12-2019

# An Epidemiologic Study of Antimicrobial Resistance

Ronita Samuels
*University of Tennessee*, pkc657@vols.utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Ronita Samuels entitled "An Epidemiologic Study of Antimicrobial Resistance." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

Agricola Odoi, Major Professor

We have read this dissertation and recommend its acceptance:

Stephen Kania, Russell Zaretzki, Robert Donnell

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

# An Epidemiologic Study of Antimicrobial Resistance

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Ronita Alysha-Val Samuels

December 2019

# Dedication

This dissertation is dedicated to my son, Grayson Samuels, whose presence, light and love have inspired me daily in my pursuit of this degree.

# Acknowledgements

I sincerely thank and acknowledge everyone who has helped and encouraged me to complete this dissertation. I would like to thank my advisor, Professor Agricola Odoi, for his dedication, expertise, and continual encouragement throughout my time at UT. I am extremely grateful to the other members of my committee Professors Stephan Kania, Russell Zaretzki, and Robert Donnell for their willingness to serve on my committee and for their academic guidance. A special thank you to Dr. David Bemis who served on my committee faithfully until his passing. Thanks are also due to Craig Carter, Jackie Smith and the team at the University of Kentucky Veterinary Diagnostic Laboratory for providing a portion of the data used in this study. I would like to acknowledge Dr. Nenene Qekwana and Dr. James Oguttu for their time and efforts with helping me to obtain survey data in South Africa. To my mother, Ronnie Wagoner, my sisters Tamara Hicks and Marysha Adams, my best friend Sheena Young and all of my other family and friends, thank you for your constant encouragement, prayers. and support.

## Abstract

The emergence of antimicrobial resistant bacteria has become a concern in veterinary medicine. In addition to this problem, appropriate usage of antimicrobials is a concern in the U.S. as well as in developing countries such as South Africa. The objectives of this study were 1) to investigate the burden and patterns of antimicrobial resistance (AMR) among equine *Staphylococcus* samples submitted to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL); 2) to investigate the opinions, knowledge and perceptions of veterinarians in Kentucky regarding AMR and antimicrobial prescription practices; and 3) to identify predictors of their knowledge and opinions; 4) to investigates the knowledge, prescription practices and attitudes towards AMR among veterinarians in the City of Tshwane, Metropolitan Municipality; and 5) to identify predictors of their knowledge and attitudes. In study 1, the proportion of resistant isolates by animal breed, species of organism, sample source, and time period were computed. Chi-square and Cochran-Armitage trend tests were used to identify significant associations and temporal trends, respectively. Logistic regression models were used to investigate predictors of AMR and multidrug resistance (MDR). In studies 2 and 3, a 30-question survey was administered to members of the Kentucky Veterinary Medical Association (KVMA) and among veterinarians in the City of Tshwane, Metropolitan Municipality. The proportion of responses to survey questions and 95% confidence intervals were computed. Predictors knowledge of antimicrobial resistance and antimicrobial prescription practices of respondents as well as their colleagues were investigated using Ordinary logistic models and multinomial logistic regression models.Study 1 found significant ($p<0.05$) associations between odds of AMR and

horse breed, species of organism and year. Similarly, significant ($p<0.05$) associations were identified between odds of MDR and breed and age. Study 2 observed no significant associations among any of the predictors. However, in study 3 veterinarians in mixed animal practice had significantly lower odds (OR=0.20; $p=0.0103$) of associating “improper use of antimicrobials” to “selection for AMR” compared to those in small animal practice. Compared to females, males were significantly more likely (Relative Risk Ratio [RRR]=10.5; $p=0.002$) to indicate that their colleagues over-prescribed antimicrobials rather than to “neither agree nor disagree” or “disagree.”

# Table of Contents

# List of Tables

# List of Figures

## List of Abbreviations

AHCF: American Horse Council Foundation

AMR: Antimicrobial Resistance

ASP: Antimicrobial stewardship programs

AVMA: American Veterinary Medical Association

BVA: British Veterinary Association

CA-MRSA: Community acquired methicillin-resistant Staphylococcus aureus

CDC: Centers for Disease Control and Prevention

CI: Confidence Interval

CLSI: Clinical Laboratory Standards Institute

CNA: colistin and nalidixic acid

CoNS: Coagulase negative staphylococci

EIP: Emerging Infections Program

E-Test: Epsilometer test

FDA - U.S. Food and Drug Administration

GLASS: Global Antimicrobial Resistance Surveillance System

HAIC: Healthcare-Associated Infections Community Interface

HA-MRSA: Hospital acquired methicillin-resistant Staphylococcus aureus

HGT: Horizontal gene transfer

IDSA: Infection Diseases Society of America

IR: Interquartile range

KVMA: Kentucky Veterinary Medical Association

MDR:  MRSA: Multidrug Resistance

MDR: Multi-drug resistant

MRS: Methicillin-resistant staphylococci

MRSP: Methicillin resistant *Staphylococcus pseudintermedius*

NARMS: National Antimicrobial Resistance Monitoring System for Enteric Bacteria

NCCLS: National Committee for Clinical Laboratory Standards

OIE: Office International des Épizooties

OR: Odds Ratio

PBP: Penicillin-binding protein

RRR: Relative Risk Ratio

SANVAD: South African National Veterinary Surveillance and Monitoring Program for Resistance to Antimicrobial Drugs

SAS: Statistical Analysis System

SHEA: Society of Healthcare Epidemiology of America

SMART: Study for Monitoring Antimicrobial Resistance Trends

U.K.: United Kingdom

UC: University of California

UKVDL: University of Kentucky Veterinary Diagnostic Laboratory

US: United States

USDA: United States Department of Agriculture

USDA: United States Department of Agriculture

VAs: Veterinary antibiotics

VFD: Veterinary Feed Directive

VMTH: Veterinary Medical Teaching Hospital

WHO: World Health Organization

# 1 Introduction

The discovery of antimicrobial agents not only revolutionized how medical professionals were able to treat infections, but is often hailed as one of the major breakthroughs in the 20th century [1]. However, with the emergence of antimicrobial resistant pathogenic bacteria, compounded by the limited number of new antibiotics entering the market, antimicrobial resistance (AMR) has become a major global health concern [2]. Antimicrobial resistance impedes effective prevention and treatment options for a variety of infections caused by bacteria, parasites, viruses and fungi [3]. A combination of factors including injudicious use of antibiotics in both human and veterinary medicine, agriculture and aquaculture [4-7], and poor infection-control practices have led to the continuing development of AMR problems worldwide [8].

In the United States alone, cost due to antibiotic-resistant infections is estimated to be between $21 billion and $34 billion [9-12]. The infections also extend hospital stays by more than 8 million days [9-12] per year. Overall per patient costs due to AMR infections differed globally. Studies have found that depending on the socioeconomic factors in a country, health care costs in patients can range from more than $10,000 in Thailand and Colombia to more than $35,000 in Turkey or less than $1,000 USD in Senegal [13] depending on length of stay. Antimicrobial resistant infections and the resulting treatment options can also have a substantial economic impact in veterinary medicine [14]. Treatment of a dog in Switzerland can amount to 176,000 Swedish crowns (around US $25,600) for a resistant infection [15]. Although the costs in the U.S.

are estimated to be much lower, similar treatment would still  be approximately $1,500–4,800 [14]. Even more concerning is AMR in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, better but more expensive agents [16].

The evolution of resistance to antimicrobials has rendered many antibiotics largely ineffective, and if replacements are not found, problems due to antimicrobial resistance will persists [17]. Resistant *Staphylococcus* infections have become major causes of concern in not only humans but in companion animals as well. More specifically, Methicillin-resistant staphylococci (MRS) have become serious emerging conditions in equine hospitals [18]. The first cases of methicillin resistant *Staphylococcus* (MRSA) in horses in a veterinary hospital in the United States was in 1999, and was soon followed by reports of MRSA infections in equine hospitals in Canada and in Central Europe only a few years later [19].

Misuse of antimicrobials is one factor that has contributed to the selection for antimicrobial resistance in both humans and animals [20]. Many antimicrobials are used in animals for therapy, prophylaxis and metaphylaxis in many countries including South Africa. Both the reported levels of antimicrobial resistance and the antimicrobial prescription practices of medical and veterinary practitioners vary among countries in Africa [21]. Inappropriate antimicrobial prescription practices among veterinarians and physicians is a contributing factor to this issue [22]. In the United States (U.S.), 80% of the of antimicrobial agents sold in 2012 were for animal use [23]. Additionally, in the

United States, the use of antibiotics in the production of food animals to enhance animal growth has been identified as contributing to antimicrobial resistance. Although antimicrobials are commonly used in the U.S. in veterinary practice, and veterinarians are concerned about antimicrobial resistance [24, 25], a lack of studies investigating veterinarians' opinions regarding antimicrobial prescription practices and usage [26] exacerbates the problem.

Appropriate use of antimicrobials is a controversial topic where opinions vary greatly amongst veterinarians in South Africa and the U.S. With the rate of counterfeiting of pharmaceuticals being so problematic, it is estimated that one in five medications being sold in South Africa, including antibiotics, are believed to be counterfeit [27]. In some cases, policies and procedures for antimicrobial prescriptions are not followed. Moreover, animal owners sometimes acquire antimicrobials without veterinary prescription or use leftover prescriptions [26]. Amidst the rising need for antimicrobial stewardship in the U.S. and judicious use practices, in 2015, a veterinary feed directive was adopted by the U.S. federal government prohibiting non-therapeutic uses of antibiotics in food animals this is expected to reduce unnecessary usage [28]. Understanding the roles that veterinary opinions and clinic policies play in prescription practices is crucial. Additionally, research into the epidemiology of resistant infections is important to guide efforts to combat the problem.

Although extensive research on the epidemiology of resistant *Staphylococcus* infections has been done in humans, far less research has been in horses [29], and yet bacterial infections present major challenges in equine medicine [30]. There are increasing reports of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and colonization in horses [31]. While MRSA has a low prevalence of nasal carriage in horses in the community, it is much higher for hospitalized horses [32].

The horse industry in Kentucky contributes $3 billion and approximately 40,000 jobs to the U.S. economy [33], while the horse industry in South Africa contributes $226 million (approximately 13%) annually to the South African GDP [34]. This makes understanding the epidemiology of infections in horses and the antimicrobial prescription practices of the veterinarians who may treat them critical. Therefore this study was designed to investigate the epidemiology of AMR in *Staphylococcus* infections in horses as well as the prescription practices of veterinarians in Kentucky and South Africa. The specific objectives were to: (i) estimate the proportion of antimicrobial resistant staphylococcal isolates among equine samples in Kentucky; (ii) evaluate the opinions of veterinarians in Kentucky and South Africa regarding antibiotic prescription practices and antimicrobial resistance and to identify predictors of their opinions. I hypothesized that not only would AMR be high among horses in Kentucky, but that although veterinarians in Kentucky and South Africa would be aware of AMR they would still report overprescribing antimicrobials. Understanding the prevalence of resistant *Staphylococcus* infections in companion animals (specifically horses) and the antimicrobial prescription practices of veterinarians are critical in guiding the development of better antimicrobial prescription policies and increasing education of both practitioners and animal owners on judicious use of antimicrobials.

This is a five chapter dissertation. The first chapter includes the introduction to the study while the second chapter contains the literature review. Chapter 3 investigates the problem of antimicrobial resistance in horses, while chapters 4 and 5 describe antimicrobial prescription practices and knowledge of AMR in veterinarians in Kentucky and South Africa. Lastly, chapter 6 summarizes key findings, provides some recommendations and conclusions.

# 2 Literature review

## 2.1 *STAPHYLOCOCCUS* INFECTIONS

### 2.1.1 Etiology

*Staphylococcus* is a genus of gram positive bacteria that takes the shape of round cocci that aggregate into cluster like formations [35]. They are facultative anaerobes that are 1μm in diameter, non-motile and non-spore forming [36, 37]. Most species of *Staphylococcus* are harmless, and can normally be found residing on the skin and in the mucous membranes of both humans and animals but they can cause opportunistic infections [38]. The genus *Staphylococcus* consists of 47 species and 23 subspecies [39]. One of the most important criteria considered when classifying *Staphylococcus* bacteria is the ability to produce coagulase. Coagulase-positive staphylococci are common commensal microorganisms and opportunistic pathogens in humans and animals [40]. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA), an important cause of nosocomial and community-associated infections in humans, has become increasingly recognized as a pathogen in companion animals [40-43]. There are several species of coagulase-positive staphylococci: *S. aureus, S. delphini, S. hyicus, S. intermedius, S. lutrae, S. pseudintermedius and S. schleiferi*. Certain staphylococci are important in both canine and feline health, including *S. intermedius, S. schleiferi subsp. coagulans, and S. pseudintermedius* [40, 44-46]. Although these staphylococi have been identified as commensal organisms, they can still cause disease such as pyoderma and otitis externa in both dogs and cats [40, 44-46]. Coagulase-negative staphylococci (CoNS) are part of the normal flora of the skin and have relatively low virulence but are increasingly being recognized as agents of clinically significant infections of the bloodstream and other sites [47]. Coagulase negative staphylococci are unable to produce coagulase and coagulate rabbit plasma. There are more than 40 recognized species of coagulase-negative staphylococci (CoNS). Species of CoNS that are most frequently associated with clinical disease are *S. epidermidis, S. lugdunensis, S. saprophyticus* and *S. haemolyticus.* Today, CoNS, as typical opportunists, represent one of the major nosocomial pathogens, having a substantial impact on human and animal health. They are particularly associated with the use of indwelling or implanted medical devices (e.g. catheters, sutures, prosthetic material, etc ), which are indispensable in modern medicine. Colonization of different parts of the skin and mucous membranes of the host is the key source of endogenous infections by CoNS *[39].*

Of the over 40 species of *Staphylococcus, Staphylococcus aureus* has become important in both animal and human health. *Staphylococcus aureus* are recognized as one of the most important pathogenic *Staphylococcus* species in veterinary medicine [48]. It is a frequent cause of serious, chronic and therapy-refractive infections in spite of susceptibility to antibiotics *in vitro* [49]. A wide variety of infections can be caused by *S. aureus*. These range from superficial skin and soft tissue infections such as boils or styes (inflammation of the eye or eyelid), to life-threatening septicemia, osteomyelitis, pneumonia and endocarditis [50]. *Staphylococcus aureus* can also cause food poisoning by releasing enterotoxins into food [51, 52] as well as causing toxic shock syndrome by releasing antigens into the blood stream [53-55].

*Staphylococcus epidermidis* is the most common source of infections on indwelling medical devices [56] and is a major cause of concern in hospital or clinic settings [57-59]. It is now the most frequent cause of nosocomial infections in humans, at a rate about as high as that of S. aureus [56]. Overall, *Staphylococcus aureus* and *Staphylococcus epidermidis* are common commensals and also have the greatest pathogenic potential (42, 43). *Staphylococcus saprophiticus* not only causes urinary tract infections and cystitis in young girls [60-63] and is second only to *E. coli* as the most frequent causative organism of uncomplicated UTI in women [63], but is also a contaminant of foods of animal origin [64] particularly foods of cattle and pig origin. There are many other species of staphylococci (*S lugdunensis, S haemolyticus, S warneri, S schleiferi, S intermedius*) that are infrequent pathogens [65].

In veterinary medicine, the most important *Staphylococcus* species are *S. aureus, S. hyicus, and S. intermedius [66, 67]* depending on the species of animal. These species are common and important causes of diseases, including abscesses, dermatitis, food poisoning, and wound infections [66]. In horses, *Staphylococcus hyicus* is often found in skin lesions or cases of dermatitis, characterized by epidermolysis, alopecia and crust formation [68]. In pigs, *Staphylococcus hyicus* causes exudative epidermitis also known as greasy pig disease, which is a relatively rare infection of the skin characterized by greasy peeling skin and blister formation [69, 70]. Its acute form can rapidly lead to the dehydration and death of suckling pigs. Furthermore, *Staphylococcus hyicus* has been isolated from cattle with septic polyarthritis and bovine mastitis [69]. *Staphylococcus intermedius* was first described in 1976 as a coagulase-positive *Staphylococcus*. It has since been identified as part of normal skin and mucosal flora in a variety of animals, including horses, dogs, cats, pigeons, minks, foxes, raccoons, goats, and gray squirrels [71]. *Staphylococcus* pseudintermedius causes infections in horses and other companion animals [72] and is one of the most common *Staphylococcus* infections in dogs.

### 2.1.2 Clinical signs

Staphylococci species can cause many forms of infection, however, skin infections are the most common. They are often associated with skin infections in many animals including horses, sheep, cattle, and dogs [73]. Bacterial folliculitis is one of the commonest causes of focal hair loss in the horse [74]. It is usually caused by coagulase positive *Staphylococcus* species. Clinical signs often include crusts, epidermal collarettes, or encrusted papules [75]. In horses, folliculitis often develops in the saddle and lumbar region, particularly in the summer, where the affected area may appear swollen and sensitive; this is followed by formation of follicular papules and pustules [75]. *Staphylococcus aureus* causes superficial skin lesions (boils, styes) and localized abscesses in other sites*. S. aureus* also has the potential to cause deep-seated infections, and is a major cause of hospital acquired (nosocomial) infection of surgical wounds. A study by Tenhagen et. al., found that *Staphylococcus aureus* represents nearly 10% to 12% of all clinical mastitis infections in cows [76].

### 2.1.3 Diagnosis

Diagnosis is made by identifying cocci on impression smears or by bacterial culture or histopathology [75]. In horses, there are a variety of outcomes resulting from *Staphylococcus* infections including skin lesions and wounds, sepsis, respiratory tract infections, and genital tract infections [77]. These conditions are diagnosed through standard methods such as catalase, coagulase, and commercial gallery testing [77], where *Staphylococcus* colonies are identified, based on morphological features, Gram stain, fermentation of maltose, polymixin B susceptibility, and results to the catalase and tube coagulase tests [78]. The coagulase test detects bound coagulase or coagulation of rabbit plasma when *Staphylococcus* bacteria is introduced and is used to differentiate coagulase negative Staphylococcus (CONS) from coagulase positive [79]. Polymyxin B, a gram-negative antibiotic, is used to identify the species of *Staphylococcus* because resistance to it is often seen in *Staphylococcus aureus* [80].

### 2.1.4 Treatment

When treating animals for confirmed Staphylococcus infections, veterinarians consider the nature of the disease in each patient to determine the best mode of therapy. For instance, in horses bacterial folliculitis caused by *Staphylococcus* infection is treated using trimethoprim sulfamethoxazole orally [75]. Antibiotics may be required in the treatment of severe cases of staphylococcal dermatitis in sheep, however, oxytetracycline and enrofloxacin have been shown to be minimally effective, whereas a lincomycin-spectinomycin combination or simply penicillin were both reported to be more effective [81]. Facial staphylococcal dermatitis will resolve within a couple of months. Another approach to treatment consists of washing the affected skin with an iodophor or chlorhexidine shampoo, followed by drying and then coating it with an antiseptic or antibiotic ointment [81]. In cows, with clinical mastitis intramammary infusion of antibiotic are used. Antimicrobial susceptibility determined *in vitro* is a prerequisite for treatment. Cure rates for mastitis caused by penicillin-resistant strains of *S. aureus* seem to be lower than those of mastitis due to penicillin-susceptible strains [82]. This may be due to the fact that *S. aureus* infections can be notoriously hard to treat in cows and infected cows must be segregated or culled [83]. Treatment of superficial pyoderma in dogs has traditionally been treated using oral clavulanate-amoxicillin, oral clavulanate-amoxicillin, clindamycin, cefadroxil, trimethoprim-sulphamethoxazole and sulfadimethoxine-ormetoprim in superficial pyoderma [84]. However, topical therapy with chlorhexidine has been reported to resolve all clinical signs of superficial pyoderma in dogs [85].

### 2.1.5 Epidemiology of *Staphylococcus* infections

#### 2.1.5.1 Prevalence

The prevalence of *Staphylococcus* in horses has been well studied worldwide. *S. aureus* has been reported to be the most prevalent cause of bloodstream infection, skin and soft-tissue infection, and pneumonia in almost all geographic areas [86]. *Staphylococcus aureus* colonizes animals, such as horses, dogs, livestock (e.g. donkeys, pigs, and sheep) or wild animals (e.g. monkeys, chimpanzees, gorillas, and bats) [87]. Colonization, wherein *S. aureus* resides at a body site without producing clinical disease, is more common than clinical infection in humans and other species [88]. In fact, multiple studies in horses have reported colonization rates ranging anywhere from 0–16% [88-93]. Infections are most commonly seen and reported in cases of mastitis in dairy animals. *S. aureus* is also seen in other food producing animals such as chickens and farmed rabbits [94].

Studies from a wide range of areas (including North America, Europe, Australia, Asia, and the Middle East) all found varying ranges (0%-12%) of nasal carriage rates of MRSA in horses with the highest rates being found in the United Kingdom (12%) [90, 93, 95-99]. An Algerian study by Agabou et. al., found the rate of *S. aureus* nasal carriage in horses to be 15.2% [100]. Peterson et. al., identified MRSA in 61% of nasal samples on a racehorse farm in the United States [101]. In Denmark, Islam et. al., found much lower rates of S. aureus (13%) and MRSA (4%) in horses [102]. In a South African study by Oguttu et. al. [103], antimicrobial resistance patterns of *Staphylococcus Spp*. isolated from horses found that 12.0% of the samples were *Staphylococcus* positive with the majority of the isolates being *Staphylococcus aureus* (41.5%) [103].

A German study by Sommerhäuser et. al., found that *S. aureus* showed a high prevalence in two dairy herds prior to the introduction of a control program [104]. Only limited data on the prevalence of *Staphylococcus aureus* is available for African countries including South Africa [105]. *S. aureus* strains have been reported in sick and healthy animals in 7 countries (Côte d'Ivoire, Egypt, Nigeria, Senegal, South Africa, Sudan, and Tunisia) [106].

#### 2.1.5.2 Transmissions

*Staphylococcus* is most commonly transmitted through direct contact or through contact with contaminated grooming or other tools. Studies have found that transmission of MRSA from humans to horses and vice versa is possible [78, 92, 93]. The transportation of horses, especially thoroughbreds and standardbreds, between Canada and the United States, may make MRSA colonization and infection more of a problem among equines [92]. Clinical mastitis infections are most commonly spread during milking and the bacteria is able to penetrate the teat canal [83].

#### 2.1.5.3 Risk factors

There are a multitude of risk factors of *Staphylococcus* infection. Animals most at risk include those with weakened immune systems, burns, or surgical wounds. Invasive devices such as urinary catheters, feeding tubes, breathing tubes, and intravascular catheters can also present a risk for *Staphylococcus* infection which can travel along the medical tubing into internal organs. Soft tissue and joint infections are most common in horses with community-associated MRSA infections, IV cathetes are the most common source of *Staphylococcus* infections seen in equine hospitals [78]. Repeated veterinary practice or hospital admissions and usage of antimicrobials such as aminoglycosides have been noted as risk factors for *Staphylococcus* infection. [107]. A polish study by Bierowiec et. al., found that having one or more owners working in the healthcare industry (human medicine or veterinary medicine), dogs being kept with the cat under investigation, treatment of the cat under investigation with antibiotics or chemotherapeutics during the previous year all to be significant risk factors for colonization of *S. aureus* in cats [108]. *Staphylococcus* bacteria can also spread easily through cuts, abrasions and skin-to-skin contact.

## 2.2 ANTIMICROBIAL RESISTANCE

### 2.2.1 Brief history of antimicrobials

The 1930s introduced the world to what is commonly referred to as the “Golden Age” of antibiotics. Antimicrobial discovery began with the identification of sulfa drugs and concluded with the emergence of quinolones in the early 1960s [109]. Around the world more than 20 novel classes of antibiotics were introduced between 1930 and 1962 [110]. Antibiotics were first prescribed to treat serious infections in the 1940s. Penicillin was successful in controlling bacterial infections among World War II soldiers. However, shortly thereafter, penicillin resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened. In response, new beta-lactam antibiotics were discovered, developed, and deployed, restoring confidence. However, the first case of methicillin-resistant *Staphylococcus* aureus (MRSA) was identified during that same decade, in the United Kingdom in 1962 and in the United States in 1968 [111]. Vancomycin was introduced into clinical practice in both human and veterinary medicine in 1972 for the treatment of methicillin resistance in both *S. aureus* and coagulase-negative staphylococci [112]. Vancomycin resistance took a while to develop and so it was believed unlikely to occur in clinical settings. However, cases of vancomycin resistance were reported in coagulase-negative staphylococci in 1979 and 1983. From the late 1960s through the early 1980s, the pharmaceutical industry introduced many new antibiotics to solve the resistance problem, but after that the antibiotic pipeline began to dry up and fewer new drugs were introduced [111].

### 2.2.2 Use of antimicrobials in veterinary medicine and humans

Arsphenamine, introduced in 1910, was the first sulfa drug and was widely used in the treatment of syphilis and trypanosomiasis [113]. Shortly after antimicrobial drugs were developed they were used in veterinary medicine to treat mastitis in dairy cows. Antibiotics are used in food animals to treat clinical disease, to prevent and control common disease events, and to enhance animal growth [114]. The antimicrobials that are most commonly used in food animals come from five of the major drug classes including β lactams, tetracyclines, aminoglycosides, macrolides, and sulphonamides [115]. Interestingly, although not one of those five major groups, quinolones have only been available in some European countries for more than 20 years. The use of antimicrobials in human medicine produced a marked interest in their use in animals. This increased interest and usage has resulted in increased resistance rates in most countries [116]. In some countries, antibiotic use in livestock requires a veterinary prescription, although individual treatment decisions are often made and administered by lay farm workers in accordance with guidelines provided by a veterinarian [114].

Despite the widespread use of antimicrobials in food animals, data about the quantity and patterns of use is sparse [117]. Of the antimicrobials available for use in animals there are "12 classes of antimicrobials—arsenicals, polypeptides, glycolipids, tetracyclines, elfamycins, macrolides, lincosamides, polyethers, beta-lactams, quinoxalines, streptogramins, and sulfonamides—that are available for use in poultry, cattle, and swine” [117]. Judicious use of antimicrobials is an important part of equine medicine. Antimicrobials are used to treat known or suspected bacterial infections and for post-operative and secondary infections. In horses, vancomycin can be used alone or in combination with an aminoglycoside to treat methicillin-resistant staphylococcal infections [118].

### 2.2.3 Why antimicrobial resistance is a problem

Antibiotic resistance is a natural occurrence in microorganisms that are exposed to antibiotic over time. Under the selective pressure of antimicrobials, susceptible bacteria are killed or inhibited, while bacteria that acquired resistance survive and multiply. Antimicrobial resistance has been attributed to combinations of microbial characteristics, selective pressures of antimicrobial use, and societal and technologic changes that enhance the transmission of drug-resistant organisms [119]. Increased AMR is the cause of severe infections, complications, longer hospital stays and increased mortality [120]. Over-prescription among veterinary and medical practitioners is associated with an increased risk of adverse effects, more frequent re-attendance and increased medicalization of self-limiting conditions [120]. Over-prescription of antimicrobials is a particular problem in primary care. An increasing number of pathogenic organisms are resistant to one or more antimicrobial drugs. Consequently, certain infections have become more difficult to treat. For instance, the treatment of equine wounds is becoming progressively difficult due to the increase of antibiotic-resistant bacterial strains, particularly MRSA [121]. In fact, the rise in multi-drug resistant (MDR) bacteria is problem found in horses worldwide, where many pathogens have become harder to treat including multidrug-resistant Salmonella, MRSA, multidrug-resistant Pseudomonas (particularly *P. aeruginosa*) and multidrug-resistant Enterococcus (e.g. vancomycin-resistant enterococci) [122]. A large number of studies focus on the consequences of emergence and spread of AMR among animals as it relates to people, and thereby a potential impact on public health [123]. This is relevant in many countries such as Egypt, where 80 % of meat production is of poultry origin, and constitutes one of the main sources of pollution with veterinary antibiotics (VAs) into the environment increasing the potential environmental risks associated with the use of VAs in these farms [124]. Research has shown that antimicrobial resistance develops in zoonotic bacteria in response to antibiotics used in food animals [125]. Increased movement of horses for trade purposes, sports, or have also effected the spread of equine diseases [126]. Antimicrobial resistance is also a problem among some foodborne bacteria including *Campylobacter* and *Salmonella* which exhibit increasing resistance, to fluoroquinolones and third generation cephalosporins [127]. However, the consequences of AMR in animals is not limited to public health, but instead often impacts antimicrobial therapy with a direct negative effect on animal health and welfare [123].

### 2.2.4 General mechanisms of antibiotic resistance

Antimicrobials are classified according to their principal mechanism of action. Mechanisms include interference with cell wall synthesis (e.g., β-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin) [128].

Resistance to only one antimicrobial class can occur through multiple biochemical pathways [129]. Overall, bacteria have developed two main adaptations to combat antimicrobials. These adaptations include both gene mutations associated with the mechanism of action of the antimicrobial compound, and horizontal gene transfer (HGT) [129]. The three main mechanisms of antimicrobial resistance include: enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics [129]. Resistance to cephalosporins is often seen with resistance to penicillins [130], where the mechanism of resistance to both antimicrobial classes is antibiotic hydrolysis mediated by the bacterial enzyme β-lactamase [131]. Methods to overcome resistance to β-lactam antibiotics include development of β-lactamase inhibitors and the compounds that keep bacteria from identifying an antibiotic as dangerous and keeping the bacteria from being able to activate resistance mechanisms [132]. Resistance to methicillin occurs through the expression of a foreign penicillin-binding protein (PBP) [133]. Bacterial enzymes play a major role in the development of AMR [134] through both enzymatic modification and synthesis of antibiotic-insensitive bacterial targets in many classes of antibiotics [131]. Penetration barriers [135] are a resistance mechanism seen in several classes of antibiotics [131].

Bacteria may be resistant to more than one class of antimicrobial agents, or may acquire resistance by mutation or through the acquisition of resistance genes from other organisms [128]. Resistance genes enable bacteria to produce enzymes that destroy the antibacterial. They also allow bacteria to express efflux systems that prevent the drug from reaching its intracellular target. These same resistance genes also give bacteria the ability to modify the drug’s target site, or to produce an alternative metabolic pathway that bypasses the action of the drug [128]. Bacteria may acquire new genetic material from resistant strains of bacteria through conjugation, transformation, or transduction, [128].

### 2.2.5 Antibiotic susceptibility testing

#### 2.2.5.1 Dilution methods

One of the earliest antimicrobial susceptibility testing methods was the macrobroth or tube-dilution method [136]. The aim of broth and agar dilution methods is to determine the lowest concentration of the antimicrobial agent, also known as the minimal inhibitory concentration (MIC) that inhibits the visible growth of the bacteria being investigated. Minimum inhibitory concentration values are used to determine susceptibilities of bacteria to antimicrobials [137]. Dilution methods are the reference methods for antimicrobial susceptibility testing. Minimum inhibitory concentration methods are used in a variety of ways from resistance surveillance to testing on organisms where disc tests may be unreliable.

Broth dilution is another testing method and uses about 1.0 ml microdilution total broth volume and can be conveniently performed in a microtiter format. Unlike in the dilution test, in broth dilution, the lowest concentration where the isolate is completely inhibited is the MIC [138]. Agar dilution is also another option and follows the same method of identifying the MIC where the lowest concentration of the serially diluted antimicrobial concentration is used where the growth of bacteria is still inhibited [138].

#### 2.2.5.2 Disk diffusion-based methods

The disk diffusion method is the most widely used method for antimicrobial susceptibility testing in veterinary clinics [139]. Known as the Kirby-Bauer disk diffusion susceptibility test it is able to determine the sensitivity or resistance of bacteria to various antimicrobial agents [139]. The bacteria for this test are grown on Mueller-Hinton agar in antimicrobial saturated filter paper disks. The growth or non-growth on these disks measures the capacity of an antimicrobial to inhibit growth [139]. The diameter of the zone are interpreted using specific criteria. The criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) or those included in the US Food and Drug Administration (FDA)-approved product inserts for the disks [140] are most commonly used. The results of the disk diffusion test are then given a category of susceptibility (ie, susceptible, intermediate, or resistant) based on the results of zone diameters. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium .

#### 2.2.5.3 E-Test

The Epsilometer test (E-test) method for antimicrobial susceptibility testing provides quantification of antimicrobial susceptibility of microorganisms [141]. This is a gradient method of testing that allows the antibiotic to diffuse freely into the agar. After 48 hours incubation [141] at 42°C [142], the MIC is read at the point where bacterial growth inhibition intersects with the MIC scale on the strip. This makes for a more convenient quantitative test [141].

#### 2.2.5.4 Automated antimicrobial testing methods

An automated testing system consists of automated inoculation of MIC panels followed by computer-assisted incubation with reading, interpretation, and reporting functions that do not require manual intervention [143]. There are several commercially available systems that are intended to reduce technical errors and lengthy preparation times. Most automated antimicrobial susceptibility testing systems although costly, are quick and more convenient in providing automated inoculation, reading and interpretation.

### 2.2.6 Epidemiology of antimicrobial resistance in *Staphylococcus* infections

#### 2.2.6.1 Prevalence

Most studies that examine AMR in horses are based on horses admitted to referral hospitals, where horses are more likely to have received antimicrobial treatment and therefore carry resistant bacteria [122]. These selection biases tend to overestimate the actual levels of antimicrobial resistance [122]. Canada, Ontario Veterinary College identified 75 horses and 27 persons colonized or infected with MRSA from October 2000 to November 2002; most isolations occurred in a 3-month period in 2002 [144]. In samples taken from both Ontario, Canada and New York state, community associated MRSA was isolated from 46 of 972 (4.7%) horses [145]. In Israel, *Staphylococcus* colonization rates were 3.8% and 51% among farm and hospital horses, respectively [18]. A German study found that, nasal MRSA colonization was found in 19.5% of veterinary personnel with occupational exposure to horses [19]. Denmark is a country with high prevalence of livestock-associated methicillin-resistant Staphylococcus aureus (MRSA). One study in that country found MRSA isolates were obtained from 54/401 (13%) of the horses originating from 30 farms [146]. An Italian study examined samples from horses on farms, at racecourses, and at slaughterhouses and found that not only were 7% positive for MRSA, but that the prevalence of MRSA in horses tested at slaughterhouses was significantly higher ($p < 0.001$) compared with those tested on farms and racecourses [147].

The prevalence of MRSA colonization has been investigated in various horse populations, with rates of 0–10.9% reported in horses in the community and upon admission to veterinary hospitals [148]. A Canadian study by Burton et. al., [149] found that MRSA colonization was not identified in any of 497 horses from Atlantic Canada. However, methicillin-susceptible *Staphylococcus aureus* (MSSA) was isolated from 7.9% of horses [149]. Colonization with MSSA is relatively common in healthy horses in Atlantic Canada, but MRSA is currently rare or absent [149]. Another Canadian study by Weese et. al., [144] identified 79 horses and 27 persons colonized or infected with MRSA from October 2000 to November 2002 [144], where clinical infections developed in 16% of the horses. A community-based study in both Ontario and New York state reported a prevalence of 4.7% [93]. These results are similar to an Ohio study that found 5.8% of the horses sampled were positive for MRSA [150].

Resistant *Staphylococcus* infections can also be seen in cats and dogs. In a study of UK cats and dogs from 2003 to 2012, a new trend of increasing resistance to important antimicrobials was identified overtime and the emergence of methicillin resistant staphylococcus pseudintermedius (MRSP) from UK clinical cases was confirmed [151]. A cross-sectional study of nasal colonization of *Staphylococcus aureus* of dogs and their owners found that almost 90% of isolates were resistant to at least one antibiotic [152]. Higher prevalence of *Staphylococcus aureus* were found in canine isolates than human isolates in this sample set [152]. A UK study by Loeffler et. al., found prevalence of MRSA in dogs to be quite low (9%) [153]. This is much lower than the resistance levels found in previous studies and does not agree with the trend of increasing resistance identified in the study by Beever et. al. which found that 90% of isolates from dogs and owners were resistant to at least one antibiotic [151]. Overall, as in many other species, MRSA can be found in a small percentage of healthy dogs, however there has been fewer investigations of colonization in cats, with rates of 0–4% reported [148].

In the United States, it is estimated that MRSA causes approximately 95,000 invasive infections and 19,000 mortality cases per year in humans [154]. MRSA infections are among the most frequently occurring of all antibiotic-resistant threats. In the United States., 11,285 deaths per year have been attributed to MRSA alone [111]. From 2001 to 2002, national *Staphylococcus aureus* and MRSA colonization prevalence estimates were 32.4%  and 0.8%, respectively. There were almost 9,000 observed cases of invasive MRSA reported from July 2004 through December 2005 [155]. Most of these cases were health care–associated (58.4%) [155]. A world health organization report on antimicrobial resistance from 2014 found that in some settings, as many as 90% of *Staphylococcus aureus* infections are reported to be methicillin-resistant [3].

In Africa, many studies have found prevalence of AMR *Staphylococcus* infections to be low particularly in MRSA infections [106] in ruminant animals, pigs, sheep and companion animals. “The prevalence of MRSA in humans varies between African countries, with prevalence reported as high as 52% in Egypt, 45% in Algeria, 44% in Botswana, and between 6 and 19% in Morocco” [156]. In fact, “even in South Africa, the prevalence of MRSA bacteraemia varies depending on the geographical location and population studied” [156].

#### 2.2.6.2 Temporal distribution

The worldwide emergence and spread of resistant *Staphylococcus* is concerning. Of even greater concern is the spread of methicillin-resistant Staphylococcus aureus (MRSA) over the last 50 years, which represents a serious challenge to health professionals and clinical scientists worldwide [157]. The first MRSA infections seen in animals were reported from cases of mastitis in dairy cattle in 1972. However, in the following years infrequent infections were reported although some did include postsurgical wound infections in horses [158]. Historically, MRSA infections in companion animals involved strains resembling human nosocomial strains, including epidemic MRSA [107]. However, this problem has developed over time to encompass strains of MRSA that are thought to have evolved in animals colonizing and infecting human attendants [159].

This global trend toward progressively more resistant bacteria is extremely evident in *Staphylococcus* bacteria, with MRSA being of major concern. In Quebec, Canada, the incidence of MRSA bacteremia increased from 0 per 100,000 person-years to 7.4 per 100,000 person-years from 1991 to 2005 [160]. “Similar trends of increasing MRSA bacteremia incidence over this time period were seen in Minnesota from 1998 to 2005 ; Calgary, Canada, from 2000 to 2006 ; and Oxfordshire, United Kingdom, from 1997 to 2003” [160]. “Since 2005, most of these same regions have experienced significant reductions in rates of MRSA bacteremia, almost certainly linked to improvements in infection control procedures” [160]. “These reductions were especially evident in the United Kingdom, where rates of MRSA bacteremia were halved between 2004 and 2011, but have also been documented in the United States, Australia, and France” [160]. A Lisbon study found that in isolates taken from companion animals over a 16 year period, that among the 38 antimicrobials analyzed, resistance increased over the period analyzed in 27 antimicrobials and the number of isolates with resistance to at least one antimicrobial or with multidrug resistance also increased over time [161]. However, a Brazillian study found no trend towards increased resistance for most antimicrobials tested in isolates taken from milk samples in cows over a 20 year period [162].

#### 2.2.6.3 Risk factors

Multiple factors including, but not limited to, the overuse and misuse of antimicrobials contribute to the increase of antibiotic resistance [163]. Risk factors for MRSA infections in animals are similar to those found in humans, where living in a household with a colonized human or animal, hospitalization and surgery are all risks factors [107]. Other risk factors identified in animals include repeated veterinary practice or hospital admissions and usage of antimicrobials such as aminoglycosides [107]. Additional issues that could contribute to the risk of MRSA such as the horizontal spread of MRSA between horses on farms and in veterinary clinics is well known. A Canadian study by Weese et. al., found that previous colonization of the horse, previous identification of colonized horses on the farm, antimicrobial administration within 30 days, admission to the neonatal intensive care unit, and admission to a service other than the surgical service were all risk factors for community-associated colonization of MRSA [164]. A Canadian study found that horses that were found to be colonized with MRSA on admission were more likely to suffer from clinical infection than non-colonized horses [31]. Another Canadian study found that admission to neonatal intensive care was a risk factor for MRSA colonization in horses [164]. A study by Anderson et. al., [165] found that both previous hospitalization and treatment with gentamicin were significantly associated with community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), while infected incision sites were associated significantly with hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA). A German study by Vincze et. al., found that the number of employees working at a veterinary setting, prior antibiotic treatment and surgical site infection are all risk factors for MRSA in companion animals [166]. Another study from the UK found that significant risk factors for MRSA infection among cats and dogs were the number of antimicrobial courses, the number of days admitted to veterinary clinics and having received surgical implants [167].

#### 2.2.6.4 Surveillance for AMR

The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) established in 1996 is a monitoring system that combines efforts from state and local public health departments, CDC, the U.S. Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA). This is a nationwide public health initiative, that monitors deviations in antimicrobial susceptibility of enteric (intestinal) bacteria through the CDC for affected people , retail meats (FDA), and food animals (USDA) across the United States [168]. There are multiple primary objectives of the NARMS system. The first objective is to provide descriptive data on the extent and temporal trends of antimicrobial drug susceptibility in Salmonella and other enteric bacteria from humans, food animals and retail foods of animal origin. Next, NARMS seeks to respond to unusual or high levels of bacterial drug resistance in humans, animals, and retail meats in order to contain or mitigate resistance dissemination. Lastly, NARMS aims to design follow-up epidemiology and research studies to better understand the emergence and transfer of antimicrobial drug resistance [169].

In South Africa surveillance efforts are also being made in veterinary medicine. The South African National Veterinary Surveillance and Monitoring Program for Resistance to Antimicrobial Drugs (SANVAD) was established in 2003 and monitors antimicrobial resistance in the country. The program, in accordance with the Office International des

Épizooties (OIE) guidelines, is based on 3 categories of bacteria: indicator bacteria, zoonotic bacteria and animal pathogenic bacteria. These categories provide the best opportunities to detect resistance where selective pressures are applied, and clinically ill animals are treated [170]. In October 2015, the Global Antimicrobial Resistance Surveillance System (GLASS) was developed to support the global action plan on antimicrobial resistance with 15 countries across the continent of Africa completing enrollment in the program. GLASS provides surveillance and laboratory guidance, tools and support to countries in developing effective AMR surveillance systems. As a part of this global initiative, a conference on antimicrobial resistance and prudent use of antimicrobial agents in animals was held in October of 2018 [171]. The objectives of this conference were to foster national AMR surveillance systems through harmonized global standards to monitor ARM trends, detect emerging resistance, and inform estimates of AMR burden .

The Study for Monitoring Antimicrobial Resistance Trends (SMART) is the premier global surveillance system on antimicrobial resistance of microbes for almost 200 countries. Data gathered from SMART studies have shown that the level of antimicrobial resistance differs by geographic region and is highest in Asia-Pacific countries [172]. A WHO report found that in the South East Asia region as of 2011, the health ministers of the region committed to combat drug resistance. Since then, there has been growing awareness of the need for appropriate tracking of drug resistance, and all countries in the report have agreed to contribute information to a regional database. Collaboration on tracking of antibiotic resistance between countries in the

WHO Western Pacific Region was established in the 1980s. However, many countries in the region have long-established national systems for tracking resistance. Recently, WHO's Regional Office for the Western Pacific has taken steps to revive the regional collaboration [3]. Major gaps in tracking of antibiotic resistance were found in the Eastern Mediterranean region. However, WHO's Regional Office for the Eastern Mediterranean has identified strategic actions to contain drug resistance and is supporting countries to develop comprehensive national policies, strategies and plans [3]. Gaps were also found in the African region, where tracking of antibiotic resistance and data gathered in a limited number of countries [3]. While it is not possible to assess the true extent of antimicrobial resistance in this area due to lack of data from many countries, with the data that are currently available the problem is concerning [3].

Across the United States, increasing cases of antimicrobial resistance are currently affecting the ability of each state's public health laboratory to keep up [173]. Funding continues to decrease for AMR education programs and surveillance. Approximately only half of state public health labs can provide some basic resistance testing [174]. In the United States, different surveillance mechanisms exist to monitor resistant *Staphylococcus* infections, focusing on resistant *Staphylococcus aureus*. In 2004 the CDC launched the Emerging Infections Program (EIP) invasive *Staphylococcus aureus* infection surveillance program [175]. The invasive *Staphylococcus aureus* infection surveillance program is an active population- and laboratory-based surveillance system. Laboratories provide reports of results among patients in defined geographic areas. This program is conducted through CDC's EIP Healthcare-Associated Infections

Community Interface (HAIC) [175]. Data from the EIP *Staphylococcus aureus* program are used to evaluate the incidence of invasive *S. aureus* infections in the population, characterize *Staphylococcus aureus* strains associated with disease, and monitor trends in disease over time [175].

## 2.3 ANTIBIOTIC STEWARDSHIP IN VETERINARY MEDICINE

### 2.3.1 History of prescription antibiotic practices

As antibiotic resistance has increased, the development of new antimicrobials has decreased dramatically over the last 30 years [172]. In order to prevent not only increased prevalence of antimicrobial resistant infections, but a return to an era before widespread use of antimicrobials, antimicrobials must be used more judiciously [172]. Antibiotic resistance can be reduced by using antimicrobials more prudently based on guidelines of antimicrobial stewardship programs (ASPs) [172]. Without antimicrobial prescription policies physicians and veterinarians often prescribe antimicrobials to patients even when there is no clear indication for their use. Strides have been made in veterinary medicine to insure judicious use of antimicrobials. The veterinary feed directive (VFD) went into effect January 1, 2017, and enacted stricter federal rules regulating how medically important antibiotics could be administered to animals in feed and drinking water [176]. In 2015 the British Veterinary Association (BVA) put in place a 7 point plan for the responsible use of antimicrobials in veterinary practice [177]: to 1) work with clients to avoid need for antimicrobials, 2) avoid inappropriate use, 3) choose the right drug for the right bug, 4) monitor antimicrobial sensitivity, 5) minimize use, 6) record and justify deviations from protocols, and 7) report suspected treatment failure [177]. Prior to 2007 there were no standard programs to instruct and provide information about antimicrobial stewardship. However as of 2007, many institutions now have Antimicrobial Stewardship Programs (ASPs) to optimize antimicrobial therapy, reduce treatment-related cost, improve clinical outcomes and safety, and reduce or stabilize antimicrobial resistance [172]. The formal guidelines for ASPs were developed in 2007 by the Infection Diseases Society of America (IDSA) and the Society of Healthcare Epidemiology of America (SHEA) [172, 178].

#### 2.3.2 How opinions affect antibiotic prescription practices

Antimicrobial stewardship seeks to improve the efficacy and reduce the adverse effects of antimicrobial use by reducing the number of inappropriate antibiotic prescriptions [179]. An Irish study by Cotter and Daly [180], used a questionnaire designed to determine attitudes and practices regarding the prescription of antibiotics of general practitioners and found that not only did 94.7% of practitioners agree that antibiotic resistance was a problem, but that 81% of practitioners felt that antibiotics were over prescribed [180]. In fact, nearly 7% of these same practitioners admitted to frequently prescribing antibiotics to patients who did not need them, while 44% admitted to sometimes doing the same [180].

A 2017 UK study by Smith et al., found that communication between veterinarians and pet owners were fraught with misunderstandings and misconceptions around antibiotics by pet owners, and veterinarians and owners had differing opinions about where the pressure to prescribe antibiotics inappropriately originated [181]. Interestingly, veterinarians reported pet owners pushed for inappropriate antibiotics, while pet owners reported that veterinarians were the ones who overprescribed without their input [181]. Additionally, there was an overall low level of understanding of AMR among pet owners [181]. A U.S. study by Ekakoro and Okafor identified culture and susceptibility test results as the most important factor in antimicrobial prescription decisions, while pressure from clients was deemed the least important factor in decision making by veterinarians [182]. An Australian study by Hardefeldt et. al., found that key barriers to the implementation of antimicrobial stewardship programs among companion animal, equine and bovine veterinarians included a lack of antimicrobial stewardship governance structures, client expectations and competition between practices, cost of microbiological testing, and lack of access to education, training and antimicrobial stewardship resources [183].

## 2.4 CONCLUSION

The global challenges in veterinary medicine presented by antimicrobial resistance are concerning. Prescription policies and guidelines need be employed both nationally and internationally in order to address some of these concerns at the clinical level. Additionally, increased surveillance efforts need be applied in order to track global increasing temporal changes in AMR infections. Lastly, research into at risk populations will give a more encompassing view of the problem in both human and animal populations.

# 3 An epidemiologic study of antimicrobial resistance of *Staphylococcus* species isolated from equine samples submitted to a diagnostic laboratory

## 3.1 DISCLOSURE

Contents of this chapter have been published in BMC Veterinary Research. Full citation is:

Adams R, Smith J, Locke S, Phillips E, Erol E, Carter C. An epidemiologic study of antimicrobial resistance of Staphylococcus species isolated from equine samples submitted to a diagnostic laboratory. BMC veterinary research. 2018 Dec;14(1):42.

My primary contribution to this paper included (a) data management (b) performing all statistical analyses and (c) interpretation as well as preparation of the manuscript draft. Agricola Odoi was involved in conceptualization of research idea, study design, data analysis and interpretation as well as extensive editing of the manuscript. Jackie Smith, Stephen Locke, Erica Phillips, Craig Carter, and Erdal Erol were involved in all laboratory analyses, provision of laboratory resources, and data curation. All authors read and approved the final manuscript.

## 3.2 ABSTRACT

**Background**: Antimicrobial resistance limits traditional treatment options and increases costs. It is therefore important to estimate the magnitude of the problem so as to provide empirical data to guide control efforts. The aim of this study was to investigate the burden and patterns of antimicrobial resistance (AMR) among equine *Staphylococcus* samples submitted to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) from 1993 to 2009. Retrospective data of 1,711 equine *Staphylococcus* samples submitted to the UKVDL during the time period 1993 to 2009 were included in the study. Antimicrobial susceptibility testing, that included 16 drugs, were performed using cultures followed by the Kirby-Bauer disk diffusion susceptibility test. The proportion of resistant isolates by animal breed, species of organism, sample source, and time period were computed. Chi-square and Cochran-Armitage trend tests were used to identify significant associations and temporal trends, respectively. Logistic regression models were used to investigate predictors of AMR and multidrug resistance (MDR).

**Results:** A total of 66.3% of the isolates were resistant to at least one antimicrobial, most of which were *Staphylococcus aureus* (77.1%), while 25.0% were MDR. The highest level of resistance was to penicillins (52.9%). Among drug classes, isolates had the highest rate of AMR to at least one type of β-lactams (49.2%), followed by aminoglycosides (30.2%). Significant ($p<0.05$) associations were observed between odds of AMR and horse breed, species of organism and year. Similarly, significant ($p<0.05$) associations were identified between odds of MDR and breed and age. While some isolates had resistance to up to 12 antimicrobials, AMR profiles featuring single

antimicrobials such as penicillin were more common than those with multiple antimicrobials.

**Conclusion:** Demographic factors were significant predictors of AMR and MDR. The fact that some isolates had resistance to up to 12 of the 16 antimicrobials assessed is quite concerning. To address the high levels of AMR and MDR observed in this study, future studies will need to focus on antimicrobial prescription practices and education of both practitioners and animal owners on judicious use of antimicrobials to slow down the development of resistance.

## 3.3 BACKGROUND

The development of antimicrobial agents has been one of the most critical advances in both human and veterinary medicine within the last century. However, due to a combination of factors, but most notably to the rise in the use of antimicrobials for treating both human and domestic species, antimicrobial resistance has become a global scientific and public health concern in both human and veterinary medicine [29, 184]. The quantity of antimicrobials used in both human and veterinary medicine as well as in aquaculture have contributed to the selection for antimicrobial resistance [185]. High rates of antimicrobial resistant bacterial infections increase morbidity, be it to a single agent, or multiple drug classes, hindering the ability to effectively treat infections. As a result, both morbidity and mortality of antimicrobial resistant infections have increased in affected populations [184]. Identification of the resistance profiles of microorganisms is a critical step in understanding antimicrobial resistance and is useful in providing information to guide treatment options and to combat the problem.

According to the World Health Organization, the frequency of resistance to first-line drugs that have traditionally been used to treat infections caused by *Staphylococcus* has increased globally [186]. Unfortunately, this resistance is not limited to human medicine, but is being seen more frequently in domestic species, and in equine medicine in particular [184]. Although the widespread use of antimicrobials among equine species in the U.S. has been addressed in multiple forums, the epidemiology of antimicrobial resistance in bacteria found in horses has not been assessed [29]. Identifying and describing the burden of antimicrobial resistance among domestic species has become even more important due to evidence of potential cross transmission of certain bacteria between humans and domestic species [187]. Both the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) have reported such findings in past years [188, 189]. Outcomes from these investigations found evidence of a potential zoonotic transfer of *Staphylococcus* bacteria and/or their genetic material between healthy humans and horses [188, 190]. Other reports suggest that resistant *Staphylococcus* infections in domestic animals may contribute to transmission seen in human contacts [191].

Understanding the burden of antimicrobial resistant *Staphylococcus* infections in horses is critical in not only being able to understand the risk to those in immediate contact with these animals, but also in effectively providing information to guide efforts for the development of antimicrobial stewardship programs. Although a number of studies have investigated mainly methicillin-resistant *S. aureus* in horses [192-196], many other *Staphylococcus* species not only exhibit resistance to antimicrobials, but are clinically relevant to understanding the epidemiology of antimicrobial resistance in horses and its zoonotic spread to humans [29]. Thus, the objective of this study was to estimate the proportion of antimicrobial resistant staphylococcal isolates among equine samples submitted to the University of Kentucky Veterinary Diagnostic Laboratory between 1993 and 2009 and to identify potential predictors of antimicrobial resistance and multidrug resistance.

## 3.4 METHODS

### 3.4.1 Data Sources, preparation & study area

Laboratory records of all samples from horses submitted to the University of Kentucky Veterinary Diagnostic Laboratory were included in this study. The records included a combination of antimicrobial sensitivity test results and animal demographic information. For the isolation of bacteria, specimens were cultured on blood agar and eosin methylene blue agar plates at 37°C in 5–10% CO2, for a minimum of 24 hr. If the specimen was from a likely contaminated site such as nasal swab, a Columbia colistin and nalidixic acid (CNA) plate with blood was also inoculated. The plates were examined for pathogenic bacteria and were incubated for an additional 24 hr at 37°C in aerobic incubators and examined again for pathogenic bacteria. The criteria used for reporting a microorganism was the isolation of the microorganism in pure culture or significant numbers from specimens (as the predominate microorganism). *Staphylococcus* isolates were identified by using colony morphology, dark-field examination, β-hemolysis on blood agar and CNA plates, and conventional biochemical tests, including coagulase, catalase, maltose, mannitol, and trehalose. Additionally, selective and differential plates with antimicrobials and indicator were used to differentiate between *S. aureus* and *S. hyicus*.

Antimicrobial susceptibility testing, that included 16 drugs, were performed using Kirby-Bauer disk diffusion susceptibility test. The laboratory followed procedures of the Clinical Laboratory Standards Institute (CLSI) testing and classification to determine the susceptibility of isolates [197-201]. Sizes of the zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant. Sizes of zones of susceptible and resistant in millimeters were as follows: bacitracin (≥13, ≤8), cephalothin (≥18, ≤14), erythromycin (≥21, ≤15), neomycin (≥17, ≤12), kanamycin (≥18, ≤13), streptomycin (≥15, ≤11), oxacillin (≥13, ≤10), lincomycin (≥19, ≤15), enrofloxacin (≥21, ≤17), amoxicillin/clavulanic acid (≥20, ≤19), nitrofurantoin (≥17, ≤14), gentamicin (≥15, ≤12), novobiocin (≥17, ≤14) penicillin (≥28, ≤19), tetracycline (≥23, ≤18), and trimethoprim and sulfamethoxazole (≥16, ≤10). Isolates were classified as either susceptible, intermediate or resistant based on the above classification procedure [197-201]. For the purpose of this study, only susceptible and resistant isolates were included for subsequent analyses. Only records from the state of Kentucky were included in the study.

### 3.4.2 Data analysis

All statistical analyses were performed in SAS 9.4 [202]. For the purpose of this study, the resistance status variable was reclassified into a binary outcome, resistant or susceptible. Thus, all isolates indicated as “intermediate” were not included in the analysis. Antimicrobial resistance (AMR) was defined as resistance to at least one antimicrobial. Additionally, multi-drug resistance (MDR) was defined as resistance to three or more antimicrobial classes [203]. The proportion of resistant isolates and 95% confidence intervals were computed by breed, sex, age, sample source, the species of *Staphylococcus*, antimicrobial agent, year (which was scaled by subtracting 1993 from each year), season and month. Season was classified as follows: summer (June-August), fall (September-November), winter (December-February), and spring (March-May). All specimen types that had frequencies of less than 1% were combined into a category called "Other". These were too many to list. Similarly, breeds with frequencies less than 1% were classified as "other breeds" and included Appaloosa, Belgian, Burro, Clydesdale, Donkey, Draft, French Warmblood, Hanover, Miniature Horse, Missouri Fox Trotter, Morgan, Other, Paint, Palomino, Percheron, and Pony.

Temporal graphs were generated in excel to visualize the temporal patterns of resistance. In addition, the Cochran-Armitage Trend test was used to identify significant temporal trends. Simple and multivariable logistic regression models were used to investigate if AMR had significant associations with breed, sex, age, sample source, species of *Staphylococcus* organism, year, season, and month. The model building process was done in two steps. In the first step, simple logistic regression models were fitted with "AMR, (1=Resistant, 0=Susceptible)" as the outcome and each of the variables in Table 1 as the explanatory variables. Variables with p-values less than 0.15 were considered for inclusion in the multivariable logistic regression model that was used in the second step. During this 2$^{nd}$ step, the multivariable logistic regression model was fitted using a manual backwards selection procedure. Confounding was assessed by comparing the change in parameter estimate of the variables in the model with and without the suspected confounding variable. A 20% change in the estimate of any of the variables already in the model was considered to be indicative of a confounder that was then retained in the final model. Odds ratios and their corresponding 95% confidence intervals were computed for all variables included in the final model. Goodness-of-fit of the final model was assessed using the Hosmer-Lemeshow goodness-of-fit test. No evidence of lack of fit was found. Steps 1 and 2 for the process above were repeated to investigate predictors of multidrug resistance (MDR). In this model, the outcome variable used was "MDR, (1=Multidrug Resistant/0=Not Multidrug Resistant)". Again, Goodness-of-fit of the final model was assessed using the Hosmer-Lemeshow goodness-of-fit test. No evidence of lack of fit was found.

## 3.5 RESULTS

### 3.5.1 Summary statistics

A total of 1,711 samples, from 26 horse breeds, were included in the study. The most common breeds were Thoroughbreds (74.3%) followed by Tennessee Walking Horses (5.6%) (Table 3.1). Overall, more samples were submitted from female horses (83.7%) than male horses (16.3%) (Table 3.1). Similarly, horses >4 years old contributed the highest proportion of samples (46.0%), followed by aborted fetuses (22.6%) and those < 1 year old (19.7%) (Table 3.1). Additionally, samples testing positive for *coagulase negative Staphylococcus* were most frequent (47.8%), followed by coagulase positive *Staphylococcus aureus* (40.3%). *S. hyicus* was the least frequent (4.4%).

Overall, 66.3% of the isolates were resistant to at least one antimicrobial. Of the samples with known breed information, the highest proportion of resistant isolates was from Thoroughbreds (70.5%) followed by the Standardbreds (68.6%) and Arabians (68.4%), while the lowest proportion of resistance was seen in mixed breeds (40.0%) (Table 3.1). Standardbreds had the highest proportion of MDR isolates (37.1%), followed by Thoroughbreds (31.1%), and Quarter Horse (18.3%). The lowest proportion of MDR was in the Tennessee Walking Horse (3.4%) (Table 3.1).  Although females seemed to have a slightly higher level of AMR (68.1%) than males (64.0%), these differences were not statistically significant. However, the same does not apply to the levels of MDR between the sexes. In fact, males had a markedly higher proportion of MDR (32.9%) than females (25.4%) (Table 3.1).

Foals (< 1 years old) showed the highest levels of AMR (75.9%), followed by horses 2–4 years old (67.3%), and yearlings (1–2 years old) (65.6%). Adult horses (> 4 years old) had the lowest levels of antimicrobial resistance (60.0%) (Table 3.1). Foals again showed the highest levels of MDR (37.6%) when compared with other age groups (Table 3.1). MDR for horses 2–4 years old (28.9%) and those 1–2 years old (18.8%) were again the next highest. The highest proportion of AMR was observed among *Staphylococcus aureus* isolates (77.1%) followed by coagulase negative *Staphylococcus* strains (60.1%) (Table 3.1).  Similarly, *Staphylococcus aureus* (38.3%) again had the highest levels of MDR, followed by coagulase negative *Staphylococcus* strains (20.0%) (Table 3.1).

**Table 3.1: Distribution and antimicrobial resistance of equine *Staphylococcus* samples submitted to the University of Kentucky veterinary diagnostic laboratory, 1993-2009**

| Variable | No. of Samples Tested | *Percentage of Samples Tested (%) | [1]95% CI | [2]AMR Samples | [2]AMR† (%) | [1]95% CI | [3]MDR Samples | [3]MDR‡ (%) | [1]95% CI |
|---|---|---|---|---|---|---|---|---|---|
| **Breed** | **n=1577** | | | **n=1046** | | | | | |
| Arabian | 19 | 1.2 | 0.7, 1.7 | 13 | 68.4 | 43.5, 87.4 | 4 | 21.1 | 6.1, 45.6 |
| American Saddlebred | 63 | 4.0 | 3.0, 5.0 | 34 | 54.0 | 40.9, 66.6 | 10 | 15.9 | 7.9, 27.3 |
| Mixed Breed | 30 | 1.9 | 1.2, 2.6 | 12 | 40.0 | 22.7, 59.4 | 3 | 10.0 | 2.1, 26.5 |
| Quarter Horse | 60 | 3.8 | 2.9, 4.8 | 28 | 46.7 | 33.8, 60.0 | 11 | 18.3 | 9.5, 30.4 |
| Rocky Mountain Saddlebred | 16 | 1.0 | 0.5, 1.5 | 7 | 43.8 | 19.8, 70.1 | 1 | 6.3 | 0.2, 30.2 |
| Standardbred | 35 | 2.2 | 1.6, 3.1 | 24 | 68.6 | 50.7, 83.2 | 13 | 37.1 | 21.5, 55.1 |
| Thoroughbred | 1172 | 74.3 | 72.2, 76.5 | 826 | 70.5 | 67.8, 73.1 | 365 | 31.1 | 28.5, 33.9 |
| Tennessee Walking Horse | 88 | 5.6 | 4.5, 6.7 | 46 | 52.3 | 41.4, 63.0 | 3 | 3.4 | 8.5, 75.5 |
| Other Breeds | 94 | 6.0 | 4.8, 7.3 | 56 | 59.6 | 49.0, 69.6 | 9 | 9.6 | 4.5, 17.4 |
| **Sex** | **n=1377** | | | **n=928** | | | | | |
| Female | 1152 | 83.7 | 81.6, 85.6 | 784 | 68.1 | 65.3, 70.7 | 293 | 25.4 | 22.9, 28.1 |
| Male | 225 | 16.3 | 14.4, 18.4 | 144 | 64.0 | 57.4, 70.3 | 74 | 32.9 | 26.6, 39.5 |
| **Age Groups** | **n=717** | | | **n=459** | | | | | |
| >4 years | 330 | 46.0 | 42.4, 49.7 | 198 | 60.0 | 54.5, 65.3 | 68 | 34.3 | 16.4, 25.4 |
| 2-4 years | 52 | 7.3 | 5.5, 9.4 | 35 | 67.3 | 52.9, 79.7 | 15 | 28.9 | 17.1, 43.1 |
| 1-2 years | 32 | 4.5 | 3.0, 6.0 | 21 | 65.6 | 46.8, 81.4 | 6 | 18.8 | 7.2, 36.4 |
| < 1 year | 141 | 19.7 | 16.8, 22.6 | 107 | 75.9 | 68.0, 82.7 | 53 | 37.6 | 29.6, 46.1 |
| Aborted Fetus (0 years) | 162 | 22.6 | 19.5, 25.7 | 98 | 60.5 | 52.5, 68.1 | 35 | 21.6 | 15.5, 28.8 |
| **Species of Organism** | **n=1711** | | | **n=1131** | | | | | |
| *CoNS*[4] | 817 | 47.8 | 45.4, 50.1 | 491 | 60.1 | 56.7, 63.5 | 163 | 20.0 | 17.3, 22.9 |
| *Staphylococcus aureus* | 689 | 40.3 | 37.9, 42.6 | 531 | 77.1 | 73.7, 80.2 | 264 | 38.3 | 34.7, 42.1 |
| *Staphylococcus hyicus* | 75 | 4.4 | 3.4, 5.4 | 31 | 41.3 | 30.1, 53.3 | 1 | 1.3 | 0.03, 7.2 |
| *Staphylococcus intermedius* | 130 | 7.6 | 6.3, 8.9 | 78 | 60.0 | 51.1, 68.5 | 16 | 12.3 | 7.2, 19.2 |

### 3.5.2 Distribution of resistance across antimicrobials

Overall, 16 antimicrobials from 10 antimicrobial classes were examined in this study (Table 3.2). Highest proportions of AMR isolates were seen among β-lactams (49.2%), with more isolates exhibiting resistance to Penicillin (52.9%) than oxacillin (15.6%) (Table 2 and Fig 1). The drug class with the second highest proportion of AMR isolates was aminoglycosides (30.2%) (Fig 3.1), with 28.9% and 22.8% of the isolates exhibiting resistance to Kanamycin and Gentamicin, respectively (Table 2). As for MDR, β-Lactams again had the highest levels (23.5%) of isolates that were MDR followed by Aminoglycosides (22.1%) (Table 3.2 and Fig 3.1). Although the majority of resistant isolates (51.3%) were only resistant to 1 or 2 antimicrobial classes, 13.4% of the resistant isolates were resistant to 5 antimicrobial classes.

**Table 3.2: Distribution of antimicrobial resistance categorized by antimicrobial class among equine *Staphylococcus* samples submitted to the University of Kentucky veterinary diagnostic laboratory, 1993-2009**

| Antimicrobial Class | Drug | [1]AMR Samples | [1]AMR % | [2]95% CI | [3]MDR Samples | [3]MDR % | [2]95%CI |
|---|---|---|---|---|---|---|---|
| Aminoglycosides | | 516/1710 | 30.2 | 28.0, 32.4 | 377/1710 | 22.1 | 20.1, 24.1 |
| | Neomycin | 53/1582 | 3.4 | 2.5, 4.4 | 46/1582 | 2.9 | 2.1, 3.9 |
| | Kanamycin | 486/1682 | 28.9 | 26.7, 31.1 | 369/1682 | 21.9 | 20.0, 24.0 |
| | Streptomycin | 59/287 | 20.6 | 16.0, 25.7 | 28/287 | 9.8 | 6.6, 13.8 |
| | Gentamicin | 369/1622 | 22.8 | 20.7, 24.9 | 270/1622 | 16.7 | 14.9, 18.6 |
| β-lactams | | 841/1710 | 49.2 | 46.8, 51.6 | 402/1710 | 23.5 | 21.5, 25.6 |
| | Penicillin | 814/1539 | 52.9 | 50.4, 55.4 | 396/1539 | 25.7 | 23.6, 28.0 |
| | Oxacillin | 254/1634 | 15.6 | 13.8, 17.4 | 235/1634 | 14.4 | 12.7, 16.2 |
| | Amox/clav. acid | 115/1644 | 7.0 | 5.8, 8.3 | 107/1644 | 6.5 | 5.4, 7.8 |
| Macrolides | | 292/1668 | 17.5 | 15.7, 19.4 | 249/1668 | 14.9 | 13.3, 16.7 |
| | Erythromycin | 292/1668 | 17.5 | 15.7, 19.4 | 249/1668 | 14.9 | 13.3, 16.7 |
| Sulfonamides | | 463/1645 | 28.2 | 26.0, 30.4 | 372/1645 | 22.6 | 20.6, 24.7 |
| | Sulfonamide | 488/1702 | 28.7 | 26.5, 30.9 | 372/1702 | 21.9 | 19.9, 24.0 |
| | Trimethoprim-sulfadiazine | 330/1355 | 24.4 | 22.1, 26.7 | 297/1355 | 21.9 | 19.7, 24.2 |
| Lincosamides | | 28/970 | 2.9 | 1.9, 4.2 | 25/970 | 2.6 | 1.7, 3.8 |
| | Lincomycin | 28/970 | 2.9 | 1.9, 4.2 | 25/970 | 2.6 | 1.7, 3.8 |
| Aminocoumarins | | 141/1578 | 8.9 | 7.6, 10.6 | 31/1578 | 2.0 | 1.3, 2.8 |
| | Novobiocin | 141/1578 | 8.9 | 7.6, 10.6 | 31/1578 | 2.0 | 1.3, 2.8 |
| Cephalosporins | | 63/1711 | 3.7 | 2.8, 4.7 | 63/1711 | 3.7 | 2.8, 17.1 |
| | Cephalothin | 48/1692 | 2.8 | 2.1, 3.7 | 48/1692 | 2.8 | 2.1, 3.7 |
| Fluoroquinolones | | 1/25 | 4.0 | 0.1, 20.4 | 1/25 | 4.0 | 0.1, 20.4 |
| | Enrofloxacin | 1/24 | 4.2 | 0.1, 21.1 | 1/24 | 4.2 | 0.1, 21.1 |
| Tetracyclines | | 451/1682 | 26.8 | 24.7, 29.0 | 326/1682 | 19.4 | 17.5, 21.4 |
| | Tetracycline | 451/1682 | 26.8 | 24.7, 29.0 | 326/1682 | 19.4 | 17.5, 21.4 |
| Polypeptides | | 45/1649 | 2.7 | 2.0, 3.6 | 36/1649 | 2.2 | 1.5, 3.0 |
| | Bacitracin | 45/1649 | 2.7 | 2.0, 3.6 | 36/1649 | 2.2 | 1.5, 3.0 |

[1]AMR: Antimicrobial Resistance
[2]95% Confidence Interval
[3]MDR: Multidrug Resistance
[4]Amoxicillin/Clavulanic Acid

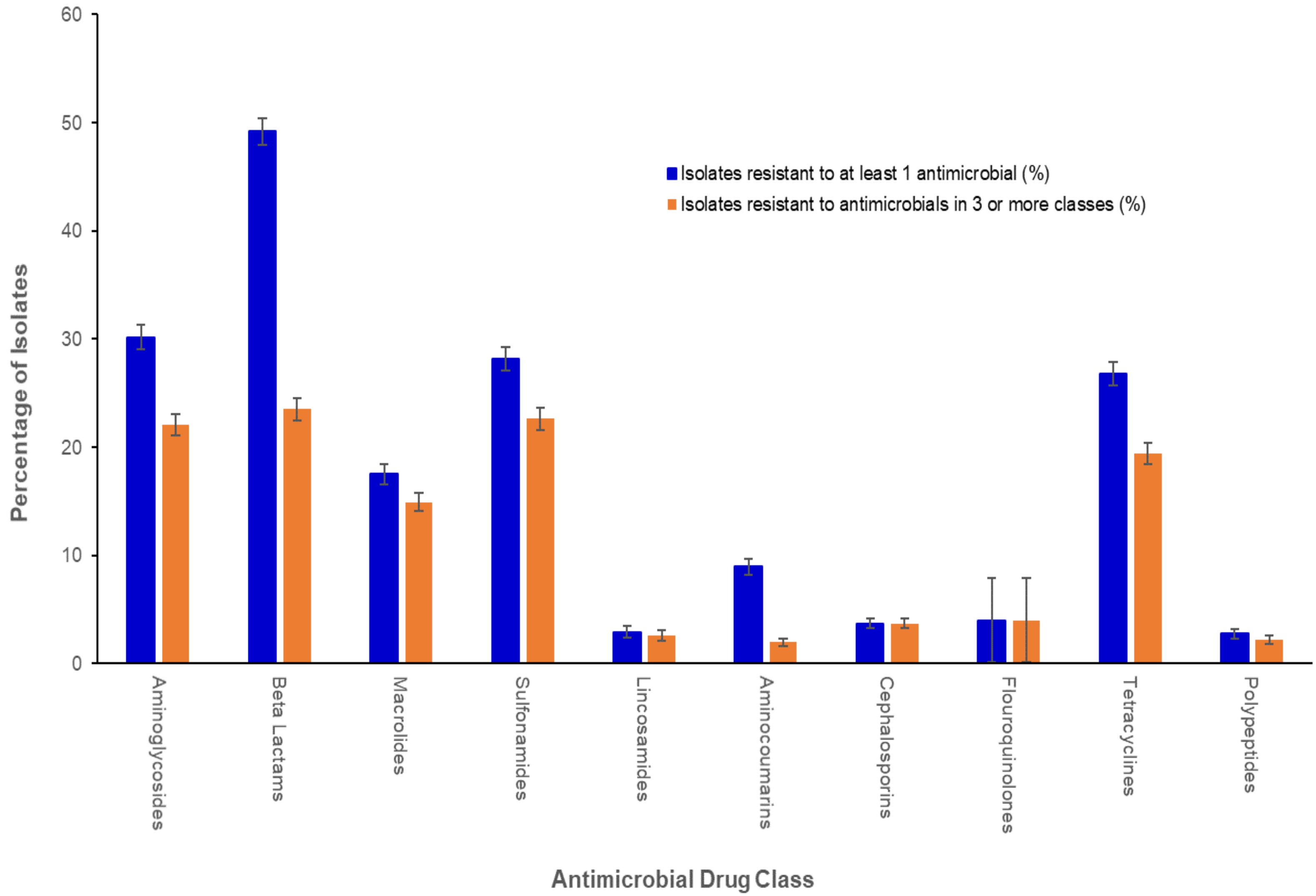


**Figure 3-1: Antimicrobial resistance and multidrug resistance by drug class from equine Staphylococcus samples submitted to the University of Kentucky veterinary diagnostic laboratory, 1993-200**

Of the isolates that were found to be MDR, 8.0% were resistant to 9 antimicrobials (Amoxycillin/clavulanic acid, Erythromicin, Gentamicin, Kanamycin, Oxacillin (Methicillin), Penicillin, Sulfonamides, Tetracycline, and Trimethoprim-sulfadiazine) belonging to 5 antimicrobial classes (Aminoglycosides, β-Lactams, Macrolides, Sulfonamides and Tetracyclines) (Table 3.3). Another 7.0% were resistant to the same profile of antimicrobials except Erythromycin. In fact, 46.0% of the isolates that were MDR, and had a sample size greater than 10, had resistance profiles that contained penicillin, kanamycin, sulfonamides, and trimethoprim-sulfadiazine (Table 3.3). Additionally, 34.0% of the MDR samples with sample sizes greater than 10 showed resistance to oxacillin (Table 3.3).

### 3.5.3 Temporal trends

There was a significant (p =0.023) decreasing temporal trend in AMR over the study period (Fig 3.2). The proportions of AMR isolates were highest in 2000 (76.0%) and reached their lowest levels by 2007 (52.4%) (Fig 3.2). On the contrary, there was an increasing temporal trend in MDR (p= 0.007) over the study period (Fig 3.2). The proportion of MDR isolates began at its lowest point in 1993 (14.4%) before reaching the highest level in 2000 (42.5%) (Fig 3.2).

***Table 3.3: Antimicrobial resistance profiles of equine resistant* Staphylococcus *samples submitted to the University of Kentucky veterinary diagnostic laboratory, 1993-2009***

| **Profile** | ***No. of Samples** | **Percent** | **[1]95% CI** |
|---|---|---|---|
| Amo-Cep-Ery-Gen-Kan-Oxa-Pen-Sul-Tet-Tri | 14 | 4.3 | 2.3, 6.9 |
| Amo-Ery-Gen-Kan-Oxa-Pen-Sul-Tet-Tri | 28 | 8.3 | 5.6, 11.8 |
| Amo-Ery-Kan-Oxa-Pen-Sul-Tet-Tri | 10 | 3.0 | 1.4, 5.4 |
| Amo-Gen-Kan-Oxa-Pen-Sul-Tet-Tri | 10 | 3.0 | 1.4, 5.4 |
| Ery-Gen-Kan-Oxa-Pen-Sul-Tet-Tri | 25 | 7.4 | 4.9, 10.8 |
| Ery-Gen-Kan-Pen-Sul-Tet-Tri | 14 | 4.2 | 2.3, 6.9 |
| Ery-Kan-Oxa-Pen-Sul-Tet-Tri | 12 | 3.6 | 1.9, 6.1 |
| Gen-Kan-Oxa-Pen-Sul-Tet-Tri | 17 | 5.0 | 3.0, 8.0 |
| Gen-Kan-Pen-Sul-Tet-Tri | 16 | 4.8 | 2.7, 7.6 |
| Gen-Kan-Pen-Sul-Tri | 10 | 3.0 | 1.4, 5.4 |

Amo: Amoxiillin/clavulanic acid
Cep: Cephalothin
Ery: Erythromycin
Gen: Gentamicin
Kan: Kanamycin
Oxa: Oxacillin
Pen: Penicillin
Sul: Sulfonamide
Tet: Tetracycline
Tri: Trimethoprim-sulfadiazine
The denominator used for each percentage was (337) after missing values were removed
[1]95% Confidence Interval

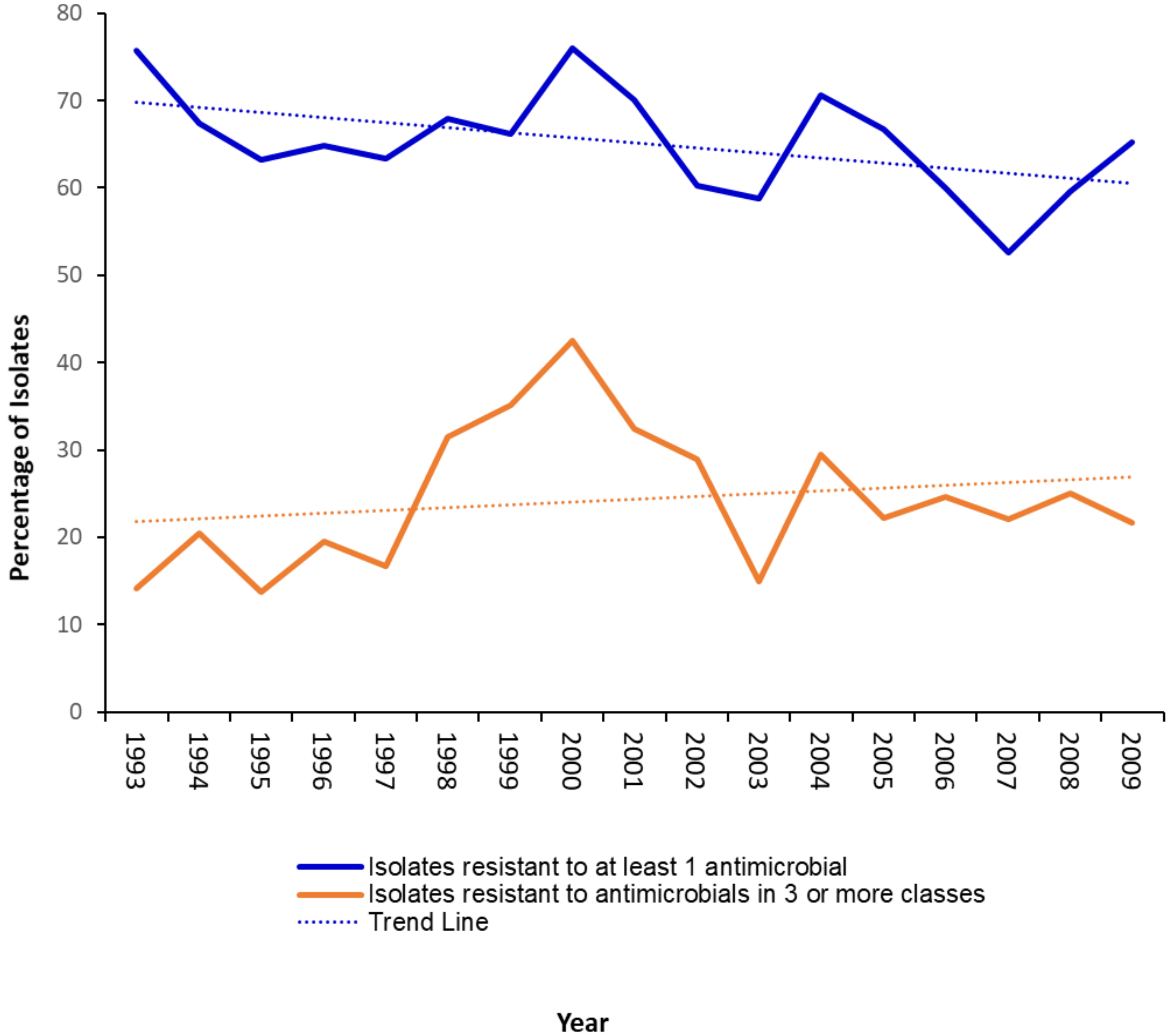


**Figure 3-2: Annual temporal distribution of antimicrobial resistance & multidrug resistance from equine *Staphylococcus* samples submitted to the University of Kentucky veterinary diagnostic laboratory, 1993-2009**

### 3.5.4 Predictors of antimicrobial resistance (AMR) and multidrug resistance (MDR)

Species of organism, breed, age, sex, season, and year all had significant simple associations with the odds of AMR at an $\alpha = 0.15$. (Table 3.4). Similarly, species of organism, breed, age, sex, and year had significant simple associations with the odds of MDR (Table 3.4). All variables found to be significant ($p \leq 0.15$) in the AMR or MDR simple models were considered for inclusion in their respective multivariable models.

Breed (p=<0.001), species of organism (p=<0.001) and year (p=0.023) were significantly associated with the odds of antimicrobial resistant *Staphylococcus* infections in horses (Table 3.5). There was a significant (p=<0.001) association between breed and AMR with Thoroughbreds having higher odds (Odds Ratio [OR] = 1.61; 95% Confidence Interval [CI] = 1.07, 2.42) of AMR than other breeds (Table 3.5). Interestingly, species of organism was a significant predictor for AMR but not MDR. The odds of AMR among *Staphylococcus aureus* isolates was significantly (p<0.0001) higher (OR=2.30; 95% CI=1.81, 2.93) than that of coagulase negative *Staphylococcus* isolates (Table 3.5), while the odds of AMR among *Staphylococcus hyicus* isolates was significantly (p<0.0001) lower (OR=0.46; 95% CI=0.27, 0.77) than that of coagulase negative *Staphylococcus* isolates. Year had a negative association with AMR (OR=0.97, 95% CI=0.95, 1.00).

**Table 3.4: Results of simple logistic models assessing predictors of antimicrobial resistance and multidrug resistance in equine *Staphylococcus* samples submitted to the Kentucky state diagnostic laboratory, 1993-2009**

| Variable | AMR[1] P-Value | MDR[2] P-Value |
|---|---|---|
| Breed | <0.001 | <0.001 |
| Age | 0.019 | 0.002 |
| Organism | <0.001 | <0.001 |
| Sex | 0.107 | 0.021 |
| Season | 0.083 | 0.781 |
| Month | 0.379 | 0.519 |
| Year | 0.046 | 0.001 |
| City | 0.390 | 0.146 |

[1]Antimicrobial Resistance
[2]Multidrug Resistance

**Table 3.5: Significant predictors of antimicrobial resistant *Staphylococcus* in equines from samples submitted to the Kentucky state diagnostic laboratory, 1993-2009**

| Variable | Odds Ratio | 95% CI[1] | P-Value |
|---|---|---|---|
| **Breed** | | | <0.001 |
| Arabian | 1.5 | 0.5, 4.3 | 0.331 |
| American Saddlebred | 0.9 | 0.5, 1.7 | 0.763 |
| Mixed Equine | 0.5 | 0.2, 1.2 | 0.057 |
| Quarter Horse | 0.6 | 0.3, 1.2 | 0.091 |
| Standard Bred | 1.5 | 0.6, 3.3 | 0.245 |
| Thoroughbred | 1.6 | 1.1, 2.4 | <.001 |
| Tennessee Walking Horse | 0.8 | 0.5, 1.5 | 0.397 |
| Other Breeds | . | . | . |
| **Species of Organism** | | | <0.001 |
| *Staphylococcus aureus* | 2.3 | 1.8, 2.9 | <.001 |
| *Staphylococcus hyicus* | 0.5 | 0.3, 0.8 | <.001 |
| *Staphylococcus intermedius* | 1.1 | 0.7, 1.8 | 0.692 |
| *Coagulase negative Staphylococcus* | . | . | . |
| **Year** | 0.97 | 0.95, 1.00 | 0.023 |

[1]95% Confidence Interval

Breed (p=<0.001) and age (p =0.020) were significantly associated with the odds of MDR of *Staphylococcus* (Table 3.6). The odds of isolates from Standardbreds being MDR were over 15 times (OR=15.0; 95% CI=3.7, 60.4) higher than those of isolates from other breeds, while the odds of MDR in isolates from Thoroughbreds were almost 7 times (OR=7.0; 95% CI=2.4, 19.8) higher than that of isolates from other breeds (Table 3.6). The odds of MDR among isolates taken from foals (< 1 year) were 63% (OR=1.6; 95% CI=1.0, 2.6) higher than that of horses >4 years old (Table 3.6).

**Table 3.6: Significant predictors of multidrug resistant *Staphylococcus* in equines from samples submitted to the Kentucky state diagnostic laboratory, 1993-2009**

| Variable | Odds Ratio | 95% CI[1] | P-value |
|---|---|---|---|
| **Breed** | | | <0.001 |
| Arabian | 3.9 | 0.6, 25.1 | 0.159 |
| American Saddlebred | 2.5 | 0.5, 12.5 | 0.257 |
| Mixed Equine | 2.3 | 0.5, 11.5 | 0.308 |
| Quarter Horse | 2.2 | 0.5, 8.7 | 0.277 |
| Standardbred | 15.0 | 3.7, 60.4 | 0.001 |
| Thoroughbred | 7.0 | 2.4, 19.8 | 0.000 |
| Tennessee Walking Horse | 0.8 | 0.2, 3.6 | 0.730 |
| Other | . | . | . |
| **Age** | | | 0.020 |
| Aborted Fetus 0 years | 0.7 | 0.4, 1.2 | 0.171 |
| < 1 year | 1.6 | 1.0, 2.6 | 0.042 |
| 1 – 2 years | 1.8 | 0.6, 4.9 | 0.266 |
| 2 – 4 years | 1.5 | 0.7, 3.1 | 0.275 |
| > 4 years | . | . | . |

[1]95% Confidence Interval

## 3.6 DISCUSSION

This study was designed to investigate the burden and patterns of both AMR and MDR among equine *Staphylococcus* samples submitted to the University of Kentucky Veterinary Diagnostic Laboratory and to investigate the predictors of AMR and MDR. The findings should provide information to guide future studies and ongoing surveillance of antimicrobial resistance. The proportion of antimicrobial resistant isolates seen in this study for both coagulase negative *Staphylococcus* infections (60.1%) and coagulase positive strains including *S. aureus* (77.1%), *S. intermedius* (60.0%), and *S. hyicus* (41.3%) suggest that the levels of AMR are high for both pathogenic and non-pathogenic *Staphylococcus* species.

### 3.6.1 Temporal trends

The temporal patterns observed in this study are interesting as a significant decreasing temporal trend was found for AMR, while an increasing temporal trend was observed for MDR. The reasons for this are unclear. However, a University of California (U.C.), Davis study that examined temporal trends in antimicrobial susceptibility patterns in equine case records from the William R. Pritchard Veterinary Medical Teaching Hospital (VMTH) from 1979 to 2010, found statistically significant increases over time in the percentage of *Staphylococcus* isolates susceptible to certain antimicrobials (chloramphenicol, ceftiofur, and penicillin) [204]. It is worth noting that, the U.C. Davis study investigated multiple organisms (*Pseudomonas* species, *Enterococcus* species, *E. coli*, *Salmonella* species., *Streptococcus* species, *Staphylococc*us species and *Actinobacillus* species) while our study was limited to *Staphylococcus* species. Findings from this study suggest that despite the significant decreasing AMR temporal trends, significant increasing MDR temporal trends in this population could have a negative impact on morbidity and mortality rates attributable to MDR infections [205, 206].

### 3.6.2 Antimicrobials

There is a paucity of published literature on antimicrobial resistance in equine *Staphylococcus* infections. Most of the work that has been published has focused only on *S. aureus* and especially MRSA. Thus the lack of literature addressing resistant *Staphylococcus* species in horses makes comparisons between the findings of this study and others difficult. Suffice it to say that although the overall proportions of AMR isolates in this study were high, MRSA levels were much lower (15.6%) than the percentage of MRSA (48%) found in a similar study done in Turkey [207]. A Belgian study, by Van den Eede et. al., that assessed occurrence of MRSA in equine nasal samples found similar MRSA levels (10.9%) to those found in our study [208]. However, studies done in Australia, Canada, and Ireland that investigated *Staphylococcus aureus* colonization in healthy horses as well as isolation rates in horses with clinical presentation of MRSA found the percentage of AMR isolates to be much lower and ranging from 4% to 8% [149, 209, 210]. These differences could be attributed to the fact that we examined a higher number of antimicrobials and species of *Staphylococcus* in this study in comparison with the above studies that only investigated methicillin resistance in *S. aureus*.

The highest levels of resistance in this study was towards β-Lactams and Aminoglycosides. This may be due to the tendency of staphylococci to adapt to the selection pressure of antimicrobial use and become resistant to antimicrobials in general and the multiple mechanisms of resistance to aminoglycosides and β-Lactams in particular [211, 212]. These findings are comparable to those of a Swiss study which reported high levels of AMR not only to β-lactams and aminoglycosides, but to tetracyclines, lincosamides and macrolides as well when compared to other drug classes [213].We also found the highest levels of AMR to be against penicillin (52.9%). Much higher levels of resistance were reported from equine hospital data in Zurich, where researchers identified AMR to penicillin in both coagulase negative staphylococci and *Staphylococcus aureus* to be around 82% and AMR to tetracycline to be 64% [196]. High levels of resistance to both penicillin (62.7%) and tetracycline (23.7%) were found in a retrospective study in France that investigated Staphylococci implicated in death or euthanasia in horses [214].The higher levels of AMR *Staphylococcus* infections reported in hospitals could explain the higher AMR levels from the Zurich study.

A German study looking at resistance profiles of MRSA in horses from veterinary hospitals and large animal clinics found that gentamicin resistance was high (85%) and mainly associated with isolates coming from equine clinics, while the majority of the isolates from all horses in the study were resistant to tetracycline (97.5 %) and fluoroquinolones (79%) with only 15.6% being resistant to erythromycin [158]. Our study found much lower levels of AMR to gentamicin (22.8%), tetracycline (26.8%) and fluoroquinolones (4.0%), than the German study. Despite our MDR profiles containing gentamicin (16.7%) and tetracycline (19.4%) resistance, these levels were still not consistent with the findings of the German study. The differences in the levels of AMR and MDR seen in our study can be explained by the fact that the isolates from our study included multiple *Staphylococcus* species.

Of the resistant isolates in this study, 25% were MDR. This is double the percentage of MDR (13%) found in a Lithuanian study by Klimienė et al. (2015) [215] and a Zurich study [196] that both reported 13% MDR. However, it is more than double that reported by Toombs-Ruane *et al.* (10.1%) in New Zealand [216]. The Swiss study mentioned previously, also found that isolates were most likely to be MDR involving β-lactams, aminoglycosides, and tetracyclines [213]. That finding is similar to that of our study where the highest proportion of MDR infections involved aminoglycosides, β-lactams, sulfonamides, cephalosporins and tetracyclines. Interestingly, a recent companion animal study done in India found that not only were the incidences of *Staphylococcus aureus* wound infections higher in equines (57.14%), but that there was 100% MDR against kanamycin, colistin, clindamycin, penicillin-G, cotrimoxazole and cefotaxime [217]. However, it is worth noting that the current study only focused on *Staphylococcus* infections in horses and not multiple companion animals as was the case in the Indian study.

### 3.6.3 Antimicrobial resistance profile

Almost half of the MDR isolates in this study had antimicrobial resistance profiles that included penicillin, kanamycin, sulfonamides, and trimethoprim-sulfadiazine. These findings are consistent with those of a similar study that found that, in isolates identified to be MDR, *Staphylococcus* isolates that were oxacillin resistant, were also resistant to kanamycin, gentamicin and penicillin [218]. In our study less than 1% of the isolates were resistant to 12 antimicrobials and antimicrobial resistance profiles showed MDR to occur most frequently among isolates resistant to aminoglycosides, β-lactams, tetracyclines, sulfonamides, and cephalosporins. These findings were different from those of a study done in Switzerland by Schnelleman et. al., (2006) [213], where 24% of the *Staphylococcus* isolates were resistant to all 12 of the antimicrobials tested, while the remainder of the isolates were resistant to a number of drug classes including β-lactams, combination β-lactam-β-lactamase-inhibitors, aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides and/or streptogramins [213]. It is important to note that isolates from the Swiss study were obtained only from horses undergoing colic surgery. A Lithuanian study by Klimienė et. al. (2015) [215], found that the *Staphylococcus* isolates that were MDR showed high levels of resistance to penicillin G, erythromycin or tetracycline. Similar to the findings of our study, they reported that 66.7 % of the isolates showed resistances to penicillin, erythromycin, tetracycline, ciprofloxacin, and gentamicin.

### 3.6.4 Distribution of resistance by host factors, species of organism and time

Thoroughbreds had the highest proportion of antimicrobial resistance (70.5%) in this study. This number is strikingly higher than the 5% AMR levels found in a similar study in Japan that examined MRSA colonization and infection in thoroughbreds [194]. However, because the Japanese study only looked at MRSA in thoroughbreds, while our study was able to examine both AMR and MDR in thoroughbreds, it is difficult to make direct comparisons between the AMR levels of the two. Nonetheless, a Canadian study looked at a mixture of draft, race, pleasure, breeding, school, and show horses and found no evidence of MRSA in thoroughbreds [219]. In this study, we found that the odds of AMR in thoroughbreds was higher than that of other breeds. The higher odds of AMR in thoroughbreds could be due to the extensive movement of this particular horse breed, increasing the risk for exposure to resistant *Staphylococcus* strains and contributing to higher resistance levels. Another Canadian study hypothesized that frequent contact with other horses, recurring and frequent travel to different sites, and the frequent use of antimicrobials in this set of horses could be associated with increased prevalence of MRSA in show and race horses [220]. Race horses, especially thoroughbreds, are moved frequently between Canada and the United States due to the large racing industry in both countries, which makes the risk of MRSA colonization and infection more widespread than seen in other breeds [221]. Horses, and thoroughbreds, in particular, are often moved between the United States, Australia, Canada, Japan, the UK, and Ireland, increasing the risk of importing infected carrier horses [194]. This could explain the high levels of resistance seen in thoroughbreds in this study.

A significant simple association was found between age and the odds of both AMR and MDR in this study. However, a significant association was only found between MDR and age in the multivariable model with age group less than 1 year showing significantly higher odds of MDR. Many past studies have focused on foals as an important population for studies of antimicrobial susceptibility [222-226]. This is likely due to the higher susceptibility of younger animals to infection resulting in higher likelihood of antimicrobial treatment and hence selection for resistance. In this study *Staphylococcus aureus* was found to have significantly higher odds of AMR when compared with other *Staphylococcus* species , which is likely due to adaptability seen in *S. aureus*, [227], as well as the high prevalence of methicillin resistance in *Staphylococcus aureus* isolates, which indicates intrinsic resistance to all other β-Lactams, aminoglycosides and macrolides [228, 229].

Year was a significant predictor of AMR but not MDR in this study, where the odds of AMR isolates decreased over time. Decreases in AMR are likely due to changes in surveillance and reporting practices for resistant *Staphylococcus* infections, as well as adherence to sound antimicrobial prescription practices and policies. A study by Weese & Rousseau (2005) [230] found that after implementation of both active surveillance cultures and infection control procedures to address endemic MRSA, there was a rapid decrease in the proportion of horses colonized with MRSA. The study done by Weese & Rousseau focused on MRSA infections so direct comparisons cannot be made. However, it does indicate that appropriate control measures can affect the proportion of resistance infections observed and reported.

## 3.7 STUDY LIMITATIONS

This retrospective laboratory-based study is not without limitations. Since data were not obtained using a statistical sampling technique, the study population should not be considered to be representative of the equine population in Kentucky. Only data available in the laboratory records could be investigated limiting the scope of investigation. For instance, information on past antimicrobial use was not available and therefore we could not assess its associations with levels of AMR or MDR. Furthermore, past medical history of the animals whose samples were used in this study was not reported.

### 3.8 CONCLUSION

The above limitations notwithstanding, the findings of this study provide useful information on the epidemiology of AMR and MDR in *Staphylococcus* infections in horses whose samples were submitted to the UKVDL. This information will be useful for guiding future primary base studies as well as efforts to address the problem. It is clear that equine *Staphylococcus* infections are exhibiting both AMR and MDR in horses. Factors such as breed and year are significant predictors of the odds of both AMR and MDR in this study, while species of staphylococci is also an important predictor of AMR and age of the horse was significantly associated with MDR. High levels of AMR and MDR could be indicative of problems in clinical prescription practices and procedures leading to selection for antimicrobial resistance. This highlights the need for a more comprehensive approach to investigating the epidemiology of AMR and MDR in horses. Future studies will need to focus on improving our understanding of antimicrobial use in horses as this will allow for more informed antimicrobial stewardship programs. Moreover, AMR surveillance in horses needs to include better record keeping and lab submission information (such as pre-treatment history). More information on risk factors may be gained through primary base observational studies that can more robustly identify risk factors that might otherwise not be investigated by retrospective lab-based studies.

# 4 Antibiotic prescription practices and opinions regarding antimicrobial resistance among veterinarians in Kentucky, USA

## 4.1 DISCLOSURE

Contents of this chapter have been submitted or publication in BMC Veterinary Research. Full citation is:

Adams R, Smith J, Carter C. Antibiotic prescription practices and opinions regarding antimicrobial resistance among veterinarians in Kentucky, USA. 2019. Submitted to BMC veterinary research.

My primary contribution to this paper included (a) data management (b) performing all statistical analyses and (c) interpretation as well as preparation of the manuscript draft. Craig Carter and Jackie Smith were involved in data collection as well as review and editing of the manuscript. Agricola Odoi was involved in conceptualization of research idea, study design, data analysis and interpretation as well as extensive editing of the manuscript. All authors read and approved the final manuscript.

## 4.2 ABSTRACT

**Background**: Appropriate usage of antimicrobials is a global concern and opinions regarding appropriate use vary greatly amongst veterinarians. Opinions of clinical veterinarians regarding antimicrobial use and its role in development of antimicrobial resistance (AMR) may influence their prescription practices and hence use of antimicrobials. It is important to understand the opinions of veterinarians regarding antimicrobial usage and its potential impact on development of AMR in order to guide efforts to curb the problem. Therefore, the objectives of this study were to investigate the opinions, knowledge and perceptions of veterinarians in Kentucky regarding AMR and antimicrobial prescription practices as well as to identify predictors of their knowledge and opinions. This cross-sectional study used a 30-question survey questionnaire administered to members of the Kentucky Veterinary Medical Association (KVMA). Survey responses from 101 participants were included in the study. The proportion of responses to survey questions and 95% confidence intervals were computed. Predictors of improper use of antimicrobials and antimicrobial prescription practices of the respondents as well as their colleagues were investigated using multinomial logistic regression models.

**Results:** Almost all (93.1%; 81/87) of the veterinarians responded that improper use of antimicrobials contributed to selection for AMR. Slightly more than half (51.7%; 47/91) of them believed that antimicrobials were appropriately prescribed, while (48.4%; 44/91) believed they were improperly prescribed. Although more than half (59.8%) of respondents worked at practices with antimicrobial prescription policies, only approximately 23.9% believed that antimicrobial prescription policies actually contributed to changes in the incidence of AMR at their facility or practice. None of the

variables investigated had significant associations with the opinion that "Improper use of antimicrobials contributes to selection for AMR", personal antimicrobial prescription practices of the respondent, or their opinions concerning their colleagues' prescription practices.

**Conclusion:** Although most veterinarians were of the opinion that improper use of antimicrobials contributed to selection for AMR, inconsistencies exist regarding the perceptions of culpability for AMR. There is critical need for increased awareness of AMR and the importance of sensible antimicrobial prescription practices to ensure judicious use of antimicrobials in veterinary practice so as to curb the development of AMR.

## 4.3 BACKGROUND

Antimicrobials are commonly used in both human and veterinary medicine to treat bacterial infections. Unfortunately, their injudicious use in both human and veterinary medicine as well as in agriculture has partly led to selection for antimicrobial resistance (AMR). Antimicrobial resistance has recently garnered more attention as it has become recognized as an increasingly important global health problem with the use of antimicrobial agents being increasingly implicated as a key risk factor in the development of AMR [231]. In veterinary medicine in particular, antimicrobial agents are used extensively for prophylaxis, metaphylaxis, therapy and growth promotion in various animal production systems [232]. The rising threat to human health from misuse of antimicrobials in food animals is critical as resistant pathogenic bacteria propagated in livestock can potentially enter the food chain and thus be widely disseminated in food products and pose health risks to humans [233].

The consumption of antibiotics is known to be higher in animals than in humans [234]. In fact, in 2017 the World Health Organization (WHO) reported that in some countries, approximately 80% of total consumption of medically important antibiotics is in the animal sector, used for growth promotion in healthy animals [235]. As a result, in 2017 WHO strongly recommended that use of all classes of medically important antibiotics in food-producing animals be reduced [236]. The organization also recommended restricting the use of antibiotics for growth promotion and disease prevention without appropriate diagnosis [236]. As of 2017 the U.S. federal government acted to address these concerns through the Veterinary Feed Directive (VFD) by mandating the manner in which medically important antibiotics are administered to animals in both feed and drinking water, while in turn the U.S. Food and Drug Administration (FDA) also now requires veterinary oversight whenever these medically important antibiotics are administered to any food animal species through feed or water. The report by Boeckel et. al., that included 228 countries, estimated that the consumption of antimicrobials in livestock was 63,151 tons in 2010 [237]. This staggering use of antimicrobials can be attributed to a multitude of factors including routine use of antimicrobials in farming and growth in consumer demand for livestock products in middle-income countries [237]. Although the problem of AMR in veterinary medicine is well known, little is known concerning the prescription practices of veterinarians or their opinions regarding antimicrobial resistance.

Despite international, national, and local efforts to encourage antimicrobial stewardship and to limit unnecessary exposure to antimicrobials, the absence of universal policies to preserve their effectiveness limits the ability of antimicrobials to combat serious and life-threatening infections [233]. In Australia, development of best-practice antimicrobial prescribing guidelines is a key component of the animal health industry's response to the issue of AMR [238]. These guidelines are intended to be used as decision making tools to assist with the rapid selection of the most appropriate antibiotic for the treatment of common infections, and/or when antimicrobial susceptibility data may not be available [238]. High volume of antimicrobial use is not always the only actionable issue in the crisis of AMR. For instance, in the Netherlands, despite the low amounts of antimicrobials used in Dutch companion animal clinics, the majority of antimicrobials prescribed are categorized as critically important for human medicine by the World Health Organization (WHO) [239]. In such instances, restraint in the use of these drugs still need attention. Furthermore, the efficacy of policies that exist in veterinary practice to control antimicrobial resistance, particularly in the United States, is not yet fully understood. Therefore, more evidence is needed to inform effective policy interventions among individual states [240].

With at least 30% of antimicrobials prescribed in the United States being deemed unnecessary by a Centers for Disease Control and Prevention (CDC) report [241], individual adherence to some sort of antimicrobial prescription policy is critical. In fact, studies have reported that not only does Kentucky have the second highest prescription rate of antibiotics in the US [242], but that it also has the highest per capita antibiotic prescription rate of any state [243]. This high level of antimicrobial prescriptions is troubling. To address these issues, it is important to understand the opinions of medical and veterinary practitioners related to use of antimicrobials and their role in the development of antimicrobial resistance. This information is useful for guiding policy and prescription guidelines to minimize or eliminate overuse and injudicious use of antimicrobials. This would help slow down the development of antimicrobial resistance. Therefore, the objectives of the study were to: (a) evaluate the opinions of veterinarians on antibiotic prescription practices and antimicrobial resistance; and (b) to identify the factors affecting their opinions.

## 4.4 METHODS

### 4.4.1 Survey setting

This cross-sectional study used a 30-item survey questionnaire designed to investigate opinions, knowledge and antimicrobial prescription practices of veterinarians in Kentucky. The survey was then uploaded to Qualtrics [244]. Veterinarians at the Kentucky Veterinary Medical Association (KVMA) were contacted by email and were requested to participate in the study. Participants were provided with a link in order to anonymously answer survey questions.

### 4.4.2 Design

This is a cross-sectional study that used a questionnaire survey administered to veterinarians in Kentucky who were members of KVMA. The questionnaire covered issues relates to opinions of veterinarians regarding antimicrobial prescription practices and how it relates to the development of antimicrobial resistance. The survey instrument was adopted from 2 previous survey questionnaires [245, 246]. The original questionnaires were improved by adding questions addressing factors associated with prescription practices, opinions regarding prescription practices, and opinions regarding antimicrobial resistance. Pretest of the questionnaire was done on a small sample of clinical veterinarians at the University of Tennessee. After the pretest, the respondents were asked a series of questions regarding the questionnaire to help identify problems with questions or the administration of the survey. This allowed identification of bias due to question design and correction of confusing, ambiguous, or misleading questions. For multiple choice questions, this allowed for the inclusion of additional of response categories identified by the pretest subjects.

#### 4.4.3 Survey administration

Administrators of the KVMA were requested to grant permission to the investigators to contact their members via their email list-serve. Upon approval of the request, the KVMA administrators sent an email (on behalf of the investigators), containing a link to the questionnaire survey to all list-serve members requesting them to participate in the study. This initial email, with a link to the online questionnaire survey, was sent to the list-serve in April 2017. The questionnaire was designed to take 20-30 minutes to complete and consisted of 30 questions divided into 6 sections: Demographic Information, Veterinary Education, Antimicrobial Prescription Practices, Factors Associated with Prescribing Habits, Opinions About Prescription Practices, and Opinions About Antimicrobial Resistance. These six sections contained both open-ended and close-ended questions consisting of a combination of yes/no questions, multiple choice questions as well as 5-point Likert scale questions (ranging from 1-strongly agree; 2-agree; 3-neither agree nor disagree; 4-disagree; 5-strongly disagree). To increase response rate, a total of 6 reminder emails were sent to the list-serve between May and October 2017 requesting list-serve members to complete the survey, if they had not done so already, and thanking those who had already completed the survey.

### 4.4.4 Data analysis

All statistical analyses were performed in SAS 9.4 [202]. The distribution of demographic variables and their 95% confidence intervals were computed. The variables considered were: sex of participant, city, veterinary practice, veterinary facility, length of time at facility, number of veterinarians at the facility, hours worked per week, and year of graduation. Frequency distributions of the responses to the survey questions were calculated. Due to small number of responses in some of the response categories, the 5-point Likert Scale variables, “Improper use of antimicrobials contributes to selection for AMR” and “My colleagues over prescribe antimicrobials” were recoded to 1-strongly agree or agree, 2-neither agree nor disagree, 3-disagree or strongly disagree. Since there were very few missing responses, all calculations excluded thee records. Multinomial logistic regression was used to investigate predictors of improper use of antimicrobials, antimicrobial prescription practices of the respondents, and antimicrobial prescription practices of their colleagues. Additionally, an ordinary logistic regression model was used to investigate predictors of whether the respondent felt they sometimes over-prescribed antibiotics (Yes/No).

For each of the models, the model building process was done in two steps. In the first step, univariable logistic regression models (multinomial or ordinary logistic regression models) were used to investigate the relationships between the potential predictors (sex, city, veterinary practice, veterinary facility, length of time at facility, number of veterinarians at the facility, hours worked per week, and year of graduation) and each of the outcomes. Potential predictors with p-values ≤ 0.20 were considered for inclusion in multivariable regression models (either multinomial or ordinary logistic as appropriate). In the 2nd step, a multivariable model (multinomial or ordinary logistic) were fit using manual backwards selection with each of the three variables above as outcomes. At this step, statistical significance was assessed at $\alpha = 0.05$. Confounding was assessed by comparing the change in model coefficients with and without the suspected confounders. If the removal of a suspected confounding variable resulted in a 20% or greater change in another variable coefficient, the removed variable was considered a confounder and retained in the model regardless of its statistical significance. Relative risk ratios and odds ratios as well as 95% confidence intervals were computed. Goodness-of-fit of the models were assessed using Hosmer-Lemeshow goodness-of-fit tests.

## 4.5 RESULTS

### 4.5.1 Summary statistics

A total of 101 veterinarians agreed to take part in the study and completed the online questionnaire. The questionnaire was completely filled out by 84% (85/101) of the respondents. The rest (16%) only responded to some of the questions. Non-responses to some of the questions may have been due to lack of knowledge/opinion to specific questions. It could also be due to the respondents stopping before completing the survey. The informed consent included information indicating that the respondents could stop taking the survey at any time, if they wished.

### 4.5.2 Participant information

Of the veterinarians that responded to the questionnaire, 57.4% were female and 42.6% were male (Table 4.1). The majority of the respondents (26.5%) were located in Lexington and Louisville (18.4%) (Table 4.1). The most common type of veterinary practices the respondents were involved in were small animal practice (58.0%), and mixed animal practice (23.0%) (Table 4.1). More than half of the veterinarians worked at primary care facilities (55.0%), while only 29.0% worked at veterinary hospitals and 16.0% were referrals (Table 4.1). The median number of years of experience of the respondents was 12 years (interquartile range: 3, 27) while the median years since graduation was 24 years (interquartile range: 9, 35). The majority of the respondents (82.8%) worked in facilities that had ≤10 veterinarians on payroll. The median number of years the respondents had worked at their practices was 3 years (interquartile range: 1, 7).

***Table 4.1: Distribution of respondent demographic information from a survey of veterinarians in Kentucky, 2017***

| Variable | Number | Percentage | 95% CI[1] |
|---|---|---|---|
| **Sex** | **n=101** | | |
| Female | 58 | 57.43 | 47.2, 67.2 |
| Male | 43 | 42.57 | 32.8, 52.8 |
| **City** | **n= 98** | | |
| Lexington | 26 | 26.53 | 18.1, 36.4 |
| Louisville | 18 | 18.37 | 11.3, 27.5 |
| Other | 54 | 55.10 | 44.7, 65.2 |
| **Veterinary Practice** | **n=100** | | |
| Large Animal | 19 | 19.00 | 11.8, 28.1 |
| Mixed | 23 | 23.00 | 15.2, 32.5 |
| Small Animal | 58 | 58.00 | 47.7, 67.8 |
| **Veterinary Facility** | **n=100** | | |
| Primary Care | 55 | 55.00 | 44.7, 65.0 |
| Referral | 16 | 16.00 | 9.4, 24.7 |
| Veterinary Hospital | 29 | 29.00 | 20.4, 38.9 |

[1]95% Confidence Interval

### 4.5.3 Veterinary education

Almost half (49.5%) of the veterinarians indicated that antibiotics were emphasized in multiple courses in their veterinary school education during non-clinical years (Table 4.2). However, the number of respondents indicating that antibiotics were emphasized in multiple classes rose to 67% during the clinical years (Table 4.2). Pharmacologist/clinical pharmacologists were mostly responsible (34.8%) for education on antibiotics, followed by clinicians (27.4%) and clinical microbiologists (18.3%) (Table 4.2). Only 5% of the respondents had post graduate education (Table 4.2).

***Table 4.2: Distribution of survey questionnaire responses from veterinarians in Kentucky, 2017***

| Question/Response | Number | Percentage | 95% CI[1] |
|---|---|---|---|
| **What was the emphasis on antibiotics in veterinary school education (non-clinical years)?** | **n=97** | | |
| Topic was not covered | 1 | 1.03 | 0.02, 5.97 |
| Light emphasis | 21 | 21.65 | 13.93, 31.17 |
| Covered thoroughly in one course | 27 | 27.84 | 19.21, 37.86 |
| Emphasized in multiple courses | 48 | 49.48 | 39.17, 59.83 |
| **What was the emphasis on antibiotics in your veterinary school education (clinical years)?** | **n=97** | | |
| Topic was not covered | 1 | 1.03 | 0.02, 5.97 |
| Light emphasis | 26 | 26.80 | 18.32, 36.76 |
| Covered thoroughly in one course | 5 | 5.15 | 1.70, 11.62 |
| Emphasized in multiple courses | 65 | 67.01 | 56.73, 76.22 |
| **What was the background of the person primarily responsible for your education on antibiotics during your veterinary education?** | n=164 | | |
| Clinical pharmacist | 20 | 12.20 | 7.61, 18.21 |
| Clinical microbiologist | 30 | 18.29 | 12.70, 25.07 |
| Clinician | 45 | 27.44 | 20.77, 34.94 |
| Pharmacologist/clinical pharmacologist | 57 | 34.76 | 27.50, 42.57 |
| Toxicologist | 7 | 4.27 | 1.73, 8.60 |
| Don't know his/her background | 5 | 3.05 | 1.00, 6.97 |
| **Do you hold any additional post graduate qualifications?** | **n=97** | | |
| Yes | 25 | 25.77 | 17.43, 35.65 |
| No | 72 | 74.23 | 64.35, 82.58 |

[1]95% Confidence Interval

### 4.5.4 Antimicrobial prescription practices

The majority (26.4%) of the veterinarians received their information regarding antimicrobials and their use from textbooks/drug handbooks and continuing professional development courses (26.0%) (Table 4.3). Peer reviewed scientific literature (18.4%) and pharmaceutical companies (15.6%) were also popular sources of information on antimicrobials for the respondents (Table 4.3). Surprisingly only 5% of the veterinarians received information regarding antimicrobials and their usage from their practice's policies (Table 4.3). However, more than half (57.6%) of them do not have a policy concerning antimicrobial prescription at their practice (Table 4.3). Almost all (92.5%) of the veterinarians were able to prescribe antimicrobials without supervision, or oversight. Interestingly, although more than half of the practices did not have antimicrobial prescription policies, 76.3% of the respondents reported prescribing antimicrobials multiple times per day (Table 4.3). Moreover, more than half (53.8%) of the respondents reported that they were not comfortable prescribing at least one type of antimicrobial (Table 4.3).

### 4.5.5 Factors influencing antimicrobial prescription practices

The most common factors that influenced the decisions of the veterinarians to prescribe antimicrobials to a patient were route of administration (26.7%), cost of antimicrobial (24.6%), and risk of potential adverse drug reaction (23.4%) (Table 4.3). The majority of veterinarians either strongly agreed (44.6%) or agreed (41.3%) with the fact that they always relied on clinical signs and symptoms to prescribe antimicrobials. However, only

**Table 4.3: Distribution of survey responses related to antimicrobial use and prescription practices among veterinarians in Kentucky, 2017**

| Question/Responses | Number | Percentage | 95% CI |
|---|---|---|---|
| **What are the main sources that you use to receive current information on antimicrobials and their use?** | | | |
| Practice policy | 14 | 4.86 | 2.68, 8.02 |
| Pharmaceutical companies | 45 | 15.63 | 11.63, 20.34 |
| Veterinary medicine directorates | 17 | 5.90 | 3.48, 9.28 |
| Peer reviewed scientific literature | 53 | 18.40 | 14.10, 23.37 |
| Textbook/drug handbook | 76 | 26.39 | 21.39, 31.88 |
| Continuing professional development courses | 75 | 26.04 | 21.07, 31.51 |
| Other | 8 | 2.78 | 1.21, 5.40 |
| **Can you prescribe antibiotics without supervision, approval, or additional oversight?** | | | |
| Yes | 86 | 92.47 | 85.11, 96.92 |
| No | 7 | 7.53 | 3.08, 14.90 |
| **Does your veterinary facility or practice have a policy concerning antibiotic prescription?** | | | |
| Yes | 39 | 42.39 | 32.15, 53.14 |
| No | 53 | 57.61 | 46.86, 67.85 |
| **On Average, how often do you prescribe antibiotics?** | | | |
| Multiple times per day | 71 | 76.34 | 66.40, 84.54 |
| Once per day | 4 | 4.30 | 1.18, 10.65 |
| Once every two days | 4 | 4.30 | 1.18, 10.65 |
| Once per week | 6 | 6.45 | 2.40, 13.52 |
| Once every two weeks | 1 | 1.08 | 0.03, 5.85 |
| Once per month | 1 | 1.08 | 0.03, 5.85 |
| Once every two to four months | 4 | 4.30 | 1.18, 10.65 |
| Quarterly | 0 | 0.00 | 0.00, 0.00 |
| Biannually | 2 | 2.15 | 0.26, 7.55 |
| Annually | 0 | 0.00 | 0.00, 0.00 |
| **Is there any antibiotic that you do not feel comfortable prescribing?** | | | |
| Yes | 50 | 53.76 | 43.12, 64.16 |
| No | 43 | 46.24 | 35.84, 56.88 |
| **Do any of the factors below affect your decision when choosing to prescribe an antibiotic to a patient?** | | | |
| Cost of antibiotic | 82 | 24.55 | 20.03, 29.53 |
| Client insurance | 3 | 0.90 | 0.19, 2.60 |
| Client expectations | 28 | 8.38 | 65.47, 93.24 |
| Route of administration | 89 | 26.65 | 21.98, 31.73 |
| Frequency of patient visits | 28 | 8.38 | 5.64, 11.89 |
| Risk of potential adverse drug reaction | 78 | 23.35 | 18.92, 28.27 |
| Other | 26 | 7.78 | 5.15, 11.20 |
| **You always rely on clinical signs and symptoms when prescribing an antibiotic.** | | | |
| Strongly agree | 41 | 44.57 | 34.19, 55.30 |
| Agree | 38 | 41.30 | 31.13, 52.05 |
| Neither agree nor disagree | 10 | 10.87 | 5.34, 19.08 |
| Disagree | 3 | 3.26 | 0.68, 9.23 |
| Strongly disagree | 0 | 0.00 | 0.00, 0.00 |

Table 4.3 Continued

| Question/Responses | Number | Percentage | 95% CI |
|---|---|---|---|
| **You rely on laboratory results before prescribing an antibiotic.** | | | |
| Strongly agree | 16 | 17.39 | 10.28, 26.70 |
| Agree | 35 | 38.04 | 28.12, 48.76 |
| Neither agree nor disagree | 29 | 31.52 | 22.23, 42.04 |
| Disagree | 8 | 8.70 | 3.83, 16.42 |
| Strongly disagree | 4 | 4.35 | 1.20, 10.76 |
| **What are your feelings concerning antibiotic prescription at your facility or practice?** | | | |
| All antibiotics are under-prescribed | 1 | 1.10 | 0.02, 5.97 |
| Some antibiotics are under-prescribed | 6 | 6.59 | 2.46, 13.80 |
| All antibiotics are appropriately prescribed | 47 | 51.65 | 40.93, 62.26 |
| Some antibiotics are over-prescribed | 34 | 37.36 | 27.44, 48.13 |
| All antibiotics are over-prescribed | 3 | 3.30 | 0.69, 9.33 |
| **Do you feel like you sometimes over-prescribe antibiotics?** | | | |
| Yes | 42 | 45.65 | 35.22, 56.37 |
| No | 50 | 54.35 | 43.63, 64.78 |
| **Your colleagues over-prescribe antibiotics.** | | | |
| Strongly agree | 6 | 6.52 | 2.43, 13.66 |
| Agree | 34 | 36.96 | 27.12, 47.66 |
| Neither agree nor disagree | 39 | 42.39 | 32.15, 53.14 |
| Disagree | 13 | 14.13 | 7.74, 22.95 |
| Strongly disagree | 0 | 0.00 | 0.00, 0.00 |
| **Veterinarians at your practice or facility always comply with antibiotic prescription policies.** | | | |
| Strongly agree | 20 | 21.74 | 13.81, 31.56 |
| Agree | 35 | 38.04 | 28.12, 48.76 |
| Neither agree nor disagree | 32 | 34.78 | 25.15, 45.43 |
| Disagree | 4 | 4.35 | 1.20, 10.80 |
| Strongly disagree | 1 | 1.09 | 0.03, 5.91 |

[1]95% Confidence Interval

slightly more than half, (17.4% strongly agreed and 38% agreed) relied on laboratory results before prescribing antimicrobials (Table 4.3).

### 4.5.6 Opinions on antimicrobial prescription practices

Overall, approximately half (51.7%) of the veterinarians believed that antimicrobials were appropriately prescribed, while 37.4% believed that some antimicrobials were over prescribed (Table 4.3). Slightly more than half of the veterinarians in this study did not believe that they ever overprescribe antimicrobials, although 45.7% of them believed that they did indeed overprescribe antimicrobials (Table 4.3). Interestingly, 43.5% either strongly agreed (6.5%) or agreed (37.0%) that their colleagues over prescribed antimicrobials (Table 4.3). Of the respondents whose practices had antimicrobial prescription policies, 59.8% (21.7% strongly agreed and 38.0% agreed) believed that their colleagues always complied with antimicrobial prescription policies (Table 4.3). However, only 24.0% (3.3% strongly agree and 20.7% agreed) believed that antimicrobial prescription policies actually contributed to a change in the incidence of antimicrobial resistance at their facility or practice (Table 4.4).

### 4.5.7 Opinions on antimicrobial resistance

Almost all respondents (93.1%) agreed that improper use of antimicrobials contributes to selection for antimicrobial resistance. Nearly 20% either strongly agreed (1.2%) or agreed (18.6%) that improper antimicrobial prescription practices among their colleagues were affecting the selection for antimicrobial resistance at their facility (Table 4.4). However, only 15.1% of the veterinarians thought that there had been an increase in the incidence of antimicrobial resistance at their practice (Table 4.4).

**Table 4.4: Distribution of responses related to opinions about antimicrobial resistance among veterinarians in Kentucky, 2017**

| Question/Response | Number | Percentage | 95% CI[1] |
|---|---|---|---|
| **Antibiotic prescription policies are contributing to a change in the frequency of antimicrobial resistance at your facility or practice.** | | | |
| Strongly agree | 3 | 3.26 | 0.68, 9.24 |
| Agree | 19 | 20.65 | 12.92, 30.36 |
| Neither agree nor disagree | 49 | 53.26 | 42.56, 63.74 |
| Disagree | 14 | 15.22 | 8.58, 24.21 |
| Strongly disagree | 7 | 7.61 | 3.11, 15.05 |
| **Improper use of antibiotics contributes to selection for antimicrobial resistance.** | | | |
| Strongly agree | 44 | 50.57 | 39.64, 61.47 |
| Agree | 37 | 42.53 | 31.99, 53.59 |
| Neither agree nor disagree | 6 | 6.90 | 2.57, 14.41 |
| Disagree | 0 | 0.00 | 0.00, 0.00 |
| Strongly disagree | 0 | 0.00 | 0.00, 0.00 |
| **How does improper use of antibiotics affect selection for antimicrobial resistance?** | | | |
| It does not affect selection for AMR[2] | 10 | 12.35 | 6.08, 21.54 |
| Improper use of antibiotics affects selection for AMR | 71 | 87.65 | 78.47, 93.92 |
| **Improper prescribing habits among your colleagues is affecting the selection for antibiotic resistance in your facility.** | | | |
| Strongly agree | 1 | 1.16 | 0.03, 6.31 |
| Agree | 16 | 18.60 | 11.02, 28.45 |
| Neither agree nor disagree | 42 | 48.84 | 37.90, 59.86 |
| Disagree | 21 | 24.42 | 15.80, 34.87 |
| Strongly disagree | 6 | 6.98 | 2.60, 14.57 |
| **There has been an increase in the number of cases of antimicrobial resistance at your facility or practice.** | | | |
| Strongly agree | 1 | 1.16 | 0.03, 6.31 |
| Agree | 12 | 13.95 | 7.42, 23.11 |
| Neither agree nor disagree | 30 | 34.88 | 24.92, 45.93 |
| Disagree | 33 | 38.37 | 28.08, 49.49 |
| Strongly disagree | 10 | 11.63 | 5.72, 20.35 |

[1]95% Confidence Interval
[2]Antimicrobial resistance

### 4.5.8 Predictors of knowledge and antimicrobial prescription practices

None of the variables investigated had significant univariable associations with "knowledge of antimicrobial resistance" (Table 4.5). Since none of the variables had significant association with the outcome, a multivariable model was not fit to the data. Additionally, none of the investigated variables had significant associations with the respondent's personal perceptions concerning antimicrobial prescription practices (Table 4.6) or with their perceptions regarding their colleagues' prescription practices. However, compared to veterinarians who work in primary care facilities, those who worked in referral clinics were significantly more likely (RRR = 6.25, 95% C.I. [1.10, 35.68], p=0.039) to strongly disagree/disagree with the idea that their colleagues overprescribe antimicrobials (Table 4.7).

**Table 4.5: Results of univariable multinomial logit model investigating predictors of veterinarian's opinion on whether "improper use of antimicrobials contributes to selection for antimicrobial resistance"**

| | | Strongly Agree/Agree | | | Strongly Disagree/Disagree | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Sample size | **RRR[1]** | **95% CI** | **P-value** | **RRR[1]** | **95% CI** | **P-value** | **Overall P-value** |
| **Gender** | 101 | | | | | | | 0.2736 |
| Male | 43 | 0.49 | 0.20, 1.22 | 0.1249 | 1.06 | 0.19, 5.93 | 0.9510 | |
| Female | 58 | ref. | ref. | ref | ref. | ref. | ref | |
| **City** | 98 | | | | | | | 0.6673 |
| Louisville | 18 | 0.90 | 0.23, 3.48 | 0.8785 | 0.75 | 0.06, 9.72 | 0.8259 | |
| Other | 54 | 1.91 | 0.68, 5.37 | 0.2222 | 1.06 | 0.15, 7.34 | 0.9538 | |
| Lexington | 26 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Veterinary practice** | 100 | | | | | | | 0.6068 |
| Large Animal | 19 | 0.47 | 0.15, 1.49 | 0.1985 | 0.53 | 0.05, 5.33 | 0.5857 | |
| Mixed | 23 | 1.43 | 0.45, 4.49 | 0.5442 | 0.88 | 0.08, 9.38 | 0.9121 | |
| Small Animal | 58 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Veterinary facility** | 100 | | | | | | | 0.6437 |
| Referral | 16 | 2.46 | 0.66, 9.21 | 0.1799 | 1.44 | 0.13, 16.41 | 0.7702 | |
| Veterinary Hospital | 29 | 1.53 | 0.56, 4.19 | 0.4041 | 0.58 | 0.06, 5.81 | 0.6392 | |
| Primary Care | 55 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Years of experience** | 93 | | | | | | | 0.9405 |
| 0-12 Years (≤Median) | 45 | 1.17 | 0.48, 2.88 | 0.7264 | <0.001 | <0.001, >999.999 | 0.9897 | |
| 13-50 Years (>Median) | 48 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Hours worked per week** | 92 | | | | | | | 0.9920 |
| 46-100 Hours (≤Median) | 45 | 1.01 | 0.41, 2.49 | 0.9807 | 1.12 | 0.20, 6.30 | 0.8997 | |
| 0-45 Hours (>Median) | 47 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Years since graduation** | 101 | | | | | | | 0.5302 |
| 26-35 Years (3rd Quartile) | 29 | 1.27 | 0.46, 3.50 | 0.6406 | 1.00. | 0.08, 12. 40 | 1.00 | |
| 36-55 Years (4th Quartile) | 20 | 1.04 | 0.32, 3.39 | 0.9494 | 4.29 | 0.59, 31.21 | 0.1508 | |
| 0-25 Years (1st Quartile & 2nd Quartile) | 52 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Antibiotic Policy** | 92 | | | | | | | 0.9032 |
| Yes | 39 | 1.12 | 0.50, 2.71 | 0.8104 | 1.47 | 0.26, 8.27 | 0.6643 | |
| No | 53 | ref. | ref. | ref. | ref. | ref. | ref. | |

[1]Relative Risk Ratios
[2]95% Confidence Interval

**Table 4.6: Results of univariable ordinary logistic regression model investigating predictors of opinions of veterinarians regarding whether they sometimes over-prescribe antimicrobials**

| Variable | Sample Size | OR[1] | 95% CI[2] | P-Value |
|---|---|---|---|---|
| **Gender** | 101 | | | |
| Male | 43 | 0.73 | 0.33, 1.63 | 0.4430 |
| Female | 58 | ref. | ref. | ref. |
| **City** | 98 | | | 0.8452 |
| Louisville | 18 | 1.02 | 0.30, 3.50 | 0.9772 |
| Other | 54 | 1.28 | 0.49, 3.33 | 0.6125 |
| Lexington | 26 | ref. | ref. | ref. |
| **Veterinary practice** | 100 | | | 0.6263 |
| Large Animal | 19 | 1.47 | 0.52, 4.19 | 0.4679 |
| Mixed | 23 | 1.50 | 0.57, 3.98 | 0.4150 |
| Small Animal | 58 | ref. | ref. | ref. |
| **Veterinary facility** | 100 | | | 0.6376 |
| Referral | 16 | 0.59 | 0.18, 1.92 | 0.3781 |
| Veterinary Hospital | 23 | 1.05 | 0.42, 2.60 | 0.9167 |
| Primary Care | 55 | ref. | ref. | ref. |
| **Years of experience** | 93 | | | 0.0844 |
| 0-12 Years (≤Median) | 45 | 2.09 | 0.91, 4.83 | 0.0844 |
| 13-50 Years (>Median) | 48 | ref. | ref. | ref. |
| **Hours worked per week** | 92 | | | 0.6583 |
| 46-100 Hours (≤Median) | 45 | 0.83 | 0.36, 1.89 | 0.6583 |
| 0-45 Hours (>Median) | 47 | ref. | ref. | ref. |
| **Years since graduation** | 101 | | | 0.3439 |
| 26-35 Years (3rd Quartile) | 29 | 0.66 | 0.26, 1.67 | 0.3794 |
| 36-55 Years (4th Quartile) | 20 | 0.46 | 0.15, 1.39 | 0.1701 |
| 0-25 Years (1st Quartile & 2nd Quartile) | 52 | ref. | ref. | ref. |
| **Antibiotic Policy** | 92 | 1.24 | 0.54, 2.84 | 0.6127 |

[1]Odds Ratios
[2]95% Confidence Interval

**Table 4.7: Results of univariable multinomial logistic regression model investigating predictors of opinions of veterinarians regarding if "they thought their colleagues over-prescribe antimicrobials"**

| | | Strongly Agree/Agree | | | Strongly Disagree/Disagree | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | | **RRR[1]** | **95% CI** | **P-value** | **RRR[1]** | **95% CI** | **P-value** | **Overall P-value** |
| **Gender** | 101 | | | | | | | 0.6115 |
| Male | 43 | 1.07 | 0.43, 2.63 | 0.8887 | 1.87 | 0.53, 6.63 | 0.3343 | |
| Female | 58 | ref. | ref. | ref | ref. | ref. | ref | |
| **City** | 98 | | | | | | | 0.7307 |
| Louisville | 18 | 0.77 | 0.20, 2.92 | 0.6997 | 2.86 | 0.41, 20.14 | 0.2921 | |
| Other | 54 | 0.73 | 0.26, 2.05 | 0.5526 | 1.67 | 0.29, 9.52 | 0.5656 | |
| Lexington | 26 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Veterinary practice** | 100 | | | | | | | 0.9956 |
| Large Animal | 19 | 0.96 | 0.31, 2.98 | 0.9414 | 0.72 | 0.13, 4.12 | 0.7110 | |
| Mixed | 23 | 0.96 | 0.31, 2.98 | 0.9414 | 1.08 | 0.23, 5.09 | 0.9243 | |
| Small Animal | 58 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Veterinary facility** | 100 | | | | | | | 0.2843 |
| Referral | 16 | 2.50 | 0.66, 9.51 | 0.1790 | 6.25 | 1.10, 35.68 | 0.0392 | |
| Veterinary Hospital | 29 | 1.50 | 0.54, 4.18 | 0.4380 | 3.13 | 0.69, 14.08, | 0.1380 | |
| Primary Care | 55 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Years of experience** | 93 | | | | | | | 0.3441 |
| 0-12 Years (≤Median) | 45 | 1.81 | 0.73, 4.53 | 0.2037 | 0.84 | 0.21, 3.37 | 0.8038 | |
| 13-50 Years (>Median) | 48 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Hours worked per week** | 92 | | | | | | | 0.3129 |
| 46-100 Hours (≤Median) | 45 | 2.01 | 0.80, 5.05 | 0.1362 | 1.17 | 0.30, 4.54 | 0.8241 | |
| 0-45 Hours (>Median) | 47 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Years since graduation** | 101 | | | | | | | 0.7609 |
| 26-35 Years (3rd Quartile) | 29 | 0.53 | 0.19, 1.49 | 0.2276 | 0.74 | 0.18, 3.02 | 0.6692 | |
| 36-55 Years (4th Quartile) | 20 | 1.05 | 0.33, 3.38 | 0.9323 | 0.74 | 0.12, 4.44 | 0.7369 | |
| 0-25 Years (1st Quartile & 2nd Quartile) | 52 | ref. | ref. | ref. | ref. | ref. | ref. | |
| **Antibiotic Policy** | 92 | | | | | | | 0.3997 |
| Yes | 39 | 1.17 | 0.48, 2.84 | 0.7272 | 0.43 | 0.10, 1.84 | 0.2564 | |
| No | 53 | ref. | ref. | ref. | ref. | ref. | ref. | |

[1]Relative Risk Ratios
[2]95% Confidence Interval

## 4.6 DISCUSSION

In this study we used a survey questionnaire to investigate antimicrobial prescription practices and opinions of Kentucky veterinarians regarding development of antimicrobial resistance. Nearly half of the veterinarians in this study indicated that antimicrobials were emphasized in multiple courses in their non-clinical years as veterinary students, this number jumped to 67% by the time they reached clinical years of study indicating an increase in antimicrobial training focus as veterinary students progressed through their curriculum. Although many studies worldwide have focused on the knowledge and perceptions of antimicrobials and AMR among medical and pharmacy students [247-254], only a small number of studies have focused on the number of courses that cover antimicrobials in veterinary students [255, 256] or examined the breadth of coverage concerning antimicrobials in both non-clinical and clinical years of study [249, 256]. Currently, there is a paucity of data investigating perceptions concerning the depth of education on antimicrobials in the non-clinical and clinical years of veterinary education. However, a nationwide study in the U.K. by Castro-Sanchez found that antimicrobial stewardship is included in the majority of undergraduate veterinary medicine courses in the U.K. [257]. Any gap in education regarding antimicrobials is dangerous because this lack of knowledge will later affect antimicrobial prescription practices once veterinarians are in clinical practice.

### 4.6.1 Antimicrobial prescription practices

This study found that the majority of veterinarians received information regarding antimicrobials and their use from textbooks/drug handbooks and continuing professional development courses and only 5% received this information from their practice's policies.

This differed from the findings of a study in the U.K. by Coyne et. al., that reported that veterinarians relied on their own experience and colleagues as well as the history of the farm [258]. However, mixed species practitioners consulted a wider variety of information sources on antimicrobials and were more likely to seek information from colleagues compared with practitioners working within specialist pig practices [258]. This information differed from our study in that we did not ask about consulting colleagues as a source of antimicrobial information. The source of information concerning antimicrobial use that veterinarians receive is important because accuracy of information ensures good antimicrobial stewardship and prescription practices as well as an implicit knowledge of the antimicrobial [259]. If the information that they rely on is outdated or inaccurate their antimicrobial prescription practices may reflect this gap in knowledge. This again highlights the need for knowledge based antimicrobial prescription policies in veterinary practice.

Almost all of the veterinarians in our study were able to prescribe antimicrobials without oversight. However, more than half of them indicated that their practice did not have a policy concerning antimicrobial prescription. This is similar to the findings of an Australian study by Hardefeldt et. al., that found that veterinary practices rarely had antimicrobial prescription policies [260]. This is concerning because lack of antimicrobial prescription policy implies that veterinarians have to rely on personal knowledge and opinions to make prescription decisions. This leaves room for variation in antimicrobial prescription practices which is a problem worldwide. This results in situations where certain antibiotic classes are preferred in certain countries or species [261]. Although no universal guideline or policy exists for antimicrobial prescription in veterinary medicine, in January of 2018 a unanimous vote by the American Veterinary Medical Association (AVMA) House of Delegates took place in order to enact a policy on antimicrobial stewardship [262]. The objective of the vote was to target veterinary antimicrobial prescription practices by working with practitioners in human medicine and regulatory agencies. This initiative and others like it are critical for efforts seeking to preserve the effectiveness of antimicrobials.

That more than 50% of the respondents were uncomfortable prescribing some antimicrobial is not uncommon and is consistent with the findings from a U.S. study by Jacob et. al., investigating opinions of clinical veterinarians at a US veterinary teaching hospital. They reported that 46% of survey respondents felt uncomfortable prescribing at least one class of antimicrobials [246]. This is a concern because the overuse of antibiotics drives the evolution of resistance [111]. It is critical to better manage concerns over improper use of antimicrobials so that injudicious use due to uncertainty does not become a widespread problem in veterinary medicine.

#### 4.6.2 Factors affecting antimicrobial prescription practices

In this study, route of administration, cost of antimicrobial, and risk of potential adverse drug reaction were the three most common factors reported to guide veterinarians' decision to prescribe antimicrobials to a patient. Understanding these factors is important in encouraging veterinarians to have responsible antimicrobial prescription practices and if possible to reduce antimicrobial use [263]. A qualitative study by Mateus et. al., in the U.K. that investigated factors associated with antimicrobial usage in small animal veterinary practices found that participants reported that antimicrobial prescription was influenced not only by veterinarian's preference for certain substances and previous experience, but by perceived efficacy, ease of administration of formulations, perceived compliance, willingness and ability to treat by pet owners, and animal characteristics as well [264]. Unlike in our study, Mateus et. al. identified cost as a factor only in low socioeconomic areas or areas of varying socioeconomic status [264]. That study only interviewed veterinarians at small animal clinics, while our study involved responses from veterinarians from various areas of practice. These differences in findings can be attributed to the differing antimicrobial prescription practices among the different veterinary practices (i.e. small animal, large animal, equine, etc). For instance, in farm animals, especially pigs and poultry, antimicrobial usage is generally directed at groups or herds of animals [115]. This is often the case when antimicrobials are used for prophylaxis. In large animal practice, antimicrobials can be given in continual low doses in order to enhance growth, feed conversion, or yield in healthy animals [115].

Our study indicated that over 80% of veterinarians either strongly agreed or agreed that they always relied on clinical signs and symptoms before prescribing an antimicrobial. However, only slightly more than half, either strongly agreed or agreed that they relied on laboratory results before prescribing an antimicrobial. It is important to note that veterinarians may request for culture and antimicrobial susceptibility tests prior to administering broad spectrum antibiotics with the option to change the antibiotic after receiving antimicrobial susceptibility test results. However, this still suggests that at least half of the respondents in this study take a responsible approach to antimicrobial prescription and supports the importance of antimicrobial prescription policies for further guidance. A U.S. study by Fowler et. al., found that only 36% of veterinarians reported ordering culture and sensitivity testing ‘often’ or ‘always’ when treating presumptive bacterial infections [265]. An Italian study by Barbarossa et. al., also identified low usage of laboratory testing, where survey respondents reported that only 7.0% made a habit of always waiting for laboratory results before starting the treatment [266]. Selecting an interim antimicrobial prior to running or receiving results from culture and sensitivity tests is not uncommon when clients often desire veterinarians to prescribe something when an animal is sick. Although more than half of the veterinarians in our study reported relying on laboratory testing, findings from the studies mentioned above did not. This could be due to accessibility to testing in Kentucky, where clinics can submit samples to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) or a commercial veterinary laboratory relatively easily.

#### 4.6.3 Opinions on antimicrobial prescription practices

Understanding the perceptions of veterinarians concerning the amount of antimicrobial use is crucial in gaining insight into the reasoning behind antimicrobial prescription practices. Although there was a pretty even split between those that believed that they did or did not overprescribe antimicrobials, approximately half of the veterinarians in this study believed that antimicrobials were appropriately prescribed. Less than 40% of respondents in the study believed that some antimicrobials were over prescribed. This is comparable to findings by Ekakoro and Okafor in Tennessee that reported that 51.6% of the respondents believed antimicrobials are being over-prescribed [267]. Antimicrobial over-prescription was identified by 88% of respondents in another U.S. study by Jacob et. al [25].

#### 4.6.4 Opinions on antimicrobial resistance

Personal beliefs regarding over prescription of antimicrobials differ among veterinarians. Research has shown that a combination of “patients, food animal producers, physicians and veterinarians have all played a part in misusing antimicrobials, often because of mistaken beliefs” [268]. The majority (93%) of the veterinarians in our study felt that improper use of antimicrobials contributes to selection for antimicrobial resistance. By contrast, an Australian study by Hardefeldt et. al., found that over 50% of respondents indicated that veterinary antimicrobial use had a moderate contribution to overall AMR [260].

#### 4.6.5 Predictors of knowledge and prescription antimicrobial practices

Gender, type of veterinary practice, years of experience, and years since graduation had no associations with either knowledge about the selection for antimicrobial resistance or respondents’ antimicrobial prescription practices. Of the veterinarians that responded to the questionnaire, women (58.0%) were represented about 16% higher than men (42.0%). An Australian study that examined opportunities and challenges to improving antibiotic prescribing practices also found that among veterinarians, women were over-represented (65.0%) [269]. Knowledge of how selection for antimicrobial resistance occurs was included in the education of most veterinarians.

## 4.7 STUDY LIMITATIONS

A limitation of this study is the relatively low number of respondents (n = 101). Despite several reminders requesting participation in the survey, we were unable to increase the total number of respondents beyond this number. This low response could have compromised the generalizability of study findings. Unfortunately, low participation rate is not uncommon in surveys involving veterinarians. Despite these limitations, the results from this study offer valuable information regarding antimicrobial prescription practices and opinions of veterinarians.

## 4.8 CONCLUSIONS

This study provides useful information on the level of knowledge and perceptions regarding prescription practices among veterinarians in Kentucky. However, discrepancies began to arise when trying to ascertain exactly who was to blame for AMR in their facilities. Despite the fact that the majority of veterinarians believed that improper use of antimicrobials was responsible for the development of AMR, very few believed that their colleagues were contributing to the problem, or even had increases of AMR in their facilities.

# 5 Antibiotic prescription practices and attitudes towards antimicrobial resistance among veterinarians in the City of Tshwane Metropolitan Municipality, South Africa

## 5.1 DISCLOSURE

Contents of this chapter have been submitted for publication to BMC Veterinary Research. Full citation is:

Adams R, Qekwana N, Oguttu J, Odoi A. Antibiotic prescription practices and attitudes towards antimicrobial resistance among veterinarians in the City of Tshwane Metropolitan Municipality, South Africa. 2019. Submitted to BMC veterinary research.

My primary contribution to this paper included (a) data management (b) performing all statistical analyses and (c) interpretation as well as preparation of the manuscript draft. Nenene Qekwana was involved in data collection as well as review of the manuscript. James Oguttu was involved in review and editing of the manuscript. Agricola Odoi was involved in conceptualization of research idea, study design, data analysis and interpretation as well as extensive editing of the manuscript. All authors read and approved the final manuscript.

## 5.2 ABSTRACT

**Background**: Antimicrobial prescription practices vary widely amongst veterinarians in South Africa. Therefore, understanding the prescription practices and attitudes of these veterinarians towards antimicrobial resistance (AMR) is critical for guiding efforts to curb AMR. Thus, this study investigated the knowledge, prescription practices and attitudes towards AMR among veterinarians in the City of Tshwane Metropolitan Municipality. A 30-question survey was administered to 54 respondents, which constituted a response rate of 65% (54/83). The percentages of responses to survey questions and their 95% confidence intervals were computed. Ordinary logistic models were used to investigate predictors of knowledge of antimicrobial resistance and antimicrobial prescription practices of respondents. Predictors of antimicrobial prescription practices of respondents' colleagues were investigated using multinomial logistic models.

**Results:** The majority (88%; 95% Confidence Interval (CI): 77.0-95.7) of respondents indicated that improper use of antimicrobials contributed to selection for AMR. As many as 32% (95% CI: 19.9-46.3) indicated that they tended to over-prescribe antimicrobials, while 37.8% indicated that their colleagues over-prescribed antimicrobials. The majority (68.6%; 95% CI:54.1-80.9) of the respondents worked at practices with antimicrobial prescription policies and 40% believed that antimicrobial prescription policies contributed to changes in the incidence of AMR at their practice. Veterinarians in mixed animal practice had significantly lower odds (OR=0.20; p=0.0103) of associating "improper use of antimicrobials" to "selection for AMR" compared to those in purely small animal practice. Compared to females, males were significantly more likely (Relative Risk Ratio [RRR]=10.5; p=0.002) to indicate that their colleagues over-prescribed antimicrobials rather than to "neither agree nor disagree" or "disagree."

**Conclusion:** Veterinarians in the study area have a reasonable understanding of the contribution of prescription practices to AMR. They were also aware of their own antimicrobial over-prescription practices as well as those of their colleagues. The fact that veterinarians in small animal practice tended to associate the problem of AMR with improper prescription practices more than their counterparts in mixed practice may indicate disparities in this knowledge. However, further studies are warranted to further investigate this. This study's findings are useful for guiding future studies and efforts to curb the problem.

## 5.3 BACKGROUND

Due to a combination of factors, but most notably the rise in use of antibiotics to treat both human and domestic animals, antimicrobial resistance (AMR) has become a global scientific and public health concern [29, 184]. The quantities of antimicrobials used in both human and veterinary medicine have, "resulted in the selection of pathogenic bacteria resistant to multiple drugs" [185]. There is evidence that widespread and indiscriminate use of antimicrobials in animals fosters the emergence of antimicrobial resistant zoonotic pathogens that inhibit the efficacy of current antibiotic therapies [270]. Moreover, the development and spread of AMR impedes both preventative and therapeutic uses of antibiotics. Worse still, the problem is becoming increasingly important in low-income African countries [271].

Levels of antimicrobial resistance vary greatly between countries, as do the antimicrobial prescription practices of medical and veterinary practitioners [21]. Unfortunately, one of the defining factors of inappropriate use of antimicrobials is prescription practices among veterinarians and physicians [22]. Compounding these variations and issues of misuse, are the lack of studies investigating attitudes of veterinarians towards antimicrobial prescription practices and usage [26].

Appropriate usage of antimicrobials is a controversial topic where opinions vary greatly amongst physicians and veterinarians worldwide including South Africa. Moreover, the rate of counterfeiting of pharmaceuticals has been recognized as being problematic in South Africa, where it is estimated that 1 in 5 medications on the market, including antibiotics are counterfeit [27]. This is exacerbated by the fact that, policies and procedures for antimicrobial prescriptions are rare and even when they are in place, they may not always be followed by clinicians [26]. Understanding the roles that opinions of veterinarians and clinic policies play in antimicrobial prescription practices is crucial for fully comprehending the problem of AMR. The fact that some veterinarians have reported feeling uncomfortable prescribing certain antibiotics [272] implies that veterinary clinics/hospitals need to take a closer look at the potential role of prescription practices of their veterinarians on the development of AMR. Furthermore, they may need to consider development and implementation of guidelines for antimicrobial use in their practices to help curb the development of AMR.

Several studies from the U.S., China, Italy, and Belgium have focused on assessing the knowledge and attitudes of medical students regarding antimicrobial resistance [273-276]. However, very few studies have addressed opinions and prescription practices of veterinarians. Furthermore, the majority of studies of veterinarians have largely focused on the antimicrobial prescription habits of veterinarians in Europe and Canada. The few studies that have been done in South Africa, have mainly investigated antimicrobial usage patterns [26]. Therefore, there is scarcity of information regarding antimicrobial prescription practices and opinions of veterinarians towards development of AMR in South Africa and yet this information is critical for guiding programs to slow down and/or curb the development of AMR. Therefore, the objectives of this study were to: (a) assess the knowledge, antimicrobial prescription practices, and attitudes towards AMR among veterinarians in the City of Tshwane Metropolitan Municipality (South Africa); and (b) identify predictors of their attitudes towards AMR.

## 5.4 METHODS

### 5.4.1 Survey setting and design

This is a cross-sectional questionnaire survey of practicing veterinarians in the City of Tshwane Metropolitan Municipality, South Africa. A 30-item questionnaire, adopted from two previous survey questionnaires [245, 246] was used to collect data on the opinions, knowledge, and antimicrobial prescription practices of veterinarians in the City of Tshwane Metropolitan Municipality. The original questionnaires, which had questions on opinions of clinical veterinarians regarding antimicrobial use and antimicrobial-resistant infections as well as antimicrobial prescribing patterns, were modified by adding questions on prescription practices, opinions about prescription practices, and antimicrobial resistance.

The final questionnaire was designed to take 20-30 minutes to complete and covered areas related to opinions of veterinarians regarding their antimicrobial prescription practices and how their prescription practices relate to the development of antimicrobial resistance. The questions were grouped into six sections: demographics, veterinary education, antimicrobial prescription practices, factors associated with prescribing habits, opinions about prescription practices, and opinions about antimicrobial resistance. The six sections contained both open-ended and close-ended questions consisting of a combination of yes/no questions, multiple choice questions as well as 5-point Likert scale questions (ranging from “strongly agree” to “strongly disagree”).

The questionnaire was pretested on a small sample of clinical veterinarians at the Faculty of Veterinary Medicine, University of Pretoria. After the pretest, the respondents were further asked a series of questions regarding the questionnaire to help identify problems with questions or the administration of the survey. This allowed identification and correction of ambiguous or misleading questions. For multiple choice questions, it also allowed identification and addition of response categories previously omitted.

### 5.4.2 Survey administration

The study was approved by both the University of Tennessee Institutional Review Board (number: 619622) and the Ethics Review Board of the University of South Africa (number: 2017/CAES/017). Heads of departments at the Faculty of Veterinary Medicine, University of Pretoria were requested for permission to distribute the survey to their clinical veterinary faculty. Faculty members were initially contacted, via email, by their respective department heads and requested to participate in the study. The respondents were again contacted by the investigators via email, and again requested to participate in the study.

An online version of the questionnaire was uploaded to Qualtrics [277] and participants were provided with a web link to access the survey and provide responses anonymously. Additionally, the survey was printed out and distributed in person to veterinarians working at 28 clinics in the City of Tshwane Metropolitan Municipality. To improve the response rate, paper copies of the survey questionnaire were also distributed to veterinary faculty members at the University of Pretoria. A reminder e-mail was sent to potential survey respondents to encourage them to complete the survey questionnaire. Phone calls were also made to remind veterinarians about the surveys. Of the 83 survey questionnaires that were distributed, a total of 54 were completed and returned between April and July 2017 resulting in a response rate of 65%.

### 5.4.3 Data analysis

All statistical analyses were performed in SAS 9.4 [278]. The distributions of demographic variables and their 95% confidence intervals were computed. The variables considered were the sex of the respondents, type of animal species treated at veterinary practice, the level of veterinary service, length of time at the facility, number of veterinarians at the facility, hours worked per week, and the year of graduation. Due to a small number of responses in some of the response categories of the question “Improper use of antimicrobials contributes to selection for AMR”, the answers “strongly agree” and “agree” were re-categorized into “agree” while “strongly disagree” and “disagree” into “disagree”.

Additionally, the 5-point Likert scale variable “My colleagues over-prescribe antimicrobials” was recoded “agree”, “disagree”, and “neither agree nor disagree”.

Shapiro-Wilk test of normality was used for evaluation of the distributions of the variables: number of years of work experience, years since graduation, and the number of veterinarians working or employed at any given practice. These variables were found to be non-normally distributed and hence median and interquartile ranges were reported.

Ordinary logistic regression models were used to investigate predictors of the outcome variables “improper use of antimicrobials contributes to selection for AMR” (Yes/No) and “Do you sometimes over-prescribed antibiotics” (Yes/No). Potential predictors considered for these models were gender, veterinary practice, veterinary facility, years of experience, hours worked per week, years since graduation and antibiotic policy. A Multinomial logistic regression model was used to investigate predictors of the outcome “Your colleagues over-prescribe antimicrobials” that had three possible responses: “agree”, “disagree”, and “neither agree nor disagree”. Potential predictors considered were the same as those for the ordinary logistic models.

For each of the models, the model building process was done in two steps. The first step entailed building a univariable logistic regression model (ordinary logistic and multinomial models). The univariable models were used to investigate the relationships between each potential predictor and each of the outcomes stated above. Potential predictors with p-values $\leq 0.20$ were considered for inclusion in multivariable regression models (ordinary logistic or multinomial). In the 2nd step, a multivariable model (ordinary logistic or multinomial) was fit using manual backward selection for each of the three outcome variables outlined above. Statistical significance for all multivariable models were assessed at $\alpha \leq 0.05$.

Confounding was assessed by comparing the change in model coefficients with and without the suspected confounders. If the removal of a suspected confounding variable resulted in a 20% or greater change in the coefficient of another variable, the variable that was removed was considered a confounder and retained in the model regardless of its statistical significance. However, no confounders was identified.

Odds ratios (ORs) and their 95% confidence intervals were computed for ordinary logistic models, while relative risk ratios (RRRs) as well as their 95% confidence intervals were computed for multinomial models. Hosmer-Lemeshow goodness-of-fit tests were used to assess the goodness-of-fit of the ordinary logistic regression models. For the multinomial logistic model, the goodness-of-fit was assessed by fitting ordinary logistic regression models to each pairwise combination of the three potential outcome categories as recommended by Dohoo, Martin and Stryhn [279]. Hosmer-Lemeshow goodness-of-fit was then checked for each of the binomial models separately. The reason for adopting this approach was that currently there are no available multinomial model fit assessment tests in SAS, the statistical software used in this study.

## 5.5 RESULTS

### 5.5.1 Respondent information

Of the 83 survey questionnaires that were distributed, a total of 54 were completed and returned between April and July 2017 resulting in a response rate of 65%. Eight of the respondents completed the online questionnaire while 46 completed the paper copies of the questionnaire.

Out of the 54 veterinarians who participated in the study, 53.7% (29/54) were females and 46.3% (25/54) were males (Table 5.1). Most (71.7%; 38/53) of the respondents were in small animal practice, while the rest (28.3%;15/53) were involved in mixed animal practice (Table 5.1). Slightly more than half (55.6%; 30/54) of the veterinarians worked at veterinary hospitals while the remaining 44.4% (24/54) worked at primary care facilities (Table 5.1).

The median number of years of work experience of the respondents was 3 years (Interquartile Range (IR):2, 7) while the median years since graduation was 10 years (IR: 0, 26). The median number of veterinarians working or employed at any given practice was 4 (IR: 1, 14).

***Table 5.1: Demographics of veterinarians in the City of Tshwane Metropolitan Municipality, South Africa (2017)***

| Variable | Number | Percent (%) | 95% CI[1] |
|---|---|---|---|
| **Sex** | **n=54** | | |
| Female | 29 | 53.7 | 39.6, 67.4 |
| Male | 25 | 46.3 | 32.6, 60.4 |
| **Veterinary Practice** | **n=53** | | |
| Mixed | 15 | 28.3 | 16.8, 42.4 |
| Small Animal | 38 | 71.7 | 57.7, 83.2 |
| **Veterinary Facility** | **n=54** | | |
| Primary Care | 24 | 44.4 | 30.9, 58.6 |
| Veterinary Hospital | 30 | 55.5 | 41.4, 69.1 |

[1]95% Confidence Interval

### 5.5.2 Veterinary education

Over half (55.6%; 30/54) of the veterinarians in practice indicated that antibiotics were emphasized in multiple courses during the pre-clinical years of their veterinary education, while 64.8% indicated that antibiotics were emphasized in courses taught during the clinical years of their veterinary training. Pharmacologist or clinical pharmacologists constituted the largest number of people (72.2%; 39/54) who were responsible for antibiotics training. This was followed by clinicians (29.6%; 16/54) (Table 5.2). With regard to the postgraduate training, just under half (42.6%; 23/54) of the respondents indicated that they had completed post graduate training (Table 5.2).

### 5.5.3 Antimicrobial prescription practices

The majority of the veterinarians (81.5%; 44/54) received their information regarding antimicrobials and their use from textbooks/drug handbooks. This was followed by continuing professional education courses (70.4%; 38/54), and peer reviewed scientific literature (55.6%; 30/54). Only 24% (13/54) of the veterinarians indicated that they had received information regarding antimicrobials and their usage from the policies of the practices where they worked (Table 5.3).

The majority (92.3%; 48/52) of the veterinarians were able to prescribe antimicrobials without supervision, or oversight. Only 31% (16/51) of the respondents indicated that their practices did not have antimicrobial prescription policies. However, 77.6% (38/49), reported prescribing antimicrobials multiple times per day. More than half (60.4%; 21/53) of the respondents reported that they were not comfortable prescribing some antibiotics (Table 5.3).

***Table 5.2: Antibiotics training among veterinarians in the City of Tshwane Metropolitan Municipality, South Africa (2017)***

| **Question/Response** | **Number** | **Percent** | **95% CI[1]** |
|---|---|---|---|
| **What was the emphasis on antibiotics in veterinary school education (non-clinical years)?** | **n=54** | | |
| Topic was not covered | 0 | 0.0 | 0.0, 6.6 |
| Light emphasis | 4 | 7.4 | 2.1, 17.9 |
| Covered thoroughly in one course | 21 | 38.9 | 25.9, 53.1 |
| Emphasized in multiple courses | 30 | 55.6 | 41.4, 69.1 |
| **What was the emphasis on antibiotics in your veterinary school education (clinical years)?** | **n=54** | | |
| Topic was not covered | 0 | 0.0 | 0.0, 0.1 |
| Light emphasis | 12 | 22.2 | 12.0, 35.6 |
| Covered thoroughly in one course | 7 | 13.0 | 5.4, 24.9 |
| Emphasized in multiple courses | 35 | 64.8 | 50.6, 77.3 |
| **What was the background of the person primarily responsible for your education on antibiotics during your veterinary education?** | **n=54** | | |
| Clinical pharmacist | 7 | 13.0 | 5.4, 24.9 |
| Clinical microbiologist | 6 | 11.1 | 4.2, 22.6 |
| Clinician | 16 | 29.6 | 18.0, 43.6 |
| Pharmacologist/clinical pharmacologist | 39 | 72.2 | 58.4, 83.5 |
| Toxicologist | 4 | 7.4 | 2.1, 17.9 |
| Don’t know his/her background | 1 | 1.9 | 0.1, 9.9 |
| **Do you hold any additional post graduate qualifications?** | **n=54** | | |
| Yes | 23 | 42.6 | 29.2, 56.8 |
| No | 31 | 57.4 | 43.2, 70.8 |

[1]95% Confidence Interval

***Table 5.3: Prescription practices among veterinarians in the City of Tshwane Metropolitan Municipality, South Africa (2017)***

| Question/Responses | Number | Percentage | 95% CI |
|---|---|---|---|
| **What are the main sources that you use to receive current information on antimicrobials and their use?** | **n=54** | | |
| Practice policy | 13 | 24.0 | 13.5, 37.6 |
| Pharmaceutical companies | 24 | 44.4 | 30.9, 58.6 |
| Veterinary medicine directorates | 12 | 22.2 | 12.0, 35.6 |
| Peer reviewed scientific literature | 30 | 55.6 | 41.4, 69.1 |
| Textbook/drug handbook | 44 | 81.5 | 68.6, 90.8 |
| Continuing professional development courses | 38 | 70.4 | 56.4, 82.0 |
| **Can you prescribe antibiotics without supervision, approval, or additional oversight?** | **n=52** | | |
| Yes | 48 | 92.3 | 81.5, 97.9 |
| No | 4 | 7.7 | 2.1, 18.5 |
| **Does your veterinary facility or practice have a policy concerning antibiotic prescription?** | **n=51** | | |
| Yes | 35 | 68.6 | 54.1, 80.9 |
| No | 16 | 31.4 | 19.1, 45.9 |
| **On average, how often do you prescribe antibiotics?** | **n=49** | | |
| Multiple times per day | 38 | 77.6 | 63.4, 88.2 |
| Once per day | 4 | 8.2 | 2.3, 19.6 |
| Once every two days | 2 | 4.1 | 0.5, 13.9 |
| Once per week | 2 | 4.1 | 0.5, 13.9 |
| Once per month | 2 | 4.1 | 0.5, 13.9 |
| **Is there any antibiotic that you do not feel comfortable prescribing?** | **n=53** | | |
| Yes | 21 | 39.62 | 26.5, 54.0 |
| No | 32 | 60.38 | 46.0, 73.6 |
| **Do any of the factors below affect your decision when choosing to prescribe an antibiotic to a patient?** | n=54 | | |
| Cost of antibiotic | 39 | 72.2 | 58.4, 83.5 |
| Client insurance | 2 | 3.7 | 0.5, 12.8 |
| Client expectations | 9 | 16.7 | 7.9, 29.3 |
| Route of administration | 44 | 81.5 | 68.6, 90.8 |
| Frequency of patient visits | 16 | 29.6 | 17.9, 43.6 |
| Risk of potential adverse drug reaction | 43 | 79.6 | 66.5, 89.37 |
| **You always rely on clinical signs and symptoms when prescribing an antibiotic.** | **n=54** | | |
| Strongly agree | 28 | 51.8 | 37.8, 65.7 |
| Agree | 20 | 37.0 | 24.3, 51.3 |
| Neither agree nor disagree | 2 | 3.7 | 0.5, 12.8 |
| Disagree | 3 | 5.6 | 1.2, 15.4 |
| Strongly disagree | 6 | 11.1 | 4.2, 22.6 |

Table 5.3 Continued

| Question/Responses | Number | Percentage | 95% CI |
|---|---|---|---|
| **You rely on laboratory results before prescribing an antibiotic.** | **n=53** | | |
| Strongly agree | 4 | 7.6 | 2.1, 18.2 |
| Agree | 19 | 35.9 | 23.1, 50.2 |
| Neither agree nor disagree | 15 | 28.3 | 16.8, 42.3 |
| Disagree | 11 | 20.8 | 10.8, 34.1 |
| Strongly disagree | 4 | 7.6 | 2.1, 18.2 |
| **What are your feelings concerning antibiotic prescription at your facility or practice?** | **n=52** | | |
| All antibiotics are under-prescribed | 0 | 0.0 | 0.0, 0.0 |
| Some antibiotics are under-prescribed | 6 | 11.5 | 4.4, 23.4 |
| All antibiotics are appropriately prescribed | 32 | 61.5 | 47.0, 74.7 |
| Some antibiotics are over-prescribed | 14 | 26.9 | 15.6, 41.0 |
| All antibiotics are over-prescribed | 0 | 0.0 | 0.0, 6.8 |
| **Do you feel like you sometimes over-prescribe antibiotics?** | **n=53** | | |
| Yes | 17 | 32.1 | 19.9, 46.3 |
| No | 36 | 67.9 | 53.7, 80.1 |
| **Your colleagues over-prescribe antibiotics.** | **n=53** | | |
| Strongly agree | 4 | 7.6 | 2.1, 18.2 |
| Agree | 16 | 30.2 | 18.3, 44.3 |
| Neither agree nor disagree | 18 | 34.0 | 21.5, 48.3 |
| Disagree | 13 | 24.5 | 13.8, 38.3 |
| Strongly disagree | 2 | 3.8 | 0.5, 12.9 |
| **Veterinarians at your practice or facility always comply with antibiotic prescription policies.** | **n=53** | | |
| Strongly agree | 5 | 9.4 | 3.1, 20.7 |
| Agree | 23 | 43.4 | 29.8, 57.7 |
| Neither agree nor disagree | 16 | 30.2 | 18.3, 44.3 |
| Disagree | 9 | 17.0 | 8.0, 29.8 |
| Strongly disagree | 0 | 0.0 | 0.0, 6.7 |

[1]95% Confidence Interval

### 5.5.4 Factors influencing antimicrobial prescription practices

The most common factors that influenced decisions of the respondents on which antimicrobials to prescribe were cost of antibiotics (77.2%; 39/54), route of administration (81.5%; 44/54), and risk of potential adverse drug reaction (79.6%; 43/54) (Table 5.3). The majority of the respondents either strongly agreed (51.9%; 28/54) or agreed (37.04%; 20/54) that they always relied on clinical signs and symptoms to prescribe antimicrobials. Fewer respondents (43.5%) indicated that they either strongly agreed (7.6%) or agreed (35.9%) that veterinarians tended to base their prescription on antibiogram (Table 5.3).

### 5.5.5 Opinions on antimicrobial prescription practices

As many as 61.5% (32/52) of the veterinarians were of the view that in general antimicrobials are often appropriately prescribed, while 26.9% (14/52) were of the view that some antimicrobials tended to be over-prescribed. However, 32.1% (17/53) believed that they sometimes over-prescribe antimicrobials. Additionally, 37.8% (20/53) agreed (strongly agreed; 7.6%; 4/53 or agreed 30.2%; 16/53) that their colleagues over-prescribed antimicrobials. Slightly over half (52.8%) of the respondents (9.4%; 5/53) strongly agreed, while under half (43.4%; 23/53) agreed that the colleagues at their practice or facility always complied with antimicrobial prescription policies (Table 5.3).

Slightly more than half of the respondents (52.8%; 28/53) either strongly agreed (9.4%; 5/53) or agreed (43.4%; 23/53) that they always comply with antibiotic prescription policies. Overall, only 39.6% (21/53) of the respondents indicated that they were aware that antimicrobial prescription policies contributed to a change in the incidence of antimicrobial resistance at their facility or practice (Table 5.4). Among the respondents whose practice did not have an antibiotic prescription policy (31.4%; 16/51), only 13.7% agreed that antimicrobial prescription policies contributed to a change in the incidence of antimicrobial resistance at their facility or practice.

***Table 5.4: Opinions on AMR among veterinarians in the City of Tshwane, Metropolitan Municipality, South Africa (2017)***

| Question/Response | Number | Percentage | 95% CI[1] |
|---|---|---|---|
| **Antibiotic prescription policies are contributing to a change in the frequency of antimicrobial resistance at your facility or practice.** | **n=53** | | |
| Strongly agree | 4 | 7.6 | 2.1, 18.2 |
| Agree | 17 | 32.1 | 19.9, 46.3 |
| Neither agree nor disagree | 23 | 43.4 | 29.8, 57.7 |
| Disagree | 7 | 13.2 | 5.5, 25.3 |
| Strongly disagree | 2 | 3.8 | 0.5, 13.0 |
| **Improper use of antibiotics contributes to selection for antimicrobial resistance.** | **n=53** | | |
| Strongly agree | 34 | 64.2 | 49.8, 76.9 |
| Agree | 13 | 24.5 | 13.8, 38.3 |
| Neither agree nor disagree | 2 | 3.8 | 0.5, 13.0 |
| Disagree | 4 | 7.6 | 2.1, 18.2 |
| Strongly disagree | 0 | 0.0 | 0.0, 6.7 |
| **How does improper use of antibiotics affect selection for antimicrobial resistance?** | **n=38** | | |
| It does not affect selection for AMR[2] | 3 | 7.9 | 1.7, 21.4 |
| Improper use of antibiotics affects selection for AMR | 35 | 92.1 | 78.6, 98.3 |
| **Improper prescribing habits among your colleagues is affecting the selection for antibiotic resistance in your facility.** | **n=53** | | |
| Strongly agree | 3 | 5.7 | 1.2, 15.7 |
| Agree | 14 | 26.4 | 15.3, 40.3 |
| Neither agree nor disagree | 27 | 50.9 | 36.8, 64.9 |
| Disagree | 7 | 13.2 | 5.5, 25. 3 |
| Strongly disagree | 2 | 3.8 | 0.5, 13.0 |
| **There has been an increase in the number of cases of antimicrobial resistance at your facility or practice.** | **n=52** | | |
| Strongly agree | 3 | 5.8 | 1.2, 16.0 |
| Agree | 10 | 19.2 | 9.6, 32.5 |
| Neither agree nor disagree | 18 | 34.6 | 22.0, 49.1 |
| Disagree | 18 | 34.6 | 22.0, 49.1 |
| Strongly disagree | 3 | 5.8 | 1.2, 16.0 |

[1]95% Confidence Interval
[2]Antimicrobial resistance

### 5.5.6 Opinions on antimicrobial resistance

The majority of respondents (92.1%; 47/53) agreed that improper use of antimicrobials contributes to selection for antimicrobial resistance. A third of these 92.1% indicated that they either strongly agreed (5.7%; 3/53) or agreed (26.4%; 14/53) that improper antimicrobial prescription practices among their colleagues were affecting the selection for antimicrobial resistance at their facility. However, 25% (13/52) of the veterinarians thought that there had been an increase in the incidence of antimicrobial resistance cases at their practice (Table 5.4). Of those that believed the antimicrobial prescription practices of their colleagues were affecting the selection for antimicrobial resistance at their facility, only 14.3% believed that there had been an actual increase in the incidence of AMR at their practice.

### 5.5.7 Predictors of knowledge and antimicrobial prescription practices

Using a relaxed critical p-value of ≤0.2 in the univariable models, gender (p= 0.0778), type of veterinary practice (p=0.0103), and years of experience (p=0.0643) were significantly associated with the opinion that "improper use of antimicrobial contributes to selection for antimicrobial resistance", and were thus included in the multivariable logistic regression model (Table 5.5). Ultimately, only veterinary facility (p=0.0103) was significantly associated with the opinion that improper use of antimicrobials contributes to selection for antimicrobial resistance (Table 5.6). Similarly, using a relaxed p-value of ≤0.2, only gender (p=0.0974) was considered for inclusion in the multivariable logistic regression model, with "Do you sometimes over-prescribe antimicrobials" as the outcome

**Table 5.5: Univariable logistic model investigating predictors of "improper use of antimicrobials contributes to AMR[1]"**

| Variable | Number | OR[2] | 95% CI[3] | P-Value |
|---|---|---|---|---|
| **Gender** | n=54 | | | |
| Male | 25 | 14.7 | 0.7, 292.8 | 0.0778 |
| Female | 29 | ref. | . | . |
| **Veterinary practice** | 53 | | | |
| Mixed | 15 | 0.2 | <0.0, 0.4 | 0.0103 |
| Small Animal | 38 | ref. | . | . |
| **Veterinary facility** | 54 | | | |
| Veterinary Hospital | 30 | 0.6 | 0.1, 3.4 | 0.5891 |
| Primary Care | 24 | ref. | . | . |
| **Years of experience** | 53 | | | |
| ≥4 | 26 | 16.8 | 0.9, 334.0 | 0.0643 |
| 0-3 | 27 | ref. | . | . |
| **Hours worked per week** | 51 | | | |
| ≤44 | 25 | 0.5 | 0.1, 2.5 | 0.3732 |
| ≥45 | 26 | ref. | . | . |
| **Years since graduation** | 54 | | | 0.1674 |
| 6-10 Years | 11 | 1.4 | 0.2, 8.5 | 0.7373 |
| 11-42 Years | 26 | 19.1 | 0.90, 406.8 | 0.0589 |
| 0-5 Years | 17 | ref. | . | . |
| **Antibiotic Policy** | 51 | | | |
| No | 16 | 4.7 | 0.2, 101.4 | 0.3219 |
| Yes | 35 | ref. | . | . |

[1]Antimicrobial Resistance
[2]Odds Ratios
[3]95% Confidence Interval

**Table 5.6: Final logistic model investigating predictors of "improper use of antimicrobials contributes to AMR[1]"**

| Variable | Number | OR[2] | 95% CI[3] | P-Value |
|---|---|---|---|---|
| **Veterinary practice** | n=53 | | | |
| Mixed | 15 | 0.2 | <0.0, 0.4 | 0.0103 |
| Small Animal | 38 | ref. | ref. | ref. |

[1]Antimicrobial Resistance
[2]Odds Ratios
[3]95% Confidence Interval

(Table 5.7). However, gender was not significantly associated with the outcome at p-value ≤0.05.

A significant association was observed between “Your colleagues over-prescribe antimicrobials” and each of the variables gender (p=0.007), veterinary practice (p=0.178), and veterinary facility (p=0.166) in the univariable model at a relaxed p-value of ≤0.2. As a result, all were assessed in the multinomial model (Table 5.8). In the final model, when compared to female respondents, male respondents were significantly more likely (RRR=10.5; p=0.002) to agree that their colleagues over-prescribed antimicrobials rather than to neither agree nor disagree. (Table 5.9).

**Table 5.7: Univariable logistic model investigating predictors of "Do you sometimes over-prescribe antimicrobials"**

| Variable | Number | OR[1] | 95% CI[2] | P-Value |
|---|---|---|---|---|
| **Gender** | n=54 | | | |
| Male | 25 | 0.4 | 0.1, 1.2 | 0.0974 |
| Female | 29 | ref. | ref. | ref. |
| **Veterinary practice** | 53 | | | |
| Mixed | 15 | 0.4 | 0.1, 0.7 | 0.2444 |
| Small Animal | 38 | ref. | ref. | ref. |
| **Veterinary facility** | 54 | | | |
| Veterinary Hospital | 30 | 1.2 | 0.4, 3.9 | 0.7434 |
| Primary Care | 24 | ref. | ref. | ref. |
| **Years of experience** | 53 | | | |
| ≥4 | 26 | 1.3 | 0.4, 4.0 | 0.6977 |
| 0-3 | 27 | ref. | ref. | ref. |
| **Hours worked per week** | 51 | | | |
| ≤44 | 25 | 0.9 | 0.3, 2.9 | 0.8283 |
| ≥45 | 26 | ref. | ref. | ref. |
| **Years since graduation** | 54 | | | |
| 6-10 Years | 11 | 1.2 | 0.3, 5.5 | 0.8233 |
| 11-42 Years | 26 | 0.3 | 0.1, 1.3 | 0.1236 |
| 0-5 Years | 17 | ref. | ref. | ref. |
| **Antibiotic Policy** | 51 | | | |
| No | 16 | 1.3 | 0.4, 4.5 | 0.6699 |
| Yes | 35 | ref. | ref. | ref. |

[1]Odds Ratios
[2]95% Confidence Interval

**Table 5.8: Univariable multinomial logistic model investigating predictors of "Do your colleagues over-prescribe antimicrobials"**

| | | Agree | | | Disagree | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Number** | **RRR[1]** | **95% CI** | **P-value** | **RRR[1]** | **95% CI** | **P-value** |
| Gender | 51 | | | | | | |
| Male | 24 | 10.5 | 2.3, 47.2 | 0.0022 | 2.2 | 0.5, 10.6 | 0.3303 |
| Female | 27 | ref. | ref. | ref | ref. | ref. | ref |
| Veterinary practice | 53 | | | | | | |
| Mixed | 15 | 0.3 | 0.1, 1.2 | 0.0868 | 0.3 | 0.1, 1.7 | 0.1843 |
| Small Animal | 38 | ref. | ref. | ref. | ref. | ref. | ref. |
| Veterinary facility | 54 | | | | | | |
| Primary Care | 30 | 0.3 | 0.1, 1.3 | 0.1118 | 0.3 | 0.1, 1.3, | 0.1015 |
| Veterinary Hospital | 24 | ref. | ref. | ref. | ref. | ref. | ref. |
| Years of experience | 53 | | | | | | |
| ≥4 | 26 | 1.5 | 0.4, 5.5 | 0.5166 | 1.8 | 0.4, 7.7 | 0.4577 |
| 0-3 | 27 | ref. | ref. | ref. | ref. | ref. | ref. |
| Hours worked per week | 51 | | | | | | |
| ≤44 | 25 | 0.6 | 0.2, 2.2 | 0.4209 | 1.8 | 0.4, 8.1 | 0.4755 |
| ≥45 | 26 | ref. | ref. | ref. | ref. | ref. | ref. |
| Years since graduation | 54 | | | | | | |
| 6-10 Years | 11 | 2.3 | 0.3, 16.2 | 0.3911 | 2.3 | 0.3, 16.2 | 0.3911 |
| 11-42 Years | 26 | 2.6 | 0.6, 12.0 | 0.2132 | 1.1 | 0.2, 5.8 | 0.9158 |
| 0-5 Years | 17 | ref. | ref. | ref. | ref. | ref. | ref. |
| Antibiotic Policy | 51 | | | | | | |
| Yes | 16 | 1.2 | 0.3, 4.8 | 0.8126 | 1.0 | 0.2, 4.8 | 0.9778 |
| No | 35 | ref. | ref. | ref. | ref. | ref. | ref. |

[1]Relative Risk Ratios
[2]95% Confidence Interval

**Table 5.9: Final multinomial logistic regression model investigating predictors of "Do your colleagues over-prescribe antimicrobials"**

| | | Agree | | | Disagree | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Number** | **RRR[1]** | **95% CI** | **P-value** | **RRR[1]** | **95% CI** | **P-value** |
| Gender | 51 | | | | | | |
| Male | 24 | 10.5 | 2.3, 47.2 | 0.0022 | 2.2 | 0.5,10.6 | 0.3303 |
| Female | 27 | ref. | ref. | ref | ref. | ref. | ref |

[1]Relative Risk Ratios
[2]95% Confidence Interval

## 5.6 DISCUSSION

This study sought to explore gaps of knowledge concerning antimicrobial use among veterinarians using a questionnaire survey to investigate antimicrobial prescription practices among veterinarians in the City of Tshwane Metropolitan Municipality and their opinions regarding development of antimicrobial resistance. Although multiple studies have investigated the issues of knowledge and perceptions of antimicrobials and AMR among medical and pharmacy students [247-254], very few have focused on the antimicrobial training received in the veterinary curriculum [255, 256]. Moreover, few studies have examined breadth of coverage concerning antimicrobials in both pre-clinical and clinical years of veterinary training [249, 256]. While more than half (55.6%) of the respondents in our study indicated that antibiotics were emphasized in multiple courses during the pre-clinical years of their curriculum, 64.8% indicated that antibiotics were also emphasized in multiple courses during the clinical years of their veterinary curriculum.

Results show that more than half of the veterinarians indicated that antimicrobials had been emphasized in multiple courses during the pre-clinical years of their veterinary training. These findings are similar to those of a Kentucky study [280] which found that just under half of the veterinarians recalled antimicrobials being emphasized in multiple courses during their pre-clinical years.

### 5.6.1 Antimicrobial prescription practices

Veterinarians in this study received information regarding antimicrobials mainly from textbooks or drug handbooks and continuing professional development courses. Only

24% of the veterinarians indicated that they had received similar information from antimicrobial prescription policies at their practices. This is concerning, because this implies that 76% of respondents received information concerning antimicrobials from sources other than an antimicrobial prescription policy at their practice. Similarly, the Kentucky study [280] found that veterinarians received information regarding antimicrobials and their use from textbooks/drug handbooks and continuing professional development courses, with only 5% of veterinarians indicating that they received information regarding antimicrobials from antimicrobial prescription policies. With just under one fourth of respondents receiving information regarding antimicrobials from antimicrobial prescription policies, the question of judicious use becomes important. As judicious use of antimicrobials and antimicrobial stewardship in clinical practice is often reliant on understanding of antimicrobials and the source of information concerning antimicrobials [259], it becomes important for antimicrobial prescription policies to not only be in place but to provide veterinarians with up to date information.

Almost 70% (35/51) of the veterinarians indicated that they had antimicrobial prescription policies at their practices. The findings from our study are dissimilar to findings from an Australian study which reported that veterinary practices rarely had antimicrobial prescription policies [260]. In contrast, slightly less than half (42%;39/92) of the veterinarians in Kentucky did have a policy on antimicrobial prescription at their practices [280]. It is concerning to note that in this study, more than 30% of veterinary practices did not have a policy on antimicrobial prescription as the presence of antimicrobial prescription policy improves prudent use of antimicrobials. It has been shown that use of prescription policies decreases preferential selection of certain antibiotic classes, which is common in some countries or species [261]. In view of this, as more practices establish antimicrobial prescription policies we expected to see more consistency in clinical decision making related to antimicrobial prescription among veterinarians in the country. A higher proportion of veterinarians in this study (39.7%) than the 23.9% reported in the Kentucky study [280] believed that antimicrobial prescription policies actually contributed to a change in the incidence of antimicrobial resistance at their facility or practice. This suggests that there is insufficient recognition of the role that antimicrobial prescription policies play in the development of antimicrobial resistance in their facilities. This emphasizes the necessity for antimicrobial prescription policies in order to ensure comprehensive antimicrobial prescription policies in veterinary practice.

It has been reported in the U.S. among veterinarians that there is a tendency for veterinarians to be uncomfortable prescribing some antimicrobials. [246]. For example in the Kentucky study it was also found that more than 50% of the respondents were uncomfortable prescribing some antimicrobials [280]. A similar observation was made among the veterinarians in this study, where more than 50% of them indicated that they were uncomfortable prescribing some antimicrobials. Although we did ask veterinarians to elaborate on why they felt uncomfortable prescribing certain antimicrobials (some indicated that they were concerned about resistance if the antimicrobial was over used while others were concerned about the number of side effects), it would be important to further investigate the reasons for this and address them appropriately.

### 5.6.2 Factors affecting antimicrobial prescription practices

In this study, cost of antimicrobial, route of administration, and risk of potential adverse drug reaction were three of the most common factors reported to influence the veterinarian's choice of antimicrobial. These are similar to findings of the Kentucky study that also reported that these three were the most common factors reported to guide veterinarians' decision to prescribe antimicrobials in the Kentucky study [280]. Mateus et. al., [264] have also reported cost as a factor in low socioeconomic areas. Therefore, it is not surprising that cost of antimicrobial is a major factor in antimicrobial prescription decisions in South Africa, a developing country as well as in Kentucky, which ranks sixth poorest state in the U.S. as of 2016 [281]. Overall, this suggests a similarity in factors that influence antimicrobial prescription practices in the veterinarians in both studies.

Less than half (44%) of the veterinarians in this study relied on laboratory results before prescribing antimicrobials. This could be attributed to the fact that few practices had antimicrobial prescription policies. It is important to note that waiting for the results of the antiprogram is not uncommon in veterinary medicine [282]. In the Kentucky study it was also observed that more than half relied on laboratory results before prescribing an antimicrobial [280]. In contrast Fowler et. al., [265] in the US reported lower proportions (36%) of veterinarians who chose to order culture and sensitivity tests before treating bacterial infections and Barbarossa et. al., [266] in Italy reported 7.0% of veterinarians who did the same.

### 5.6.3 Opinions on antimicrobial prescription practices

This study found that 27% of the veterinarians believed that some antimicrobials were over-prescribed, which is much less than the 51.6% reported among the veterinarians in the US [267]. Much higher levels (88%) have been reported by another US study done in North Carolina [25]. Although slightly higher than the findings of this study, the Kentucky study found that less than 40% of respondents believed that some antimicrobials were over-prescribed [280]. Similar to the findings of the Kentucky study, this study found that less than half of the participants believed that they did over-prescribe antimicrobials [280].

### 5.6.4 Opinions on antimicrobial resistance

This study found that almost 90% of the veterinarians were of the view that improper use of antimicrobials contributes to selection for antimicrobial resistance. Similar to the findings of a Kentucky study which reported that the majority (93%) of veterinarians agreed that improper use of antimicrobials contributes to selection for antimicrobial resistance [280]. This reflects the degree of knowledge and understanding of the problem of injudicious use of antimicrobials and antimicrobial resistance in the current study population.

### 5.6.5 Predictors of knowledge and antimicrobial prescription practices

Compared to veterinarians in small animal practice, those in mixed animal practice were less likely to be aware that improper use of antimicrobials contributes to selection for AMR. This suggests that vets in different practice types have differences in understanding of AMR probably due to the differences in the sources of information that they use at their practices concerning on antimicrobials. In contrast, a Kentucky study found no association between the type of practice and knowledge that improper use of antimicrobials contributes to selection for AMR [280]. Male respondents had higher odds (OR=9.1) of reporting that their colleagues over-prescribe antimicrobials than their female counterparts. This may imply that women were less likely to report over-prescribing practices, which could be attributed to their more empathic nature [283, 284]. It is important for practices to encourage all veterinarians to recognize and point out over prescription of antimicrobials at their practice.

### 5.7 STUDY LIMITATIONS

The low number of respondents (n = 54) in this study was a major limitation. Despite printing out and distributing the survey questionnaire in person to veterinarians in the study area, as well as offering an online version of the study questionnaire we were unable to increase the total number of respondents beyond this number. This low response could have compromised the generalizability of study findings. Unfortunately, low participation rate is not uncommon in surveys involving veterinarians. Despite these limitations, the results from this study offer valuable information regarding antimicrobial prescription practices and opinions of veterinarians.

### 5.8 CONCLUSIONS

Veterinarians in the study area have a reasonable understanding of the contribution of prescription practices to AMR. They were also aware of their own antimicrobial over-prescription practices as well as those of their colleagues. The fact that veterinarians in small animal practice tended to associate the problem of AMR with improper prescription practices more than their counterparts in mixed practice may indicate disparities in this knowledge. However, further studies are warranted to further investigate this. Antimicrobial prescription policies are not widely adopted among veterinary practices in the study area. Therefore, we recommend a drive for practices to adopt antimicrobial prescription policies to ensure judicious use of antimicrobials.

This study's findings are useful for guiding future studies and efforts to curb the problem.

# 6 Summary, conclusions and recommendations

## 6.1 SUMMARY

In this study, the proportion of resistant isolates of *Staphylococcus* in horses was found to be high for both pathogenic and non-pathogenic *Staphylococcus* species. A significant decreasing temporal trend was found for AMR, while an increasing temporal trend was observed for MDR. Thus, although there is a decreasing trend in AMR , the increasing trend in MDR among horses in this study population could have a negative impact on morbidity and mortality rates attributable to *Staphylococcus* infections. Interestingly, despite decreasing trends in AMR, the overall proportions of AMR isolates were high, while proportions of MRSA infections were much lower despite significant increasing temporal trends in overall MDR. Although relatively high prevalence of MDR has been identified and such bacteria have been the cause of infections in horses [32] decreasing trends in AMR have been linked to prudent use of antimicrobials, based on antibiotic stewardship programs, appropriate usage of diagnostic testing and antimicrobial susceptibility testing [172]. The reasons for the decreasing trend in this study are likely due to these factors. Among the antimicrobials studied, the highest levels of AMR were seen in β-Lactams and Aminoglycosides, which is likely attributable to selection pressure and the multiple mechanisms of these classes of antimicrobials. The high proportion of AMR (70.5%) seen in thoroughbreds could be due to the extensive movement of this particular horse breed due to the necessity of travel to horse race shows. This increases the risk of exposure to resistant *Staphylococcus* strains and potentially contributing to the high AMR. The higher odds of MDR observed among horses less than 1 year is likely due to the higher susceptibility of younger animals to infection resulting in higher likelihood of antimicrobial treatment and hence higher selection pressure for resistance. Among *Staphylococcus aureus* the significantly higher odds of AMR observed compared with other *Staphylococcus* species is likely due to the virulence factors that enable *S. aureus* to readily adapt to different environmental niches in diverse hosts [285], as well as the high prevalence of methicillin resistance among *Staphylococcus aureus* isolates.

Almost half of the veterinarians in Kentucky indicated that antimicrobials were emphasized in multiple courses in their pre-clinical years as veterinary students, which increased by more than 20% by the time they reached clinical years of study. In South Africa, more than half of the respondents indicated that antimicrobials had been emphasized in multiple courses during the preclinical years of their veterinary training. This increased by 10% by the clinical years of study. This suggests an increase in antimicrobial training focus in both Kentucky and South Africa, as veterinary students progressed through their curriculum. In Kentucky, 26% of veterinarians' sources of information regarding antimicrobials were textbooks/drug handbooks and continuing professional development courses, with only 5% coming from their practice's policies. Similarly in South Africa, textbooks/drug handbooks (81.5%) and continuing professional development courses (70.4%) were the most common information sources for antimicrobials. However, only 24% of respondents from South Africa received similar information from antimicrobial prescription policies at their practices. The lack of information being received from practice policies is important, because access to information is critical in the pursuit of judicious use as well as overall knowledge of antimicrobials. This emphasizes the need for antimicrobial prescription policies in veterinary practice. More than half of the veterinarians in Kentucky indicated that their practice did not have a policy concerning antimicrobial prescription, while in South Africa only 31% of veterinarians had a policy on antimicrobial prescription. This implies that many veterinarians have to rely solely on information not provided by their practice and personal experience to make prescription decisions. If more clinics/hospitals had antimicrobial prescription policies, there might be improvement in consistency in antimicrobial clinical decision making among veterinarians. More than 50% of the respondents were uncomfortable prescribing some antimicrobials in both Kentucky and South Africa, which indicates a knowledge of potential risks involved in prescribing certain antimicrobials. Route of administration, cost of antimicrobial, and risk of potential adverse drug reaction were the three most common factors reported to guide veterinarians' decision to prescribe antimicrobials to patients in both Kentucky and South Africa. This implies that both clinical factors and factors pertaining to cost play a role in antimicrobial prescription practices. Over 80% of veterinarians in Kentucky and over 90% of those in South Africa either strongly agreed or agreed that they always relied on clinical signs and symptoms before prescribing antimicrobials. However more than half of the veterinarians in Kentucky, either strongly agreed or agreed that they relied on laboratory results before prescribing an antimicrobial, while less than half in South Africa admitted to doing the same. This suggests that at least half of the respondents take a more measured approach to antimicrobial prescription while others may not have antimicrobial prescription policies for further guidance.

Less than 40% of the respondents in both South Africa and Kentucky believed that some antimicrobials were over prescribed. However, almost 40% of the respondents in South Africa believed that antimicrobial prescription policies actually contributed to a change in the incidence of antimicrobial resistance at their facility or practice, which was much higher than the 24% of respondents who felt the same in Kentucky. This indicates that respondents do credit changes in AMR to antimicrobial prescription policies in their facilities. Overall, most of the veterinarians felt that improper use of antimicrobials contributes to selection for antimicrobial resistance. This is important because it reflects the degree of knowledge and understanding of the problem of injudicious use of antimicrobials and antimicrobial resistance in our study population.

## 6.2 LIMITATIONS

The retrospective laboratory-based study data used in this study were not obtained using a statistical sampling technique, and therefore the study population should not be considered to be representative of the equine population in Kentucky. Thus, only data available in the laboratory records could be investigated limiting the scope of investigation. For instance, information on past antimicrobial use was not available and therefore associations with levels of AMR or MDR could not be assessed. Furthermore, past medical history of the animals whose samples were used in this study was not reported. Although cost effective, both survey studies have limitations. Discrepancy in recall among survey participants may have occurred. Additionally, the response rate of the Kentucky survey could not be determined because the exact number of veterinarians who received the survey was unknown.

## 6.3 RECOMMENDATIONS

Knowledge of AMR and judicious use of antimicrobials are integral components of good veterinary practice. In order to ensure that these remain priorities, promoting certain antimicrobial prescription guidelines and research initiatives geared towards practicing veterinarians is critical. Listed below are some recommendations for future efforts to address the problem of AMR infections among veterinarians.

- Focus on providing/continuing to provide annual continuing education classes or workshops concerning judicious use of antimicrobials and AMR for veterinarians at individual practices in order to keep up to date on judicious use guidelines.

- Encourage veterinarians to discuss AMR levels at their practice or facility. This could be done following the annual continuing education class or seminar. This would give veterinarians the opportunity to voice concerns regarding the problem of AMR as well as antimicrobial prescribing habits at their own practices.

- Appoint a team that will answer veterinarian questions or concerns about antimicrobial prescription at their practice or facility. This team could be made up of those who write the antimicrobial prescription policies for their facilities. A hard copy of the policy could be made available to all veterinarians in the practice. For practices without antimicrobial prescription policies, this team could consist of elected veterinarians from within the practice.

The high proportion of AMR (70.5%) seen in thoroughbreds in this study, indicates a need to better understand the burden of AMR among horses and the potential economic impact that AMR has on the equine industry. Additional research is needed concerning changing trends in AMR in equine species [286] and the economics of breeding and racing farms affected by AMR infections. This research should focus specifically on clinically relevant resistant bacteria such as (*Enterococcus faecium*, *Staphylococcus aureus*, *Clostridium difficile* (*Klebsiella pneumoniae*), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*) [287]. Successive research must then focus on understanding temporal trends in AMR in horses over time [288] and how this affects antimicrobial prescription habits of the veterinarians that are treating them.

With more than 50% of the respondents in both Kentucky and South Africa uncomfortable prescribing some antimicrobials, future studies should focus on how negative effects of antimicrobials affect antimicrobial prescription practices among veterinarians. Moreover, because route of administration, cost of antimicrobial, and risk of potential adverse drug reaction were the three most common factors reported to guide veterinarians' decision to prescribe antimicrobials to patients in both Kentucky and South Africa, these studies should focus on how the overall socioeconomic status of the geographic area affects antimicrobial prescription practices in veterinarians. It will be especially important to compare findings in developed nations such as the U.S. and in developing countries such as South Africa where economies differ greatly.

In South Africa, veterinarians in small animal practice tended to associate the problem of AMR with improper prescription practices more than their counterparts in mixed practice may indicate disparities in this knowledge. Future studies should focus on investigating the differences in prevalence of AMR among small animal and mixed animal practices in South Africa, as well as investigating the opinions of veterinarians at these practices concerning contributing factors to AMR. Additionally, because antimicrobial prescription policies were not widely adopted among veterinary practices in South Africa, future research should focus on the effect of implementing antimicrobial prescription practices on AMR levels in veterinary practices in the study area.

# References

1. Cantas L, Shah SQ, Cavaco LM, Manaia C, Walsh F, Popowska M, Garelick H, Bürgmann H, Sørum H: **A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota**. *Frontiers in Microbiology* 2013, **4**.
2. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD: **Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis**. *Bmj* 2010, **340**:c2096.
3. Organization WH: **Antimicrobial resistance: global report on surveillance**: World Health Organization; 2014.
4. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP: **Antibiotics in agriculture and the risk to human health: how worried should we be?** *Evolutionary applications* 2015, **8**(3):240-247.
5. Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ: **Human Health Consequences of Use of Antimicrobial Agents in Aquaculture**. *Clinical Infectious Diseases* 2009, **49**(8):1248-1253.
6. Marshall BM, Levy SB: **Food Animals and Antimicrobials: Impacts on Human Health**. *Clinical Microbiology Reviews* 2011, **24**(4):718-733.
7. Organization WH: **Antimicrobial use in aquaculture and antimicrobial resistance**. *Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance Seoul, Republic of Korea* 2006:5-17.
8. French G: **The continuing crisis in antibiotic resistance**. *International journal of antimicrobial agents* 2010, **36**:S3-S7.
9. Filice GA, Nyman JA, Lexau C, Lees CH, Bockstedt LA, Como-Sabetti K, Lesher LJ, Lynfield R: **Excess costs and utilization associated with methicillin resistance for patients with Staphylococcus aureus infection**. *Infection control and hospital epidemiology* 2010, **31**(4):365-373.
10. Guidos RJ, Infectious Diseases Society of A: **Combating Antimicrobial Resistance: Policy Recommendations to Save Lives**. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 2011, **52**(Suppl 5):S397-S428.
11. Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA: **Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant gram-negative bacteria**. *Antimicrob Agents Chemother* 2010, **54**(1):109-115.
12. Roberts RR, Hota B, Ahmad I, Scott RD, 2nd, Foster SD, Abbasi F, Schabowski S, Kampe LM, Ciavarella GG, Supino M *et al*: **Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009, **49**(8):1175-1184.
13. Founou RC, Founou LL, Essack SY: **Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis**. *PLoS One* 2017, **12**(12):e0189621.

14. Bengtsson B, Greko C: **Antibiotic resistance—consequences for animal health, welfare, and food production**. *Upsala journal of medical sciences* 2014, **119**(2):96-102.
15. Foster JD, Trepanier LA, Ginn JA: **Use of linezolid to treat MRSP bacteremia and discospondylitis in a dog**. *Journal of the American Animal Hospital Association* 2014, **50**(1):53-58.
16. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP: **Antimicrobial resistance in developing countries. Part I: recent trends and current status**. *The Lancet infectious diseases* 2005, **5**(8):481-493.
17. Clardy J, Fischbach MA, Currie CR: **The natural history of antibiotics**. *Current biology* 2009, **19**(11):R437-R441.
18. Tirosh-Levy S, Steinman A, Carmeli Y, Klement E, Navon-Venezia S: **Prevalence and risk factors for colonization with methicillin resistant Staphylococcus aureus and other Staphylococci species in hospitalized and farm horses in Israel**. *Preventive veterinary medicine* 2015, **122**(1-2):135-144.
19. Cuny C, Abdelbary MM, Köck R, Layer F, Scheidemann W, Werner G, Witte W: **Methicillin-resistant Staphylococcus aureus from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans**. *One Health* 2016, **2**:11-17.
20. Mathole MA, Muchadeyi, F.C., Mdladla, K., Malatji, D.P., Dzomba, E.F., Madoroba, E. : **Presence, Distribution, serotypes and antimicrobial resistance profiles of Salmonella among pigs, chickens, and goats in South Africa.** . *Food Control* 2016.
21. Ahmed MO, Clegg PD, Williams NJ, Baptiste KE, Bennett M: **Antimicrobial resistance in equine faecal Escherichia coli isolates from North West England**. *Ann Clin Microbiol Antimicrob* 2010, **9**:7.
22. Quet F, Newton PN, Longuet C: **Antibiotic prescription behaviours in Lao People's Democratic Republic: a knowledge, attitude and practice survey (vol 93, pg 219, 2015)**. *Bull World Health Organ* 2015, **93**(7):512-512.
23. Paulson JA, Zaoutis TE, Pediatrics CoIDJ: **Nontherapeutic use of antimicrobial agents in animal agriculture: implications for pediatrics**. 2015, **136**(6):e1670-e1677.
24. Fowler H, Davis MA, Perkins A, Trufan S, Joy C, Buswell M, McElwain TF, Moore D, Worhle R, Rabinowitz PM: **A survey of veterinary antimicrobial prescribing practices, Washington State 2015**. *The Veterinary record* 2016, **179**(25):651.
25. Jacob ME, Hoppin JA, Steers N, Davis JL, Davidson G, Hansen B, Lunn KF, Murphy KM, Papich MG: **Opinions of clinical veterinarians at a US veterinary teaching hospital regarding antimicrobial use and antimicrobial-resistant infections**. *J Am Vet Med Assoc* 2015, **247**(8):938-944.
26. Chipangura JK, Eagar H, Kgoete M, Abernethy D, Naidoo V: **An investigation of antimicrobial usage patterns by small animal veterinarians in South Africa**. *Preventive veterinary medicine* 2017, **136**:29-38.

27. Moyane JN, Jideani AIO, Aiyegoro OA: **Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance**. *African Journal of Microbiology Research* 2013, **7**(24):2990-2997.
28. Centner TJJEi: **Recent government regulations in the United States seek to ensure the effectiveness of antibiotics by limiting their agricultural use**. 2016, **94**:1-7.
29. Maddox TW, Clegg PD, Williams NJ, Pinchbeck GL: **Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance**. *Equine Veterinary Journal* 2015, **47**(6):756-765.
30. Panchaud Y, Gerber V, Rossano A, Perreten V: **Bacterial infections in horses: a retrospective study at the University Equine Clinic of Bern**. *Schweizer Archiv fur Tierheilkunde* 2010, **152**(4):176-182.
31. Weese JS, Caldwell F, Willey BM, Kreiswirth BN, McGeer A, Rousseau J, Low DE: **An outbreak of methicillin-resistant Staphylococcus aureus skin infections resulting from horse to human transmission in a veterinary hospital**. *Veterinary Microbiology* 2006, **114**(1):160-164.
32. Maddox T, Clegg P, Williams N, Pinchbeck GJEvj: **Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance**. 2015, **47**(6):756-765.
33. Kentucky Uo: **Kentucky's Equine Industry has $3 Billion Economic Impact** In. University of Kentucky University of Ketucky 2013: http://equine.ca.uky.edu/news-story/kentuckys-equine-industry-has-3-billion-economic-impact.
34. Africa F: **The South African Horse Industry: A Potential Billion Rand Market**. In: *Economy.* 2018: https://www.forbesafrica.com/economy/2018/2004/2010/south-african-horse-industry-potential-billion-rand-market/.
35. Foster T: **Staphylococcus**. In: *Medical Microbiology 4th edition.* edn.: University of Texas Medical Branch at Galveston; 1996.
36. Masalha M, Borovok I, Schreiber R, Aharonowitz Y, Cohen G: **Analysis of transcription of the Staphylococcus aureus aerobic class Ib and anaerobic class III ribonucleotide reductase genes in response to oxygen**. *Journal of bacteriology* 2001, **183**(24):7260-7272.
37. Namvar AE, Bastarahang S, Abbasi N, Ghehi GS, Farhadbakhtiarian S, Arezi P, Hosseini M, Baravati SZ, Jokar Z, Chermahin SG: **Clinical characteristics of Staphylococcus epidermidis: a systematic review**. *GMS hygiene and infection control* 2014, **9**(3):Doc23-Doc23.
38. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K: **Multiplex-PCR Method for Species Identification of Coagulase-Positive Staphylococci**. 2010, **48**(3):765-769.
39. Becker K, Heilmann C, Peters G: **Coagulase-Negative Staphylococci**. 2014, **27**(4):870-926.
40. Hanselman BA, Kruth SA, Rousseau J, Weese JS: **Coagulase positive staphylococcal colonization of humans and their household pets**. *The Canadian veterinary journal = La revue veterinaire canadienne* 2009, **50**(9):954-958.

41. Rich M, Roberts LJVR: **MRSA in companion animals**. 2006, **159**(16):535-536.
42. Tomlin J, Pead M, Lloyd D, Howell S, Hartmann F, Jackson H, Muir PJVR: **Methicillin-resistant Staphylococcus aureus infections in 11 dogs**. 1999, **144**(3):60-64.
43. Weese J, Dick H, Willey B, McGeer A, Kreiswirth B, Innis B, Low DJVm: **Suspected transmission of methicillin-resistant Staphylococcus aureus between domestic pets and humans in veterinary clinics and in the household**. 2006, **115**(1-3):148-155.
44. Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, De Graef E, Snauwaert C, Cleenwerck I, Dawyndt P, Swings J, Decostere AJIjos *et al*: **Staphylococcus pseudintermedius sp. nov., a coagulase-positive species from animals**. 2005, **55**(4):1569-1573.
45. Morris DO, Mauldin EA, O'Shea K, Shofer FS, Rankin SCJAjovr: **Clinical, microbiological, and molecular characterization of methicillin-resistant Staphylococcus aureus infections of cats**. 2006, **67**(8):1421-1425.
46. Morris DO, Rook KA, Shofer FS, Rankin SCJVd: **Screening of Staphylococcus aureus, Staphylococcus intermedius, and Staphylococcus schleiferi isolates obtained from small companion animals for antimicrobial resistance: a retrospective review of 749 isolates (2003–04)**. 2006, **17**(5):332-337.
47. Huebner M, Johannes, Goldmann M, Donald A %J Annual review of medicine: **Coagulase-negative staphylococci: role as pathogens**. 1999, **50**(1):223-236.
48. Peton V, Le Loir Y: **Staphylococcus aureus in veterinary medicine**. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2014, **21**:602-615.
49. Tuchscherr L, Medina E, Hussain M, Völker W, Heitmann V, Niemann S, Holzinger D, Roth J, Proctor RA, Becker KJEmm: **Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection**. 2011, **3**(3):129-141.
50. Fluit AC: **Livestock-associated Staphylococcus aureus**. *Clinical Microbiology and Infection* 2012, **18**(8):735-744.
51. Argudín MÁ, Mendoza MC, Rodicio MR: **Food poisoning and Staphylococcus aureus enterotoxins**. *Toxins (Basel)* 2010, **2**(7):1751-1773.
52. Hennekinne J-A, De Buyser M-L, Dragacci S: **Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation**. *FEMS microbiology reviews* 2012, **36**(4):815-836.
53. Chu VH: **Staphylococcal toxic shock syndrome**. *UpToDate, Baron, EL (Ed), UpToDate, Waltham, MA(Accessed on February 23, 2017)* 2014.
54. Sada R, Fukuda S, Ishimaru H: **Toxic shock syndrome due to community-acquired methicillin-resistant Staphylococcus aureus infection: Two case reports and a literature review in Japan**. *IDCases* 2017, **8**:77-80.
55. Suga H, Shiraishi T, Takushima A, Harii K: **Toxic Shock Syndrome Caused by Methicillin-Resistant Staphylococcus aureus (MRSA) After Expander-Based Breast Reconstruction**. *Eplasty* 2016, **16**:e2.
56. Otto M: **Staphylococcus epidermidis--the 'accidental' pathogen**. *Nature reviews Microbiology* 2009, **7**(8):555-567.

57. Foster T: **Staphylococcus**. Galveston, TX.: University of Texas Medical Branch at Galveston; 1996.
58. Otto M: **Staphylococcus epidermidis—the'accidental'pathogen**. *Nat Rev Microbiol* 2009, **7**(8):555.
59. Von Eiff C, Jansen B, Kohnen W, Becker K: **Infections associated with medical devices**. *Drugs* 2005, **65**(2):179-214.
60. Hovelius B, Mardh PA: **Staphylococcus saprophyticus as a common cause of urinary tract infections**. *Reviews of infectious diseases* 1984, **6**(3):328-337.
61. Marrie T, Kwan C, Noble M, West A, Duffield L: **Staphylococcus saprophyticus as a cause of urinary tract infections**. *Journal of clinical Microbiology* 1982, **16**(3):427-431.
62. Motwani B, Khayr W: **Staphylococcus saprophyticus urinary tract infection in men: A case report and review of the literature**. *Infectious Diseases in Clinical Practice* 2004, **12**(6):341-342.
63. Raz R, Colodner R, Kunin CM: **Who Are You—Staphylococcus saprophyticus?** *Clinical Infectious Diseases* 2005, **40**(6):896-898.
64. Hedman P, Ringertz O, Lindstrom M, Olsson K: **The origin of Staphylococcus saprophyticus from cattle and pigs**. *Scandinavian journal of infectious diseases* 1993, **25**(1):57-60.
65. Foster T: **Staphylococcus**. 1996.
66. Aarestrup FM: **Antimicrobial resistance in bacteria of animal origin**. 2006.
67. Peton V, Le Loir Y: **Staphylococcus aureus in veterinary medicine**. *Infection, Genetics and Evolution* 2014, **21**:602-615.
68. Devriese LA, Vlaminck K, Nuytten J, De Keersmaecker P: **Staphylococcus hyicus in skin lesions of horses**. *Equine veterinary journal* 1983, **15**(3):263-265.
69. Casanova C, Iselin L, von Steiger N, Droz S, Sendi P: **Staphylococcus hyicus bacteremia in a farmer**. *Journal of clinical microbiology* 2011, **49**(12):4377-4378.
70. Park J, Friendship RM, Poljak Z, Weese JS, Dewey CE: **An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of Staphylococcus hyicus and Staphylococcus aureus isolated from clinical cases**. *The Canadian Veterinary Journal* 2013, **54**(2):139-144.
71. Pottumarthy S, Schapiro JM, Prentice JL, Houze YB, Swanzy SR, Fang FC, Cookson BTJJocm: **Clinical isolates of Staphylococcus intermedius masquerading as methicillin-resistant Staphylococcus aureus**. 2004, **42**(12):5881-5884.
72. Stull JW, Slavić D, Rousseau J, Weese JS: **Staphylococcus delphini and methicillin-resistant S. pseudintermedius in horses, Canada**. *Emerg Infect Dis* 2014, **20**(3):485-487.
73. Courter RD, Galton MM: **Animal staphylococcal infections and their public health significance**. *Am J Public Health Nations Health* 1962, **52**(11):1818-1827.
74. Pilsworth R, Knottenbelt DJEVE: **Bacterial folliculitis**. 2007, **19**(6):324-325.
75. White SD, Yu A: **Equine dermatology**. In: *Proceedings of the annual convention of the AAEP: 2006*; 2006.

76. Tenhagen B-A, Hansen I, Reinecke A, Heuwieser W: **Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition**. *Journal of Dairy Research* 2009, **76**(2):179-187.
77. Haenni M, Targant H, Forest K, Sévin C, Tapprest J, Laugier C, Madec J-YJJovdi: **Retrospective study of necropsy-associated coagulase-positive staphylococci in horses**. 2010, **22**(6):953-956.
78. Burton S, Reid-Smith R, McClure JT, Weese JSJTCVJ: **Staphylococcus aureus colonization in healthy horses in Atlantic Canada**. 2008, **49**(8):797.
79. Chamberlain N: **Coagulase Test for Staphylococcus Species**. 2009.
80. Chang VS, Dhaliwal DK, Raju L, Kowalski RP: **Antibiotic Resistance in the Treatment of Staphylococcus aureus Keratitis: a 20-Year Review**. *Cornea* 2015, **34**(6):698-703.
81. Roberson JR, Baird A, Pugh DJS, Goat Medicine. 2nd ed. Eds Pugh. D. G BAMH, Mo: Saunders Elsevier: **Diseases of the integumentary system**. 2012:256-290.
82. Pyörälä S: **Treatment of mastitis during lactation**. *Ir Vet J* 2009, **62 Suppl 4**(Suppl 4):S40-S44.
83. Petersson-Wolfe CS, Mullarky IK, Jones GM: **Staphylococcus aureus mastitis: cause, detection, and control**. 2010.
84. Beco L, Guaguere E, Méndez CL, Noli C, Nuttall T, Vroom MJTVR: **Suggested guidelines for using systemic antimicrobials in bacterial skin infections: part 2—antimicrobial choice, treatment regimens and compliance**. 2013, **172**(6):156.
85. Bajwa J: **Canine superficial pyoderma and therapeutic considerations**. *The Canadian veterinary journal = La revue veterinaire canadienne* 2016, **57**(2):204-206.
86. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M: **Survey of Infections Due to Staphylococcus Species: Frequency of Occurrence and Antimicrobial Susceptibility of Isolates Collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program, 1997–1999**. *Clinical Infectious Diseases* 2001, **32**(Supplement_2):S114-S132.
87. Schaumburg F, Alabi A, Peters G, Becker K: **New epidemiology of S taphylococcus aureus infection in A frica**. *Clinical Microbiology and Infection* 2014, **20**(7):589-596.
88. Burton S, Reid-Smith R, McClure JT, Weese JS: **Staphylococcus aureus colonization in healthy horses in Atlantic Canada**. *The Canadian veterinary journal = La revue veterinaire canadienne* 2008, **49**(8):797-799.
89. Baptiste KE, Williams K, Willams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CAJEid: **Methicillin-resistant staphylococci in companion animals**. 2005, **11**(12):1942.
90. Busscher J, Van Duijkeren E, van Oldruitenborgh-Oosterbaan MSJVm: **The prevalence of methicillin-resistant staphylococci in healthy horses in the Netherlands**. 2006, **113**(1-2):131-136.

91. Vengust M, Anderson M, Rousseau J, Weese JJLiam: **Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community**. 2006, **43**(6):602-606.
92. Weese JS, Archambault M, Willey B, Dick H, Hearn P, Kreiswirth B, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JJEid: **Methicillin-resistant Staphylococcus aureus in horses and horse personnel, 2000–2002**. 2005, **11**(3):430.
93. Weese JS, Rousseau J, Traub-Dargatz JL, Willey BM, McGeer AJ, Low DEJJotAVMA: **Community-associated methicillin-resistant Staphylococcus aureus in horses and humans who work with horses**. 2005, **226**(4):580-583.
94. Smyth DS, Hartigan PJ, Meaney WJ, Fitzgerald JR, Deobald CF, Bohach GA, Smyth CJ: **Superantigen genes encoded by the egc cluster and SaPIbov are predominant among Staphylococcus aureus isolates from cows, goats, sheep, rabbits and poultry**. *Journal of Medical Microbiology* 2005, **54**(4):401-411.
95. Boyle AG, Rankin SC, Duffee LA, Morris D: **Prevalence of Methicillin-Resistant Staphylococcus aureus from Equine Nasopharyngeal and Guttural Pouch Wash Samples**. *J Vet Intern Med* 2017, **31**(5):1551-1555.
96. Kuroda T, Kinoshita Y, Niwa H, Shinzaki Y, Tamura N, Hobo S, Kuwano AJVR: **Meticillin-resistant Staphylococcus aureus colonisation and infection in Thoroughbred racehorses and veterinarians in Japan**. 2016:vetrec-2015-103576.
97. Sieber S, Gerber V, Jandova V, Rossano A, Evison JM, Perreten VJMdr: **Evolution of multidrug-resistant Staphylococcus aureus infections in horses and colonized personnel in an equine clinic between 2005 and 2010**. 2011, **17**(3):471-478.
98. Tirosh-Levy S, Steinman A, Carmeli Y, Klement E, Navon-Venezia SJPvm: **Prevalence and risk factors for colonization with methicillin resistant Staphylococcus aureus and other Staphylococci species in hospitalized and farm horses in Israel**. 2015, **122**(1-2):135-144.
99. Weese JS, Archambault M, Willey BM, Hearn P, Kreiswirth BN, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JF, Low DE: **Methicillin-resistant Staphylococcus aureus in horses and horse personnel, 2000-2002**. *Emerg Infect Dis* 2005, **11**(3):430-435.
100. Agabou A, Ouchenane Z, Ngba Essebe C, Khemissi S, Chehboub MTE, Chehboub IB, Sotto A, Dunyach-Remy C, Lavigne J-P: **Emergence of Nasal Carriage of ST80 and ST152 PVL+ Staphylococcus aureus Isolates from Livestock in Algeria**. *Toxins (Basel)* 2017, **9**(10):303.
101. Peterson AE, Davis MF, Awantang G, Limbago B, Fosheim GE, Silbergeld EK: **Correlation between animal nasal carriage and environmental methicillin-resistant Staphylococcus aureus isolates at U.S. horse and cattle farms**. *Vet Microbiol* 2012, **160**(3-4):539-543.
102. Islam MZ, Espinosa-Gongora C, Damborg P, Sieber RN, Munk R, Husted L, Moodley A, Skov R, Larsen J, Guardabassi L: **Horses in Denmark Are a Reservoir of Diverse Clones of Methicillin-Resistant and -Susceptible Staphylococcus aureus**. 2017, **8**(543).

103. Oguttu JW, Qekwana DN, Odoi A: **An Exploratory Descriptive Study of Antimicrobial Resistance Patterns of Staphylococcus Spp. Isolated from Horses Presented at a Veterinary Teaching Hospital**. *BMC veterinary research* 2017, **13**(1):269.
104. Sommerhäuser J, Kloppert B, Wolter W, Zschöck M, Sobiraj A, Failing K: **The epidemiology of Staphylococcus aureus infections from subclinical mastitis in dairy cows during a control programme**. *Veterinary Microbiology* 2003, **96**(1):91-102.
105. Kolawole DO, Adeyanju A, Schaumburg F, Akinyoola AL, Lawal OO, Amusa YB, Köck R, Becker K: **Characterization of colonizing Staphylococcus aureus isolated from surgical wards' patients in a Nigerian university hospital**. *PLoS One* 2013, **8**(7):e68721.
106. Lozano C, Gharsa H, Ben Slama K, Zarazaga M, Torres C: **Staphylococcus aureus in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent**. *Microorganisms* 2016, **4**(1):12.
107. Morgan M: **Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis?** *Journal of Antimicrobial Chemotherapy* 2008, **62**(6):1181-1187.
108. Bierowiec K, Płoneczka-Janeczko K, Rypuła KJBri: **Prevalence and risk factors of colonization with Staphylococcus aureus in healthy pet cats kept in the city households**. 2016, **2016**.
109. Dougherty TJP, M. J. : **Antibiotic discovery and development**. New Haven, CT: Springer Science & Business Media; 2011.
110. Coates ARM, Halls G, Hu Y: **Novel classes of antibiotics or more of the same?** *British Journal of Pharmacology* 2011, **163**(1):184-194.
111. Ventola CL: **The Antibiotic Resistance Crisis: Part 1: Causes and Threats**. *Pharmacy and Therapeutics* 2015, **40**(4):277-283.
112. Wijesekara P, Kumbukgolla W, Jayaweera J, Rawat DJVs: **Review on usage of vancomycin in livestock and humans: maintaining its efficacy, prevention of resistance and alternative therapy**. 2017, **4**(1):6.
113. Zaffiri L, Gardner J, Toledo-Pereyra LH: **History of antibiotics. From salvarsan to cephalosporins**. *Journal of Investigative Surgery* 2012, **25**(2):67-77.
114. Landers TF, Cohen B, Wittum TE, Larson EL: **A review of antibiotic use in food animals: perspective, policy, and potential**. *Public health reports (Washington, DC : 1974)* 2012, **127**(1):4-22.
115. Johnston AM: **Use of antimicrobial drugs in veterinary practice**. *BMJ : British Medical Journal* 1998, **317**(7159):665-667.
116. Collignon P: **Fluoroquinolone Use in Food Animals**. *Emerging Infectious Diseases* 2005, **11**(11):1789-1792.
117. Sarmah AK, Meyer MT, Boxall ABJC: **A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment**. 2006, **65**(5):725-759.
118. Orsini JA, Snooks-Parsons C, Stine L, Haddock M, Ramberg CF, Benson CE, Nunamaker DM: **Vancomycin for the treatment of methicillin-resistant**

**staphylococcal and enterococcal infections in 15 horses**. *Can J Vet Res* 2005, **69**(4):278-286.
119. Cohen MLJS: **Epidemiology of drug resistance: implications for a post—antimicrobial era**. 1992, **257**(5073):1050-1055.
120. Llor C, Bjerrum L: **Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem**. *Ther Adv Drug Saf* 2014, **5**(6):229-241.
121. Olofsson TC, Butler É, Lindholm C, Nilson B, Michanek P, Vásquez A: **Fighting Off Wound Pathogens in Horses with Honeybee Lactic Acid Bacteria**. *Current microbiology* 2016, **73**(4):463-473.
122. Weese JS, Baptiste KE, Baverud V, Toutain P-LJGtauia: **Guidelines for antimicrobial use in horses**. 2008:161-182.
123. Bengtsson B, Greko C: **Antibiotic resistance--consequences for animal health, welfare, and food production**. *Ups J Med Sci* 2014, **119**(2):96-102.
124. Dahshan H, Abd-Elall AMM, Megahed AM, Abd-El-Kader MA, Nabawy EEJEm, assessment: **Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt**. 2015, **187**(2):2.
125. Wegener HC, Aarestrup FM, Gerner-Smidt P, Bager FJAVSS: **Transfer of antibiotic resistant bacteria from animals to man**. 1999, **92**:51-57.
126. Kumar B, Manuja A, Gulati BR, Virmani N, Tripathi BN: **Zoonotic Viral Diseases of Equines and Their Impact on Human and Animal Health**. *Open Virol J* 2018, **12**:80-98.
127. Angulo FJ, Nunnery J, Bair HJRSeT-OIdE: **Antimicrobial resistance in zoonotic enteric pathogens**. 2004, **23**(2):485-496.
128. Tenover FC: **Mechanisms of Antimicrobial Resistance in Bacteria**. *The American Journal of Medicine* 2006, **119**(6, Supplement 1):S3-S10.
129. Munita JM, Arias CA: **Mechanisms of Antibiotic Resistance**. *Microbiol Spectr* 2016, **4**(2):10.1128/microbiolspec.VMBF-0016-2015.
130. Grimm H: **[Parallel resistance between cephalosporins and penicillins]**. *Arzneimittel-Forschung* 1984, **34**(1):62-65.
131. Dever LA, Dermody TSJAoim: **Mechanisms of bacterial resistance to antibiotics**. 1991, **151**(5):886-895.
132. Worthington RJ, Melander C: **Overcoming resistance to beta-lactam antibiotics**. *The Journal of organic chemistry* 2013, **78**(9):4207-4213.
133. Stapleton PD, Taylor PW: **Methicillin resistance in Staphylococcus aureus: mechanisms and modulation**. *Sci Prog* 2002, **85**(Pt 1):57-72.
134. Egorov AM, Ulyashova MM, Rubtsova MY: **Bacterial Enzymes and Antibiotic Resistance**. *Acta Naturae* 2018, **10**(4):33-48.
135. Singh R, Sahore S, Kaur P, Rani A, Ray P: **Penetration barrier contributes to bacterial biofilm-associated resistance against only select antibiotics, and exhibits genus-, strain- and antibiotic-specific differences**. *Pathog Dis* 2016, **74**(6).
136. Jorgensen JH, Turnidge JD: **Susceptibility test methods: dilution and disk diffusion methods**. In: *Manual of Clinical Microbiology, Eleventh Edition.* edn.: American Society of Microbiology; 2015: 1253-1273.

137. Wiegand I, Hilpert K, Hancock RE: **Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances**. *Nature protocols* 2008, **3**(2):163-175.
138. **Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution**. *Clinical Microbiology and Infection* 2003, **9**(8):ix-xv.
139. Hudzicki J: **Kirby-Bauer disk diffusion susceptibility test protocol**. 2009.
140. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ: **Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices**. *Clinical Infectious Diseases* 2009, **49**(11):1749-1755.
141. Nachnani S, Scuteri A, Newman MG, Avanessian AB, Lomeli SL: **E-test: a new technique for antimicrobial susceptibility testing for periodontal microorganisms**. *Journal of periodontology* 1992, **63**(7):576-583.
142. Varela NP, Friendship R, Dewey C, Valdivieso A: **Comparison of agar dilution and E-test for antimicrobial susceptibility testing of Campylobacter coil isolates recovered from 80 Ontario swine farms**. *Can J Vet Res* 2008, **72**(2):168-174.
143. Evangelista AT, Karlowsky JAJMoCMiCMIE: **Automated and Manual Systems for Antimicrobial Susceptibility Testing of Bacteria**. 2016:414-432.
144. Weese JS, Archambault M, B.M.Willey, Dick H, Hearn P, Kreiswirth BN, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JF *et al*: **Methicillin-resistant Staphylococcus aureus in Horses and Horse Personnel, 2000–2002**. *Emerging Infectious Diseases* 2005, **11**(3):430-435.
145. Weese JS, Rousseau J, Traub-Dargatz JL, Willey BM, McGeer AJ, Low DE: **Community-associated methicillin-resistant Staphylococcus aureus in horses and humans who work with horses**. *Journal of the American Veterinary Medical Association* 2005, **226**(4):580-583.
146. Islam MZ, Espinosa-Gongora C, Damborg P, Sieber RN, Munk R, Husted L, Moodley A, Skov R, Larsen J, Guardabassi L: **Horses in Denmark are a reservoir of diverse clones of methicillin-resistant and-susceptible Staphylococcus aureus**. *Frontiers in microbiology* 2017, **8**:543.
147. Parisi A, Caruso M, Normanno G, Latorre L, Miccolupo A, Fraccalvieri R, Intini F, Manginelli T, Santagada G: **High Occurrence of Methicillin-Resistant Staphylococcus aureus in Horses at Slaughterhouses Compared with Those for Recreational Activities: A Professional and Food Safety Concern?** *Foodborne pathogens and disease* 2017, **14**(12):735-741.
148. Weese JS, van Duijkeren E: **Methicillin-resistant Staphylococcus aureus and Staphylococcus pseudintermedius in veterinary medicine**. *Veterinary microbiology* 2010, **140**(3-4):418-429.
149. Burton S, Reid-Smith R, McClure JT, Weese JS: **Staphylococcus aureus colonization in healthy horses in Atlantic Canada**. *The Canadian Veterinary Journal* 2008, **49**(8):797-799.
150. van Balen J, Mowery J, Piraino-Sandoval M, Nava-Hoet RC, Kohn C, Hoet AE: **Molecular epidemiology of environmental MRSA at an equine teaching hospital: introduction, circulation and maintenance**. *Vet Res* 2014, **45**(1):31.
151. Beever L, Bond R, Graham P, Jackson B, Lloyd D, Loeffler A: **Increasing antimicrobial resistance in clinical isolates of Staphylococcus intermedius**

**group bacteria and emergence of MRSP in the UK**. *The Veterinary record* 2015, **176**(7):172-172.
152. Boost M V, O'Donoghue M M, James A: **Prevalence of Staphylococcus aureus carriage among dogs and their owners**. *Epidemiology and Infection* 2008, **136**(7):953-964.
153. Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH: **Prevalence of methicillin-resistant Staphylococcus aureus among staff and pets in a small animal referral hospital in the UK**. *Journal of Antimicrobial Chemotherapy* 2005, **56**(4):692-697.
154. Saba CKS, Amenyona JK, Kpordze SW: **Prevalence and pattern of antibiotic resistance of Staphylococcus aureus isolated from door handles and other points of contact in public hospitals in Ghana**. *Antimicrobial Resistance & Infection Control* 2017, **6**(1):44.
155. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM: **Invasive methicillin-resistant Staphylococcus aureus infections in the United States**. *Jama* 2007, **298**(15):1763-1771.
156. Fortuin-de Smidt MC, Singh-Moodley A, Badat R, Quan V, Kularatne R, Nana T, Lekalakala R, Govender NP, Perovic O: **Staphylococcus aureus bacteraemia in Gauteng academic hospitals, South Africa**. *Int J Infect Dis* 2015, **30**:41-48.
157. Strommenger B, Bartels MD, Kurt K, Layer F, Rohde SM, Boye K, Westh H, Witte W, De Lencastre H, Nübel U: **Evolution of methicillin-resistant Staphylococcus aureus towards increasing resistance**. *Journal of Antimicrobial Chemotherapy* 2014, **69**(3):616-622.
158. Cuny C, Abdelbary MMH, Köck R, Layer F, Scheidemann W, Werner G, Witte W: **Methicillin-resistant Staphylococcus aureus from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans**. *One Health* 2016, **2**:11-17.
159. Van Belkum A, Melles DC, Peeters JK, Van Leeuwen WB, Van Duijkeren E, Huijsdens XW, Spalburg E, De Neeling AJ, Verbrugh HA, MRSA DWPoSRo: **Methicillin-resistant and-susceptible Staphylococcus aureus sequence type 398 in pigs and humans**. 2008, **14**(3):479.
160. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG: **Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management**. *Clinical microbiology reviews* 2015, **28**(3):603-661.
161. Couto N, Monchique C, Belas A, Marques C, Gama LT, Pomba C: **Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period**. *Journal of Antimicrobial Chemotherapy* 2016, **71**(6):1479-1487.
162. Kowalski AP, Maboni G, Espindola JP, Foletto A, Bandeira Junior G, Pötter L, Botton SdA, Vargas ACdJCR: **Temporal profile of antimicrobial resistance exhibited by strains of Staphylococcus spp. isolated from cases of bovine mastitis for 20 years (1992-2011)**. 2015, **45**(6):1035-1041.
163. Prestinaci F, Pezzotti P, Pantosti A: **Antimicrobial resistance: a global multifaceted phenomenon**. *Pathogens and Global Health* 2015, **109**(7):309-318.

164. Weese JS, Lefebvre SL: **Risk factors for methicillin-resistant Staphylococcus aureus colonization in horses admitted to a veterinary teaching hospital**. *Can Vet J* 2007, **48**(9):921-926.
165. Anderson M, Lefebvre S, Rankin S, Aceto H, Morley P, Caron J, Welsh R, Holbrook T, Moore B, Taylor DJEvj: **Retrospective multicentre study of methicillin-resistant Staphylococcus aureus infections in 115 horses**. 2009, **41**(4):401-405.
166. Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lubke-Becker A, Walther B: **Risk factors for MRSA infection in companion animals: results from a case-control study within Germany**. *International journal of medical microbiology : IJMM* 2014, **304**(7):787-793.
167. Soares Magalhães RJ, Loeffler A, Lindsay J, Rich M, Roberts L, Smith H, Lloyd DH, Pfeiffer DU: **Risk factors for methicillin-resistant Staphylococcus aureus (MRSA) infection in dogs and cats: a case-control study**. *Vet Res* 2010, **41**(5):55-55.
168. by NARMS AT, Data H: **National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS)**.
169. White DG, Fedorka-Cray P, Chiller C: **The national antimicrobial resistance monitoring system (NARMS)**. In: *NMC-Annual Meeting Proceedings: 2006*; 2006: 56-60.
170. Henton MM, Eagar HA, Swan GE, Van Vuuren MJSSAMJ: **Part VI. Antibiotic management and resistance in livestock production**. 2011, **101**(8):583-586.
171. Organization WH: **Global Antimicrobial Resistance Surveillance System (GLASS)**. In.; 2019.
172. Lee C-R, Cho IH, Jeong BC, Lee SH: **Strategies to Minimize Antibiotic Resistance**. *International Journal of Environmental Research and Public Health* 2013, **10**(9):4274-4305.
173. Knobler S, Lemon S, Najafi M, Burroughs T: **Institute of Medicine (US). Forum on Emerging Infections. The resistance phenomenon in microbes and infectious disease vectors: implications for human health and strategies for containment**. In: *Workshop summary Washington, DC: National Academies Press: 2003*; 2003.
174. Infectious Diseases Society of A: **KENTUCKY: A Summary of Select Antimicrobial Resistance**

**Data**.
175. Prevention CfDCa: **Invasive Staphylococcus aureus (MRSA/MSSA) Infection Tracking**. In.; 2016.
176. Association AVM: **Veterinary Feed Directive (VFD) Basics**. In.; 2019.
177. Association BV: **Responsible use of antimicrobials in veterinary practice: The 7-Point Plan**. In.; 2015.
178. Dellit TH, Owens RC, McGowan JE, Gerding DN, Weinstein RA, Burke JP, Huskins WC, Paterson DL, Fishman NO, Carpenter CF: **Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship**. *Clinical infectious diseases* 2007, **44**(2):159-177.

179. Yeo JM: **Antimicrobial stewardship: Improving antibiotic prescribing practice in a respiratory ward**. *BMJ Open Quality* 2016, **5**(1):u206491. w203570.
180. Cotter M, Daly L: **Antibiotic prescription practices of general practitioners**. *Irish medical journal* 2007, **100**(9):598-601.
181. Smith M, King C, Davis M, Dickson A, Park J, Smith F, Currie K, Flowers PJAR, Control I: **Pet owner and vet interactions: exploring the drivers of AMR**. 2018, **7**(1):46.
182. Ekakoro JE, Okafor CC: **Antimicrobial use practices of veterinary clinicians at a veterinary teaching hospital in the United States**. *Veterinary and Animal Science* 2019, **7**:100038.
183. Hardefeldt LY, Gilkerson J, Billman-Jacobe H, Stevenson M, Thursky K, Bailey K, Browning GJJovim: **Barriers to and enablers of implementing antimicrobial stewardship programs in veterinary practices**. 2018, **32**(3):1092-1099.
184. Bryan J, Leonard N, Fanning S, Katz L, Duggan V: **Antimicrobial resistance in commensal faecal Escherichia coli of hospitalised horses**. *Irish Veterinary Journal* 2010, **63**(6):373-379.
185. Nikaido H: **Multidrug Resistance in Bacteria**. *Annual Review of Biochemistry* 2009, **78**:119-146.
186. WHO: **Antimicrobial Resistance Fact Sheet**. In*.* Edited by Organization WH: World Health Organization; 2015.
187. Epstein JH, Price JT: **The Significant but Understudied Impact of Pathogen Transmission from Humans to Animals**. *Mount Sinai Journal of Medicine* 2009, **76**(5):448-455.
188. Johnson YJ, Myinit MS, Herrmann JA, Mathewson A, Maddox CW, Troutt HF, Opacich K: **Preliminary findings on the prevalence of methicillin-resistant Staphylococcus species in healthy horses and their human contacts**. In*.*: United States Food and Drug Administration; 2007: 128.
189. Ruthland BE, Weese, J. S., Bolin C. , Au J. , Malani A.N. : **Human-to-dog transmission of methiciliilin-resistant Staphylococcus aureus**. In*.*, vol. 15: Emerging Infectious Diseases; 2009.
190. Van Duijkeren E. THL, Wagenaar JA., De Bruijn M., Laarhoven L., Verstappen K, et al: **Emerging Infectious Diseases**. In: *Suspected horse-to-human transmission of MRSA ST398.* vol. 17: Emerging Infectious Disease Journal; 2011.
191. Bramble M, Morris D, Tolomeo P, Lautenbach E: **Potential Role of Pet Animals in Household Transmission of Methicillin-Resistant Staphylococcus aureus: A Narrative Review**. *Vector-Borne and Zoonotic Diseases* 2011, **11**(6):617-620.
192. Bergstrom K, Bengtsson B, Nyman A, Andersson UG: **Longitudinal study of horses for carriage of methicillin-resistant Staphylococcus aureus following wound infections**. *Veterinary Microbiology* 2013, **163**(3-4):388-391.
193. Carfora V, Caprioli A, Grossi I, Pepe M, Alba P, Lorenzetti S, Amoruso R, Sorbara L, Franco A, Battisti A: **A methicillin-resistant Staphylococcus**

**aureus (MRSA) Sequence Type 8, spa type t11469 causing infection and colonizing horses in Italy**. *Pathogens and Disease* 2016, **74**(4).
194. Kuroda T, Kinoshita Y, Niwa H, Shinzaki Y, Tamura N, Hobo S, Kuwano A: **Meticillin-resistant Staphylococcus aureus colonisation and infection in Thoroughbred racehorses and veterinarians in Japan**. *Veterinary Record* 2016, **178**(19):473-U444.
195. Schwaber MJ, Navon-Venezia S, Masarwa S, Tirosh-Levy S, Adler A, Chmelnitsky I, Carmeli Y, Klement E, Steinman A: **Clonal transmission of a rare methicillin-resistant Staphylococcus aureus genotype between horses and staff at a veterinary teaching hospital**. *Veterinary Microbiology* 2013, **162**(2-4):907-911.
196. van Spijk JN, Schoster A, Wittenbrink MM, Schmitt S: **A retrospective analysis of antimicrobial resistance in bacterial pathogens in an equine hospital (2012-2015)**. *Schweizer Archiv Fur Tierheilkunde* 2016, **158**(6):433-442.
197. National Committee for Clinical Laboratory Standards: **Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard M2-A5**, vol. 14, 5th edn; 1993.
198. National Committee for Clinical Laboratory Standards: **Performance Standards for Antimicrobial Disk Susceptibility Tests – Fifth Informational Supplement. Approved Standard M100-S5**, vol. 14; 1994.
199. National Committee for Clinical Laboratory Standards: **Performance Standards for Antimicrobial Susceptibility Tests. Approved Standard M2-A6.** , 6th edn; 1997.
200. National Committee for Clinical Laboratory Standards: **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically**, 3rd edn; 1997.
201. National Committee for Clinical Laboratory Standards: **Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard, M100-S8**. 1997.
202. Institute S: **Base SAS 9.4 Procedures Guide**: SAS Institute; 2014.
203. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B *et al*: **Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance**. *Clinical Microbiology and Infection* 2012, **18**(3):268-281.
204. Theelen MJP, Wilson WD, Edman JM, Magdesian KG, Kass PH: **Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979-2010**. *Equine Veterinary Journal* 2014, **46**(2):161-168.
205. Leekha S, Diekema DJ, Perencevich EN: **Seasonality of staphylococcal infections**. *Clinical Microbiology and Infection* 2012, **18**(10):927-933.
206. van Balen J, Mowery J, Piraino-Sandoval M, Nava-Hoet RC, Kohn C, Hoet AE: **Molecular epidemiology of environmental MRSA at an equine teaching hospital: introduction, circulation and maintenance**. *Vet Res* 2014, **45**:17.
207. Aslantas O, Turkyilmaz S, Yilmaz MA, Erdem Z, Demir C: **Isolation and molecular characterization of methicillin-resistant staphylococci from**

**horses, personnel and environmental sites at an equine hospital in Turkey**. *J Vet Med Sci* 2012, **74**(12):1583-1588.
208. Van den Eede A, Martens A, Lipinska U, Struelens M, Deplano A, Denis O, Haesebrouck F, Gasthuys F, Hermans K: **High occurrence of methicillin-resistant Staphylococcus aureus ST398 in equine nasal samples**. *Veterinary Microbiology* 2009, **133**(1):138-144.
209. Abbott Y, Leggett B, Rossney AS, Leonard FC, Markey BK: **Isolation rates of meticillin-resistant Staphylococcus aureus in dogs, cats and horses in Ireland**. *Vet Rec* 2010, **166**(15):451-455.
210. Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, Kiehne J, Coombs G: **Methicillin-resistant Staphylococcus aureus in a population of horses in Australia**. *Aust Vet J* 2011, **89**(6):221-225.
211. Chambers HF, DeLeo FR: **Waves of Resistance: Staphylococcus aureus in the Antibiotic Era**. *Nature reviews Microbiology* 2009, **7**(9):629-641.
212. Priyantha R, Gaunt MC, Rubin JE: **Antimicrobial susceptibility of Staphylococcus pseudintermedius colonizing healthy dogs in Saskatoon, Canada**. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 2016, **57**(1):65-69.
213. Schnellmann C, Gerber V, Rossano A, Jaquier V, Panchaud Y, Doherr MG, Thomann A, Straub R, Perreten V: **Presence of new mecA and mph (C) variants conferring antibiotic resistance in Staphylococcus spp. isolated from the skin of horses before and after clinic admission**. *Journal of clinical microbiology* 2006, **44**(12):4444-4454.
214. Haenni M, Targant H, Forest K, Sevin C, Tapprest J, Laugier C, Madec JY: **Retrospective study of necropsy-associated coagulase-positive staphylococci in horses**. *J Vet Diagn Invest* 2010, **22**(6):953-956.
215. Klimienė I, Ružauskas M, Mockeliūnas R, Šiugždinienė R, Virgailis M, Špakauskas V: **Antimicrobial resistance of *Staphylococcus* spp. isolated from riding-horses nasal mucosa**. *Veterinarija ir Zootechnika* 2015, **71**(93).
216. Toombs-Ruane LJ, Riley CB, Kendall AT, Bolwell CF, Benschop J, Rosanowski SM: **Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses**. *Journal of Veterinary Internal Medicine* 2015, **29**(6):1700-1706.
217. Tiwari R, Yadav SK, Singh S: **Methicillin Resistant Staphylococcus aureus Isolated from Wounds of Livestock and Companion Animals of Uttar Pradesh India: A Preliminary Study**. *Int J Pharmacol* 2016, **12**(8):821-829.
218. Sieber S, Gerber V, Jandova V, Rossano A, Evison JM, Perreten V: **Evolution of Multidrug-Resistant Staphylococcus aureus Infections in Horses and Colonized Personnel in an Equine Clinic Between 2005 and 2010**. *Microbial Drug Resistance* 2011, **17**(3):471-478.
219. Burton S, Reid-Smith R, McClure JT, Weese JS: **Staphylococcus aureus colonization in healthy horses in Atlantic Canada**. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 2008, **49**(8):797-799.
220. Tokateloff N, Manning ST, Weese JS, Campbell J, Rothenburger J, Stephen C, Bastura V, Gow SP, Reid-Smith R: **Prevalence of methicillin-resistant Staphylococcus aureus colonization in horses in Saskatchewan, Alberta,**

**and British Columbia**. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 2009, **50**(11):1177-1180.
221. Weese JS, Archambault M, Willey BM, Dick H, Hearn R, Kreiswirth BN, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JF *et al*: **Methicillin-resistant Staphylococcus aureus in horses and horse personnel, 2000-2002**. *Emerging Infectious Diseases* 2005, **11**(3):430-435.
222. Giguere S, Lee E, Williams E, Cohen ND, Chaffin MK, Halbert N, Martens RJ, Franklin RP, Clark CC, Slovis NM: **Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in Rhodococcus equi isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of R equi**. *Journal of the American Veterinary Medical Association* 2010, **237**(1):74-81.
223. Marsh PS, Palmer JE: **Bacterial isolates from blood and their susceptibility patterns in critically ill foals: 543 cases (1991-1998)**. *Journal of the American Veterinary Medical Association* 2001, **218**(10):1608-1610.
224. Russell CM, Axon JE, Blishen A, Begg AP: **Blood culture isolates and antimicrobial sensitivities from 427 critically ill neonatal foals**. *Australian veterinary journal* 2008, **86**(7):266-271.
225. Theelen MJ, Wilson WD, Edman JM, Magdesian KG, Kass PH: **Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979-2010**. *Equine Veterinary Journal* 2014, **46**(2):161-168.
226. Theelen MJ, Wilson WD, Edman JM, Magdesian KG, Kass PH: **Temporal trends in prevalence of bacteria isolated from foals with sepsis: 1979-2010**. *Equine Vet J* 2014, **46**(2):169-173.
227. Lowy FD: **Antimicrobial resistance: the example of Staphylococcus aureus**. *Journal of Clinical Investigation* 2003, **111**(9):1265-1273.
228. Guignard B, Entenza JM, Moreillon P: **Beta-lactams against methicillin-resistant Staphylococcus aureus**. *Curr Opin Pharmacol* 2005, **5**(5):479-489.
229. Sahebnasagh R, Saderi H, Owlia P: **The Prevalence of Resistance to Methicillin in Staphylococcus aureus Strains Isolated from Patients by PCR Method for Detec-tion of mecA and nuc Genes**. *Iranian Journal of Public Health* 2014, **43**(1):84-92.
230. Weese JS, Rousseau J: **Attempted eradication of methicillin-resistant Staphylococcus aureus colonisation in horses on two farms**. *Equine Veterinary Journal* 2005, **37**(6):510-514.
231. Singleton DA, Sánchez-Vizcaíno F, Dawson S, Jones PH, Noble PJM, Pinchbeck GL, Williams NJ, Radford AD: **Patterns of antimicrobial agent prescription in a sentinel population of canine and feline veterinary practices in the United Kingdom**. *Veterinary Journal (London, England : 1997)* 2017, **224**:18-24.
232. da SILVA KC, Knöbl T, Moreno AM: **Antimicrobial resistance in veterinary medicine**. *Braz j vet res anim sci* 2013, **50**(3):171-183.
233. Landers TF, Cohen B, Wittum TE, Larson EL: **A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential**. *Public Health Reports* 2012, **127**(1):4-22.

234. Daeseleire E, De Graef E, Rasschaert G, De Mulder T, Van den Meersche T, Van Coillie E, Dewulf J, Heyndrickx M: **Antibiotic use and resistance in animals: Belgian initiatives**. *Drug testing and analysis* 2016, **8**(5-6):549-555.
235. Lindmeier C: **Stop using antibiotics in health animals to prevent the spread of antibiotic resistance**. In*.* Geneva: World Health Organization 2017.
236. Organization WH: **Stop using antibiotics in healthy animals to prevent the spread of antibiotic resistance**. In*.*: World Health Organization; 2017.
237. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R: **Global trends in antimicrobial use in food animals**. *Proceedings of the National Academy of Sciences* 2015, **112**(18):5649-5654.
238. Bennett C, Australia AM: **Antibiotic prescribing guidelines for livestock and horses**. In*.*: WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA; 2017.
239. Hopman NE, Hulscher ME, Graveland H, Speksnijder DC, Wagenaar JA, Broens EM: **Factors influencing antimicrobial prescribing by Dutch companion animal veterinarians: a qualitative study**. *Preventive veterinary medicine* 2018.
240. Dar OA, Hasan R, Schlundt J, Harbarth S, Caleo G, Dar FK, Littmann J, Rweyemamu M, Buckley EJ, Shahid M: **Exploring the evidence base for national and regional policy interventions to combat resistance**. *The Lancet* 2016, **387**(10015):285-295.
241. Control CfD, Prevention: **CDC: 1 in 3 antibiotic prescriptions unnecessary**. *Atlanta: CDC Newsroom* 2016.
242. Smith M, Vidwan N, Myers J, Woods C: **Recurrent Antibiotic Prescriptions in the Kentucky Medicaid Population**. *Open Forum Infectious Diseases* 2017, **4**(Suppl 1):S498-S498.
243. Smith M, Vidwan N, Feygin Y, Myers J, Woods C: **Antibiotic Prescribing in the Pediatric Kentucky Medicaid Population**. In: *Open Forum Infectious Diseases: 2016*: Oxford University Press; 2016.
244. Qualtrics LJU, USA: Qualtrics: **Qualtrics [software]**. 2014.
245. Hughes LA, Williams N, Clegg P, Callaby R, Nuttall T, Coyne K, Pinchbeck G, Dawson S: **Cross-sectional survey of antimicrobial prescribing patterns in UK small animal veterinary practice**. *Preventive veterinary medicine* 2012, **104**(3–4):309-316.
246. Jacob ME, Hoppin JA, Steers N, Davis JL, Davidson G, Hansen B, Lunn KF, Murphy KM, Papich MG: **Opinions of clinical veterinarians at a US veterinary teaching hospital regarding antimicrobial use and antimicrobial-resistant infections**. *Journal of the American Veterinary Medical Association* 2015, **247**(8):938-944.
247. Ahmad A, Khan MU, Moorthy J, Jamshed SQ, Patel I: **Comparison of knowledge and attitudes about antibiotics and resistance, and antibiotics self-practicing between Bachelor of Pharmacy and Doctor of Pharmacy students in Southern India**. *Pharmacy practice* 2015, **13**(1).
248. Ahmad A, Khan MU, Patel I, Maharaj S, Pandey S, Dhingra S: **Knowledge, attitude and practice of B. Sc. Pharmacy students about antibiotics in Trinidad and Tobago**. *Journal of research in pharmacy practice* 2015, **4**(1):37.

249. Dyar OJ, Hills H, Seitz L-T, Perry A, Ashiru-Oredope D: **Assessing the knowledge, attitudes and behaviors of human and animal health students towards antibiotic use and resistance: a pilot cross-sectional study in the UK**. *Antibiotics* 2018, **7**(1):10.
250. Haque M, Rahman NIA, Zulkifli Z, Ismail S: **Antibiotic prescribing and resistance: knowledge level of medical students of clinical years of University Sultan Zainal Abidin, Malaysia**. *Therapeutics and clinical risk management* 2016, **12**:413.
251. Hoque R, Mostafa A, Haque M: **Insight of Medical Students of Clinical Years to Antimicrobials Prescribing and Resistance in Private Medical School, Chittagong, Bangladesh**. *Journal of Young Pharmacists* 2016, **8**(4).
252. Scaioli G, Gualano MR, Gili R, Masucci S, Bert F, Siliquini R: **Antibiotic use: a cross-sectional survey assessing the knowledge, attitudes and Practices amongst Students of a School of Medicine in Italy**. *PLoS One* 2015, **10**(4):e0122476.
253. Seid MA, Hussen MS: **Knowledge and attitude towards antimicrobial resistance among final year undergraduate paramedical students at University of Gondar, Ethiopia**. *BMC infectious diseases* 2018, **18**(1):312.
254. Yang K, Wu D, Tan F, Shi S, Guo X, Min Q, Zhang X, Cheng H: **Attitudes and perceptions regarding antimicrobial use and resistance among medical students in Central China**. *SpringerPlus* 2016, **5**(1):1779.
255. Anyanwu MU, Okorie-Kanu OJ, Anyaoha CO, Kolade OA: **Veterinary Medical Students' Perceptions, Attitude and Knowledge about Antibiotic Resistance and Stewardship: How Prepared Are Our Future Prescribers?** *Notulae Scientia Biologicae* 2018, **10**(2):156-174.
256. Hardefeldt L, Nielsen T, Crabb H, Gilkerson J, Squires R, Heller J, Sharp C, Cobbold R, Norris J, Browning G: **Veterinary Students' Knowledge and Perceptions About Antimicrobial Stewardship and Biosecurity—A National Survey**. *Antibiotics* 2018, **7**(2):34.
257. Castro-Sánchez E, Drumright LN, Gharbi M, Farrell S, Holmes AH: **Mapping antimicrobial stewardship in undergraduate medical, dental, pharmacy, nursing and veterinary education in the United Kingdom**. *PLoS One* 2016, **11**(2):e0150056.
258. Coyne L, Latham S, Williams N, Dawson S, Donald I, Pearson R, Smith R, Pinchbeck G: **Understanding the culture of antimicrobial prescribing in agriculture: a qualitative study of UK pig veterinary surgeons**. *Journal of Antimicrobial Chemotherapy* 2016, **71**(11):3300-3312.
259. Mateus A, Brodbelt D, Stärk K: **Evidence-based use of antimicrobials in veterinary practice**. *In Practice* 2011, **33**(5):194.
260. Hardefeldt LY, Gilkerson JR, Billman-Jacobe H, Stevenson MA, Thursky K, Bailey KE, Browning GF: **Barriers to and enablers of implementing antimicrobial stewardship programs in veterinary practices**. *J Vet Intern Med* 2018, **32**(3):1092-1099.
261. De Briyne N, Atkinson J, Pokludová L, Borriello SP: **Antibiotics used most commonly to treat animals in Europe**. *The Veterinary record* 2014, **175**(13):325-325.

262. Veterinarian A: **AVMA Establishes Antimicrobial Policy for Veterinary Medicine**. In: *American Veterinarian.* 2018.
263. De Briyne N, Atkinson J, Pokludová L, Borriello S, Price S: **Factors influencing antibiotic prescribing habits and use of sensitivity testing amongst veterinarians in Europe**. *The Veterinary record* 2013, **173**(19):475.
264. Mateus AL, Brodbelt DC, Barber N, Stärk KD: **Qualitative study of factors associated with antimicrobial usage in seven small animal veterinary practices in the UK**. *Preventive veterinary medicine* 2014, **117**(1):68-78.
265. Fowler H, Davis M, Perkins A, Trufan S, Joy C, Buswell M, McElwain T, Moore D, Worhle R, Rabinowitz P: **A survey of veterinary antimicrobial prescribing practices, Washington State 2015**. *Veterinary Record* 2016:vetrec-2016-103916.
266. Barbarossa A, Rambaldi J, Miraglia V, Giunti M, Diegoli G, Zaghini A: **Survey on antimicrobial prescribing patterns in small animal veterinary practice in Emilia Romagna, Italy**. *Veterinary Record* 2017, **181**(3):69-69.
267. Ekakoro JE, Okafor CC: **Determinants of antimicrobial use practices among veterinary clinicians at The University of Tennessee Veterinary Medical Center**. In.: PeerJ Preprints; 2017.
268. McIntosh W, Dean W: **Factors associated with the inappropriate use of antimicrobials**. *Zoonoses Public Health* 2015, **62 Suppl 1**:22-28.
269. Zhuo A, Labbate M, Norris JM, Gilbert GL, Ward MP, Bajorek BV, Degeling C, Rowbotham SJ, Dawson A, Nguyen K-A: **Opportunities and challenges to improving antibiotic prescribing practices through a One Health approach: results of a comparative survey of doctors, dentists and veterinarians in Australia**. *BMJ open* 2018, **8**(3):e020439.
270. Zishiri OT, Mkhize N, Mukaratirwa S: **Prevalence of virulence and antimicrobial resistance genes in Salmonella spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil**. *Onderstepoort J Vet Res* 2016, **83**(1):11.
271. Adefisoye MA, Okoh AI: **Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa**. *MicrobiologyOpen* 2016, **5**(1):143-151.
272. Samuels R, Carter C, Smith J, Odoi A: **Antibiotic prescription practices and opinions regarding antimicrobial resistance among veterinarians in Kentucky, USA**. In. Edited by Tennessee Uo; 2019.
273. Abbo LM, Cosgrove SE, Pottinger PS, Pereyra M, Sinkowitz-Cochran R, Srinivasan A, Webb DJ, Hooton TM: **Medical Students' Perceptions and Knowledge About Antimicrobial Stewardship: How Are We Educating Our Future Prescribers?** *Clinical Infectious Diseases* 2013, **57**(5):631-638.
274. Huang Y, Gu JR, Zhang MY, Ren Z, Yang WD, Chen Y, Fu YM, Chen XB, Cals JWL, Zhang FM: **Knowledge, attitude and practice of antibiotics: a questionnaire study among 2500 Chinese students**. *BMC Med Educ* 2013, **13**:9.

275. Scaioli G, Gualano MR, Gili R, Masucci S, Bert F, Siliquini R: **Antibiotic Use: A Cross-Sectional Survey Assessing the Knowledge, Attitudes and Practices amongst Students of a School of Medicine in Italy**. *PLoS One* 2015, **10**(4):12.
276. Thriemer K, Katuala Y, Batoko B, Alworonga JP, Devlieger H, Van Geet C, Ngbonda D, Jacobs J: **Antibiotic Prescribing in DR Congo: A Knowledge, Attitude and Practice Survey among Medical Doctors and Students**. *PLoS One* 2013, **8**(2):8.
277. Qualtrics IJP, UT, USA: **Qualtrics**. 2013.
278. Inc SI: **SAS® 9.4 [Computer software]**. In*.*: Author Cary, NC; 2013.
279. Dohoo IR, Martin W, Stryhn H: **Veterinary epidemiologic research**: AVC Incorporated Charlottetown, Canada; 2003.
280. Samuels RC, Craig Smith, Jackie Odoi, Agricola: **Antibiotic prescription practices and opinions regarding antimicrobial resistance among veterinarians in Kentucky, USA**. In*.* Edited by Tennessee Uo; 2019.
281. Zahng Q, Nene G: **The Impacts Entrepreneurship has on Economic Growth in Georgia, New Mexico, Kentucky Counties**. 2016.
282. Wayne A, McCarthy R, Lindenmayer J: **Therapeutic antibiotic use patterns in dogs: observations from a veterinary teaching hospital**. *J Small Anim Pract* 2011, **52**(6):310-318.
283. Schulte-Rüther M, Markowitsch HJ, Shah NJ, Fink GR, Piefke MJN: **Gender differences in brain networks supporting empathy**. 2008, **42**(1):393-403.
284. Toussaint L, Webb JRJTJosp: **Gender differences in the relationship between empathy and forgiveness**. 2005, **145**(6):673-685.
285. Tuchscherr L, Loffler B, Buzzola FR, Sordelli DO: **Staphylococcus aureus adaptation to the host and persistence: role of loss of capsular polysaccharide expression**. *Future Microbiol* 2010, **5**(12):1823-1832.
286. Bowen IM, Clegg PD: **Antimicrobial resistance in the horse - EVJ's online collection**. *Equine veterinary journal* 2015, **47**(6):745-746.
287. Argudín MA, Deplano A, Meghraoui A, Dodémont M, Heinrichs A, Denis O, Nonhoff C, Roisin S: **Bacteria from Animals as a Pool of Antimicrobial Resistance Genes**. *Antibiotics (Basel)* 2017, **6**(2):12.
288. Papich M, Acvcp D: **Current Concepts in Antimicrobial Therapy for Horses**. *Proc Am Assoc Equine Pract* 2001.

# Appendices

## APPENDIX A

## Survey distributed to veterinarians in the Kentucky Veterinary Medical Association (KVMA)

**Survey Investigation of Opinions and Antibiotic Prescription Practices Among Veterinarians in Kentucky**

**INTRODUCTION LETTER**

**Investigation of Opinions and Antibiotic Prescription Practices Among Veterinarians**

You are invited to participate in a survey of veterinarians that is part of research into the use of antimicrobials and prescription practices in veterinary medicine. We are requesting both small and large animal veterinarians to complete a questionnaire to collect information on antibiotic prescription practices and opinion on antimicrobial resistance. Your participation in this study is important and will help us better understand antimicrobial use in small and large animal veterinary practices.

**All responses are anonymous and completely confidential.**

The information that you provide in this questionnaire will not be made available to third parties. Participation in this study is entirely voluntary.

**Thank you for your assistance**

For any questions or concerns please contact:
Ronita Adams
Department of Biomedical and Diagnostic Sciences,
University of Tennessee, 2407 River Drive Knoxville TN 37996
Email: pkc657@vols.utk.edu

**CONSENT FORM**

Ronita Adams  University of Tennessee Department of Biomedical and Diagnostic Sciences  Email: pck657@vols.utk.edu

**Consent Form**

Again, you have been invited to take part in a research survey investigating the antimicrobial prescription practices among veterinarians and opinions regarding antimicrobial resistance. The survey will take approximately 15-20 minutes to complete. Your participation in this survey is important and will allow us to better understand the link between prescription practices and opinions regarding development of antimicrobial resistance. Taking part in this study is completely voluntary. If you choose to participate in this study, you can withdraw at any time. Your responses will be kept strictly confidential and anonymous. Any reports or publications that result from this research will be done at an aggregated level.

If you have questions or want a copy or summary of this study's results, you can contact us at the email address above. If you have questions about your rights as a participant, you may contact the University of Tennessee IRB Compliance Officer at utkirb@utk.edu or (865) 974-7697. Please feel free to print a copy of this consent page to keep for your records.

- ○ I have read the above information and I agree to participate in this study

**DEMOGRAPHICS**

1). What is your gender?

- ○ Male
- ○ Female

2). What city do you work in?

______________________________________________

3). Is your veterinary practice:

- ○ Small Animal
- ○ Large Animal
- ○ Equine
- ○ Mixed

4). What type of veterinary facility do you practice at?

- ◯ Primary Care
- ◯ Referral
- ◯ Veterinary Hospital
- ◯ Charity Clinic
- ◯ Academic

5). How long have you worked at your practice?(in years) ___________________________

6). What is the total number of veterinarians employed at your facility or practice?

______________________________________________________________________________

7). How many hours per week do you work?

______________________________________________________________________________

8). What year did you graduate with your veterinary degree?

______________________________________________________________________________

**VETERINARY EDUCATION**

9). What was the emphasis on antibiotics in your veterinary school education (non-clinical years)?

- ◯ a. Topic was not covered
- ◯ b. Light emphasis
- ◯ c. Covered thoroughly in one course
- ◯ d. Emphasized in multiple courses

10). What was the emphasis on antibiotics in your veterinary school education (clinical years)?

- ◯ a. Topic was not covered
- ◯ b. Light emphasis
- ◯ c. Covered thoroughly in one course
- ◯ d. Emphasized in multiple courses

11). What was the background of the person primarily responsible for your education on antibiotics during your veterinary education? (Please select all that apply)

- ◯ a. Clinical pharmacist
- ◯ b. Clinical microbiologist
- ◯ c. Clinician
- ◯ d. Pharmacologist/clinical pharmacologist
- ◯ e. Toxicologist
- ◯ f. Don't know what his/her background was

12). Do you hold any additional post graduate qualifications?

- ◯ a. No
- ◯ b. Yes
- ◯ If yes, please list your post graduate qualifications below

______________________________________________

**ANTIMICROBIAL PRESCRIPTION PRACTICES**

13). What are the main sources that you use to receive current information on antimicrobials and their use? (Please select all that apply)

- ○ a. Practice policy
- ○ b. Pharmaceutical companies
- ○ c. Veterinary Medicine Directorates
- ○ d. Peer reviewed scientific literature
- ○ e. Textbook/Drug handbook
- ○ f. Continuing Professional Development courses
- ○ g. Other (Please specify)

---

14). Can you prescribe antibiotics without supervision, approval, or additional oversight?

- ○ a. Yes
- ○ b. No (Please explain)

---

- ○ Never

15). Does your veterinary facility or practice have a policy concerning antibiotic prescription?

- ○ a. Yes
- ○ b. No

16). On Average, how often do you prescribe antibiotics?

- a. Multiple times per day
- b. Once per day
- c. Once every two days
- d. Once per week
- e. Once every two weeks
- f. Once per month
- g. Once every two to four months
- h. Quarterly
- i. Biannually
- j. Annually

17). Is there any antibiotic that you do not feel comfortable prescribing?

- a. No
- b. Yes,  please explain below

______________________________________________

**FACTORS POTENTIALLY ASSOCIATED WITH PRESCRIPTION PRACTICES**

18). Do any of the factors below affect your decision when choosing to prescribe an antibiotic to a patient? (Please select all that apply)

- ○ a. Cost of antibiotic
- ○ b. Client insurance
- ○ c. Client expectations
- ○ d. Route of administration
- ○ e. Frequency of patient visits
- ○ f. Risk of potential adverse drug reaction
- ○ g. Other (Please specify below)

______________________________________________

19). You always rely on clinical signs and symptoms when prescribing an antibiotic?

- ○ a. Strongly agree
- ○ b. Agree
- ○ c. Neither agree nor disagree
- ○ d. Disagree
- ○ e. Strongly disagree

20). You rely on laboratory results before prescribing an antibiotic?

- ○ a. Strongly agree
- ○ b. Agree
- ○ c. Neither agree nor disagree
- ○ d. Disagree
- ○ e. Strongly disagree

**OPINIONS ABOUT PRESCRIPTION PRACTICES**

21). What are your feelings concerning antibiotic prescription at your facility or practice?

- ◯ a. All antibiotics are under-prescribed
- ◯ b. Some antibiotics are under-prescribed (Please list them below)

---

- ◯ c. All antibiotics are appropriately prescribed
- ◯ d. Some antibiotics are over-prescribed (Please list them below)

---

- ◯ e. All antibiotics are over-prescribed

22). Do you feel like you sometimes over-prescribe antibiotics?

- ◯ a. No
- ◯ b. Yes

23). Your colleagues over-prescribe antibiotics?

- ◯ a. Strongly agree
- ◯ b. Agree
- ◯ c. Neither agree nor disagree
- ◯ d. Disagree
- ◯ e. Strongly Disagree

24). Veterinarians at your practice or facility always comply with antibiotic prescription policies.

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

25). Antibiotic prescription policies are contributing to a change in the frequency of antimicrobial resistance at your facility or practice?

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

**OPINIONS ABOUT ANTIMICROBIAL RESISTANCE**

26). Improper use of antibiotics contributes to selection for antimicrobial resistance.

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

27). How does improper use of antibiotics affect selection for antimicrobial resistance?

○ a. It does not affect selection for antimicrobial resistance

○ b. Improper use of antibiotics affects selection for antimicrobial resistance in the following ways:

______________________________________________________________________

28). Improper prescribing habits among your colleagues is affecting the selection for antibiotic resistance in your facility.

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

29). There has been an increase in the number of cases of antimicrobial resistance at your facility or practice.

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

30). In your opinion, what percentage of your clients are compliant with the instructions for prescribed antibiotics? ___________

Thank you for completing this survey. Please submit your responses by clicking the "Submit" button below.

○ Submit

## APPENDIX B

## Survey distributed to veterinarians in the city of Tshwane, Metropolitan Municipality

### INTRODUCTION LETTER

**Investigation of Opinions and Antibiotic Prescription Practices Among Veterinarians in city of Tshwane, Metropolitan Municipality**

You are invited to participate in a survey of veterinarians that is part of research into the use of antimicrobials and prescription practices in veterinary medicine. We are requesting both small and large animal veterinarians to complete a questionnaire to collect information on antibiotic prescription practices and opinions on antimicrobial resistance. Your participation in this study is important and will help us better understand antimicrobial use in small and large animal veterinary practices. All responses are anonymous and completely confidential. The information that you provide in this questionnaire will not be made available to third parties. Participation in this study is entirely voluntary.

Thank you for your assistance

For any questions or concerns please contact:
Nenene Qekwana
Lecturer
University of Pretoria,
cnr Lynnwood Road and Roper Street,
Hartfield South Africa.
Email: Nenene.Qekwana@up.ac.za

Dr James W.Oguttu, Ph.D
College of Agriculture and Environmental Sciences
Department of Agric & Animal Health
UNISA Florida Campus
E-mail: joguttu@unisa.ac.za

**CONSENT FORM**

Nenene Qekwana
University of Pretoria
Email: Nenene.Qekwana@up.ac.za

You have been invited to take part in a research survey investigating the antimicrobial prescription practices among veterinarians and opinions regarding antimicrobial resistance. The survey will take approximately 15-20 minutes to complete. Your participation in this survey is important and will allow us to better understand the link between prescription practices and opinions regarding development of antimicrobial resistance. Taking part in this study is completely voluntary. If you choose to participate in this study, you can withdraw at any time. Your responses will be kept strictly confidential and anonymous. Any reports or publications that result from this research will be done at an aggregated level.

If you have questions or want a copy or summary of this study's results, you can contact us at the email address above. If you have questions about your rights as a participant, you may contact the research ethics office at the University of Pretoria at 012 356 3084 or 012 356 3085. Please feel free to print a copy of this consent page to keep for your records.

○ I have read the above information and I agree to participate in this study

**DEMOGRAPHICS**

1). What is your gender?

◯ Male

◯ Female

2). What city do you work in?

______________________________________________________

3). Is your veterinary practice:

◯ Small Animal

◯ Large Animal

◯ Equine

◯ Mixed

4). What type of veterinary facility do you practice at?

◯ Primary Care

◯ Referral

◯ Veterinary Hospital

◯ Charity Clinic

◯ Academic

5). How long have you worked at your practice? _______________________years

6). What is the total number of veterinarians employed at your facility or practice?

________________________________________________________________________

7). How many hours per week do you work?

________________________________________________________________________

8). What year did you graduate with your veterinary degree?

________________________________________________________________________

**VETERINARY EDUCATION**

9). What was the emphasis on antibiotics in your veterinary school education (non-clinical years)?

◯ a. Topic was not covered

◯ b. Light emphasis

◯ c. Covered thoroughly in one course

◯ d. Emphasized in multiple courses

10). What was the emphasis on antibiotics in your veterinary school education (clinical years)?

◯ a. Topic was not covered

◯ b. Light emphasis

◯ c. Covered thoroughly in one course

◯ d. Emphasized in multiple courses

11). What was the background of the person primarily responsible for your education on antibiotics during your veterinary education? (Please select all that apply)

◯ a. Clinical pharmacist

◯ b. Clinical microbiologist

◯ c. Clinician

◯ d. Pharmacologist/clinical pharmacologist

◯ e. Toxicologist

◯ f. Don’t know what his/her background was

12). Do you hold any additional post graduate qualifications?

- ◯ a. No
- ◯ b. Yes
- ◯ If yes, please list your post graduate qualifications below

  ______________________________________________

**ANTIMICROBIAL PRESCRIPTION PRACTICES**

13). What are the main sources that you use to receive current information on antimicrobials and their use? (Please select all that apply)

- ◯ a. Practice policy
- ◯ b. Pharmaceutical companies
- ◯ c. Veterinary Medicine Directorates
- ◯ d. Peer reviewed scientific literature
- ◯ e. Textbook/Drug handbook
- ◯ f. Continuing Professional Development courses
- ◯ g. Other (Please specify)

  ______________________________________________

14). Can you prescribe antibiotics without supervision, approval, or additional oversight?

- ◯ a. Yes
- ◯ b. No (Please explain)

  ______________________________________________

- ◯ Never

15). Does your veterinary facility or practice have a policy concerning antibiotic prescription?

- ◯ a. Yes
- ◯ b. No

16). On Average, how often do you prescribe antibiotics?

- ○ a. Multiple times per day
- ○ b. Once per day
- ○ c. Once every two days
- ○ d. Once per week
- ○ e. Once every two weeks
- ○ f. Once per month
- ○ g. Once every two to four months
- ○ h. Quarterly
- ○ i. Biannually
- ○ j. Annually

17). Is there any antibiotic that you do not feel comfortable prescribing?

- ○ a. No
- ○ b. Yes, please explain below

**FACTORS POTENTIALLY ASSOCIATED WITH PRESCRIPTION PRACTICES**

18). Do any of the factors below affect your decision when choosing to prescribe an antibiotic to a patient? (Please select all that apply)

○ a. Cost of antibiotic

○ b. Client insurance

○ c. Client expectations

○ d. Route of administration

○ e. Frequency of patient visits

○ f. Risk of potential adverse drug reaction

○ g. Other (Please specify below)

____________________________________________

19). You always rely on clinical signs and symptoms when prescribing an antibiotic?

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

20). You rely on laboratory results before prescribing an antibiotic?

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

**OPINIONS ABOUT PRESCRIPTION PRACTICES**

21). What are your feelings concerning antibiotic prescription at your facility or practice?

○ a. All antibiotics are under-prescribed

○ b. Some antibiotics are under-prescribed (Please list them below)

______________________________________________________________

○ c. All antibiotics are appropriately prescribed

○ d. Some antibiotics are over-prescribed (Please list them below)

______________________________________________________________

○ e. All antibiotics are over-prescribed

22). Do you feel like you sometimes over-prescribe antibiotics?

○ a. No

○ b. Yes

23). Your colleagues over-prescribe antibiotics?

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly Disagree

24). Veterinarians at your practice or facility always comply with antibiotic prescription policies.

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

25). Antibiotic prescription policies are contributing to a change in the frequency of antimicrobial resistance at your facility or practice?

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

**OPINIONS ABOUT ANTIMICROBIAL RESISTANCE**

26). Improper use of antibiotics contributes to selection for antimicrobial resistance.

- a. Strongly agree
- b. Agree
- c. Neither agree nor disagree
- d. Disagree
- e. Strongly disagree

27). How does improper use of antibiotics affect selection for antimicrobial resistance?

○ a. It does not affect selection for antimicrobial resistance

○ b. Improper use of antibiotics affects selection for antimicrobial resistance in the following ways: ______________________________

28). Improper prescribing habits among your colleagues is affecting the selection for antibiotic resistance in your facility.

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

29). There has been an increase in the number of cases of antimicrobial resistance at your facility or practice.

○ a. Strongly agree

○ b. Agree

○ c. Neither agree nor disagree

○ d. Disagree

○ e. Strongly disagree

30). In your opinion, what percentage of your clients are compliant with the instructions for prescribed antibiotics? __________

Thank you for completing this survey.

# Vita

Ronita Alysha-val Samuels was born in Knoxville, Tennessee on October 8, 1988. She graduated from Oak Ridge High School in 2007. Ronita attended the University of Kentucky in Lexington, KY and received her BS in Biology in 2011. She attended graduate school at the Tennessee State University in Nashville, TN and received her Masters in Public Health with a focus in behavioral science. In 2015 she moved to the University of Tennessee in Knoxville, TN where she received both her PhD in Comparative and Experimental Medicine with a focus in Epidemiology and her Masters in statistics in 2019.