Streptococcus*	*	S	*	*	*	S	R	S	R	*	*	*	S	R	R
259	*Streptococcus*	*	R	*	R	R	S	R	*	R	*	S	S	*	*	*
260	*Streptococcus*	*	S	*	*	S	S	R	S	R	*	*	*	S	*	R
261	*Streptococcus*	*	R	*	*	*	S	R	R	R	*	*	S	S	*	R
262	*Streptococcus*	*	R	*	*	*	S	S	S	R	*	*	S	R	*	R

KEY: Pen: Penicillin, Amp: Ampicillin, Ery: Erythromicin, Apc: Ampicillin/cloxacillin, Cef: Cefaclor,Gen: Gentamicin, Cot: Cotrimoxazole, Chl: Chrolamphenicol, Tet: Tetracycline, Kan: Kanamycin, Str: Streptomycin, Nor: Norfloxacin, Acv: Amoxillin/clavulanic acid, Axy: Amoxicillin, Sul: Sulfamethoxazole; Strep: *Streptococcus* spp., *S. aureus*: *Staphylococcus aureus*, Staph: Other *Staphylococcus* spp., *E. coli*: *Escherichia coli*, R: Resistant, S: Susceptible,

*Not tested against that antimicrobial agent.

Appendix 3: Sample collection form

Sample Collection Form For dogs

Section A

Date: ……..

Name: ……

Breed: ……

Owner's Name: ……

Lab I.D:……..

Case No: …….

Age: ……

Sex: ……

Section B

Tick the Appropriate answer

1. Presenting Complaint
   a) Wound
      Yes ☐ No ☐
   b) If yes to (a) above, how long has the wound been there
      <1wk ☐
      >1wk ☐
      N/A ☐
2. Has the dog been treated with antimicrobials/antibiotics
   Yes ☐ No ☐
3. If yes to (2), when was the last treatment administered
   Past one week ☐
   Past one month ☐
   3months ago ☐

Place of Residence……

Section C: TO BE FILLED BY THE ATTENDING VETERINARIAN

Reason for Visit to clinic

Vaccination ☐

Routine Check-up ☐

Clinical Case ☐

Surgical Case ☐

Physical Examination Findings

Diagnosis

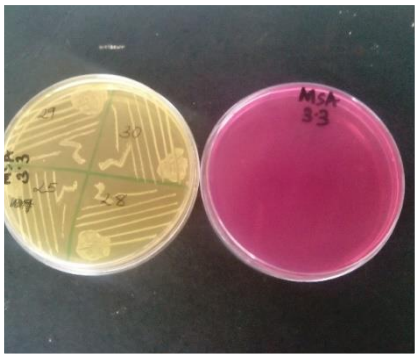
Attending Clinician

Name……..

Signature……

Appendix 4: Tests for Differentiation of Staphylococcus spp.

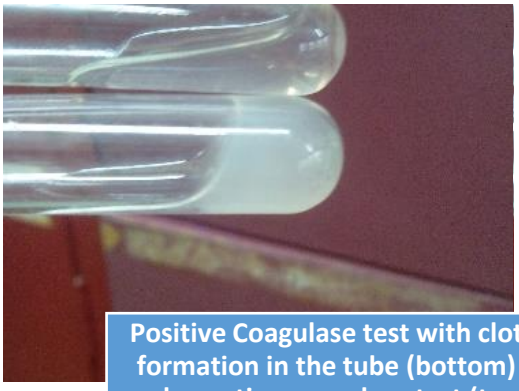
Figure 4*:* Appearance of presumptive *Staphylococcus aureus* isolates on mannitol salt agar



6 A: Growth of Staph on MSA(left) showing fermentation of mannitol (colour change to yellow) and right plate showing MSA agar prior to incubation

6 B : Standard organism on MSA ATCC 25923

Figure 5: Coagulase test



Positive Coagulase test with clot formation in the tube (bottom) and negative coagulase test (top)

Appendix 5: Oxacillin disk diffusion antimicrobial susceptibility testing zone diameter readings for presumptive coagulase positive Staphylococcus isolates.

Sample No.	ZOIA	ZOIB	Score
52	**6**	**6**	**R**
58	21	22	S
59	23	27	S
65	**6**	**6**	**R**
71	**6**	**6**	**R**
79	19	19	S
82	**6**	**6**	**R**
83	**6**	**6**	**R**
86	**6**	**6**	**R**
89	**6**	**6**	**R**
100	**6**	**6**	**R**
147	23	22	S
148	**6**	**6**	**R**
149	19	19	S
154	23	23	S
155	25	25	S
162	19	19	S
166A	25	26	S
167B	19	20	S
174	19	21	S
183	21	22	S
186A	**11**	**10**	**R**
186B	**11**	**11**	**R**
187	**15**	**15**	**R**
188	**14**	**15**	**R**

Key: ZOI- Zone of Inhibition

S- Susceptible I- Intermediate R- Resistant

Appendix 6: Nucleotide and amino acid sequences of the resistant genes

Sample No. 1 I.D: CS 100 Gene: *mecA* Primers: *mecA* F/ *mecA* R

Nucleotide sequence

TTCCGGTTATTTTATAACTTGTTTTGTCGTCTAATGTCTTATTATTTAAGCCAATCA
TAGCTGTTAATATTTTTTGAGTAGAACCTGGTGATGTTGTGATTTGGAACTTATTA
AGAAGTGGCTCTTTATCATCTTCCGTTAATTTCTTATAATCTTCATCACTCATACC
ATTCATAAATGGATAAATATCATAAGATGGTGTGCTGACAAGTGCTAACAATTCG
CCTGTTTGAGGGTGGATAGCAAAACTTGTCAGCACACATCTTATGATATTTATCC
ATTTATGAATGGTATGAGTGATGAAGATTATAAGAAATTAACGGAAGATGATAA
AGAGCCACTTCTTAATAAGTTCCAAATCACAACATCACCGGTTCTACTCAAAAAT
ATTAACAGCTATGATTGGCTTAATAATAAACATTACACGACTTATACAGTTATAA
AATTAACGGAAAAGCTGGCAAAAGATAAATCTGGGGGTGGTTACCACCTTTA

Amino acid sequence

TSMTSIHLCMAVTKNIINPKIKKNLCSTSSRLQLHQVQLKKYQQLGITKHTIKQVIKS
MVKVGKKINLGVVTTL

Sample No. 2 I.D.: CS 148 Gene: *mecA* Primers: *mecA* F/ *mecA* R

Nucleotide Sequence

CACGCTTTACCTCGATTTTATAACTTGTTTTATCGTCTAATGTTTTGTTATTTAACC
CAATCATTGCTGTTAATATTTTTTGAGTTGAACCTGGTGAAGTTGTAATCTGGAAC
TTGTTGAGCAGAGGTTCTTTTTTATCTTCGGTTAATTTATTATATTCTTCGTTACTC
ATGCCATACATAAATGGATAGACGTCATATGAAGGTGTGCTTACAAGTGCTAATA
ATTCACCTGTTTGAGGGTGGATAGCAACA

Amino acid sequence

LLSTLKQVNYHLAHLHMTSIHLCMAVTKNIINPKIKKNLCSTSSRLQLHQVQLKKYQ
QLGITKHTIKQVIKSRSV

Sample submitted to GenBank sequencing

Nucleotide Sequence: CS100 Primers: $mecA_2$ F/ $mecA_2$ R Size: 886bp

AWGCATTAGGCGTTAAGATATAAACATTCAGGATCGTAAAATAAAAAAAGTATC
TAAAAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGTAA
CATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTA
GATTGGGATCATAGCGTCATTATTCCAGGAATGCAGAAAGACCAAAGCATACAT
ATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAA
TTGGCCAATACAGGAACAGCATATGAGATAGGCATCGTTCCAAAGAATGTATCT
AAAAAAGATTATAAAGCAATCGCTAAAGAACTAAGTATTTCTGAAGACTATATC
AAACAACAAATGGATCAAAATTGGGTACAAGATGATACCTTCGTTCCACTTAAA
ACCGTTAAAAAAATGGATGAATATTTAAGTGATTTCGCAAAAAAATTTCATCTTA
CAACTAATGAAACAGAAAGTCGTAACTATCCTCTAGAAAAAGCGACTTCACATC
TATTAGGTTATGTTGGTCCCATTAACTCTGAAGAATTAAAACAAAAAGAATATAA
AGGCTATAAAGATGATGCAGTTATTGGTAAAAAGGGACTCGAAAAACTTTACGA
TAAAAAGCTCCAACATGAAGATGGCTATCGTGTCACAATCGTTGACGATAATAG
CAATACAATCGCACATACATTAATAGAGAAAAAGAAAAAAGATGGCAAAGATA
TTCAACTAACTATTGATGCTAAAGTTCAAAAGAGTATTTATAACAACATGAAAAA
TGATTATGGCTCAGGTACTGCTATCCACCCTCCAAACAGGTGA