



A survey of antimicrobial usage patterns by veterinarians treating dogs and cats in South Africa

By

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DECLARATION

I declare that the dissertation hereby submitted to the University of Pretoria for the degree Master of Science Veterinary Industrial Pharmacology has not been previously submitted by me for a degree at this University or any other University, that it is my own work in design, execution and that all assistance in the study has been duly acknowledged

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ABSTRACT

The emergence of antimicrobial resistant bacteria in animal health that may affect humans directly or transfer resistant genes to human pathogens is a cause for major concern. As a result both the human and veterinary use of antimicrobials has come under increased scrutiny. The aim of this research project was to characterise antimicrobial usage patterns in dogs and cats in South Africa and to confirm whether South African veterinarians treating companion animals were making use of the proper selection guidelines in optimizing their antimicrobial use. To meet this objective, a survey was undertaken through an online questionnaire sent to 2880 registered South African veterinarians. The questionnaire covered general use principles; scope of extra-label use of antimicrobials; Section 21 applications for unregistered antimicrobials; use of antibiograms; adverse drug reactions; owner compliance; disposal of expired stock and length of use of multi-dose vials.

Questionnaires were completed by 181 veterinarians representing a response of 16% from the 1120 small animal veterinarians to which the questionnaire was sent. Use of antimicrobials without laboratory diagnosis, off label prescriptions and compounding of antimicrobials by small animal veterinarians in South Africa was reported. When presented with first time cases, 91.16% (n=165) of the respondents selected their antimicrobial empirically before undertaking laboratory testing. Antimicrobial compounding was practiced by 13.26% (n=24) of the respondents, with the following preparations being compounded; enrofloxacin (baytril) + saline + silver sulfadiazine (flamazine); gentamicin + dimethyl sulfoxide (DMSO); enrofloxacin + DMSO; enrofloxacin (baytril) + ear preparations. A high proportion of respondents also used



antimicrobials off label (86.19%; n=156). All the major classes of antimicrobials were in use by small animal veterinarians.

This descriptive study is an important first stage in investigating small animal antimicrobial usage patterns in South Africa. The descriptive information gained from this study will play a major role in the development of appropriate hypotheses that can be tested in future studies linked with the emergence of antimicrobial resistant bacteria. It is also envisaged that this project will help in the review of antimicrobial prudent usage guidelines and facilitate better veterinary antimicrobial stewardship.



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LIST OF ABBREVIATIONS

ADR	-	Adverse drug reaction
AUC	-	Area under the curve
BVSc	-	Bachelor of Veterinary Science
CBC	-	Complete blood count
cGMP	-	current Good Manufacturing Practice
C _{max}	-	Maximum concentration of antimicrobial reached in the serum
CNS	-	Central Nervous System
CPD	-	Continued professional development
DAFF	-	Department of Agriculture, Forestry and Fisheries
DHFR	-	Dihydrofolate reductase
DHPS	-	Dihydropteroate synthase
DNA	-	Deoxyribonucleic acid
FIV	-	Feline Immunodeficiency Virus
GIT	-	Gastrointestinal tract
MCC	-	Medicine Control Council of South Africa
MIC	-	Minimum Inhibitory Concentration
MMedVet	-	Master of Veterinary Medicine
mRNA	-	messenger Ribonucleic Acid
MRSA	-	Methicillin-resistant <i>Staphylococcus aureus</i>
MRSP	-	Methicillin-resistant <i>Staphylococcus pseudointermedius</i>
NAG	-	N-acetyl glucosamine



NAM	-	N-acetyl muramic acid
OM	-	Outer membrane
OTC	-	Over the counter
PABA	-	Para-aminobenzoic acid
PAE	-	Post-antibiotic effect
PBP	-	Penicillin-binding proteins
PD	-	Pharmacodynamics
PEP	-	Phosphoenolpyruvate
PK	-	Pharmacokinetics
pKa	-	Acid dissociation constant of a solution
SAVA	-	South African Veterinary Association
SAVC	-	South African Veterinary Council
tRNA	-	transfer Ribonucleic Acid
UDP	-	Uridine diphosphate



CHAPTER: 1 INTRODUCTION

Antimicrobials are extremely important in veterinary medicine as they are used extensively in different animal species for the treatment and prevention of diseases. With time their continuous and repeated use has resulted in the selection of resistant bacterial populations, which is a natural and unavoidable phenomenon (Hughes et al. 2012). Antimicrobial resistance is the capability of bacteria to survive exposure to a defined concentration of an antimicrobial, be it therapeutically, prophylactically, for growth promotion or for disinfection. While a number of factors have been linked to the development of antimicrobial resistance, the most common factor linked to its development is their incorrect use in cases where they are not warranted (e.g. viral infections), and/or when they are used widely for prophylaxis. Resistance to antimicrobial poses a serious and growing problem, as infectious diseases are becoming more difficult to treat (e.g. extreme drug resistant tuberculosis). Resistant bacteria possess mechanisms of changing their internal structure so that the antimicrobial no longer works, develop ways to inactivate or neutralize the antimicrobial. In addition, bacteria can transfer the genes coding for antimicrobial resistance between them, making it possible for bacteria to acquire resistance to agents they themselves have never been exposed to.

Resistance development can be minimised, if prudent or judicious usage measures are implemented to prolong the efficacy of antimicrobials in both human and veterinary medicine (Phillips et al. 2004). Antimicrobial prudent usage is characterized by optimizing the therapeutic effects while minimizing the risk for the development of resistance in order to preserve the efficacy of available drugs. In this practice, the antimicrobial should not be viewed in isolation,



but a part of the discipline of good veterinary practice, which includes other aspects of animal health such as animal husbandry, animal welfare, hygiene, nutrition, and vaccination (OIE 2004, OIE 2009). It cannot be over-emphasised that antimicrobials should always be part of, and not a replacement for, integrated disease control programs (OIE 2004).

With the development of resistance being inevitable, there is need for the regular assessment of the effectiveness of antimicrobial usage trends in disease control programmes as resistance is associated with continuous usage. While efforts to establish veterinary antimicrobial usage patterns have been made in different parts of the world, the extent to which antimicrobials usage in small companion animal medicine in South Africa contributes to resistance is yet to be fully quantified. This research project is intended to establish the trends of antimicrobial use patterns in small animal medicine in order to enhance awareness of veterinarians to the problem of antimicrobial resistance.

1.1 Hypothesis

South African small companion animal veterinarians have an established method of selecting and optimizing their antimicrobial selection and use.

1.2 Benefits arising from the project

- This project will present baseline information on antimicrobial usage patterns in small animal medicine in South Africa. This will help facilitate better antimicrobial use in South Africa.



- The project will contribute towards the publication of an MSc dissertation.

1.3 Aims/Objectives

- To obtain a detailed insight into the exposure of dogs and cats to antimicrobials.
- To explore the clinical use of antimicrobials in dogs and cats in South Africa.
- To identify factors leading to antimicrobial resistance.
- To increase the awareness of veterinarians to the problem of antimicrobial resistance.
- To review prudent antimicrobial use and come to judicious and informed policy decisions with respect to antimicrobial resistance in small companion animal health.



CHAPTER: 2 LITERATURE REVIEW

2.1 Introduction

Companion animals (dogs, cats and horses) form an important part of the life of millions of people worldwide by providing emotional support, reducing stress and increasing social contact to their owners, especially in an age of increased urbanization and decreased social contact (OIE 2009). As with people, animals are not immune to infectious disease and as-such require veterinary care to return to full health. Veterinary chemotherapeutic drugs are important tools for the management of bacterial, viral, fungal and parasitic diseases in companion animals. When these diseases are left untreated, in addition to becoming a welfare concern due to unnecessary suffering, some companion animal diseases can be transmitted to humans through close contact or, alternatively from the environment for example salmonellosis, toxoplasmosis, ringworm, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudointermedius* (MRSP) (Okusu, Ma & Nikaido 1996).

2.2 Bacteria

Bacteria are prokaryotic, single celled microscopic living organisms that can be spherical (cocci) or rod (bacilli) shaped existing independently or as parasites (Margulis, Chapman 2009). Bacteria have detrimental effects to animal health when they cause diseases for example *Bacillus anthracis*, which causes a serious disease in livestock, or beneficial for example Streptomycetes used for the production of the antibiotic streptomycin. The aim of antimicrobial chemotherapeutics is to target and destroy pathogenic bacteria without attacking the host cells, a



phenomenon called selective toxicity. Antimicrobial selective toxicity is possible because bacterial cells have unique structures and pathways that do not exist in eukaryotic cells for example the peptidoglycan cell wall (Figure 1) (Ryan, Ray 2010).

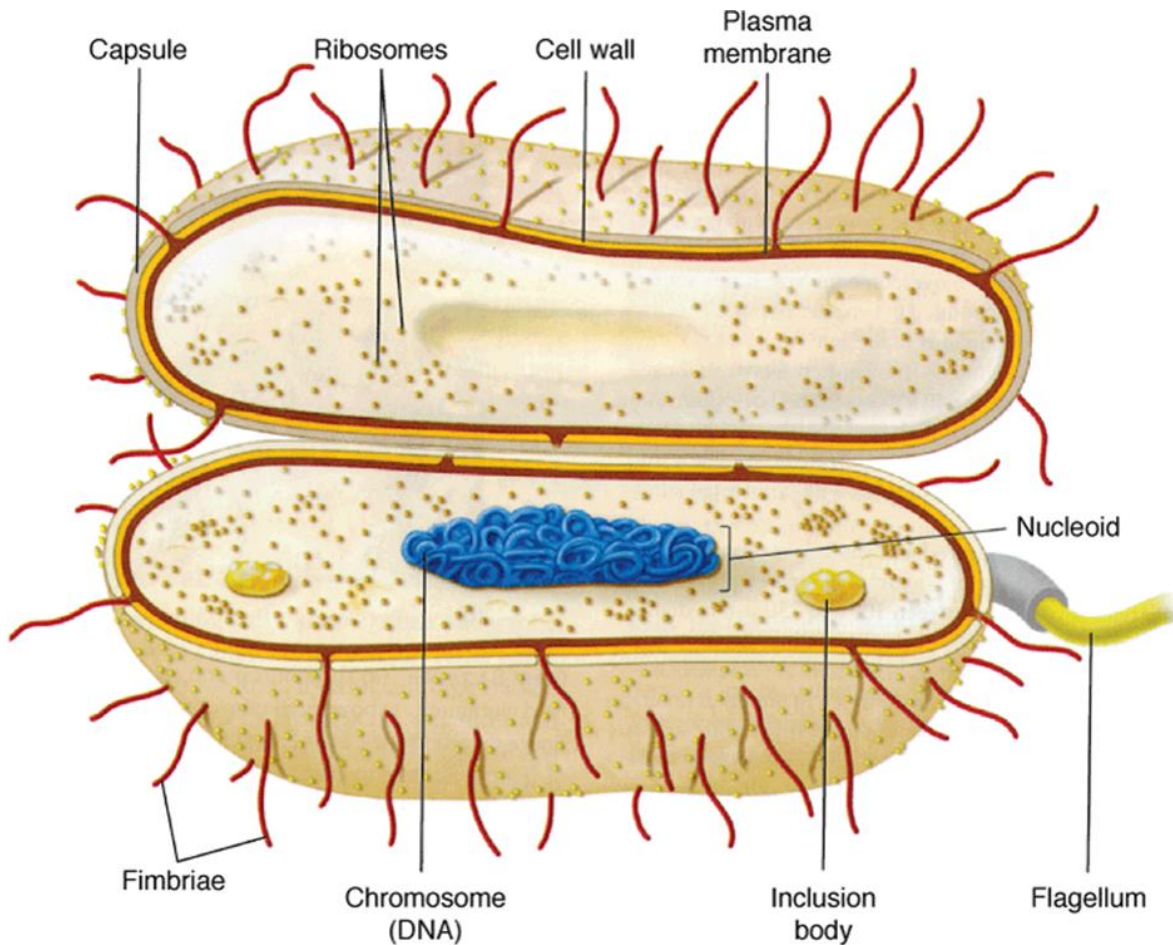


Figure 1: Prokaryotic bacterial cell structure.

The figure above shows the unique features of the bacterial cell, which is the absence of a nuclear membrane, the presence of a cell wall and free ribosomes in the cytoplasm (Source: Sherris medical Microbiology, 5th edition (Ryan, Ray 2010))

2.2.1 Bacterial extracellular structures

The bacterial cell wall present on the outside of the cytoplasmic membrane is essential to the survival of bacteria by preventing osmotic damage (Nanninga 2009). The cell wall building



blocks comprise of peptidoglycan layers made from polysaccharide chains cross-linked by peptides containing D-amino acids (Krell, Beveridge 1987). There are two types of bacterial cell walls, a thick one in the Gram-positives and a thinner one in the Gram-negatives (Figure 2) (Levinson 2014). The names originate from the reaction between the bacterial cell wall and the Gram stain. Gram-positive bacteria possess a thick peptidoglycan layer that retains crystal violet during Gram staining, giving the bacteria a purple colour when viewed under the microscope (Margulis, Chapman 2009, Nanninga 2009). In contrast, Gram-negative bacteria have a relatively thin peptidoglycan layer surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins, which prevents retention of crystal violet. On counterstaining Gram negative bacteria will take the pink colour of safranin. The cell wall is a major distinguishing factor to that of mammalian cells and therefore is the main target pathway for bacterial-specific killing.

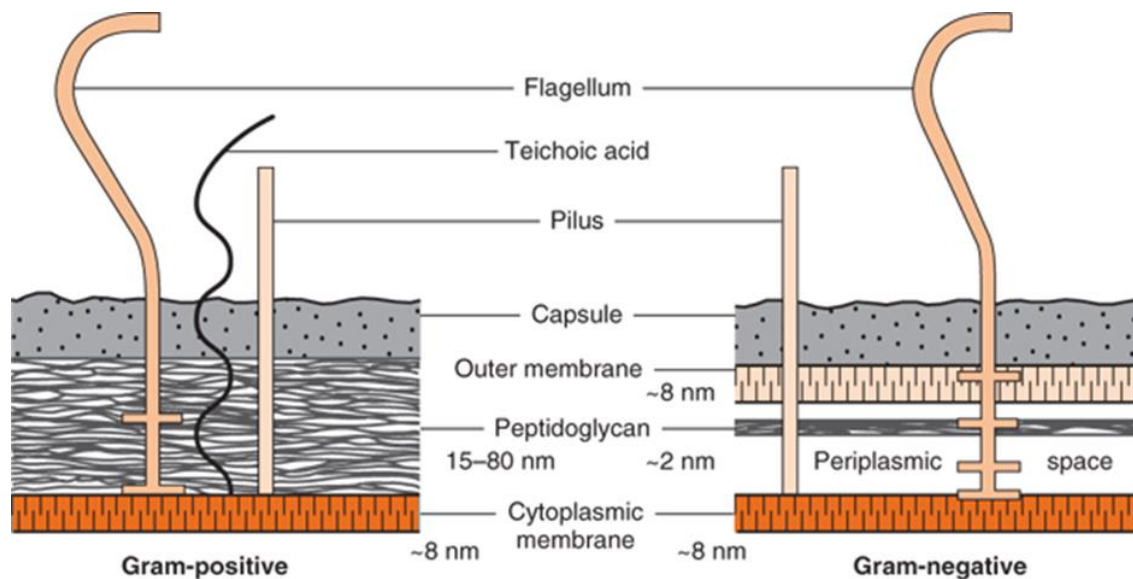


Figure 2 : The cell wall structures for Gram-Positive and Gram-negative bacteria
The figure above shows the structure of bacterial cell walls and compares the thick cell wall of Gram-positive bacteria with the comparatively thin cell wall of Gram-negative bacteria (Source: Review of Medical Microbiology and Immunology (Levinson 2014))



2.2.2 Bacterial intracellular structures

Bacteria do not have intracellular membrane-bound organelles, for example, they lack a true nucleus and mitochondria present in eukaryotic cells (Margulis, Chapman 2009). Since bacterial intracellular structures differ from those of eukaryotic cells, this makes it possible for selective inhibition of bacterial intracellular function without concomitant inhibition of the equivalent mammalian cell targets. The genetic material of bacteria is free in the cytoplasm and bacterial DNA can be transferred from one bacterium to another during conjugation or by viruses (Margulis, Chapman 2009, Krell, Beveridge 1987). Bacteria also have a different ribosomal unit from that of eukaryotic cells. Bacteria have (70S) while animals have (80S) ribosomes (Margulis, Chapman 2009). The bacterial 70S ribosome is made up of the 50S and 30S subunits; with the 50S subunit containing the 23S and 5S rRNA while the 30S subunit contains the 16S rRNA (Nanninga 2009). These rRNA molecules differ in size in eukaryotes and antimicrobial drugs that inhibit bacterial protein synthesis exploit the difference between these ribosomes.

2.3 History of antimicrobials

Infections have always been a major cause of death in people and animals mainly due to a poor understanding of the mechanisms of disease transmission (Podolsky 2010, Clardy, Fischbach & Currie 2009). The idea that diseases are caused by tiny creatures invisible to the naked eye dates back to the sixteenth century when Fracastoro hypothesised that there were "seeds" in the environment that could multiply in the body and produce disease (Lewinsohn 1998, Hudson, Morton 1996). It was during the nineteenth century when it became clear that bacteria were a leading cause of infections after the work by Louis Pasteur and Robert Koch (Clardy, Fischbach



& Currie 2009, Zaffiri, Gardner & Toledo-Pereyra 2012). Louis Pasteur established the germ theory of disease stating that microbes such as bacteria, fungi, viruses and protozoans were already present in organic material and multiplied when conditions became favourable for them. Robert Koch reinforced the ‘germ theory’ explanation when he postulated how a specific microorganism could cause a particular disease. During his experiments, Koch successfully isolated and cultured the *Bacillus anthracis*, latter reintroducing it into healthy sheep and mice (Blevins, Bronze 2010). As he had predicted, the healthy animals he injected developed anthrax clinical signs. Koch achieved similar results for tuberculosis and cholera. In 1890, Koch’s work led to the development of the ‘Koch’s Postulates’, which consist of the following rules (Blevins, Bronze 2010, Münch 2003):

- The microorganism must be present in all diseased animals.
- The microorganism must be capable of being isolated and identified.
- When healthy animals are inoculated with the microorganism they must develop symptoms of the original disease.
- The microorganism must be recoverable from these newly diseased animals. It must then be isolated and identified as being the same as the microbe in the original infected animals.

Even with a better understanding of the causes of infectious diseases, it took several years before a breakthrough with regards to therapy was discovered, despite pressing needs such as the World War I (1914 – 1918) where infection control was identified as a priority. While not confirmed, it has been suggested that up to 93% of mortalities recorded where as a result of secondary wound infections e.g. soldiers who initially survived an abdominal wound later died from bacterial infection and the subsequent septicaemia (Clardy, Fischbach & Currie 2009). In 1935, Gerhard



Domagk at the Bayer Laboratories (I.G. Farben) in Germany started a breakthrough that brought about the era of antibacterial drugs. Domagk et al., discovered and developed the first sulphonamide, popularly known by the trade name Prontosil and it was the first commercially available antibacterial (Hawking 1963). Unfortunately it was not until the World War II (1939 – 1945) that antibiotics entered into the main stream, with the isolation of the first significant antibiotic, penicillin, by Florey following on the description of a Staphylococcus inhibitory substance produced by the penicillium species in culture by Fleming in 1928 (Bennett, Chung 2001, Fraenkel 1999). Through Florey's fermentation process, the Pfizer Company was able to mass-produce penicillin that was later used to treat secondary wound infections in the allied power soldiers (Bennett, Chung 2001, Petri 2011a).

The inspiration from the ground breaking work of Domagk and Florey has since led to a number of subsequent antimicrobial discoveries. In 1944, Waksman isolated streptomycin and subsequently found agents such as chloramphenicol, tetracyclines, and erythromycin in soil samples (Hawking 1963). To this day, improvements in fermentation techniques and advances in medicinal chemistry have permitted the synthesis of natural, synthetic and semisynthetic antimicrobials (Figure 3) (Giguère 2013a).

With the large number of drugs that affect growth and survival of bacterial and other pathogenic organisms, the available drugs can be classified into the following groups:

- Antibiotics are defined as chemicals produced by or derived from one microorganism, usually fungi, which inhibit the growth of or kill other microorganisms such as bacteria and fungi (Golan 2012).



- Antimicrobials are a broader grouping of compounds that include the antibiotics, substances derived from a biological source or their modifications, and the synthetically produced drugs such as the quinolones and sulphonamides (Gumbo 2011, Giguère 2013c).
- Chemotherapeutics are the final grouping and includes the antimicrobial drugs, as well as drugs that are effective against other microorganisms (fungi, rickettsia and protozoa) and larger parasitic organisms (helminth, insect and arthropod infestations) (Gumbo 2011).



2.4 Classification of antimicrobials

Even with the broad classification mentioned above, the antimicrobial groups (each with several antimicrobials therein) can be further broadly classified into three criteria, based on features important for their optimal therapeutic selection (Gumbo 2011).

2.4.1 Spectrum of activity

The spectrum refers to the number of the bacterial species affected by the same drug. There are narrow, intermediate and broad-spectrum antimicrobials (Giguère 2006). Narrow spectrum antimicrobials act against a specific microorganism/microorganisms e.g. cloxacillin, which targets gram-positive bacteria. Narrow spectrum antimicrobials are recommended for use when a specific pathogen is known and such use decreases the risk of development of antimicrobial resistance. Intermediate spectrum antimicrobials are drugs that are effective predominantly against Gram-negative organisms but also having some secondary activity against Gram-positive bacteria and *vice versa* (Giguère 2013c). The broad-spectrum antimicrobials interfere with the life cycle of several types of bacteria in a less specific manner across the Gram spectrum and administered in cases where the specific type of microorganism is unknown in empirical medicine (Morley et al. 2005).

2.4.2 Mode of Action

This mode of action refers to the final effect on the bacteria under in vitro culture conditions, which may be bacteriostatic, bactericidal or bacteriolytic.



- Bacteriostatic antimicrobials inhibit bacterial growth thereby keeping them in a stationary phase of growth e.g. the tetracyclines, macrolides and amphenicols. For bacteriostatic antimicrobials to be effective, the host immune system must be active in order to remove the bacteria from the body (Ungemach, Muller-Bahrdrdt & Abraham 2006). If the immune system is not active and the antimicrobial concentration falls below the minimum inhibitory concentration (MIC), the suppressed bacteria are able to once again divide and establish viable colonies.
- Bactericidal antimicrobials kill the bacterial pathogens. For example, cell wall synthesis inhibitors (penicillins and cephalosporins) cause bacterial lysis when bacteria grow in or are exposed to hypertonic or hypotonic environment (Prescott 2013a). Bactericidal drugs can be used with success in immune compromised patients.
- Bacteriolytic antimicrobials kill bacteria by activating autolysin-mediated autolysis leading to bacterial lysis. Autolysins naturally produced by peptidoglycan containing bacteria, function to break down the peptidoglycan layer for the insertion of new building blocks in preparation for bacterial division (Giguère 2013a, Lambert, Allison & Gilbert 2002).

While the above principles are well accepted, they are an *in vitro* microbiological determinant and dependent on drug concentration, growth conditions, bacterial density, test duration and extent of reduction in bacterial numbers (Giguère 2006). As a result, most antimicrobials are better described as potentially being both bactericidal and bacteriostatic, as this is yet to be a proven effect clinically (Rubin 2013, Mueller, de la Peña & Derendorf 2004).



2.4.3 Mechanism of Action

The third classification of antimicrobials is according to their mechanism of action (Figure 4). The goal of antimicrobial drug therapy is selective toxicity to the bacterial cell while at the same time being non-lethal to mammalian host cells (Vardanyan, Hraby 2006). In principle, drugs exhibit the least host toxicity when they target unique target common pathways of the pathogenic organism. Selective toxicity can be realised through the following mechanisms; targeting the genetic or biochemical pathway that is unique to the pathogen; targeting protein isoform that is unique to the pathogen or targeting metabolic requirement that is specific to the pathogen (Lambert, Allison & Gilbert 2002, Vardanyan, Hraby 2006, Toussaint, Gallagher 2014). Antimicrobial agents typically function through one of the five mechanisms of action (see section 2.4.3.1- 2.4.3.5) (Davies 2006, Midtvedt 2007, Baron 1996).

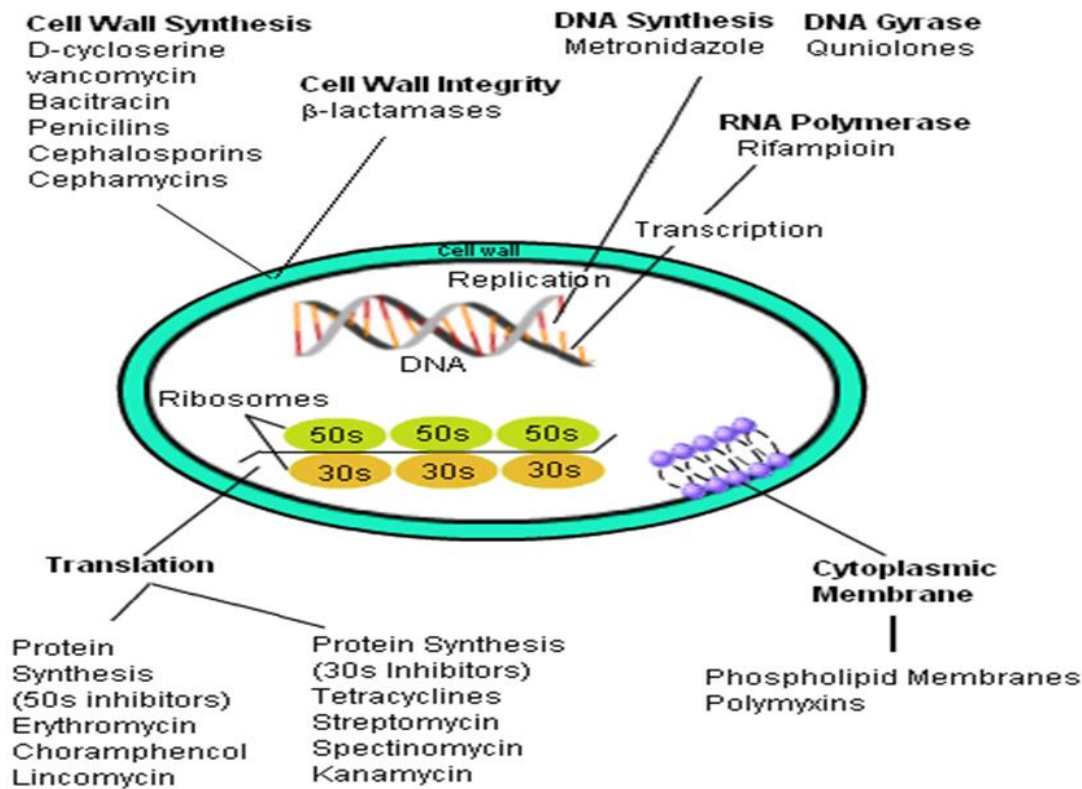


Figure 4: Antimicrobial mechanisms of action

The figure above is a schematic representation of the target and mechanism of action of certain antimicrobials and examples of drugs. (Adapted from Medical Microbiology (Baron 1996))

2.4.3.1 Inhibition of cell wall synthesis

The bacterial cell wall is an important heteropolymeric component that provides rigid mechanical stability for maintaining an osmotic balance for bacterial normal growth due to its highly cross-linked peptidoglycan lattice structure (Davies 2006). The cell wall of bacteria can be divided into two broad groups, the Gram-positive and the Gram-negative (see section 2.2). The biosynthesis of the peptidoglycan involves several bacterial enzymes and occurs in different stages (Giguère 2006). The first stage occurs in the cytosol and involves the synthesis of murein monomers from amino acids and sugar building blocks. The murein monomer is a disaccharide



comprising N-acetyl glucosamine (NAG) connected via a beta linkage to the C4 hydroxyl of N-acetyl muramic acid (NAM), functionalised on the C3 lactate moiety with a peptide (Lambert, Allison & Gilbert 2002). The first two enzymes in this process, MurA and MurB, convert the C3 hydroxyl of NAG to lactate. MurA, also known as enolpyruvate transferase, transfers enolpyruvate from phosphoenolpyruvate (PEP) to UDP-NAG to form UDP-NAG pyruvate enol ether (Nanninga 2009). The flavoenzyme MurB a UDP-NAG-enolpyruvate reductase, reduces the double bond to produce UDP-NAM, which has a free carboxylate to serve as the handle for the peptide chain (Midtvedt 2007). UDP-NAM is a sugar unique to bacteria, and its biosynthesis thus provides opportunities for selective antimicrobials. One clinically used antimicrobials that blocks the biosynthesis of UDP-NAM is fosfomycin, a PEP analog that inhibits MurA (Golan 2012, Michalopoulos, Livaditis & Gougoutas 2011) .

The second and third stages involve the export of these murein monomers to the surface of the inner membrane, followed by their polymerization into linear peptidoglycan polymers (Petri 2011a). The terminal reaction in its synthesis is the addition of a dipeptide, D-alanine-D-alanine, and involves the completion of the cross-linkages in the peptidoglycan lattice structure (Midtvedt 2007). The enzyme responsible for catalysing the crosslinking of the peptidoglycan lattice structure is the peptidoglycan glycosyltransferases outside the cell membrane of gram positive and within the periplasmic space of gram-negative bacteria (Petri WA 2011b). The transpeptidase enzymes catalyse the formation of the peptide cross-bridges. Glycopeptides and β -lactam compounds inhibit the glycosyltransferases reaction (Prescott 2013a). Penicillin acetylates the transpeptidase enzyme to form a penicilloyl enzyme and when bound the reaction is usually irreversible (Petri 2011a, Toussaint, Gallagher 2014). The disruption of the cell wall



formation weakens the wall allowing fluid to transgress into the hyperosmotic internal environment, thereby causing osmotic rupture of the bacterial cell and consequently death (Lambert, Allison & Gilbert 2002).

2.4.3.2 **Inhibition of cell membrane function**

Bacterial plasma membranes are composed of a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bilayer (Lambert, Allison & Gilbert 2002). The plasma membrane acts as a diffusion barrier for nutrients, water, ions and for transport systems (Levinson 2014). A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic and neutral agents that disrupt the structure of cell membrane lipid bilayer increasing cell permeability through a detergent-like action (Giguère 2006). These drugs specifically disrupt the outer membrane of susceptible gram-negative bacteria by binding with lipopolysaccharides through the interaction of cationic drugs with the anionic lipid region in the outer membrane of the gram-negative bacteria (Lambert, Allison & Gilbert 2002). Polymyxin B and polymyxin E are the best known antimicrobials that disrupt cell membrane permeability leading to leakage of cell contents (nucleic acid and nutrients) and death.

2.4.3.3 **Inhibition of protein synthesis**

Protein synthesis occurs through translation of the genetic information coded in the mRNA (Toussaint, Gallagher 2014). This process takes place on the ribosomes, and consists of three stages: initiation, elongation and termination (Davies 2006). In general, the functional unit of bacterial protein synthesis is the 70S ribosome, which consists of the 30S and 50S subunits. For



translation to result, the 30S subunit, a tRNA with a start codon and the specific mRNA need to attach to start the sequence (Strohl 1997). Once this sequence is initiated, which is completely random, the 50S ribosomal subunit is bound initiating the process of protein formation. Hereafter the amino acid chain elongates by the binding of a correct tRNA (codon to anti-codon matching) at the peptidyl site of the 50S subunit, peptide bond-formation via the activity of the peptidyltransferase and subsequent translocation of the 70S ribosomal unit along the mRNA chain (MacDougall, Chambers 2011b, MacDougall, Chambers 2011a).

A protein synthesis inhibitor is an antimicrobial that stops, slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins (Davies 2006). The aminoglycosides, amphenicols, lincosamides, macrolides, streptogramins and the tetracyclines inhibit protein synthesis, through various mechanisms in the protein translation process. They bind to ribosomes and prevent normal peptide chain formation at one or more of the points, which include, the interference with the initiation of polysome construction; misreading of mRNA on the recognition region of the ribosome; insertion of wrong amino acid in growing a peptide chain; breakup of polysomes into monosome; blocking the attachment of charged aminoacyl-tRNA thereby preventing introduction of new amino acids into a growing peptide chain; inhibition of peptidyltransferase causing interruption of binding new amino acids and interference with aminoacyl translocation reaction (Golan 2012, MacDougall, Chambers 2011b).



2.4.3.4 **Inhibition of nucleic acid synthesis or prevention of repair**

Deoxyribonucleic acid (DNA) replication is the process of producing two identical replicas from one original DNA molecule (Arnott et al. 1974). This biological process occurs in all living organisms and is the basis for biological inheritance. Cellular proof reading and error-checking mechanisms ensure near perfect fidelity for DNA replication (Frank-Kamenetskii, Prakash 2014). A number of proteins are associated with the replication fork that helps in terms of the initiation and continuation of DNA synthesis. DNA gyrase and topoisomerase I act in concert to maintain an optimum supercoiling state of DNA in the cell (Arnott et al. 1974). In this capacity, DNA gyrase is essential for relieving torsional strain during replication of circular chromosomes in bacteria. The antimicrobials that function against these target sites interfere with nucleic acid synthesis and function by complexing with DNA or by inactivating/competing with the other components involved in nucleic acid replication reactions (Strohl 1997). The rifamycins interfere with DNA-directed RNA polymerase; the quinolones disrupt DNA synthesis by interference with DNA gyrase and topoisomerase IV during replication, while the nitroimidazoles bind to DNA, causing the reduction of the nitro moiety to the nitro anion radical leading to a loss of the helical structure, strand breakage, and impairment of DNA function (Giguère, Dowling 2013).

2.4.3.5 **Inhibition of metabolic pathways**

Unlike mammals, bacteria lack a transport system to take up preformed folic acid from the environment and must synthesize their own folates. Bacteria need PABA (*para*-aminobenzoic acid) to form dihydrofolic acid, a precursor of folic acid (Petri 2011b). Folic acid is required for purine and pyrimidine synthesis and hence nucleic acid synthesis. The first stage in the synthesis



of purines and pyrimidine involves the reaction between PABA and pteroylglutamic acid to form dihydropteroic acid, catalysed by the enzyme pteridine synthetase (Prescott 2013b). The second stage catalysed by the enzyme dihydrofolate synthetase involves the formation of dihydrofolic acid from dihydropteroic acid and L-glutamate. The last stage involves the formation of tetrahydrofolic acid from dihydrofolic acid and NADPH catalysed by the enzyme dihydrofolate reductase. Tetrahydrofolic acid can then be used for the synthesis of purines and pyrimidines (Prescott 2013b). Sulphonamides are structural analogues of PABA and thus act as competitive antagonists in bacterial cells (Davies 2006). Sulphonamides not only block formation of folic acid but can also be incorporated into the precursors, forming a pseudo metabolite that is reactive and antimicrobial. Mammalian cells are not susceptible to sulphonamides as they absorb and use preformed folic acid, resulting in a wide therapeutic index (Strohl 1997). The combination of sulphonamides with diaminopyrimidines (trimethoprim) has a potentiated activity (Petri 2011b). Diaminopyrimidines enter bacteria and inhibit bacterial dihydrofolic acid reductase, thus acting on the same metabolic pathway as sulphonamides in a synergistic manner (Giguère 2006). The binding affinity of trimethoprim is very much greater for the bacterial enzyme than for mammalian enzyme; therefore, selective bacterial toxicity occurs (Petri 2011b).

2.5 Antimicrobial usage in animal health

Antimicrobial use in veterinary medicine is for the treatment and control of diseases in a variety of animal species. They are also used in livestock feed in very low doses as growth promoters. Veterinary medical advances made it possible to locate, treat, and prevent catastrophic animal health diseases that could be of major concern to animal owners (OIE 2004). In a normal



scenario, when the animal owner first notices signs of animal disease, the owner should refer the case to the veterinarian, who will then discern the course of treatment, if necessary. If the veterinarian decides antimicrobials are necessary, the main objective of their use should be to limit the progression of disease as quickly as possible in a population of animals, (Junker *et al* 2009). Antimicrobial use in animals is divided into three categories of therapeutic, prophylactic or metaphylactic management of diseases:

2.5.1 Therapeutic

Antimicrobial therapeutics is the administration of antimicrobials to animals with the objective to limit disease progression (Anthony et al. 2001, van den Bogaard, Stobberingh 2000). Successful antimicrobial therapy begins with the decision to treat and subsequent decision on selecting the most appropriate antimicrobial for use in a particular animal or infectious agent. The decision to use antimicrobials relies on the advances in veterinary diagnostics, which makes it possible to make a definitive bacterial diagnosis. With current problems with antimicrobial resistance, veterinarians should avoid administering antimicrobials for therapeutic purposes unless the evidence of infection justifies treatment (Morley et al. 2005).

2.5.2 Prophylactic

Antimicrobial prophylaxis is the administration of antimicrobials to a group of animals at risk as a measure taken to maintain health and prevent disease (Freeman 1980). Prophylactic use of antibacterial agents is important in preventing the establishment of bacterial infectious diseases (Contopoulos-Ioannidis, Wiedermann 1999). For example, perioperative antimicrobial



prophylaxis indicated after dental procedures and contaminated surgery where the consequences of infection would be disastrous (Golan 2012, Freeman 1980). Antimicrobials are also used prophylactically to prevent infections in animals with weakened immune systems such as feline immunodeficiency virus (FIV) in cats (Morley et al. 2005).

2.5.3 Metaphylactic

Antimicrobial metaphylaxis refers to a particular form of prophylaxis in the presence of a pathogen with a high likelihood of disease; for example administration of antimicrobials to clinically healthy animals belonging to the same group as animals with clinical signs (Schwarz, Kehrenberg & Walsh 2001). In this case, infections are treated before their clinical appearance.

2.6 Factors affecting the success or efficacy of antimicrobial therapy

The efficacy of any antimicrobial agent is dependent on the biophasic drug concentration (its pharmacokinetics), the drug's mechanisms of action (pharmacodynamics) and the drug's relation with the bacterial MIC (referred to as pharmacokinetic-pharmacodynamic interaction) (Gumbo 2011, Giguère 2013c).

2.6.1 Pharmacokinetics

Pharmacokinetics deals with the movement of an antimicrobial from the administration site to the place of its pharmacologic activity and its elimination from the body (Giguère 2006). The pharmacokinetic phase describes how the antimicrobial agent reaches the site of infection and the duration of time the drug spends at the area of infection before being eliminated from the



body. The pharmacokinetics of a drug is determined by the dose rate and its interaction with a number of other factors that affect the absorption, serum concentration and disposition of a drug after administration (Buxton, Benet 2011). Important factors are the drug's physico-chemical characteristics, such as lipid solubility, molecular size, pKa and protein binding of the drug and physiological factors such as pH, perfusion, distribution barriers, tissue composition and size of fluid compartments in the body (Buxton, Benet 2011). To be effective, an antimicrobial agent must be distributed to the site of infection and come into contact with the infecting organism in adequate concentrations of the active drug form (Weese 2006). In tissues with adequate blood supply, free drug concentrations achieved in plasma are directly related to or equal to the concentration in the extracellular space (Buxton IO 2011). However, drug distribution to the central nervous system (CNS), eye, epithelial lining of the lung, prostate and mammary gland is limited by decreased membrane permeability, as the lipid membrane forms a barrier to drug diffusion (Golan 2012, Buxton IO 2011). Intracellular bacteria e.g. Brucella and Mycobacterium will not be affected by antimicrobial agents that remain in the extracellular space and must therefore be treated with antimicrobials that accumulate in cells, for example fluoroquinolones, lincosamides and macrolides (Giguère, Dowling 2013). Local factors that restrict access of antimicrobial agents to the site of infection include abscess formation, pus, necrotic debris (inactivates aminoglycosides and sulphonamides) and oedema fluid (Dowling 2013a). Environmental conditions can be manipulated to enhance antibacterial activity for example, in lower urinary tract infections it is desirable for urine to be acidic when using tetracyclines and alkaline when using aminoglycosides (Petri WA 2011a). The ability of a molecule to penetrate membranes increases with decreasing electrostatic charge; thus antimicrobial drugs that are weak



acids work best in an acidic environment and those that are weak bases work best in an alkaline environment (Baggot & Giguère 2013) .

The most important pharmacokinetic parameters are C_{max} , AUC, half-life of elimination, and T_{max} (Norris, Spyker 1984b). The C_{max} is the maximum plasma drug concentration achieved after drug administration (Mueller, de la Peña & Derendorf 2004). The T_{max} is the time of maximum drug concentration and is reached when drug elimination rate equals absorption rate (Baggot, Giguère 2013). The AUC is the area under the plot of plasma concentration of drug against time after drug administration (Norris, Spyker 1984a). The AUC is determined by the “trapezoidal rule” and is of particular use in estimating the extent of bioavailability of drugs. Bioavailability is a measurement of the rate and extent at which the active moiety of the administered drug reaches systemic circulation thereby accessing the site of action (Baggot, Giguère 2013). Half-life is the period of time required for the concentration of drug in the body to be reduced by one-half and is usually considered in relation to the amount of the drug in plasma or serum (Mueller, de la Peña & Derendorf 2004). A drug molecule that leaves plasma may have any of several fates. It can be eliminated from the body, or it can be translocated to another body fluid compartment such as the intracellular fluid or it can be destroyed in the blood. The removal of a drug from the plasma is known as clearance and the distribution of the drug in the various body tissues is known as the volume of distribution. Both of these pharmacokinetic parameters are important in determining the drug dosage and dosing frequency (Norris, Spyker 1984b).



2.6.2 Pharmacodynamics

Pharmacodynamics correlates the concentration of the antimicrobial with its clinical effects at the site of action. For an antimicrobial, this correlation refers to the ability of the drug to kill or inhibit the growth of microorganisms (Martinez, Toutain & Turnidge 2013). Antimicrobials elicit their activity against bacteria by binding to a specific protein(s) or structure(s) in the bacteria (Gumbo 2011, Martinez, Toutain & Turnidge 2013). For an antimicrobial to eradicate an organism, it must bind to its target site, occupy an adequate number of binding sites, and remain at the binding site for a sufficient period of time in order for the metabolic processes of the bacteria to be sufficiently inhibited (Gumbo 2011, Buxton, Benet 2011). The most important PD parameter is the MIC. The MIC is defined as the lowest concentration of an antimicrobial that completely inhibits the growth of bacteria in vitro (Mueller, de la Peña & Derendorf 2004). The MIC is determined using agar plates, tubes or microtitre trays with serial dilutions of antimicrobials that are inoculated with the bacteria and incubated. After a day of incubation the MIC is recorded as the lowest concentration of antimicrobial agent with no visible growth (Mueller, de la Peña & Derendorf 2004).

2.6.3 Pharmacokinetic-Pharmacodynamic Interactions

The pharmacodynamic parameter, MIC, is a good indicator of the potency of an antimicrobial, but indicates nothing about the time course of antimicrobial activity (Mueller, de la Peña & Derendorf 2004). On the other hand pharmacokinetic parameters (C_{max} , AUC and T_{max}) quantify the serum concentration time course, but do not describe the killing activity of antimicrobials. The integration of the pharmacokinetic (PK) with pharmacodynamic parameters



(PD) quantifies the activity of antimicrobials (Figure 5) and is crucial in the successful treatment of infections and prevention of antimicrobial resistance (Gumbo 2011, Martinez, Toutain & Turnidge 2013). Using the MIC approach, antimicrobials are frequently divided into two major groups: those that exhibit time-dependent killing and those that exhibit concentration-dependent killing (Mueller, de la Peña & Derendorf 2004). The success of time dependent antimicrobials (e.g. penicillins, macrolides) depends on the length of time that the antimicrobial concentration in body fluids and in contact with the bacteria is above the MIC ($T > MIC$). Their effect will increase with increasing concentrations until a finite point is reached. After that point, increasing concentrations will not produce a corresponding increase in the effect; therefore, high peak concentration will not help. Maximum killing has been seen to occur at concentrations approximately four to five times the MIC (Mueller, de la Peña & Derendorf 2004). The time above MIC needs to exceed 40 – 50% of the dosing interval to prevent the regrowth of resistant sub-populations of bacteria (Martinez, Toutain & Turnidge 2013). The clinical implication for time-dependent killing antimicrobials is that they should be dosed more frequently or if possible have their half-life prolonged by other drugs so that the concentration persists above the MIC as long as possible (Mueller, de la Peña & Derendorf 2004, Buxton, Benet 2011, Norris, Spyker 1984a).

Concentration dependent killing antimicrobials, which include the aminoglycosides and fluoroquinolones, exhibit a different killing pattern. In their case, the bacterial rate of killing is seen to increase with increasing concentrations of the antimicrobial (MacDougall, Chambers 2011a). The goal in this case is to maximize the drug concentration. The efficacy of concentration-dependent killing antimicrobials is dependent on the ratio of AUC and maximum



concentration of antimicrobial reached in the serum (C_{\max}) to the MIC (Gumbo 2011, Buxton, Benet 2011, Baggot, Giguère 2013). The AUC to MIC ratio ($AUC > MIC$) of concentration-dependent antimicrobials will determine the overall outcome of therapy (Golan 2012, Martinez, Toutain & Turnidge 2013). The C_{\max} to MIC ratio (C_{\max}/MIC) is very important in preventing the regrowth of a resistant sub-population of bacteria (Lloyd 2007). The C_{\max}/MIC linked antimicrobials (e.g. rifampin) can be administered less frequently because of their long duration of the post-antibiotic effect (PAE). The PAE is when the antimicrobial effect continues long after the antimicrobial concentrations declines below the MIC (Mueller, de la Peña & Derendorf 2004, Martinez, Toutain & Turnidge 2013). Less frequent dosing with concentration dependent killing antimicrobials may decrease the chances of drug related adverse effects.

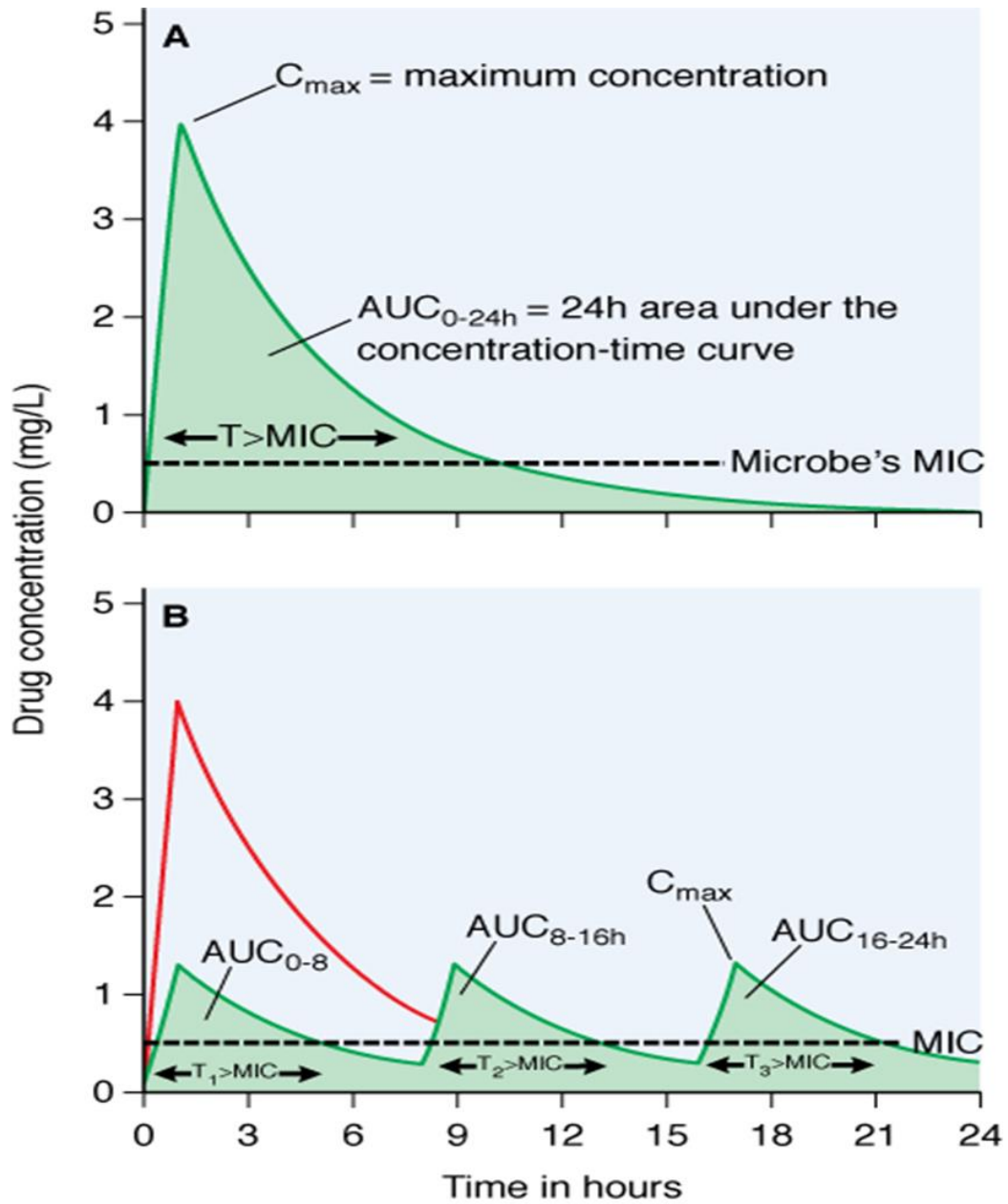


Figure 5: Pharmacokinetic-pharmacodynamic parameters

The figure above shows graphs that integrate the pharmacokinetic (PK) with pharmacodynamic parameters (PD) quantifying the activity of antimicrobials and the effect of different dose schedules on shape of concentration-time curve. (Source: Goodman and Gilman's The Pharmacological Basis of Therapeutics).



2.7 Antimicrobial resistance

Antimicrobial resistance is the capability of bacteria to survive exposure to a defined concentration of an antimicrobial, be it therapeutically, prophylactically, for growth promotion or for disinfection (Boerlin, White 2013, Milani et al. 2012). Resistance can be an intrinsic property of the bacteria themselves or it can be acquired (van den Bogaard, Stobberingh 2000, Okeke et al. 2005b). Acquired antimicrobial resistance can result from a mutation of cellular genes, the acquisition of foreign resistance genes or a combination of these two mechanisms (Boerlin, White 2013, Cain 2013). Thus, there are two main ways of acquiring antimicrobial resistance: through mutation in different chromosomal loci and through horizontal gene transfer (*i.e.* acquisition of resistance genes from other microorganisms) (Boerlin, White 2013).

Although the manner of acquisition of resistance may vary among bacterial species, resistance is created by only a few mechanisms:

- i. Antimicrobial inactivation: direct inactivation of the active antimicrobial molecule for example, enzymatic deactivation of Penicillin G by β -lactamase in penicillin-resistant bacteria (Cain 2013).
- ii. Target modification: Target modification is the modification of the antimicrobial target site so that the antimicrobial is unable to bind properly for example, alteration of PBP, the binding target site of penicillins in MRSA and other penicillin-resistant bacteria (Boerlin, White 2013).
- iii. Efflux pumps: Efflux pumps are membrane bound proteins that export antimicrobials out of the cell and keep its intracellular concentrations at low levels. Efflux pumps affect almost



all classes of antimicrobials, especially tetracyclines, fluoroquinolones and macrolides because these antimicrobials inhibit intracellular processes like protein and DNA biosynthesis and therefore must be intracellular to exert their effect (Okusu, Ma & Nikaido 1996, Périchon, Courvalin 2009, Singer et al. 2003).

- iv. Outer membrane (OM) permeability changes and target bypass: some bacteria become resistant to antimicrobials by circumventing inactivation by enzymes. This mode of resistance is common in many trimethoprim- and sulphonamide-resistant bacteria. An example is the bypassing inhibition mechanism of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) enzymes. Trimethoprim and sulphonamides inhibit DHFR and DHPS enzymes respectively (Périchon, Courvalin 2009, Andersson, Hughes 2012).

There is a great variety of antimicrobial resistance mechanisms within each of the four classes and a single bacterial strain may have several types of resistance mechanisms. The mechanism that dominates depends on the antimicrobial class, bacterial species, target site and whether it is mediated by a plasmid or by a chromosomal mutation (Gumbo 2011). The problem of antimicrobial resistance is facilitated by poor therapeutic practices as well as indiscriminate use of antimicrobials for agricultural and animal husbandry purposes, for example when they are used to treat disorders against which they have no efficacy and when they are used widely for prophylaxis (Escher et al. 2011). The big concern with the development of antimicrobial resistance in both human and animal health is that there is then cross-resistance between antimicrobials used in animal health and antimicrobials applied in the treatment of people (Lloyd 2007). This may lead to fewer efficacious and safe antimicrobials for the treatment of diseases in



humans, increasing the risk of treatment failure (Morley et al. 2005). These antimicrobials may also be more difficult or inconvenient to administer (for example intravenous administration) in that their use may necessitate hospitalization for treatment and thereby incur higher costs for treatment as well as more suffering for the patient (Eagar, Swan & Van Vuuren 2012). Companion animals live in close proximity to humans and the risk of transmission of resistant bacteria or genetic material-conferring resistance cannot be overlooked (Pleydell 2012). Therefore, it is of utmost importance that veterinarians are aware of and recognize the importance of judicious antimicrobial use when treating small companion animals.

2.8 Prudent use of antimicrobials

The prudent or judicious use of antimicrobials has been recommended in order to optimize the therapeutic effects while minimizing the risk for resistance development (Anthony et al. 2001). In this system, the use of the antimicrobials is combined with principles of animal good management and husbandry in order to reduce the need for antimicrobials (Morley et al. 2005). Numerous professional groups have published prudent use guidelines on antimicrobial use in animals. In the following sections (2.8.1– 2.8.7), a brief overview of the basic principles is given.

2.8.1 Veterinarian-client-patient relationship

The use of prescription antimicrobials or extra label use requires a good veterinarian animal owner relationship. This relationship exists when all of the following conditions have been met (Ungemach, Muller-Bahrtdt & Abraham 2006, Escher et al. 2011, Guardabassi et al. 2009, Törneke, Boland 2013):



- i. The veterinarian has accepted the responsibility for clinical judgments regarding the health of the animal and the need for medical treatment, and the animal owner has agreed to follow the veterinarian's instructions.
- ii. The veterinarian has knowledge of the animal to initiate a tentative diagnosis of the medical condition of the animal.
- iii. The veterinarian should be readily available for follow-up evaluation or should have arrangements for emergency coverage in cases of adverse reactions.

2.8.2 Use of narrow spectrum antimicrobials

Generally, broad-spectrum antimicrobials can lead to the development of resistance in non-targeted bacterial populations more rapidly compared to narrow spectrum antimicrobials because they exert a selection pressure on a greater number of microorganisms (Rodríguez-Rojas et al. 2013). Narrow spectrum antimicrobials should be the first line of treatment, as they do not have much effect in non-target bacterial populations (Weese 2006). The theory is that narrow spectrum antimicrobials will have a less of an effect on non-target species of bacteria hence less chances of resistance development in commensal bacteria (Andersson, Hughes 2012; Rich, Perry 2011).

2.8.3 Use of culture and sensitivity tests to select antimicrobials

Susceptibility profiles can vary between different herds and flocks. Periodic culture and sensitivity testing can provide historical data on which to base future empirical treatment as well as assist with refractory infections (Guardabassi et al. 2009). Ideally, the susceptibility profile of



the causal organism should be determined before starting therapy. The veterinarian has a responsibility to determine the applicability to the specific disease indication of the breakpoints used by the laboratory (Weese 2006). In disease outbreaks involving high mortality or where there are signs of rapid spread of disease, treatment may be started on the basis of a clinical diagnosis before susceptibility results are obtained (Ungemach, Muller-Bahrtdt & Abraham 2006). Even so, the susceptibility of the suspected causal organism should be determined so that if treatment fails it can be changed in the light of the results of susceptibility testing. Antimicrobial susceptibility trends should be monitored over time, and such monitoring used to guide clinical judgment on antimicrobial usage. The latter is not always possible as it is sometimes difficult to send samples for cultures and sensitivity for financial reasons or poor availability of nearby laboratories (Rich, Perry 2011). In such cases the veterinarian has no option other than treating empirically.

2.8.4 Minimise therapeutic exposure

Theoretically, infections should be treated with antimicrobials only until the host's defence system is competent to resolve the infection (Weese 2006, Monnet 2010). Since this period is difficult to judge, antimicrobials should be used for a limited duration of time as determined by the veterinarian to minimize the exposure of non-target bacterial population to the antimicrobial. However, treatment for too short a period is discouraged as it can lead to recrudescence of the infection, which in turn promotes the development antimicrobial resistance as bacterial populations are exposed to sub therapeutic doses (Sykes 2013).



2.8.5 Limit antimicrobial treatment to sick animals only

The use of antimicrobials in the absence of clinical disease or pathogenic infections should be restricted to situations where experience indicates that the group of animals has a high risk of developing a disease if not treated (Törneke, Boland 2013). In addition, long-term administration to prevent disease should not be done without a clear medical justification.

2.8.6 Minimise environmental contamination

Dispose of unused antimicrobials properly and responsibly in line with national laws (Guardabassi et al. 2009). Some antimicrobials may be environmentally stable in manure, water and pasture. This will lead to non-pathogenic environmental bacteria being exposed to sub lethal antimicrobial doses and consequent development of resistant genes (Gaze et al. 2008). The resistant genes can later on be transferred to pathogenic human and animal bacteria. Consideration maybe needed to develop and enforce proper disposal methods that will not recycle resistant organisms to humans or animals.

2.8.7 Regulatory authorities and legislation

National regulatory authorities are responsible for granting marketing authorisations (Bartoloni 2007). The terms under which these authorisations should be granted are an important tool for promoting subsequent prudent use and for providing appropriate information to the veterinarians administering the antimicrobials. In most countries, national regulatory authorities are responsible for establishing systems for the surveillance of antimicrobial consumption, draw up



national guidelines on the prudent use of antimicrobials and elaborate national regulations to ensure adherence to them (Franklin et al. 2001). The regulatory authorities are also responsible for carrying out surveys to ensure that labels and advertising materials are accurate and in accordance with approved claims. Advertising materials should have clear explanations about the drug, indications and contraindications, dosage, and frequency of medication.

Veterinary or health-related teaching institutions should offer courses on prudent use of antimicrobials and this will be an effective investment, as graduates will apply knowledge on antimicrobial best practices and thereby avoiding unnecessary use of antimicrobials. Over the counter (OTC) sales without a prescription should be controlled and personnel selling OTC antimicrobials should be informed and educated on antimicrobial drug resistance. Veterinarians play a central role in ensuring the prudent use of antimicrobials in the management of animal diseases and in encouraging good hygienic and infection control practices, to reduce the need for antimicrobials (Guardabassi et al. 2009). Furthermore, the use of antimicrobials as growth promoters should be strictly controlled by making these antimicrobial growth promoters available for food animal use only by veterinary prescription. If possible, this practice should be completely stopped.

There is need for better dissemination of information to users of antimicrobials on knowledge about resistance patterns in target animal organisms and on the optimum choice of antimicrobial and dosage regime per indication (Scott, Page & Prescott 2013). Continued professional development (CPDs) is an important communication tool for reaching out to veterinarians and influences their prescribing habits. Early and continuous engagement of stakeholders is essential



for successful implementation of prudent use guidelines and this can be achieved by holding workshops that include veterinarians, manufacturers and animal owners.

In South Africa antimicrobials are regulated by the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, (Act 36 of 1947), administered by the Department of Agriculture, Forestry and Fisheries and the Medicines and Related Substances Control Act, (Act 101 of 1965), administered by the Medicine Control Council (MCC) under the National Department of Health (Eagar, Swan & Van Vuuren 2012). Antimicrobials registered under Act 36 of 1947 as stock remedies are available over the counter (OTC). Antimicrobials intended for use in animals and registered under Act 101 of 1965 may only be administered and/or prescribed by a veterinarian (Eagar, Swan & Van Vuuren 2012). The free access and indiscriminate use of OTC antimicrobials under Act 36 makes it difficult to implement the principles of prudent use and this will contribute to the development of antimicrobial resistance. There is also dichotomy in the marketing of veterinary medicines in South Africa, with more control by Act 101 of 1965 but no prescriptive measures by Act 36 of 1947. This has implications for the availability and access of antimicrobials in the various sectors of animal health. It is strongly believed that fragmented legislation leads to conflicting views on the implementation of prudent use of veterinary antimicrobials.

2.9 International initiative on veterinary antimicrobial usage

Efforts to establish veterinary antimicrobial usage patterns have been made in different parts of the world, with limited success and variations between regions and countries in their surveillance



capacity (Hughes et al. 2012, Holso et al. 2005). Data on total antimicrobial utilization in particular areas are unfortunately limited, unreliable or many times non-existent. Many countries do not have data on the antimicrobial usage patterns; those that have them use different systems of data collection, making comparisons difficult or impossible to establish (Escher et al. 2011, Rantala et al. 2004). The ultimate goal of surveillance on antimicrobial usage should therefore aim at providing tools, information and direction needed to guide policy on the appropriate use of antimicrobials and to evaluate resistance containment interventions at local and international levels (Naidoo, Sykes 2006). Systematic surveillance is still lacking in many parts of the world particularly in developing countries due to constraints related to infrastructure, trained personnel, networking and coordination (Woodward 2005). In countries with effective surveillance, political support and strong health systems appear to be critical for success (Woodward 2005). Below is a list of examples on the currently prevailing global status of surveillance for veterinary antimicrobials.

- In 1995 the Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) was established. SVARM is a surveillance system for the volumes of veterinary antimicrobials consumed in Sweden (Björn, Christina & Catarina 2003).
- In the United States, the non-profit organization Alliance for the Prudent Use of Antibiotics (APUA) formed a panel in 1999 to investigate issues concerning antimicrobial resistance and the use of antimicrobials in animals (APUA 2014).
- In Denmark the Ministry of Food, Agriculture and Fisheries funds a monitoring system based on antibiotic usage information at herd level. Since 1996 trends on antibiotic usage patterns in food animals have been published and the data has been collected using a system called VETSTAT (DANMAP 2003).



- In Finland a condition based use of antimicrobials in dogs was undertaken (Rantala et al. 2004), followed by another similar study in cats in 2009 (Thomson et al. 2009).
- In Australia antibiotic usage patterns is assessed from import records. All antibiotic importers are requested to declare the indication of the antibiotics they are importing to the Therapeutic Goods Administration (TGA).
- In South Africa, a study was undertaken on the establishment and standardization of veterinary antimicrobial resistance surveillance and monitoring in 2004 (Nel, van Vuuren & Swan 2004).
- In South Africa, the South African National Veterinary Surveillance and Monitoring programme was established for monitoring resistance to antimicrobial drugs (Van Vuuren, Picard & Greyling 2007).
- In 2008 a survey of antimicrobial usage in animals in South Africa with specific reference to food animals was conducted (Eagar, Swan & Van Vuuren 2012).
- In 2011 a cross sectional survey of antimicrobial prescribing patterns in UK small animal veterinary practice was undertaken (Hughes et al. 2012).

2.10 Conclusion

Antimicrobials are deemed necessary for the treatment and prevention of infectious diseases in animals. The main concern regarding the use of antimicrobials in animals has been the emergence of antimicrobial resistant bacteria that could affect humans directly or the transfer of resistant genes to human pathogens. Data on antimicrobial usage patterns is still lacking in many parts of the world particularly in developing countries due to constraints related to infrastructure,



trained personnel, networking and coordination. Effective surveillance and political support are critical for the successful data collection on antimicrobial usage. It is therefore of great importance in South Africa that patterns associated with the prescription and use of antimicrobials are optimised. The ultimate goal of surveillance on antimicrobial usage should aim at providing tools, information and direction needed to guide policy on the appropriate use of antimicrobials and to evaluate resistance containment interventions at local and national levels.



CHAPTER: 3 MATERIALS AND METHODS

3.1 Data collection

The following study was undertaken through the use of a survey. The survey consisted of 20 closed and 5 open questions loaded onto Google forms. The questionnaire was delivered to participants via an email that provided the link to the online survey tool (see addendum). On opening the link, respondents had access to the questionnaire where closed questions were compulsory and respondents would not be able to send back the questionnaire if they had not responded to any of the closed questions. Open-ended questions were optional.

3.2 Population and sample size

The source population for the survey was registered veterinarians with the South African Veterinary Council (SAVC). The questionnaire was sent out electronically to 2880 veterinarians after a request was made to SAVC to have access to their email addresses. At the time of emailing the questionnaire, it was not possible to identify email addresses specific to veterinarians in small animal practice and still practicing in South Africa, hence the questionnaire was sent to all registered veterinarians with SAVC with a request for those in small animal practice and practicing in South Africa to complete the online survey.

3.3 Statistical analysis

All the data from the returned questionnaires was transferred to a Microsoft excel 2007 spreadsheet and evaluated by descriptive statistics in the form of a histogram and pie charts.



CHAPTER: 4 RESULTS

During the time (October 2013) that the survey questionnaire was sent, there were 1120 registered small animal veterinarians. With 181 completed questionnaires the survey had a 16.16% response rate. Responses were received from eight provinces as follows; Eastern Cape 7.02% (n=12), Free State 4.09% (n=7), Gauteng 39.18% (n=67), KwaZulu Natal 20.46% (n=35), Mpumalanga 1.75% (n=3), North West 3.51% (n=6), Northern Cape 0.58% (n=1), Western Cape 23.39% (n=40); (Figure 6). The majority of the responses were from veterinarians with a bachelor's degree (63.54%, n=115), 21.55% (n=39) with BVSc (Hons), 3.31% (n=6) with MSc (Vet), 1.66% (n=3) with MSc (Non Vet), 7.18% (n=13) MMedVet specialists and 3% (n=5) PhD holders. Respondents that worked in full time small animal practice constituted 77.9% (n=141) while 22.1% (n=40) were part time small animal veterinarians. In this study, 55.25% (n=100) of the respondents work in small animal practice only, 22.1% (n=40) predominantly small animal and 19.34% (n=35) in mixed practice. Table 1 is a summary of the respondents' answers.


Table 1: Questionnaire responses by number and percentages for the 181 respondents

Question	Response	Respondents	
		Number	(%)
Gender	Male	89	49.17
	Female	92	50.83
Highest level of qualification	Bachelors	115	63.54
	BVSc(Hons)	39	21.55
	MSc(Vet)	6	3.31
	MSc(Non-Vet)	3	1.66
	MMedVet	13	7.18
	PhD	5	2.76
Are you employed full-time or part-time	Full-time	141	77.90
	Part-time	40	22.10
Practice Type	Small animal only	100	55.25
	Predominantly small animal	40	22.10
	Mixed practice	35	19.34
	Other	6	3.31
Does the practice have set protocols for treating sick animals with antimicrobials	Yes	48	26.52
	No	133	73.48
How often do you encounter owner-initiated treatments before a case is presented to the practice?	Frequently	23	12.71
	Sometimes	127	70.17
	Never	31	17.13
How often do you encounter owner compliance challenges?	Always	17	9.39
	Sometimes	162	89.50
	Never	2	1.10



Question	Response	Respondents	
		Number	(%)
When a patient presents for the first time and antimicrobials are needed, what is your preferred method of selecting your antimicrobial?	Empirical First	165	91.16
	Antibiogram first,	1	0.55
	Empirical whilst awaiting antibiogram	15	8.29
How often do you get patients returning due to a lack of efficacy of the antimicrobial?	Never	7	3.87
	Sometimes (1-3 times a year)	147	81.22
	Frequently (more than 3 times a year)	27	14.92
Do you routinely undertake any antibiograms in cases of therapeutic failure?	Yes	130	71.82
	No	51	28.18
Have you ever needed to make use of an unregistered antimicrobial via an MCC issued Section 21 permit.	Yes	0	0.00
	No	181	100.00
How often does the practice compound antimicrobial?	Never	157	86.74
	Sometimes (1-3 times a year)	19	10.50
	Frequently (more than 3 times a year)	5	2.76
How does the practice dispose of expired antimicrobial?	Waste disposal contract	69	38.12
	Through another medical waste disposal facility	65	35.91
	Other	47	25.97
For how long do you use a multidose vial once it is broached?	Less than 4 weeks	79	43.65
	4 - 8 weeks	56	30.94
	9 - 11 weeks	18	9.94
	More than 12 weeks	28	15.47



Question	Response	Respondents	
		Number	(%)
How often do you prescribe the extra-label use of antimicrobial?	Never	25	13.81
	Sometimes	133	73.48
	Frequently	23	12.71
How often do you encounter adverse drug reactions associated with antimicrobial use?	Never	58	32.04
	Sometimes	121	66.85
	Frequently	2	1.10
What action do you take in such cases, for which an adverse reaction is seen?	Report to MCC	27	14.92
	Report to veterinary council	9	4.97
	Report to Registrar of Stock Remedies	7	3.87
	No action	138	76.24

Percentage, is the percentage response per category from the 181 respondents

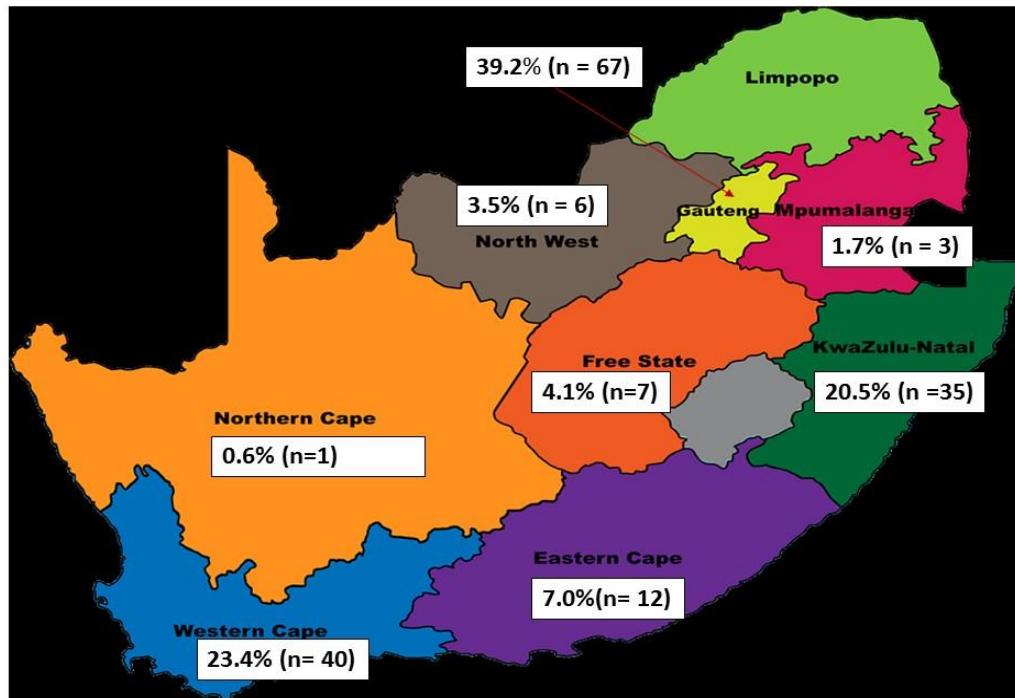


Figure 6: Map showing the percentage distribution of responses from 171 veterinarians across the 9 provinces of South Africa.

4.1 Owner compliance and owner initiated antimicrobial treatments

During the course of their duties, 82.88% (n=150) of the respondents encountered cases of owner initiated treatment before a case was presented to the practice. Respondents encountering cases of owner-initiated treatment had qualifications as follows; BVSc - 53.1% (n = 96); BVSc (Hons) - 18.8% (n=34); MSc (Vet) - 2.8% (n=5); MSc (Non-Vet) - 1.7% (n = 3); MMedVet - 6.11% (n = 11); and PhD - 0.6% (n = 1). A small proportion 17.13% (n=31) reported that they have never encountered cases of owner initiated treatments. In cases of owner initiated treatments, it was reported that owners use the following antimicrobials; Flagyl (metronidazole), Synulox (amoxicillin), Baytril (enrofloxacin), Purbac (sulfamethoxazole and trimethoprim), Terramycin (tetracycline), Augmentin (clavulanate-amoxicillin), Surolan (Miconazole, polymyxin B,



prednisolone), Hitet (tetracycline), doxycycline, penicillins and cephalixin mainly left over from previous treatments.

The majority of the respondents (98.89%; n=179) faced challenges with owner compliance with antimicrobial treatments. They also indicated that they did try to convince their clients on the importance of completing the course of prescribed antimicrobials, followed-up telephonically and during subsequent examinations by asking the owner if they had given the medication as prescribed.

4.2 Patient returns due to lack of efficacy

The majority of respondents (96.14%; n=174) reported that they encountered cases where patients returned to the practice due to antimicrobial therapeutic failure, whilst only 3.87% (n=7) never experience patient returns. Respondents experiencing patient returns had qualifications as follows; BVSc - 62.9% (n = 114); BVSc (Hons) - 21% (n=38); MSc (Vet) - 3.4% (n=6); MSc (Non-Vet) - 1.1% (n = 2); MMedVet - 5.6% (n = 10); and PhD - 2.2% (n = 4).

4.3 Preferred method of antimicrobial selection

The practices that did not have set protocols for treating sick animals with antimicrobials constituted 73.48 % (n = 133) while 26.52% (n = 48) used fixed protocols. Respondents selected antimicrobials for first-time cases as follows; Empirical treatment - 91.16% (n = 165); Antibigram before treatment - 0.6% (n = 1); Empirical treatment whilst waiting for antibiogram



results - 8.29% (n = 15); Antibiogram in case of therapeutic failure - 71.82% (n = 130) and Never use antibiograms in cases of therapeutic failure - 28.18% (n = 51).

4.4 Antimicrobial compounding

Antibiotic compounding was practiced by 13.26% (n=24) of the respondents while 86.74% (n=157) were not in the practice of compounding antimicrobials. The following preparations were compounded: Baytril +Saline + flamazine; Gentamicin + DMSO; Enrofloxacin + DMSO; Baytril + ear preparations.

4.5 Adverse drug reactions with antimicrobial use

Even though antimicrobial adverse drug reactions (ADR) were commonly encountered (67.95%; n = 123) most of the respondents (76.24%; n = 138) took no action after encountering an ADR. Those who did report reactions did so to the Registrar of Act 36 of 1947 (3.87%; n = 7); the South African Veterinary Council (SAVC) (4.97%; n=9) and to the Medicines Control Council (MCC) (14.92%; n = 27).

4.6 Section 21 and extra label use of antimicrobials

All the respondents (100%; n=181) had never made use of an unregistered antimicrobials through the MCC section 21 approval process. In contrast extra-label use of antimicrobials was common practice across the different levels of qualifications. The proportion of respondents using antimicrobials extra- label was 86.19% (n = 156).



4.7 Antimicrobial usage with respect to the specific organ systems

In the form of a table respondents were asked to indicate their preferred antimicrobial by infected organ system by selecting only one box per antimicrobial row (the program as a default allowed only one selection). Figure 7 below lists the most preferred antimicrobial class used by organ system in this survey. A clear trend was per drug and is reported below per antimicrobial class using pie charts.

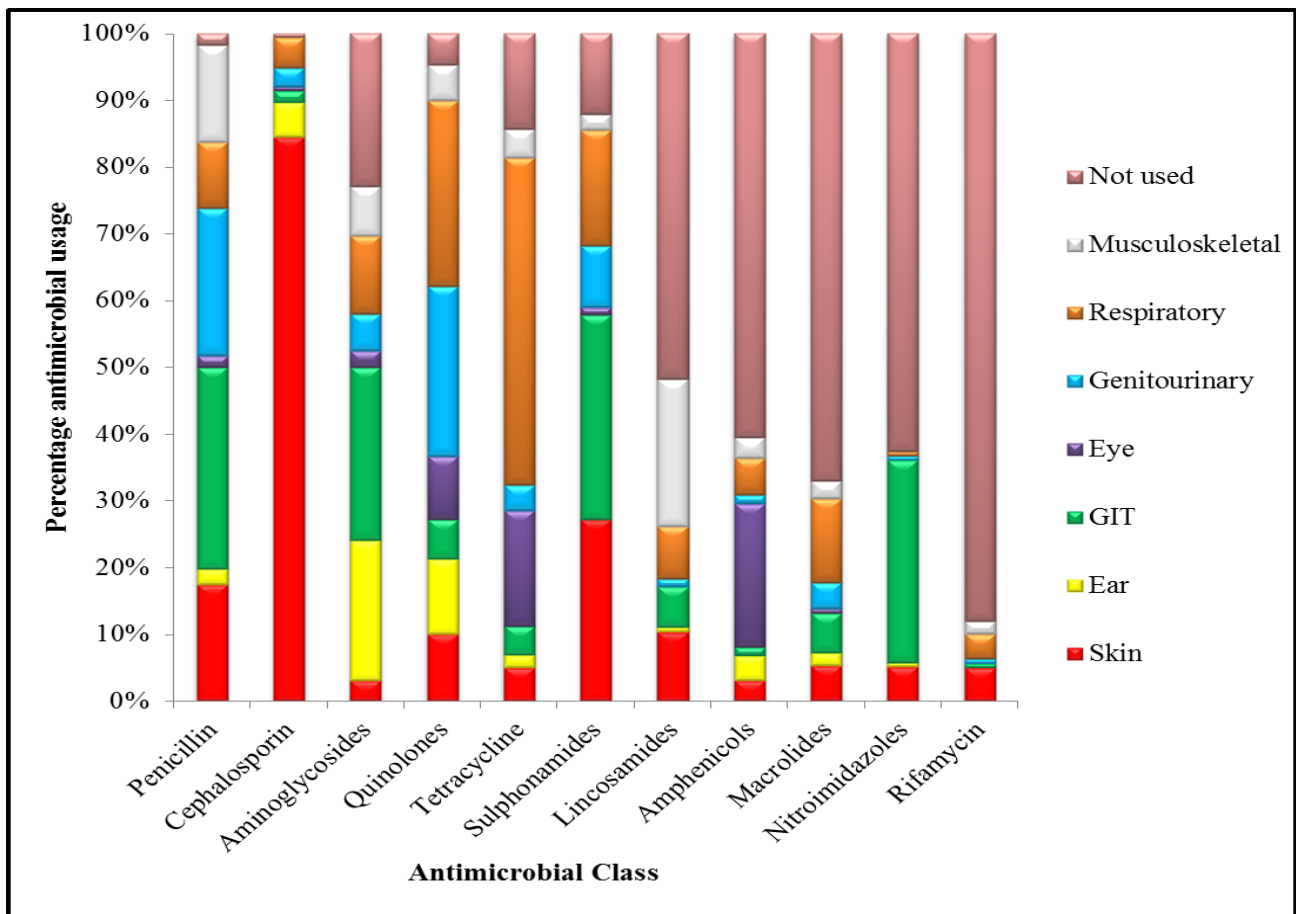


Figure 7: Percentage antimicrobial usage for different organs systems



4.7.1 Aminoglycosides usage pattern on different organ systems

A small proportion of the respondents (23%) did not use aminoglycosides in small animal practice (Figure 8). The remaining 77% of the respondents used the aminoglycosides for the treatment of infection of following organ systems; Skin (3%); Ear (21%); Gastrointestinal tract (GIT) (26%); Eye (2%); Genitourinary (6%); Respiratory (12%) and Musculoskeletal (7%).

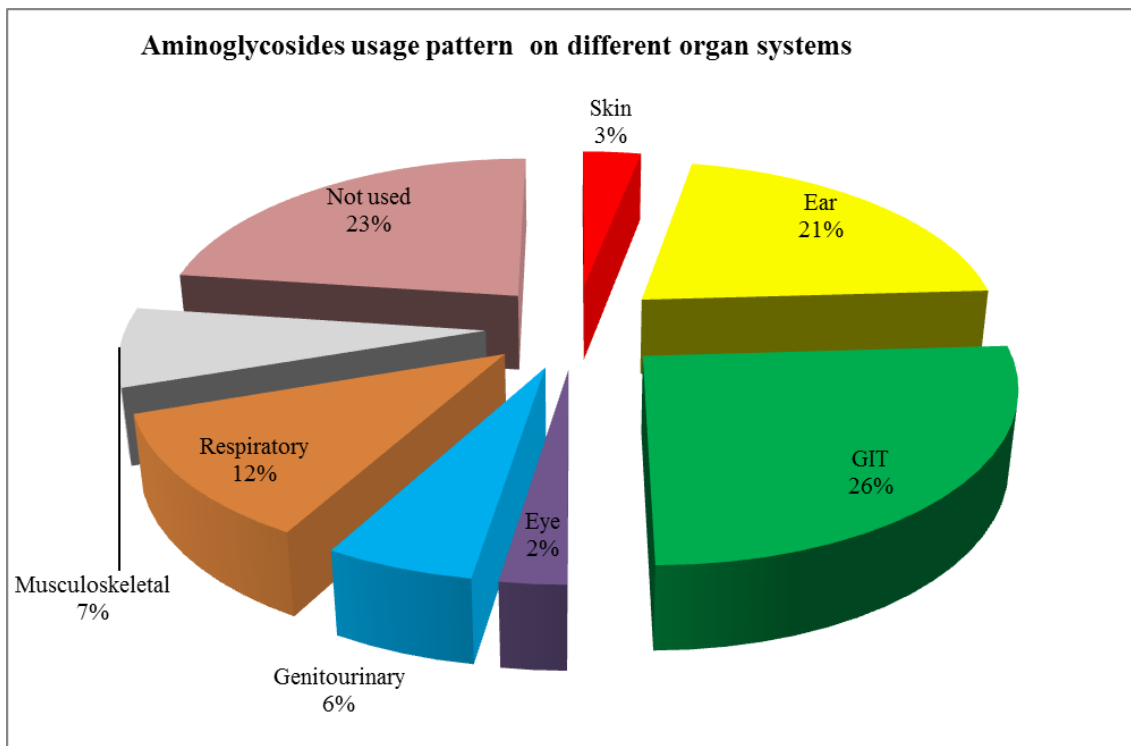


Figure 8: Aminoglycosides usage pattern on different organ systems



4.7.2 Penicillins usage pattern on different organ systems

A small proportion of the respondents did not use the penicillins (2%) (Figure 9). The other respondents made use of penicillins for infections of various organ systems as follows; Skin (17%); Ear (2%); GIT (30%); Eye (2%); Genitourinary (22%); Respiratory (10%) and Musculoskeletal (15%).

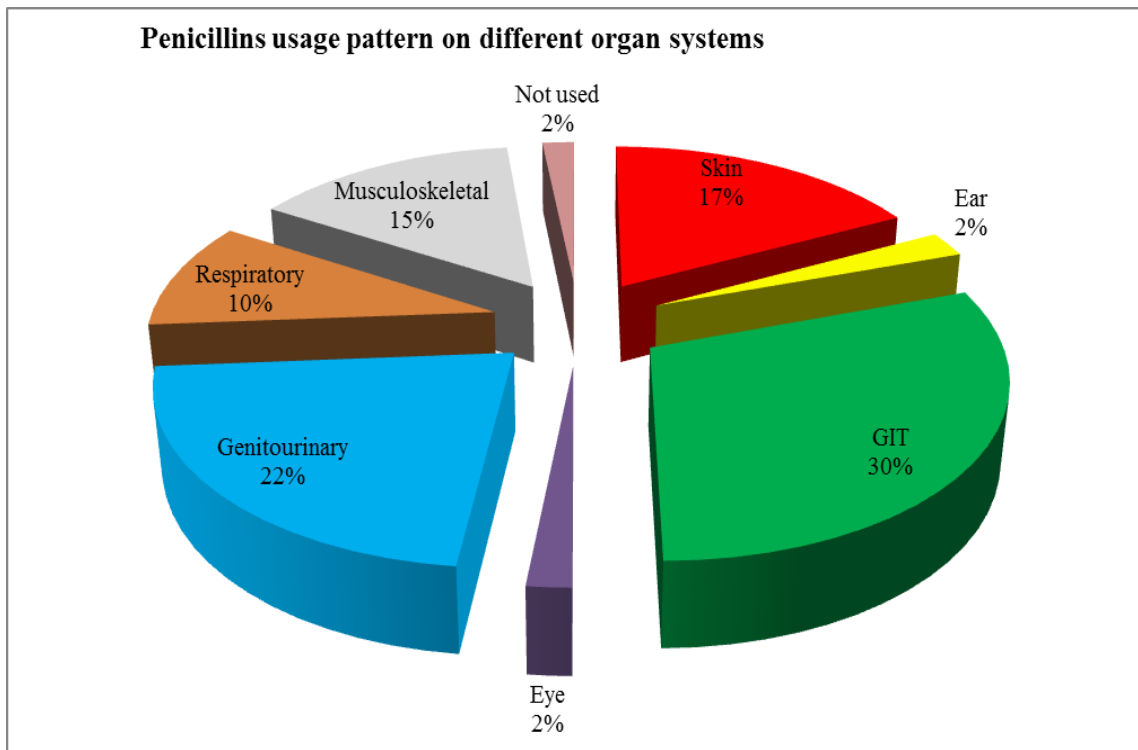


Figure 9: Penicillin usage pattern on different organ systems



4.7.3 Cephalosporins usage pattern on different organ systems

All the respondents have made use of cephalosporins (Figure 10) for infections of various organ systems as follows; Skin (85%); Ear (5%); GIT (2%); Genitourinary (3%) and Respiratory (5%).

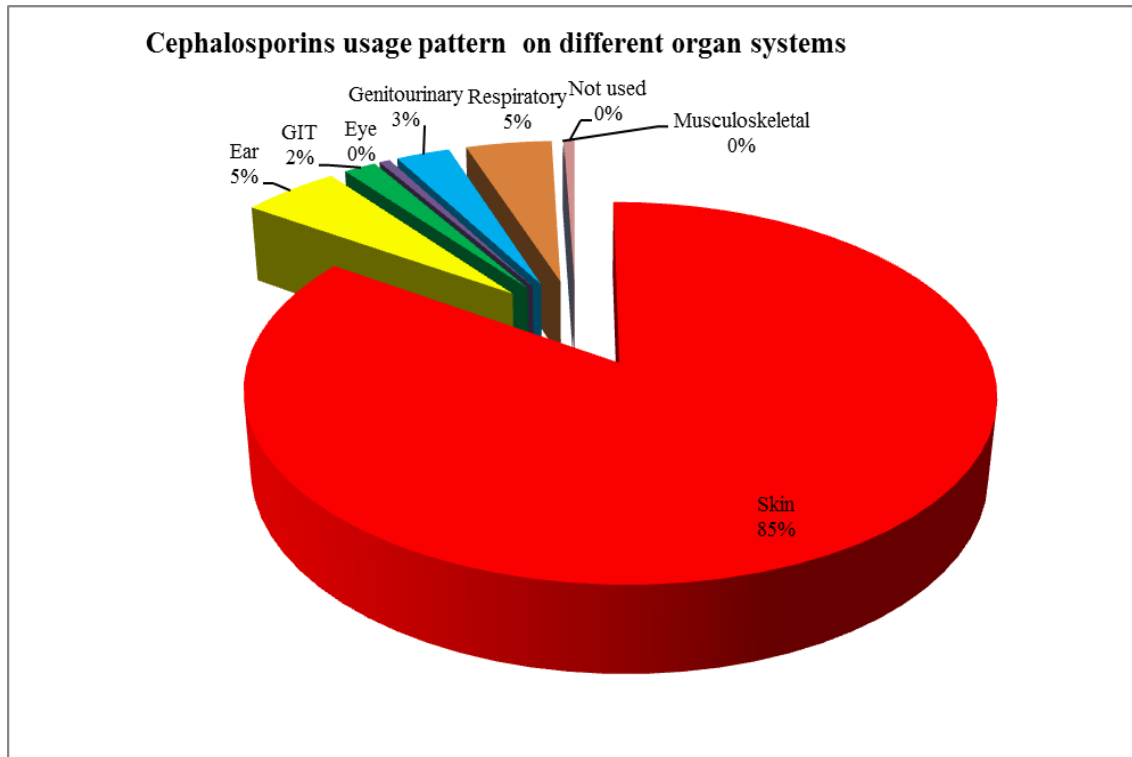


Figure 10: Cephalosporins usage pattern on different organ systems



4.7.4 Quinolones usage pattern on different organ systems

A small proportion of the respondents did not use the quinolones (5%) (Figure 11). The other respondents made use of quinolones for infections of various organ systems as follows; Skin (10%); Ear (11%); GIT (6%); Eye (10%); Genitourinary (25%); Respiratory (28%) and Musculoskeletal (5%).

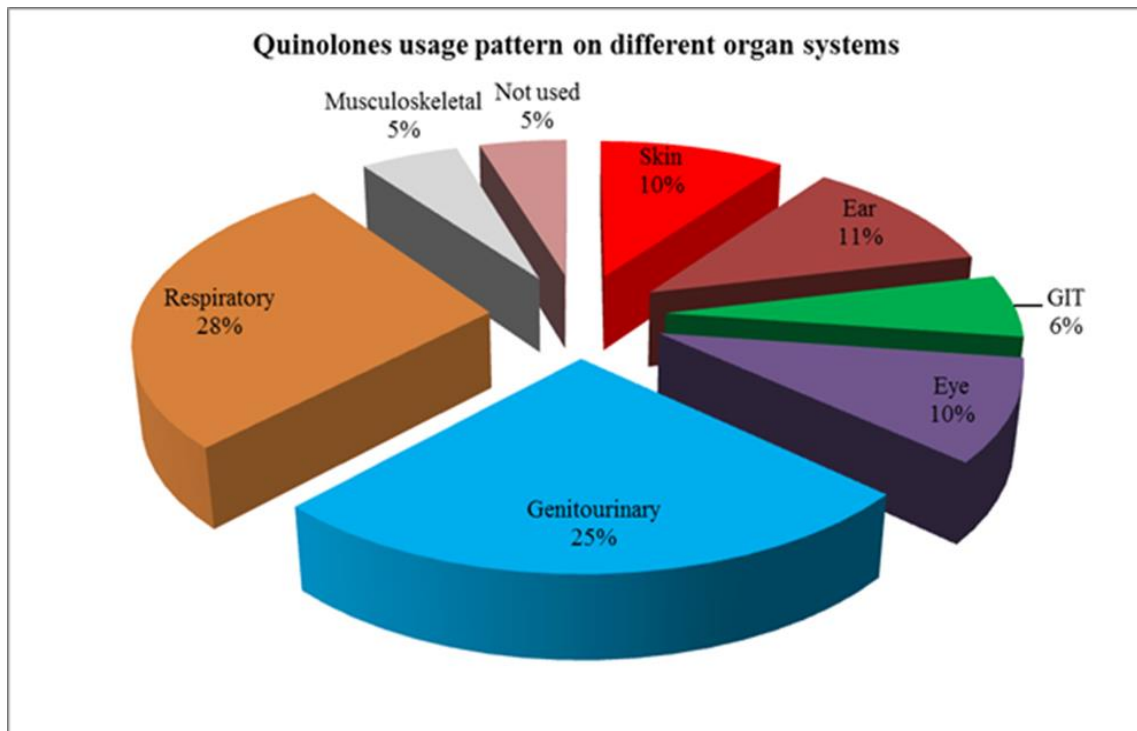


Figure 11: Quinolones usage pattern on different organ systems



4.7.5 Tetracyclines usage pattern on different organ systems

A small proportion of the respondents did not use the tetracyclines (14%) (Figure 12). The other respondents made use of tetracyclines for infections of various organ systems as follows; Skin (5%); Ear (2%); GIT (4%); Eye (18%); Genitourinary (4%); Respiratory (49%) and Musculoskeletal (4%).

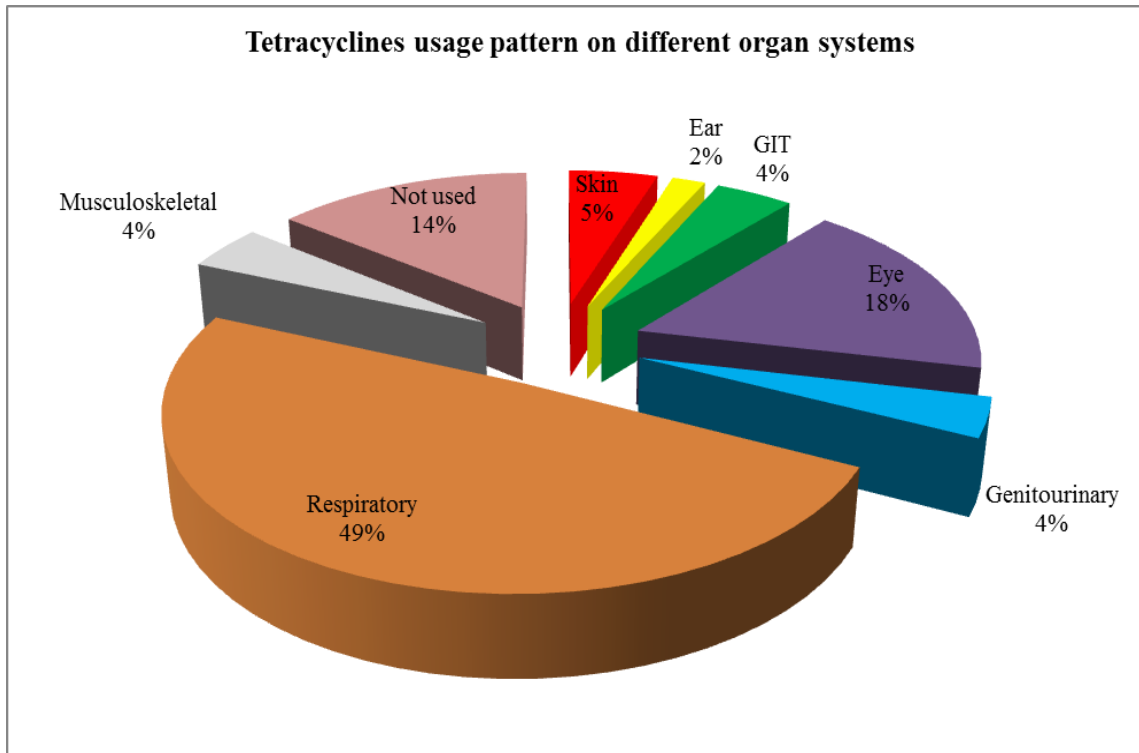


Figure 12: Tetracyclines usage pattern on different organ systems



4.7.6 Sulphonamides usage pattern on different organ systems

A small proportion of the respondents did not use the sulphonamides (12%) (Figure 13). The other respondents made use of sulphonamides for infections of various organ systems as follows; Skin (27%); GIT (31%); Eye (1%); Genitourinary (9%); Respiratory (18%) and Musculoskeletal (2%).

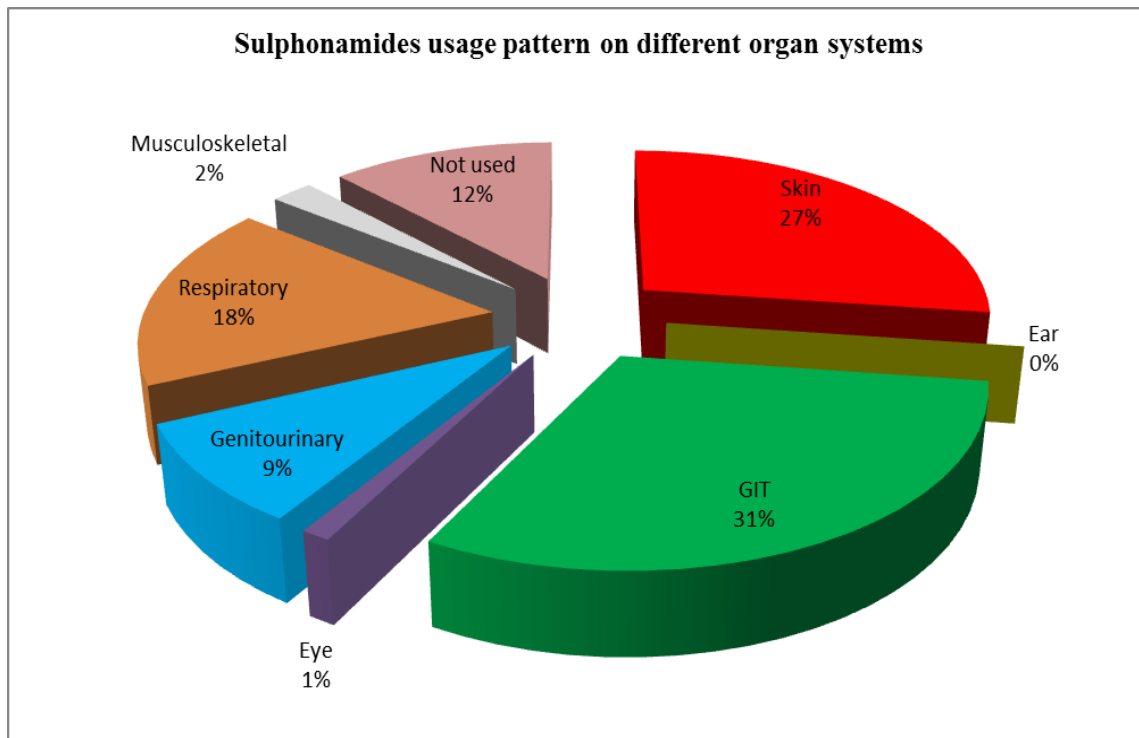


Figure 13: Sulphonamides usage pattern on different organ systems



4.7.7 Lincosamides usage pattern on different organ systems

A big proportion of the respondents did not use the lincosamides (52%) (Figure 14). The other respondents made use of lincosamides for infections of various organ systems as follows; Skin (10%); Ear (1%); GIT (6%); Genitourinary (1%); Respiratory (8%) and Musculoskeletal (22%).

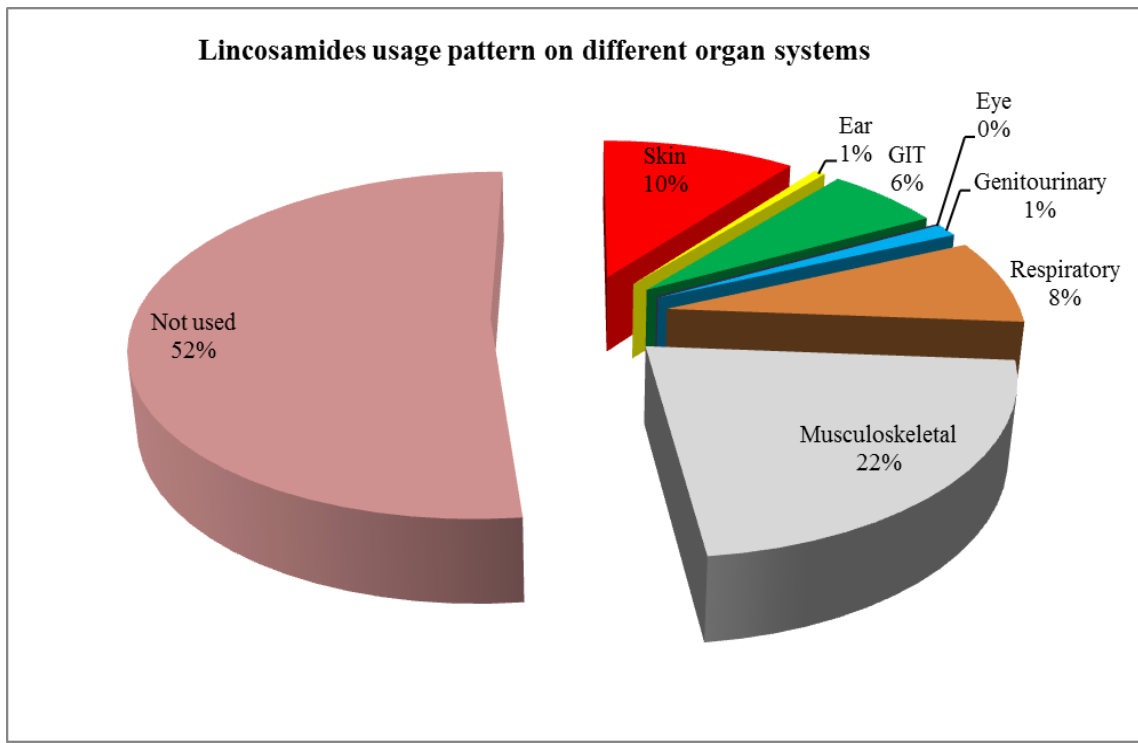


Figure 14: Lincosamides usage pattern on different organ systems



4.7.8 Macrolides usage pattern on different organ systems

A big proportion of the respondents did not use the macrolides (67%) (Figure 15). The other respondents made use of macrolides for infections of various organ systems as follows; Skin (5%); Ear (2%); GIT (6%); Eye (1%); Genitourinary (4%); Respiratory (12%) and Musculoskeletal (3%).

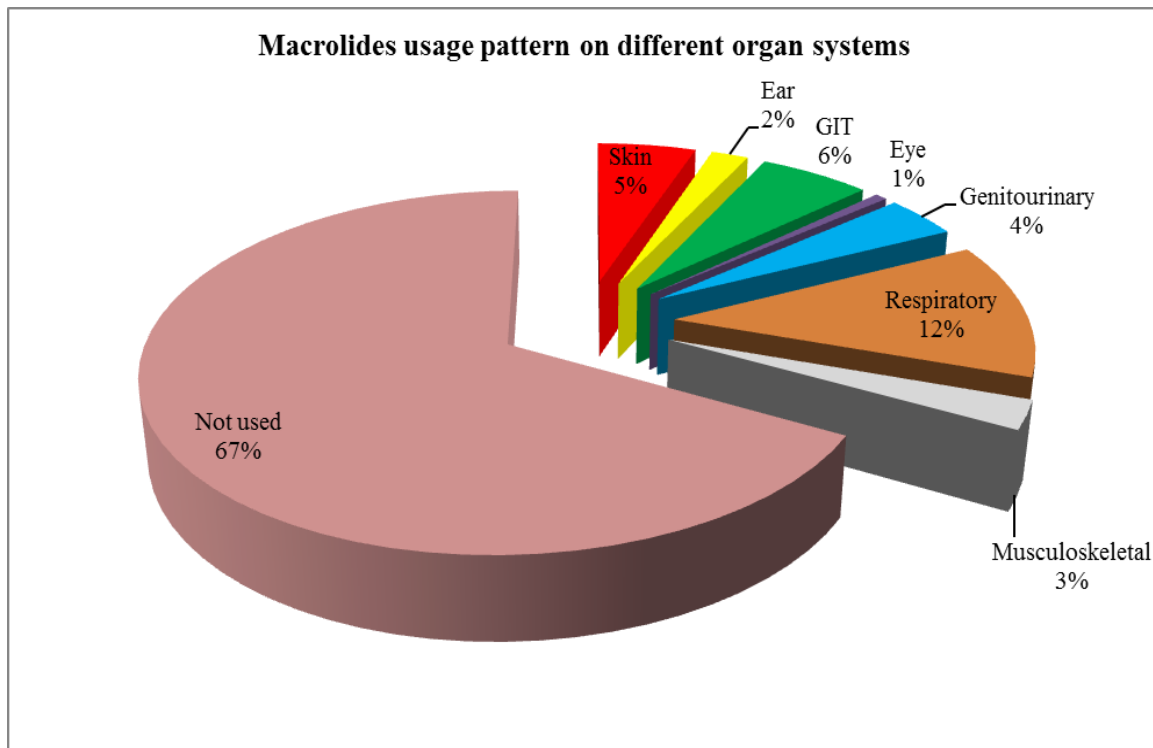


Figure 15: Macrolides usage pattern on different organ systems



4.7.9 Amphenicols usage pattern on different organ systems

A big proportion of the respondents did not use the amphenicols (60%) (Figure 16). The other respondents made use of amphenicols for infections of various organ systems as follows; Skin (3%); Ear (4%); GIT (1%); Eye (22%); Genitourinary (1%); Respiratory (6%) and Musculoskeletal (3%)

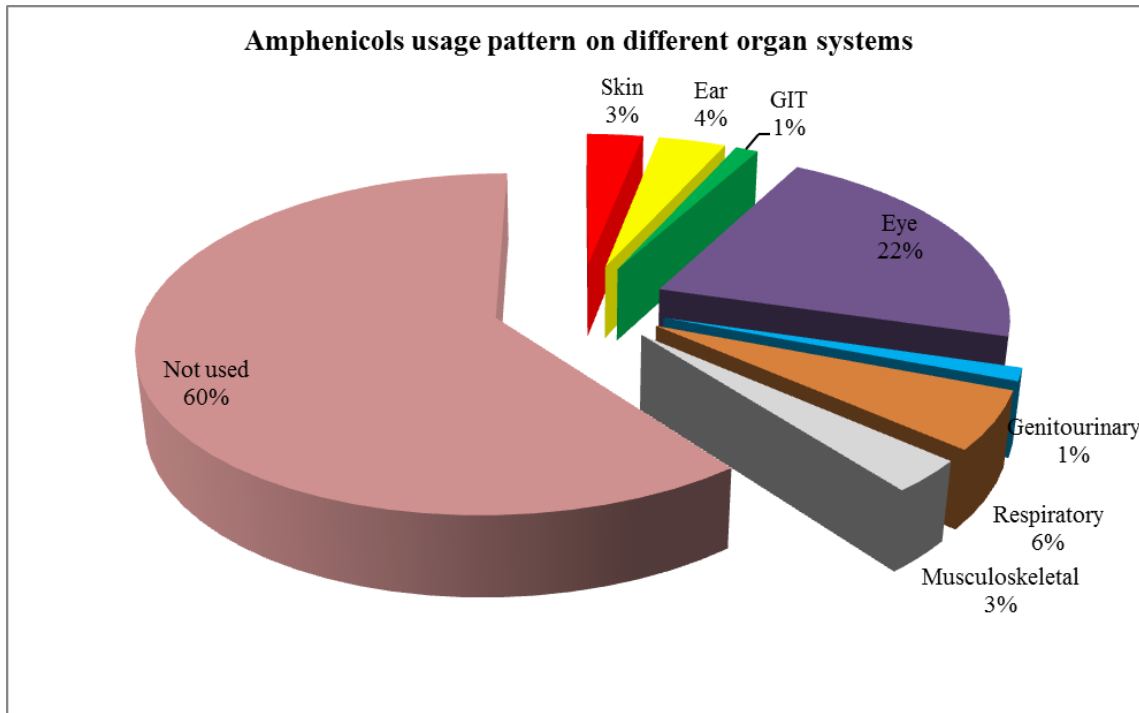


Figure 16: Amphenicols usage pattern on different organ systems



4.7.10 Nitroimidazoles usage pattern on different organ systems

A big proportion of the respondents did not use the nitroimidazoles (63%) (Figure 17). The other respondents made use of nitroimidazoles for infections of various organ systems as follows; Skin (5%); GIT (30%); Genitourinary (1%) and Respiratory (1%)

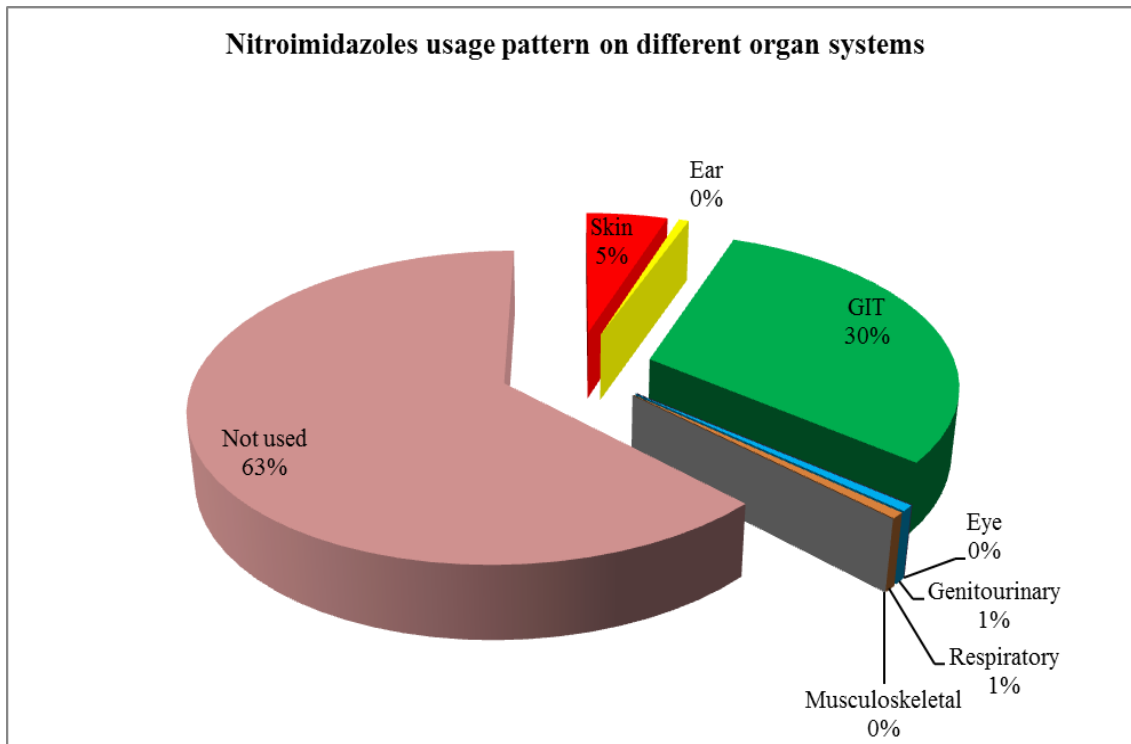


Figure 17: Nitroimidazoles usage pattern on different organ systems



4.7.11 Rifamycins usage pattern on different organ systems

A big proportion of the respondents did not use the rifamycins (88%) (Figure 18). The other respondents made use of rifamycins for infections of various organ systems as follows; Skin (5%); Genitourinary (1%); Respiratory (4%) and Musculoskeletal (2%).

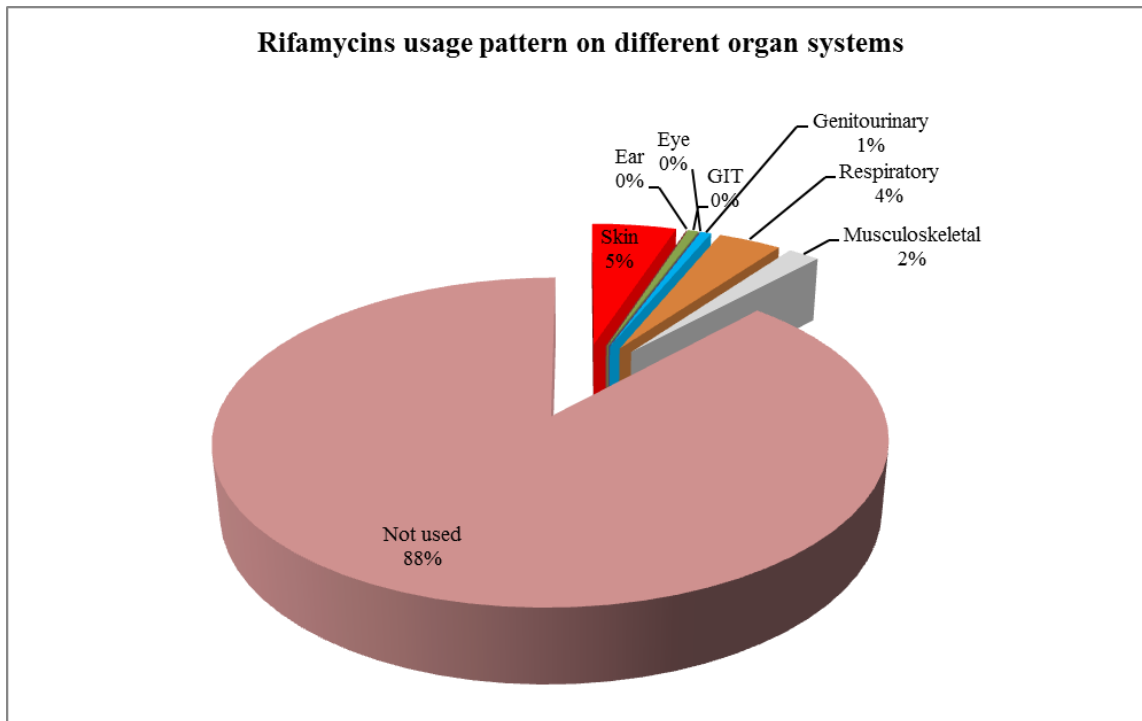


Figure 18: Rifamycins usage pattern on different organ systems

4.8 Treatment duration and use of multidose vials

The respondents provided an indication of the duration they treated infections of the various organ systems (Table 2). None of the respondents treated skin and respiratory infections for less than 3 days and GIT infections for over 21 days. There was no trend observed with the treatment



of other organ systems as the treatment duration varied from less than 3 days to more than 21 days.

Table 2: Duration of treatment for different organ systems

Organ system	Treatment length				
	Less than 3 days	4-7 days	8 - 14 days	15 - 21 days	Over 21 days
Skin	0 (0)	16 (8.84)	48 (26.52)	71 (39.23)	46(25.41)
GIT	13 (7.18)	159 (87.85)	8 (4.42)	1 (0.55)	0 (0.00)
Ear	0 (0)	51 (28.18)	91 (50.28)	25 (13.81)	14 (7.73)
Eye	11 (6.08)	139 (76.80)	27 (14.92)	2(1.1)	2 (1.10)
Genitourinary	1 (0.55)	54 (29.83)	83 (45.86)	34 (18.78)	9 (4.97)
Respiratory	0 (0)	81 (44.75)	84 (46.41)	13 (7.18)	3 (1.66)
Musculoskeletal	2 (1.10)	97 (53.59)	37 (20.44)	27 (14.92)	18 (9.94)

With regards to the period of use of multi-dose vials, various periods of use were recorded once the vial was broached: 4 weeks use - 43.65% (n = 79); 4 to 8 weeks use - 30.94% (n = 56); 9 – 11 weeks use - 9.94% (n = 18) and more than 12 weeks use 15.47% (n = 28).



CHAPTER: 5 DISCUSSION

5.1 Survey Response

The aim of this study was to identify antimicrobial use patterns in small animal veterinary medicine in South Africa and to find out whether veterinarians treating dogs and cats were making use of antimicrobial prudent use guidelines in optimizing their antimicrobial usage, via a survey. Responses were received from all the provinces except from Limpopo province; with the highest proportion coming from Gauteng province (39.18%), followed by Western Cape (23.39%) and then KwaZulu Natal (20.46%). The distribution of the responses across the provinces most likely is representative of the responding small animal veterinarians being based in major cities (Johannesburg, Pretoria, Durban and Cape Town) where the most affluent societies are found. A poor response rate of only 16.16% was obtained for this study. This compared poorly in comparison to a similar study undertaken in the United Kingdom (UK), which had a 51% response (Hughes et al. 2012). Even when compared to other South African studies, the response was low as one study had a response rate of 27% (162 completed questionnaires) (Joubert 2000). Whilst the cause of the poor response was not known at this stage, the format of the survey may have played a role as it was very detailed. The response rate could also have been influenced by the topic of the study, as antimicrobial usage in animals is a controversial topic with veterinarians having different opinions. It is also possible that veterinarians who did not respond were unwilling to share their opinions.

Despite the few respondents, the study results are similar to findings of Hughes *et al.* (2012), who concluded that antimicrobial use guidelines were rare, while off license prescriptions with



antimicrobials by small animal veterinarians occurred in the UK. In addition to these findings, this study shows that compounding of antimicrobials, acquiring of antimicrobials without veterinary prescription and animal owners using left over antimicrobials are other problems encountered in South Africa.

5.2 Owner compliance and owner initiated antimicrobial treatments

Veterinarians play a big role in overseeing and directing antimicrobial use in animals as they are responsible for making key treatment decisions for example; clinical diagnosis, selecting the correct antimicrobials for use, collecting samples for laboratory analysis and most importantly communicating the decision to the animal owner (Anthony et al. 2001). However, from this study it was clear that owners do not see their veterinarians as the first step in the treatment of their pets as they were attempting to treat their animals without veterinary consultation or attempted to use leftover antimicrobials as a first step in treating sick animals. Whilst many reasons may have contributed to this, the most likely reason is the cost of veterinary treatment, as the owner may be attempting to treat their animals as a means of saving money, by hoping that their animals recover without significant financial input. The problem with their manner of use is that they are using the antimicrobials as a uniform group without taking into consideration that the different drugs have different spectrums and resistance profiles i.e. to them all antimicrobials are identical.

Also of concern is their ability to source antimicrobials even though most are prescription drugs in terms of Act 101 of 1965. One such source would be left-over antimicrobials from their



animal's previous veterinary visit or even from their visits to their doctor. Another source would be the acquiring of antimicrobials through other channels, such as the tetracyclines, sulphonamides and tylosin which are registered through Act 36 of 1947 for over the counter (OTC) use in production animals. The problem with this use is that these drugs are often used at subtherapeutic drug concentration (Scott, Page & Prescott 2013) as they are not matching the disease with the correct antimicrobial, they use the antimicrobials for the incorrect duration or they simple use the incorrect dose. Subtherapeutic drug concentrations are below the MIC, and hence will not have a beneficial effect as the concentration will be too low to inhibit bacterial growth (Baggot, Giguère 2013, Martinez, Toutain & Turnidge 2013). Exposed bacteria can take advantage of concentrations below the MIC and develop ways to coexist with the antimicrobial thereby developing resistance (Aminov 2009).

An intervention to prevent owners from incorrectly using the antimicrobials is needed. These would include the removal of all the farm animal antimicrobials from over-the-counter use. Efforts should be made by the South African government to provide information to the South African public on the importance of antimicrobial resistance and the reason for veterinary supervision when being used. The level of such training should perhaps be a part of the school biology curriculum. It is also incumbent on the veterinarian to explain to the animal owner in layman's language on why an antimicrobial is given, importance of completing treatment, signs to look for in case of an ADR, how antimicrobial resistance develops and its impact on public health. Educating the client about antimicrobial resistance together with consideration of specific factors of owner compliance, as mentioned above will decrease the likelihood of poor owner compliance. Such communication and cooperation will also decrease the risk of



occurrence of antimicrobial resistance. According to the survey, South African veterinarians must be praised for implementing the latter intervention of trying to educate animal owners about antimicrobial resistance.

5.3 Preferred method of antimicrobial selection

The majority of the respondents (91.16%) reported that they treated animals empirically before undertaking an antibiogram. This may have resulted from two reasons:

- Economics: The majority of the South African population are middle income earners who are not prepared to spend a lot of money on their pet animals. In most of the cases veterinarians are left with no laboratory diagnostic option as clients tend to opt for the more economical course of action. Taking into consideration the costs involved with confirmatory diagnostics laboratory tests, veterinarians will end up treating empirically.
- Underestimation of the importance of antibiograms: This could be an indication that veterinarians underestimate the importance of antimicrobial susceptibility testing and are not fully aware of the risks posed by antimicrobial-resistant bacteria. It may also indicate that South African veterinarians are not taking into account the development of resistance in human pathogenic bacteria. A similar conclusion was made in a New Zealand study by Pleydell et al., (2012) that the broad-spectrum antimicrobials considered by the World Health Organisation to be critical for human health (e.g. fluoroquinolones and clavulanate-amoxicillin), were frequently prescribed antimicrobials in companion animal medicine without susceptibility testing.



Current prudent use guidelines recommend that culture and susceptibility tests of the causal organism be done as this helps determine the type of antimicrobial most effective in treating the infection and thereby decreasing the risk of selection for antimicrobial resistance in the pathogenic bacterial population (Giguère 2006, Scott, Page & Prescott 2013). This may be facilitated by the veterinarian collecting a sample from a suspected infected organ, secretion or excretion e.g. lungs, urine or faeces. The samples are then sent to the microbiology laboratory where culture and susceptibility testing of the bacterial pathogen will be done. It is further recommended that veterinarians should not start treatment before receiving the results of culture and susceptibility testing, unless it is a life threatening condition whereupon treatment can be based on surveillance data of the local pathogens (Escher et al. 2011), in a process called empirical treatment (Giguère 2013c).

Despite arguments that past experience can ensure that a veterinarian chooses the correct antimicrobial, there is no evidence to support this statement as antimicrobial resistance is on the increase. In supporting the use of antibiograms, we have also taken into account that the young veterinarians do not have any past experience to work on, as none of this wealth of past experience is published, as well taking into account that antimicrobials susceptibility testing is itself flawed, as it fails to take into account *in vivo* conditions such as the dilution effect of tissue fluid, a functional immune system and a non-static environment that may alter the concentration of the antimicrobial achieved at the biophase (Rubin 2013). Nonetheless it remains the only monitoring tool that both the veterinarian and epidemiologist can use to monitor individual and population resistance.



5.4 Antimicrobial compounding

Antibiotic compounding was practiced by 13.26% (n=24) of the respondents. The following preparations were compounded: enrofloxacin (baytril) + saline + silver sulfadiazine (flamazine); gentamicin + dimethyl sulfoxide (DMSO); enrofloxacin + DMSO; enrofloxacin (baytril) + ear preparations. Compounding is the preparation, mixing, combining and altering ingredients of a drug to create a medication tailored for the needs of an individual patient in accordance with a licensed practitioner's prescription (Spenser 2004). Compounding is important as a response to unmet species-specific needs for veterinary drugs where species-specific formulations have not been developed. While compounding drugs into more dilute solutions or different formulations facilitates appropriate dosing and client administration, it may compromise stability, potency and absorption; raising health and safety issues as quality of pharmaceuticals is built into the products through compliance with current good manufacturing practice (cGMP) (Spenser 2004, Sánchez-Regaña et al. 2013).

Veterinarians should be cautious when prescribing compounded drugs and monitor carefully for response to therapy and development of adverse events. The compounded drug may not have the anticipated therapeutic effect. Since there is paucity of pharmacokinetic data for compounded antimicrobials, it is difficult to guarantee that bacterial populations will be exposed to antimicrobial concentrations above the MIC and this maybe the source for the emergence of resistant bacteria populations (Spenser 2004, Sánchez-Regaña et al. 2013). It will also be difficult to guarantee that the compounded antimicrobial will be stable for the length of time of recommended treatment. Veterinarians must be made aware of compliance with cGMP when



compounding medicines and should only compound medicines when there is no other alternative registered veterinary antimicrobial or extra label human formulation for treatment.

5.5 Adverse drug reactions with antimicrobial usage

Adverse drug reactions (ADR), including patient returns due to lack of efficacy need to be reported to regulatory authorities, as a means of monitoring drugs post-registration (Freeman 1980, Naidoo, Sykes 2006). The latter is important as testing in the registration process is limited to a relatively small sample size compared to the final target population size. In South Africa the reporting of adverse reaction is a condition of registration in terms of Act No. 36 of 1947 and obligatory in terms of Regulation 37 of Act No. 101 of 1965 to report suspected ADRs. In 2006, Naidoo and Sykes concluded that the reporting of ADRs in South Africa was on the decline (Naidoo, Sykes 2006), which supports the current response by the majority of clinicians (n= 138; 72.24%) who did not report ADRs.

Adverse reaction monitoring and reporting for the antimicrobial drugs is particularly important for the surveillance on the emergence of antimicrobial resistance and has an impact on future use of an antimicrobial (Woodward 2005). It is also of assistance to the veterinarian as it provides an overview of the adverse reaction profiles that might be expected with individual drugs or products in a particular species, or even in some cases, breeds of animal, during or following the treatment of animals with antimicrobials. Ideally, the animal owner who first notes the ADR reports it to the veterinarian who will then take it up with the company that supplied the drug or with the relevant regulatory authority (Woodward 2005). The regulatory authority will collate all



the data and make a decision concerning the continued availability of the drug. In some developed countries, ADRs reporting is through an online submission tool where veterinarians enter details of a suspected event (Woodward 2005).

It is recommended that all stakeholders in animal health such as the Department of Agriculture, Forestry and Fisheries (DAFF), MCC, SAVC, South African Veterinary Association (SAVA), veterinary students, Industry, Faculty of Veterinary Science, research institutions and small animal clinicians collaborate and coordinate together to establish the right infrastructure for the reporting of adverse drug reactions and thereby facilitate an active surveillance and reporting system that will be of benefit to all stakeholders.

5.6 Section 21 and extra label use of antimicrobials

The extra-label use of antimicrobials was common practice across the different levels of qualifications. The proportion of respondents who used antimicrobials extra-label was 86.19% (n = 156). The frequent use of antimicrobials licensed for human use represents another critical point of the prescription and/ or dispensing trends observed in the study, and compares with the Norwegian study where 80% and 50% of antimicrobials used respectively for cats and dogs were products registered for use in people (Grave et al. 1992). Despite the wide scale use of human preparations in animals, care is advocated in that antimicrobials in companion animals should be strictly limited to those products properly authorized to treat specific conditions in the target species (Morley et al. 2005). The extra label use of products licensed for other animal species or for the treatment of other conditions in the same species, while necessary, should only be



practised whenever no authorized products or alternative antimicrobials for veterinary use are available (Hughes et al. 2012). The reason being that the pharmacokinetic parameters of a specific formulation are optimised to the registered species e.g. bioavailability, tissue distribution, half-life and tissue kinetics ensure that the selected agent reaches the site of infection in adequate concentrations (Baggot, Giguère 2013). Another concern with the use of human antimicrobials is that one may not have considered their overall importance in the management of human disease. From this survey, it can be concluded that veterinarians are prescribing antimicrobials, i.e. quinolones without the use of antibiograms. From the WHO guidelines, it is recommended that these drugs be reserved for use in humans and animals for life threatening conditions in conjunction with antimicrobial sensitivity tests that prove their possible effectiveness against the isolated bacteria (Escher et al. 2011).

In this study, none of the respondents has requested for the importation of antimicrobials using Section 21 through MCC. This is most likely as a result of human formulation being used extra-label as well as the veterinarians having the right to compound. This is far easier and cheaper than the section 21 route, which comes with an additional cost of R250-00 for the permit, an administration process, as well as the importing costs of bringing an antimicrobial from Europe.

5.7 Antimicrobial disposal

From this survey 25.97% of the respondents reported that they used other methods of antimicrobial disposal other than through a waste disposal company. Prudent use guidelines encourage the proper disposal of antimicrobials through waste disposal companies, as other



disposals may result in environmental bacteria being exposed to sub therapeutic antimicrobial concentrations thereby leading to the development of resistance (Scott, Page & Prescott 2013). These resistant environmental bacteria may then be transferred to animals through water, food and contact with the environment (Gaze et al. 2008, Aminov 2009). Once animals are exposed to resistant environmental bacteria, resistant genes may be transferred to pathogenic bacteria leading to antimicrobial resistance.

5.8 Antimicrobial usage with respect to the specific organ systems:

The structure and formatting of the online questionnaire was such that tables only allowed respondents to mark one box per row and this had an effect on question 25 where respondents could only indicate one organ system they treated with a particular class of each antimicrobial, for example if a veterinarian most commonly uses penicillin for skin infection, they could tick skin in the penicillin row.

5.8.1 Skin infections

From this survey, cephalosporins (85%; n=148) (Figure 10) were commonly used for skin infections in similarity to the UK study, where a large proportion of veterinarians surveyed preferred to prescribe a cephalosporin or clavulanate-amoxicillin for pyoderma (Hughes et al. 2012). From literature staphylococcus, streptococcus, E.coli, pseudomonas and pasteurilla spp are commonly isolated from skin and subcutaneous infections (Thomson et al. 2009). These microbes are generally susceptible to clindamycin, first generation cephalosporins, clavulanate-amoxicillin, trimethoprim sulphonamide, lincomycin, and erythromycin (Sykes 2013). Most of



the isolated bacteria from skin infections are susceptible to clindamycin but only a small proportion of the respondents (10%, n=17) (Figure 14) use antimicrobials in this class.

While the empirical use of the cephalosporins appears prudent by international standard, we still advise care in using these products without an antibiogram. Previous studies have shown that skin infection may be caused by methicillin -resistant *staphylococcus aureus* (MRSA) and methicillin -resistant *staphylococcus pseudointermedius* (MRSP)(Rankin et al. 2005), and that the MRSA clones isolated in dogs and cats being the same as those frequently isolated in human infections (Boerlin, White 2013). Considering that MRSA and MRSP transmission is by direct contact with infected skin (Rankin et al. 2005), veterinarians and animal owners may be at risk of contracting these MRSA and MRSP if antimicrobial prudent use guidelines are not followed.

5.8.2 Ear infections

From Figure 7, the aminoglycosides (21%; n=34) were the preferred antimicrobial class, followed by the quinolones (11%; n= 19) for management of ear infections. The most commonly isolated bacterial species from ear infections are staphylococcus and less commonly streptococcus, pseudomonas and *E. coli* (Sykes 2013). These bacteria are susceptible to topical enrofloxacin, aminoglycosides, polymyxin B and ticarcillin-clavulanate (Sykes 2013). The currently registered topical ear formulations in South Africa contain mainly the aminoglycosides and this would explain their common use. However, with the absence of topical quinolones preparations in South Africa and the veterinarians resorting to compounding (Baytril + Saline + flumazine; Enrofloxacin + DMSO or Baytril + ear preparations); this tends to suggest that some



veterinarians prefer quinolones for ear infections and do not use the readily available aminoglycosides probably due to their ototoxic potential.

5.8.3 Eye infections

From Figure 7, the majority of respondents were using amphenicols (22%, n=35), followed by tetracyclines (18%; n=28), which was an unexpected finding. From published literature, the most common superficial infections of the eye are due to *Staphylococcus*, *Streptococcus*, *E. coli*, *Chlamydomphila*, *Mycoplasma*, *Pseudomonas* and *Proteus* spp, which are best responsive to neomycin, polymyxin B and bacitracin (Sykes 2013, Dowling 2013b). While chloramphenicol is widely used for eye infections, its lipid and water solubility make it the treatment of choice for anterior chamber infections and corneal stromal abscess covered by intact epithelium (Dowling 2013c). Chloramphenicol also has an excellent activity against *Chlamydomphila* and *Mycoplasma* spp., while being less effective than aminoglycosides and fluoroquinolones against some gram-negative bacteria especially *Pseudomonas* spp (Dowling 2013c). Tetracyclines were the second most commonly used, probably because they are broad-spectrum and lipid soluble with a good activity against most pathogens that cause conjunctivitis. It can thus be speculated, that the wide-scale use of chloramphenicol and tetracyclines was linked to the fact that most registered eye preparations contain these antimicrobials.

5.8.4 Gastrointestinal (GIT) infections

From Figure 7, the penicillins (30.23%; n=52), aminoglycosides (25.93%; n=42), sulphonamides (30.64%; n=53) and nitroimidazoles (30.38%; n=48) were the most commonly used



antimicrobials for GIT infections, and was very similar to results from an Italian study that found the three most frequently used antimicrobials to be the penicillins, metronidazole and fluoroquinolones (Escher et al. 2011). From literature *Salmonella*, *Campylobacter*, *Clostridium*, *Helicobacter* and *E. coli* were the most common bacterial species isolated from GIT bacterial infections (Sykes 2013). These microbes are susceptible to a combination of amoxicillin, clarithromycin and bismuth salicylate or amoxicillin, metronidazole and bismuth salicylate (Sykes 2013). If a systemic infection is present (i.e. fever), parenteral fluoroquinolones can also be used (Rantala et al. 2004, Hansson, Sternberg & Greko 1997).

While the choice of antimicrobials was similar to the Italian study, it is believed that the South African epidemiology needs to be taken into consideration, as parvo-viral enteritis is an extremely prevalent disease in the country.

5.8.5 Genitourinary infections

In the treatment of genitourinary infections a high proportion of respondents used the quinolones (25%; n=43) (Figure 11) followed by the penicillins (22%, n=38) (Figure 9), in contrast to a Finish study where trimethoprim, clavulanate-amoxicillin and fluoroquinolones were the most commonly prescribed antimicrobials (Thomson et al. 2009). The most commonly isolated bacterial species from urinary infections are *E. coli*, staphylococcus, streptococcus, enterococcus, enterobacter, klebsiella and pseudomonas (Sykes 2013), which are susceptible to trimethoprim sulphamide, clavulanate-amoxicillin and fluoroquinolones (Dowling 2013b). Respondents from this survey were making the right antimicrobial selection for the management of



genitourinary infections considering the bacteria that are normally isolated; however it is recommended that culture and sensitivity be routinely performed so that development of resistance is picked at an early stage.

5.8.6 Respiratory infections

From this study the tetracyclines (Figure 12) were mainly used for respiratory infections (49%; n=79) followed by quinolones (28%; n= 47). This was similar to reported literature, whereby doxycycline was seen as the treatment of choice against the most commonly isolated bacteria (*Bordetella*, streptococci and *Mycoplasma*) (Sykes 2013). While clavulanate-amoxicillin is a good alternative, it is not active against *Mycoplasma* spp (Giguère 2013b). The choice of antimicrobial is however dependent on the severity of the infection as complicated cases may respond better to combination treatment such as clindamycin with enrofloxacin for aerobic infections, while anaerobes are more responsive to a combination of a potentiated penicillin and trimethoprim sulphonamide (Gumbo 2011, Giguère 2013b).

5.8.7 Musculoskeletal infections

For musculoskeletal infections the highest number of respondents used the lincosamides (22%; n=36) (Figure 14) followed by the penicillins (15%; n=25) (Figure 9). With the most common bacterial infection of the musculoskeletal system being osteomyelitis and septic arthritis, this is supported by literature which recommends the use of clindamycin, clavulanate-amoxicillin or cephalexin (cephalosporin) (Dowling 2013b). In case of high prevalence of methicillin resistant *Staphylococcus* spp, chloramphenicol may be a suitable alternative (Sykes 2013). Nonetheless



culture and susceptibility is highly recommended for this organ system and treatment should not commence before getting laboratory results unless the condition is life threatening. If prior treatment is considered essential, then clindamycin or clindamycin and an aminoglycoside can be used (Dowling 2013b). This is considered necessary as treatment is long-term and as a result can promote the development of resistance if drug selection is not based on an antibiogram.

5.9 Treatment duration

An optimum duration of antimicrobial therapy should eradicate infection whilst minimising adverse drug reactions, resistance and costs (Monnet 2010). However, there is a paucity of data guiding veterinarians on the appropriate duration of therapy. Current practice states that treatment should be continued until resolution of clinical signs or absent of pathology visible on diagnostic aids (Pleydell et al. 2012). The success of antimicrobial therapy depends upon the administration of the correct dose, an appropriate dosing interval and the correct duration of treatment to get rid of the causative pathogenic bacteria at the site of the infection (Ungemach, Muller-Bahrtdt & Abraham 2006, Guardabassi et al. 2009).

From the survey there was a large degree of variation in the duration of treatment per organ systems. Some of the respondents were treating infections for short periods, less than three days (GIT 7.18%, eye 6.08%, musculoskeletal 1.1%) while others were treating for prolonged periods of over 21 days (skin 25.41%, ear 7.73%, eye 1.10%, genitourinary 4.97%, respiratory 1.66% and musculoskeletal 9.94), leading to the conclusion that the duration of treatment was largely



empirical. A trend was only evident for the GIT and ocular systems which were being managed for around one week.

It is imperative that antimicrobial therapy be maintained for an adequate duration, which should be based upon monitoring the response both by clinical assessment of the patient and bacterial culture (Giguère 2013c). While determining the duration of treatment by monitoring the resolution of clinical signs can be challenging the benefits of the correct period are numerous as too short a duration of treatment can lead to recurrence of the infection and increase the likelihood of selection for bacteria with reduced sensitivity to the antimicrobial (Holso et al. 2005). The veterinarian should also consider the additional expense to the pet owner associated with a longer duration of treatment. A longer than necessary course of antimicrobial treatment also has its dangers, such as an increased incidence of side effects, as well as interference with the natural flora. In some cases extended courses of treatment are unavoidable for example in immunocompromised animals or in infections where the causative microorganism is relatively inaccessible (e.g. osteomyelitis, FIV) (Sykes 2013).

5.10 Multidose vial

Antimicrobial stewardship encourages the use of a multidose vial for less than 4 weeks once broached (Scott, Page & Prescott 2013). Clamoxyl® (Amoxicillin) and Convenia® (Cefovecin) package inserts recommend that the products be used for a maximum of 28 days from the day of first use while that for Baytril® 100 (enrofloxacin) recommends 3 months. From the study there were variable responses on the use of multidose vials once they were broached with only 30.94%



(n = 56) using a multidose vial for less than the recommended 4 weeks. With some antimicrobials (e.g. clavulanate-amoxicillin) that do not contain an antimicrobial preservative, prolonged use of the vial once broached can lead to antimicrobial contamination and loss of potency with increased risk of the development of resistant bacterial population (Toussaint, Gallagher 2014). Prolonged and repeated use after broaching can also result in increased amount of air introduced into the vial causing oxidation thereby affecting the stability of the drug, which can also result in a less potent antimicrobial. Multidose clavulanic based antimicrobials are susceptible to hydrolysis in the presence of water that could be introduced into the vial with wet needles (Petri 2011a). The resulting hydrolysed antimicrobial will be less potent, thereby promoting antimicrobial resistance when used. Some package inserts for multi-use vials [e.g. Snylox® RTU (clavulanate-amoxicillin), Baytril® 5% (enrofloxacin), Obermycin® 125 (Oxytetracycline), Engemycin® 10% (Oxytetracycline) and Terramycin® LA (Oxytetracycline)] are silent on the length of time the vial should be used from the time of first use. This could explain why there was a wide variation in the length of time that the respondents used multidose vials once broached.



CHAPTER: 6 RECOMMENDATIONS AND CONCLUSION

Prudent use of antimicrobials is an integral part of good veterinary practices and a guide for optimal use of antimicrobials. The main aim of promoting prudent antimicrobial usage is to slow down the rate of development of drug resistance, prolong the usefulness of antimicrobials and promote responsible use of antimicrobials. Veterinarians are on the frontline of antimicrobial usage in animals and this research focused on the small companion animal veterinarian although the concept of antimicrobial prudent use involves many other elements and stakeholders. Policy makers must implement antimicrobial usage guidelines that will bridge the current gap between the problem of resistance and eventual development of new antibiotics and other new approaches to control infection.

From this study, it can be concluded that antimicrobial usage in South Africa is influenced by the interplay of knowledge, expectations, and interactions of prescribers and animal owner's economic incentives, the regulatory environment and availability of resources. Given these results, it can be concluded that South African small animal veterinarians do experience obstacles preventing them from properly implementing antimicrobial prudent usage guidelines. While they do not make use of culturing for identification of infectious agents, they do appear to have a protocol when selecting antimicrobials for use in their patients. However, with the current level of antimicrobial resistance, more can be done to optimise the use of the antimicrobial drugs.



It is recommended that antimicrobial prudent use guidelines be frequently included in small animal Continuous Professional Development (CPD) courses where veterinarians and dispensers should be educated on the importance of appropriate antimicrobial use and containment of antimicrobial resistance. With problems of owner compliance, veterinary professionals should be encouraged to educate animal owners on appropriate use of antimicrobials and the importance of adherence to prescribed treatments. Compliance with guidelines maybe a challenge because of inadequate communication, differences in opinion or failure to adopt guidelines to reflect cultural differences and to overcome this challenge, it is recommended that policy makers make sure that guidelines are effectively distributed and all stakeholders are made aware of them.

It is also recommended that all antimicrobials used in animals be available only through a veterinarian as this will prevent cases of owner initiated antimicrobial treatment, will facilitate accurate monitoring of antimicrobial drug use, thereby reducing incidences of misuse and resistance development. This can be achieved through an integrated regulatory framework with one organization administering legislation for the control of all veterinary antimicrobials unlike in the current dual system where some antimicrobials are governed through Act 101/1965 and others Act 36/1947

This descriptive study is an important first stage of investigation into small companion animal antimicrobial usage patterns in South Africa. The descriptive information gained from this study should play a vital role in the development of future studies for example; retrospective determination of antimicrobial susceptibility profiles using antibiogram records, determination of prescribing patterns by going through nationwide prescription records, a study to find the



association between emergence of antimicrobial resistant bacterial population and use of antimicrobial prudent use guidelines.



CHAPTER: 7 REFERENCES

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CHAPTER: 8 ADDENDUM

8.1 Questionnaire