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ANTIMICROBIAL RESISTANCE IN BACTERIA OF CLINICAL ORIGIN FROM SOUTH AFRICAN FEEDLOT CATTLE DURING 2002-2016.

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DECLARATION

The work reported in this dissertation was carried out in the section of Pharmacology and Toxicology of the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, under the adept supervision of Professor Vinny Naidoo.

The mini-dissertation has not previously been submitted at this University or any other academic institution of learning for consideration. It is the result of my own investigations, except where the inputs of others are acknowledged.

I, Dr Mgabadeli Sikhokhele Ncube, declare the statements above to be correct.

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Prof. Vinny Naidoo (Supervisor)

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ABSTRACT

Antimicrobial resistance is a global challenge that risks rendering currently available antimicrobial drugs ineffective. Antimicrobials are routinely used in feedlot systems as prophylactic, metaphylactic and therapeutic drugs. Feedlot cattle are an important source of animal protein in South Africa accounting for 75% to 90% of total beef production. Unfortunately, feedlot cattle derived beef is also potentially a public health hazard from the drugs used during farming. This study investigates the level of antimicrobial resistance in South African feedlots from samples (*n=16 599*) collected from infected weaner cattle in various feedlots in South Africa from 2002 to 2016 from the clinical records of a single veterinary practice. The susceptibility data was evaluated by means of descriptive statistics. The chi-square ($\chi 2$) test was undertaken to test for significant changes in antimicrobial resistance for the different variables and *p*-values < 0.05 were considered significant. Logistic regression was used to quantify the effect of different covariates on resistance giving odds ratios as effect measures.

The results showed that resistance increased by 4.7% between 2002 and 2016. Gauteng had the highest cumulative resistance (27.1%), resistance was highest towards aminoglycosides (45.6%) and *E. coli* showed the highest (55.3%) resistance towards antimicrobials. The study raises concern as resistance was not only increasing but higher than data from other countries. To combat the increase in antimicrobial resistance will require a coordinated national programme that has a defined veterinary antimicrobial use policy, appropriate resistance monitoring programmes supported by relevant stakeholders; and the implementation of legislation limiting antimicrobial use.

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LIST OF ABBREVIATIONS

AGP Antimicrobial Growth Promoters

AMR Antimicrobial Resistance

AST Antimicrobial Susceptibility Testing

BRD Bovine Respiratory Disease

BVDV Bovine Viral Diarrhoea Virus

CDDEP Center for Disease Dynamics, Economics & Policy

CAFO Concentrated Animal Feeding Operations

DAFF Department of Agriculture, Forestry and Fisheries

DALRRD Department of Agriculture, Land Reform and Rural Development

DNA Deoxyribonucleic Acid

EU European Union

FAO Food and Agriculture Organisation

FDA Food and Drug Administration

ILO Intensive Livestock Operations

KZN-DARD KwaZulu-Natal Department of Agriculture and Rural Development

MRSA Methicillin-Resistant *Staphylococcus aureus*

NARMS National Antimicrobial Resistance Monitoring System

NGS Next Generation Sequencing

OIE Office International des Épizooties

SAAHA South African Animal Health Association

SANAS South African National Accreditation System

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

SAVC South African Veterinary Council

USA United States of America

UTI Urinary Tract Infections

WHO World Health Organisation

1 INTRODUCTION

1.1 Introduction

A feedlot is a type of herd management system. It is an intensive animal farming system mainly used to fatten and finish beef cattle prior to slaughter but can also be used for pigs and other ruminants like goats and sheep (Campagnolo *et al.,* 2002). Feedlots contain hundreds of animals in an assemblage of pens as intensive livestock operations (Gilchrist *et al.,* 2007). The idea behind the feedlot system is to produce meat of high quality that can be marketed profitably. In feedlots, cattle are fed a high energy and high protein diet that encourages muscle growth and intramuscular fibre fat deposition, known as marbling, to allow for faster weight gains than is possible on pastures (Hersom *et al.,* 2004; Zinn, 2004). In addition to faster growth, marbling improves meat tenderness and flavouring making feedlot produced meat more desirable to consumers (Glitsch, 2000). Feedlots are thus of economic value as they bolster trade in meat and meat products, provide employment opportunities and more importantly ensure food security (Brandebourg *et al.,* 2013). Due to the ever increasing global population and demand for food, feedlots are becoming more and more common in developing and developed countries (McAlpine *et al.,* 2009).

Medicinal and clinical practices are an integral part of feedlot systems. Due to the feedlot setup of movement, restricted and overcrowded animals, feedlot conditions are stressing to animals and the rate of bacterial proliferation and disease propagation is high. This prompts most feedlot operators to engage in the use of antimicrobials in an effort to prevent, treat or control infections (Ferber, 2003). While antimicrobials are beneficial in the treatment of disease, their use is not without concern due to scientifically incorrect use practices. As far back as the early

eighties, close to half of all antibiotics produced in the United States were directly added to the farm animal feed (Novick, 1981) as prophylactic treatment and/or growth promoters. It is this use of sub-therapeutic antimicrobial doses that has contributed to the incremental development of resistance together with other factors such as poor stewardship policies and unregulated use of antimicrobials among others.

1.2 Aim

Ascertain level of antimicrobial drug use and resistance over the past fifteen years in feedlot cattle.

1.3 Objectives

- To determine the extent of bacterial resistance to antimicrobial agents in feedlot animals in South Africa from retrospective data and to compare results to other countries
- To explore the effect of antimicrobial resistance in feedlot cattle.

2 LITERATURE REVIEW

2.1 Feedlot cattle and their importance

2.1.1 Introduction

According to the Food and Agriculture organisation (FAO)(Ilea, 2009) approximately 56 billion land animals are raised yearly for human consumption with this number expected to double by 2050 (Webb, 2013). Global meat production excluding fish and other aquatic animals will increase from 229 million tons at the turn of the millennium to 465 million tons in 2050 (Ilea, 2009). Current beef production is unevenly distributed worldwide with approximately 39% being in Europe, 44% in North and South America, 9 % in Africa and 6% in Asia and the Oceania (Herrero *et al.,* 2013). Cattle production systems are broadly classified into extensive and intensive cattle production systems with the former being a low input system and the latter a high input system (Dikeman, 1984). Under intensive herd management, the feedlot system is the most intensive cattle production system (Nguyen *et al.,* 2010), and is the animal farming system mainly used to fatten and finish beef cattle prior to slaughter but can also be used for pigs and other ruminants like goats and sheep (Campagnolo *et al.,* 2002).

Australia has approximately 400 accredited feedlots that contribute about a third of total beef production, while the world's largest beef producer, the USA, has over 729,000 beef cattle operations (Harrington & Lu, 2002), and China had 31,685 feedlots in 2013 (Han *et al.,* 2016). Feedlots in Egypt contribute approximately 70% to the annual production of over 330 000 metric tons of beef annually (Shapouri *et al.,* 1985) and South Africa has over 650,000 cattle in the feedlot system accounting for 75 - 90% of South Africa's total beef production (Meissner *et al.,*

2013; Anderson & McLachlan, 2012). The largest feedlot south of the equator is located in South Africa, boasting a one-time-capacity of 130,000 cattle (Meissner *et al.,* 2013).

Cattle stay an average of 150-180 days in the feedlot in Canada and USA (Andrews, 2018) and gain an average of 1.2kg per day whilst the weight gain in grazing steers averages 0.5 – 0.8kg per day depending on the quality and availability of pastures (Du *et al.,* 2010). In the South African province of KwaZulu-Natal, cattle remain at the feedlots for 90-120 days (Meissner *et al.,* 2013). As a result most commercial farmers find it more cost effective to transfer their weaners to feedlots for final fattening (Thomson *et al.,* 2015).

2.2 Feedlot diseases

Despite the benefits of fast growth from high density feeding, the crowding of a thousands of animals into a confined environment leads to an increase in the prevalence of diseases. A review by Smith (2004) found that respiratory infections were the most common clinical and necropsy findings in feedlot cattle with peak disease incidence occurring within three to four weeks post-arrival in the feedlots. Metabolic and digestive disorders such as bloat, ruminal acidosis and liver abscesses are fewer and their prevalence tends to increase later in the feeding period (Smith, 1998). Elanco Animal Health, a major veterinary pharmaceutical company, reported that the liver abscess condemnation rate in USA in 1995 averaged 12.9% for steers and 11.5% for heifers (Casewell *et al.,* 2003). In the same year, Beef Quality Audit results (Smith, 1998) showed that the liver condemnation rate was 22%, not exclusively due to liver abscesses but also due to telangiectasis and sawdust livers with
multiple foci of nonsuppurative necrosis.

Numerous organisms have been identified as causative infectious agents. For the most part the cause of disease tends to be viral caused by bovine viral diarrhoea virus exclusively or in combination with bovine respiratory syncytial virus and/or parainfluenza-3 virus (Gagea *et al.*, 2006). These viral infections then predispose the animal to the development of bacterial infections. In a study by Haines *et al* (2001) lung and joint tissue from 49 feedlot cattle showed the presence of *Mycoplasma bovis* (45% of joints and 71% of lungs tested), *Histophilus somni* (14% lungs only), *Pasteurella (Mannheimia) hemolytica* (23% lungs only), and bovine viral diarrhoea virus (BVDV) (40% lungs only).

2.2.1 Management of diseases in feedlots

Medicinal and clinical practices are an integral part of feedlot systems. Routine vaccinations are undertaken, and while dependent on disease prevalence in the particular regions, they are usually aimed at clostridia organisms (blackleg, enterotoxaemia, malignant oedema) and respiratory diseases (shipping fever, bovine respiratory disease, bovine viral disease, bovine respiratory syncytial virus) (Smith, 2004). Routine deworming is also undertaken to optimize the feed conversion ratio and attain a good average daily weight gain of between 0.9-1.2 kg per day (Henrickson *et al.*, 1965). However it is the management of Bovine Respiratory Disease (BRD) that requires the greatest attention due to the high mortality and morbidity associated with the disease (Taylor *et al.*, 2010).

Gallo and Berg (1995) noted that the use of in-feed antimicrobials both treats and prevents BRD. In high-risk animal groups such as calves and weaners, prophylactic use of antimicrobials in the feed reduces BRD linked morbidity whilst improving feed conversion efficiency and the daily average weight gain (Harland *et al.*, 1991; Van Donkersgoed, 1992).

Since then, much research has demonstrated the herd health and economic benefits associated with infeed antimicrobial use in feedlots (Gibb, 2006; Hughes and Heritage, 2004).

2.3 Growth Promoting in Feedlots

Despite the fast growth seen under feedlot conditions, the high price of animal feed, especially the protein component and cracked maize, has the inherent danger that the cost of production exceeds the price of the meat. To overcome this, operators make use of pharmaceutical products that enhance growth commonly known as growth promoters. Tetracyclines, erythromycin, virginiamycin, quinoxalines, flavophospholipol, avoparcin, avilamycin, salinomycin, monensin and arsenical compounds are some of the growth promoters used in animal production (Hughes and Heritage, 2004). Antimicrobial growth promoters (AGPs) improve the feed conversion ratio (Foka *et al.,* 2018) and overall animal productivity (Van Duijkeren *et al.,* 2014). First used in the mid-1950s, AGPs are small, sub therapeutic antibiotic doses (a fraction of a therapeutic dose), delivered in-feed to enhance the feed conversion ratio in livestock (Marshall and Levy, 2011).

In South Africa, most available in feed antimicrobials for use in food animals are legislated by Act 36 of 1947; the Stock Remedies Act (Eager *et al.,* 2008). Antimicrobials from the pleuromutilin and macrolide classes are the most frequently used followed by tetracyclines, sulphonamides and penicillins (Eager *et al.,* 2008).

2.3.1 Concern with use of AGPs in feedlots

Despite the valuable contribution of the feedlot industry to food security, the use of antimicrobials in such a larger number of animals for therapeutic, prophylactic or growth promoting effects has raised concerns. Concerns associated with the risk of development of

drug-resistant pathogenic bacterial strains (Gilchrist *et al.,* 2007) and other food safety concerns. Global antimicrobial use in food animals is heterogeneously distributed, depending on country and national/bloc legislation, degree of animal production systems industrialization and market preferences for animal products (Ronquillo and Hernandez, 2017; Von Boeckel *et al.,* 2015). An association exists between most intensive farming practices and extent of AGP utilisation. China is the largest global user of antimicrobials for food animal production at 23% followed by Brazil and the United States of America both at 13% while India and Germany hold the fourth place jointly at 3% (Ronquillo and Hernandez, 2017). Countries with dense food animal populations and more intensive farming practices are commonly associated with significantly high AGP utilisation (Ronquillo and Hernandez, 2017). Cully (2014) found that close to 80% of antibiotics used by volume in the USA are used in food production animals mainly as growth promoters whereas in Spain, a heavy European user of antimicrobials, utilization is two thirds of that used in the United States (Cully, 2014).

Van Boeckel *et al* (2015) approximated food animal production global use of antimicrobials at 63 thousand tons with a 67% increase to 105 569 tons by the year 2030. Antimicrobials authorised for use as in feed- growth promoters in South Africa include tylosin, tilmicosin, josamycin, olaquindox, virginiamycin, chlortetracycline, oxytetracycline, kitasamycin, tiamulin, lasalocid, flavophospholipol, avilamycin, monensin, bacitracin, poly 2-propenal 2-propenoic acid, streptogramins and phosphonic acids under *Act 36 of 1947* and *Act 101 of 1965*. Many if not all of these are banned as in-feed growth promoters in the European Union (EU) (Eager *et al.,* 2008).

The use of AGPs definitely has the prospect to surge the selection for and consequent development of antimicrobial-resistant commensal and pathogenic bacteria (Alexander *et al.,*

2010; Salyers *et al.,* 2004). As an example *Escherichia coli* (*E. coli*) is one organism in which resistance conferring genes are transferable from livestock to humans. *E coli*, the reservoir of resistance genes, has been shown to exchange genetic matter with other bacteria via conjugation and plasmid transfer (Blake *et al.,* 2003; Alexander *et al.,* 2010). The public health concern and pressure from politico-consumer groups (Alexander *et al.,* 2010) thus prompted the EU to ban AGPs in 1999 on the eve of the millennium (Casewell *et al.,* 2003).

2.4 Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is when a microorganism develops resistance to a dose of antimicrobial agent to which it was previously susceptible (Acar and Rostel, 2001). As a result, the once effective recommended dose becomes ineffective and infections caused by these microbes persist and spread (Wenzel and Emond, 2000). Resistant infections drastically increase veterinary costs, morbidity and mortality rates (Laxminarayan, 2010).

2.4.1 Emergence of Antimicrobial Resistance

AMR develops naturally as a selection pressure and survival adaptation strategy by microbes (Laxminarayan, 2010). Systematic mutation of the bacterial genome and resistant gene acquisition confers antimicrobial resistance (Wenzel and Edmond, 2000). However, antimicrobial abuse is hastening the rate of AMR development (Acar and Rostel, 2001). The world over, antibiotics are overused and misused in the treatment of humans and animals. They are often prescribed without professional oversight, in many cases for the management of viral infections such as influenza and as AGPs in animal and fish husbandry (Oluwasile *et al.,* 2014).

The transfer of antibiotic resistance genes and subsequent selection of antibiotic resistant bacteria (Ronquillo and Hernandez, 2017) is a two staged process as described by Roe and Pillai

(2003) and Mathew *et al.* (2007). The first stage is the introduction of the resistance gene into the bacteria while the second stage is when the antimicrobial resistance gene is expressed allowing the cells containing the resistance gene to survive and propagate even in the presence of the antimicrobial agent whilst cells lacking the resistance gene are vulnerable and die (Mathew *et al.*, 2007). The introduction of the genetic change can occur by mutations or antibiotic-resistance gene transfer from a resistant bacterium to a susceptible bacterium rendering the once susceptible bacterium resistant like the former. Resistance gene transfer may occur in one of three ways: transduction (via bacteriophages), conjugation (via plasmid transfer) or transformation (free DNA taken in) (Martinez and Baquero, 2000). Studies suggest that complex genetic and environmental factors such as specific diet, age and ecological selection pressures (Fairchild *et al.*, 2005; Berge *et al.*, 2005) possibly play a role in perpetuating resistance (Marshall and Levy, 2011). As a result of the above, in-feed antimicrobial use has been associated with AMR (Bager *et al*., 1997) and in the early eighties in Europe, avoparcin use as an AGP in poultry and pigs was linked with the emergence of a *Enterococcus faecium* strain that was vancomycin resistant (Marshall and Levy, 2011).

2.4.2 Public health and veterinary implications of Antimicrobial Resistance

Veterinarians, and farm and abattoir personnel are at risk of infection by resistant bacterial strains because of their close exposure with colonised or infected animals (Mølbak *et al.*, 1999). This type of bacterial transmission may initially seem insignificant as a population-level health threat (Marshall and Levy, 2011) but it is an entryway of resistance genes into the local area and surrounding geo-communities, where more propagation of resistance genes occurs (Voss *et al.*, 2005). Initially drug resistant microbes were more common in medical facilities and

hospitals because of the extensive use of antimicrobials, but are now also prevalent in the community (Okeke *et al.,* 2005)

Occupationally exposed individuals have a markedly greater risk of colonisation by multidrug-resistant bacterial strains (Hashmi *et al.,* 2017). A study by Price *et al.* (2007) found the risk for carrying gentamicin-resistant strains of *E. coli* was thirty two times more in poultry farmworkers compared to the general populace (Hashmi *et al.,* 2017). Earlier studies by Van Den Bogaard *et al.* (2002), Aubry-Damon *et al.* (2004) and Katsunuma *et al.* (2007) consistently reported a higher prevalence of resistant gastro-intestinal tract bacteria among farm workers compared to the general public (Marshall and Levy, 2011).

Of public health concern in the veterinary field is resistance development in zoonotic microbes (Ronquillo and Hernandez, 2017). The World Health Organisation (WHO and FAO, 2015) highlights that antimicrobial abuse or misuse in human medicine is a major cause of AMR. AMR emanating from the use of AGPs in food animals can worsen this global health hazard (Ronquillo and Hernandez, 2017; WHO and FAO, 2015). Resistant bacteria are selected for, through antimicrobial usage (Ronquillo and Hernandez, 2017), thereby establishing a correlation between usage extent and prevalence of resistance (Jensen *et al.,* 2002).

The notion of animal origin of bacteria that infect humans is supported by gene-based methods of analysis as correlative relationships have been established (Voss *et al.,* 2005). Homologous relationships between resistance genes for *E. coli* and *Salmonella,* for several *Enterococcus* and for methicillin-resistant *Staphylococcus aureus* (MRSA) species have been identified (Katsunuma *et al.,* 2007). Zhang *et al* (2009) found apramycin resistant strains of *E. coli* in Chinese farm workers yet apramycin is used solely in veterinary and not in human

medicine. This suggests a relationship between use of in-feed AGPs in animals and the human problem of multidrug-resistant microbial infections.

Veterinary antimicrobials and their residues also reach the environment through animal excreta and wastewater, posing a detrimental threat to aquatic flora and fauna (Huyghebaert *et al.,* 2011). Residues of veterinary antimicrobials also remain in animal products consumed by humans, predisposing humans to antimicrobial associated allergic reactions, multi-drug resistant bacterial infections and various other health issues (Tasho and Cho, 2016). As an example, lincomycin in dairy feed has been reported to have toxicological effects ranging from anorexia to diarrhoea and ketosis in humans (McEvoy, 2002).

2.4.3 Prevention of Antimicrobial Resistance

Antimicrobials should only be used for their approved and intended uses, while medically essential antibiotics should be banned for use as AGPs (Hughes and Heritage, 2004). Practitioners and subscribers should adhere to internationally and nationally established antimicrobial stewardship guidelines (Anthony *et al.,* 2001). Such recommendations aim to reduce and possibly prevent the selection of antimicrobial resistant bacterial strains (Uchil *et al.,* 2014). Professional veterinary associations should draft species-distinct clinical regulations on rational antimicrobial use, specific to product choice and treatment guideline (Anthony *et al.,* 2001). Eager and Naidoo (2017) also suggest good hygiene practices and establishment of pre-registration conditions for all antimicrobials to limit the use of antimicrobials.

The South African Veterinary Strategy 2016-2026 (DAFF, 2016) highlights the need for an integrated approach by all relevant stakeholders and suggests considerations such as prohibition of compounded medicines, restriction of antimicrobials to therapeutic utilisation only, utilisation

of mandatory drug registers and re-evaluation of direct drug sales to farmers (Eager and Naidoo, 2017).

Antimicrobial use training and sensitisation to AMR in collaboration with relevant regulatory and professional organisations, the pharmaceutical industry, medical and veterinary schools and research institutes with a prime focus on infection management and prevention protocols to minimise antimicrobial prescription and usage should be embarked on (Anthony *et al.,* 2001). Veterinarians and animal scientists should educate farmers on eco-conservative farming practices and alternative strategies to prevent or control disease such as vaccination (Uchil *et al.,* 2014) and the all-in-all-out infection prevention strategy commonly used in pig production (Hughes and Heritage, 2004).

2.5 Antimicrobial Resistance surveillance and monitoring programs

2.5.1 Purpose and effectiveness

The fundamental goals of surveillance and monitoring programs are to improve detection and identification of emerging antimicrobial resistance. By understanding the susceptibility of organisms, the best drug can be chosen thus extending the effective life of antimicrobials and aiding new drug development (Mathew *et al.,* 2007). Secondly such programs can also help investigate associations between antimicrobial use and AMR prevalence especially among bacteria of zoonotic and public health importance in a bid to provide information in good time to veterinarians (Mathew *et al.,* 2007), physicians and dispensers regarding resistance profiles of pathogens (Aarestrup *et al.,* 2010).

Successful programs have been developed and implemented in Denmark and USA among other countries and have proven to be highly beneficial. USA focused studies, using the

National Antimicrobial Resistance Monitoring System (NARMS) data, have highlighted that *Campylobacter jejuni* isolates from poultry have become more ciprofloxacin resistant. Resistance was reported at 15% in 2005 compared to 9% of isolates in 1998 (NARMS, 2005; Mathew *et al.,* 2007), attributed to the use of veterinary enrofloxacin, prompting the FDA to ban veterinary enrofloxacin. The World Health Organisation (WHO) used the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) data to show that avilamycin resistant *E. faecium* poultry and pig isolates have declined significantly after the ban on AGPs in 2000 (Mathew *et al.,* 2007), indicating that interventions are successful. The impact and success of such programs is unquestionable. Improved surveillance and monitoring, legal regulation, transparency among prescribers and users and public education are crucial for the success of frameworks for curtailing the propagation of AMR (Edwards *et al.,* 2018).

Drug resistance monitoring programs require scientific methods that detect molecular changes or isolate (parasite) sensitivity indicating alterations in either drug target or intra-parasite active drug levels (Croft, 2001). Changes in susceptibility of microbial agents to recommended drug dosages can be arbitrarily monitored by taking baseline susceptibility tests of the microbes to the drug prior to use of the drug and then carrying out subsequent susceptibility tests at specified intervals, followed by comparisons with the baseline values (Albonico *et al.,* 2004). More importantly, *invitro* testing may provide an early warning of impending resistance before it becomes clinically apparent by randomly sampling organisms in animals (Bennett *et al.,* 2008).

Besides routine resistance surveillance with the genetic determinants of resistance being well understood, next generation sequencing (NGS) can be used to determine the DNA sequence of the entire bacterial genome (Motro and Moran-Gilad, 2017). Based on the bacterial genome,

information on resistance and virulence is obtained and can be used during outbreak investigations to develop outbreak-specific screening tests (Jackson *et al.*, 2016)

In addition to monitoring resistance within organisms, methodical bioanalysis of stock feed and animal products using methods such as liquid chromatography with ultraviolet detection and mass spectrophotometry to detect molecular additives is a vital step in scanning for use (Ronquillo and Hernandez, 2017). To ensure sustainability of livestock production systems, WHO and FAO (2015) advocate for legislated monitoring of AMR, public awareness and education on the dangers of antimicrobial residues in food animal products and the controlled use of antibiotics with the aim to reduce the use of antibiotics and AGPs in livestock.

2.5.2 The African scenario

The high prevalence of transmittable diseases in African countries fosters substantial use of antimicrobials and subsequent development of resistance with wide ranging health and socio-economic implications (Essack *et al.*, 2016). In 2016, two out of 54 African countries (3.7%) had AMR master plans, 13% (7 countries) had comprehensive disease prevention and control strategies, 44 countries had essential medicines lists and 79.6% (43 countries) had national policies and treatment protocols advocating for principled use of antimicrobials. None had representative surveillance systems and none did subsidised new medicines research and development (Essack *et al.*, 2016).

2.6 AMR in South Africa and other developing countries

A South African study by Jonker and Picard (2010) showed that *Campylobacter jejuni* isolates mainly of poultry origin were more resistant to fluoroquinolones, macrolides and tetracyclines whereas *Campylobacter coli* strains were more resistant to the macrolides

and lincosamides. In the Eastern Cape, Adefisoye and Okoh (2016) working on *E. coli* resistance, reported that meropenem and imipenem were effective on all isolates, one isolate exhibited resistance to gentamycin and the highest frequency of resistance was shown against tetracycline (60.1%), then ampicillin (55.6%) and cephalexin (51.1%).

While AMR surveillance is a key aspect of global action against AMR, the WHO Africa region has a paucity of data on AMR prevalence (WHO, 2014) due to limited laboratory resources, expertise and surveillance networks. AMR rates are likely to be heterogeneous within individual African countries, especially between urban and rural contexts, hence contextually diverse studies within individual countries are key in order to ascertain accurate estimates of AMR (Bernabe *et al.,* 2017). An independent report revealed major shortfalls in antimicrobial susceptibility testing (AST) in most African countries (Frean *et al.*, 2012), potentially compromising empirical treatments of day-to-day bacterial infections (Bernabe *et al.,* 2017).

In West Africa, Bernabe (2017) revealed soaring rates of ampicillin resistance by *E. coli* and *Klebsiella* species. Isolates from human urinary tract infections (UTIs) showed low resistance to fluoroquinolones while ciprofloxacin was only moderately active against *E. coli*, *Klebsiella* species and *P. aeruginosa* UTIs (Bernabe *et al.,* 2017). Resistance against penicillin was low among *S. pneumoniae* at 12.3% and no notable resistance against cefotaxime and ceftriaxone was reported (WHO, 2013). Chloramphenicol is an effective option upon failure of safer first line antibiotics due to low resistance rates of *N. meningitidis, S. pneumoniae* and *H. influenza* (Bernabe *et al., 2017*). *Streptococcus pneumoniae* is highly susceptible to third generation cephalosporins hence they are the drugs of choice in *S. pneumoniae* caused bacterial meningitis in the region (Bernabe *et al.,* 2017).

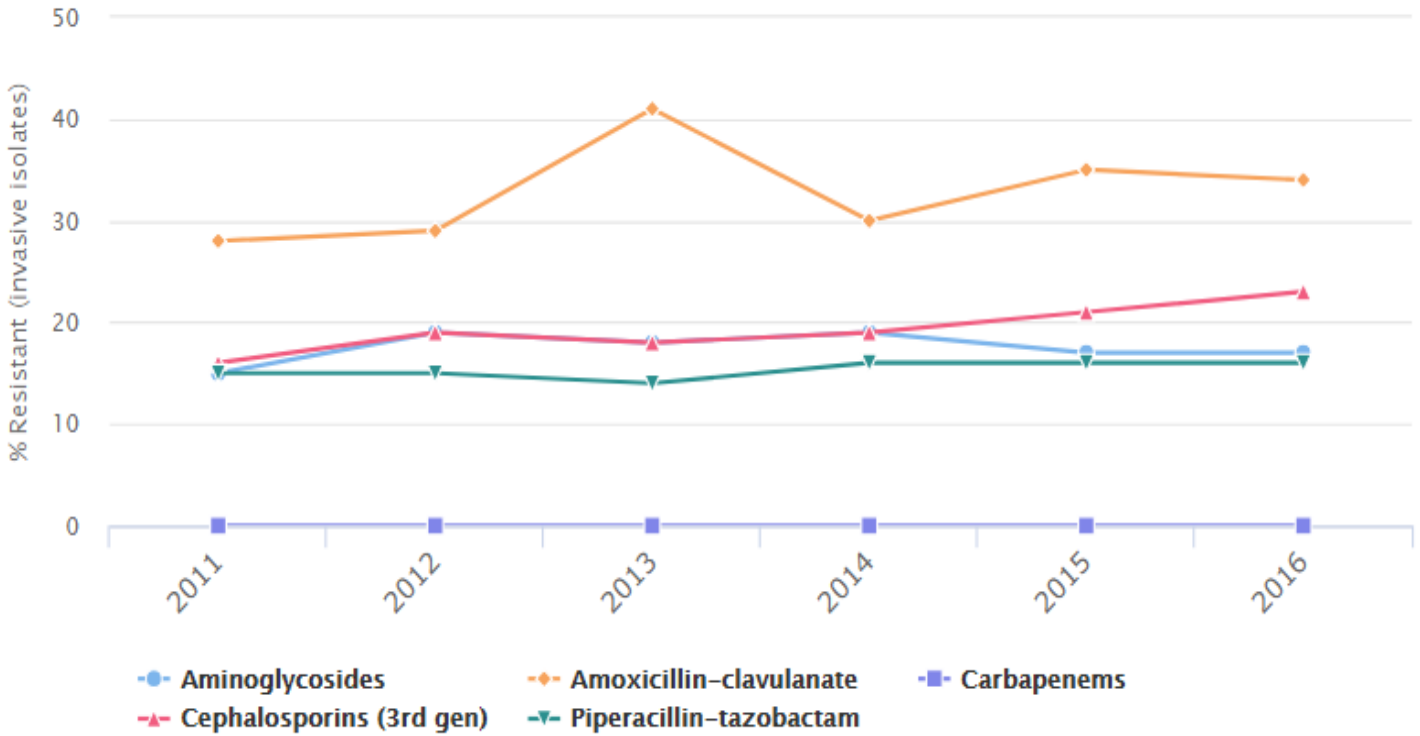


Figure 2.1: Antibiotic resistance of clinical Escherichia coli (E. coli) isolates in South Africa from 2011 -2016. (CDDEP, 2017)

Data from the South African National Health Laboratory Services was used to draft a resistance survey map by the Center for Disease Dynamics, Economics & Policy (CDDEP) for five antibiotic classes. Figure 2.1 shows that there was significant resistance against common drug classes such as aminoglycosides, third generation cephalosporins, amoxicillin-clavulanate and piperacillin-tazobactam among the *E. coli* isolates tested from 2011 to 2016. Resistance was low to none against carbapenems (imipenem, meropenem, ertapenem) for which resistance was 0% over the same period. *E. coli* resistance against aminoglycosides increased from 15% to 17%, whilst resistance against amoxicillin-clavulanate and third generation cephalosporins increased from 28% to 34% and 16% to 23% respectively. These facts substantiate the notion that antimicrobial use fosters antimicrobial resistance, the more commonly used an antimicrobial drug the higher the chances of microbes developing resistance and actually being resistant to the antimicrobial drug (Marshall and Levy, 2011).

A proposal in the South African national approach to antibiotic stewardship discourages empirical prescription (Goff *et al.*, 2017). The antimicrobial use surveillance system, coupled with an AMR surveillance and monitoring plan of action aid in curbing the rate of AMR development and early detection of problems associated with resistance (Eager *et al.*, 2008). From the veterinary perspective, the South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs (SANVAD), created in 2003, is the domestic program that seeks to manage AMR (Eager & Naidoo, 2017) in accordance to international standards and the Office International des Épizooties (OIE) guidelines. The national South African surveillance and reporting systems on antibiotic use are strong and effective and serve to advise antibiotic selection for the national essential drugs project (Goff *et al.*, 2017).

Antimicrobial use and AMR are worldwide issues due to the global presence of bacterial infections (Mather *et al*., 2012). The South Africa livestock industry has a high burden of infectious diseases, largely of bacterial origin (Van den Hornet *et al.,* 2018). AMR is proliferated by several factors, mainly inappropriate antibiotic use and management. Despite South Africa having the most functional surveillance for veterinary AMR in Africa, high levels of AMR exist. Regulatory framework and legislation, veterinary services presence, farmer expectations and demands collectively influence antimicrobial use (Gelband and Duse, 2011).

The economic impact of AMR on production is largely unknown and is yet to be evaluated. As antibiotics become less effective and AMR spreads, the growing South African population and consequently protein demand, will be compelled to pay more for animal products due to increasing rearing and veterinary costs. Depending on current antimicrobial stewardship policy and implementation this can happen in the near or distant future. AMR emergence and growth can be halted and upon great lengths reversed thereby enhancing livestock and public health (Gelband and Duse, 2011).

Building on the knowledge base already available and going forward, it is important that affordable, effective and not only South African but African scenario relevant interventions are drafted and adopted to solve African and developing countries problems as has been initiated in Kenya and India. Finally, South Africa's progress in AMR research and developing strategies to minimise AMR is not limited to a national level. The problems and relevant solutions that are discovered will definitely be of relevant interest and application in other African countries (Duse, 2011). It is thus important for academia and industry to work together in finding lasting and sustainable solutions against a common but very important threat to human and animal health, AMR.

3 METHODOLOGY

3.1 Animals

The study analysed past antimicrobial susceptibility records from bovine samples from feedlots in all South African provinces except the Eastern Cape and Western Cape provinces. The data was obtained from the client records of one feedlot consultant in the country and is representative of 90% of the over 70 feedlots in the country. The percentage contribution of these feedlots to total beef production in South Africa is 70-90%.

3.2 Sample handling

Samples were collected from sick animals and were submitted to veterinary diagnostic laboratories in South Africa; namely Idexx (Johannesburg), Deltamune (Pretoria), Golden Veterinary laboratory (Johannesburg), University of Pretoria (Pretoria) and Vet Diagnostix (Johannesburg). Animal samples submitted for bacterial culture and susceptibility testing included faecal, joint swab, lung, various organ and trans-tracheal aspirate samples. The samples were cultured using standard diagnostic bacteriology procedures. The total number of antibiogram conducted was 16 559.

The study covered the period of years 2002, 2007 to 2012 and 2014 to 2016. Bacterial culture and antimicrobial susceptibility testing (AST) were performed using standardised methods according to the general guidelines for South African laboratories. Results of each antibiogram were captured into Microsoft Excel indicating the sampling date, laboratory name and laboratory number where the AST was performed, the veterinarian who collected the sample and from which species the sample was collected, the feedlot and province from which the

sample originated from, the sample type, age of animal, antimicrobials for which bacterial susceptibility was tested and the result of the test whether the bacterium was sensitive or resistant to the antimicrobial.

3.3. Statistical analysis

Data editing and cleaning was performed using Microsoft Excel. The data was imported into SPSS software, IBM SPSS Statistics version 21, and evaluated by descriptive statistics. Statistical analysis was performed using the SPSS software. The chi-square ($\chi 2$) test was undertaken to test for significant changes in antimicrobial resistance for the different variables. For these analyses, p-values < 0.05 were considered significant.

Multivariable statistics for binary outcome of resistance namely logistic regression was used to quantify the effect size of different covariates on resistance giving odds ratios as effect measures. Odds ratios were used as a measure of effect and the null value for odds ratios used was 1, meaning odds ratios below 1 where protective against resistance while those above 1 show the size of risk associated with the variable. For significance, p values less than 0.05 were considered significant and odds ratio confidence intervals which did not include the null value (1) were considered significant.

4 RESULTS

4.1 Bacterial species resistance to antimicrobials in feedlots

Following the evaluation of microbial susceptibility to antimicrobials from records of South African feedlots over a fifteen year period, Figure 4.1, the antimicrobial resistance for the period under study increased steadily from 18.5% in 2002 to 26.9% in 2008. Hereafter the resistance fluctuated randomly between a low of 18% and a high of 27.4%. When comparing the degree of resistance in 2002 to the 2016, resistance had increased by 5%, indicating a minor trend for increased total resistance over the 15 year monitoring period.

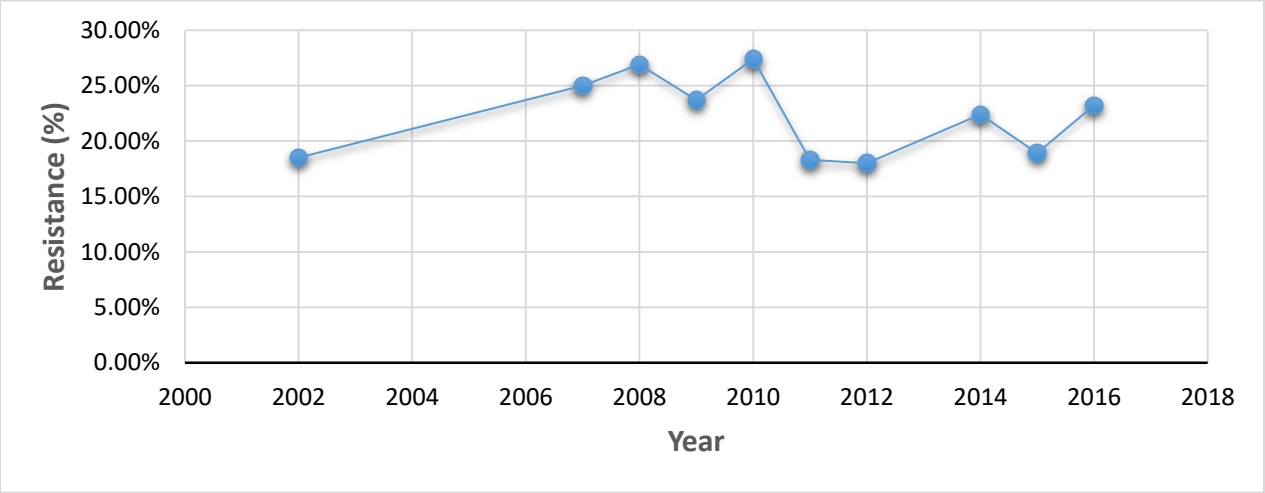
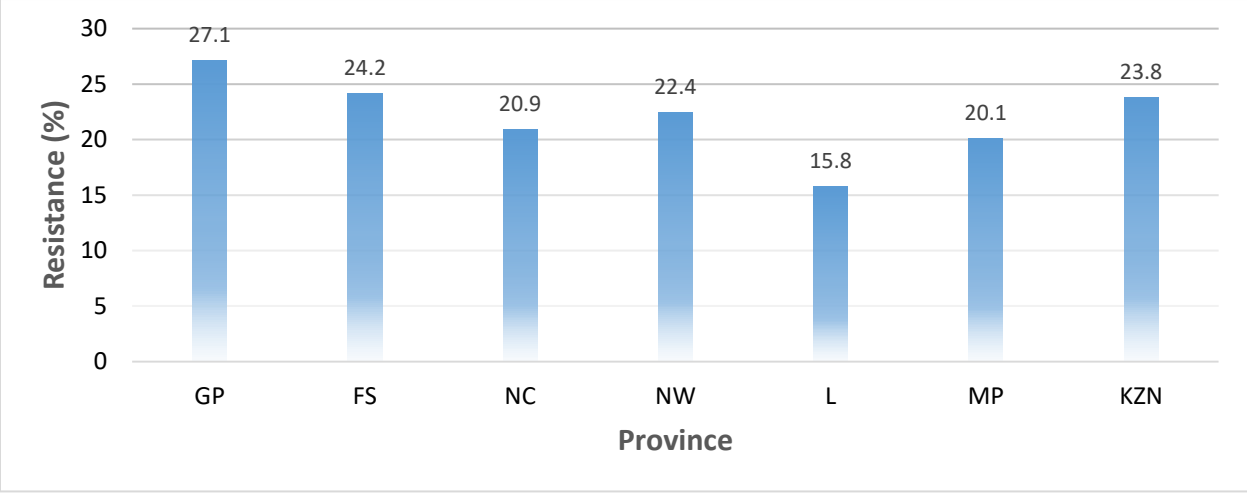


Figure 4.1: Line graph of year by year change in percentage resistance from 2002 to 2016.

Using logistic regression and adjusted for the effect of province, antimicrobial class and bacterium, the likelihood for resistance in 2009 [*p = 0.003; 1.08 - 1.46*] and 2010 [*p = 0.013; 1.09 – 2.09*] was 26% and 51% respectively more than in 2008 where most samples were tested. Compared to 2008, resistance in 2012 [*p = 0.000; 0.58 – 0.79*] and 2015 [*p = 0.002; 0.66 – 0.91*] was 32% and 22% less likely. Resistance in 2002, 2007, 2011, 2014 and 2016 was not significantly different from 2008.

4.2 Resistance by province

With resistance over the entire period pooled, of the seven provinces (Figure 4.2) from where samples originated, Gauteng has the highest cumulative resistance at 27.1% followed by the Free State and KwaZulu Natal at 24.2% and 23.8% respectively. The North West province had a cumulative resistance of 22.4% followed by Northern Cape and Mpumalanga at 20.9% and 20.1%. Limpopo has the lowest recorded resistance of 15.8%.



Key: GP: Gauteng, FS: Free State, NC: Northern Cape, NW: North West, L: Limpopo, MP: Mpumalanga, KZN: KwaZulu-Natal.

Figure 4.2: Bar graph representing cumulative resistance (%) for each province for the period under study 2002 to 2016.

Using logistic regression and adjusting for the effect of year, antimicrobial drug class and microbial species, resistance in Free State, Northern Cape and KwaZulu Natal was not significantly different from North West province where the highest number of samples were collected. Limpopo and Mpumalanga provinces were 33% and 18% respectively less resistant while Gauteng was 21.0% [*p = 0.01; 1.05 – 1.39*] more resistant compared to the North West province.

4.3 Resistance per antimicrobial drug class

The resistance per evaluated antimicrobial class is presented in Table 4.1. Resistance for macrolides, sulphonamides, cephalosposrins, tetracyclines and fluoroquinolones increased over the period of study whilst for aminoglycosides, penicillins, lincosamides, polymixins, phenicols decreased.

Table 4.1: Resistance (%) per antimicrobial drug class from 2002 to 2016, and the cumulative resistance over the same period.

Antimicrobial Class	**Year**										**Total**
	2002	*2007*	*2008*	*2009*	*2010*	*2011*	*2012*	*2014*	*2015*	*2016*	
Macrolides	0.0	42.9	45.4	42.1	67.9	25.0	23.4	33.7	36.5	33.3	***37.0***
Sulphonamides	0.0	14.9	9.0	5.1	7.1	25.0	4.2	11.6	7.1	28.0	***9.1***
Aminoglycosides	40.0	58.7	59.7	43.6	78.6	50.0	43.3	21.7	20.2	-	***45.6***
Penicillins	18.2	14.8	10.3	4.2	2.4	7.7	3.1	6.2	5.7	4.6	***6.9***
Tetracyclines	20.0	37.0	28.4	15.4	14.3	0.0	29.8	18.8	13.1	32.4	***22.8***
Fluoroquinolones	30.0	39.5	42.3	33.2	39.3	29.2	27.9	40.2	40.7	57.0	***37.8***
Lincosamides	-	15.6	6.7	11.5	3.6	0.0	3.0	4.4	5.5	0.0	***6.7***
Cephalosporins	0.0	17.0	23.7	29.6	28.9	20.0	12.7	26.2	14.8	12.6	***20.1***
Polymixins	10.0	6.7	3.6	2.3	0.0	0.0	0.0	0.0	-	-	***2.6***
Phenicol	-	-	-	25.0	0.0	0.0	42.5	2.4	6.1	0.0	***15.4***
Total											**22.5**

Results all showed significant differences over time (with $p = 0.001$). Using logistic regression and adjusting for the effect of year, province and antimicrobial species, resistance against sulphonamides, penicillins, tetracyclines and fluoroquinolones was 86% [*0.11 – 0.18*], 89% [*0.09 – 0.13*], 59% [*0.35 - 0.49*] and 99.5% [*0.04 – 0.07*] respectively less likely compared to aminoglycosides, the most used antimicrobial drug class. Resistance against lincosamides was 14 times [*11.02 – 17.47*] more likely compared to aminoglycosides. Resistance against cephalosporins, polymixins, phenicols and chloramphenicols was 99.7% [*0.03 – 0.05*], 99.2% [*0.05 – 0.12*], 99.3% [*0.05 – 0.10*] and 84% [*0.11 – 0.22*] less likely compared to

aminoglycosides. Resistance against macrolides was not significantly different from aminoglycosides.

The resistance of the individual drugs was as follows:

4.3.1 Macrolides

For the macrolide class, resistance increased marginally from 42.9% in 2007 to 45.4% in 2008 and peaked at a high of 67.9% in the year 2010. The resistance then decreased to 25.0% in 2011 and 23.4% in 2012 then rose to 36.5% in 2015 and lastly 33.3% in 2016 during the last year of the study. The total cumulative microbial resistance against macrolides antimicrobials for the period under study was 37.0%.

4.3.2 Sulphonamides

For the sulphonamide class resistance was 0.0% for the first year of study, increased to 14.9% in 2007 then decreased during the next three years to a low of 7.1% in 2010. In 2011 the resistance against sulphonamide antimicrobials increased to 25.0% then decreased to 7.1% over the next three years of the study to end on a high of 28% for 2016. The total cumulative microbial resistance against sulphonamide class antimicrobials for the period under study was 9.1%.

4.3.3 Aminoglycosides

For the aminoglycoside class, resistance for the first year of study, 2002, was 40.0% and rose to 59.7% in 2008 and to a high of 78.6%. The resistance then decreased to 50.0% in 2011, 21.7% in 2014 and to 20.2% in 2015. The total cumulative microbial resistance against the aminoglycoside class antimicrobials, for the period under study, was 45.6%.

4.3.4 Penicillins

For the penicillin class, resistance decreased during the period of study from an initial high of 18.2% to 10.3% in 2008 and 2.4% in 2010. Resistance then increased to 6.2% in 2014 and decreased to 5.7% in 2015 and 4.6% in 2016 the last year of the study. Cumulative resistance against the penicillin antimicrobials was 6.9% for the whole period under study.

4.3.5 Tetracyclines

For the tetracyclines resistance increased during the first 3 years of the study from 20.0% in 2002 to 37.0% in 2007 and 28.4% in 2008. It then decreased the following 3 years reaching a low of 0.0% in the year 2011 then increased to 29.8% in 2012 and decreased to 18.8% in 2014 and 13.1% in 2015. In 2016, resistance against tetracyclines was 32.4% and the total cumulative resistance against tetracyclines for the period under study was 22.8%.

4.3.6 Fluoroquinolones

For the fluoroquinolones resistance increased from 30.0% in 2002 to 39.5% in 2007, 42.3% in 2008. Resistance decreased to 39.3% in 2010, 29.2% in 2011 and 27.9% in 2012. Resistance then increased to 40.2% in 2014 and 57.0% in 2016, the highest for all drugs recorded for the study, while the total cumulative resistance for the period under study was 37.8%.

4.3.7 Lincosamides

For the lincosamides resistance decreased from 15.6% in 2007 to 11.5% in 2009 and 0.0% in 2011. It then increased to 3.0% in 2012, 4.4% in 2014 and 5.5% in 2015 then decreased to a low of 0.0% for the last year of study, 2016. The cumulative microbial resistance against the lincosamides was 6.7% for the period under study.

4.3.8 Cephalosporins

For the cephalosporins resistance was 0.0% in the first year of study 2002, rose to 17.0% in 2007 then to 23.7% in 2008 and 29.6% in 2009. In the following year, 2010 resistance decreased to 28.9% then to 20.0% in 2011 and 12.7% in 2012. Resistance rose to 26.2% in 2014 and decreased to 14.8% and 12.6% in 2015 and 2016 respectively recording a total cumulative resistance against cephalosporins of 20.1% for the whole period under study.

4.3.9 Polymixins

For the polymixin group, resistance started on a high of 10.0% during the first year of the study and decreased to 6.7% in 2007, 3.6% in 2008, and 2.3% in 2009 and maintained 0.0% from 2010 to 2014 with no entries for 2015 and 2016. The total cumulative microbial resistance against polymixin class antimicrobials for the period under study was 2.6%.

4.3.10 Phenicols

For the phenicol class, resistance started at a high of 25.0% for the year 2009, then decreased to 0.0% in 2010 and 2011. In 2012, microbial resistance against phenicol antimicrobials rose to 42.5% then decreased to 2.4% in 2014, 6.1% in 2015 and 0.0% in 2016. The total cumulative resistance against phenicols for the period under study was 15.4%.

4.4 Bacterial species resistance

As for the antimicrobial drugs, the different bacteria identified during the study, showed varying degrees of resistance over the years of study. These results are summarised in Table 4.2 as follows:

Table 4.2: Resistance (%) data per bacterial species from 2002 to 2016, and the cumulative resistance over the same period

Bacterium	**Year**										**Total**
	2002	2007	2008	2009	2010	2011	2012	2014	2015	2016	
Trueperella pyogenes	0.0	13.7	5.0	18.4	21.7	0.0	3.5	18.8	-	-	***11.2***
Escherichia coli	-	52.3	47.9	81.3	-	-	43.5	68.8	52.1	-	***55.3***
Histophilus somni	15.0	6.50	7.3	7.7	-	-	8.7	17.5	10.3	20.4	***11.3***
Klebsiella pneumoniae	-	100.00	-	68.8	-	-	34.8	-	34.4	-	***47.4***
Mannheimia species	15.4	24.9	21.9	28.5	28.8	-	16.9	22.0	16.0	20.1	***20.8***
Pasteurella species	24.2	23.5	30.9	22.4	24.6	24.3	20.4	20.1	17.2	33.6	***23.4***
Pseudomonas aeruginosa	-	84.0	60.9	15.3	-	-	52.2	6.3	77.5	40.0	***53.8***
Streptococcus species	-	38.1	37.5	37.5	-	-	47.8	-	53.1	0.0	***39.0***
Total	***18.5***	***25.0***	***26.9***	***23.7***	***27.4***	***18.3***	***18.0***	***22.4***	***18.9***	***23.2***	**22.5**

Using logistic regression and adjusting for effect of year, province and antimicrobial drug class, resistance of *Trueperella pyogenes*, and *Histophilus somni* was 62% [*0.29 – 0.50*] and 65% [*0.29 – 0.41*] less likely compared to the most isolated bacterial species, *Mannheimia* species. *Escherichia coli* and *Klebsiella pneumoniae* were 8 times [*5.81 – 10.66*] and 7 times [*3.93 – 12.4*] more likely to be resistant compared to *Mannheimia* species. *Pasteurella* species and *Pseudomonas aeruginosa* were 41% and 8 times respectively more likely to be resistant than *Mannheimia* species whilst *Streptococcus* species were 4 times [*2.64 – 4.93*] more likely to be resistant than *Mannheimia* species. [*Escherichia coli and Pseudomonas aeruginosa > Klebsiella pneumoniae> Streptococcus* species *>Pasteurella* species > *Mannheimia* species > *Trueperella pyogenes > Histophilus somni*]

4.4.1 Trueperella pyogenes

Trueperella pyogenes resistance against antimicrobials increased from 0.0% in 2002 to 13.7% in 2007, decreased to 5.0% in 2008 and increased to 18.4% in 2009, 21.7% in 2010. In

2011, *T. pyogenes* resistance was 0.0%, increased to 3.5% in 2012 and 18.8% in 2014. The total cumulative resistance of *T. pyogenes* for the period under study was 11.2%.

4.4.2 Escherichia coli

Escherichia coli resistance against antimicrobial drugs was 52.3% in 2007, decreased by 4.4% the following year and increased to 81.3% the next year. The resistance then decreased to 43.5% in 2012, increased to 68.8% in 2014 then decreased to 52.1% in 2015 and recorded a total cumulative average of 55.3% for the period under study.

4.4.3 Histophilus somni

Histophilus somni resistance against antimicrobials was 15.0% in 2002, decreased to 6.5% in 2007, and increased to 7.3% in 2008, 7.7% in 2009, 8.7% in 2012 and 17.5% in 2014. In 2015 the resistance decreased to 10.3% then increased and was 20.45 in the last year of study. The total cumulative resistance for the period under study was 11.3%.

4.4.4. Klebsiella pneumoniae

Klebsiella pneumoniae resistance against antimicrobials was 100.0% in 2007, decreased to 68.8% in 2009, 34.8% in 2012 and a low of 34.4% in 2015. The total cumulative resistance was 47.4% for the period under study.

4.4.5 Mannheimia species

Mannheimia species antimicrobial resistance was 15.4% in 2002, rose to 24.9% in 2007, 28.5% in 2009 and 28.8% in 2010. It then decreased to 16.9% in 2012 and 16.0% in 2015 then

rose in the last year of study to 20.1%. The total cumulative resistance for the period under study was 20.8%.

4.4.6 Pasteurella species

Pasteurella species resistance against antimicrobials was 24.2% in 2002, rose through 2007 to 30.9% in 2008 then decreased to 22.4% in 2009 and rose to 24.3% in 2011. It then decreased to 20.1% in 2014, 17.2% in 2015 and was 33.6% in the last year of the study with a total cumulative value of 23.4% for the whole period under study.

4.4.7 Pseudomonas aeruginosa

Pseudomonas aeruginosa resistance against antimicrobials was 84.0% in 2007 and decreased in the following two years to 60.9% in 2008 and 15.3% in 2009. It then rose to 52.2% in 2012, decreased to 6.3% in 2014 and rose gain to 77.5% in 2015. In the last year of the study it decreased to 40.0% and the total cumulative for the period under study was 53.8%.

4.4.8 Streptococcus, Salmonella and Staphylococcus species

Streptococcus species resistance against antimicrobials was 38.1% in 2007 and 37.5% in 2008 and 2009. Resistance rose to 47.8% in 2012 and 53.1% in 2015 then decreased to 0.0% in 2016 with a total cumulative of 39.0% for the period under study. *Salmonella* species resistance against antimicrobials was 41.7% in 2008, rose to 56.3% in 2009. In 2014 it decreased to 29.2% and the total cumulative resistance for the period under study was 45.8%. *Staphylococcus* species recorded a total cumulative resistance of 17.5% with the resistance in 2007 being 12.5% and in 2014 being 25.0%.

5 DISCUSSION

5.1 Selection pressure

Antimicrobial resistance is of great public health concern as it may compromise therapy for people suffering from multi-resistant bacterial infections. The phenomenon of resistance is of grave concern the world over but more so in developing countries. In the veterinary industry contributors to resistance includes the large scale use of drugs in farming either therapeutically, metaphylactically or prophylactically. Van Boeckel *et al* (2015) projects that due to increasing consumer demand for animal products and by-products in rising middle-income economies and a shift to intensive large-scale farming operations with routine use of antimicrobials, overall antimicrobial usage will increase 66.6% by the year 2030, with a twofold increase in India, Brazil, China and South Africa thus potentially escalating the selection pressure for resistant bacteria strains (Van Boeckel *et al*., 2015). The paramount selection pressure for AMR is drug use and respective amount of drug used (Van De Sande-Bruinsma *et al.,* 2008; Austin *et al.,* 1999).

The phenomenon of selection pressures as a driver of resistance is not a new concept. Studies as early as Levy (1982) noted that introduction of new antimicrobials has always been followed by emergence of resistance against those new antimicrobials (Aarestrup, 2015). In Germany, nalidixic acid resistant bacterial strains increased following enrofloxacin licensing (Malorny *et al.,* 2003). Taiwanese studies have shown the emergence of pig derived quinolone resistant *Salmonella* strains that subsequently spread to the human population (Su *et al.,* 2004; Wang *et al.,* 2006). With regard to usage, Germany, Denmark and Argentina have proven that reduced AGP usage significantly decreases resistance (Aarestrup, 2005). Nonetheless it is worth

noting that other studies documented cases where the prevalence of resistant bacteria remained significantly high without antimicrobial drug use or other notable selection pressures (Call *et al.*, 2008) thereby suggesting no correlation between prevalence of resistant bacteria and antimicrobial use.

5.2 Initiatives for resistance monitoring and surveillance

To mitigate antimicrobial resistance, many countries have adopted local mitigation guidelines. In some cases such as for the Scandinavian countries, antimicrobial use guidelines have been in place for over three decades. These guidelines recommend narrow spectrum antimicrobial use against the most likely causative bacteria (Aarestrup, 2005). These seemingly elementary guidelines have proven effective, with the implementing countries having significantly low antimicrobial consumption levels and consequently low AMR prevalence rates compared to the majority of other countries (WHO, 2018).

In South Africa, State Veterinary Services in the Department of Agriculture, Land Reform and Rural Development (DALRRD) formerly the Department of Agriculture, Forestry and Fisheries (DAFF) have rolled out the South African Veterinary Strategy 2016 to 2026. This initiative seeks to help fulfill particular mandates as required of State Veterinary Services such as the right to adequate and healthy animal derived food, via State policies, strategies and Acts which regulate veterinary services; Animal Diseases Act of 1984 and the Meat Safety Act of 2000. The Veterinary Strategy also highlights the need for a unified AMR monitoring programme between DALRRD and the Department of Health, since antimicrobial resistance in South Africa (SANVAD, 2007) is seen to be on the increase (Eager *et al.*, 2012).

Antimicrobial resistance monitoring helps to assess the magnitude of resistance. Several factors have to be considered when drafting and implementing a monitoring programme such as sampling strategies, target bacterial species and their isolation, susceptibility testing techniques, data analyses and reporting. An ideal method is to sample randomly selected clinically healthy subjects, hinging on epidemiological data relevant to the target population (Aarestrup, 2005). However due to the expense, this method is not always easy to implement. As a result, many surveillance programmes rather rely on samples collected from clinical practice, which is not only cheaper but random. A drawback of using random susceptibility testing results is bias because the requisition basis for susceptibility testing varies among veterinarians and medical doctors alike. In the veterinary field, samples are often collected post empirical treatment and often includes various isolates from the same herd (Aarestrup, 2005). Owing to the above, reporting of findings from such data has to be done cautiously.

Early initiatives in South Africa to combat antimicrobial resistance began with the antimicrobial resistance congress held in Durban in October 2003 that sought to determine a medical and veterinary national policy on antimicrobial resistance. The local veterinary monitoring programme, SANVAD, conducted a pilot study in 2001 based on OIE guidelines.

The first SANVAD report was released in 2007 illustrating higher *E. coli* and *Enterococcus species* resistance rates than those reported for Europe (SANVAD, 2007). After the adoption of the OIE established international standards for the monitoring of AMR in 2002, the total resistance increased by an overall 8.4% over six years to 26.9% attributed to lack of antimicrobial awareness, poor antimicrobial stewardship and increased antimicrobial use. It was also found that antimicrobial resistance awareness campaigns were on the rise and training of personnel on usage of standard surveillance methodologies was being carried out during this

period. The first notable decline is recorded between 2008 and 2009 and decreases in 2008 and 2011 which could be attributed to the establishment of the SANVAD, the consequent SANVAD report published in 2007 and an increased awareness and monitoring of antimicrobial use.

Since the 2007 SANVAD report in South Africa, no focused veterinary surveillance programme has been active in South Africa. As a result it remains unknown if resistance is on the increase in the country. As a surrogate for this study, we evaluate clinical samples from feedlots even though the method likely over estimates resistance. The reason for selection of feedlots is that the system is both organized and a known high user of antimicrobial drugs.

5.3 Resistance by province

The amount of beef produced per province in South Africa depends on feedlots and abattoirs infrastructure (DAFF, 2015). The Eastern Cape province accounts for the largest share of beef production, 24% in 2014, followed by KwaZulu-Natal at 20%, the Free State at 17%, North West at 12% and Mpumalanga at 10% (DAFF, 2015). Gauteng has the highest cumulative resistance of 27.1% yet accounts for 2% of total beef production. This could be linked to higher stocking densities in Gauteng feedlots, bioaccumulation and an interaction with other features of a densely populated province. Further investigations are recommended to establish why KwaZulu-Natal came second on beef production but third highest cumulative resistance after the Free State which is third on beef production but has second highest cumulative resistance. North West was fourth on beef production and had the fourth highest cumulative resistance as well. At present the reason for the difference in the province is unknown. Possible reasons that need further investigation could be climatic as respiratory disease may be more severe and require use of more drugs if the area has dryer winters or is dustier. It may be possible that resistance is

linked to densities of human settlement, which may be indicatory of shared resistance determinants between veterinary and human pathogens.

5.3.1 Fluctuations in resistance

While the resistance fluctuated during the study period, this was not an unexpected finding. Beukers *et al.* (2018) reported fluctuations in resistance as being a result of microbial genotypes changing significantly over time with fluctuations in resistance reflecting a net change in microbial genotypes. Lahra *et al.* (2017), reported that persisting genotypes and fluctuations are the most important determinants of antimicrobial resistance. Lahra *et al.* (2016) found that *Neisseria gonorrhoeae* genotypes circulating in the population change within a 3 year time period while several other numerous though less common genotypes appeared or disappeared. The sum effect of microbial genotype alterations determines the levels of microbial resistance within a population.

5.4 Resistance per antimicrobial drug class

In the South African study by Eager *et al.* (2008), macrolides and pleuromutilins were reported as the antimicrobial class with the largest sales in terms of weight, with specific reference to tylosin and tiamulin respectively. In the 2018 Department of Health report on antimicrobial use, the most commonly used drugs were growth promoters, accounting for 62% of antimicrobials used for animal health (Department of Health, 2018). According to this report the AGPs most commonly used are flavophospholipol, olaquindox, zinc bacitracin, tylosin phosphate and the ionophores, monensin sodium and salinomycin (Department of Health, 2018). With the accepted principle that the level of use is a main driver of resistance, it is not surprising that the cumulative resistance against macrolides was 37% and the third highest to the

aminoglycosides at 45.6%. The macrolides (tylosin, tilmicosin and tulathromycin) are indicated as treatment of *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* associated bovine respiratory diseases which are frequently isolated and are common in feedlots (MSD Vet Manual., 2018). It is more difficult to explain why the aminoglycosides had a cumulative resistance of 45.6%. Gentamicin, amikacin, tobramycin, streptomycin and kanamycin are used to treat local and systemic Gram-negative aerobic bacterial infections of the respiratory tract, abdomen and urinary tract, as well as bacteraemia and endocarditis (DeDonder, 2016; Rinchen, 2016). It is possible that their broad indication translated to high usage and hence high resistance against them by microbes. A positive outcome from this study, was that resistance against aminoglycosides was on the decline from of 58.7% in 2007 and 78.6% in 2010 to 21.7% in 2014 and 20.2% in 2015. This could/may be attributed to increased awareness and improved antimicrobial use stewardship.

The fluoroquinolones recorded a cumulative resistance of 37.8%, second only to aminoglycosides with resistance increasing from 30% in 2002 to 39.3% in 2010 and 57% in 2016. Studies have revealed an increasing prevalence of fluoroquinolone resistant *Campylobacter* strains sampled from livestock and humans subsequent to the initiation of fluoroquinolones as an alternative drug in food animals (Engberg *et al.*, 2001). Danofloxacin and enrofloxacin are routinely indicated for the treatment and control of respiratory disease in cattle, which due to the nature of feedlot conditions, are fairly common (Food and Drug Administration, 2018). The increase in resistance is a worrying trend of increasing concern as fluoroquinolones are among the drugs of choice in several countries for treatment of gut infections (Dalhoff, 2012). It should also be noted that with the removal of colistin from use in animals, the quinolones are suggested as possible alternatives (Gharaibeh and Shatnawi, 2019). The finding

from this study tends to suggest that in South Africa, the quinolones need to be as well protected as colistin.

Eager *et al* (2008) noted that tetracyclines are the second largest group of antimicrobials sold in South Africa and the Department of Health estimated animal consumption of tetracyclines at 27% (278 tons) of total antimicrobial sales (Department of Health, 2018), compared to the OIE reported 640 tons (63%) average for African countries (OIE, 2016; OIE, 2017) demonstrating that South Africa's agro-patterns differ from farming practices of most African countries. In Kenya, tetracyclines account for 55% of the antimicrobial consumption in food animals (Mitema and Kikuvi, 2004). In terms of resistance tetracyclines was fourth at 22.8% with fluctuations over the study period recording a high of 37% in 2002 and a low of 0% in 2011 and 13.1% in 2015. Tetracyclines are effective against *Mycoplasma* and *Chlamydophila* species and are also active against *Erhlichia*, rickettsia and anaplasma (Li *et al.*, 2017). A study by Dargatz *et al. (2002)* found that microbial resistance to tetracyclines and sulfamethoxazole is common in feedlots. A large number of sulphonamides (trimethoprim sulfadiazine/sulfamethoxazole) products are registered under Act 36 of 1947 making them readily available. Sulphonamides are wide spectrum antimicrobials active against Gram-positive bacteria, *Chlamydophila* species and protozoa (Prescott, 2013; Act 36 of 1947; Act 101 of 1965). However, sulphonamides recorded a relatively low cumulative average resistance of 9.1% over the study period with a low of 0% in 2002, 5.1% in 2009 and a high of 28% in 2016.

Penicillins, although being the fourth largest group of antimicrobials sold (Eagar *et al.*, 2008), are primarily used for bovine mastitis treatment with 63% of the South African registered penicillins being intra-mammary preparations used in dairy operations for the treatment of Gram-positive infections and anaerobes. It follows that their use in beef feedlot operations is lower

compared to dairy operations and hence the low resistance of 6.9% when compared to other drug classes (Prescott, 2013). Of note too, is that resistance against the penicillins decreased over the study period from 18.2% in 2002, 14.8% in 2007 to 5.7% in 2015 and 4.6% in 2016. This may be attributed to a general shift from broad to narrow spectrum antimicrobials.

Polymixins have a low resistance of 2.6% owing to their low usage in beef feedlots. Sulfate salts of polymixin B and colistin are clinically limited to topical and oral use because of their systemic toxicity (Oh *et al.,* 2017). Resistance to polymixin decreased from 10% in 2002 to 2.3% in 2009 and was 0% from 2010 to 2014. Polymixin susceptible bacteria develop resistance slowly except when plasmid resistance is present as polymixins are bactericidal surface-active cationic detergents that interfere with cell membrane phospholipids, destabilizing their integrity and disrupting bacterial cell membrane structure (Oh *et al.,* 2017).

5.5 Drug use and resistance

Due to the sensitivity of medical records and information, it is a challenge to obtain accurate and valid information about the consumption volumes and use patterns of antimicrobial agents for medical and growth promoting purposes (Eager *et al.,* 2012; SANVAD, 2007).

DALRRD reports that between 2014 and 2015 the dominant antibiotic classes used in animal health are AGPs, tetracyclines and macrolides at 62%, 27% and 11% respectively. According to DALRRD, AGPs include ionophores, flavophospholipol, olaquindox, zinc bacitracin and tylosin. Tylosin and tetracycline by Act 36 of 1947 (The Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act) are registered as AGPs whilst ionophores are registered as antiparasitics but interpreted as “growth promoters” hence are being reported as growth promoters. Attempts to ameliorate the reporting of this class are being sought. As

reported by the South African DALRRD, of the 4.35 tons of antimicrobials imported in 2014, 77% was estimated for human use and 23% for animal use. In contrast, the animal sector in the USA accounts for 70% of antimicrobial consumption (O Neill, 2016). The South Africa figure estimate is akin to estimates of middle-income countries except India and China (O Neill, 2016).

Locally, on the backdrop of increased use and hence demand, an estimated increment of 58.1% in animal use imports and 38.3% in human use imports was recorded, giving a total estimated import bill of 6,3 tonnes between 2014 and 2015. The total cumulative resistance in feedlots followed this trend and increased the following year from 18.9% in 2015 to 23.2% in 2016. Humans consumed 97.8% of penicillins hence the low usage in animals. There was also a huge decrease in penicillin and fluoroquinolones use, 49% and 26% respectively. In the same period resistance to penicillins decreased from 6.2% to 5.7% yet resistance to fluoroquinolones remained relatively unchanged at 40.2% in 2014 and 40.7% in 2015 despite a 26% decrease in use. Over the same period, sulphonamides imports increased by 398% with a corresponding increase in resistance from 11.6% in 2014 to 28% in 2016 whilst macrolides imports increased by 120% without a significant change in resistance, 33.7% in 2014 and 33.3% in 2016.

The resistance to the quinolones remains a concern, as mentioned above, they are important for medical use. As seen for quinolones, resistance may not decrease with decreased use. Resistance pattern studies suggest that antimicrobial resistance impacts a cost on microbial fitness in such a way that reducing antimicrobial use promotes the selection of sensitive microbial strains over antimicrobial resistant strains by reducing the selection pressure and the subsequent dilution effect (Levin, 2001; Lipsitch, 2001). Furthermore, antimicrobial use is not the sole driver of AMR as the specific classes of antimicrobials used are crucial and resistance mutations may differ between bacterial strains (Dagan *et al.*, 2008).

5.6 Resistance per microbial species

The calculated magnitude of AMR in South African feedlot cattle, 22.5% is relatively high compared to feedlot operations in developed countries where levels of less than 2.5% for Canadian feedlots (Brault *et al.,* 2019), and low to absent levels for Australian feedlots (Abraham *et al.,* 2014; Barlow *et al.,* 2015) was reported. Due to paucity of data on AMR levels in feedlots especially in other African countries we could not compare the results from this study to other countries on the continent (Silbergeld *et al.,* 2008).

Catry *et al.* (2006) reported relatively low levels of antimicrobial resistance in Canadian feedlots of less than 10% for *Pasteurella* species whilst South African herds recorded 23.4% for *Pasteurella* species. In Canadian feedlots, respiratory pathogen isolates showed low resistance levels, save for resistance to sulfamethoxazole in *P. multocida* and *M. haemolytica* and to ampicillin in *M. haemolytica* (Feyen *et al.,* 2005). *Mannheimia* species in South African feedlots recorded a cumulative average of 20.8% with a low of 15.4% in 2002, a high of 28.8% in 2010 and 20.1% in 2016. Thus while resistance has increased steadily from 2002 to 2010, this has plateaued at 20.1% in 2016 suggestive that prudent principles in the use of antimicrobials may be taking effect. Nonetheless the level of resistance is almost double of that reported by Klima *et al.* (2011) for southern Alberta feedlots for *M. haemolytica*.

In the USA, most commensal *Escherichia coli* and *Salmonella* species sampled from dairy cattle faeces on farms in twenty one states had low to none resistance to a wide range of antimicrobials (Lowrance *et al.,* 2007). In South African feedlots, *E. coli* is highly resistant to recommended antimicrobials with a cumulative average of 55.3%, a high of 81.3% in 2009 and a low of 43.5% in 2012. *Escherichia coli* faecal shedding in feedlots is not uncommon and is a public health hazard as the foodborne transmission hazard can lead to fatal disease in humans

(Beauvais *et al.*, 2018). Further support that the resistance seen in feedlots is linked to drug use is evident when results are compared to the results of Mupfunya (2018) in Mnisi rural community, Mpumalanga province. In this study, Mupfunya (2018) found that *E. coli* isolates had low resistance towards colistin 16%, chlortetracycline 8% and were 100% susceptibility to gentamicin.

Klebsiella pnuemoniae and *Pseudomonas aeruginosa* recorded cumulative resistances of 47.4% and 53.8%. This is significantly higher than the total average of 22.5% but it is noted that for both bacteria the resistance decreased from 100% for *K. pneumoniae* in 2007 to 34.4% in 2015 and 84% in 2007 for *P. aeruginosa* to 40% in 2016. In comparison to results from Iran, Azimi *et al.* (2019) found strong association between genotypes of *P. aeruginosa* and *K. pneumoniae*, and resistance levels between 62% and 100% to all tested antimicrobials except colistin and tetracyclines indicative of multi-drug resistance. *Trueperella pyogenes* and *Histophilus somni* recorded cumulative average resistances of 11.2% and 11.3% respectively, the lowest for the bacteria isolated. A Canadian study by Timsit *et al.* (2017) found record levels of oxytetracycline (67%) and penicillin (52%) resistance in *H. somni* isolates. A Brazilian study identified and described *H. somni* as a potential threat to Brazilian beef feedlots Headley *et al.* (2014). An American study by Jost *et al.* (2003), found *T. pyogenes* to be the leading cause of liver abcessation in feedlot cattle. 22,9% of the isolates tested demonstrated inducible or constitutive resistance to tylosin.

This pioneer study has provided a gateway into further research on antimicrobial resistance in food animals and their products. The major limitation of our study was lack of valid scientific data, or its inaccessibility thereof, locally and from other African countries for a clearer regional or continental picture of AMR in feedlot cattle.

6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Antimicrobial resistance in clinical isolates from feedlot cattle increased within the period of study. The cumulative average resistance for the period under study (2002 to 2016) was relatively higher in comparison with other first world countries with established antimicrobial use legislation and strict antimicrobial use policies and guidelines. The antimicrobial resistance in South African feedlot cattle may thus pose a problem to public health and further initiatives to curb and reduce resistance need to be implemented. The tetracyclines, quinolones, sulphonamides and penicillins were of most concern as in addition to being extensively used in animal health, these were the drugs to which the most relevant feedlot bacteria have a statistically significant level of resistance. The public health concern is that these specific antimicrobial classes have analogues that are immensely essential in human medicine (Mitema and Kikuvi, 2004).

6.2 Recommendations

Considering the findings of this study, it is clear that stewardship can have an impact on antimicrobial use. However, the study also showed that resistance mitigation strategies applied in South Africa are not as effective as for other countries. For this we would recommend:

6.2.1 Policy strategy

Drafting and implementing a veterinary antimicrobial use policy that takes into account resistance development and the value of using distinct antimicrobials for medical and veterinary practice.

6.2.2 Adherence to legislation

Adherence to the SAVC Section 10 rules and regulations on compounding and use of veterinary medicines (Veterinary and Para-veterinary Professions Act, 1982). Update and implement a single veterinary medicine Act to allow for stricter control of antimicrobial drugs.

6.2.3 Standardisation of antimicrobial sensitivity tests

Expansion of SANAS accredited veterinary laboratory services to better support detection and surveillance of resistance using harmonized standard operating procedures.

6.2.4 Monitoring of antimicrobial use

Programmes by government in conjunction with relevant stakeholders to continuously monitor the development and prevalence of resistance, and gross consumption of antimicrobials especially in food animal operations in a more proactive manner. Sharing of information on resistance prevalence on all levels so as to advise policy and spot notable changes that necessitate pro-action.

7 REFERENCES

AARESTRUP, F. M. 2015. The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 370, 20140085.

AARESTRUP, F. M., JENSEN, V. F., EMBORG, H.-D., JACOBSEN, E. & WEGENER, H. C. 2010. Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. *American Journal of Veterinary Research,* 71, 726-733.

AARESTRUP, F. M. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic & Clinical Pharmacology & Toxicology,* 96, 271-281.

ABRAHAM, S., GROVES, M. D., TROTT, D. J., CHAPMAN, T. A., TURNER, B., HORNITZKY, M. & JORDAN, D. 2014. Salmonella enterica isolated from infections in Australian livestock remain susceptible to critical antimicrobials. *International Journal of Antimicrobial Agents*, 43, 126-130.

ACAR, J. & ROSTEL, B. 2001. Antimicrobial resistance: an overview. *Revue Scientifique et Technique-Office International des Epizooties,* 20, 797-810.

ADEFISOYE, M. A. & OKOH, A. I. 2016. Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa. *Microbiology Open,* 5, 143-151.

ALBONICO, M., ENGELS, D. & SAVIOLI, L. 2004. Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *International Journal for Parasitology*, 34, 1205-1210.

ALEXANDER, T., INGLIS, G., YANKE, L., TOPP, E., READ, R., REUTER, T. & MCALLISTER, T. 2010. Farm-to-fork characterization of Escherichia coli associated with feedlot cattle with a known history of antimicrobial use. *International Journal of Food Microbiology*, 137**,** 40-48.

ANDERSON, C. R. & MCLACHLAN, S. M. 2012. Exiting, enduring and innovating: Farm household adaptation to global zoonotic disease. *Global Environmental Change*, 22, 82-93.

ANDREWS, R. *Cattle feedlot: Behind the scenes* [Online]. Precision Nutrition. Available: https://www.precisionnutrition.com/cattle-feedlot-visit [Accessed 04 December 2018].

ANTHONY, F., ACAR, J., FRANKLIN, A., GUPTA, R., NICHOLLS, T., TAMURA, Y., THOMPSON, S., THRELFALL, E., VOSE, D. & VAN VUUREN, M. 2001. Antimicrobial resistance: responsible and prudent use of antimicrobial agents in veterinary medicine. *Revue Scientifique et Technique-Office International des Epizooties*, 20, 829-837.

AUBRY-DAMON, H., GRENET, K., SALL-NDIAYE, P., CHE, D., CORDEIRO, E., BOUGNOUX, M.-E., RIGAUD, E., LE STRAT, Y., LEMANISSIER, V. & ARMAND-LEFÈVRE, L. 2004. Antimicrobial

resistance in commensal flora of pig farmers. *Emerging Infectious Diseases,* 10, 873.

AUSTIN, D. J., KRISTINSSON, K. G. & ANDERSON, R. M. 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proceedings of the National Academy of Sciences,* 96, 1152-1156.

AZIMI, L., ALAGHEHBANDAN, R., ASADIAN, M., ALINEJAD, F. & LARI, A. R. 2019. Multi-drug resistant Pseudomonas aeruginosa and Klebsiella pneumoniae circulation in a burn hospital, Tehran, Iran. *GMS hygiene and infection control,* 14.

BAGER, F., MADSEN, M., CHRISTENSEN, J. & AARESTRUP, F. M. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish poultry and pig farms. *Preventive Veterinary Medicine,* 31, 95-112.

BARLOW, R. S., MCMILLAN, K. E., DUFFY, L. L., FEGAN, N., JORDAN, D. & MELLOR, G. E. 2015. Prevalence and antimicrobial resistance of Salmonella and Escherichia coli from Australian cattle populations at slaughter. *Journal of Food Protection,* 78, 912-920.

BEAUVAIS, W., GART, E. V., BEAN, M., BLANCO, A., WILSEY, J., MCWHINNEY, K., BRYAN, L., KRATH, M., YANG, C.-Y. & ALVAREZ, D. M. 2018. The prevalence of Escherichia coli O157: H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: A randomized controlled trial. *PloS ONE,* 13**,** e0192149.

BENNETT, D. E., BERTAGNOLIO, S., SUTHERLAND, D. & GILKS, C. F. 2008. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antiviral Therapy,* 13, 1.

BERGE, A., ATWILL, E. & SISCHO, W. 2005. Animal and farm influences on the dynamics of antibiotic resistance in faecal Escherichia coli in young dairy calves. *Preventive Veterinary Medicine,* 69, 25-38.

BERNABE, K. J., LANGENDORF, C., FORD, N., RONAT, J.-B. & MURPHY, R. A. 2017. Antimicrobial resistance in West Africa: a systematic review and meta-analysis. *International Journal of Antimicrobial Agents,* 50, 629-639.

BEUKERS, A. G., ZAHEER, R., COOK, S. R., CHAVES, A. V., WARD, M. P., TYMENSEN, L., MORLEY, P. S., HANNON, S., BOOKER, C. W. & READ, R. R. 2018. Comparison of antimicrobial resistance genes in feedlots and urban wastewater. *Canadian Journal of Veterinary Research,* 82, 24-38.

BLAKE, D. P., HUMPHRY, R. W., SCOTT, K. P., HILLMAN, K., FENLON, D. R. & LOW, J. C. 2003. Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within commensal Escherichia coli populations. *Journal of Applied Microbiology*, 94, 1087-1097.

BRANDEBOURG, T.D., WOLFE, D.F. & FORADORI, C.D., 2013. US beef industry: a sustainable success story, challenges and priorities. *Journal of Fisheries and Livestock Production*, *1*.

BRAULT, S. A., HANNON, S. J., GOW, S. P., WARR, B. N., WITHELL, J., SONG, J., WILLIAMS, C. M., OTTO, S. J., BOOKER, C. W. & MORLEY, P. S. 2019. Antimicrobial use on 36 beef feedlots in Western Canada: 2008–2012. *Frontiers in Veterinary Science,* 6**,** 329.

CALL, D. R., DAVIS, M. A. & SAWANT, A. A. 2008. Antimicrobial resistance in beef and dairy cattle production. *Animal Health Research Reviews,* 9, 159-167.

CAMPAGNOLO, E. R., JOHNSON, K. R., KARPATI, A., RUBIN, C. S., KOLPIN, D. W., MEYER, M. T., ESTEBAN, J. E., CURRIER, R. W., SMITH, K., THU, K. M. & MCGEEHIN, M. 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. *Science of the Total Environment*, 299, 89-95.

CASEWELL, M., FRIIS, C., MARCO, E., MCMULLIN, P. & PHILLIPS, I. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy*, 52, 159-161.

CATRY, B., DECOSTERE, A., SCHWARZ, S., KEHRENBERG, C., DE KRUIF, A. &HAESEBROUCK, F. 2006. Detection of tetracycline-resistant and susceptible Pasteurellaceae in the nasopharynx of loose group-housed calves. *Veterinary Research Communications*, *30*(7), 707-715.

CENTER FOR DISEASE DYNAMICS, ECONOMICS & POLICY (CDDEP). 2017. Resistance Map. Washington DC.

CROFT, S. L. 2001. Monitoring drug resistance in leishmaniasis. Tropical Medicine & International Health, 6, 899-905.

CULLY, M. 2014. The politics of antibiotics. Nature, 509, 16e17.

DAGAN, R., BARKAI, G., GIVON-LAVI, N., SHARF, A. Z., VARDY, D., COHEN, T., LIPSITCH, M. & GREENBERG, D. 2008. Seasonality of antibiotic-resistant Streptococcus pneumoniae that causes acute otitis media: a clue for an antibiotic-restriction policy? *The Journal of Infectious Diseases,* 197, 1094-1102.

DALHOFF, A. 2012. Global fluoroquinolone resistance epidemiology and implications for clinical use. *Interdisciplinary perspectives on infectious diseases,* 2012.

DANMAP (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. 2005. Available at www.danmap.org/pdfFiles/Danmap_2005.pdf . Accessed 10 April 2007.

DARGATZ, D. A., FEDORKA-CRAY, P. J., LADELY, S. R., FERRIS, K. E., GREEN, A. L. & HEADRICK, M. L. 2002. Antimicrobial susceptibility patterns of Salmonella isolates from cattle in feedlots. *Journal of the American Veterinary Medical Association,* 221**,** 268-272.

DEDONDER, K. D. 2016. Antimicrobial resistance and bovine respiratory disease; a pharmacokinetic/pharmacodynamic approach to macrolide resistance. Kansas State University.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES. 2016. National Veterinary Strategy 2016. Online at: http://www.nda.agric.za/docs/media/Vet%20strategy%20final%20signed.pdf . Website accessed May 2017.

DEPARTMENT OF HEALTH. 2018. Surveillance for Antimicrobial Resistance and Consumption of Antibiotics in South Africa, *p* 4-5

DIKEMAN, M. E. 1984. Cattle Production Systems to Meet Future Consumer Demands. *Journal of Animal Science*, 59, 1631-1643.

DU, J., LIANG, Y., XIN, H., XUE, F., ZHAO, J., REN, L. & MENG, Q. 2010. Evaluation of dry matter intake and average daily gain predicted by the Cornell Net Carbohydrate and Protein System in crossbred growing bulls kept in a traditionally confined feeding system in China. *Asian-Australasian Journal of Animal Sciences,* 23**,** 1445-1454.

DUSE, A. G. 2011. The global antibiotic resistance partnership (GARP). *South African Medical Journal*, 101, 551-551.

EAGAR, H. A., SWAN, G. & VAN VUUREN, M. 2008. *A survey of antimicrobial usage in animals in South Africa with specific reference to food animals*. University of Pretoria.

EAGAR, H., SWAN, G. & VAN VUUREN, M. 2012. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. *Journal of the South African Veterinary Association,* 83, 15-23.

EAGAR, H. & NAIDOO, V. 2017. Veterinary antimicrobial stewardship in South Africa. *International Biology Review,* 1.

EDWARDS, S. E., MOREL, C. M., BUSSE, R. & HARBARTH, S. 2018. Combatting antibiotic resistance together: how can we enlist the help of industry? *Antibiotics,* 7**,** 111.

ENGBERG, J., AARESTRUP, F. M., TAYLOR, D. E., GERNER-SMIDT, P. & NACHAMKIN, I. 2001. Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases,* 7**,** 24.

ESSACK, S., DESTA, A., ABOTSI, R. & AGOBA, E. 2016. Antimicrobial resistance in the WHO African region: current status and roadmap for action. *Journal of Public Health,* 39, 8-13.

FAIRCHILD, A., SMITH, J., IDRIS, U., LU, J., SANCHEZ, S., PURVIS, L., HOFACRE, C. & LEE, M. 2005. Effects of orally administered tetracycline on the intestinal community structure of chickens and on tet determinant carriage by commensal bacteria and Campylobacter jejuni. *Applied and Environmental Microbiology,* 71, 5865-5872.

FERBER, D., 2003. WHO advises kicking the livestock antibiotic habit. *Science*, *301*(5636), pp.1027-1028.

FEYEN, B., VANROBAEYS, M., OPSOMER, G., SCHWARZ, S. & DE KRUIFA, A. 2005. Variability in acquired resistance of pasteurella and mannheimia isolates. *Differentiation and Antimicrobial Resistance,* p.81.

FOKA, F. E. T., KUMAR, A. & ATEBA, C. N. 2018. Emergence of vancomycin-resistant enterococci in South Africa: Implications for public health. *South African Journal of Science,* 114, 1-7.

FREAN, J., PEROVIC, O., FENSHAM, V., MCCARTHY, K., GOTTBERG, A. V., GOUVEIA, L. D., POONSAMY, B., DINI, L., ROSSOUW, J. & KEDDY, K. 2012. External quality assessment of national public health laboratories in Africa, 2002-2009. *Bulletin of the World Health Organization,* 90, 191-199.

GAGEA, M. I., BATEMAN, K. G., VAN DREUMEL, T., MCEWEN, B. J., CARMAN, S., ARCHAMBAULT, M., SHANAHAN, R. A. & CASWELL, J. L. 2006. Diseases and pathogens associated with mortality in Ontario beef feedlots. *Journal of Veterinary Diagnostic Investigation,* 18**,** 18-28.

GALLO, G. F. & BERG, J. L. 1995. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. *The Canadian Veterinary Journal,* 36**,** 223.

GELBAND, H. & DUSE, A. 2011. Executive summary. *South African Medical Journal,* 101, 551-551.

GHARAIBEH, M. H. & SHATNAWI, S. Q. 2019. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global

responses, and the alternatives to colistin: A review. *Veterinary World,* 12**,** 1735.

GIBB, D., SCHWARTZKOPF-GENSWEIN, K., MCALLISTER, T., GENSWEIN, B. & STREETER, M. 2006. Effect of sub-therapeutic antibiotics and auction exposure on health, performance, and feeding behavior of weaned calves. *Canadian Journal of Animal Science,* 86, 457-460.

GILCHRIST, M. J., GREKO, C., WALLINGA, D. B., BERAN, G. W., RILEY, D. G. & THORNE, P. S. 2007. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives,* 115, 313-316.

GLITSCH, K. 2000. Consumer perceptions of fresh meat quality: cross-national comparison. *British Food Journal,* 102, 177-194.

GOFF, D. A., KULLAR, R., GOLDSTEIN, E. J., GILCHRIST, M., NATHWANI, D., CHENG, A. C., CAIRNS, K. A., ESCANDÓN-VARGAS, K., VILLEGAS, M. V. & BRINK, A. 2017. A global call from five countries to collaborate in antibiotic stewardship: united we succeed, divided we might fail. *The Lancet Infectious Diseases,* 17, e56-e63.

HAINES, D. M., MARTIN, K. M., CLARK, E. G., JIM, G. K. & JANZEN, E. D. 2001. The immunohistochemical detection of Mycoplasma bovis and bovine viral diarrhea virus in tissues of feedlot cattle with chronic, unresponsive respiratory disease and/or arthritis. *The Canadian Veterinary Journal,* 42, 857.

HAN, X., HUBBERT, B., HUBBERT, M. & REINHARDT, C. 2016. Overview of the beef cattle industry in China: The widening deficit between demand and output in a vicious circle. *Journal of Fisheries & Livestock Production*, 1-6.

HARLAND, R. J., JIM, G. K., GUICHON, P. T., TOWNSEND, H. G. & JANZEN, E. D. 1991. Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves. *The Canadian Veterinary Journal*, 32, 163.

HARRINGTON, L. M. & LU, M. 2002. Beef feedlots in southwestern Kansas: local change, perceptions, and the global change context. *Global Environmental Change*, 12, 273-282.

HASHMI, M. Z., STREZOV, V. & VARMA, A. 2017. *Antibiotics and Antibiotics Resistance Genes in Soils: Monitoring, Toxicity, Risk Assessment and Management*, Springer.

HEADLEY, S., ALFIERI, A., OLIVEIRA, V., BEUTTEMMÜLLER, E. & ALFIERI, A. 2014. Histophilus somni is a potential threat to beef cattle feedlots in Brazil. *Veterinary Record*, 175, 249-249.

HENRICKSON, R., POPE, L. & HENDRICKSON, R. 1965. Effect of Rate of Gain of Fattening Beef Calves on Carcass Composition 1. *Journal of Animal Science*, 24, 507-513.

HERRERO, M., HAVLÍK, P., VALIN, H., NOTENBAERT, A., RUFINO, M. C., THORNTON, P. K., BLÜMMEL, M., WEISS, F., GRACE, D. & OBERSTEINER, M. 2013. Biomass use, production, feed efficiencies,

and greenhouse gas emissions from global livestock systems. *Proceedings of the National Academy of Sciences*, 110, 20888-20893.

HERSOM, M., HORN, G., KREHBIEL, C. & PHILLIPS, W. 2004. Effect of live weight gain of steers during winter grazing: I. Feedlot performance, carcass characteristics, and body composition of beef steers. *Journal of Animal Science*, 82, 262-272.

https://www.msdvetmanual.com/pharmacology/antibacterial-agents/macrolides#targetText=Tilmicosin%2C%20gamithromycin%2C%20and%20tulathromycin%20are%20approved%20for%20use%20in%20treatment,Pasteurella%20multocida%2C%20and%20Histophilus%20somni.

https://www.fda.gov/animal-veterinary/antimicrobial-resistance/extralabel-use-and-antimicrobials#targetText=Two%20fluoroquinolone%20drugs%20are%20approved,high%2Drisk%20of%20developing%20disease.

HUGHES, P. & HERITAGE, J. 2004. Antibiotic growth-promoters in food animals. *FAO Animal Production and Health Paper***,** 129-152.

HUYGHEBAERT, G., DUCATELLE, R. & VAN IMMERSEEL, F. 2011. An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, 187, 182-188.

ILEA, R. C. 2009. Intensive livestock farming: Global trends, increased environmental concerns, and ethical solutions. *Journal of Agricultural and Environmental Ethics*, 22, 153-167.

JACKSON, B. R., TARR, C., STRAIN, E., JACKSON, K. A., CONRAD, A., CARLETON, H., KATZ, L. S., STROIKA, S., GOULD, L. H. & MODY, R. K. 2016. Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak detection and investigation. *Reviews of Infectious Diseases,* 63, 380-386

JENSEN, L. B., HAMMERUM, A. M., HASMAN, H. & AARESTRUP, F. M. 2002. Effects of the termination of antibiotic growth promoters use on presence of resistance genes in bacterial isolates from production animals. *Beyond Antimicrobial Growth Promoters in Food Animal Production,* 32.

JONKER, A. & PICARD, J. 2010. Antimicrobial susceptibility in thermophilic Campylobacter species isolated from pigs and chickens in South Africa. *Journal of the South African Veterinary Association,* 81, 228-236.

JOST, B. H., FIELD, A. C., TRINH, H. T., SONGER, J. G. & BILLINGTON, S. J. 2003. Tylosin resistance in Arcanobacterium pyogenes is encoded by an Erm X determinant. *Antimicrobial Agents and Chemotherapy,* 47, 3519-3524.

KATSUNUMA, Y., HANAZUMI, M., FUJISAKI, H., MINATO, H., HASHIMOTO, Y. & YONEMOCHI, C. 2007. Associations between the use of antimicrobial agents for growth promotion and the occurrence of antimicrobial-resistant Escherichia coli and enterococci in the feces of livestock and livestock farmers in Japan. *The Journal of General and Applied Microbiology,* 53, 273-279.

KLIMA, C., ALEXANDER, T., READ, R., GOW, S., BOOKER, C., HANNON, S., SHEEDY, C., MCALLISTER, T. & SELINGER, L. 2011. Genetic characterization and antimicrobial susceptibility of Mannheimia haemolytica isolated from the nasopharynx of feedlot cattle. *Veterinary microbiology,* 149, 390-398.

LAHRA, M.M., TREMBIZKI, E., BUCKLEY, C., DONOVAN, B., CHEN, M., GUY, R., KUNDU, R.L., REGAN, D.G., & WHILEY, D.M. 2017. Changes in the rates of Neisseria gonorrhoeae antimicrobial resistance are primarily driven by dynamic fluctuations in common gonococcal genotypes. *Journal of Antimicrobial Chemotherapy, 72*(3), pp.705-711.

LAXMINARAYAN, R. ed., 2010. *Battling resistance to antibiotics and pesticides: an economic approach*. Routledge.

LEVIN, B. R. 2001. Minimizing potential resistance: a population dynamics view. *Clinical Infectious Diseases,* 33, S161-S169.

LEVY, S.B., 1982. Microbial resistance to antibiotics. An evolving and persistent problem. *Lancet, 2,* pp.83-88.

LI, M., ZHANG, X., HUANG, K., QIU, H., ZHANG, J., KANG, Y. & WANG, C. 2017. Presence of Chlamydia trachomatis and Mycoplasma spp., but not Neisseria gonorrhoeae and Treponema pallidum, in women undergoing an infertility evaluation: high prevalence of tetracycline resistance gene tet (M). *AMB Express,* 7**,** 1-9.

LIPSITCH, M., 2001. The rise and fall of antimicrobial resistance. *Trends in Microbiology, 9*(9), pp.438-444.

LOWRANCE, T. C., LONERAGAN, G. H., KUNZE, D. J., PLATT, T. M., IVES, S. E., SCOTT, H. M., NORBY, B., ECHEVERRY, A. & BRASHEARS, M. M. 2007. Changes in antimicrobial susceptibility in a population of Escherichia coli isolated from feedlot cattle administered ceftiofur crystalline-free acid. *American Journal of Veterinary Research,* 68, 501-507

MALORNY, B., SCHROETER, A., GUERRA, B. AND HELMUTH, R., 2003. Incidence of quinolone resistance in strains of Salmonella isolated from poultry. cattle and pigs in Germany between 1998 and 2001. *Veterinary Record*, *153*(21), pp.643-648.

MARSHALL, B. M. & LEVY, S. B. 2011. Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews,* 24, 718-733.

MARTINEZ, J. & BAQUERO, F. 2000. Mutation frequencies and antibiotic resistance. *Antimicrobial Agents and Chemotherapy,* 44, 1771-1777.

MATHER, A. E., MATTHEWS, L., MELLOR, D. J., REEVE, R., DENWOOD, M. J., BOERLIN, P., REID-SMITH, R. J., BROWN, D. J., COIA, J. E. & BROWNING, L. M. 2012. An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations. *Proceedings of the Royal Society of London B: Biological Sciences,* 279, 1630-1639.

MATHEW, A. G., CISSELL, R. & LIAMTHONG, S. 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathogens and Disease,* 4, 115-133.

MCALPINE, C.A., ETTER, A., FEARNSIDE, P.M., SEABROOK, L. & LAURANCE, W.F., 2009. Increasing world consumption of beef as a driver of regional and global change: A call for policy action based on evidence from Queensland (Australia), Colombia and Brazil. *Global Environmental Change*, *19*(1), pp.21-33.

McEVOY, J. 2002. Contamination of animal feedingstuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. *Analytica Chimica Acta*, 473, 3-26.

MEISSNER, H., SCHOLTZ, M. & PALMER, A. 2013. Sustainability of the South African livestock sector towards 2050 Part 1: Worth and impact of the sector. *South African Journal of Animal Science*, 43, 282-297.

MITEMA, E. & KIKUVI, G. 2004. Surveillance of the overall use of antimicrobial drugs in humans over a 5 year period (1997–2001) in Kenya. *Journal of Antimicrobial Chemotherapy,* 54, 966-967.

MØLBAK, K., BAGGESEN, D. L., AARESTRUP, F. M., EBBESEN, J. M., ENGBERG, J., FRYDENDAHL, K., GERNER-SMIDT, P., PETERSEN, A. M. & WEGENER, H. C. 1999. An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype Typhimurium DT104. *New England Journal of Medicine*, 341, 1420-1425.

MOTRO, Y. & MORAN-GILAD, J. 2017. Next-generation sequencing applications in clinical bacteriology. *Biomolecular Detection and Quantification*, 14, 1-6.

MUPFUNYA, C. R. 2018. *Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community Mpumalanga South Africa.* University of Pretoria.

NARMS (National Antimicrobial Resistance Monitoring System). Annual veterinary isolates data. Centers for Disease Control. U.S. Department of Health and Human Services. Washington, DC: 2005. Available at www.ars.usda.gov/Main/docs.htm?docid_6750 . Accessed 16 June 2018

NGUYEN, T. L. T., HERMANSEN, J. E. & MOGENSEN, L. 2010. Environmental consequences of different beef production systems in the EU. *Journal of Cleaner Production,* 18, 756-766.

NOVICK, R. P. 1981. The development and spread of antibiotic-resistant bacteria as a consequence of feeding antibiotics to livestock. *Annals of the New York Academy of Sciences,* 368, 23-60.

OFFICE INTERNATIONAL DES EPIZOOTIES. 2016. Annual report on the use of antimicrobial agents in animals; Better understanding of the global situation.

OFFICE INTERNATIONAL DES EPIZOOTIES. 2017. Annual report on the use of antimicrobial agents in animals; Better understanding of the global situation.

OH, Y. J., PLOCHBERGER, B., RECHBERGER, M. & HINTERDORFER, P. 2017. Characterizing the effect of polymyxin B antibiotics to lipopolysaccharide on Escherichia coli surface using atomic force microscopy. *Journal of Molecular Recognition,* 30**,** e2605.

OKEKE, I. N., LAXMINARAYAN, R., BHUTTA, Z. A., DUSE, A. G., JENKINS, P., O'BRIEN, T. F., PABLOS-MENDEZ, A. & KLUGMAN, K. P. 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet Infectious Diseases,* 5, 481-493.

OLUWASILE, B., AGBAJE, M., OJO, O. & DIPEOLU, M. 2014. Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto Journal of Veterinary Sciences,* 12, 45-50.

O'NEILL, J., 2016. Review on Antimicrobial Resistance: Tackling drug-resistant infections globally. *Wellcome Trust and the Department of Health of UK Government*.

PRESCOTT, J. F. 2013. Sulfonamides, diaminopyrimidines, and their combinations. *Antimicrobial therapy in veterinary medicine,* 5**,** 279-94.

PRICE, L. B., GRAHAM, J. P., LACKEY, L. G., ROESS, A., VAILES, R. & SILBERGELD, E. 2007. Elevated risk of carrying gentamicin-resistant Escherichia coli among US poultry workers. *Environmental Health Perspectives,* 115, 1738-1742.

RINCHEN, S., 2016. Non-Patent Drug Usage In Veterinary Practice Of Bhutan

ROE, M. & PILLAI, S. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. *Poultry science,* 82**,** 622-626.

RONQUILLO, M. G. & HERNANDEZ, J. C. A. 2017. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. *Food Control,* 72, 255-267.

SALYERS, A. A., GUPTA, A. & WANG, Y. 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in Microbiology,* 12, 412-416.

SHAPOURI, S., WHITE, T. K. & KHEDR, H. 1985. Egyptian feedlot practices, costs, and returns. ERS staff report (USA).

SILBERGELD, E. K., GRAHAM, J. & PRICE, L. B. 2008. Industrial Food Animal Production, Antimicrobial Resistance, and Human Health. *Annual Review of Public Health,* 29, 151-169.

SMITH, R. A. 1998. Impact of disease on feedlot performance: a review. *Journal of Animal Science,* 76**,** 272-274.

SMITH, R. A. 2004. Feedlot diseases and their control. *Medecin Veterinaire Du Quebec*., 34, 50-51.

SOUTH AFRICAN NATIONAL VETERINARY SURVEILLANCE AND MONITORING PROGRAMME FOR RESISTANCE TO ANTIMICROBIAL DRUGS (SANVAD), 2007. University of Pretoria and ARC-Onderstepoort Veterinary Institute. Pretoria.

SU, L.H., CHIU, C.H., CHU, C. AND OU, J.T., 2004. Antimicrobial resistance in nontyphoid Salmonella serotypes: a global challenge. *Clinical infectious diseases*, *39*(4), pp.546-551.

TASHO, R. P. & CHO, J. Y. 2016. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment,* 563, 366-376.

TAYLOR, J.D., FULTON, R.W., LEHENBAUER, T.W., STEP, D.L. & CONFER, A.W., 2010. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors?. *The Canadian Veterinary Journal*, *51*(10), p.1095.

THOMSON, D., EISENBARTH, J., SIMROTH, J., FRESE, D., LEE, T., STEPHENS, M. & SPARE, M. Beef cattle transportation issues in the United States. *Proceedings of the American association of bovine practitioners 2015 Conference*, New Orleans, LA. 2017.

TIMSIT, E., HALLEWELL, J., BOOKER, C., TISON, N., AMAT, S. & ALEXANDER, T. W. 2017. Prevalence and antimicrobial susceptibility of Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni isolated from the lower respiratory tract of healthy feedlot cattle and those diagnosed with bovine respiratory disease. *Veterinary Microbiology*, 208, 118-125.

UCHIL, R.R., KOHLI, G.S., KATEKHAYE, V.M. & SWAMI, O.C., 2014. Strategies to combat antimicrobial resistance. *Journal of clinical and diagnostic research: JCDR*, *8*(7), p.ME01.

VAN BOECKEL, T. P., BROWER, C., GILBERT, M., GRENFELL, B. T., LEVIN, S. A., ROBINSON, T. P., TEILLANT, A. & LAXMINARAYAN, R. 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, 112, 5649-5654.

VAN DE SANDE-BRUINSMA, N., GRUNDMANN, H., VERLOO, D., TIEMERSMA, E., MONEN, J., GOOSSENS, H., FERECH, M. & SYSTEM, E. A. R. S. 2008. Antimicrobial drug use and resistance in Europe. *Emerging Infectious Diseases*, 14, 172.

VAN DEN BOGAARD, A., WILLEMS, R., LONDON, N., TOP, J. & STOBBERINGH, E. 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, 49, 497-505.

VAN DEN HONERT, M.S., GOUWS, P.A. & HOFFMAN, L.C., 2018. Importance and implications of antibiotic resistance development in livestock and wildlife farming in South Africa: A Review. *South African Journal of Animal Science, 48*(3), pp.401-412.

VAN DONKERSGOED, J. 1992. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. *The Canadian Veterinary Journal,* 33, 786.

VAN DUIJKEREN, E., GREKO, C., PRINGLE, M., BAPTISTE, K. E., CATRY, B., JUKES, H., MORENO, M. A., POMBA, M. C. M. F., PYÖRÄLÄ, S. & RANTALA, M. 2014. Pleuromutilins: use in food-producing animals in the European Union, development of resistance and impact on human and animal health. *Journal of Antimicrobial Chemotherapy,* 69, 2022-2031.

Veterinary and Para-veterinary Professions Act, 1982 (Act no. 19 of 1982), as amended 2015. Rules relating to the practice of veterinary professions.

Online at: http://www.savc.org.za/pdf_docs/act_19_of_1982.pdf Website accessed November 2019.

VOSS, A., LOEFFEN, F., BAKKER, J., KLAASSEN, C. & WULF, M. 2005. Methicillin-resistant Staphylococcus aureus in pig farming. *Emerging Infectious Diseases,* 11.

WANG, J.-Y., HWANG, J.-J., HSU, C.-N., LIN, L.-C. & HSUEH, P.-R. 2006. Bacteraemia due to ciprofloxacin-resistant Salmonella enterica serotype Choleraesuis in adult patients at a university hospital in Taiwan, 1996–2004. *Epidemiology & Infection,* 134**,** 977-984.

WEBB, E. C. 2013. The ethics of meat production and quality-a South African perspective. *South African Journal of Animal Science,* 43, S2-S11.

WENZEL, R.P. & EDMOND, M.B., 2000. Managing antibiotic resistance. *New England Journal of Medicine*, *343*, pp.1961-1963.

WHO & FAO. 2015. Codex alimentarius: Codex texts on foodborne antimicrobial resistance (p. 223). Food and Agriculture Organization of the United Nations and World Health Organization

WORLD HEALTH ORGANIZATION, 2013. *Pocket book of hospital care for children,* Geneva, Switzerland, WHO.

WORLD HEALTH ORGANIZATION, Antimicrobial Resistance. 2014. Global Report on Surveillance. *Antimicrobial Resistance, Global Report on Surveillance*.

WORLD HEALTH ORGANIZATION, 2018. *Antimicrobial resistance and primary health care* (No. WHO/HIS/SDS/2018.57). World Health Organization.

ZHANG, X. Y., DING, L. J. & FAN, M. Z. 2009. Resistance patterns and detection of aac (3)-IV gene in apramycin-resistant Escherichia coli isolated from farm animals and farm workers in northeastern of China. *Research in Veterinary Science*, 87, 449-454.

ZINN, R. 2004. A guide to feed mixing. University of California, Davis.