Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa

by

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DECLARATION

I Charlotte R Mupfunya, declare that this dissertation hereby presented to the University of Pretoria for the Master of Veterinary Science degree is my own work and I have not presented it for any degree or award in another university. All secondary material used was acknowledged and referenced as required by the University of Pretoria.
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ABSTRACT

Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa

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Containing antimicrobial resistance is crucial to mitigate against a post-antimicrobial era given the current limited production of novel antimicrobial drugs. Surveillance of antimicrobial use and resistance is a critical component of antimicrobial resistance containment strategies because it provides the background information needed to guide decisions regarding policy changes and therapeutic regimes. A substantial percentage of the livestock in South Africa are reared by communal farmers yet there is a paucity of published research on antimicrobial use practices and antimicrobial resistance profiles in communal farming systems. Given the substantial contribution of communal livestock to the national herd, it is vital to include them in any surveillance work including antimicrobial resistance surveillance.

The aim of this study was to determine the resistance of the indicator bacteria, *Escherichia coli* and *Enterococcus* species, isolated from communal cattle, and to determine the knowledge on and use practices of antimicrobial drugs by farmers in the Mnisi community Ward B1 in Mpumalanga province of South Africa.
Seventy farmers were interviewed at five dip tanks on antibiotics they have used and where they source the drugs; observation of withdrawal periods; disposal of expired antibiotics and knowledge on antimicrobial resistance. Rectal swabs were collected from cattle (n=100) belonging to the interviewed farmers for culture of *E. coli* and enterococci on MacConkey (without crystal violet) and sheep blood agar. The bacterial isolates were presumptively identified using primary biochemical tests, while confirmatory identification was done using API 10S for *E. coli*, and a streptococcal grouping kit and differential substrate utilisation for the *Enterococcus* species. Susceptibility of the isolates to selected antimicrobial drugs belonging to different classes was determined using a broth micro-dilution method.

The farmers indicated that they source their drugs from the local animal clinic (60%) and from an agricultural retailer (34%) in the nearby town. Among the various listed antibiotics, farmers (87%) indicated having used tetracyclines yet worryingly nearly all of them (99%) neither know what an antimicrobial drug is nor understand the concept of antimicrobial resistance. Only 1% of the farmers adhere to the recommended treatment duration while 29% keep treatment records. Forty five percent of the farmers indicated that observation of withdrawal periods was necessary because drug residues in treated animals can affect humans.

In total, 150 (79 *E. coli* and 71 *Enterococcus* species) bacterial isolates were obtained. The enterococci species isolated were *E. faecium* (46%, 33/71), *E. faecalis* (4%, 3/71), *E. durans* (4%, 3/71), *E. avium* (4%, 3/71) and non-specified *Enterococcus* species (41%, 29/71). The *Enterococcus* isolates were resistant to amoxycillin (3%, 2/71) and enrofloxacin (55%, 39/71), intermediate to erythromycin (38%), and completely susceptible to chlortetracycline and vancomycin. *E.coli* isolates exhibited resistance to colistin (16%, 13/79), amoxycillin (8%, 6/79), chlortetracycline (8%, 6/79) and enrofloxacin (3%, 2/79), and complete susceptibility (100%, 79/79) to gentamicin. The level of colistin resistance detected was an unexpected finding considering that the study focused on a rural communal farming area. It is thus suspected that technical limitations in performing minimum inhibitory concentration tests for polymixins, may be contributory to the high resistance levels.
Antimicrobial resistance in the indicator bacteria was generally low. However, given that colistin is a last resort drug for the treatment of multidrug resistant Gram-negative infections, the detection of colistin resistance warrants further research. A critical outcome of this study is the identification of the need for tailor made farmer education to raise awareness on antimicrobial resistance and to promote prudent use of antimicrobial drugs in the community. The findings of this study can be compared with follow up studies in this community to assess the impact of implemented awareness programs.
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AHT:</td>
<td>Animal health technician</td>
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<td>AMR:</td>
<td>Antimicrobial resistance</td>
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<tr>
<td>ATCC:</td>
<td>American Type Culture Collection</td>
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<tr>
<td>CLSI:</td>
<td>Clinical and Laboratory Standards Institute</td>
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<td>DANMAP:</td>
<td>Danish Integrated Antimicrobial Resistance Monitoring and Research Program</td>
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<tr>
<td>DNA:</td>
<td>Deoxyribonucleic acid</td>
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<td>ECV:</td>
<td>Epidemiological cut off values</td>
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<td>EU:</td>
<td>European Union</td>
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<td>FAO:</td>
<td>Food and Agriculture Organization of the United Nations</td>
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<tr>
<td>FMD:</td>
<td>Foot and Mouth Disease</td>
</tr>
<tr>
<td>GARP:</td>
<td>Global Antibiotic Resistance Partnership</td>
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<tr>
<td>MIC:</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MRSA:</td>
<td>Methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>OIE:</td>
<td>World Organisation for Animal Health</td>
</tr>
<tr>
<td>SANVAD:</td>
<td>South African National Veterinary Surveillance and Monitoring Program</td>
</tr>
<tr>
<td>SAASP:</td>
<td>South African Antibiotic Stewardship Program</td>
</tr>
<tr>
<td>SPSS:</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SVARM:</td>
<td>Swedish Veterinary Antimicrobial Resistance Monitoring</td>
</tr>
<tr>
<td>VRE:</td>
<td>Vancomycin resistant enterococci</td>
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<td>WHO:</td>
<td>World Health Organization</td>
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CHAPTER 1: INTRODUCTION

The development of antimicrobial drugs for both human and animal use was among the greatest accomplishments of the 20th century (Aarestrup, 2004). Antimicrobials began playing a crucial role in preventing infections and in alleviating suffering of diseased animals and humans (Nel, van Vuuren and Swan, 2004). Infections that would normally have been fatal during the pre-antimicrobial era became manageable (Aarestrup, 2015). Since their introduction over sixty years ago, antimicrobial drugs used for treatment, prophylaxis and growth promotion in livestock have made a significant contribution to the sustainability and profitability of the food animal production industry (Eagar, Swan and Van Vuuren, 2012).

Unfortunately the effectiveness of antimicrobials is diminishing due to the emergence and spread of antimicrobial resistant organisms and there are growing fears of a “post antibiotic era” in the near future (Okeke and Sosa, 2010; Aarestrup, 2015). Antimicrobial resistance is a growing threat to both animal and human health and must be prioritized at local, regional and international level (National Department of Health, 2014; Roca et al., 2015). In 2010, the “World Health Organization” (WHO ), the “World Organisation for Animal Health” (OIE), and the “Food and Agriculture Organisation of the United Nations” (FAO) formed an alliance with the aim of sharing the responsibility of fighting against critical diseases of which antimicrobial resistance was listed as one of the top three priorities of the tripartite (OIE, 2015).

Containing antimicrobial resistance requires joint and coordinated efforts among human and animal health practitioners, farmers, pharmacists, drug manufacturers, regulatory authorities and other stakeholders (EAGAR, 2006). Given the current limited development of novel antimicrobial drugs, especially for the treatment of infections caused by resistant Gram-negative microbes, it is pertinent to safeguard the usefulness of existing efficacious antimicrobial drugs through antimicrobial stewardship (Rice, 2009; Aarestrup, 2015). WHO in collaboration with OIE and FAO drafted a Global Action Plan to tackle antimicrobial resistance (WHO, 2015a). In September 2016, country leaders convened at the United Nations General Assembly to discuss at length the
challenge of antimicrobial resistance and resolved to develop national plans based on the Global Action Plan to tackle resistance (WHO, 2016).

Measures to be taken to fight antimicrobial resistance outlined in the Global action plan include; improving awareness and understanding of antimicrobial resistance, conducting antimicrobial resistance monitoring and surveillance, improving disease prevention and control so as to minimize antimicrobial treatment of animals, and developing more efficient diagnostic tools to ensure use of the correct medicines (Roca et al., 2015; WHO, 2015; Holmes et al., 2016). Surveillance is one of the critical control measures because it provides the background information needed to guide decisions regarding policy changes and therapeutic regimes for antimicrobial drugs (EAGAR, 2006).

When designing resistance surveillance programmes in animals, there are certain targeted microorganisms tested for, namely important veterinary pathogens, zoonotic pathogens and indicator bacteria. E. coli and enterococci have been selected in several studies as indicator bacteria for monitoring resistance because they easily develop resistance (Caprioli et al., 2000; van Vuuren, Picard and Greyling, 2007). Secondly, these bacteria are present as commensals in both healthy and sick individuals and thus give a truer picture of the level of antimicrobial resistance in the population compared to pathogenic isolates (Caprioli et al., 2000; OIE, 2003). Indicator bacteria are also reservoirs of antimicrobial resistance genes which are transferrable to pathogenic bacteria of animals and humans (Caprioli et al., 2000; Varga et al., 2008). The emergence of Enterococcus species as one of the top three nosocomial pathogens in human hospitals in recent years also necessitates their inclusion in resistance surveillance programs (Miller et al., 2015).

Antimicrobial resistance surveillance was selected as one of the mainstays of the South African Antimicrobial Resistance Strategy framework due to the recognition of its critical role in the fight against antimicrobial resistance. Nonetheless, there are several knowledge gaps in veterinary antimicrobial resistance surveillance and monitoring in South Africa (Mendelson and Matsoso, 2015). Another concern is that most of the publications on antimicrobial resistance surveillance focus on commercial farms with limited data available from communal farming systems. Globally,
information on risk factors for the emergence and spread of antimicrobial resistance and the levels of antimicrobial resistance in small scale and communal farming systems is scanty especially in low and middle income countries (Caudell et al., 2017; Graham et al., 2017). In many countries, including South Africa, a substantial percentage of livestock are reared by small scale and communal farmers yet majority of research work and developments mainly focus on commercial farms (Waters-Bayer and Bayer, 1992). Meissner, Scholtz and Palmer (2013) reported that approximately 67% of goats, 41% of cattle and 28% of pigs in South Africa were reared by communal and small scale farmers. With communal herds contributing substantially to the national herd, it is important to include them in any surveillance system including antimicrobial resistance surveillance. In addition, surveillance is critical because small scale production units are somewhat ubiquitous and thus serve as potential sources and recipients of antimicrobial resistant organisms from surrounding environments (FAO, 2016).

There is a shortage of veterinary professionals in the country especially in rural areas and as a compensatory measure, the government through the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947) permits over the counter availability of certain antimicrobial drugs including tetracyclines to allow timely treatment of easily recognisable endemic livestock diseases (Naidoo, 2009; Henton et al., 2011). Given the current global recognition of resistance as a critical threat to health, if farmers are allowed to handle antimicrobial drugs without veterinary supervision, it is crucial to conduct surveillance on their knowledge of antimicrobial drugs, antimicrobial resistance and the roles they can play to mitigate it (Katakweba et al., 2012). Such surveillance is even more important in communal areas where the literacy level of farmers is generally poor and as such compliance with antimicrobial use instructions and awareness on resistance may be poor.

This study seeks to address some of the existing aforementioned knowledge gaps in communal areas by determining antimicrobial use practices of farmers and their knowledge on antimicrobial drugs and resistance, and determining the prevalence of resistance in the indicator bacteria *E. coli* and *Enterococcus* species isolated from cattle managed under a communal farming system in the
Mnisi community (Bushbuckridge Municipality) in Mpumalanga Province of South Africa where the University of Pretoria is involved in an animal health programme in the community.

1.1 Aims

- The first aim of the project was to isolate *E. coli* and *Enterococcus* species from cattle in Mnisi community Ward B1 and determine susceptibility of these isolates to selected antimicrobial drugs.
- The second aim was to determine the knowledge on veterinary antimicrobial drugs and usage practices of farmers in this community to identify areas that need to be improved on.

1.2 Objectives

- Objective 1: To isolate *E.coli* and *Enterococcus* species from rectal swabs collected from apparently healthy cattle in Mnisi Ward B1.
- Objective 2: To determine the susceptibility of the isolated *E. coli* and *Enterococcus* species to selected antibiotics using the broth micro-dilution method.
- Objective 3: To determine the knowledge on veterinary antimicrobial drugs and usage practices of selected farmers in Mnisi Ward B1.

1.3 Benefits arising from the project

- The project will provide baseline data on antimicrobial resistance in cattle in the study area. The baseline data can be used as a reference in future programs on antimicrobial resistance.
- The questionnaire survey will allow the identification of knowledge gaps that need to be addressed through tailor made farmer education programs.
- The results will also make a contribution towards data required to guide policy changes and therapeutic guideline changes to promote prudent use of antimicrobial drugs in the Mnisi community.

CHAPTER 2: LITERATURE REVIEW
2.1 Communal livestock farming in South Africa

Livestock rearing in communal areas in developing countries including South Africa is an important means of diversifying livelihoods and alleviating poverty (Dovie, Shackleton and Witkowski, 2006; Meissner, Scholtz and Palmer, 2013). Livestock play various economic, social and cultural roles in the upkeep of families in communal areas. Many families opt to engage in livestock rearing to enjoy the multiple benefits it offers (Dovie, Shackleton and Witkowski, 2006). The benefits include; household use and subsistence consumption of animal products; income generation; provision of manure and draught power for ploughing and for transportation (Mutibvu et al., 2012; Meissner, Scholtz and Palmer, 2013; Tavirimirwa et al., 2013; Bettencourt et al., 2015). In some areas, livestock rearing is the only viable agricultural practice.

Despite the multiple benefits of livestock to the rural economy and family wellbeing, farming under this system is not easy. Diseases are a major constraint to production due to poor growth, reduced fertility and the high mortality rates associated with disease (Meltzer, 1995; Mutibvu et al., 2012). The disease burden in livestock in communal areas is in part due to the relatively poor or complete absence of extension veterinary services in these areas (Mutibvu et al., 2012). In an effort to compensate for the poor provision of veterinary services, South Africa effected a dual regulatory system for veterinary drugs. In this system, certain drugs are classified as “stock remedies” under the “Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947)” while others are classified as veterinary medicines under the “Medicines and Related substances Act (Act 101 of 1965)”. “Veterinary medicines” should only be administered or prescribed by veterinarians. Stock remedies are relatively safe and easy to administer over-the-counter drugs that are freely available to farmers from agricultural retail shops to manage common diseases which are presumably easy for lay persons to diagnose (Naidoo, 2009; Henton et al., 2011). The categories of stock remedies available are:

- Nutritional Supplements: mainly vitamins and mineral supplements for the support of animal production, e.g. multivitamin stress packs, mineral salt licks.
- Anthelminthic drugs: agents administered to eliminate worms, e.g. fenbendazole, nitroxynil and albendazole.
- Ectoparasiticides: agents administered to kill external parasites such as ticks and fleas, e.g. amitraz and cypermethrin.
- Anti/protozoal: agents administered to treat or prevent protozoal infections, e.g. imidocarb dipropionate and buparvaqone.
- Anti-rickettsia drugs: agents used to kill rickettsial organisms, e.g. oxytetracycline.
- Antimicrobials: These are the antimicrobials used for therapeutic purposes, e.g. tetracyclines and sulphonamides.
- Growth promoters: These include the antimicrobial drugs such as bacitracin, avoparcin and tylosin used to enhance production as well as non-antimicrobial growth promoters such as probiotics.
- Vaccines: biological preparations administered to an animal to confer active immunity against a specific disease condition, e.g. New castle disease vaccine for poultry birds (Naidoo, 2009)

Despite the need for these over the counter drugs, allowing certain antimicrobial drugs to be available for direct use by farmers without much restriction does create problems. To begin with, the level of knowledge of the farmer may not necessarily be sufficiently high to allow for the correct diagnosis and management of disease. This may result in selection of an ineffective antimicrobial for the condition or worse still result in unwarranted antimicrobial use. Another concern is the tendency for farmers to use incorrect doses and to treat animals over an inappropriate duration of time (Katakweba et al., 2012; FAO, 2016). These practices pose a problem because the incorrect use of antimicrobial drugs is known to be the key driver of acquired resistance development.

2.2 The emergence and impact of antimicrobial resistance

Acquired antimicrobial resistance is the ability of a microorganism to survive in the presence of a concentration of an antimicrobial drug that previously would have killed or at least inhibited its growth (OIE, 2003). Not long after the development of penicillin, antimicrobial resistance was first confirmed in 1940 through the detection of a bacterial penicillinase (Abraham, E.P and Chain,
Despite resistance being a known phenomenon for decades now, it is the rapid escalation in development and spread of resistance in recent times that has raised alarm. Resistant organisms and resistance genes are increasingly spreading to new geographical locations and even more worrying is the development of multidrug resistant pathogens (OIE, 2003; Masterton, 2008; Roca et al., 2015). Magiorakos et al define ‘multidrug resistant organisms’ as organisms with acquired resistance to at least three drugs belonging to different antimicrobial classes. The detection of a *Klebsiella pneumoniae* isolate resistant to nearly all commonly used antibiotics in the urine of a human cardiac surgery patient in South Africa is a clear indicator that multidrug resistance is a serious problem even in South Africa

Multidrug resistance is a great cause for concern for various reasons. Firstly, common infections that were previously easy to treat are becoming increasingly difficult to manage. This has led to an increase in healthcare expenses due to use of more expensive second and third line drugs and prolonged hospitalization; and increased mortalities more so in developing countries were use of the more current and effective drugs may be limited by financial constraints (Okeke *et al.*, 2005; Tenover, 2006; Masterton, 2008; Eagar, Swan and Van Vuuren, 2012). Prolonged hospitalisation subsequently causes increased incidence of hospital acquired infections many of which are drug resistant (WHO, 2001). In some cases, clinicians are left with no treatment option at all or have to use more toxic antimicrobial drugs to treat multidrug resistant infections (Falagas, Kasiakou and Saravolatz, 2005). In Europe alone, resistant bacteria account for more than 25,000 deaths in humans per annum (Kostić *et al.*, 2015). In America, resistance was estimated to account for 21 to 34 billion dollars in healthcare bills and 8 million days of prolonged hospitalisation per year (WHO, 2014). In 1999, multidrug resistance was estimated to increase the treatment cost of human tuberculosis in South Africa more than tenfold (from 215 rands to 26 354 rands) (Okeke *et al.*, 2005).

Secondly, multidrug resistance is alarming because the development of novel antimicrobial drugs is very much outpaced by the development of resistance against existing drugs (Tenover, 2006). Pharmaceutical companies in affluent countries have shifted their production priorities from agents against infectious diseases to agents for the currently more prevailing lifestyle diseases (WHO,
The retarded development of novel agents is particularly worrying considering that it takes at least a decade to develop and place an antimicrobial drug on the market. Sadly, resistance is already being detected in some of the newer antimicrobials (OIE, 2003). Aarestrup (2015) recently reported that resistance has reduced the efficacy lifespan of new antimicrobial drugs to less than twenty years. This has further reduced the incentive to invest in development of new antimicrobial drugs.

Antimicrobial resistance in food animals is also a threat to food security. Resistant infections in livestock result in reduced productivity due to prolonged treatment periods and withdrawal periods; and in worse scenarios, increased mortalities due to treatment failure. The food chain is a known means for dissemination of resistance from animals to humans. Media reports of increasing resistance in food animals have had a negative economic impact on the agricultural sector due to reduced confidence of the consumers in the safety of certain animal food products resulting in decreased sales especially in developed countries (WHO, 2001).

2.3 Types of antimicrobial resistance

Antimicrobial resistance is broadly classified into intrinsic, acquired and adaptive, but the greatest cause for concern is acquired resistance (Tenover, 2006).

- Intrinsic (inherent or innate) resistance occurs due to natural functional or structural properties of a microbe. The microbes either naturally lack the target site(s) of the antimicrobial agent(s), or have membranes that are poorly permeated by the antimicrobial agent(s) (Cox and Wright, 2013). Genes encoding for properties that confer intrinsic resistance are carried on chromosomes. A particular intrinsic resistance mechanism is thus often exhibited by all microbes belonging to a certain species or genus since chromosomes are inherited by all progeny of a microbe (Courvalin, 2008). For example, several Gram-negative genera are intrinsically resistant to antibiotics such as older generation penicillins due to the partial impermeability of the outer membrane of these bacteria (Cox and Wright, 2013).
With acquired resistance, a microbe becomes insensitive to an antimicrobial agent it was previously susceptible to (Tenover, 2006). Acquired resistance may arise due to:

- Mutations in chromosomal genes that result in structural or functional changes that interfere with the action of the antimicrobial agent.
- Acquisition of mobile genetic elements from other bacteria. Mobile genetic elements include transposons (DNA sequences that can change position within a genome) and plasmids (DNA strands that can replicate independent of chromosomal DNA) encoding for resistance (Courvalin, 2008; van Hoek et al., 2011). Acquisition of mobile genetic elements may occur via conjugation (transfer of DNA coding for resistance between cells in physical contact mediated by conjugative elements); transduction (a bacteriophage i.e. a virus that infects bacteria, transfers DNA coding for resistance from one cell to another); or transformation (uptake of free DNA that codes for resistance) (Hirsch and Zee, 1999; Holmes et al., 2016) as illustrated in Fig 2:1 below. Plasmids carrying resistance genes are named R plasmids (where “R” denotes resistance). A bacterium may contain several R plasmids and each R plasmid may contain two or more resistance genes thereby resulting in multidrug resistance. Some R plasmids have a wide host range while others have a relatively narrow host range such that they occur only in a specific bacterial genus or species (Hirsch and Zee, 1999).
Adaptive resistance involves temporary activation of certain genes that code for phenotypes (e.g. reduced cell membrane permeability, active efflux of drugs) that render the microbe more resistant to an antimicrobial agent. Adaptive resistance usually occurs in response to stressful stimuli including antimicrobial drugs and heavy metals in the environment (Motta, Cluzel and Aldana, 2015; Jahn et al., 2017). It differs from conventional acquired resistance in that it is usually reversed once the stimulus is removed. It has been demonstrated in certain bacteria such as *Salmonella enterica* and *E. coli* (Motta, Cluzel and Aldana, 2015).

2.4 Mechanisms of antimicrobial resistance

Most antimicrobial drugs have intracellular target sites which require the drug to traverse cell membranes to access those targets. Inside the cell, the drugs have to accumulate to sufficient concentrations at the target sites so as to exert their effects (Zhou et al., 2015). Structural integrity
of the target site is thus vital for effective interaction of the antimicrobial drug and the target. Microbes naturally possess or develop mechanisms to interfere with the aforementioned processes to prevent the drug from having an effect on them. Resistance to a particular antimicrobial drug may be achieved by employing two or more resistance mechanisms. On the other hand, certain resistance mechanisms confer resistance against many or all antimicrobial drugs of a certain class. Some mechanisms even confer resistance to antimicrobial drugs of different classes (Zhou et al., 2015). The mechanisms that confer antimicrobial resistance are numerous and some examples are included below:

- Reduced permeability of the bacterial cell resulting in restricted access to target sites.
  - Gram-negative bacteria have an outer membrane which is impermeable to several antibiotics. A noteworthy example is vancomycin which cannot permeate the outer membrane and is thus not active against Gram-negative bacteria (Munita and Arias, 2016).
  - Most hydrophilic antimicrobial agents rely on specific pores (porins) to permeate the outer membrane. Porin mediated resistance can occur due to: down-regulation of porin expression; alterations in porin structure; or a switch in the type of porin expressed (Pagès, James and Winterhalter, 2008).
  - In some cases, bacteria produce biofilms (microbial cells adherent to each other and enclosed by a polysaccharide substance they secrete) which limit antibiotic penetration through: hydrophobic or electrostatic interactions (since the exopolysaccharide is negatively charged); size exclusion; or directly reacting with the antimicrobial agents (Lewis et al., 2001; Zhou et al., 2015). There can also be synergy between the limited antibiotic penetration conferred by biofilms and another resistance mechanism such as degradation of the drug thereby resulting in more effective resistance as observed in Beta lactamase producing Pseudomonas aeruginosa biofilms (Lewis et al., 2001).

- Active extrusion of the antimicrobial drug via efflux channels resulting in failure of the drug to concentrate sufficiently at the target site(s) to exert an effect.
  - Some bacteria may possess efflux channels that can actively transport a specific drug or structurally different drugs thereby resulting in multi-drug resistance (Courvalin,
2008; Zhou et al., 2015). These pumps either use adenosine triphosphate (ATP) or the membrane electrochemical potential as a source of energy (Zhou et al., 2015). An example is Tet efflux pumps which extrude tetracycline and are particularly common in Gram-negative bacteria (Roberts, 2005). *Pseudomonas aeruginosa* has multidrug efflux pumps which are encoded for by genes on chromosomes and confer resistance to different antimicrobials including chloramphenicol and tetracycline (Cox and Wright, 2013).

- Production of enzymes that modify the antimicrobial agent through hydrolysis, oxidation or group substitution.
  - Group substitution reduces the avidity (overall strength of binding) of the antimicrobial drug for its target due to steric hindrance (Munita and Arias, 2016). An example is acetylation, adenylation or phosphorylation of aminoglycosides by aminoglycoside modifying enzymes (Ramirez and Tolmasky, 2010)
  - Production of hydrolytic enzyme(s) which deactivate the antimicrobial drug. For example, Beta lactamases hydrolyse the Beta lactam ring thereby inactivating Beta lactam antibiotic agents such as penicillins, cephalosporins, carbapenems and monobactams (Shaikh et al., 2015).

- Target site modification
  - Mutations or enzymatic modifications of the target site result in structural changes that reduce the affinity of the antimicrobial drug for the target (Munita and Arias, 2016).
  - Protection of the target site hinders the antimicrobial drug from interacting with the target. For example, ribosomal protection proteins render certain microbes tetracycline resistant by causing allosteric changes in the target site for tetracyclines resulting in dislodgment of the drug from the ribosome (Connell et al., 2003).

- Replacement of the target i.e. production of molecules that can perform the function of the target but are not affected by the antimicrobial drug (Munita and Arias, 2016). For example, production of altered penicillin binding proteins (encoded for by a foreign gene) with
reduced affinity for penicillins confers certain bacteria, including methicillin resistant *Staphylococcus aureus*, with resistance against penicillins (Stapleton and Taylor, 2002).

- Overproducing the target so as to overwhelm the drug (Munita and Arias, 2016). For example, overproduction of dihydrofolate reductase (the target for trimethoprim) due to mutational changes in the promoter of the gene of this enzyme (Flensburg and Skold, 1987).

### 2.5 Dissemination of resistant organisms and resistance genes

Antimicrobial resistant organisms and resistance genes can be disseminated in various ways. Resistance genes can be disseminated within a clone through vertical transfer (Courvalin, 2008), or be transferred horizontally from one cell to another through transduction, transformation or conjugation (Holmes *et al.*, 2016). Conjugation is the most common method of horizontal transfer of resistance genes (Hirsch and Zee, 1999). Common means of transfer of antimicrobial resistant microbes from one place to another include trade of animals and animal products, water bodies and wildlife migration. International trading of animals and animal products facilitates the rapid and efficient global dissemination of resistant organisms and genes. Examples of resistant bacteria that have spread globally are, methicillin resistant *Staphylococcus aureus* CC398; various Salmonella clones, for example DT104 (Aarestrup, 2015); “New Delhi metallo-beta-lactamase-1 producing” Gram-negative bacteria; and “*Klebsiella pneumoniae* carbapenemase” from America (Mendelson and Matsoso, 2014).

Resistant microbes can be transferred from animals to humans indirectly through the consumption of animal products such as meat, milk and eggs (Founou, Founou and Essack, 2016); drinking water contaminated by animal excreta; or consuming raw fruits and vegetables contaminated by untreated animal manure (Harris, Cormican and Cummins, 2012). Resistant microbes can also be transmitted from animals to humans directly through contact owing to poor hygiene, particularly in farmworkers, abattoir workers and in veterinarians due to occupational exposure (Founou, Founou and Essack, 2016).
Some antimicrobials may stay in meat, milk or eggs for long periods resulting in antimicrobial residues remaining in the animal product. Some cooking methods may reduce certain antimicrobial residues depending on time and temperature but nonetheless do not increase the margin of safety for consumers since they may not fully break down the residues. Furthermore there are some antimicrobial residues like quinolones and nitrofurans, which are generally stable to most conventional cooking methods (Heshmati, 2015). Continuous consumption of foods with antimicrobial residues results in replacement of normal gut flora with strains resistant to the antimicrobial residues due to the selection pressure they exert on the microbes over time (Beyene, 2016).

2.6 Antimicrobial use as a driver of antimicrobial resistance

Despite the focus on antimicrobial resistance for decades now, there are still several gaps in our understanding of antimicrobial resistance emergence and spread. What is known with certainty is that antimicrobial use in humans and animals has been the most significant driver of antimicrobial resistance emergence and spread (Aarestrup, 2015; O’Neill, 2015a). This principle in itself is not a new concept. A study conducted by Datta and Hughes (1983) showed that a substantial proportion of the plasmids during the pre-antibiotic era fell in the same group with the current resistance plasmids but they did not contain resistance genes. This suggests that prior to the introduction of antibiotics in clinical practice, bacterial isolates were rarely resistant to antimicrobial drugs and that acquisition of resistance was mainly through resistance gene insertion into plasmids. The use of antimicrobials exerts significant selection pressure which favours the survival of resistant organisms over susceptible ones (O’Neill, 2015a; Holmes et al., 2016).

The converse to this principle was demonstrated in some countries including the United Kingdom, Denmark, Sweden and Netherlands, where surveillance studies showed that reduced use of certain antimicrobial drugs (Hammerum et al., 2007; Moyane, Jideani and Aiyegoro, 2013) can decrease the presence of resistant strains. Some examples are the reduction in tetracycline resistant Salmonella in pigs following the prohibition of use of tetracycline for growth promotion in
Netherlands (van Leeuwen et al., 1979); and reduction in glycopeptide resistant *Enterococcus* species following the banning of the use of macrolides for growth promotion in food animals in Denmark (Aarestrup, 2005; Hammerum et al., 2007; Holmes et al., 2016).

An antimicrobial use practice that seems to be a critical driving factor in resistance development is the use of broad spectrum antibiotics. These antibiotics are active against a large variety of bacterial species compared to narrow spectrum antibiotics and hence they tend to select for resistance in several bacterial species. In contrast, narrow spectrum antimicrobials are preferred because they are more target specific and hence select for resistance in less bacterial species. They are also less likely to cause collateral damage of commensal bacteria and subsequent loss in colonization resistance (i.e. superinfection by potentially pathogenic microbes due to loss of normal flora) (Vollaard and Clasener, 1994; Blaser, 2011). While it is well known that ideally narrow spectrum agents should be used, it is unfortunate that the need to start antimicrobial treatments without conducting culture and sensitivity tests due to inadequate diagnostic services, the costs of sensitivity testing or the need to start therapy while culture results are pending often prompts the use of broad spectrum antimicrobial drugs in South Africa more so in animals (National Department of Health, 2014).

Another antimicrobial use practice that promotes development of resistance is the use of large volumes of antimicrobial drugs in production animals. The use of these agents in production animals may not only contribute to the development of resistance in animals, but in humans as well because the antimicrobial drugs used in animals are similar to or closely resemble those used in humans. The use of antimicrobial drugs in some countries is higher in production animals compared to humans (Klima et al., 2014; O’Neill, 2015a). This is due to the fact that in production animals, particularly those under intensive rearing, apart from therapeutic use, antimicrobial drugs may also be routinely administered to the whole flock or entire groups for other purposes such as growth promotion, prophylaxis, eradication and metaphylaxis, all of which are not practiced in human medicine (Aarestrup, 2015).

- Prophylaxis involves administration of antimicrobial drugs in clinically healthy animals to prevent disease occurrence at a time when the animals are highly susceptible to contracting
the disease. Such times include periods when the animals are stressed, for example at the time of weaning, and when there are increased numbers of vectors in the case of vector transmitted diseases (Aarestrup, 2015).

- Metaphylaxis involves administration of antimicrobial drugs in both sick and healthy animals within a flock when the risk of disease occurrence in the entire herd is high so as to cure the sick animals and to prevent overt disease occurrence in the clinically healthy animals (Johnston, 1998).

- Growth promotion involves the administration of antimicrobial drugs at sub-therapeutic levels for extended time periods to reduce incidence of disease and to promote gut health thereby improving feed conversion efficiency (Modi et al., 2011).

This high antimicrobial use in animals promotes resistance in that;

- It directly exerts selection pressure on microbes thereby promoting development of resistant strains.
- It is associated with increased excretion of antimicrobial residues which contaminate the environment and exert selective pressure on environmental micro-organisms with subsequent transfer of resistance genes from these environmental microbes to veterinary and human pathogens (O’Neill, 2015a).

2.7 Antimicrobial resistance drivers in communal farming systems

Small scale and communal farming systems are generally thought to have lower antimicrobial resistance levels compared to commercial farms where there is intensive use of antimicrobials (FAO, 2016). This might partially explain why those that conduct surveillance and monitoring work have a bias towards commercial farms since high antimicrobial usage has been identified as the key driver of resistance emergence and spread (O’Neill, 2015b). However, it is important to conduct more research in small scale and communal farming areas to objectively assess the magnitude and trends in antimicrobial resistance in these areas (Graham et al., 2017).
First and foremost, selection pressure occurs regardless of the manner or volume of antimicrobial use thus it is necessary to conduct resistance surveillance in different antimicrobial use practice settings (FAO, 2016). Secondly, the association between the use of antimicrobial drugs and the development of resistance is not a simple correlation. Besides use of high volumes of antimicrobial drugs, factors such as wrong route of administration, under-dosing and use of sub-standard agents are also important drivers of resistance (WHO, 2001). Although the volume of antimicrobials used in small scale farms is relatively less compared to commercial farms, antimicrobial abuse is likely higher due to several factors (FAO, 2016).

There are fewer trained veterinary personnel in these remote areas to make disease diagnoses and institute appropriate antimicrobial treatment, and to supervise and promote appropriate use of antimicrobial drugs. Appropriate dose estimation is usually problematic due to unavailability of scales to weigh the animals resulting in under or over-dosing of animals (Caudell et al., 2017). Sub-therapeutic doses or use of inappropriate antimicrobials can result in selection of resistant bacteria leading to increased levels of resistant bacteria in the excreta of animals (Katakweba et al., 2012). Due to scarcity of drug stores in rural areas, there can be individuals that engage in the selling of drugs some of which may be counterfeits (FAO, 2016). The concern with counterfeit antimicrobial drugs is that they often have lower doses or no active ingredient at all resulting in accelerated development of resistance or ineffective treatment respectively (Kelesidis and Falagas, 2015).

Another important point to consider is that antimicrobial resistance spread is poorly studied and monitored, thus prevalence of antimicrobial resistance may be higher than anticipated in certain environments, including rural areas. The livestock graze at communal pastures with animals from different households and frequently closely interact with humans (FAO, 2016). An important environmental exposure pathway for transmission of resistance in households rearing livestock is direct exposure to animal waste especially for children who are in the habit of eating soil as they play in the yard (Graham et al., 2017). The construction and maintenance of blair toilets and sewer systems may be substandard resulting in environmental contamination with human excreta.
harbouring antimicrobial residues or resistant bacteria (FAO, 2016). Antimicrobial resistance can thus easily spread between households, and from livestock to humans and vice versa due to poor sanitation and lack of biosecurity measures.

In rural areas, animals are frequently slaughtered at home without any formal food-safety controls like those employed at abattoirs (FAO, 2016). In a study conducted on traditional slaughter (i.e. home slaughter) of goats in Gauteng by Qekwana et al., (2017), a substantial percentage of the respondents indicated that; the health status of slaughtermen was unknown (77.5%) and they did not put on protective clothing during slaughter (37%). In addition, the animals were slaughtered directly on the ground (39%); they did not wash knives regularly during flaying; and none of them conducted meat inspection. Such practices pose as risk factors for transmission of food borne pathogens some of which may harbour resistance genes. In small scale farming, animal waste is commonly used as manure in crop fields without any prior treatment hence there is a potential for contamination with resistant bacteria of the crops and soil and eventually ground water and water bodies due to leaching and surface run off (Berglund, 2015). Observation of antimicrobial withdrawal periods may also be poor due to ignorance resulting in consumption of antimicrobial residues in meat and milk (Katakweba et al., 2012; Caudell et al., 2017). The spread of resistant organisms via the food chain in communal farming systems can thus be quite significant.

Livestock in communal areas also frequently come into close contact with wildlife during their grazing excursions. Exposure of wildlife to the excreta of livestock and humans which are treated with antimicrobial drugs allows transmission of resistant microbes and resistance determinants to wildlife. Spill over of antimicrobial resistance to wildlife animals is a great cause for concern because there is potential for them to disperse antimicrobial resistance genes to new areas because of their long range movements. This is particularly true for migratory birds which travel beyond national boundaries, and for animals that are trans-located to new game parks (Arnold, Williams and Bennett, 2016). A number of studies conducted detected antimicrobial resistance in areas relatively remote and uninhabited by humans (Brown and Balkwill, 2009; Toth et al., 2010; Bhullar et al., 2012). This highly suggests that wildlife may naturally harbour genetic determinants for antimicrobial resistance or may acquire them from environmental microbes. These resistance
determinants may then spill over to livestock and humans (Arnold, Williams and Bennett, 2016). The human/livestock/wildlife interface is thus a critical area for antimicrobial resistance surveillance.

2.8 Resistance Studies in South African communal livestock

As already highlighted, there are a few studies on resistance that were conducted in the communal farming systems and majority of these focused on pathogenic bacteria rather than the commensal bacteria which are better indicators of antimicrobial resistance in a population. These include a study conducted in Mafikeng to determine the antimicrobial susceptibility profiles of *E. coli* and *Enterococcus* species in communal and commercial pigs (Moneoang and Bezuidenhout, 2009). All the bacteria isolated from the communal pigs exhibited resistance to sulphamethoxazole while resistance of the *E. coli* isolates and *E. faecium* isolates to oxytetracycline was 89.1% and 100% respectively. The farmers in this area indicated that they predominantly used tetracyclines for animal disease treatment and control since they were readily available over the counter antibiotics and relatively cheap.

In another study, Ateba *et al* (2010) isolated *Staphylococcus aureus* from milk from cattle from both commercial and communal farms. The isolates from the communal farms had high (39 to 100%) resistance to several antibiotic agents (penicillin G, ampicillin, methicillin, oxytetracycline, erythromycin, sulphamethoxazole, and nitrofurantoin) and moreover they were more resistant in comparison to the isolates from the commercial farms. Vancomycin resistance though very low (3.4% and 4.7%), was detected in the two communal farms while the commercial isolates were all susceptible to the antibiotic. Multidrug resistance was detected in 82.3% and 60.9% of the isolates in the two communal farms.

A study conducted in the North West Province involved characterisation of *E. coli* O157 isolated from pigs and cattle from commercial and communal farms, as well as humans (Moneoang and Bezuidenhout, 2009). High resistance to tetracycline (100%), sulphamethoxazole (95.7%), erythromycin (69.7%) and streptomycin (52.2%) was detected in the pigs (*n* = 23) from the communal farm in Tlapeng. Similarly, high resistance to tetracycline (100%), sulphamethoxazole
(100%), erythromycin (100%) and streptomycin (50%) was detected in the cattle from the communal farm in Mogosane.

A study was conducted in Vhembe rural district to determine Salmonella contamination and antimicrobial susceptibility patterns of cattle slaughtered at the local abattoirs. About 72% of the isolates were resistant to one or more antibiotics with the highest resistance (51.9%) being towards oxytetracycline (Madoroba, Kapeta and Gelaw, 2016). A trend that can be observed from all the aforementioned studies in the communal set ups is the high resistance to tetracyclines and sulphonamides.

### 2.9 Importance of antimicrobial resistance monitoring and surveillance

Surveillance is a critical component of the ‘WHO Global Strategy for Containment of Antimicrobial Resistance’ (WHO, 2001) and was adopted as one of the three pillars of the ‘South African Antimicrobial Resistance Strategy framework’ (Mendelson and Matsoso, 2015). Surveillance should be on both antimicrobial use and antimicrobial resistance (EAGAR, 2006) and must be conducted at local, regional and international level on a regular basis (Aarestrup, 2005).

Comprehensive monitoring and auditing of antimicrobial use is important for the following reasons:

- It provides data for the risk analysis required for the registration of and extension of use options of specific antimicrobial drugs.
- It also allows identification of areas of high antimicrobial usage as key targets for intervention programs at both local and national level (EAGAR, 2006).
- Evaluation of antimicrobial use trends and subsequent correlation with antimicrobial resistance data allows identification of necessary changes in prescribing practices and prudent use recommendations (OIE, 2003; Masterton, 2008).

Antimicrobial resistance surveillance work is important for the following reasons;
• It allows for timely detection of emerging resistance; risk factors for development of resistance and appropriate intervention strategies thereby limiting the extent and severity of outbreaks and reducing treatment costs.
• Long term surveillance allows evaluation of the effectiveness of any interventions employed (EAGAR, 2006; Aarestrup, 2015).
• Reports on antimicrobial resistance monitoring and surveillance are a useful guide in; making necessary legislation and therapeutic guideline changes to promote more judicious use of antimicrobial drugs (EAGAR, 2006).
• Surveillance guides the selection of antimicrobial drugs to be added to the list of “critical medicines” on the basis of observed resistance patterns and trends (WHO, 2017).
• Surveillance on resistance mechanisms is critical in guiding pharmacological research in the development of novel and more efficacious antimicrobial drugs (Masterton, 2008).

Despite the importance of surveillance, the process is not always easy to standardize. As an example, some studies rely on clinical isolates and this tends to create bias (Aarestrup, 2004) since veterinarians usually submit for resistance testing the samples of severe or recurrent cases unresponsive to initiated antimicrobial treatment (OIE, 2003; Aarestrup, 2004). Other studies only survey zoonotic pathogens such as Salmonella enterica and Campylobacter because of their public health significance (Caprioli et al., 2000).

2.10 Use of indicator bacteria in resistance surveillance

While no perfect surveillance system exists, it has been suggested that resistance indicator bacteria should be enteric commensals such as E. coli and Enterococcus species (Aarestrup, 2004; Varga et al., 2008). Both bacterial species have been employed as resistance indicators in various antimicrobial resistance surveillance systems including the “South African National Veterinary Surveillance and Monitoring Program” (SANVAD), “Danish Integrated Antimicrobial Resistance Monitoring and Research Programme” (DANMAP) and the “Swedish Veterinary Antimicrobial Resistance Monitoring” (SVARM) (van Vuuren, Picard and Greyling, 2007). The advantage of selecting these bacteria is that by virtue of being resident in the gastrointestinal tract, they are
exposed to orally administered antimicrobial drugs and antimicrobial residues eliminated in bile and thus they easily develop resistance (OIE, 2003). Secondly, these indicator bacteria can be isolated from healthy individuals and hence they give a truer picture of the level of antimicrobial resistance in the whole population compared to pathogenic isolates (Caprioli et al., 2000; Aarestrup, 2004). Due to their presence in diverse animal species, studying these indicator bacteria allows comparison of resistance in various animal species and in animals raised under different antimicrobial usage practices (Caprioli et al., 2000; Varga et al., 2008).

Indicator bacteria are also crucial because they are reservoirs of antimicrobial resistance genes that can be transmitted to pathogenic bacteria in both humans and animals (Caprioli et al., 2000; Varga et al., 2008). This is particularly so for Enterococcus species which have even been nicknamed “resistance gene traffickers” (Werner et al., 2013). Mobile genetic elements, some of which encode resistance, may constitute up to 25% of the entire genome of Enterococcus faecalis (Paulsen et al., 2003), and up to 38% of the genome in Enterococcus faecium (Lam et al., 2012). Indicator bacteria in animals can be transmitted to humans via the food chain and then subsequently transfer resistance genes to other microbes in the gastrointestinal tract. In some instances, they may cause opportunistic infections in immunocompromised individuals (Bortolaia, Espinosa-Gongora and Guardabassi, 2016).

2.10.1 Enterococcus species

Enterococcus species are facultative anaerobic Gram-positive cocci that are part of the resident gastrointestinal flora of various animal hosts and humans, and also occur in the environment (Hammerum, 2012; Miller et al., 2015). They are harmless in animals but some are potentially pathogenic in humans. Enterococcus species have multiple resistance determinants which allow them to proliferate and dominate other intestinal micro-organisms in the face of antimicrobial selective pressure (Miller et al., 2015). They are intrinsically resistant to several first line antibiotic drugs, are capable of forming biofilms and have a very malleable genome with the capacity to take up and transfer resistance genes with ease (Marothi, Agnihotri and Dubey, 2005).
Transfer of enterococcal mobile genetic elements harbouring resistance genes has been demonstrated both within species and to bacteria belonging to different genera (Palmer, Kos and Gilmore, 2010). An example of transfer occurring within *E. faecalis* species is the transfer of pheromone responsive plasmids such as pCF10 which encodes tetracycline resistance (Hirt *et al.*, 2005), and pMG2200 which encodes vancomycin resistance (Zheng *et al.*, 2009). Broad host range plasmids include incompatibility group 18 plasmids such as pAMβ1 and pIP501 in *E. faecalis* which are transferrable to *Listeria* species, *Lactococcus* species, *Streptomyces lividans* and other Gram-positive bacteria. These two plasmids encode resistance to lincosamides, macrolides and streptogramin B (Palmer, Kos and Gilmore, 2010).

In addition to possessing multiple resistance determinants, enterococci are hardy bacteria that can withstand harsh environmental conditions such as extreme temperature and pH, (Hürlimann *et al.*, 2016) ionizing radiation, disinfectants and oxidative stress. They are resistant to desiccation such that they can survive on dry surfaces for several months (Bale *et al.*, 1993). These properties make enterococci highly adapted to the hospital environment and have led to their emergence as one of the top three causes of life threatening hospital acquired infections in immunosuppressed human patients in Europe and America (Miller *et al.*, 2015), particularly *E. faecalis* and *E. faecium* (Marothi, Agnihotri and Dubey, 2005). Hospital acquired enterococcal infections include urinary tract infections, endocarditis, wound infections (Nishioka *et al.*, 2009; Hammerum, 2012; Hürlimann *et al.*, 2016) and meningitis. More often than not, these infections are very resistant to several antibiotics (Arsène and Leclercq, 2007).

Of the various antibiotics that enterococci are resistant to, vancomycin resistance has raised the greatest alarm. This is because vancomycin is one of the last resort drugs for the treatment of multi-drug resistant Gram-positive infections in humans including methicillin resistant *Staphylococcus aureus* (MRSA) (Boneca and Chiosis, 2003). Vancomycin resistant enterococcal infections are thus extremely difficult to treat and are associated with increased mortality (Iweriebor, Obi and Okoh, 2015). A study by DiazGranados *et al* (2005) demonstrated that vancomycin resistance increased mortality rate of patients with enterococcal blood stream infections by more than two fold. There has been an increase in the prevalence of nosocomial vancomycin resistant
enterococcal infections in human hospitals over the years (Simner et al., 2015). There were several outbreaks of vancomycin resistant enterococcal infections in human hospitals in South Africa in 2012 (Mendelson and Matsoso, 2015).

Studies using mice models as well as comparison of plasmids derived from animal and human hosts have demonstrated the feasibility of transfer of vancomycin resistance genes between animal and human derived enterococci (Hammerum, 2012). Horizontal transfer of vancomycin resistance genes from enterococci to MRSA has been detected in experimental models (de Niederhäusern et al., 2011). Vancomycin resistant E. faecium appear to be very host specific. In contrast, there is no evolutionary distinction between animal and human E. faecalis strains and as such there are chances that E. faecalis ingested in contaminated animal food may cause opportunistic infections in immunocompromised humans (Bortolaia, Espinosa-Gongora and Guardabassi, 2016). Vancomycin resistant enterococci of animal origin may thus be a threat to public health.

2.10.2 Escherichia coli

E. coli is a Gram-negative bacterium belonging to the family Enterobacteriaceae. It is ubiquitous in nature and is one of the dominant commensal flora of the gastrointestinal tract of various animals and humans. Most E. coli strains in animals are harmless commensals but some are pathogenic in humans and of public health significance. Pathogenic strains are broadly classified into enteric and extra-intestinal on the basis of their associated clinical signs. Enteric pathogenic E. coli generally cause diarrhoeas of varying severity and in some cases vomiting. Extra-intestinal pathogenic E. coli cause various disease conditions such as neonatal meningitis, urinary tract infections, and septicaemia in immunocompromised or hospitalised individuals (Bélanger et al., 2011; Allocati et al., 2013). Enterohemorrhagic E. coli O157:H7 is a recognised zoonotic strain with ruminants, especially cattle, being reservoir hosts. The pathogen is transmitted directly through consumption of contaminated food or water and indirectly through contact. Disease outbreaks due to the strain have been reported in several countries (Bélanger et al., 2011; Ferens and Hovde, 2011). Uropathogenic E. coli is the top cause of urinary tract infections in humans, accounting for more than three quarters of the cases (Allocati et al., 2013).
The incidence of multidrug resistant Gram-negative bacterial infections including resistance of Enterobacteriaceae such as *E. coli* and *Klebsiella* to third-generation cephalosporins due to extended spectrum beta-lactamase production is reportedly on the increase in human hospitals in South Africa (Mendelson and Matsoso, 2015; Coetzee *et al*., 2016). Carbapenems are one of the antimicrobial drugs commonly employed for the management of these drug resistant infections. Unfortunately, growing resistance to carbapenems due to carbapenemase production coupled with lack of efficacious novel drugs has prompted clinicians to recall the parenteral use of colistin (Osei Sekyere, 2016; Hadjadj *et al*., 2017). Colistin had been shelved for decades due to its renal toxicity and neurotoxicity. It had only continued being used in ophthalmic and topical preparations with systemic use being limited to cystic fibrosis patients (Falagas, Kasiakou and Saravolatz, 2005). Colistin is now a last resort drug for the treatment of multidrug resistant Gram-negative bacterial infections (Falagas, Kasiakou and Saravolatz, 2005). Noteworthy examples of Gram-negative superbugs that necessitate the use of colistin include “New Delhi metallo-beta-lactamase-1 producing” Gram-negative bacteria and “*Klebsiella pneumoniae* carbapenemase” which have spread globally and are reportedly on the increase in South Africa (Mendelson and Matsoso, 2014).

Until recently, colistin resistance was previously thought to arise only due to chromosomal mutations. The European Medicines Agency in a meeting set in 2013, recommended continued investigation of the presence of mobile genetic elements encoding for colistin resistance in resistant isolates which once detected would lead to review of the policy regarding the veterinary and medical use of the drug. Following these recommendations, surveillance for colistin resistance in humans and in food animals then became compulsory for European Union member states as of 2014 (European Medicines Agency, 2016). Detection of plasmid mediated colistin resistance (*mcr-1*) in food animals, raw meat and human patients in China (Liu *et al*., 2016) steered surveillance studies in several other countries which also detected *mcr-1*. The discovery of *mcr-1* prompted the review and change in policies regarding colistin use in veterinary medicine and in humans (Poirel and Nordmann, 2016). In May 2016, the European Medicines Agency’s “Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health” which banned use of colistin in livestock unless
justified by susceptibility testing as the only effective treatment option was published (European Medicines Agency, 2016).

In contrast to human medicine where colistin is a last resort drug, colistin has been used for decades mainly as a feed additive in livestock because it is cheap and not toxic in animals due to poor intestinal absorption of the drug. It is mainly used in poultry and pigs for growth promotion, prophylaxis and group treatment of Gram-negative gastrointestinal infections (South African Veterinary Council, 2016). The use of high volumes of colistin in production animals is implicated as the most probable cause for the emergence of plasmid mediated colistin resistance in humans. Detection of mcr-1 in more animal isolates compared to human isolates in several studies; identification of an insertion sequence (ISAplI) similar to one in Pasteurella multocida (a well-recognised animal pathogen) upstream the mcr-1 gene; and co-expression of an antibiotic resistance gene floR for florfenicol (a drug exclusively used in animals) in a human E. coli isolate in Switzerland further suggest a high probability of the animal origin of plasmid mediated colistin resistance (Poirel and Nordmann, 2016).

Plasmid mediated colistin resistance is a great cause for concern because plasmids are mobile genetic elements hence the resistance genes can disseminate rapidly via the food chain and through environmental contamination. Worryingly, plasmid mediated colistin resistance is increasingly being detected in several countries including South Africa (Coetzee et al., 2016). To date, up to five (mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5) plasmid borne colistin resistance genes have been identified in different hosts and in the environment (Li et al., 2018). A relevant example in veterinary medicine in South Africa which sparked concern is the case of colistin resistance in avian pathogenic E. coli. A study by Perreten et al (2016) detected an increase in colistin resistance in avian pathogenic E. coli isolates from 2.3 to 4.4% between 2008 and 2013, to 7.6% in 2014 then up to 13.6% in 2015. The mcr-1 gene was detected in 19 out of 20 strains among the colistin resistant isolates obtained in 2015. Multidrug resistance in avian pathogenic E. coli which causes airsacculitis in poultry led to increased use of colistin in the management of the disease and promoted development of colistin resistance. The detection of mcr-1 gene by Coetzee et al (2016) in E. coli isolates from nine human patients all of which had no previous exposure to colistin
suggest a probable link between the use of colistin in animals and the subsequent development of colistin resistance in animals which is then transferred to humans via the food chain.

Based on several studies conducted on animal, human and environmental samples, it seems *E. coli* is the dominant reservoir of *mcr-1* colistin resistance. Even the plasmid mediated colistin resistance in wild birds in various countries was detected in *E. coli* isolates (Liakopoulos *et al.*, 2016; Ruzauskas and Vaskeviciute, 2016; Bachiri *et al.*, 2018). This is a cause for concern because the ubiquitous occurrence and easy transfer of *E. coli* between hosts and the environment allows for spillover of resistance from animals to humans and vice versa (Poirel and Nordmann, 2016). The above findings indicate the need for stricter control for colistin use in livestock and the need to conduct colistin resistance surveillance in diverse ecological niches including different livestock production systems to elucidate reservoirs and transmission routes of resistance determinants (Liakopoulos *et al.*, 2016; Perreten *et al.*, 2016).

### 2.11 Milestones and drawbacks in antimicrobial resistance mitigation in South Africa

Due to the risk of antimicrobial resistance and the potential for spread at both local and global level, over the years much attention has been given to drafting policies and strategies to slow down resistance. South Africa is the African country that is relatively ahead in antimicrobial resistance related work. Since 2010, South Africa is a member of the Global Antibiotic Resistance Partnership (GARP), in collaboration with Kenya, India and Vietnam. GARP’s mandate is to engage in antimicrobial resistance work in the four countries with the aim of reducing resistance (Moyane, Jideani and Aiyegoro, 2013). The South African Antibiotic Stewardship Program (SAASP) was established in 2012. It brings together various professionals including medical practitioners, veterinarians, pharmacists, pharmacologists, microbiologists and epidemiologists, with the aim of promoting good prescribing practices and continued education on antimicrobial resistance. SAASP has an “Antimicrobial Resistance Strategy Framework” which compromises of three main pillars, namely antimicrobial surveillance, infection prevention and control, and antimicrobial stewardship (Mendelson and Matsoso, 2015).
Nel, van Vuuren and Swan (2004) conducted and completed a study in 2001 to come up with a standardized method for monitoring the levels of resistance in bacteria isolated from animals in South Africa. The findings were to provide guidelines for the methodology to be employed in the then soon to be established “South African National Veterinary Surveillance and Monitoring Program for Resistance to Antimicrobial Drugs” (SANVAD). The SANVAD program was conducted in 2005-2007 in response to OIE’s appeal to its member states to carry out national antimicrobial resistance surveillance and risk assessment programs. For the program, the University of Pretoria Faculty of Veterinary Science collaborated with the Swedish National Veterinary Institute Department of Antibiotics so as to benefit from the knowledge and expertise they have accrued since the start of the Swedish Veterinary Antimicrobial Resistance Monitoring Program in 1980 (van Vurren, Picard and Greyling, 2007). The SANVAD program looked at resistance in; indicator organisms (E.coli, and enterococci), veterinary pathogens (Mannheimia hemolytica, Staphylococcus aureus, Beta hemolytic Streptococci, E. coli) and zoonotic pathogens (Salmonella enterica). High resistance to tetracyclines and sulphonamides was detected in E. coli, S. enterica and Enterococcus species (van Vuuren, Picard and Greyling, 2007; Henton et al., 2011).

The South African Veterinary Association’s Medicines Committee and the University of Pretoria Department of Para-clinical Sciences Faculty of Veterinary Science staff drafted a booklet on guidelines on judicious use of antimicrobial drugs in animals in South Africa in accordance with the OIE International Standards on Antimicrobial Resistance published in 2003 in the OIE Terrestrial Animal Health Code (National Department of Health, 2014). The booklet was distributed to all members of the South African Veterinary Association at the time. The Faculty also drafted another booklet entitled, ‘Veterinary drug control and management for the practicing veterinarian in South Africa’ (National Department of Health, 2014; Mendelson and Matsoso, 2015). In October 2014, an Antimicrobial Resistance summit was held at Birchwood hotel in South Africa. The emphasis was on the roles to be played by various stakeholders including veterinarians, farmers, pharmacists and regulatory authorities, to contain antimicrobial resistance in the country (van Vuuren, 2014). Act 101 of 1965 was amended in Act 14 of 2015 to make it a requirement for veterinarians to have a “veterinary medicines” dispensing license to allow improved monitoring of the use of these drugs (Eagar and Naidoo, 2017). The latter is however yet to be enacted.
Mendelson et al (2018) most recently reported that the increased incidence of multidrug resistant infection in human hospitals necessitating the use of colistin, detection of a high prevalence (95%) of the *mcr*-1 gene in selected colistin resistant *E. coli* isolates in South African poultry by Perreten et al (2016) and the need to tackle antimicrobial resistance from a One Health perspective necessitated the creation of the South African Colistin Working Group. The group comprises of various stakeholders such as representatives from the Ministerial Advisory Committee for Antimicrobial Resistance, various veterinary boards including the South African Medicines Control Council and the department of Sector-Wide Procurement; the registrar of Medicines and the adviser of the Stock remedies registrar. The group first convened in April 2016 and reviewed the volumes and preparations of colistin used; indications for use; legislation governing the use of the drug; and trends in colistin resistance, in both human and veterinary medicine in the country.

Following the publication of “Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health” in May 2016, the Colistin Working Group in its second meeting in July 2016 adopted European Medicines Agency’s recommendations for colistin use on the premise that the country did not have the capacity to conduct its own surveillance in a timely manner to determine the course of action to take regarding colistin use in livestock (Mendelson et al., 2018). The South African Veterinary Council on the fourteenth of November 2016 sent a letter to all its members recommending colistin not to be used in food animals unless sensitivity testing justifies it as the only treatment option (South African Veterinary Council, 2016). The plan of the Colistin Working Group is to extend such antimicrobial stewardship efforts from a one health perspective to all drugs used in both animals and humans (Mendelson et al., 2018).

Despite the achievements made in South Africa thus far, the country is lagging behind in implementation of some strategies recommended by WHO and other boards.

- Unrestricted use of antimicrobial drugs: Some countries have long since banned over the counter selling of antimicrobial drugs which are used for therapeutic purposes in humans
and animals and stopped the use of antimicrobial growth promoters. However, the use of antimicrobial growth promoters, including some like tylosin and bacitracin which were recommended for banning by WHO, is still allowed in South Africa under the Stock Remedies Act (Act 36 or 1947). There are some antimicrobial drugs, including tetracyclines, registered under the Stock Remedies Act (Act 36 or 1947) which can be bought over the counter without a prescription (National Department of Health, 2013). There is need for a comprehensive review of antimicrobial feed additives registered for use in South Africa (van Vuuren, 2014).

- Counterfeit drugs: Another major concern is that there is a heavy presence of counterfeit medicines on the South African market and that includes antimicrobial drugs (Henton et al., 2011). According to the United Nations Office on Drugs and Crime, about 30% of pharmaceutical agents on the African market are counterfeits. The WHO reports that half of the counterfeit medicines worldwide are antimicrobial drugs (Kelesidis and Falagas, 2015). Approximately 20% of the drugs sold in South Africa are believed to be counterfeits (Moyane, Jideani and Aiyegoro, 2013). A good transport network, poor customs monitoring and the relatively high buying power attract international traffickers to South Africa (Knudsen and Nickels, 2015). Online buying, including that of medicines, is increasingly becoming popular in South Africa and at the same time online sites selling illegal and counterfeit medicines are also on the increase (Child, 2015).

- Deficient monitoring of the use of antimicrobial drugs: There is very limited data available on antimicrobial volumes and antimicrobial use patterns in production animals in South Africa. Lack of a standardized national electronic system for data capture in pharmacies and drug stores makes it difficult to make a comprehensive estimate of volumes of antimicrobials used (National Department of Health, 2013). Pharmaceutical companies are yet to divulge information on their antimicrobial sales in open press, as they regard it sensitive information that may prejudice their sales. Furthermore no record is kept of the use of stock remedies and illegal importation of drugs certainly leads to underestimation of antimicrobial volumes whenever efforts are made to add up the volumes of antimicrobials used (Moyane, Jideani and Aiyegoro, 2013).
Deficient routine culture and sensitivity testing: Monitoring and surveillance of antimicrobial resistance in animals in South Africa falls short of international standards. Only a small fraction of health practitioners send clinical samples for culture and sensitivity testing before prescribing antimicrobials. This makes it difficult to evaluate the national burden of antimicrobial resistance. There are also a few qualified personnel to conduct antimicrobial resistance monitoring and surveillance work. Another setback is limited funds to conduct nationwide surveillance and monitoring programs and to raise public awareness on prudent use of antimicrobials and antimicrobial resistance. A good example is the SANVAD which was conducted once. Had it not been for financial constraints, this surveillance program would have continued running annually (National Department of Health, 2013).

2.12 General Conclusion

Antimicrobial resistance is a globally recognized threat to human and animal health. Resistance has increased treatment costs and mortalities due to treatment failure (Tenover, 2006). Resistance development cannot be halted given that mutations are a natural phenomenon in microorganisms. However measures can be taken to at least reduce the rate of resistance development (Courvalin, 2008) and as such global and local actions have been taken and plans put in place towards this goal. Surveillance is a critical component of resistance containment strategies and should be conducted on both antimicrobial use and resistance profiles of microorganisms to allow holistic risk assessment (Mendelson and Matsoso, 2015). Continuous surveillance provides data that is essential in making informed policy changes and therapeutic guideline changes (EAGAR, 2006).

Most surveillance studies are conducted in commercial production systems while communal and small scale systems are generally neglected probably because of the intensive use of antimicrobial drugs in commercial farms as opposed to the extensive use of antimicrobial drugs in communal areas. However, it is necessary to conduct resistance surveillance in different antimicrobial use practices even use that is in line with prudent use guidelines because resistance selection pressure occurs regardless of the manner or volume of antimicrobial use (FAO, 2016). Furthermore, given
the substantial contribution of communal and small scale herds to the national herd in South Africa, it is important to boost surveillance in communal and small scale farming systems. The increased contact between livestock, humans and wildlife, reduced biosecurity and poor sanitation in communal areas facilitates spread of antimicrobial resistance which makes it pertinent to conduct surveillance in these communities. Given the over the counter availability of some antimicrobial drugs to laymen in South Africa, a significant percentage of which are barely literate in the communal and rural areas, it is important to conduct surveillance not just on resistance profiles of organisms, but also on antimicrobial use practices of farmers. Such surveillance work will give informed insights on areas that need to be addressed to raise awareness of farmers on antimicrobial drugs and prudent use practices.
Chapter 3: MATERIALS AND METHODS

3.1 The study area

The study was conducted in the Mnisi community located in the north-eastern part of the Bushbuckridge Municipal Area in Mpumalanga Province, South Africa (Figure 3.1). Animal husbandry is the major agricultural activity in the area, with cattle being the most common livestock (Musoke, 2016). Cattle are thus likely the most exposed to antimicrobial drugs. The cattle graze at communal pastures and thus have relatively similar environmental exposure factors for antimicrobial resistance development and moreover resistance and infectious diseases can easily spread between herds. Over two thirds of the Mnisi community shares a border with surrounding wildlife conservation parks including the Kruger National Park, Sabi sands, and Manyelethi game reserves (Simela, 2012). Foot and Mouth Disease (FMD) and other tick borne diseases such as Corridor disease are endemic in the surrounding wildlife. The climate in Mnisi is conducive for proliferation of different ticks and as such tick borne diseases occur in the area (Choopa, 2015).

The Mnisi community is subdivided into four wards (B1, B2, B3 and B4) each comprising of a number of villages. There are 21 dip tank sites in total distributed across the different wards in the Mnisi community and each ward is serviced by one animal health technician (AHT). Animal health technicians are the primary contact veterinary personnel for the communal farmers (Simela, 2012). They carry out animal health activities such as ear tagging, vaccination, routine cattle dipping and inspection for surveillance of important diseases such as FMD. They also work alongside the state veterinarians in animal inspection prior to slaughter and for granting of transportation permits among other activities.

The community is privileged to have a local animal clinic where farmers can get veterinary assistance at subsidized fees. The clinic is an integral part of the University of Pretoria Mnisi Community Program which was established to boost veterinary service provision to farmers in the area since state veterinary officials are overwhelmed. University of Pretoria Veterinary students together with veterinarians from the Hluvukani animal clinic or from the University of Pretoria...
Faculty of Veterinary Science regularly visit the dip tanks to assist farmers with disease diagnosis and treatment of their sick animals among other veterinary services.

3.2 Study population

The study focused on farmers and their cattle as they presented them at the dip tanks for routine dipping of cattle and inspection for FMD as required by the law. Apparently healthy cattle (as determined by the absence of clinical signs of disease) above six months of age and belonging to interviewed farmers were included in the study.

3.3 Sample collection sites

Due to the poor condition of the roads in the area during the time of sample collection, sample collection was limited to Ward B1. Samples were collected from all the dip tanks in Ward B1, namely; Thlavekisa, Welverdiend A, Welverdiend B, Clare A and Clare B. Each village has a designated dip tank. All cattle in Mnisi are required by law to be assigned to a specific dip tank and to be inspected once weekly for FMD at the allocated dip tank. The dip tanks are thus representative of the cattle in the area.
Figure 3.1: Map of dip tanks in the Mnisi community and surrounding game parks. Samples were collected from Thlavekisa, Welverdiend A, Welverdiend B, Clare A and Clare B dip tanks (map courtesy of Mnisi Community Program, University of Pretoria)

3.4 Enrolment of participating farmers

Invitation to participate in the study was extended to each farmer at the dip tanks. Each farmer signed a consent form before participating in the study.
3.5 **Questionnaire survey**

**Farmer questionnaire**

- Face to face interviews based on a standardized questionnaire were conducted with participating farmers with the assistance of a local environmental health monitor who was translating. The farmers were interviewed using Shangaan, the local language. The questionnaire included both open and close ended questions on; demographic data, livestock species kept, duration of cattle rearing, cattle herd size, antibiotics used on the farm and where they source them, disposal of expired antibiotics, knowledge on antimicrobial resistance and importance of observing antimicrobial withdrawal periods. The full questionnaire is presented in the addendum.

**Animal health technician questionnaire**

- Questionnaires were also administered to willing animal health technicians that work in Mnisi to obtain data on antimicrobial drugs commonly used and antimicrobial use malpractices of farmers in the community. The full questionnaire is presented the addendum.

- A few veterinarians who had worked at the local Hluvukani animal clinic in 2017 were contacted to get more information on veterinary antimicrobial drugs they had commonly prescribed for use in the area.

3.6 **Animal sample collection and processing**

Sampling of cattle to isolate and determine antimicrobial resistance profiles of enteric *E. coli* and *Enterococcus* species was undertaken.

3.6.1 **Animal selection**

The number of cattle to be sampled at each dip tank (34 at Welverdiend A, 19 at Welverdiend B, 22 at Clare B, 13 at Clare A and 12 at Thlavekisa to obtain a total of 100 samples) was determined
proportionally according to the number of cattle assigned to a particular dip tank over the total number of cattle in the Ward (i.e. proportional stratified sampling approach) to ensure a good geographical spread of the sample. The selection of cattle participating in the study was done by randomly picking the fifth or (the last animal for herds with less than 5 cattle), then the tenth or and fifteenth animal depending on the number of cattle sampled per herd. One to three cattle per interviewed farmer were selected for sample collection.

### 3.6.2 Sample collection and transportation

Sample collection was conducted between January and February 2018 in the morning (between 05:45 and 7am) as the cattle were presented for routine inspection at dip tanks. One fecal sample was collected rectally from each animal using sterile dry culture swabs. On each day, samples were transported to the laboratory on ice within three hours of collection and then stored at – 80°C awaiting processing at the BSL2+ laboratory in the Department of Veterinary Tropical Disease at the Faculty of Veterinary Science, University of Pretoria.

![Fig 3.2: The cattle were manually restrained while in the race to allow sample collection by rectal swabbing.](image)

### 3.6.3 Bacterial isolation

At the laboratory, *E. coli* and *Enterococcus* species were cultured from the fecal samples using standard procedures. The fecal swabs were inoculated onto sheep blood agar (Thermo Fischer...
Scientific, South Africa) and then onto MacConkey agar without crystal violet (Thermo Fischer Scientific). The blood agar plates were incubated at 37°C in 5% carbon dioxide for 24 hours while MacConkey agar plates were incubated at 37°C for 24 hours in an aerobic incubator. Suspect *E. coli* and *Enterococcus* species colonies based on colony morphology, hemolysis and lactose fermentation were subcultured on sheep blood agar and MacConkey agar without crystal violet and incubated as previously described to obtain pure cultures. Selected suspect colonies on the blood agar were further subjected to gram staining, catalase test and oxidase test (Davies Diagnostics, South Africa). Swab samples from which *E. coli* were not recovered on first attempt were enriched with peptone buffered water and the culture process repeated.

Gram-positive, catalase negative cocci occurring singly or in pairs and producing pin point red colonies on MacConkey agar without crystal violet (i.e. lactose fermenters) and positive for aesculin (Thermo Fischer Scientific) hydrolysis were subjected to Streptococcal grouping using a commercial test kit, Streptex kit (Thermo Fischer Scientific). Colonies falling in Lancefield group D were subjected to further sugar tests (Thermo Fischer Scientific) to allow differentiation of some of the *Enterococcus* species according to the criteria described by Quinn et al (1994) shown in Table 3.1 below. Some enterococci could not be identified to species level due to the limited array of sugars tested.

**Table 3.1:** Differentiation of *E. faecium*, *E. faecalis*, *E. durans* and *E. avium* based on biochemical tests.

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Lactose</th>
<th>Arabinose</th>
<th>Sorbitol</th>
<th>Mannitol</th>
<th>Growth in 6.5% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>E. durans</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>E. avium</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Gram-negative, catalase positive and oxidase negative, medium sized rods producing large pink colonies on MacConkey agar (i.e. lactose fermenters) were tested for indole (Merck, South Africa) production and presumed to be Enterobacteriaceae if positive for indole production and then subjected to API 10S test (BioMerieux, South Africa). The four digit numerical profile obtained for the grouped tests on the API 10S series was entered into APIWEB® to identify *E. coli* isolates. The *E. coli* and enterococci isolates were stored in cryovials (Aec Amershan, South Africa) at -80°C.

### 3.7 Antimicrobial susceptibility testing

#### 3.7.1 Method overview

A microtitre broth dilution method was used to determine the antimicrobial susceptibility profile of each isolate following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008).

The *E. coli* isolates were subjected to antimicrobial susceptibility testing to the following agents; colistin sulphate, amoxicillin, enrofloxacin, chlortetracycline and gentamicin (Sigma Aldrich, Germany).

The *Enterococcus* isolates were subjected to antimicrobial susceptibility testing to the following agents; amoxicillin, chlortetracycline, erythromycin, vancomycin and enrofloxacin (Sigma Aldrich).

#### 3.7.2 Preparation of antibiotics

Water soluble antibiotic powders were used. The weight of each antibiotic powder to be used was calculated based on the percentage purity (assay potency) of the antibiotic and the antibiotics dissolved in sterile water. The Mueller Hinton broth (Oxoid, South Africa) with 0.5% glucose and a phenol red indicator (Thermo Fischer Scientific) was prepared and autoclaved to ensure sterility of the media. The antibiotic stock solutions were further diluted with the Mueller Hinton broth as necessary.
One hundred micro-litres of Mueller Hinton broth was added to all wells of the 96 well plates (Aec Amershan) except for the first row. 100µl of the diluted antibiotics were added to the first two rows of the plate at double the strength of the required highest concentration and twofold serial dilutions made down the rest of the plate. Each antibiotic agent was tested in duplicate at concentrations ranging from 0.25µg/ml to 32µg/ml.

3.7.3 Preparation of inoculum and plating into wells

Bacterial isolates were inoculated onto blood agar and incubated at 37°C for 24 hours to obtain fresh cultures for the antimicrobial susceptibility testing. To prepare the inoculum, a few colonies were inoculated into 3ml Mueller Hinton broth in a screw cap tube until a turbidity equivalent to that of a 0.5 McFarland standard (1-2 x10⁸ cfu/ml) was reached. The bacterial suspension was mixed by gently inverting the tube and thereafter an aliquot diluted with broth (1:150) to obtain approximately 1x10⁶ cfu/ml. 100 µl of this diluted suspension was added to each well with antibiotics thereby achieving a final inoculum density of approximately 5 x 10⁵ cfu/ml. The plates were then sealed in a plastic to prevent vaporization and incubated at 37°C for 24 hours. The remainder of each bacterial inoculum suspension was incubated at 37°C for 24 hours to serve as a growth control.

To check for inoculum density and purity of each isolate tested, 10 µl of the final inoculum preparation was diluted using 10ml of normal saline. 100 µl of this mixture was then withdrawn and spread on to sheep blood agar using a sealed and bent pipette flamed in absolute alcohol. Following incubation, the plates were checked for presence of mixed cultures and colony count done to check if there were between 10 to 50 colonies. *E. coli* ATCC #25922 and *E. faecalis* ATCC # 29212 were used as controls.

3.7.4 Reading and Interpretation of results

Plates were read under a viewing mirror using naked eyes to check for bacterial growth. A yellow color or increased turbidity was indicative of bacterial growth. The lowest antibiotic concentration
completely inhibiting visible growth was recorded as the minimum inhibitory concentration (MIC).

MIC breakpoints used were based on Clinical Laboratory Standards Institute breakpoints guidelines (CLSI, 2008; CLSI, 2018) as shown in Table 3.2. On the basis of these breakpoints, isolates were categorized as susceptible, intermediate or resistant. Isolates that were resistant to at least one antimicrobial drug were defined as “resistant” while those resistant to three or more antimicrobial classes were defined as “multi drug resistant” (Magiorakos et al., 2012).

Table 3.2: Minimum inhibitory concentration (MIC) breakpoints used for resistance classification of bacterial isolates (adapted from CLSI 2018).

<table>
<thead>
<tr>
<th>MIC Interpretive criteria (µg/ml)</th>
<th>E. coli</th>
<th>Enterococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>susceptible</td>
<td>intermediate</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>≤ 8</td>
<td>16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤ 4</td>
<td>8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤ 4</td>
<td>8</td>
</tr>
<tr>
<td>Enrofloxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤ 0.25</td>
<td>0.5 - 1</td>
</tr>
<tr>
<td>Colistin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≤ 2</td>
<td></td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>≤ 8</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤ 4</td>
<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤ 0.5</td>
<td>1 - 4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤ 4</td>
<td>8 - 16</td>
</tr>
<tr>
<td>Enrofloxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤ 0.25</td>
<td>0.5 - 1</td>
</tr>
</tbody>
</table>

a. enrofloxacin breakpoint for bovine respiratory disease was used
b. colistin breakpoint for <i>Pseudomonas aeruginosa</i> was used
3.8 Data analysis

Questionnaire responses and susceptibility profiles of the isolates were coded and entered into SPSS (IBM SPSS statistics 25) for descriptive analysis (i.e. generation of frequencies and bar graphs).

3.9 Permit applications

Ethical approval for the questionnaire survey and for animal use was obtained from the University of Pretoria Faculty of Humanities (reference number: 17406201 (GW20171117HS)) and the University of Pretoria Faculty of Veterinary Science Animal Ethics Committee respectively (project number V103-17). A red cross permit (reference number: 12/11/1/1/6 (619)) for transportation of the cattle rectal swab samples was obtained from the Department of Agriculture, Forestry and Fisheries Republic of South Africa. The permits are presented in the addendum.
CHAPTER 4: RESULTS

4.1 Isolated bacteria

A total of 150 bacterial isolates were obtained, 79 *E. coli* and 71 *Enterococcus* species (Table 4.1). Of the *E. coli* and *Enterococcus* isolates obtained, (15%; 12/79 and 17%; 12/71) were sourced from Clare A, (23%, 18/79 and 20%; 14/71) from Clare B, (33%; 26/79 and 23%; 16/71) from Welverdiend A, (22%, 17/79 and 25%; 18/71) from Welverdiend B, and (8%; 6/79 and 15%; 11/71) from Thlavekisa respectively.

![Table 4.1: Distribution of *E. coli* and *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality by sampling sites.](image)

Among the enterococci isolated, *E. faecium* (47%; 33/71) was the dominant species followed by *E. faecalis* (4%; 3/71), *E. durans* (4%; 3/71) and *E. avium* (4%; 3/71). Forty one percent (29/71) of the enterococci isolates were not identified to species level (Figure 4.1).
Figure 4.1: Proportions of *Enterococcus* species isolated from rectal swabs of healthy cattle at dip tanks in Mnisi community Ward B1, Bushbuckridge Municipality.

4.2 Antimicrobial susceptibility of isolates

4.2.1 *Enterococcus* species

4.2.1.1 Prevalence of antimicrobial resistant *Enterococcus* species

Of the 71 enterococci isolates tested, (55%; 39/71) were resistant to at least one of the antibiotics tested. The highest level of resistance detected was against enrofloxacin (55%; 39/71) followed by amoxycillin (3%; 2/71). Intermediate susceptibility to erythromycin was detected in (38%; 27/71) of the enterococci isolates. All (100%, 71/71) the enterococci isolates were susceptible to chlortetracycline and vancomycin.
Figure 4.2: Antimicrobial susceptibility profile to five antimicrobial drugs of Enterococcus species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

Among the Enterococcus species, only E. faecium isolates (6%; 2/33) were resistant to amoxycillin. All (3/3; 100%) E. durans isolates were resistant to enrofloxacin, followed by (91%; 30/33) of E. faecium isolates. None (0%; 0/3) of the E. faecalis isolates were resistant to enrofloxacin. None (0%; 0/71) of the different Enterococcus species were resistant to chlortetracycline or vancomycin.

Table 4.2: Antimicrobial susceptibility profile of Enterococcus species from healthy cattle in Mnisi community Ward B1.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Amoxycillin</th>
<th>Enrofloxacin</th>
<th>Chlortetracycline</th>
<th>Vancomycin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>E. faecium</td>
<td>33</td>
<td>94</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>29</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>62</td>
<td>28</td>
</tr>
<tr>
<td>E. avium</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>E. durans</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are presented as percentage prevalence. S = susceptible, I = intermediate, R = resistant, n = number of isolates.
Resistance to enrofloxacin was detected at all dip tanks with more than half of the *Enterococcus* isolates from Clare B (57%; 8/14); Thlavekisa (73%; 8/11) and Welverdiend A (75%; 12/16) being resistant to enrofloxacin. Resistance to amoxycillin was detected at Welverdiend A (6%; 1/16) and Thlavekisa (9%; 1/11) (Table 4.3).

Table 4.3: Antimicrobial susceptibility of *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality according to dip tank sites.

<table>
<thead>
<tr>
<th>Dip tank</th>
<th>n</th>
<th>Amoxycillin</th>
<th>Enrofloxacin</th>
<th>Chlortetracycline</th>
<th>Vancomycin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
</tr>
<tr>
<td>Clare A</td>
<td>12</td>
<td>10 0 0</td>
<td>67 0 33</td>
<td>100 0 0</td>
<td>100 0 0</td>
<td>67 33 0</td>
</tr>
<tr>
<td>Clare B</td>
<td>14</td>
<td>10 0 0</td>
<td>14 29 57</td>
<td>100 0 0</td>
<td>100 0 0</td>
<td>71 29 0</td>
</tr>
<tr>
<td>Welverdiend A</td>
<td>16</td>
<td>94 0 6</td>
<td>25 0 75</td>
<td>100 0 0</td>
<td>100 0 0</td>
<td>44 56 0</td>
</tr>
<tr>
<td>Welverdiend B</td>
<td>18</td>
<td>10 0 0</td>
<td>67 0 33</td>
<td>100 0 0</td>
<td>100 0 0</td>
<td>88 12 0</td>
</tr>
<tr>
<td>Thlavekisa</td>
<td>11</td>
<td>91 0 9</td>
<td>27 0 73</td>
<td>100 0 0</td>
<td>100 0 0</td>
<td>36 64 0</td>
</tr>
</tbody>
</table>

Results are presented as percentage prevalences. S = susceptible, I = intermediate, R = resistant, n = number of isolates

4.2.1.2 MIC distributions of the *Enterococcus* species isolates

The most frequently observed MIC for enrofloxacin, amoxycillin, vancomycin and erythromycin was 0.25µg/ml. Vancomycin and chlortetracycline had very narrow MIC distributions, ≤0.25 - 0.5µg/ml and 0.5µg/ml respectively. Enrofloxacin had the widest MIC range (≤0.25 - ≥32 µg/ml) which had a bimodal distribution with a cluster of susceptible/intermediate strains and a cluster of resistant strains with peaks at 0.25µg/ml and 8µg/ml respectively (Figures 4.3 to 4.7). The MIC$_{50}$ for amoxycillin, vancomycin and erythromycin was 0.25µg/ml while that for enrofloxacin was 4µg/ml. The MIC$_{90}$ for amoxycillin, vancomycin, erythromycin, chlortetracycline and enrofloxacin were 0.5, 0.25, 2, 0.5 and 16 µg/ml respectively.
**Figure 4.3:** Frequency of erythromycin MIC distributions for *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

**Figure 4.4:** Frequency of amoxycillin MIC distribution for *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.
**Figure 4.5:** Frequency of enrofloxacin MIC distribution for *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

**Figure 4.6:** Frequency of vancomycin MIC distribution for *Enterococcus* species isolated from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.
4.2.1.3 Phenotypic antimicrobial resistance patterns of the *Enterococcus* species

Two phenotypic antimicrobial resistance patterns were observed among the *Enterococcus* isolates with resistance to enrofloxacin only 52% (37/71) being dominant while enrofloxacin-amoxycillin resistance was detected in just 3% (2/71) of the isolates (Table 4.4). No multidrug (3 or more antibiotics) resistant strains were detected.

**Table 4.4:** Phenotypic antimicrobial resistance patterns of enterococci isolates from healthy cattle in Mnisi community in Ward B1 Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>Number of isolates</th>
<th>Percentage of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>37</td>
<td>52%</td>
</tr>
<tr>
<td>Enrofloxacin-amoxycillin</td>
<td>2</td>
<td>3%</td>
</tr>
</tbody>
</table>
4.2.2 *Escherichia coli* isolates

4.2.2.1 Prevalence of antimicrobial resistant *E. coli*

Of the 79 *E. coli* isolates tested, (27%; 21/79) were resistant to at least one of the antibiotics tested. The highest level of resistance detected was against colistin (16%; 13/79) followed by chlortetracycline (8%; 6/79) and amoxycillin (8%; 6/79) while only (3%; 2/79) of the *E. coli* isolates were resistant to enrofloxacin. All (100%, 79/79) of the *E. coli* isolates were susceptible to gentamicin (Figure 4.8).

![Antimicrobial Susceptibility](image)

**Figure 4.8:** Antimicrobial susceptibility to five antimicrobial drugs of *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

Colistin resistance in *E. coli* isolates was detected at all dip tanks; Thlavekisa (33%; 2/6), Clare A (17%, 2/12), Clare B (17%; 3/18), Welverdiend A (12%; 3/26) and Welverdiend B (6%; 1/17). Resistance to amoxycillin was detected at Welverdiend B (12%; 2/17), Clare A (8%, 1/12) and Clare B (11%; 2/18) while resistance to enrofloxacin was detected only at Welverdiend A (4%; 1/26) and Clare A (8%, 1/12) (Table 4.5).
Table 4.5: Antimicrobial susceptibility profiles of *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality according to dip tank sites.

<table>
<thead>
<tr>
<th>Dip Tank</th>
<th>N</th>
<th>Amoxycillin</th>
<th>Enrofloxacin</th>
<th>Chlortetracycline</th>
<th>Gentamicin</th>
<th>Colistin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Clare A</td>
<td>1</td>
<td>92</td>
<td>0</td>
<td>8</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clare B</td>
<td>1</td>
<td>89</td>
<td>0</td>
<td>11</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welverdiend A</td>
<td>2</td>
<td>96</td>
<td>4</td>
<td>0</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welverdiend B</td>
<td>1</td>
<td>88</td>
<td>0</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thlavekisa</td>
<td>6</td>
<td>83</td>
<td>17</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are presented as percentage prevalences. S = susceptible, I = intermediate, R = resistant; n = total number of isolates.

4.2.2.2 MIC distributions of the *E. coli* isolates

The most frequently observed MIC for chlortetracycline and gentamicin was 0.5µg/ml and also happened to be the MIC<sub>50</sub> for both antibiotics. The most frequently observed MIC and MIC<sub>50</sub> for amoxycillin, colistin and enrofloxacin were 2µg/ml, 1µg/ml and 0.25µg/ml respectively. The MIC<sub>90</sub> for enrofloxacin, amoxycillin, colistin, gentamicin and chlortetracycline was 0.25, 8, 4, 1 and 4µg/ml respectively (Figures 4.9 to 4.13). All the antibiotics had a wide MIC distribution with the exception of enrofloxacin which had 97% of the isolates having the same MIC (0.25µg/ml) and the remaining isolates having an MIC ≥32µg/ml.
Figure 4.9: Frequency of amoxycillin MIC distributions for *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

Figure 4.10: Frequency of chlortetracycline MIC distributions for *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.
Figure 4.11: Frequency of colistin MIC distributions for *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

Figure 4.12: Frequency of enrofloxacin MIC distributions for *E. coli* isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.
Figure 4.13: Frequency of gentamicin MIC distributions for E. coli isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

4.2.2.3 Phenotypic antimicrobial resistance patterns of the E. coli isolates

Seven antimicrobial resistance phenotypes were detected in the E. coli isolates (Table 4.6). Four of these phenotypes comprised of resistance to just one antibiotic agent namely; amoxycillin, chlortetracycline, colistin and enrofloxacin. Three co-resistance phenotypes were identified among the E. coli isolates namely, amoxycillin-chlortetracycline (5.1%; 4/79); chlortetracycline-colistin (1.3%; 1/79) and enrofloxacin-colistin (1.3%; 1/79) resistance. No multidrug resistant strains were detected.

Table 4.6: Phenotypic antimicrobial resistance patterns of E. coli isolates from healthy cattle in Mnisi community Ward B1, Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>Number of isolates</th>
<th>Percentage of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlortetracycline</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>amoxycillin</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>enrofloxacin</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>colistin</td>
<td>11</td>
<td>14%</td>
</tr>
<tr>
<td>chlortetracycline-colistin</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>chlortetracycline-amoxycillin</td>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>enrofloxacin-colistin</td>
<td>1</td>
<td>1%</td>
</tr>
</tbody>
</table>
4.3 Farmer Questionnaire Responses

4.3.1 Demographic data of participating farmers

The farmers who participated in the questionnaire survey (n=70) were predominantly males (80%). More than half (59%) of the respondents were over 45 years old (Table 4.6).

Table 4.7: Demographic data of participating farmers in Mnisi community Ward B1, Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number (n=70)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>56</td>
<td>80</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Number (n=63)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-25yrs</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>26-35yrs</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>36-45yrs</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Over 45yrs</td>
<td>37</td>
<td>59</td>
</tr>
</tbody>
</table>

4.3.2 Livestock reared and duration of cattle rearing at households

All (100%) of the participating farmers were cattle owners. Besides cattle, some of the farmers indicated rearing poultry (61%), goats (24%) and pigs (4%), but none (0%) reared sheep. Majority of the farmers (91%) indicated that they had been rearing cattle for more than 5 years. Among the farmers that indicated making additions to their herd in the past year, none acquired those cattle outside the Mnisi community.

4.3.3 Knowledge on and sourcing of antimicrobial drugs for animal use

One percent of the farmers indicated knowing what an antimicrobial agent is but they could not provide an example. Despite not being able to identify what an antimicrobial agent is, some farmers did indicate that they had used Terramycin® (86%) and Hitet® (43%) in the last year. Ninety three percent of the farmers that used Hitet® had used both Hitet® and Terramycin® during the course of the year. The farmers indicated the local Hluvukani Animal clinic (60%) as the most
common place where they buy their veterinary drugs followed by a co-operative (an agricultural store) in Hoedspruit (34%).

Table 4.8: Knowledge on and source of antimicrobial drugs of selected farmers in Mnisi community Ward B1, Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th>Do you know what an antimicrobial agent is (n=70)</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>69</td>
<td>99</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

| Could give an example of an antibiotic         | 0      | 0          |

<table>
<thead>
<tr>
<th>Antibiotics farmers used in the last year (n=70)</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terramycin®</td>
<td>60</td>
<td>86</td>
</tr>
<tr>
<td>Hitet®</td>
<td>30</td>
<td>43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of drugs (n=67)</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corporative</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Local animal clinic</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Veterinarian</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Animal health technicians</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Villagers that sell drugs</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Farmers were also asked to indicate if they had used drugs to treat for conditions such as diarrhea, fever, coughing, mastitis, abscess, or to prevent tick borne diseases in the last year. Six percent (4/70) of the farmers indicated having treated for diarrhea, 1% (1/70) for fever, 1% for tick borne disease prevention and 1% (1/70) for coughing. Each one of these farmers indicated that response to treatment was good but only 14% (1/7) of them could remember the drug they had used which happened to be Terramycin®.

4.3.4 Antimicrobial use practices of farmers

Twenty nine percent of the farmers indicated that they keep a record of the treatments they give to their livestock. Only 1% of the farmers indicated treating their livestock for the duration stated on the instruction label of the drug, with 70% of the farmers indicating treating the animals till the drug was finished. The farmers disposed of expired drugs in various ways including throwing away in the garbage pit (32%) or toilet (24%), returning the drug where they purchased it (27%), and burning (2%).
The farmers indicated waiting for various periods before slaughtering their animals for food after treating them. Some of the reported withdrawal periods fell below the withdrawal periods recommended for the oxytetracyclines the farmers commonly use. Seventy two percent of the farmers indicated that they were aware of the importance of observing a withdrawal period. Of these, 63% highlighted that it was important to do so because the drug will still be in the body of the animal and may affect them.

Table 4.9: Antimicrobial use practices of selected farmers in Mnisi community Ward B1, Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keep record of treatments given to their livestock</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46</td>
<td>71</td>
</tr>
<tr>
<td>Duration of treatment of sick animals</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Until clinical signs stop</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Three days</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>As indicated on the medicine use instructions</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Until the drug is finished</td>
<td>46</td>
<td>70</td>
</tr>
<tr>
<td>Disposal of expired drugs</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Throw in the bin or garbage pit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Throw in the toilet</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Return to place of purchase</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>Throw away (not specified where)</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Do you know the importance of observing a withdrawal period</td>
<td>48</td>
<td>72</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>Reasons given for observing a withdrawal period</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>Because the drug may affect them</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>To check if animal has fully recovered</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>To prevent contracting the disease</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Simply because vets and AHTs instruct them to</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
4.3.5 Knowledge on antimicrobial resistance (AMR)

Forty two percent of the farmers indicated that they have heard about antimicrobial resistance either from health workers (82%), television or radio programs (11%), or from a farmers’ day workshop (7%). Of the farmers that had heard about AMR, only 4% (1/28) indicated that antimicrobial resistance involves microorganisms becoming resistant to treatment. However the same farmer indicated that the body of the animal becomes resistant to treatment hence none of the farmers have a clear understanding of the concept of antimicrobial resistance. None of the farmers that had heard about AMR were aware of antimicrobial use practices that promote resistance development All the farmers indicated interest in learning more on prudent use of antimicrobial drugs.

Table 4.10: Knowledge on antimicrobial resistance (AMR) of selected farmers in Mnisi community Ward B1, Bushbuckridge Municipality.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Have you heard about AMR</strong></td>
<td>(n=67)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
<td>58</td>
</tr>
<tr>
<td><strong>Source of information on AMR</strong></td>
<td>(n=28)</td>
<td></td>
</tr>
<tr>
<td>Health workers (veterinarians and AHTs)</td>
<td>23</td>
<td>82</td>
</tr>
<tr>
<td>Television/radio</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Farmer’s day talk</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td><strong>Know antimicrobial use practices that promote AMR development</strong></td>
<td>(n=28)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>96</td>
</tr>
<tr>
<td><strong>With AMR, the body becomes resistant to treatment</strong></td>
<td>(n=28)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>96</td>
</tr>
<tr>
<td><strong>With AMR, microorganisms become resistant to treatment</strong></td>
<td>(n=28)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>96</td>
</tr>
</tbody>
</table>

4.4 Animal Health Technician and Veterinarian responses

The two animal health technicians interviewed indicated the tetracyclines and penicillins as the commonly used antibiotics in the area. All the interviewed veterinarians (n=3) indicated that
tetracyclines were commonly used while penicillins, sulphonamides and enrofloxacin were only used occasionally.

The animal health technicians rated owner compliance to the recommended antimicrobial use practices as poor. Both technicians indicated that they rarely encounter cases of failed antimicrobial treatment failure. When asked how often they encountered cases of owner initiated treatment, one of the animal health technicians highlighted that he did so sometimes while the other rarely did. When asked to highlight antibiotic use practices of farmers in the Mnisi community that favor development of resistance the technicians listed the following:

- Use of wrong route of administration
- Under dosing
- Inappropriate storage of drugs
- Use of expired drugs

The technicians indicated interest in continued professional education on antimicrobial stewardship.
CHAPTER 5: DISCUSSION

The effectiveness of antimicrobial drugs is decreasing due to the development and rapid spread of antimicrobial resistant organisms. Strategies to contain resistance to conserve the efficacy of existing antimicrobial drugs are very much needed given that development of novel antimicrobial drugs is currently very limited (Pál, Papp and Lázár, 2015). It is important to conduct antimicrobial resistance surveillance in micro-organisms to detect emerging resistance to allow for timely intervention (Aarestrup, 2015) and to guide necessary legislation and therapeutic guideline changes for prudent use of antimicrobial drugs (EAGAR, 2006).

According to our literature search, in South Africa much focus has thus far been placed on the commercial farming sector with little antimicrobial resistance surveillance conducted in rural communal farming systems. This bias has also been observed in other countries (Mubita et al., 2008; Graham et al., 2017). Usually the communal farming sector is believed to be of low risk in terms of resistance development on the basis that antimicrobial drug use in the sector is low. However, there are several factors in rural communal farming settings which may drive the development and spread of antimicrobial resistance thereby making resistance surveillance in these areas important. These factors include; imprudent use of antimicrobial drugs partly due to low literacy levels which result in poor understanding of drug use instructions; limited veterinary services resulting in farmer initiated treatment of diseases and use of drugs of compromised quality (Katakweba et al., 2012; FAO, 2016); poor sanitation; reduced access to clean water; and increased human, livestock and wildlife contact (FAO, 2016; Graham et al., 2017). One of the objectives of this study was to determine the antimicrobial susceptibility of *E. coli* and *Enterococcus* species isolated from communally grazed apparently healthy cattle in the Mnisi community, a rural community in Mpumalanga province in South Africa.
5.1 Antimicrobial resistance in the indicator bacteria

In routine resistance surveillance, *E. coli* is selected to be representative of Gram-negative bacteria while enterococci are representative of Gram-positive bacteria (Caprioli *et al*., 2000). The advantage of using these bacteria in resistance surveillance is that they are commensals of the gastrointestinal tract that can be isolated from both healthy and sick animals. They have been shown to provide a better indication of the burden of resistance in a given population compared to pathogenic isolates. These indicator bacteria often harbour antibiotic resistance genes on plasmids and hence they serve as reservoirs of resistance genes that can be transferred to veterinary and human pathogens (Caprioli *et al*., 2000; Varga *et al*., 2008).

The isolation rate of *E. coli* was lower than expected. *E. coli* is one of the dominant commensal flora of the gastrointestinal tract of various animals including cattle. Thus ideally, one should be able to isolate *E. coli* from nearly all faecal samples. We thus suspect that this may have been due to the sampling procedure. The study made use of dry swabs without transport medium and this might have led to drying of the specimens (Centre for Disease Control and Prevention, 1994). In future, we would recommend that sampling carried out in remote areas be done using swabs with transport media.

*E. faecium* was the most common *Enterococcus* species isolated in this study. Other investigators have had similar findings in fecal samples from cattle at dairy farms in South Africa (53%) and on farms in Nigeria (52%) (Ngbede *et al*., 2017). In contrast, *E. hirae* was the dominant *Enterococcus* species isolated from commercial dairy cattle in South Africa (78%) (Tanih, 2016) and from pastured cattle in America (40%) (Anderson *et al*., 2008). In other studies conducted in cattle, *E. casseliflavus* predominated (39%) (United States Department of Agriculture, 2009). *E. faecalis* was isolated at a low frequency in our study and this is consistent with findings of other studies in cattle including studies in South Africa (2%) (Tanih, 2016), (9%) (Iweriebor, Obi and Okoh, 2016), Nigeria (2%) (Ngbede *et al*., 2017) and America (3%) (Anderson *et al*., 2008; United States Department of Agriculture, 2009). The predominance of *E. faecium* in this study is of public health importance since *E. faecium* and *E. faecalis* are the top causes of enterococcal infections in humans. While *E. faecalis* accounts for more clinical disease cases in humans compared to *E.
faecium (Marothi, Agnihotri and Dubey, 2005), *E. faecium* is more commonly associated with antimicrobial resistance compared to *E. faecalis* (Hollenbeck and Rice, 2012; Miller *et al*., 2015). *E. durans* and *E. avium* species which were isolated in low proportions in our study have also been implicated as causes of infections in humans (Byappanahalli *et al*., 2012). Differences in the relative abundance of the *Enterococcus* species in different studies may be due to differences in the diet (Anderson *et al*., 2008) and variation in the environmental microbiomes to which the different cattle populations are exposed.

The bacterial isolates were subjected to antimicrobial susceptibility testing using broth micro-dilution, which is the recommended method for resistance surveillance more so for baseline studies like ours. The advantage of using broth micro-dilution is that it is a precise quantitative method as it gives the actual minimum inhibitory concentration values unlike disk diffusion methods which rely on extrapolation of minimum inhibitory concentrations from zone diameters (Caprioli *et al*., 2000; Nel, van Vuuren and Swan, 2004).

For all the tested antibiotics in this study, *E. faecium* was more resistant compared to *E. faecalis* and the other *Enterococcus* species. This is in agreement with the aforementioned reports that *E. faecium* is associated more with antimicrobial resistance compared to *E. faecalis* (Hollenbeck and Rice, 2012; Miller *et al*., 2015). Complete susceptibility to vancomycin and chlortetracycline, and low resistance to amoxycillin was detected in the enterococci isolates in this study. Similarly, complete susceptibility or low resistance of enterococci to these antibiotics was reported in Australia (Barlow *et al*., 2017) and in America (United States Department of Agriculture, 2009) (Table 5.1). In contrast, higher levels of resistance to the same antibiotics were detected in South Africa at selected commercial dairy farms (Tanih, 2016) and in the South African National Veterinary Surveillance and Monitoring Program (SANVAD) (van Vuren, Picard and Greyling, 2007); and in pastoral cattle in Zambia (Mubita *et al*., 2008) (Table 5.1).

Resistance of the enterococci isolates, particularly *E. faecium*, to enrofloxacin was very high as observed in the SANVAD in South Africa and in America (Table 5.1). The high enrofloxacin resistance may partly be due to intrinsic rather than acquired resistance mechanisms since
enrofloxacin is only used occasionally in this community. In both *E. faecium* and *E. faecalis*, intrinsic resistance to fluoroquinolones may be conferred by chromosomally encoded Qnr (pentapeptide repeat proteins) like proteins which protect DNA gyrase, the target site for fluoroquinolones (Arsène and Leclercq, 2007; Jacoby and Hooper, 2013).

Table 5.1: Resistance Surveillance studies for enterococci isolated from healthy cattle.

<table>
<thead>
<tr>
<th>Surveillance study/citation</th>
<th>Enterococcus species</th>
<th>number of isolates</th>
<th>Percentage resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercial farms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tanih, 2016)</td>
<td><em>E. faecium</em></td>
<td>15</td>
<td>26.7 20 n/t 33 6.7</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>6</td>
<td>16.7 0 n/t 0 0</td>
</tr>
<tr>
<td>(Iweriebor, Obi and Okoh, 2016)</td>
<td>All isolated enterococci</td>
<td>341</td>
<td>n/t 99 n/t 100 12</td>
</tr>
<tr>
<td><strong>Production system not specified</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SANVAD 2007</td>
<td><em>E. faecium</em></td>
<td>10</td>
<td>50 40 40 20 90</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>9</td>
<td>100 88.9 11.1 0 88.9</td>
</tr>
<tr>
<td>(Ngbede <em>et al.</em>, 2017)</td>
<td><em>E. faecium</em></td>
<td>20</td>
<td>20 30 50 0 10</td>
</tr>
<tr>
<td>(Barlow <em>et al.</em>, 2017)</td>
<td><em>E. faecium</em></td>
<td>120</td>
<td>11.7 8.3 0 0 n/t</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>96</td>
<td>7.3 10.4 0 0 n/t</td>
</tr>
<tr>
<td>(United States Department of Agriculture, 2009)</td>
<td><em>E. faecium</em></td>
<td>135</td>
<td>12.6 0.7 n/t 0 45.9</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>38</td>
<td>2.6 0 n/t 0 0</td>
</tr>
<tr>
<td><strong>Pastured cattle/communal cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mubita <em>et al.</em>, 2008)</td>
<td><em>E. faecium</em></td>
<td>29</td>
<td>51.7 72.4 65.5 n/t n/t</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>62</td>
<td>41.9 56.5 62.9 n/t n/t</td>
</tr>
<tr>
<td>This study</td>
<td><em>E. faecium</em></td>
<td>33</td>
<td>0 0 6.1 0 90.9</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>3</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

Freq = frequency, tet = tetracyclines, ery = erythromycin, amo/amp = amoxycillin or ampicillin, van = vancomycin, enro/cip = enrofloxacin or ciprofloxacin, n/t = not tested

An association between the use of glycopeptide growth promoters such as avoparcin and the occurrence of vancomycin resistance has been reported. Vancomycin resistant enterococci were common in animals in countries that use avoparcin but never isolated in countries where its use was forbidden or not practiced (van den Bogaard, 2000; Ngbede *et al.*, 2017). In addition, bans in
the use of avoparcin as a growth promoter in countries such as Denmark, Netherlands, Taiwan and Germany were followed by substantial decreases in prevalence of vancomycin resistant enterococci (VRE) in livestock (Bager et al., 1997; van den Bogaard, 2000; Nilsson, 2012). The absence of vancomycin resistance in this study was thus not surprising given that the use of growth promoters including glycopeptides is not a common practice in rural areas. On the other hand, avoparcin was used on commercial farms in South Africa before its ban in the 1990s and thus detection of vancomycin resistance in the SANVAD survey is not an unusual finding (van Vuuren, Picard and Greyling, 2007; Henton et al., 2011). Contrary to the aforementioned observed association of glycopeptide use and occurrence of VRE, in more recent times, VRE have been detected in the absence of glycopeptide growth promoter use. For example, glycopeptides were never allowed for growth promotion in livestock in the United States yet vancomycin resistant enterococci were isolated from pigs (11%; 6/55 isolates) in a surveillance study in Michigan (Donabedian et al., 2010). Hence, for this study it was important to include vancomycin in the antibiotic panel tested to determine if vancomycin resistance was present in this rural community.

The level of resistance to amoxycillin, enrofloxacin, and gentamicin in E. coli isolates in this study was low. In the SANVAD study in South Africa, similarly low levels of resistance to ampicillin, enrofloxacin and gentamicin were detected in E. coli isolated from healthy cattle (van Vurren, Picard and Greyling, 2007). Our results also concur with the low levels of resistance to ampicillin, tetracycline and gentamicin detected in E. coli isolates from healthy dairy cattle (Mainda et al., 2015) and pastoral cattle (Mubita et al., 2008) in Zambia, cattle in the DANMAP in Denmark (Danmap, 2016) and SVARM in Sweden (Swedres-Svarm, 2015), (Table 5:2) and cattle at Australian abattoirs (Barlow et al., 2015). In contrast to the low tetracycline resistance detected in the E. coli isolates in our study, high tetracycline resistance levels were reported in South Africa in the SANVAD (34%) (van Vurren, Picard and Greyling, 2007) and in Pennsylvania (Sawant et al., 2007) (Table 5.2).
Table 5:2: Resistance Surveillance studies for *E. coli* isolated from healthy cattle.

<table>
<thead>
<tr>
<th>Surveillance study/citation</th>
<th>Percentage resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of isolates</td>
</tr>
<tr>
<td></td>
<td>tet</td>
</tr>
<tr>
<td>Production system not specified</td>
<td></td>
</tr>
<tr>
<td>(van Vurren, Picard and Greyling, 2007)</td>
<td>119</td>
</tr>
<tr>
<td>DANMAP 2016</td>
<td>121</td>
</tr>
<tr>
<td>(Swedres-Svam, 2015)</td>
<td>101</td>
</tr>
<tr>
<td>(Sawant <em>et al.</em>, 2007)</td>
<td>152</td>
</tr>
<tr>
<td>(Barlow <em>et al.</em>, 2015)</td>
<td></td>
</tr>
<tr>
<td>beef</td>
<td>223</td>
</tr>
<tr>
<td>dairy</td>
<td>106</td>
</tr>
<tr>
<td>Small scale/communal farming</td>
<td></td>
</tr>
<tr>
<td>(Mubita <em>et al.</em>, 2008)</td>
<td>83</td>
</tr>
<tr>
<td>(Mainda <em>et al.</em>, 2015)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>This study</td>
<td>79</td>
</tr>
</tbody>
</table>

Freq = frequency, tet = tetracyclines, col = colistin, amo/amp = amoxycillin or ampicillin, gen = gentamicin, enro/cip = enrofloxacin or ciprofloxacin, n/t = not tested

Though tetracyclines were the most popular antibiotic used by the farmers in our study, resistance to tetracyclines was low and similar observations were made in other studies. In a study conducted in small scale dairy farms in Zambia, majority of the farmers indicated using tetracyclines yet the *E. coli* isolates were highly susceptible to tetracycline and all the antibiotics tested. However tetracycline resistance was the predominant resistance phenotype (Mainda et al., 2015). In another study conducted in pastoral cattle at a wildlife/livestock interface in Zambia, all farmers indicated using tetracyclines yet tetracycline resistance was very low (3.6%) in the *E. coli* isolates but high (over 40%) in the enterococci isolates (Mubita et al. 2008). One of the limitations of this study was that we did not investigate the volumes and frequency of antibiotic use. Perhaps the use of the tetracyclines was not frequent enough to exert adequate selective pressure for resistance.
development. Financial constraints may contribute to infrequent antibiotic use in the rural community. In the study by Mainda et al (2015) in Zambia, financial constraints were cited as a possible reason for low antibiotic use. Infrequent use coupled with the short term nature of oxytetracycline resistance demonstrated by Stabler et. al (1982) might explain the low tetracycline resistance detected.

Detection of colistin resistance is a potential cause for concern considering that colistin is one of the drugs of last resort for the treatment of multidrug resistant gram negative infections in humans (Falagas, Kasiakou and Saravolatz, 2005). Until recently, colistin resistance was thought to arise only due to chromosomal mutations and as such surveillance of colistin resistance was not a priority. For this reason, colistin resistance was not tested for during the SANVAD in 2007. The discovery of plasmid mediated colistin resistance turned the tables, and surveillance of colistin resistance has become crucial and is even mandatory for European Union member states because resistance genes borne on plasmids can disseminate rapidly (European Medicines Agency, 2016).

The detection of colistin resistance in cattle in a rural communal farming area was unexpected. However, colistin resistance has previously been detected in E. coli isolates from hosts not previously exposed to colistin or any antimicrobial treatment. For example, in Japan, colistin resistance was detected in 9.3% of E. coli isolates from two farms with no history of colistin use. Colistin resistance was also detected in E. coli isolates from wildlife including; wild macaques (1%; 1/86 isolates) in Algeria (Bachiri et al., 2018), migratory European herring gulls in Lithuania (7%; 8/117 isolates) (Ruzauskas and Vaskeviciute, 2016), and Kelp gulls in Argentina (5 isolates from 50 fecal samples) (Liakopoulos et al., 2016).

In comparison to our findings, lower levels of colistin resistance were detected in enteric E. coli isolates from cattle at an abattoir in Spain (4%) (Hernández et al., 2017), cattle with suspected enteric infections or mastitis in Europe (2%) (Brennan et al., 2016) and from healthy cattle on farms in China (0.9%) (Huang et al., 2017) and in the 2015 SVARM in Sweden (1%) (Swedres-Svarm, 2017). The colistin resistance levels in our study are similar to results from a South African laboratory report from 1997 to 1999 were E. coli isolates from piglets and calves with enteritis
exhibited 17.2% resistance to colistin. However the latter resistance level may be an overestimate since the isolates were from clinical referral cases and the Kirby Bauer method used in that study can give falsely elevated MICs (Virbac).

Colistin resistance results should always be interpreted with caution because colistin susceptibility determination has limitations for the various testing methods available. The CLSI currently recognizes broth micro-dilution as the most reliable method but it also has its limitations (Matuschek et al., 2018). Studies have shown that colistin adsorbs to various materials such as plastic which make up laboratory apparatus resulting in reduced availability of the antibiotic in assays including broth micro-dilution antimicrobial susceptibility testing (Humphries, 2015; Karvanen et al., 2017; Bakhavatchalam et al., 2018). Some authors advice the use of surfactants such as polysorbate 80 to prevent adherence of colistin to the laboratory ware (Humphries, 2015; Karvanen et al., 2017). In a particular study, the minimum inhibitory concentrations for colistin reduced four to eightfold when polysorbate 80 was added to the broth (Humphries, 2015). However, given the concerns that the surfactants may have antimicrobial properties and may act synergistically with colistin thereby producing falsely low MICs (Humphries, 2015; Karvanen et al., 2017), no surfactants were used in this study.

Eleven out of the thirteen isolates that were deemed resistant in this study had an MIC of 4ug/l which is the lowest concentration of colistin which is interpreted as resistant. If we take into account the reduced availability of colistin due to adsorption to plastic, the detected level of resistance may be an overestimate. Furthermore, lack of an intermediate susceptibility category for colistin increases the likelihood of false susceptible and false resistant categorization of isolates with minimum inhibitory concentrations close to the resistance breakpoint (Matuschek et al., 2018). Therefore further studies using molecular techniques are recommended.

The most common co-resistance phenotype in the E. coli isolates in this study was amoxycillin-chlortetracycline resistance as was the case in the study in dairy cattle in Zambia (Mainda et al., 2015). In a study by Shin et al (2015) in South Korean beef cattle, nearly half of the E. coli isolates exhibited resistance to both tetracycline and ampicillin. In a study by Sawant et al. (2007)
tetracycline resistant *E. coli* isolates from dairy cattle in Pennsylvania were also resistant to other antibiotics tested with tetracycline-florfenicol-ampicillin resistance and tetracycline-ampicillin resistance being some of the predominant co-resistance phenotypes. Detection of co-resistance in our study may suggest that the resistance is most likely conferred by resistance genes borne on the same mobile genetic elements such as plasmids. Co-resistance is a setback as it necessitates the use of second line drugs which are more expensive but fortunately it was detected at low levels in this study.

It is important to take note that comparisons of resistance data from different studies have limitations due to differences in study design ranging from sampling strategies and sample sizes, to the method used for antimicrobial susceptibility testing and the breakpoints used for interpretation of results (Mainda *et al*., 2015). Nonetheless, the similarities and differences in the levels of resistance observed in our study and the aforementioned studies and national surveillance programs are probably partially attributed to differences and similarities in antimicrobial use practices in the different locations. While in communal settings such as our study area, poverty limits the use of antimicrobial drugs (FAO, 2016), in Europe and most developed countries, strict legislation prohibiting use of antimicrobial growth promoters and allowing use of antibiotics by prescription only limits antibiotic use thereby reducing resistance development in both settings (Barlow *et al*., 2015; Speksnijder *et al*., 2015).

In South Africa, tetracycline use is popular in both commercial and small scale/communal farming due to over the counter availability and the relatively low cost of the drugs (EAGAR, 2006; Henton *et al*., 2011) hence the similarity in dominance of tetracycline resistance in the SANVAD and our study. The differences in levels of tetracycline resistances detected in our study and the SANVAD may in part be due to the higher antimicrobial use in commercial farms due to the use of antibiotics for growth promotion and prophylaxis, practices which are rare in communal farming areas; higher disease burden associated with the stress factors of intensive production; and higher buying power. We believe that this indicates that restricted use of antimicrobials, more importantly avoiding their use for growth promotion and prophylaxis, can be an important measure in retarding development of resistance.
The sampling strategy of one or two animals per farm and a single isolate per animal used allows for wide geographical coverage and reduces the effect of clustering (“likeness amongst observations close in time or space”) of isolates thereby increasing isolate diversity but unfortunately no conclusions on resistance at animal or herd level can be made (Reynolds, Lambert and Burton, 2008; Persoons et al., 2011).

5.2 Questionnaire survey

Containing antimicrobial resistance requires the input of everyone in society (WHO, 2015b) with farmers being among the individuals with a significant role to play (EAGAR, 2006). It is thus essential for farmers to know how to use antimicrobial drugs prudently, and to understand antimicrobial resistance and the roles they have to play in its containment. The other objective of this study was to establish baseline knowledge on antimicrobial resistance and antimicrobial usage practices of cattle farmers.

From the survey results, it was very worrying to note that only 1% of the farmers knew what an antimicrobial agent was, despite many of the farmers making use of antibiotics. This finding was similar to the low awareness recorded in a study among small scale dairy farmers in rural Peru where only 0.6% of the respondents knew what an antibiotic was and that antibiotics are used to manage bacterial infections (Redding et al., 2014). Failure of the farmers in our study to define what an antimicrobial agent is may have been partly due to lack of a Shangani (the local language) term that denotes these drugs. It was previously noted by Chauhan et al (2018) that farmers in rural India where there was also no vernacular term to denote antibiotics could not define antibiotics.

If the farmers are not aware that the drugs they are using are antimicrobial drugs, chances are that they might not take the necessary precautions when using them and this is a cause for concern. In addition, farmers may at times use antibiotics for diseases they are not indicated for, a practice observed in the Mnisi community by one of the animal health technicians. In Khartoum Sudan, more than two thirds of the respondents highlighted nonbacterial livestock diseases as examples of diseases requiring antibiotic treatment (Eltayb et al., 2012). Unwarranted use of antibiotics by
the farmers may perhaps be due to poor understanding of disease etiology. In a study conducted in Tanzania, the authors believed that the Maasai’s use of antibiotics even when not warranted was likely due to poor knowledge on disease etiology (Caudell et al., 2017).

Among the various antibiotics listed, the farmers only indicated using tetracyclines to treat their cattle. This was in agreement with the response of the animal health technicians and veterinarians who highlighted that tetracyclines were the most commonly used agents in the community. Tetracyclines have been identified as the most commonly used antibiotic by communal or small scale livestock farmers in different African countries including South Africa (Moneoang and Bezuidenhout, 2009; Eagar, Swan and Van Vuuren, 2012); Zambia (Mubita et al., 2008; Mainda et al., 2015); in Tanzania (Nonga et al., 2009; Katakweba et al., 2012; Caudell et al., 2017). The popularity of tetracyclines among livestock farmers is not surprising because they are used for the treatment of various clinical disease conditions including tick borne diseases such as heartwater which is caused by *Ehrlichia (Cowdria) ruminantium* and Anaplasmosis, and they are relatively inexpensive and readily available to farmers without prescription. However, over reliance on tetracyclines may promote development of resistance to tetracyclines as well as other antibiotics due to co-selection.

It was a positive finding that the respondents sourced their veterinary drugs from legally established distributors since the drugs sold in these establishments are most likely quality assured (FAO, 2016) and as such the risk of development of resistance due to use of poor quality agents is significantly reduced in this community. Similar findings were made in small scale dairy farms in Zambia and in rural Peru were 91% and 87.8% of the farmers respectively, indicated that they purchase their drugs from veterinarians and veterinary drug stores (Redding et al., 2014; Mainda et al., 2015).

Antimicrobial use can negatively impact human health through the effect of drug residues on bacteria within the human gastrointestinal tract. The observation of antimicrobial withdrawal periods is crucial to reduce human exposure to antimicrobial residues in animal products (Beyene, 2016). A high proportion of the respondents in this study were aware of the need to observe drug
withdrawal periods. Katakweba et al. (2012) and Nonga et al (2009) reported that 77.5% and 95% of the respondents in small scale farms in Tanzania were aware of the need to observe withdrawal periods. In contrast, 93% of the Maasai pastoralists in Tanzania indicated not observing antimicrobial withdrawal periods (Caudell et al., 2017). The downside is that some of the withdrawal periods the farmers in our study stated fell short of the recommended withdrawal periods for the oxytetracyclines which were used by the farmers. This highlights the need to stress to farmers to religiously check the duration of withdrawal periods on the product label since withdrawal periods differ from one product to another and from one animal species to another.

On further questioning, only 46% of the respondents were aware of the public health importance of observing antimicrobial withdrawal periods. They indicated that the drug will still be in the body of the animal and may affect them but did not specify the effects residues have on their health. This represents a knowledge gap that needs to be addressed given that knowledge on effects of antimicrobial residues on human health is the main motivator for compliance with recommended antimicrobial withdrawal periods (Chen, Wu and Xie, 2018). Chen, Wu and Xie (2018) reported that the probability of improper use of antibiotics by rural pig farmers ignorant of the public health effects of veterinary antibiotic residues was a staggering 90%.

It is noteworthy that in our study a significant number of the farmers that were aware of the public health importance of observing antimicrobial withdrawal periods cited the veterinarians and animal health technicians as the ones providing this information to them. This once again shows the importance of the influence of veterinary professionals in rural areas where other means of animal health and veterinary public health information dissemination are poor.

Besides understanding the importance of withdrawal periods, adherence to withdrawal periods is also reliant on proper record keeping since exact treatment dates are needed to determine the dates of lapse of the withdrawal period. In addition, treatment records are helpful in tracking general drug use and disease conditions occurring on the farm to guide future preventative measures and primary animal health initiatives (Speksnijder et al., 2015). Poor treatment record keeping by majority of the farmers is thus a cause for concern.
The animal health technicians highlighted concerns that the farmers did not necessarily follow treatment instructions and this potentially negates the effectiveness of observation of the withdrawal period since the withdrawal period is specific to a particular drug formulation, the route of administration and maximum registered dose (Khatun et al., 2018). This highlights the importance of farmer education on importance of drug use instruction compliance. The concerns raised by the animal health technicians on use instruction non-compliance were supported by the responses given by the farmers with only 1% of the farmers indicating that they treat their animals for the recommended time duration. A substantial percentage (70%) of the farmers indicated that they treat their animals until the medicine is finished. Depending on the amount of drug, the required dosage and duration of treatment, such a treatment regimen may mean overmedication or under medication of which both practices promote resistance development. Under medication can result in “target selected resistance” while overmedication promotes resistance selection in commensal flora because the longer the exposure to antimicrobial drugs, the greater the resistance selection pressure exerted on the bacteria (Llewelyn et al., 2017). In addition, overmedication renders the withdrawal period stipulated by the manufacturer ineffective in protecting the public from drug residues (Khatun et al., 2018).

Another danger of antimicrobial use is the impact of drug residues on the environment. Safe disposal of antimicrobial drugs is imperative to avoid environmental contamination and increased selection pressure for resistance development in environmental organisms. The majority of the respondents in our study indicated that they throw away expired drugs in the garbage or in the toilet. In rural areas were dogs and other wild animals scavenge for food in garbage bins or pits, disposing antimicrobial drugs with garbage can result in exposure of the animals to these drugs with subsequent resistance development. The containers can also break resulting in seepage of the drugs into the soil promoting development of resistance in microorganisms in the environment. Previously, flushing drugs in the toilet or disposing them down drains was considered safe until research exposed the impact of these practices on the aquatic environment (Bound and Voulvoulis, 2005). When there is need, the safest way of disposing expired drugs is burning them or returning
them to drug stores where they can subsequently be disposed according to waste management regulations.

In this study, 42% of the farmers indicated that they had heard about antimicrobial resistance before. While the level of awareness is close to that of the dairy farmers in rural South Carolina (over 40%) (Kramer et al., 2017), it was substantially lower than the level (70%) recorded in small scale livestock farmers in Tanzania (Katakweba et al., 2012). The low resistance awareness may be correlated with low education level of farmers in the Mnisi area (Simela, 2012). Eltayb et al (2012) reported an association between level of education and knowledge on resistance among farmers in Khartoum Sudan.

Hearing about resistance does not necessarily translate to understanding the principle as was observed in our study. A clear understanding on antimicrobial resistance and its consequences in animals and humans is a significant motivator for prudent use of antimicrobial drugs (Eltayb et al., 2012; Sirdar et al., 2012). Therefore it is worrying that all the farmers interviewed did not understand what antimicrobial resistance involves nor were they aware of inappropriate antimicrobial use practices that promote development of resistance.

Despite concerns raised on the level of knowledge of farmers in the area, a positive finding was farmers identifying veterinarians and animal health technicians as their main source of information on antimicrobial resistance. This highlights that the veterinary and paraveterinary professionals can serve as important information channels in resistance mitigation and other animal health education programs. Similar to the finding of our study, in the survey conducted in small scale farmers in Tanzania animal health workers were cited as the most common source of information on resistance (Katakweba et al., 2012). Similar findings were also observed among dairy farmers in rural South Carolina (Friedman et al., 2007) and among cattle and pig farmers in Netherlands (Kramer et al., 2017). The contribution of media in disseminating information on resistance in the Mnisi community is low as was the case in the survey in small scale farmers in Tanzania (Katakweba et al., 2012). This is perhaps due to limited media resources inherent in rural settings in addition to low media coverage of this topic in the country more so in vernacular. Jin et al (2011)
was of the opinion that information tools such as the media are less likely to impact poorer individuals because they tend to trust advice from individuals they have relationships with more than advice given through formal education.

Despite the important findings from the questionnaire survey, it has some shortcomings. The responses in this questionnaire survey were self-reported and as such they may be subject to recall bias and in some instances farmers might have given a response that they deem acceptable rather than an honest response. Use of both the interview data and inspection of households for drugs in stock is encouraged in future studies (Caudell et al., 2017). Another source of bias is the convenient sampling of the participating farmers since only farmers willing to participate could be interviewed. Considering that the farmers are not aware what antimicrobial drugs are, the responses given may not pertain to antimicrobial drugs only but rather cover various veterinary drugs they use.
6.1 Conclusion

Overall, the level of antimicrobial resistance detected in this study was not substantial. Low resistance to tetracyclines works to the advantage of the farmers given that tetracyclines are relatively more accessible and affordable compared to other antibiotics. In addition, the efficacy of tetracyclines and penicillins reduces the need to use second line drugs some of which are critical medicines for humans and should be of limited use in animals. The level of colistin resistance detected given that this was a rural communal farming area was unexpectedly high. Technical limitations in the performing of minimum inhibitory concentration tests for polymixins may have resulted in overestimation of the level of colistin resistance. The detection of colistin resistance is worrying since colistin is a last resort drug for the treatment of multidrug resistant Gram-negative infections (Falagas, Kasiakou and Saravolatz, 2005). Furthermore, the colistin resistance may potentially be plasmid mediated of which colistin resistance genes borne on plasmids can disseminate rapidly via the food chain and through environmental contamination. A follow up evaluation with molecular techniques is thus recommended.

Some of the critical findings from the interviews were poor knowledge on purpose of antimicrobial drugs, non-prudent antimicrobial use practices such as treatment duration incompliance, ignorance on what antimicrobial resistance involves, and the critical role of veterinarians and animal health technicians in farmer education. The information obtained in the survey provides useful insights on the knowledge gaps that need to be addressed through tailor made education programs to raise awareness on prudent antimicrobial use practices and antimicrobial resistance so as to influence behavioral changes among communal farmers. The findings of this study can be compared with follow up studies in this community to assess the impact of implemented awareness programs.
6.2 Recommendations

- The detection of colistin resistance warrants further investigation to check for plasmid mediated colistin resistance genes.

- It is also important to investigate human antimicrobial use practices and resistance levels in bacteria isolated from humans and from the environment to obtain a more holistic picture of antimicrobial resistance in this community and other communal areas.

- It is critical that the government’s efforts to increase access to antimicrobial drugs through over the counter availability be complemented by stewardship programs to promote responsible use so as to limit resistance development (Årdal et al., 2016). These stewardship programs include the following:
  - As a first step, there is a need to introduce vernacular terms that delineate antimicrobial drugs from other medicines and vaccines in the country. There should be seminars and campaigns to educate farmers and the general public on basic information on antimicrobial drugs, their intended uses, prudent use practices including treatment record keeping and observation of withdrawal periods, and the impact of antimicrobial resistance. The positive impact of appropriate farmer education cannot be underestimated and is evident from simulation experiments conducted on rural pig farmers in China where the more knowledgeable farmers were on veterinary use of antibiotics, the more likely they were to use antibiotics appropriately (Chen, Wu and Xie, 2018).
  - Adequate training of all veterinary drug salesmen through a nationally recognized tertiary course which emphasizes prudent drug use practices since these persons can at times be the only source of information available to farmers when buying drugs.
  - Improved monitoring and compulsory recording of antimicrobial sales including over the counter antimicrobial drugs (Eagar and Naidoo, 2017).
Farmers must be encouraged to avoid stocking up antimicrobial drugs to reduce the incidences of drug expiration due to prolonged storage and thus reduce the amounts of drugs to be disposed. Drug inserts should also include safe disposal instructions that are relevant to laymen. Another option the government may consider in rural areas is to have state veterinary service personnel collect drugs for disposal from farmers during routine dipping sessions.

- With key points of veterinary drug purchase being known, these establishments can be target sites for dissemination of educational material on prudent antimicrobial use practices to farmers in this community.

- Since animal health technicians are key informants on animal health in communal areas, it is essential to boost their education levels through continuous professional development programs and by improving their diploma learning curriculum.

- Human health practitioners also have a role to play in emphasizing the possible impact of resistance in animals on public health to augment efforts made by animal health professionals given that farmers are more likely to take advice on human health from medical practitioners more seriously compared to advice from veterinarians.

- The positive impact of the University of Pretoria Mnisi community health program is evident. It would be great to extend such programs to other rural farming communities.
CHAPTER 7: BIBLIOGRAPHY


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Virbac, Colistine 1200: E.coli and Salmonella specialist, Virbac RSA (Pty) Ltd, Halfway House, South Africa


ADDENDUM

FARMER QUESTIONNAIRE

Antimicrobial Resistance in Small Scale Farms

Farm questionnaire

Purpose of survey

This survey is being conducted as part of my research studies at the University Of Pretoria’s Department Of Veterinary Tropical Diseases. The purpose of this survey is to assess how antimicrobials are used in your region. The answers you provide will be helpful in identifying the areas where we may be of assistance. Our aim is to help put together a treatment guide that will make treatment of animals in your area easier. Your personal details will be kept strictly confidential and we will prepare a report from the responses provided. Your participation in this survey is greatly appreciated.

Instructions

For questions with ready-made answers, please tick the relevant answer(s).

Date

Stock card number

Sex

female [ ]

male [ ]

Age group

18 – 25 years [ ] 26 – 35 years [ ] 36 – 45 years [ ] over 46 years [ ]

1. State the number of years that production animals have been kept on your household.

2.a) What production animal species are currently kept on the household?

   Cattle [ ]

   Goats [ ]

   Sheep [ ]

   Pigs [ ]

   Poultry [ ]
b) How many cattle are kept on your household?

c) Where did you source additions to your cattle herd if any were made in the last year?

- Within the same village
- Other villages in Mnisi
- Outside Mnisi
- No additions were made

3. a) Do you know what an antimicrobial drug is? Yes□ No□

b) If your response to 3.a) is yes, list three (3) antimicrobial drugs that you know

1. ............................................................

2. ............................................................

3. ............................................................

c) Tick the drugs you have used on your animals in the last year;

1) Terramycin □

2) Depocillin □

3) Depomycin □

4) Oxytetracycline □

5) Hitet □

6) Sulfatrim □

7) Trivetin □

8) Intertrim □

9) Dofatrin □
4. Where do you source the drugs you use on your animals? *(Tick all the relevant answers)*

<table>
<thead>
<tr>
<th>Source</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacy/drug store</td>
<td>☐</td>
</tr>
<tr>
<td>Local animal clinic</td>
<td>☐</td>
</tr>
<tr>
<td>Mobile salesman</td>
<td>☐</td>
</tr>
<tr>
<td>Veterinary doctor</td>
<td>☐</td>
</tr>
<tr>
<td>Animal Health Technician</td>
<td>☐</td>
</tr>
</tbody>
</table>
5.a If you have treated any of your cattle for one of the following conditions in the table below in the last year, which drugs did you use and what were the routes of administration and the outcomes of the treatments?

<table>
<thead>
<tr>
<th>Condition/indication</th>
<th>Drug(s) used</th>
<th>Route of administration</th>
<th>Outcome of the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animals recovered</td>
</tr>
<tr>
<td>cough</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fever</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mastitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swelling or abscess</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To prevent infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To prevent tick borne diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
b. Of the drugs in 5.a, list those that required a prescription for purchase.

..................................................................................................................................................
..................................................................................................................................................
..................................................................................................................................................
..................................................................................................................................................

6. Do you keep a record of the treatments you give to your animals
   Yes    No

7. How long do you wait from the last treatment to slaughtering the animals for food
   ............................................................................................................................................... 

8. Do you know the importance of waiting for a while from the last treatment to slaughtering animals for food or drinking milk from the animals?   Yes    No

9. If your response to 8 is yes, give one reason why it is important to wait from the last treatment to slaughtering the animals for food.
   ............................................................................................................................................... 

10. How long do you usually use the antibiotics for when treating animals?
    until clinical signs stop
    up to the end of the indicated antibiotic treatment course on the box or label
    For three days
    Until the bottle is empty

11. How do you dispose of expired antibiotics?
    ............................................................................................................................................... 
    ...........
12. Have you heard/read about antimicrobial resistance?

No  

If Yes, tick all the relevant answers below

From health workers  
On tv/radio  
From a pamphlet or newspaper or magazine  
From farmers’ day  
From veterinary students who come to the farm  

13. If you have heard/read about antimicrobial resistance, answer the following questions,

a) Antimicrobial resistance involves;

i) Infections becoming resistant to treatment by antimicrobial drugs.  Yes  No  

ii) The body of the animal becoming resistant to antimicrobial drugs.  Yes  No  

b) State any two antibiotic use practices that promote antimicrobial resistance.

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........................................................................................................................................
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14. Are you interested in short courses on safe use of antimicrobial agents?

Yes  No  

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AHT QUESTIONNAIRE

Antimicrobial Resistance in Small Scale Farms

AHT questionnaire

Purpose of survey
This survey is being conducted as part of my research studies at the University Of Pretoria’s Department Of Veterinary Tropical Diseases. The purpose of this survey is to assess how antimicrobials are used in your region. The answers you provide will be helpful in identifying the areas where we may be of assistance. Our aim is to help put together a treatment guide, that will make treatment of animals in your area easier. Your personal details will be kept strictly confidential and we will prepare a report from the responses provided. Your participation in this survey is greatly appreciated.

Instructions
For questions with ready-made answers, please tick the relevant answer(s).

Date.................................................................

Sex  female □  male □

Age group
18 – 25 years □ 26 – 35 years □ 36 – 45 years □ over 46 years □

1. State the number of years that you have been serving in Muisi as an AHT.

2. Name six antibiotics you use the most to treat animals in Muisi.

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3. If you have treated animals with antimicrobials in the last 3 months, which drugs did you use and what were the routes of administration and the outcomes of the treatments?

<table>
<thead>
<tr>
<th>Condition/indication</th>
<th>Drug(s) used</th>
<th>Route of administration</th>
<th>Outcome of the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animals recovered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animals still sick</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animals died</td>
</tr>
</tbody>
</table>

4. Do you encounter cases of antimicrobial treatment failure?

- Never
- Sometimes
- Often
- Rarely
5. How often do you attend to cases of owner initiated antimicrobial treatment?

Never ☐  Sometimes ☐

Often ☐  Rarely ☐

6. How do you rate owner compliance to recommended antimicrobial use practices?

Poor ☐  satisfactory ☐  good ☐

7. Are you interested in short courses on safe use of antimicrobial agents?

Yes ☐  No ☐

8. Highlight factors that you have identified that can lead to spread of antimicrobial resistance in Mnisi.

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## Animal Ethics Certificate

### Project Title
Prevalence of Antimicrobial resistance in Escherichia coli and Enterococcus species in cattle in Mzini, South Africa

### Project Number
V103-17

### Researcher/Principal Investigator
Dr. C Mupfunya

### Student Number (where applicable)
U_17406201

### Dissertation/Thesis Submitted For
MSc

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Animals</td>
<td>384</td>
</tr>
</tbody>
</table>

Approval period to use animals for research/testing purposes: September 2017 - September 2018

### Supervisor
Prof. V Naidoo

### Kindly Note:
Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment.

### Approved
Date: 26 September 2017

**Chairman: UP Animal Ethics Committee**
HUMAN ETHICS CERTIFICATE

11 November 2017

Dear Ms Mupfunya

Project: Prevalence of antimicrobial resistance in Escherichia coli and Enterococci species in cattle in Mnisi, South Africa
Researcher: C Mupfunya
Supervisor: Prof V Naidoo
Department: Veterinary Tropical Diseases
Reference number: 17466201 (GW201711176S)

Thank you for the application that was submitted for ethics review.

I have pleasure in informing you that the Research Ethics Committee formally approved the above study at an ad hoc meeting held on 11 December 2017. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should your actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely

Prof Maxi Sohoman
Deputy Dean: Postgraduate and Research Ethics
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

cc: Prof V Naidoo (Supervisor)
RED CROSS PERMIT

agriculture, forestry & fisheries
Department
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X120, Pretoria 0001

Enquiries: Mr Harry Goldo - Tel: +27 12 310 7032 - Fax: +27 12 310 7476 - E-mail: HarryG@dlr.gov.za
Reference: 1211/11/06/019

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Co: Ms Jeannette Wentzel (jeannette.wentzel@up.ac.za)
Dr Bjorn Reininghaus (sjverjan@gmail.com)

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Dr Mupfunya,

Your application sent with the email on 2 November 2017 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:
1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HarryG@dlr.gov.za;
3. Samples collected from cloven-hooved animals in the FMD infected and protection zones are routinely not allowed to be sent to any other laboratory except for TADP in the FMD free zone. As this study only involves faecal samples (rectal swabs) and
the collection will be conducted under state veterinary supervision, the samples may 
be sent to the DVT D BSL2+ laboratory under the conditions specified;

4. Rectal swabbing may only be conducted in the Mnisi community under supervision 
of State Veterinary Services. All cattle entering the depot site need to be examined 
for any clinical signs of FMDV to confirm the absence of such signs prior to rectal 
swabbing;

5. Samples collected have to be packaged in compliance with the National Road Traffic 
Act, 1996 (Act No. 93 of 1996), where after these samples may be sent to the 
BSL2+ laboratory at the Department of Veterinary Tropical Diseases. All samples 
must travel under the cover of a Red Cross Permit issued by the State Veterinarian 
of the area of origin and in full compliance with all the conditions of such a permit;

6. All potentially infectious material utilised, collected or generated during the study is 
to be destroyed at the completion of the study using the specified waste contractor. 
Records must be kept for five years for auditing purposes.

7. If required, an application for an extension must be made by the responsible 
researcher at least one month prior to the expiry of this Section 20 approval;

Title of research/study: “Prevalence of Antimicrobial resistance of Escherichia coli and 
Enterococci in cattle in Mnisi, Mpumalanga, South Africa”
Researcher: Dr Charlotte Ropafadzo Mupfunya
Institution: Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, 
University of Pretoria
Our ref Number: 12/11/1/1/6 (619)
Your ref: V1103-17
Expiry date: March 2018

Kind regards,

[Signature]

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2017-11-17

SUBJECT: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES
ACT, 1984 (ACT NO. 35 OF 1984)