ANTIMICROBIAL RESISTANCE PROFILES OF SELECTED COMMENSAL BACTERIA ISOLATED FROM IMPALA (*AEPYCEROS MELAMPUS*) AND THEIR WATER SOURCES IN THE KRUGER NATIONAL PARK

By

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Submitted in partial fulfilment of the requirements for the degree of
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Date submitted: February 2013

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and gratitude to:

- Ms J Greyling for her valuable guidance, patience, friendly support and technical assistance in the laboratory. Without her support this study would not have been possible.

- Dr J Picard, Dr A Jenkins and Prof. M van Vuuren for their guidance, patience and their financial contributions to this study.

- Danny Govender, At Dekker and Onica the game guard at Kruger National Park Scientific Services for their assistance with the sampling trip.

- Bacteriology Laboratory, Department of Veterinary Tropical Diseases for the use of their facilities.

- My family and Byron for their love, patience, encouragement and keen support.

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LIST OF ABBREVIATIONS

µg	Microgram
µm	Micrometre
AMP	Ampicillin
BHI	Brain-heart infusion
CBA	Columbia blood agar
CFU	Colony Forming Units
CLI	Clindamycin
CLSI	Clinical Laboratory Standards Institute
CTET	Chlortetracycline
DANO	Danofloxacin
DWA	Department of Water Affairs
E. cloacae	*Enterobacter cloacae*
E. coli	*Escherichia coli*
E. durans	*Enterococcus durans*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
ENRO	Enrofloxacin
et al.	*Et alii* (and others)
FFN	Florfenicol
g	Gram
GEN	Gentamicin
GPS	Global Positioning System
i.e.	*Id est* (that is)
km	Kilometre
KNP	Kruger National Park
MAC	MacConkey agar
MIC	Minimum inhibitory concentration
mℓ	Millilitre
NEO	Neomycin
OIE	World Organisation for Animal Health
OXY	Oxytetracycline
PEN	Penicillin
SANVAD	South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs

SDM	Sulphadimethoxine
SPE	Spectinomycin
Spp.	Species
SXT	Trimethoprim/Sulfamethoxazole
TIA	Tiamulin
TIL	Tilmicosin
TIO	Ceftiofur
TUL	Tulathromycin
TYLT	Tylosin tartrate
WHO	World Health Organisation

SUMMARY

Antimicrobial resistance profiles of selected commensal bacteria isolated from impala (*Aepyceros melampus*) and their water sources in the Kruger National Park

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Worldwide there is a growing concern of the emergence and evolution of antimicrobial resistance among bacterial pathogens, which poses a threat to human and animal health. The extensive use and misuse of antimicrobials in human and veterinary clinical therapy and agricultural practices have been a major selective force for the emergence, selection, and dissemination of antimicrobial resistant bacteria and resistant genes.

Commensal bacteria constitute a reservoir of resistant genes and their level of resistance is considered to be a good indicator for resistance problems to be expected in pathogens. The monitoring of the prevalence of resistance in indicator bacteria such as faecal *Escherichia coli* and enterococci in different human and animal populations allows the comparison of the prevalence of resistance and to detect transfer between animals and humans and vice versa.

Antimicrobial resistance has however, been found in the bacteria of wildlife not exposed to antimicrobials and living in remote areas of this earth. This has implications for resistance control strategies. Previous studies on antimicrobial resistance in wildlife have yielded contrasting results, such as an almost complete absence of resistance in enterobacteria isolated from moose, deer and vole in Finland compared to a high prevalence of resistance in faecal bacteria from wild rodents living in northwest England, which are possibly due to

differences in the ecological systems and the proximity to anthropogenic activities. This study further investigates the phenomenon of antimicrobial resistance in wildlife.

A previous study conducted in the conservancy area of Kruger National Park (KNP) within South Africa showed that surface water could be a possible source of antimicrobial resistance in unexposed animal populations and that impala (*Aepyceros melampus*) were good sentinel animals for the documentation of antimicrobial resistance through rivers. This current study followed on this hypothesis and investigated the prevalence of resistance in commensal bacteria isolated from impala and their water sources in KNP.

The following four perennial river systems were selected: the Olifants, the Letaba, the Crocodile, the Sabie-Sand Rivers. Samples of river water (n=11) and faeces (n=165) were collected at 11 different sites along these rivers. Samples were directly plated and resistant colonies were selected by means of discs containing antimicrobials (direct plating method). Resistant colonies that grew in the presence of antimicrobials were cultured and identified. Isolates of *E. coli* (n=12), *Enterobacter cloacae* (n=49), *Pantoea* species (n=9), *Enterococcus faecalis* (n=59), *Enterococcus faecium* (n=4) and *Enterococcus durans* (n=64) were tested for susceptibility to a selection of commonly used veterinary antimicrobial drugs. Susceptibility to 18 antimicrobial drugs was determined by means of minimum inhibitory concentrations (MIC) using a commercial MIC test (Sensititre® Bovine/Porcine plate format BOP06F).

Our results allow us to give further support to our working hypothesis that antimicrobial resistance, as evidenced in the impala faeces, may have been due to the impala drinking from the polluted rivers, knowing full well that impala are not routinely subjected to any form of antimicrobial treatment. Although the isolates obtained from the water sources were not as many as those obtained from the faecal samples, a degree of resistance was also observed across all the four river systems that we isolated bacteria from, and this was also evident in the faecal samples as well. Our results also further add to the importance of wildlife as sentinels in environmental antimicrobial resistance studies.

CHAPTER 1

1. INTRODUCTION

Antimicrobial resistance is a worldwide problem. There is a growing concern about the increased prevalence of antimicrobial resistance and in recent years it has been the topic of many scientific papers. Antimicrobial drugs are used for therapy and prophylaxis of infectious diseases in humans and animals and have been used extensively as growth promoters in livestock. The general consensus is that the increased use and misuse of antimicrobial drugs are the main risk factors for the emergence, selection, and dissemination of antimicrobial resistant bacteria and resistance genes (Van den Bogaard & Stobberingh 2000; Sayah *et al*. 2005). The risk of the spread of antimicrobial resistance between species in different environments has led the World Organisation for Animal Health (OIE) and the World Health Organisation (WHO) to see antimicrobial resistance as a significant threat to animal and human health (WHO 2002; OIE 2011). The emergence of multi-drug resistant bacteria coupled with the decrease in the research and development of new antimicrobial drugs by pharmaceutical companies - as much as 56% in the past 20 years - is cause for major concern (Spellberg *et al*. 2004; Bartoloni *et al*. 2008). To avoid this serious threat to animal and human health, academia, biotechnology, the pharmaceutical industry, regulators, healthcare providers and veterinarians must find solutions to this problem (Norrby, Nord & Finch 2005).

Antimicrobial resistance has come about due to a variety of circumstances, including the adaptive resistance mechanisms of bacteria, farming practices and the extensive use of human and veterinary medicines producing selective pressure and the transmission of bacteria amongst humans and animals. Originally, antimicrobial resistance was solely nosocomial, but a gradual shift from the hospital setting to the community at large has occurred. Presently, the commensal and environmental bacterial flora are considered to be a reservoir of antimicrobial resistance, and resistance appears to have spread to remote areas with only limited antimicrobial pressure. Commensal bacteria, especially intestinal commensal bacteria in humans and animals have been suggested to play a major role in the spread of antimicrobial resistance (Skurnik *et al*. 2006). Evidence has shown that antimicrobial resistance is exchanged between bacteria and is already present in bacteria in natural environments (Davison 1999; Kümmerer 2004). Bacteria are either naturally resistant (bacterial defence against natural antibiotics), have become resistant by the use of antimicrobials (selective pressure) or by the uptake and exchange of genetic material

encoding resistance in the environment and from other bacteria of similar species (Kümmerer 2004).

Water has been shown to play a role in the dissemination of resistant bacteria among human and animal populations and also as the route by which resistance genes are introduced into natural bacterial ecosystems in the environment. In natural environments non-pathogenic bacteria could serve as a reservoir of resistance genes and platforms (Bacquero, Martínez & Cantón 2008). Resistant bacteria and resistance genes in the environment are now being seen as an ecological problem (Kümmerer 2004). Commensal bacteria isolated from humans and wild animals living in remote places and that have not been exposed to antimicrobials in any great quantity have showed high rates of acquired antimicrobial resistance (Bartoloni *et al*. 2008). The origins of antimicrobial resistance in the environment is relevant to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens (Allen *et al*. 2010).

Programmes for the surveillance of antimicrobial resistance have been implemented in many countries worldwide (Aarestrup 2006). In South Africa the monitoring of antimicrobial resistance in bacteria of animal origin was implemented by the creation of the South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs (SANVAD). In accordance with OIE guidelines, SANVAD is based on three categories of bacteria, namely indicator bacteria, zoonotic bacteria and animal pathogenic bacteria (SANVAD 2007). The monitoring of the spread of antimicrobial resistance into environments where antimicrobials are not used has not yet been well researched in South Africa (Mariano *et al*. 2009).

Large nature reserves in South Africa provide good study areas for the dissemination of antimicrobial resistance in nature; this is because the wildlife populations have never been treated with antimicrobials, mostly live in low population densities and often have no direct contact with domesticated animals and human populations. Theoretically commensal bacteria, especially those found in the intestine of wild animals, should have low levels of resistance to antimicrobials hence these bacteria can be used to study the role that environmental and specifically, water pollution play in the dissemination of antimicrobial resistance. A study found that impala (*Aepyceros melampus*) drinking from polluted waters were 19.3 times more likely to carry enteric *Escherichia coli* that had phenotypic resistance to tetracycline than those drinking from waters that did not contain tetracycline resistant *E. coli*. The study further showed that in the Kruger National Park (KNP) impala were good

sentinel animals for the documentation of antimicrobial resistance through rivers (Mariano *et al*. 2009).

The objectives of this research project were to monitor for antimicrobial resistance in an ecological system not known to be under any antimicrobial pressure and to compare the antimicrobial resistance in impala, a sentinel animal, and their water sources along four perennial river systems in KNP.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Definition of antimicrobial resistance

The phenomenon of antimicrobial resistance is complex and involves many different antimicrobial drugs, species of bacteria, resistance genes, and mechanisms of resistance (Aarestrup 2006).

The word “antimicrobial” is from the Greek *anti* which means “against”, *micros* which means “little”, and *bios* which means “life”, and therefore antimicrobial drugs are substances against life of microorganisms. “Antibiotics” is from the Greek *anti* and *biotikos*, “concerning life” and may be defined as “chemical substances that are produced by microorganisms and that have the capacity, in dilute solution, to selectively inhibit the growth of and even to destroy other microorganism” (Aarestrup 2006).

Both professional and non-professional people often refer to substances used for the treatment of bacterial diseases in humans and animals as “antibiotics”. The terms “antimicrobial drug” and “antibiotic”, however, are not the same as the definition of the term “antibiotic” pertains only to substances of microbial origin acting on microorganisms. This should not be used for synthetic or semi-synthetic compounds or substances of plant or animal origin, and substances active against animal cells (Aarestrup 2006).

The definitions generally used for antimicrobial resistance are based on microbiological (*in vitro* resistance) and clinical (*in vivo* resistance) criteria. A strain is defined as resistant if it grows in the presence of higher concentrations of the drug compared with phylogenetically related strains, according to the microbiological definition. A strain is defined as resistant when it survives antimicrobial therapy, according to the clinical definition (Aarestrup 2006). The pharmacological definition of antimicrobial resistance is where the bacteria survive a range of concentrations expressing the various amounts of an antimicrobial drug present in the different compartments of the body when the antimicrobial drug is administered at the recommended dose (Acar & Röstel 2001). In epidemiological terms it is any group of bacterial strains that can be distinguished from the normal (Gauss) distribution of minimum inhibitory concentrations (MIC) to an antimicrobial drug (Acar & Röstel 2001).

Under laboratory conditions, resistance can be quantified by determining the MIC of a given drug (Aarestrup 2006). MIC is defined as the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test (CLSI 2008). A strain may be classified as resistant, intermediate or susceptible based on published breakpoints (Aarestrup 2006).

2.2 Mechanisms of resistance to antimicrobial drugs

In order for bacteria to survive in the presence of antimicrobial drugs, they must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial drug (MSU 2011). Bacteria have developed various mechanisms to resist the action of antimicrobial drugs (Figure 1). These mechanisms include:

- Preventing access - impermeable barrier
- Use of efflux pumps
- Drug modification
- Target modification

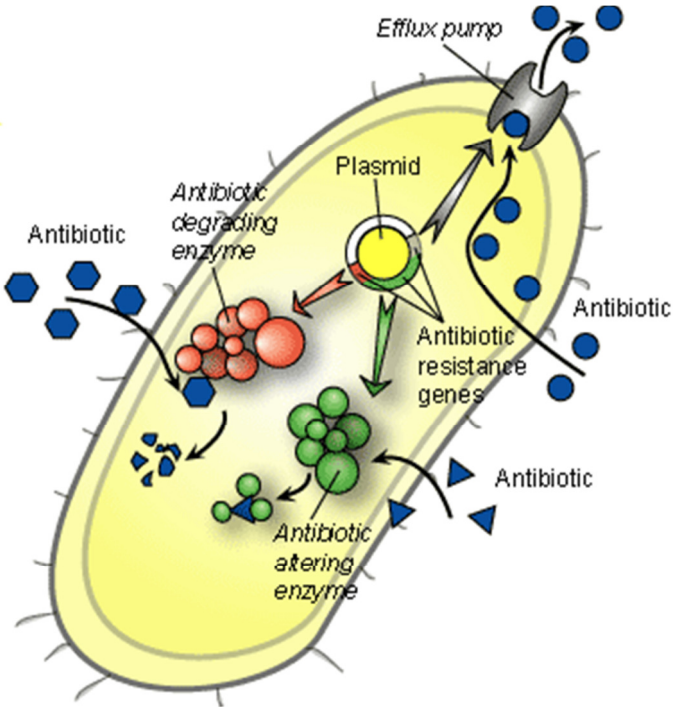

Figure 1: Mechanisms of resistance to antimicrobial drugs (Todar 2009).

Bacterial resistance can be of two types; intrinsic or acquired. Intrinsic resistance is the natural ability of an organism to resist activity of a particular antimicrobial drug through its inherent structural or functional characteristics (MSU 2011). For example, an organism lacks

the target of the antibiotic molecule, or in the case of Gram-negative bacteria, the cell wall has an impermeable barrier against the antimicrobial drug (Todar 2009). Intrinsic resistance is specific to a species or genus (Murray *et al*. 1995).

Acquired resistance occurs when a particular bacterium obtains the ability to resist the activity of a particular antimicrobial drug to which it was previously susceptible. Unlike intrinsic resistance, acquired resistance is present in only certain strains or subpopulations of a particular species or of a genus (Murray *et al*. 1995). Acquired resistance can result from the mutation of genes or from the acquisition of foreign resistance genes (MSU 2011).

Horizontal gene transfer is the process of swapping genetic material between neighbouring bacteria. Many of the resistance genes are carried on plasmids, transposons or integrons which act as vectors for the transfer of resistance genes from one bacterial cell to another. Horizontal gene transfer occurs via three main mechanisms; transformation, conjugation or transduction, as shown in Figure 2 (MSU 2011).

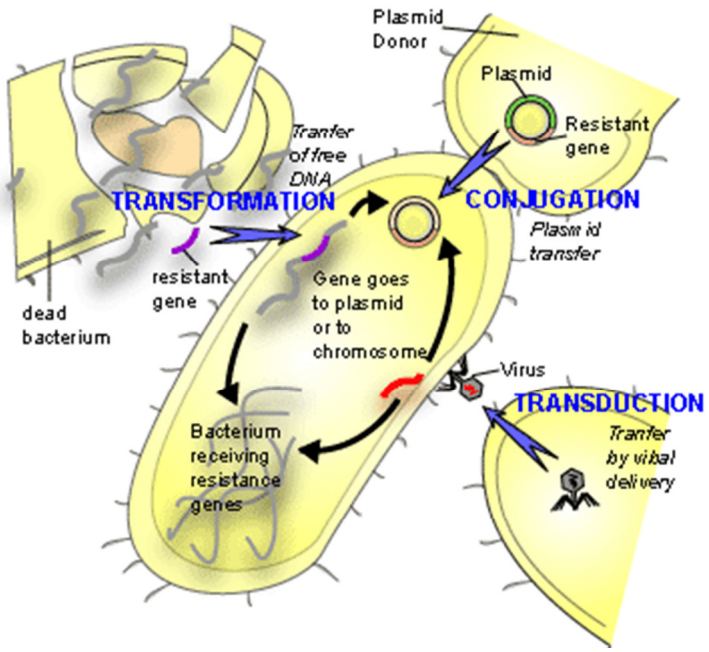

Figure 2: Mechanisms of horizontal gene transfer (Todar 2009).

Transformation is the direct uptake of DNA from a cell's environment. Conjugation is the direct transfer of DNA from one bacterial cell to another. Transduction is the indirect transfer of DNA from one bacterial cell to another via a virus (Fairbanks & Andersen 1999).

The genetic processes of mutation and selection, the ability to exchange genes, and the fast growth rate of bacteria are evolutionary tools which have facilitated bacteria in their adaptation and resistance to the presence of antimicrobial drugs in the environment (Todar 2009).

2.3 The emergence of antimicrobial resistance

Since their discovery during the 20th century, antimicrobial drugs have substantially reduced the threat posed by infectious diseases (WHO 2002). These "wonder drugs" helped lead to a drop in mortality from infectious diseases during the latter part of the last century. However, the benefits of these "wonder drugs" are now being jeopardized by another recent development: the emergence of antimicrobial resistance. The emergence is most evident in bacterial infections which contribute the greatest to human disease: diarrhoeal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital-acquired infections with severe consequences. Infections caused by resistant microbes fail to respond to treatment resulting in longer periods of infectivity and exposure to the general population and also a greater risk of mortality (WHO 2002). Of major concern is where resistance is developing for virtually all currently available drugs, thus raising the spectre of a multi-drug resistant era where antimicrobial drugs are no longer effective (WHO 2002). Recognition of the antimicrobial drug resistance problem has therefore intensified the need for surveillance of antimicrobial use and resistance.

2.4 Antimicrobial drugs and their use in livestock animals

Antimicrobial drugs are used in the treatment of infectious diseases in humans and also for a number of non-human applications such as agriculture, animal husbandry, aquaculture and veterinary medicine (Mazel & Davies 1999). Apart from use in therapy, antimicrobial drugs are used in animal feed as growth promoters; to increase growth rates, improve feed conversion and to reduce morbidity and mortality. There is concern that these growth promoters could select for resistance (Aarestrup *et al*. 1998).

Food animals may serve as a reservoir of resistant bacteria and resistant genes. As such, one of the first consequences of antimicrobial usage in agriculture was the transfer of resistance genes from animal isolates to human pathogens through the food chain (e.g. *Salmonella enterica* subsp. *enterica* serotype Typhimurium) (Mazel & Davies 1999). A growing number of multi-drug resistant organisms which are pathogenic to both animals and humans, such as *Salmonella* Typhimurium definitive phage type (DT) 104 and *Escherichia*

coli O157:H7, have been identified in animals (Mazel & Davies 1999). It has been accepted generally that the therapeutic use of antimicrobials has increased the number of resistant pathogenic bacteria.

A study conducted in Australia found that although there are no products registered for use in aquaculture, antimicrobial resistance is present in isolates from aquaculture and aquaculture environments. This supports the view that there is a risk of transfer of resistant bacteria to humans from consumption of aquaculture products (Akinbowale, Peng & Barton 2006).

In the 1960s the Swann Committee, concerned over the emergence of resistant bacteria in domestic animals, recommended that antimicrobials that were of value in the treatment of humans should not be approved for growth promotion in food animals. The ban and voluntary withdrawal of antimicrobial growth promoters was based on the expectation that removal of the selective antimicrobial pressure in animals would reduce the exposure of humans, via food, to resistant bacteria from animals (Aarestrup *et al*. 2001).

2.5 Sources of antimicrobials and resistance in the environment

It has been estimated that more than a million metric tons of antimicrobials have been released into the biosphere during the last 50 years (Mazel & Davies 1999). Resistance found in different environmental compartments is summarized (Kümmerer 2004):

- Hospital effluent: Antimicrobials used in medicine for the treatment of infections and prophylaxis are released into the aquatic environment via waste water. Unused therapeutic drugs are sometimes disposed of down drains. Antimicrobials and disinfectants are present in the effluent of hospitals. Antimicrobial concentrations calculated and measured in hospital effluents are in the same order of magnitude as the MICs for sensitive pathogenic bacteria. Another important source of resistance is the input of bacteria already resistant because of the use of antimicrobials in medical treatment. It has been said that the widespread use of biocides such as triclosan and quaternary ammonium compounds used in hospitals and homes could select for antimicrobial resistant bacteria.

- Municipal sewage and activated sludge of sewage treatment plants: Antimicrobials and disinfectants have been detected in sewage water at concentrations of a few micrograms per litre. Resistant bacteria are present in municipal sewage because of

the use of antimicrobials at home and it is thought that the general community is responsible for this main input of resistant bacteria into sewage treatment plants. In the United Kingdom, rivers receive a large proportion of treated sewage and there is limited regulation on microbiological quality of discharge from many sewage works (Avery *et al*. 2008).

- Surface water: Antimicrobial residues in surface water, such as rivers and lakes, are in the low microgram per litre concentration range. Antimicrobial resistance has been found in marine bacteria and bacteria living in estuaries. Bacteria resistant to antimicrobials are present in surface water. The survival of *Escherichia coli* O157:H7 in surface waters may increase the potential for dissipation of the organism to facilitate cycles of livestock re-infection and lead to human infection (Avery *et al*. 2008).

- Ground water: Antimicrobial residues are rarely found in ground water and are detected far below the microgram per litre range. Inputs of antimicrobials and resistant bacteria in ground water include: leaching from fields fertilized with animal manure, manure runoff from fields, leakage from septic tanks and broken sewage pipes. High incidences of resistant *E. coli* has been found in rural ground water.

- Drinking water: As early as the 1980s, antimicrobial resistant bacteria were detected in drinking water. The bacteria were detected using classical microbiological methods. It was concluded that treatment of the raw water selects for antimicrobial resistant bacteria.

- Sediments: The antimicrobials used in fish farming enter sediments directly from the water without undergoing any purification process, and thus becoming locally concentrated. These high concentration loads in sediments are sufficiently potent to inhibit the growth of bacteria. A sensitive environmental indicator of past antibacterial use is an increased resistance in sedimentary bacteria.

- Soil: Antimicrobials used in veterinary medicine or as growth promoters are excreted by animals and end up in manure. The manure is then used as a fertilizer where the antimicrobials in the manure are passed into the soil. These antimicrobials accumulate and reach levels in the MIC range. It is important to note that antibiotics occur naturally in soils.

2.6 The movement of antimicrobial resistance genes

The overall problem of antimicrobial resistance is one of genetic ecology. Over half a century of antimicrobial use has undoubtedly influenced all aspects of microbial genetic information (horizontal gene transfer) between microbes; this has been a period of strong evolutionary pressure and extensive selection. The genetic ecology of antimicrobial resistance involves the acquisition, dissemination, and organization of resistance genes within bacterial communities (Mazel & Davies 1999). Antimicrobial resistant strains are associated with many different environments. There are four main genetic reactors in which antimicrobial resistance evolves:

- human and animal microbiota;
- hospitals, long-term care facilities, farms, aquaculture, overcrowded places;
- wastewater, effluents, sewage treatment plants;
- soil, surface or ground water, environmental organisms (Baquero *et al*. 2008).

Resistance genes occur naturally in the environment. Humans apply selective pressure on the resistance genes due to large quantities of antimicrobials we produce, consume and apply in human and veterinary medicine and in agriculture. The widespread dissemination of resistance genes throughout many environments is due to physical and biological forces, as shown in Figure 3 (Allen *et al*. 2010).

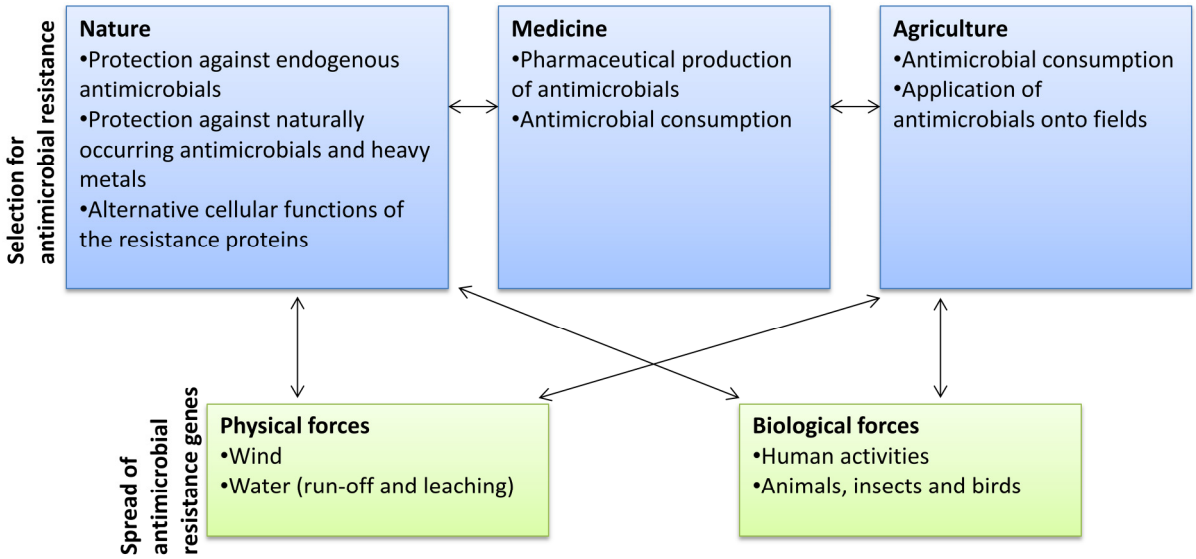

Figure 3: Sources and movement of antimicrobial resistance genes in the environment (Allen *et al*. 2010).

Physical forces, such as water and wind, are important drivers of the spread of antimicrobial resistance genes (Allen *et al*. 2010). Water is a crucial agent in all four genetic reactors and this provides a suitable environment for the bacteria from human, animal and environmental origins to mix. Based on the presence of antimicrobial resistance genes in some of these bacteria, the possibilities of sharing may occur. The evolution of resistance is therefore the result of the shuffling of these genes, genetic platforms (plasmids, transposons, and integrons) and genetic vectors. Antimicrobials, disinfectants and heavy metals are also released into the water and exert selective pressures on the evolution of antimicrobial resistance (Baquero *et al*. 2008). The forces of nature can move bacteria from static environments, such as soil; one example is the intercontinental transport of bacteria on desert dust (Allen *et al*. 2010).

Biological forces, such as wild animals aid the spread of antimicrobial resistance genes. The antimicrobial resistance profiles of the commensal bacteria of wild animals are influenced by the proximity to human activities. Wild birds inhabit a wide variety of environments and carry a reservoir of antimicrobial resistant bacteria with the potential for long distance dissemination (Allen *et al*. 2010).

Humans are an important biological force. Even in the most secluded communities, antimicrobial resistant bacteria have been found – high levels of antimicrobial resistance were found in *E. coli* from an isolated human population in Bolivia (Allen *et al*. 2010).

2.7 Studies on antimicrobial resistance in commensal bacteria from wild animals

In pristine systems, where no antimicrobials are used, it is possible to evaluate the transmission of antimicrobial resistance through the environment and to understand the dynamics of the various input sources. Several studies evaluating the acquired antimicrobial resistance in commensal bacteria from different species of wild animals including mammals, birds, amphibians and reptiles that have not been directly exposed to antimicrobial drugs have been conducted. Comparison of the results is not straight forward as there are differences in the methodology used and the species under study. Information regarding possible contact with human activity is often lacking (Bartoloni *et al*. 2008).

The prevalence of antimicrobial resistance in wildlife differs in different ecosystems. One of the first studies of resistance in commensal bacteria from wild animals and humans living in surroundings free of antimicrobial drugs was conducted in southern Africa in the 1960s. The study investigated 47 Kalahari Bushmen, 334 wild animals (mostly impala and blue

wildebeest) in the Kruger National Park in South Africa and 201 animals (mostly warthog and kudu) in the Pohwe river valley in the then, Rhodesia. Specimens from the intestines of the freshly killed wild animals and fresh faeces of Bushmen were directly plated onto MacConkey agar containing the antimicrobials sulphonamide, streptomycin, ampicillin, chloramphenicol and tetracycline in concentrations of 10µg/mℓ. Colonies which arose after incubation within the zones of inhibition were considered drug-resistant and were considered for further investigation. Fifty seven (10%) of the 582 specimens contained drug-resistant Gram-negative bacteria which is low compared to 78% of 207 humans investigated in Pretoria (Maré 1968).

A study done in Portugal on the characterization of enterococci in wild animals showed that 28.6% were resistant to tetracycline and 20.7% to erythromycin (Poeta *et al*. 2005). A study done in Spain, on quinolone resistance in *E. coli* from wild birds, found that all nine *E. coli* strains isolated from wild birds with septicaemia, were resistant to nalidixic acid (Gómez *et al*. 2004). A study conducted on faecal samples from wild mammals living in the Stelvio National Park in Italy recorded antimicrobial resistant strains of *E. coli* in 17 of 121 faecal specimens examined. A second survey of the same area examined *E. coli* strains isolated from 81 faecal samples of red deer, roe deer, chamois and alpine marmot. Direct plating of specimens on media containing antimicrobial drugs allowed isolation of resistant strains of *E. coli* from 10 out of 59 (17%) specimens. Twenty-nine percent of the specimens from red deer contained resistant strains (Caprioli *et al*. 1991).

In Michigan, USA, in the Red Cedar River, a study was done to investigate the pattern of antimicrobial resistance in *E. coli* originating from human sewage, wildlife, domestic animals, farm environments and surface water. Of the *E. coli* isolated from 34 farmed deer, 2.94% were resistant to tetracycline and 11.76% to cephalothin, while in 54 wild waterfowls sampled 1.85% of the *E. coli* isolates were resistant to tetracycline and 11.11% to cephalothin (Sayah *et al*. 2005). In general, the study showed that *E. coli* isolated from domestic animals showed resistance to the highest number of antimicrobials in comparison to isolates from human sewage, surface water and wildlife.

In a study done in Finland, an almost complete absence of acquired resistance traits in enterobacteria from the ungulate faecal flora of moose, white-tailed deer and bank voles was found. Their results disagreed with those from a study of enterobacteria from wild English rodents, where extremely high resistance was found (Österblad *et al*. 2001). The two studies combined suggest that the gut flora of wildlife populations with no known direct contact with

anthropogenic antimicrobials are influenced by the proximity to humans; the closer the proximity to humans, the higher the levels of antibiotic resistance (Gilliver *et al*. 2001).

Commensal bacteria constitute a reservoir of resistance genes and their level of resistance is considered to be a good indicator for resistance problems to be expected in pathogens. It is feasible to compare the prevalence of resistance and to detect dissemination of resistant bacteria and their resistance genes from animals to humans and vice versa by monitoring the prevalence of resistance in indicator bacteria such as faecal *Escherichia coli* and enterococci (Van den Bogaard & Stobberingh 2000). Our understanding of the origins and roles of antibiotic resistance genes in natural intestinal microbial communities will increase with more complete profiles of antimicrobial resistance in wild animals. This will help us manage emerging zoonotic diseases (Allen *et al*. 2010).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Experimental design

Impala faeces and water sources frequented by impala were monitored for the presence of bacteria resistant to antimicrobial drugs. The study area selected for this research was the Kruger National Park (KNP) within South Africa (Figure 4). KNP was chosen as it is a pristine environment and it has free-ranging animals that have never been treated with antimicrobials. Four river systems within KNP were chosen for the study, namely: Olifants, Letaba, Crocodile and Sabie-Sand River systems (Figure 4). The choice of rivers was based on location as the rivers needed to be associated with a high density of impala. Perennial rivers were chosen, so that for future studies, samples could be taken at different times of the year.

The study design and sampling is based on the study done by Mariano (2009). A total of 11 collection points along these rivers were sampled; two on the Olifants, two on the Letaba, three on the Crocodile, three on the Sabie, and one on the Sand river (Figure 4). To avoid overlap between the highly territorial impala herds, the collection points were selected so that there was a minimum distance of 10km between them or a physical barrier. When the water samples were collected, impala herds were selected within a 5km radius of the water samples. Considering the home range of impala, a 5km range was selected. For each water sampling point, 15 faecal samples were collected (sample size of 176).

Water and faecal samples were processed for bacterial culture on growth media. The preparations were streaked onto the media and antimicrobial disks placed on the surface of the media. Colonies growing within zones of inhibition on the growth media were selected. A representative of each colony type was streaked onto Columbia Blood and MacConkey agar. The isolates were identified and then stored.

Indicator bacteria (*Escherichia coli* and *Enterococcus* species) and bacteria isolated in significant numbers were considered for further testing. Susceptibility to antimicrobial drugs was measured using a commercial MIC microtitre plate system and the results expressed as minimum inhibitory concentrations (MICs).

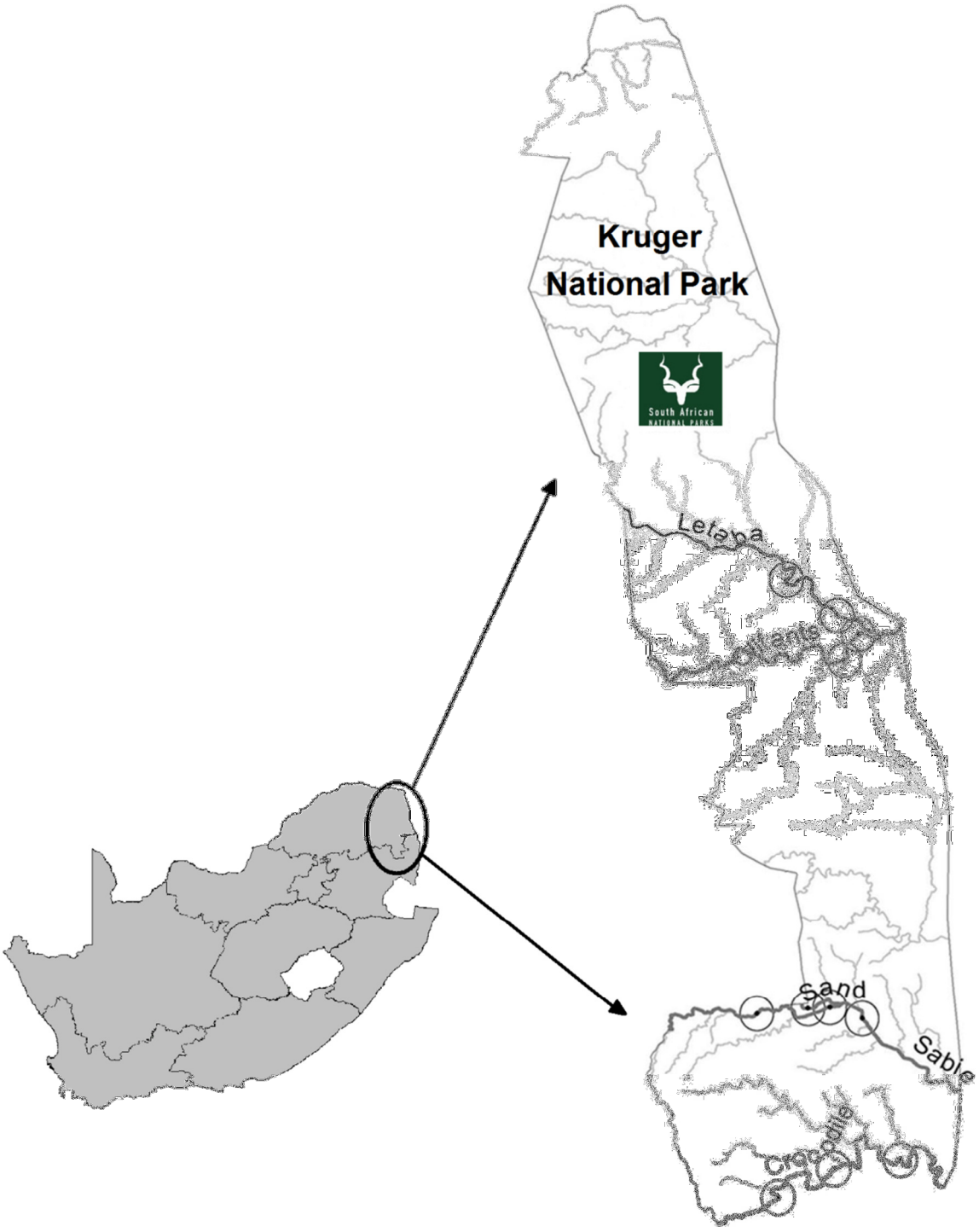

Figure 4: Location of KNP within South Africa, sampled rivers and sampling points.

3.2 Study rivers

The four perennial river systems chosen for the study are associated with a high density of impala and include the Olifants River (Figure 5), Letaba River (Figure 6), Crocodile River (Figure 7) and Sabie-Sand Rivers (Figure 8).

Figure 5: Olifants River.

Figure 6: Letaba River.

Figure 7: Crocodile River.

Figure 8: Sabie-Sand Rivers.

3.3 Study animal

The impala (*Aepyceros melampus*) was chosen as the study animal (Figure 9) as they are distributed widely, dependent on water, highly social and highly territorial.

Figure 9: Male impala (*Aepyceros melampus*).

3.4 Sample collection

Sampling occurred during the month of November 2010. At each sampling point, Global Positioning System (GPS) coordinates were recorded with a portable GPS device (eTrex Garmin®)[1]. For the water samples, 500 mℓ of flowing river water was collected into sterile glass bottles[2]. A five-step sampling technique was used, as described by the Department of Water Affairs (DWA 2000). First the bottle cap was removed whilst wearing sterile latex gloves. The water sample was collected by holding the bottle at the bottom and plunging it below the water surface. The mouth of the bottle was positioned towards the oncoming water current to allow the water to flow into the bottle. The cap was replaced and the bottle labelled.

The faecal samples were collected within a 5km radius of the water sampling sites. Fifteen fresh impala faecal samples per water sampling site were collected from the impala middens. Approximately 10g of fresh faecal pellets were collected from the top of the impala middens, to avoid contamination from soil bacteria. Sterile latex gloves were used for each sample collection to avoid cross-contamination. The faecal pellets were stored in plastic

[1] Garmin®: www.garmin.co.za
[2] Schott Duran® Laboratory glass bottle. Supplied by: Lasec SA, PO Box 2110, Cape Town WC, 8000

bags (Whirl-pak®)[3] containing buffered peptone water (Oxoid)[4] to prevent the samples drying out. The samples were transported in a cooler bag at ±4℃ until their arrival at the laboratory. A total of 176 samples (11 water and 165 faecal) were collected.

3.5 Direct plating method

The samples were screened for resistant isolates by a rapid method, similar to one employed in studies of community resistance (Lester *et al*. 1990; Bartoloni *et al*. 2004; Bartoloni *et al*. 2006).This approach was used as it is known to correlate well with those based on testing of randomly collected colonies from primary faecal culture and because it is more sensitive (Lester *et al*. 1990; Bartoloni *et al*. 2004; Bartoloni *et al*. 2006).

The water samples were filtered through a 0.45µm filter, using a Millipore vacuum pump filtration system (Millipore)[5]. Each filter membrane was then placed into 10mℓ of buffered peptone water (Oxoid)[6] and incubated overnight at 37℃ before testing. The faecal samples were crushed by hand and mixed thoroughly with a sterile throat swab before testing.

A sterile throat swab was used to directly plate the sample onto Mueller-Hinton agar (Oxoid)[7] to form a lawn of growth. The antimicrobial susceptibility test discs (Oxoid)[8] were immediately applied at equidistance onto the seeded plates. A total of nine antimicrobial drugs (belonging to six classes) were tested (Table 1). The antimicrobial drugs chosen were from antimicrobial classes commonly used in both human and veterinary medicine.

[3] Whirl-pak Bags B01020WA. Supplied by: Guth SA – Gauteng, PO Box 58070, Newville GT, 2114
[4] Oxoid buffered peptone water CM0509B. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[5] Millipore- Sterifil® aseptic system and vacuum pump. www.millipore.com
[6] Oxoid Buffered peptone water CM0509B. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[7] Oxoid Mueller-Hinton agar CM0337. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[8] Oxoid Antimicrobial Susceptibility Test Discs. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160

Table 1: Antimicrobial drugs used in the direct plating method.

Antimicrobial class	**Antimicrobial subclass**	**Antimicrobial drugs (disc potency)**
Aminoglycosides		gentamicin (10µg)
		neomycin (30µg)
β-lactam/β-lactamase inhibitors combinations		amoxicillin- clavulanic acid (30µg)
Cephalosporins	third generation cephalosporins	cefotaxime (30µg)
		ceftiofur (30µg)
Folate pathway inhibitors		sulphamethoxazole and trimethoprim (25µg)
Quinolones	quinolone	nalidixic acid (30µg)
	fluoroquinolone	enrofloxacin (5µg)
Tetracyclines		doxycycline (30µg)

The plates, after being incubated overnight (24hr) at 37℃, were inspected for growth (Figure 10). One the following situations could be observed around each disc: (1) No inhibition zone (Figure 11a), (2) Inhibition zone with internal colonies (Figure 11b) and (3) Inhibition zone without internal colonies (Figure 11c).

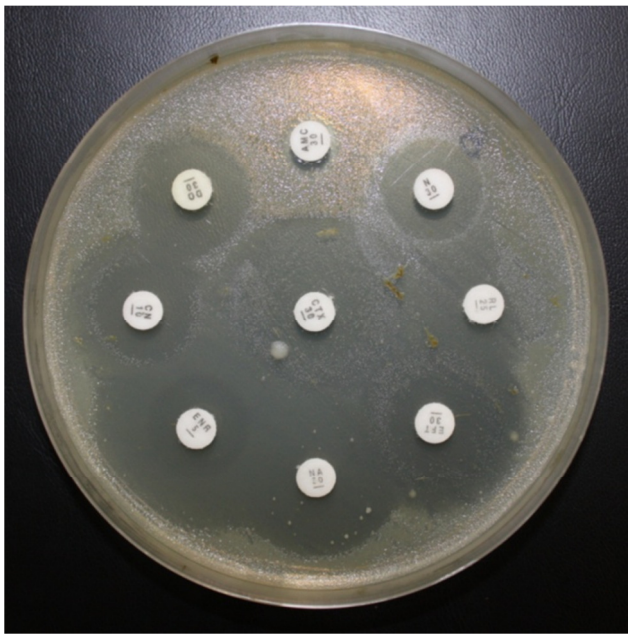

Figure 10: Direct plating method on a Mueller-Hinton agar plate.

a) **b)** **c)**

Figure 11: Illustrations of inhibition zones around antimicrobial discs.

Colonies growing within the zones were considered resistant and selected for further testing. In order to identify each colony type within the zones, an inoculum was streaked onto Columbia agar with 5% horse blood (CBA)[9] and MacConkey agar (MAC)[10] and incubated overnight at 37°C. The bacterial growth was examined for purity and viability. Colonies that were not pure were re-streaked onto CBA and MAC before further tests were attempted.

3.6 Preliminary identification

All isolates were subjected to Gram's staining method[11], catalase[12], oxidase[13] and spot indole[14] tests. Microscopic appearance, colony morphology on CBA plates and lactose production (pink colonies) on MAC plates was recorded. From these results, the isolates were divided into Gram-negative and Gram-positive organisms.

3.7 Biochemical characterization of the microflora

1) Enterobacteriaceae: The Gram-negative isolates that were oxidase negative were subjected to the commercial API® 10S identification system (bioMérieux® SA)[15] and the results read with the APIWEB® programme.

2) Enterococci: The Gram-positive coccal isolates giving catalase negative and oxidase negative results were subjected to the Lancefield streptococcal grouping test (Streptex™

[9] Oxoid Columbia blood agar base CM0331. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[10] Oxoid MacConkey agar base CM0507(CM7b). Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[11] Gram's Stain. Supplied by: L&T Diagnostics CC, PO Box 32, Rosettenville GT, 2130
[12] Hydrogen peroxide. Medicolab CC, 5 Bessemer Road, Amalgam
[13] 11330, Bactident® Oxidase. www.merckmillipore.com
[14] 109293 KOVACS' Indole reagent. www.merckmillipore.com
[15] API 10S. Supplied by: Biomérieux South Africa PTY LTD, PO Box 2316, Randburg GT, 2125

test)[16]. Enterococci belong to Lancefield Group D, tolerate the bile salts in MacConkey agar and appear as small pinpoint colonies on this medium. For the differentiation of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans* the following biochemical tests were used: lactose, arabinose, sorbitol, mannitol and growth in 6.5% NaCl (Quinn *et al*. 1994). The criteria are indicated in Table 2.

Table 2: Differential characteristics of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans.*

Biochemical tests	***Enterococcus faecalis***	***Enterococcus faecium***	***Enterococcus durans***
Lactose	+	+	+
Arabinose	-	+	-
Sorbitol	+	-	-
Mannitol	+	+	-
6.5% NaCl	+	+	+

After all the isolates had been identified, they were stored in 2 mℓ cyrovials®[17] containing 1mℓ brain-heart infusion broth (BHI)[18] (Oxoid) at - 86℃.

3.8 Minimum inhibitory concentration determinations

Susceptibility of the isolates to a selection of commonly used veterinary antimicrobial drugs was determined by broth microdilution as recommended by the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) (CLSI 2008). The isolates were tested using a commercial MIC test (Sensititre® Bovine/Porcine plate format BOP06F)[19] which contained the antimicrobial drugs shown in Table 3.

[16] Streptex Streptococcal Grouping Kit DR0585A. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[17] Cyrovial 2ml T309-2A. Supplied by: J-Plast, PO Box 6715, Weltevredenpark GT, 1715
[18] Oxoid Brain-heart infusion broth CM1135. Supplied by: Quantum Biotechnologies (PTY) LTD. PO Box 215, Ferndale GT, 2160
[19] Trek Diagnostic Systems. Supplied by: Separation Scientific

Table 3: Antimicrobial drugs used in the Sensititre® Bovine/Porcine MIC plate.

Antimicrobial class	Antimicrobial subclass	Antimicrobial drugs and dilution ranges (µg/mℓ)
Aminocyclitols		spectinomycin (64-8)
Aminoglycosides		gentamicin (16-1)
		neomycin (32-4)
Cephalosporins	third generation cephalosporins	ceftiofur (8-0.25)
Folate pathway inhibitors		sulphadimethoxine (256)
		trimethoprim/sulfamethoxazole (2/38)
Lincosamides		clindamycin (16-0.25)
Macrolides	triamilide	tulathromycin (64-1)
		tilmicosin (64-4)
		tylosin tartrate (32-0.5)
Penicillins	penicillin	penicillin (8-0.12)
	aminopenicillin	ampicillin (16-0.25)
Phenicols		florfenicol (8-0.25)
Quinolones	fluoroquinolone	danofloxacin (1-0.12)
		enrofloxacin (2-0.12)
Tetracyclines		chlortetracycline (8-0.5)
		oxytetracycline (8-0.5)
Pleuromutilins		tiamulin (32-0.5)

The stored isolates were plated onto CBA and incubated for 24 hours at 37℃ to provide fresh colonies. One colony of the isolate from the CBA plate was inoculated into a 4mℓ tube of sterile distilled water producing a turbidity of 0.5 MacFarland standard. After it was vortexed well for ten seconds, 10µℓ of the suspension was transferred into 11 mℓ Sensititre® Mueller-Hinton broth[20] to obtain a concentration of $1x10^5$ Colony Forming Units (CFU)/mℓ. For enterococcal isolates, 30µℓ of suspension was transferred to help improve endpoint determination. The Sensititre® Mueller-Hinton broth containing the inoculum was poured into sterile plastic Petri dishes to facilitate the dispensing of the bacterial suspension into the microtitre plates.

[20] Trek Diagnostic Systems. Supplied by: Separation Scientific

50 µℓ of the bacterial suspension was inoculated into each well of the microtitre plate using a multi-channel automatic pipette. Each microtitre plate was covered with the adhesive seal provided to prevent drying out of the wells. From the bacterial suspension 10µℓ was uniformly streaked out onto CBA to check for purity. The plates were incubated for 18-24 hours at 37℃ in an aerobic incubator in stacks of not more than three plates to ensure that even incubation temperatures were kept. After incubation, the inoculum control was checked for purity and a colony count performed (around 10-50 colonies per plate). The results for the microtitre plates were read using a viewer mirror and the MIC was recorded in table format as the lowest concentration of antimicrobial drug that inhibited visible growth. Growth appeared as turbidity or as a deposit of cells at the bottom of a well. If there was no growth in the growth control wells, the results were deemed invalid.

Quality control procedures performed simultaneously with the MIC tests included an inoculum density/purity control for each isolate and growth control wells were checked on each plate. Reference organisms obtained from the American Type Culture Collection (ATCC)[21] were tested to see if the strains fell between the required ranges according to the requirements of the CLSI. Reference organisms tested included *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212.

3.9 Data analysis

All the data was entered into Microsoft® Excel 2007 spreadsheets. The prevalence of resistance was calculated for “breakpoint” MICs to reflect its clinical significance. The breakpoints selected for each antimicrobial drug tested are shown in Table 4. The breakpoints are derived from CLSI, Table 2, Document M31-A3, Volume 28, Number 8, February 2008. Since there are no published breakpoints for wild animals, the CLSI-approved MIC breakpoints for cattle were used first choice whenever available. The MIC values classified as resistant were expressed as percentages, and percentage of distribution of MIC tables for each species was made.

[21] American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA

Table 4: Minimum inhibitory concentration (MIC) breakpoints for animal pathogens.

Antimicrobial Drug	MIC Breakpoint (µg/mℓ)		
	Susceptible	Intermediate	Resistant
Spectinomycin[1]	≤32	64	≥128
Gentamicin[2]	≤4	8	≥16
Neomycin[3]	≤16	32	≥64
Ceftiofur[1]	≤2	4	≥8
Sulphadimethoxine[4]	≤256		≥512
Trimethoprim/Sulfamethoxazole[5]	≤2/38		≥4/76
Clindamycin[6]	≤0.5	1-2	≥4
Tulathromycin[1]	≤16	32	≥64
Tilmicosin[1]	≤8	16	≥32
Tylosin tartrate[7]	≤8	16	≥32
Penicillin[8]	≤8		≥16
Ampicillin[8]	≤8		≥16
Florfenicol[1]	≤2	4	≥8
Danofloxacin[9]	≤0.25	0.5-1	≥2
Enrofloxacin[1]	≤0.25	0.5-1	≥2
Chlortetracycline[10]	≤4	8	≥16
Oxytetracycline[10]	≤4	8	≥16
Tiamulin[11]	≤16		≥32

[1] CLSI-approved MIC breakpoints for cattle respiratory disease were used. [2] CLSI-approved MIC breakpoints for humans were used. [3] CLSI-approved MIC breakpoints of kanamycin were used as the reference for neomycin. [4] CLSI-approved MIC breakpoints for the class representative for sulfonamides were used. [5] CLSI-approved MIC breakpoints for *Enterobacteriaceae* were used. [6] CLSI-approved MIC breakpoints for *Staphylococcus* species were used. [7] CLSI-approved MIC breakpoints of tilmicosin were used as the reference for tylosin tartrate. [8] CLSI-approved MIC breakpoints for enterococci were used. [9] CLSI-approved MIC breakpoints of enrofloxacin were used as the reference for danofloxacin. [10] CLSI-approved MIC breakpoints for the class representative for tetracyclines were used. [11] CLSI-approved MIC breakpoints for swine respiratory disease were used.

CHAPTER 4

4. RESULTS

4.1 Direct plating method

Faecal samples were collected from 165 impala middens and water samples collected from 11 points along the perennial rivers in KNP. All 176 samples that were directly plated on Mueller-Hinton agar revealed a lawn of bacterial growth. Resistant bacteria, with growth right up to the antimicrobial disc (no inhibition zone) were detected mostly with nalidixic acid (74.4%) and trimethoprim-sulfamethoxazole (67.6%). Resistant bacteria, growing as single colonies within the inhibition zones of the antimicrobial discs, were detected in all samples. Colonies growing within the inhibition zone were detected mostly with cefotaxime (67.6%), ceftiofur (57.4%), amoxicillin-clavulanic acid (47.2%) and enrofloxacin (43.2%). Bacteria were most sensitive to neomycin (82.4%), gentamicin (76.1%) and doxycycline (64.8%) respectively as evidenced by complete inhibition zones without internal colonies (Figure 12).

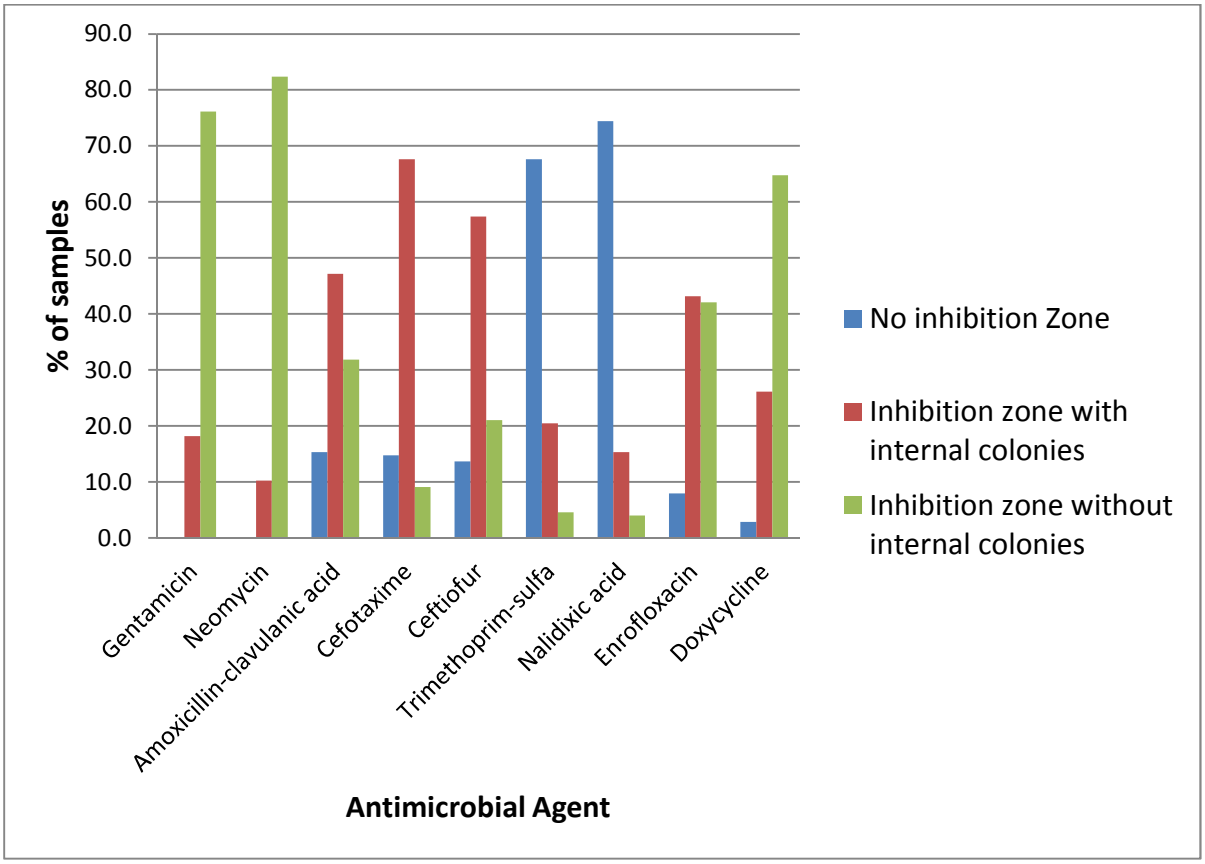

Figure 12: Percentage of antimicrobial sensitivity/resistance of the faecal and water samples to a range of antimicrobials.

4.2 Nature of the microflora isolated and identified

Colonies that grew within the inhibition zones of the antimicrobial discs from the direct plating method were selected for culture and identification. From the 176 samples processed, a total of 280 isolates were obtained. The Gram-negative bacteria identified include: *Acinetobacter baumannii*, *Citrobacter farmeri*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia*, *Pantoea* species, *Proteus* species, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Stenotrophomonas maltophilia*. The dominant Gram-negative bacteria cultured were *Enterobacter cloacae* (17.5%) followed by *Escherichia coli* (4.3%). The Gram-positive bacteria identified include: *Bacillus* species, *Corynebacterium* species, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium* and *Streptococcus* species. The dominant Gram-positive bacteria cultured were *Enterococcus durans* (22.9%), *Enterococcus faecalis* (21.1%) and *Bacillus* species (20.4%). The number of bacterial isolates cultured and their percentage prevalence is summarised in Table 5.

Table 5: Total number of isolates cultured and their percentage prevalence.

Bacterial Species	Number of Isolates	% Prevalence
Gram-negative bacteria		
Acinetobacter baumannii	2	0.7
Citrobacter farmeri	2	0.7
Enterobacter aerogenes	5	1.8
Enterobacter cloacae	49	17.5
Escherichia coli	12	4.3
Klebsiella pneumoniae	1	0.4
Pantoea species	9	3.2
Proteus species	7	2.5
Pseudomonas aeruginosa	1	0.4
Serratia marcescens	1	0.4
Stenotrophomonas maltophilia	1	0.4
Gram-positive bacteria		
Bacillus species	57	20.4
Corynebacterium species	5	1.8
Enterococcus durans	64	22.9
Enterococcus faecalis	59	21.1
Enterococcus faecium	4	1.4
Streptococcus species	1	0.4
TOTAL	280	

The composition of the commensal microflora isolated from impala faeces was similar in the four perennial river systems in KNP. *Enterobacter cloacae* was the dominant Gram-negative species, and the highest number was found in the Sabie-Sand Rivers (n=24). *E. coli* was isolated from the four river systems at a much lower rate compared to *Enterobacter cloacae* and the highest number was found in the Letaba River (n=4) and Sabie-Sand Rivers (n=4). *Pantoea* species was only isolated from the Crocodile River (n=9). *Bacillus* species, *Enterococcus durans* and *Enterococcus faecalis* were the dominant Gram-positive species across the four river systems.

A low rate of bacteria was isolated from the water samples. Only one *E. coli* was isolated from the Olifants River. *Proteus* species was found in the Letaba, Crocodile and Sabie-Sand Rivers. *Enterococcus faecalis* was found in the Letaba, Crocodile and Sabie-Sand Rivers. Notably so, *Enterococcus durans* was the only species found in faecal and water samples in all four locations. The number and percentage prevalence of the bacterial species isolated from impala faeces and the water sources of the animals along four perennial river systems in KNP are shown in Table 6.

4.3 MIC test results

All *E. coli*, *Enterobacter cloacae*, *Pantoea* species, and *Enterococcus* species isolates (a total of 197 isolates) were subjected to antimicrobial susceptibility testing and included 12 strains of *E. coli*, 49 strains of *Enterobacter cloacae*, 9 strains of *Pantoea* species, 59 strains of *Enterococcus faecalis*, 4 strains of *Enterococcus faecium*, and 64 strains of *Enterococcus durans*. The individual MIC values in µg/mℓ recorded for tests for *E. coli*, *Enterobacter cloacae*, *Pantoea* species, *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus durans* are given in Appendix A. The MIC range of each organism tested against each antimicrobial drug is indicated in Table 7. *E.coli*, *Enterobacter cloacae* and *Pantoea* species gave similar MIC ranges whilst the *Enterococcus* isolates gave similar MIC ranges.

Table 6: Number and prevalence (% of samples) of bacterial species isolated from impala faeces and river water along four perennial river systems in KNP.

	Olifants River				Letaba River				Crocodile River				Sabie-Sand Rivers			
	Faeces n=43		Water n=8		Faeces n=42		Water n=10		Faeces n=64		Water n=11		Faeces n=88		Water n=14	
Gram-negative bacteria																
Acinetobacter baumannii	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.6)	0	(0.0)	0	(0.0)	1	(7.1)
Citrobacter farmeri	1	(2.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(9.1)	0	(0.0)	0	(0.0)
Enterobacter aerogenes	3	(7.0)	0	(0.0)	1	(2.4)	0	(0.0)	1	(1.6)	0	(0.0)	0	(0.0)	0	(0.0)
Enterobacter cloacae	6	(14.0)	0	(0.0)	7	(16.7)	0	(0.0)	12	(18.8)	0	(0.0)	24	(27.3)	0	(0.0)
Escherichia coli	2	(4.7)	1	(12.5)	4	(9.5)	0	(0.0)	1	(1.6)	0	(0.0)	4	(4.5)	0	(0.0)
Klebsiella pneumoniae	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(7.1)
Pantoea species	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	9	(14.1)	0	(0.0)	0	(0.0)	0	(0.0)
Proteus species	1	(2.3)	0	(0.0)	0	(0.0)	2	(20.0)	0	(0.0)	2	(18.2)	0	(0.0)	2	(14.3)
Pseudomonas aeruginosa	0	(0.0)	0	(0.0)	1	(2.4)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Serratia marcescens	0	(0.0)	1	(12.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Stenotrophomonas maltophilia	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(7.1)
Gram-positive bacteria																
Bacillus species	10	(23.3)	0	(0.0)	10	(23.8)	0	(0.0)	13	(20.3)	1	(9.1)	23	(26.1)	0	(0.0)
Corynebacterium species	0	(0.0)	0	(0.0)	1	(2.4)	0	(0.0)	1	(1.6)	0	(0.0)	3	(3.4)	0	(0.0)
Enterococcus durans	7	(16.3)	6	(75.0)	9	(21.4)	4	(40.0)	12	(18.8)	6	(54.5)	13	(14.8)	7	(50.0)
Enterococcus faecalis	12	(27.9)	0	(0.0)	9	(21.4)	4	(40.0)	12	(18.8)	1	(9.1)	19	(21.6)	2	(14.3)
Enterococcus faecium	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	2	(3.1)	0	(0.0)	2	(2.3)	0	(0.0)
Streptococcus species	1	(2.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

Table 7: The ranges of the minimum inhibitory concentrations (MICs) recorded for antimicrobial drugs against the different isolates.

	MIC ranges in µg/mℓ					
Antimicrobial Drug	***E. coli***	***E. cloacae***	***Pantoea* spp.**	***E. faecalis***	***E. faecium***	***E. durans***
Spectinomycin	16 - 32	16 - 64	16	16 - >64	32 - 64	16 - >64
Gentamicin	<1	<1	<1	<1 - 8	<1 - 8	<1 - >16
Neomycin	<4	<4	<4	<4 - >32	<4 - 32	<4 - >32
Ceftiofur	<0.25 - 1	0.5 - 1	0.5	4 - >8	8 - >8	<0.25 - >8
Sulphadimethoxine	<256 - >256	<256 - >256	>256	>256	>256	>256
Trimethoprim/sulfamethoxazole	<2/38 - >2/38	<2/38 - >2/38	>2/38	<2/38	<2/38	<2/38 - >2/38
Clindamycin	>16	>16	>16	<0.25 - 16	2 - 16	1 - 16
Tulathromycin	4 - 32	8 - >64	4 - 8	2 - 32	2 - 32	<1 - 64
Tilmicosin	32 - >64	>64	64	<4 - 32	16 - 32	<4 - 64
Tylosin tartrate	>32	>32	>32	<0.5 - 8	>32	1 - 16
Penicillin	>8	>8	>8	0.5 - 4	0.5 - 2	0.25 - 4
Ampicillin	2 - >16	4 - >16	2 - 4	0.5 - 1	0.5 - 1	<0.25 - 1
Florfenicol	2 - >8	1 - 8	1 - 2	2 - 4	2 - 4	2 - 8
Danofloxacin	<0.12 - >1	<0.12	<0.12	0.5 - >1	>1	0.5 - >1
Enrofloxacin	<0.12 - >2	<0.12	<0.12	0.5 - >2	1 - >2	0.5 - >2
Chlortetracycline	2 - >8	2 - >8	>8	<0.5 - 1	<0.5 - 1	<0.5 - >8
Oxytetracycline	1 - >8	2 - >8	>8	<0.5 - 2	<0.5 - 1	<0.5 - >8
Tiamulin	>32	>32	>32	1 - >32	>32	8 - >32

4.4 Occurrence of resistance to antimicrobial drugs

The percentage of MIC values in the dilution ranges for each antimicrobial drug tested against each bacterium, are shown in Tables A - K in Appendix B. These tables indicate the distribution of the MICs in each dilution range, for each antimicrobial drug. For each species investigated, the data is organised into which river the samples came from and if it was a faecal or water sample.

The shaded areas depict the dilution range of each antimicrobial drug. When the results were higher than the range given, the MIC percentage was shown outside the shaded area and when the results were equal or lower than the lowest concentration tested, the MIC percentage was given as the lowest tested concentration. The bold vertical lines indicate the breakpoint for resistance and MICs equal and above this line are considered resistant ensuring the percentage resistance for each antimicrobial drug to be calculated. The overall percentage resistance observed for each antimicrobial drug tested against the bacterial strains is shown graphically in Figures 13 and 14.

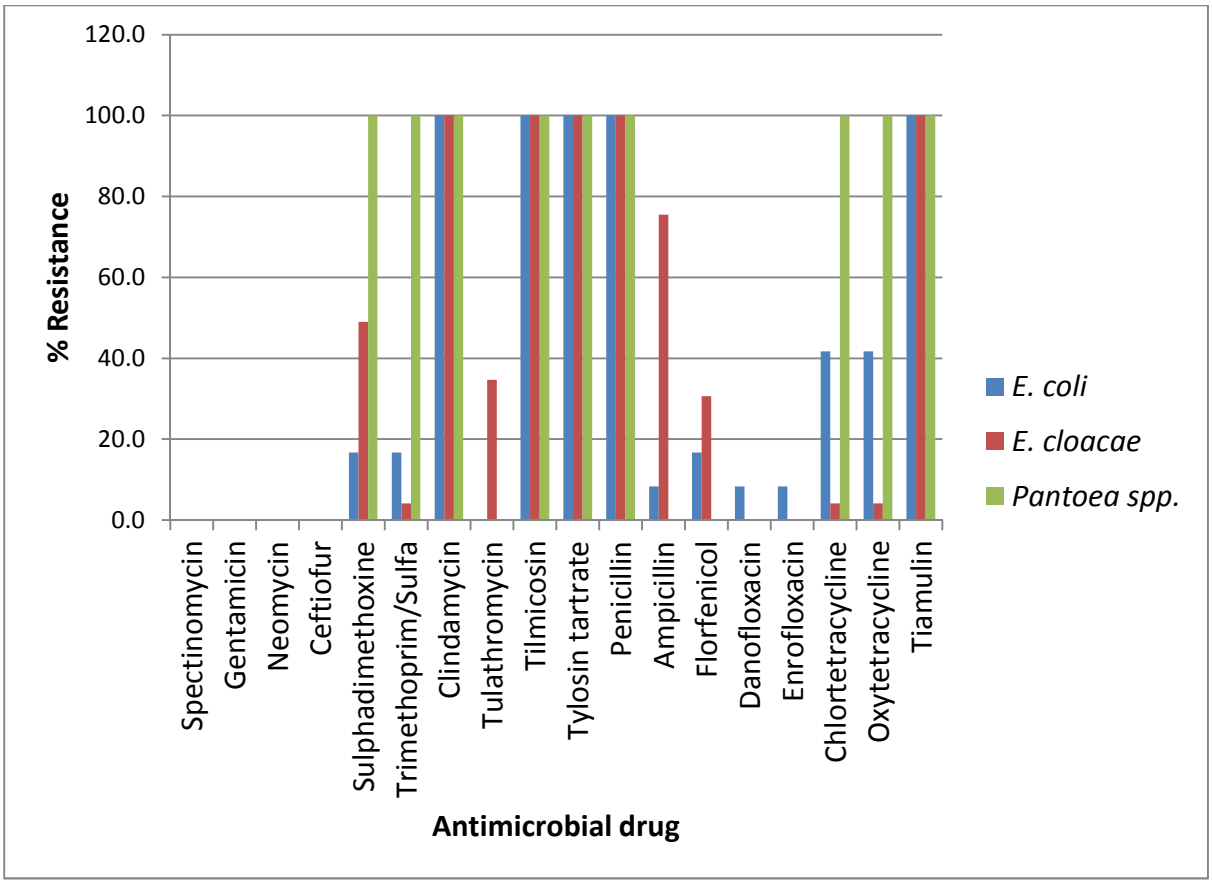

Figure 13: Percentage antimicrobial resistance observed against three Gram-negative bacteria tested.

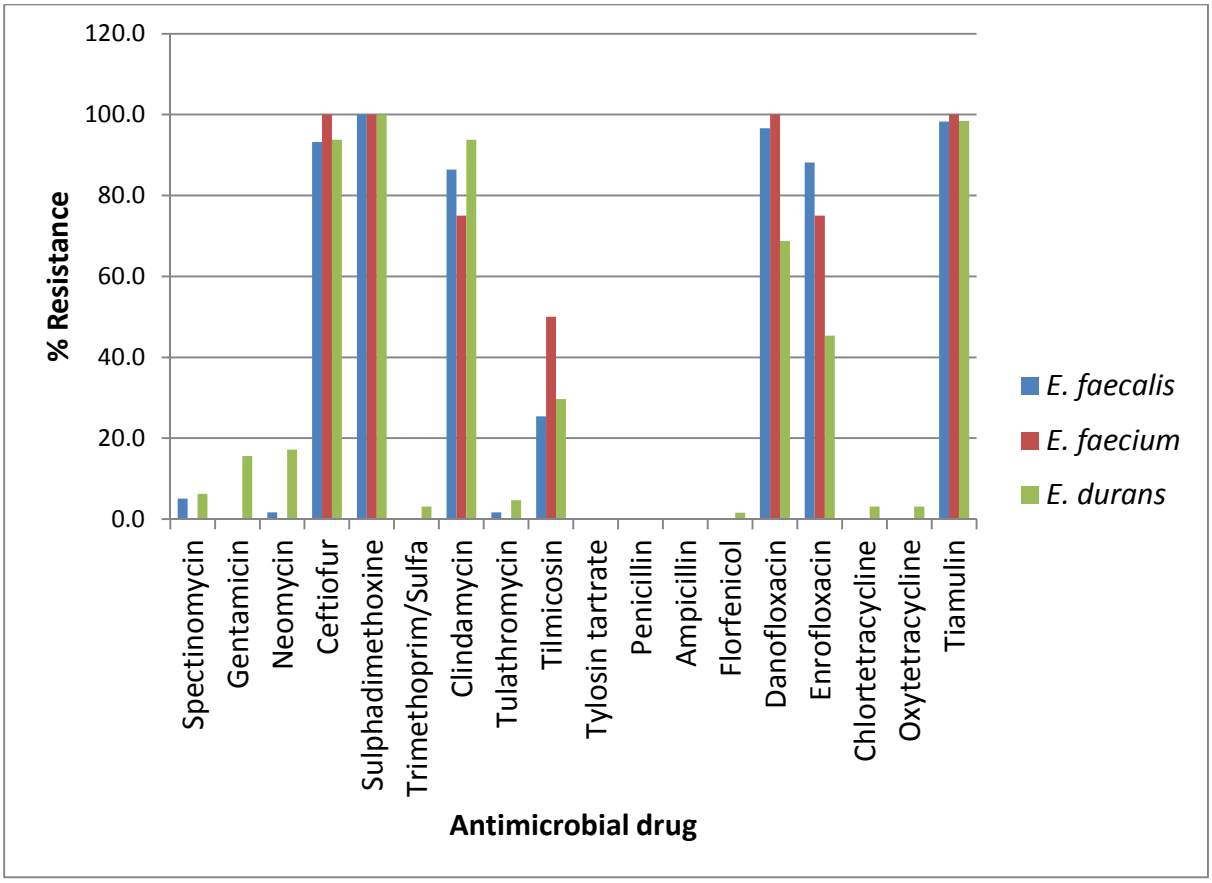

Figure 14: Percentage antimicrobial resistance observed against three *Enterococcus* strains tested.

4.4.1 *Escherichia coli*

Twelve *E. coli* isolates were tested in total and they included 11 isolates from the impala faecal samples, *i.e.*, 2 isolates from Olifants River, 4 isolates from Letaba River, 1 isolate from Crocodile River and 4 isolates from Sabie-Sand Rivers. Only one isolate was obtained from the water samples, from the Olifants River. The percentage resistance of *E. coli* isolated from the four perennial river systems in KNP is given in Table 8.

Across all four river systems *E. coli* was susceptible to spectinomycin, gentamicin, neomycin, ceftiofur and tulathromycin and totally resistant to clindamycin, tilmicosin, tylosin tartrate, penicillin and tiamulin (Figure 13). Approximately 16.7% of the *E. coli* isolates were resistant to the sulphonamides (sulphadimethoxine and trimethoprim/sulfamethoxazole). The sulphonamide resistance was only found in the water sample from the Olifants River and the faeces collected near the Letaba River. For the macrolides, *E. coli* was susceptible to tulathromycin but totally resistant to tilmicosin and tylosin tartrate.

Table 8: Percentage resistance of *E.coli* isolated from the four perennial river systems in KNP.

	Olifants River		Letaba River	Crocodile River	Sabie-Sand Rivers	All samples
	Faeces	Water	Faeces	Faeces	Faeces	
Antimicrobial Drug	**n=2**	**n=1**	**n=4**	**n=1**	**n=4**	**n=12**
Spectinomycin	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0
Neomycin	0.0	0.0	0.0	0.0	0.0	0.0
Ceftiofur	0.0	0.0	0.0	0.0	0.0	0.0
Sulphadimethoxine	0.0	**100.0**	25.0	0.0	0.0	16.7
Trimethoprim/Sulfamethoxazole	0.0	**100.0**	25.0	0.0	0.0	16.7
Clindamycin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Tulathromycin	0.0	0.0	0.0	0.0	0.0	0.0
Tilmicosin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Tylosin tartrate	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Penicillin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Ampicillin	0.0	**100.0**	0.0	0.0	0.0	8.3
Florfenicol	0.0	0.0	25.0	0.0	25.0	16.7
Danofloxacin	0.0	**100.0**	0.0	0.0	0.0	8.3
Enrofloxacin	0.0	**100.0**	0.0	0.0	0.0	8.3
Chlortetracycline	0.0	**100.0**	**100.0**	0.0	0.0	41.7
Oxytetracycline	0.0	**100.0**	**100.0**	0.0	0.0	41.7
Tiamulin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

Resistance to penicillin and general susceptibility to ampicillin was also observed with the *E. coli* isolates, however the only isolate from the water sample from the Olifants River was found to be resistant to ampicillin. The same isolate from the Olifants River was also resistant to danofloxacin and enrofloxacin. Resistance to florfenicol (16.7%) was found in two isolates from faecal samples, one each, from the Letaba River and the Sabie-Sand Rivers respectively. Of the *E. coli* isolates, 41.7% were resistant to the tetracylines (chlortetracycline and oxytetracycline) with the majority of these originating from faecal samples collected near the Letaba River, and one water isolate from the Olifants River. *E. coli* was totally resistant to tiamulin. The resistance in *E. coli* to antimicrobials known to be effective against this bacterium came mainly from the water sample from the Olifants River and the faecal samples collected near the Letaba River.

4.4.2 *Enterobacter cloacae*

A total of 49 *Enterobacter cloacae* isolates were tested and they were all of faecal origin. Six isolates from Olifants River, 7 isolates from Letaba River, 12 isolates from Crocodile River and 24 isolates from Sabie-Sand Rivers were obtained. The percentage resistance of *Enterobacter cloacae* isolated from the four perennial river systems in KNP is given in Table 9.

Across all four river sampling areas, *Enterobacter cloacae* was widely susceptible to spectinomycin, gentamicin, neomycin and ceftiofur and moderately resistant to the sulphonamides. Resistance was higher to sulphadimethoxine (49.0%) compared to trimethoprim/sulfamethoxazole (4.1%). Partial resistance to sulphadimethoxine was found in all four river systems; with the highest seen in the Crocodile River and the lowest in the Letaba River. The resistance to trimethoprim/sulfamethoxazole was found in the Crocodile River. *Enterobacter cloacae* were totally resistant to clindamycin and penicillin but displayed a high level of resistance to ampicillin (75.5%). For the macrolides, moderate resistance to tulathromycin (34.7%) and total resistance to tilmicosin and tylosin tartrate was observed.

Enterobacter cloacae was moderately resistant to florfenicol (30.6%) and this was the same resistance pattern observed across all four river systems. *Enterobacter cloacae* was susceptible to danofloxacin and enrofloxacin. A very low resistance (4.1%) was found to the tetracyclines (chlortetracycline and oxytetracycline) and this resistance originated from the faecal samples obtained near the Crocodile River. Across all four river systems *Enterobacter cloacae* was totally resistant to tiamulin.

Table 9: Percentage resistance of *Enterobacter cloacae* isolated from the four perennial river systems in KNP.

	Olifants River	Letaba River	Crocodile River	Sabie-Sand Rivers	All samples
	Faeces	Faeces	Faeces	Faeces	
Antimicrobial Drug	**n=6**	**n=7**	**n=12**	**n=24**	**n=49**
Spectinomycin	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0	0.0
Neomycin	0.0	0.0	0.0	0.0	0.0
Ceftiofur	0.0	0.0	0.0	0.0	0.0
Sulphadimethoxine	33.3	14.3	91.7	41.7	49.0
Trimethoprim/Sulfamethoxazole	0.0	0.0	16.7	0.0	4.1
Clindamycin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Tulathromycin	66.7	28.6	25.0	33.3	34.7
Tilmicosin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Tylosin tartrate	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Penicillin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Ampicillin	100.0	85.7	50.0	79.2	75.5
Florfenicol	33.3	14.3	25.0	37.5	30.6
Danofloxacin	0.0	0.0	0.0	0.0	0.0
Enrofloxacin	0.0	0.0	0.0	0.0	0.0
Chlortetracycline	0.0	0.0	16.7	0.0	4.1
Oxytetracycline	0.0	0.0	16.7	0.0	4.1
Tiamulin	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

4.4.3 *Pantoea* species

A total of 9 *Pantoea* species isolates were tested for resistance and were all obtained from faeces collected near the Crocodile River. The percentage resistance of *Pantoea* spp. is given in Table 10. *Pantoea* spp. was susceptible to spectinomycin, gentamicin, neomycin, ceftiofur, tulathromycin, ampicillin, florfenicol, danofloxacin and enrofloxacin. *Pantoea* spp. was resistant to sulphadimethoxine, trimethoprim/sulfamethoxazole, clindamycin, tilmicosin, tylosin tartrate, penicillin, chlortetracycline, oxytetracycline and tiamulin.

Table 10: Percentage resistance of *Pantoea* species isolated from the Crocodile River in KNP.

	Crocodile River
	Faeces
Antimicrobial Drug	**n=9**
Spectinomycin	0.0
Gentamicin	0.0
Neomycin	0.0
Ceftiofur	0.0
Sulphadimethoxine	100.0
Trimethoprim/Sulfamethoxazole	100.0
Clindamycin	100.0
Tulathromycin	0.0
Tilmicosin	100.0
Tylosin tartrate	100.0
Penicillin	100.0
Ampicillin	0.0
Florfenicol	0.0
Danofloxacin	0.0
Enrofloxacin	0.0
Chlortetracycline	100.0
Oxytetracycline	100.0
Tiamulin	100.0

4.4.4 *Enterococcus faecalis*

A total of 59 *Enterococcus faecalis* isolates were tested for resistance. Twelve isolates were from the Olifants River, 9 from Letaba River, 12 from Crocodile River and 19 from Sabie-Sand Rivers that were of faecal origin, whilst the water samples comprised of 4 isolates from Letaba River, 1 isolate from Crocodile River and 2 isolates from Sabie-Sand Rivers. The percentage resistance of *Enterococcus faecalis* isolated from the four perennial river systems in KNP is given in Table 11.

Table 11: Percentage resistance of *Enterococcus faecalis* isolated from the four perennial river systems in KNP.

	Olifants River	Letaba River		Crocodile River		Sabie-Sand Rivers		All samples
	Faeces	Faeces	Water	Faeces	Water	Faeces	Water	
Antimicrobial Drug	**n=12**	**n=9**	**n=4**	**n=12**	**n=1**	**n=19**	**n=2**	**n=59**
Spectinomycin	8.3	11.1	25.0	0.0	0.0	0.0	0.0	5.1
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neomycin	0.0	0.0	25.0	0.0	0.0	0.0	0.0	1.7
Ceftiofur	100.0	88.9	100.0	91.7	100.0	89.5	100.0	93.2
Sulphadimethoxine	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Trimethoprim/Sulfamethoxazole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Clindamycin	91.7	100.0	25.0	83.3	100.0	89.5	100.0	86.4
Tulathromycin	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.3**	**0.0**	**1.7**
Tilmicosin	**8.3**	**44.4**	**50.0**	**16.7**	**0.0**	**26.3**	**50.0**	**25.4**
Tylosin tartrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ampicillin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Florfenicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Danofloxacin	100.0	100.0	75.0	100.0	100.0	100.0	50.0	96.6
Enrofloxacin	100.0	88.9	50.0	91.7	100.0	89.5	50.0	88.1
Chlortetracycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oxytetracycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tiamulin	100.0	100.0	100.0	91.7	100.0	100.0	100.0	98.3

The resistance pattern of the *Enterococcus faecalis* isolates was similar across all four river systems and between the faecal and water samples.*Enterococcus faecalis* was totally susceptible to gentamicin, trimethoprim/sulfamethoxazole, tylosin tartrate, penicillin, ampicillin, florfenicol, chlortetracycline and oxytetracycline. *Enterococcus faecalis* was totally resistant to sulphadimethoxine and had a high resistance to danofloxacin (96.6%) and enrofloxacin (88.1%). *Enterococcus faecalis* was mostly resistant to tiamulin (98.3%).

Enterococcus faecalis was generally widely susceptible to the aminoglycosides spectinomycin and neomycin with only 5.1% and 1.7% of the isolates respectively showing resistance and this was seen at the Olifants River and Letaba River, however total susceptibility to gentamicin was observed. Across all four river systems *Enterococcus faecalis* had a very high resistance to ceftiofur (93.2%). For the sulphonamides, *Enterococcus faecalis* was totally resistant to sulphadimethoxine and susceptible to trimethoprim/sulfamethoxazole. *Enterococcus faecalis* was highly resistant to clindamycin (86.4%) across the river systems except for the water samples from Letaba River which had a low resistance (25%).

Enterococcus faecalis had a low resistance to the macrolides. The resistance to tulathromycin was very low (1.7%) which was only found in the isolates from faecal samples collected near the Sabie-Sand Rivers. There was resistance to tilmicosin (25.4%) across all four river systems, with the highest seen in the water and faecal samples of the Letaba River and the Sabie-Sand Rivers. *Enterococcus faecalis* was however, totally susceptible to tylosin tartrate.

4.4.5 *Enterococcus faecium*

A total of 4 *Enterococcus faecium* isolates were tested and they were all of faecal origin from the Crocodile River (n=2) and the Sabie-Sand Rivers (n=2). The percentage resistance of *Enterococcus faecium* isolated from the Crocodile and Sabie-Sand Rivers in KNP is given in Table 12.

Table 12: Percentage resistance of *Enterococcus faecium* isolated from the Crocodile and Sabie-Sand Rivers in KNP.

	Crocodile River	Sabie-Sand Rivers	All samples
	Faeces	Faeces	
Antimicrobial Drug	**n=2**	**n=2**	**n=4**
Spectinomycin	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Neomycin	0.0	0.0	0.0
Ceftiofur	**100.0**	**100.0**	**100.0**
Sulphadimethoxine	**100.0**	**100.0**	**100.0**
Trimethoprim/Sulfamethoxazole	0.0	0.0	0.0
Clindamycin	100.0	50.0	75.0
Tulathromycin	0.0	0.0	0.0
Tilmicosin	50.0	50.0	50.0
Tylosin tartrate	0.0	0.0	0.0
Penicillin	0.0	0.0	0.0
Ampicillin	0.0	0.0	0.0
Florfenicol	0.0	0.0	0.0
Danofloxacin	**100.0**	**100.0**	**100.0**
Enrofloxacin	100.0	50.0	75.0
Chlortetracycline	0.0	0.0	0.0
Oxytetracycline	0.0	0.0	0.0
Tiamulin	**100.0**	**100.0**	**100.0**

In the two river systems where *Enterococcus faecium* was found, all isolates were susceptible to spectinomycin, gentamicin, neomycin, trimethoprim/sulfamethoxazole, tulathromycin, tylosin tartrate, penicillin, ampicillin, florfenicol, chlortetracycline and oxytetracycline. All isolates were resistant to ceftiofur, sulphadimethoxine, danofloxacin and tiamulin. Resistance of *Enterococcus faecium* to tilmicosin was 50% for both river systems. Resistance to clindamycin and enrofloxacin was 75%. The resistance pattern was the same for both river systems except for clindamycin and enrofloxacin, where the Crocodile River isolates (100%) had a higher resistance than the isolates from the Sabie-Sand Rivers (50%).

4.4.6 *Enterococcus durans*

A total of 64 *Enterococcus durans* isolates were tested. Seven isolates from the Olifants River, 9 from the Letaba River, 12 from Crocodile River and 13 from the Sabie-Sand Rivers were obtained that were of faecal origin. Six isolates from the Olifants River, 4 from the Letaba River, 6 from the Crocodile River and 7 from the Sabie-Sand Rivers were obtained from the water samples. The percentage resistance of *Enterococcus durans* isolated from the four perennial river systems in KNP is given in Table 13.

Table 13: Percentage resistance of *Enterococcus durans* isolated from the four perennial river systems in KNP.

	Olifants River		Letaba River		Crocodile River		Sabie-Sand Rivers		All samples
	Faeces	Water	Faeces	Water	Faeces	Water	Faeces	Water	
Antimicrobial drug	**n=7**	**n=6**	**n=9**	**n=4**	**n=12**	**n=6**	**n=13**	**n=7**	**n=64**
Spectinomycin	0.0	0.0	11.1	0.0	0.0	0.0	7.7	28.6	6.3
Gentamicin	0.0	16.7	11.1	0.0	25.0	16.7	23.1	14.3	15.6
Neomycin	0.0	33.3	11.1	0.0	25.0	0.0	23.1	28.6	17.2
Ceftiofur	100.0	100.0	88.9	100.0	100.0	100.0	84.6	85.7	93.8
Sulphadimethoxine	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Trimethoprim/Sulfamethoxazole	0.0	0.0	11.1	0.0	0.0	0.0	7.7	0.0	3.1
Clindamycin	85.7	100.0	88.9	75.0	100.0	100.0	92.3	100.0	93.8
Tulathromycin	14.3	0.0	0.0	25.0	8.3	0.0	0.0	0.0	4.7
Tilmicosin	14.3	33.3	55.6	50.0	33.3	16.7	15.4	28.6	29.7
Tylosin tartrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ampicillin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Florfenicol	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	1.6
Danofloxacin	85.7	100.0	66.7	75.0	58.3	83.3	46.2	71.4	68.8
Enrofloxacin	42.9	100.0	44.4	50.0	41.7	33.3	15.4	71.4	45.3
Chlortetracycline	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	3.1
Oxytetracycline	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	3.1
Tiamulin	100.0	100.0	88.9	100.0	100.0	100.0	100.0	100.0	98.4

Enterococcus durans was totally susceptible to tylosin tartrate, penicillin and ampicillin. A low resistance to spectinomycin was observed in isolates mainly in the faecal samples of the Letaba River and the faecal and water samples of the Sabie-Sand Rivers. Similarily a low level resistance to florfenicol (1.6%), chlortetracycline (3.1%) and oxytetracycline (3.1%) was observed and was only found in isolates from the water samples from the Crocodile River. Resistance in *Enterococcus durans* to gentamicin (15.6%) and neomycin (17.2%) was found in all four river systems. *Enterococcus durans* was highly resistant to ceftiofur (93.8%).

Enterococcus durans was totally resistant to sulphadimethoxine and had a low resistance to trimethoprim/sulfamethoxazole (3.1%) originating from the faecal samples obtained near the Letaba River and the Sabie-Sand Rivers. *Enterococcus durans* was mostly resistant to clindamycin (93.8%) across all four river systems. For the macrolides, resistance to tulathromycin (4.7%) was observed and originated from the faecal samples obtained near the Olifants River and Crocodile River and the water samples of the Letaba River. Resistance to tilmicosin (29.7%) was found across all four river systems. Resistance to danofloxacin (68.8%), enrofloxacin (45.3%) and tiamulin (98.4%) was found in all river sampling points.

CHAPTER 5

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Antimicrobial resistance in wildlife is receiving more prominence following the emergence of antimicrobial resistance in human and veterinary medicine and the risk of zoonotic infections with resistant bacteria (Acar & Röstel 2001; Bengis *et al*. 2004; Middleton & Ambrose 2005; Wheeler *et al*. 2012). The increased prevalence of antimicrobial resistance is thought to be due to the use and/or misuse of antimicrobial drugs (Van Den Bogaard & Stobberingh 2000). Interestingly, a lot of research has been has been done into understanding the ecology of antimicrobial resistance in pathogens, however there remains a need for studies on resistance in commensal bacteria and in natural populations (Allen *et al*. 2010). Investigating the likely sources, reservoirs and mechanisms of persistence of antimicrobial resistance in wildlife, which have not been treated with antimicrobials, is important in interpreting the role of wildlife as sources of resistant bacteria, or in the transmission of resistant bacteria to humans and domestic animals. Establishing antimicrobial resistance surveillance and monitoring programmes within pristine areas will further allow for monitoring for emerging antimicrobial resistance. Studies in different parts of the world have yielded conflicting results of the prevalence of antimicrobial resistance in the normal enteric bacterial flora of wildlife, such as an almost complete absence of resistance in enterobacteria isolated from moose, deer and vole in Finland compared to a high prevalence of resistance in faecal bacteria from wild rodents living in northwest England (Gilliver *et al*. 2001; Österblad *et al*. 2001). The purpose of this research project was to investigate the antimicrobial resistance patterns in commensal bacteria isolated from impala and from their water sources in the Kruger National Park (KNP), South Africa.

An antimicrobial resistance surveillance and monitoring programme should be based on three categories of bacteria: indicator or commensal bacteria, e.g. *E. coli, Enterococcus faecalis*, *E. faecium*; zoonotic bacteria, e.g. *Salmonella enterica*; and animal pathogens, e.g. *Mannheimia haemolytica* (OIE 2011). Indicator bacteria are selected as they are common in the intestinal tract of both humans and animals and represent a reservoir of resistance genes for pathogenic bacteria. Commensal bacteria and their resistance genes are easily transferred between humans and animals and this highlights the concern about these organisms as potential emerging zoonoses (Epstein *et al*. 2009). Zoonotic bacteria are included because the resistance developed has implications for therapy of infected humans. Monitoring animal pathogens is also important because knowledge of resistance of animal

pathogens to antimicrobial drugs is important as a guide to veterinarians in their prescription decisions (OIE 2011). This is especially important from a veterinary public health perspective for the drugs used in food animals – as an increased rate of resistance to these antimicrobials is vital should alternative drugs that are crucial to human health be used to treat animals (Marshall & Levy 2011).

For this environmental study of emerging antimicrobial resistance in an ecological system not known to be under any antimicrobial pressure, commensal bacteria from impalas and their water sources in the KNP were selected. From the indicator bacteria group, *E. coli*, *Enterococcus faecalis*, and *E. faecium* were cultured and analysed further. *Enterococcus durans* (22.9%) and *Enterobacter cloacae* (17.5%) were cultured from the impala faeces and their water sources, and thus were included in this study. *Pantoea* species was isolated only from the Crocodile River, and was selected to see whether it had any peculiar resistance patterns. Interestingly, zoonotic bacteria and primary animal pathogens were not isolated, as certain zoonotic bacteria would not have grown using the direct plating method in this study. This should however not detract from the results as commensal bacteria represent the best study material for determining the spread of resistant bacteria from industrialised areas to pristine regions (McArthur & Tuckfield 2000). Antimicrobial resistance was shown to a wide range of structurally different antimicrobials including daptomycin, an antimicrobial of last resort in the treatment of drug resistant Gram-positive pathogens in a microbiome, a cave in New Mexico that has been isolated for over 4 million years (Bhullar *et al*. 2012).

The direct plating method was used to isolate resistant bacteria by means of discs containing antimicrobials and allows for screening of greater than 10 000 bacteria per sample (Lester *et al*. 1990). Other studies using this method used MacConkey agar which selects for Gram-negative organisms (Maré 1968; Lester *et al*. 1990; Bartoloni *et al*. 2004; Bartoloni *et al*. 2006; Thaller *et al*. 2010). In this study, Mueller-Hinton agar was used so that Gram-positive and Gram-negative bacteria could be cultured. A problem found with the method of directly plating the faecal sample, is that fungi also grew within the zones of inhibition, leading to unreadable zones. Several plates were infested with fly larvae and with their movement around the plate, gave mixed cultures. In the pure cultures, colonies that grew within the zones of inhibition were considered to be resistant. Further analysis of these bacteria allowed analysis of the extent of the antimicrobial resistance.

To quantitatively study the resistance in the commensal bacteria, the microdilution inhibitory concentration (MIC) antimicrobial susceptibility test was chosen because it overcomes several limitations of the disc diffusion test. There are however no commercial MIC plates for

wild animals, hence, the commercial MIC plate (Sensititre® Bovine/Porcine plate format BOP06F) was chosen, as it was reasonable to assume that the impala as a ruminant is closely related to members of the Family Bovidae. All the plates tested conformed to the quality control measures put in place.

Historically impala occupied the Lowveld, mopane veld, sweet and mixed bushveld, mountain or sour bushveld and Kalahari (Bothma 2002). Impala prefer woodland areas especially those associated with *Acacia*, *Colophospermum mopane*, *Baikiaea*, *Combretum* and *Terminalia*. Cover and availability of water are essential for them. They generally avoid open grasslands and floodplains. They have a tendency to congregate on heavily over-utilised veld (Skinner & Chimimba 2005). Impala browse and graze. Impala are dependent on the availability of water, remain within 2 km of it and drink daily. They require 2.5 litres per day. Some of their moisture can be obtained from succulent food when necessary in the dry season (Bothma 2002).

Impala are gregarious, occurring in small herds of 6-20 animals and in larger congregations of 50-100 animals during the wet season. Their social organisation consists of males, which are territorial only during the rut, and bachelor and breeding herds. The average annual home range for female impalas was 5.16 km^2 and for males it varied depending on their age (Skinner & Chimimba 2005).

The Olifants River system covers an area of approximately 54 570 km^2 and is divided into nine secondary catchments. The total mean annual runoff is approximately 2400 million cubic metres per year. There are thirty large dams in the Olifants River catchment. The Olifants River originates in the Highveld grasslands and major tributaries include the Klein Olifants River, Elands River, Wilge River and Bronkhorstspruit. The Olifants River meanders through the Drakensberg, descending the escarpment where the Steelpoort and Blyde tributaries join the river before it enters the KNP (RHP 2001a).

The upper part of the Olifants River catchment is characterised by activities, such as mining for coal, and industrial activities. Acid leaching from mines in the Witbank area result in poor water quality. Agricultural activities have limited influence in the area of the Bronkhorstspruit Dam and Loskop Dam. However, the water quality of the Olifants River is negatively impacted by the high acidity and high concentrations of dissolved salts in some of the tributaries, especially the Klip River. The area from Loskop Dam through to Marble Hall is in unacceptable condition due the intensive irrigation of crops and fruit trees. The heavy abstraction of water impacts negatively on the river ecology through changes to flow regime.

Runoff from commercial agricultural areas causes eutrophication and contamination of the water. The middle section is characterised by deforestation, informal settlements and subsistence farming result in overgrazing and overutilization of the area. As a result, riverbanks are collapsing due to erosion. After heavy rains the Olifants River has a red-brown colour from all the suspended sediments. The health of the Olifants River improves downstream at the confluence of the Blyde River, as the water coming in from the Blyde River is of better quality than that of the Olifants River. The Blyde River is part of the "Kruger to Canyon" biosphere reserve which aims to manage the river in a natural state in order to preserve water quality downstream in the KNP. Mining, industrial activities and farming in the Phalaborwa area negatively impacts the Olifants River. Heavy metals and chlorides may reach unacceptable levels during the low flow periods. Large quantities of sediment and irregular release of water from storage dams has a negative impact on the ecosystem in the lower part of the Olifants River System (RHP 2001a). Within the KNP, sewage from the Olifants camp enters the river after treatment (Mariano 2007).

The Letaba River system covers an area of approximately 13 670 km^2. The mean annual runoff is 574 million cubic metres per year. There are more than twenty dams in the Letaba River catchment. The Groot Letaba River originates in the Northern Drakensberg Mountains and flows down in an easterly direction where it confluences with the Klein Letaba River at the KNP border. The Letaba River passes through the KNP where it joins the Olifants River near the Mozambique border. Major tributaries of the Letaba River include: the Klein Letaba, the Middle Letaba, the Nsama and the Molotosi Rivers (RHP 2001b).

The natural grasslands of the Groot Letaba headwater have been replaced by commercial forestry. Other land-use activities in this area include: trout farming, sand mining, agriculture and informal settlements. The Groot Letaba catchment between Tzaneen Dam and KNP has been transformed into commercial agriculture, of which more than 42% is under irrigation. Impoundment and abstraction for agriculture reduce the flow of the Groot Letaba River. Rural communities in the eastern part of the Letaba River practise subsistence farming and over-utilize the vegetation through cutting and grazing with their cattle. The biggest threat to the water quality of the western section of the Groot Letaba River is the use of agricultural pesticides and fertilisers. The land-use of the Klein Letaba River consists of subsistence farming by rural communities and large commercial banana, papino, paw-paw and mango plantations. An irrigation scheme feeds the commercial fruit farms. The Klein Letaba carries high sediment loads because of erodible soils and poor land management in the catchment. The lower end of the river is in quite good condition due to low population densities near the river. The Letaba River within the KNP is a wilderness area and tourist impacts are minimal

(RHP 2001b). Within the KNP, sewage from the tourist camps of Shimuweni and Letaba camps, after treatment, are discharged into the river (Mariano 2007).

The Crocodile River system covers an area of approximately 10 450 km^2. The mean annual runoff is 1200 million cubic metres per year. The Crocodile River originates in the Steenkampsberg Mountains at an altitude of more than 2000 m above sea level. Tributaries in the Drakensberg Mountains join the Upper Crocodile River. The Crocodile River then drops into the Lowveld at an altitude range of 800-1000 m. The river forms the southern boundary of the KNP where it is 40-50m wide, slow flowing and with mostly large sandy pools (RHP 2001a).

In the Upper Crocodile River catchment, agriculture, forestry and infrastructure development are the drivers of ecological change. The land-use activity in the Dullstroom and Machadodorp area is trout farming which has a negative impact on the river system due to the construction of weirs and dams which damage the wetlands. Agricultural activities (irrigated and dry-land cropping, cattle and sheep grazing) in the Kwena Basin area result in natural habitat loss. The water quality is poor due to the application of fertilisers and the resulting contamination and eutrophication of the water. The lowveld area is under intense pressure due to agricultural (citrus, vegetable, tobacco and sugar cane), industrial and urban land uses. Water is abstracted from the river for irrigation and domestic use. Domestic effluent and urban and industrial waste from Nelspruit have a negative impact on the water quality of the Crocodile River. In the KNP region, the northern bank of the river has a high conservation status. However, the southern bank is heavily utilised by citrus and sugar cane farming as well as tourist lodges. The water abstraction for irrigation often results in a lower than desired flow which in turn has a negative impact on the overall water quality (RHP 2001a). Within the KNP, sewage from the tourist camps of Malelane, Berg en Dal and Crocodile Bridge, after treatment, discharge into the Crocodile River (Mariano 2007).

The Sabie-Sand River system covers an area of approximately 6320 km^2. The mainstream of the catchment is the Sabie River, with the Sand and Marite Rivers acting as major tributaries. The source of the Sabie River is in the Drakensberg Escarpment at 2130 m above sea level. The Sabie River then drops into the Lowveld and joins the Sand River inside the KNP. The mean annual rainfall in this river system varies between 2000 mm on the Escarpment to around 600 mm in the Lowveld. Rainfall is mostly between November and March, in the form of tropical storms. The summer maximum temperatures are high and evaporation averages at 1700 mm per year in the Lowveld region. Mean annual runoff in the Sabie-Sand River system is approximately 762 million cubic metres. Low flows in the Sabie

River are experienced at the end of the winter dry season and flows peak in the summer. No-flow conditions have never been recorded (RHP 2001a).

The Sabie-Sand River system is in a natural state due to the KNP and private conservation activities. However, urbanisation and other land uses threaten the protected areas. The main land use activity upstream is forestry. The activities in the middle section of the river system include subsistence and small scale farming of livestock and fruit. Overgrazing in this area has caused extensive erosion and sedimentation. The demand for water and the generation of wastewater are relatively high. The lower part of the river system is protected by the conservation activities of KNP (RHP 2001a). Inside KNP, sewage from tourist camps (Kruger Gate, Skukuza, Nkuhlu and Lower Sabie) are discharged into the river after going through sewage purification plants (Mariano 2007). With Skukuza being the main camp of KNP, there is a high impact from tourists.

The members of the *Enterobacteriaceae* are geographically widespread and are found in the intestines of animals and humans and also widely distributed throughout the environment in soil, water and on plants (Quinn *et al*. 1994). *E. coli* is considered to be the predominant facultative anaerobe in the bowel and thought to have low virulence in adult animals and to be beneficial to the host, as part of the normal microflora. However there are many pathogenic strains of *E. coli* that cause a variety of diseases in animals and humans (Aarestrup 2006). *E. coli* is an important “indicator bacterium” used in the tracking of the evolution of antimicrobial resistance in different ecosystems (Costa *et al*. 2008). The majority of studies carried out on antimicrobial resistance in healthy wild animals have focused on *E. coli* as the study organism (Pagano *et al*. 1985; Caprioli *et al*. 1991; Cole *et al*. 2005; Sayah *et al*. 2005; Costa *et al*. 2008; Sjölund *et al*. 2008; Adesiyun *et al*. 2009; Literak *et al*. 2010; Alroy & Ellis 2011; Williams *et al*. 2011). In this study *E. coli* was isolated at a low rate with significant resistance against the sulphonamides, florenicol, danofloxacin, enrofloxacin, and tetracyclines are noticeable and suggest that these strains must have arrived in the KNP via water from industrialized and populated areas. *Enterococcus* species are usually found in the intestines of humans and other animals but can also be found free-living in soil, on plants, or in dairy products (Manero & Blanch 1999). Our results are similar to the findings of Poeta (2005) in a study conducted in Portugal investigating antimicrobial resistance in enterococci.

Antimicrobial drugs of the aminoglycosides and aminocyclitols include, among others, streptomycin, amikacin, apramycin, gentamicin, kanamycin, neomycin, netilmicin, tobramycin, and spectinomycin. Members of this group inhibit bacterial protein synthesis at

the ribosomal level (Prescott & Baggot 1988; CLSI 2008). These antimicrobial drugs are active primarily against aerobic Gram-negative bacilli and if used in synergistic combination with cell-wall active compounds are active against some resistant Gram-positive bacteria, such as enterococci (Murray *et al*. 1995; CLSI 2008). Gentamicin has greater activity than neomycin (Prescott & Baggot 1988). This was also reported in South Africa, SANVAD report (2007) which indicated resistance against gentamicin: *E. coli* (0-6.5%), *E. faecium* (20%), *E. faecalis* (29.2%) and neomycin: *E. coli* (0-19.6%), *E. faecium* (40%), *E. faecalis* (66.7%). Our studies showed that the Gram-negative *E. coli*, *Enterobacter cloacae* and *Pantoea* species were mostly susceptible to spectinomycin, gentamicin and neomycin whilst the Gram-positive enterococci showed a low resistance to spectinomycin, gentamicin and neomycin. *E. faecium* was susceptible to the three drugs. *E. durans* was slightly more resistant to these three drugs compared to *E. faecalis*. The resistance levels for gentamicin and neomycin in this research project were much lower compared to the SANVAD report (2007).

Cephalosporins which fall under the β-lactam antimicrobial drugs all share a common, central, four-membered β-lactam ring and their mode of action is the inhibition of cell wall synthesis (CLSI 2008). These drugs are often referred to as “first-”, “second-“, “third-” or “fourth-generation” based on the extent of their activity. Third-generation (broad-spectrum) cephalosporins have slightly reduced activity than the narrow-spectrum drugs against Gram-positive cocci, but they are much more active against the *Enterobacteriaceae* (Prescott & Baggot 1988; Murray *et al*. 1995). Enterococci are considered resistant to cephalosporins and currently the test results of enterococci against the cephalosporins should not be reported (CLSI 2008). In this research report the Gram-negative bacteria were sensitive to ceftiofur, whereas the resistance reported for *E. coli* in SANVAD (2007) was 0-8.8%. Our results reported the enterococci were mostly resistant to ceftiofur, much higher compared to the SANVAD report (2007): *E. faecium* (60%) and *E. faecalis* (33.4%).

Sulphonamides are broad-spectrum antimicrobial drugs, inhibiting bacteria, toxoplasma and other protozoal drugs such as coccidia, but their antibacterial activity is significantly limited by the extensive resistance that has developed after over 50 years of use (Prescott & Baggot 1988). *E. coli* strains were initially susceptible to the sulphonamides; however increasing bacterial resistance has limited their efficacy. Enterococci are usually resistant to the sulphonamides (Murray *et al*. 1995) and this is commonly reported in bacteria isolated from animals, thus reflecting use of these drugs over many years (Prescott & Baggot 1988). Many Gram-positive cocci and most Gram-negative bacilli are susceptible to trimethoprim/sulfamethoxazole. The drug combination has variable bactericidal effects on enterococci (Murray *et al*. 1995). In this study *E. coli* was mostly susceptible to the

sulphonamides; with resistant strains originating from the Olifants and Letaba River. Sulphadimethoxine was less active against *Enterobacter cloacae* compared to *E. coli*. Resistance to trimethoprim/sulfamethoxazole in *Enterobacter cloacae* was present only in the Crocodile River. However, *Pantoea* species was totally resistant to sulphadimethoxine and trimethoprim/sulfamethoxazole. The enterococci were totally resistant to sulphadimethoxine and only *E. durans* originating from the Letaba and Sabie-Sand Rivers had a low resistance to trimethoprim/sulfamethoxazole. Results from this study showed a similar pattern to the SANVAD report (2007) where resistance was higher for sulphadiamethoxine compared to trimethoprim/sulfamethoxazole.

The lincosamides include lincomycin, clindamycin and pirlimycin. Their mode of action involves inhibiting protein synthesis at the ribosomal level (CLSI 2008). The lincosamides have a broad spectrum of activity against the aerobic Gram-positive cocci and anaerobes. However, the *Enterobacteriaceae* and enterococci are resistant to the lincosamides (Murray *et al*. 1995) and hence clindamycin should not be tested for activity against enterococci because of lack of clinical correlation (CLSI 2008). The Gram-negative bacteria are resistant because of impermeability and methylation of the ribosomal binding site of lincosamides (Prescott & Baggot 1988). The results from this study also support this general notion where it showed that the Gram-negative bacteria were also resistant to clindamycin, thus reflecting on the intrinsic resistance nature of the organisms to this group of antimicrobials. The enterococci were on the other hand, representing Gram-positive bacteria, were reported to be mostly resistant to clindamycin.

Antimicrobial drugs which consists of aivlosin, erythromycin, tylosin, tilmicosin and tulathromycin are referred to as macrolides and have been approved for use in animals. They inhibit bacterial protein synthesis at the ribosomal level (CLSI 2008) and hence are broad-spectrum antimicrobials, with activity against Gram-positive and some Gram-negative bacteria, mycoplasmas, chlamydiae, treponemes and rickettsiae (Murray *et al*. 1995). Many enterococci are resistant to all macrolides and *Enterobacteriaceae* are generally resistant (Prescott & Baggot 1988; Murray *et al*. 1995). Results from this study showed that *E. coli*, *Enterobacter cloacae* and *Pantoea* spp. were completely resistant to tylosin tartrate and tilmicosin and only *Enterobacter cloacae* showed some resistance to tulathromycin. The enterococci showed a low resistance to tilmicosin, susceptiblity to tylosin tartrate and were mostly susceptible to tulathromycin.

Penicillins are primarily active against non-β-lactamase-producing, Gram-positive and some fastidious Gram-negative bacteria, like the *Pasteurellaceae* family. Aminopenicillins

(ampicillin and amoxicillin) are active against some members of the *Enterobacteriacae* (CLSI 2008). The major antibacterial action of penicillins is derived from their ability to inhibit a number of bacterial enzymes, namely, penicillin-binding proteins that are essential for peptidoglycan synthesis (Murray *et al*. 1995). SANVAD (2007) reported resistance against ampicillin: *E. coli* (0-28.3%), *E. faecium* (40%) and *E. faecalis* (20.8%). In this research report the enterococci are reported to have no resistance to ampicillin. Ampicillin resistance in *E. coli* was within the range of the SANVAD (2007) report. The resistance to ampicillin in *Enterobacter cloacae* was high in our results.

Phenicols are broad-spectrum drugs which inhibit bacterial growth by binding to the peptidyltransferase centre of the ribosomes and prevention of peptide chain elongation. Antimicrobial drugs of the phenicols include: chloramphenicol, florfenicol and thiamphenicol (CLSI 2008). Chloramphenicol is active against Gram-positive and Gram-negative bacteria chlamydiae, mycoplasmas and rickettsiae but variably active against enterococci. Most *Enterobacteriaceae* are susceptible and activity against *Enterobacter* isolates is variable (Murray *et al*. 1995). SANVAD (2007) reported resistance to chloramphenicol as: *E. coli* (0-23.9%), *E. faecium* (0%) and *E. faecalis* (20.8%). Results from this study were lower than SANVAD (2007) except for *Enterobacter cloacae*.

Quinolones include the older quinolones, e.g. nalidixic acid, and the newer fluoroquinolones, such as danofloxacin, difloxacin, enrofloxacin, marbofloxacin, and orbifloxacin. This group of antimicrobial drugs function by inhibiting DNA-gyrase and/or topoisomerase IV activity of many Gram-positive and Gram-negative bacteria (CLSI 2008). Nalidixic acid has limited clinical applications as a result of the widespread emergence of bacterial resistance (Murray *et al*. 1995). Fluoroquinolones possess excellent activity against *Enterobacteriaceae*, however, activity against enterococci is lower (Murray *et al*. 1995). SANVAD (2007) reported resistance to enrofloxacin as: *E. coli* (0-65.9%), *E. faecium* (90%) and *E. faecalis* (95.8%). Results from this study indicate 8.3% resistance to danofloxacin and enroflaxacin in *E. coli*. The resistance in enterococci to the fluoroquinolones in our study was of a similar level to that reported in SANVAD (2007). *E. durans* had a lower resistance compared to *E. faecium* and *E. faecalis*.

Tetracyclines are broad-spectrum and drugs include: chlortetracycline, oxytetracycline, doxycycline and minocycline (Murray *et al*. 1995). Tetracyclines inhibit protein synthesis at the ribosomal level (CLSI 2008). Tetracyclines have activity against many Gram-positive and Gram-negative bacteria, mycoplasmas, chlamydiae, rickettsiae, and some protozoa. Although many *E. coli* isolates are susceptible to tetracyclines, pseudomonads and many

Enterobacteriaceae are resistant (Murray *et al*. 1995). SANVAD (2007) reported resistance to oxytetracycline as: *E. coli* (0-93.5%), *E. faecium* (50%) and *E. faecalis* (75%). In our research report resistance by *E. coli* to tetracycline is noticeable. Of the enterococcal isolates, only *E. durans* was reported to have resistance to the tetracyclines.

Pleuromutilins, such as tiamulin and valnemulin, inhibit protein synthesis (CLSI 2008). Tiamulin is used in veterinary practice for the control and specific therapy of infections in swines (Jones *et al*. 2002). Tiamulin is active against mycoplasma and anaerobic bacteria (Murray *et al*. 1995). Jones (2002) found that enterococci and enteric and nonfermentative Gram-negative bacilli were resistant to tiamulin, with MICs being >32µg/mℓ. Our results agree with this finding where *E. coli*, *Enterobacter cloacae* and *Pantoea* were resistant to tiamulin and the enterococci were 98.3 - 100% resistant.

5.1 Conclusion and Recommendations

Contrary to the hypothesis that pristine ecosystems often present with a low degree of antimicrobial resistance, our results are in tandem with the hypothesis that suggests that antimicrobial resistance occurs independent of the ecosystem, primarily due to evolutionary mechanisms possessed by microbes to evolve in nature. Furthermore in a setting such as KNP, whilst the wildlife may not be directly exposed to resistant bacteria, as a consequence of the use/misue of antimicrobials, they can be exposed via environmental contamination.

There have been several studies published on the detection of antimicrobial resistance in the environment, but the results are not comparable due to differences in sampling and laboratory methods used. Most studies used the disc diffusion method however, as has been shown in human and veterinary antimicrobial resistance monitoring and surveillance programmes, but a well standardized MIC method is preferable. Despite the differences in methods used, our results strongly suggest the effect of potentially polluted river systems on wildlife population. Whilst we were not able to show a strong correlation between the water samples and faecal samples, as just a few bacteria were isolated from water in comparison to faecal samples, the level of resistance showed by the bacteria isolated from water and faecal samples were indistinguishable. The difficulty in isolating bacteria from water compared to the relative ease from faecal samples as highlighted in this study further lends support to the importance of wildlife as sentinels in antimicrobial resistance tracking studies either as indicators of potential exposure of a local area to outside resistance factors (Sayah *et al*. 2005).

In the light of the above, and knowing full well that impalas are not subjected in any way to antimicrobial treatment, one can assume that they acquired the bacteria from the river systems in the KNP. Furthermore, based on reports on the river systems in the KNP, there is a significant level of pollution upstream in most of the rivers as a reasonable level of human activity *i.e.* farming systems and industrialization are known to occur. A proper evaluation of the river system in a tier system *i.e.* upper, mid and lower stream and a full assessment of coliform count may be necessary to fully ascertain the extent of the bacterial pollution of the rivers systems and to further justify the source of pollution as either local or ascending from areas of high human activity.

In general our data are significantly comparable to the SANVAD document despite the differences in the animal species tested; bearing in mind also the adjustments that had to be made in selecting appropriate breakpoints. National antimicrobial resistance monitoring and surveillance programmes should expand their surveillance to include environmental samples and wildlife samples, as the presence of resistant bacteria in the environment is now becoming increasingly relevant and the value of wildlife as indicators or sentinels of antimicrobial resistance cannot be underestimated.

In the final analysis, it must be emphasized that limited background levels of resistance from natural factors could be expected in bacterial isolates from impala and water sources. Antimicrobial drugs exist in nature, providing protection to fungi and plants that produce them. Bacteria had therefore developed resistance long before the advent of pharmaceuticals. However, during this study resistant phenotypes were observed that are very similar to those described from domestic animals in the country. Antimicrobial drugs have never been used on impala in the KNP suggesting that the resistance found in bacteria from impala was acquired from domestic animals most likely via water sources.

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APPENDIX A: MIC values for *Escherichia coli*, *Enterobacter cloacae*, *Pantoea* species, *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans* determined for all isolates

MICs for *Escherichia coli*			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Oliphants	W1.1	Water	32	<1	<4	<0.25	>256	>2/38	>16	8	64	>32	>8	>16	2	>1	>2	>8	>8	>32
	F1.14.1	Faeces	16	<1	<4	0.5	<256	<2/38	>16	16	64	>32	>8	4	4	<0.12	<0.12	4	2	>32
	F2.12.2.1	Faeces	32	<1	<4	0.5	<256	<2/38	>16	8	64	>32	>8	4	4	<0.12	<0.12	2	1	>32
Letaba	F3.6.2	Faeces	16	<1	<4	<0.25	<256	<2/38	>16	8	>64	>32	>8	2	8	<0.12	<0.12	>8	>8	>32
	F4.1.2	Faeces	16	<1	<4	0.5	<256	<2/38	>16	16	>64	>32	>8	4	4	<0.12	<0.12	>8	>8	>32
	F4.2.1	Faeces	32	<1	<4	0.5	>256	>2/38	>16	32	>64	>32	>8	4	4	<0.12	<0.12	>8	>8	>32
	F4.7.1	Faeces	16	<1	<4	0.5	<256	<2/38	>16	8	64	>32	>8	8	4	<0.12	<0.12	>8	>8	>32
Sand	F5.14.1	Faeces	16	<1	<4	<0.25	<256	<2/38	>16	8	64	>32	>8	2	2	<0.12	<0.12	2	1	>32
Sabie	F6.2.2	Faeces	16	<1	<4	0.5	<256	<2/38	>16	8	64	>32	>8	2	4	<0.12	<0.12	2	4	>32
	F9.5.3.1	Faeces	16	<1	<4	<0.25	<256	<2/38	>16	4	32	>32	>8	2	4	<0.12	<0.12	4	2	>32
	F10.12.2	Faeces	16	<1	<4	1	<256	<2/38	>16	16	>64	>32	>8	8	>8	<0.12	<0.12	8	4	>32
Crocodile	F11.9.2	Faeces	16	<1	<4	<0.25	<256	<2/38	>16	8	>64	>32	>8	4	4	<0.12	<0.12	4	2	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *Enterobacter cloacae*			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Oliphants	F1.12.1	Faeces	32	<1	<4	0.5	<256	<2/38	>16	64	>64	>32	>8	16	4	<0.12	<0.12	8	4	>32
	F2.6.1	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
	F2.8.2	Faeces	16	<1	<4	1	>256	<2/38	>16	64	>64	>32	>8	>16	4	<0.12	<0.12	4	2	>32
	F2.9.1	Faeces	16	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	8	4	>32
	F2.10.1	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
	F2.11.1	Faeces	32	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
Letaba	F3.1.3	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	16	4	<0.12	<0.12	4	2	>32
	F3.5.1	Faeces	32	<1	<4	1	<256	<2/38	>16	>64	>64	>32	>8	>16	4	<0.12	<0.12	4	2	>32
	F3.6.1	Faeces	16	<1	<4	0.5	<256	<2/38	>16	32	>64	>32	>8	16	4	<0.12	<0.12	4	4	>32
	F3.9.1	Faeces	32	<1	<4	0.5	<256	<2/38	>16	64	>64	>32	>8	>16	4	<0.12	<0.12	2	4	>32
	F3.11.3	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F3.14.1	Faeces	16	<1	<4	0.5	<256	<2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	4	4	>32
	F3.15.2	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
Sand	F5.1.1.2	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
	F5.2.1	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	4	2	>32
	F5.3.2.1	Faeces	16	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	16	4	<0.12	<0.12	4	4	>32
	F5.4.1	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	4	4	<0.12	<0.12	8	4	>32
	F5.5.1	Faeces	32	<1	<4	0.5	<256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F5.6.1.1	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	4	4	>32
	F5.6.1.2	Faeces	32	<1	<4	1	>256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
	F5.9.1	Faeces	32	<1	<4	0.5	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F5.13.1	Faeces	32	<1	<4	1	<256	<2/38	>16	16	>64	>32	>8	16	4	<0.12	<0.12	4	4	>32
	F5.15.1	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	8	8	<0.12	<0.12	8	4	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *E. cloacae* (continued)			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Sabie	F6.5.1	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	4	<0.12	<0.12	4	2	>32
	F6.6.2	Faeces	32	<1	<4	1	<256	<2/38	>16	64	>64	>32	>8	>16	8	<0.12	<0.12	4	2	>32
	F6.15.2	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	>16	8	<0.12	<0.12	8	4	>32
	F9.1.1	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F9.2.1	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	2	2	>32
	F9.7.1	Faeces	32	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	2	4	>32
	F9.8.3	Faeces	16	<1	<4	1	<256	<2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	4	4	>32
	F9.9.1.1	Faeces	32	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	16	8	<0.12	<0.12	8	4	>32
	F9.9.1.2	Faeces	32	<1	<4	1	>256	<2/38	>16	64	>64	>32	>8	8	4	<0.12	<0.12	4	2	>32
	F9.10.1	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	8	8	<0.12	<0.12	4	4	>32
	F10.2.1	Faeces	32	<1	<4	0.5	<256	<2/38	>16	64	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F10.3.1	Faeces	16	<1	<4	0.5	>256	<2/38	>16	32	>64	>32	>8	16	4	<0.12	<0.12	4	4	>32
	F10.4.3	Faeces	32	<1	<4	0.5	<256	<2/38	>16	64	>64	>32	>8	16	4	<0.12	<0.12	4	4	>32
	F10.6.2	Faeces	32	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	8	4	>32
Crocodile	F7.3.2	Faeces	64	<1	<4	0.5	<256	<2/38	>16	>64	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F7.9.2	Faeces	16	<1	<4	1	>256	>2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	>8	>8	>32
	F7.9.3	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	>64	>32	>8	4	1	<0.12	<0.12	>8	>8	>32
	F8.2.1	Faeces	32	<1	<4	2	>256	<2/38	>16	32	>64	>32	>8	>16	8	<0.12	<0.12	4	4	>32
	F8.5.3	Faeces	32	<1	<4	1	>256	<2/38	>16	>64	>64	>32	>8	8	8	<0.12	<0.12	4	4	>32
	F8.6.2	Faeces	32	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	4	4	>32
	F8.6.3	Faeces	16	<1	<4	0.5	>256	<2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	4	4	>32
	F8.7.1	Faeces	32	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	8	<0.12	<0.12	4	2	>32
	F11.2.1	Faeces	32	<1	<4	0.5	>256	<2/38	>16	32	>64	>32	>8	8	4	<0.12	<0.12	4	2	>32
	F11.4.2	Faeces	32	<1	<4	1	>256	<2/38	>16	64	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F11.13.2	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32
	F11.15.1	Faeces	16	<1	<4	1	>256	<2/38	>16	32	>64	>32	>8	>16	4	<0.12	<0.12	4	4	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *Pantoea* spp.			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Crocodile	F7.4.3	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	2	1	<0.12	<0.12	>8	>8	>32
	F7.5.3	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	4	1	<0.12	<0.12	>8	>8	>32
	F7.6.2	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	2	1	<0.12	<0.12	>8	>8	>32
	F7.7.2	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	2	2	<0.12	<0.12	>8	>8	>32
	F7.8.2	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	4	2	<0.12	<0.12	>8	>8	>32
	F7.11.2	Faeces	16	<1	<4	0.5	>256	>2/38	>16	4	64	>32	>8	2	2	<0.12	<0.12	>8	>8	>32
	F7.12.1	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	2	1	<0.12	<0.12	>8	>8	>32
	F7.13.2	Faeces	16	<1	<4	0.5	>256	>2/38	>16	4	64	>32	>8	2	1	<0.12	<0.12	>8	>8	>32
	F8.14.1	Faeces	16	<1	<4	0.5	>256	>2/38	>16	8	64	>32	>8	2	2	<0.12	<0.12	>8	>8	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *Enterococcus faecalis*			Antimicrobial Drugs (µg/mℓ)																	
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Oliphants	F1.3.2	Faeces	>64	8	16	>8	>256	<2/38	16	2	8	8	2	0.5	2	>1	>2	1	<0.5	>32
	F1.4.3	Faeces	64	8	16	>8	>256	<2/38	16	2	8	8	2	0.5	4	>1	>2	<0.5	<0.5	>32
	F1.5.2	Faeces	64	8	16	>8	>256	<2/38	16	4	16	4	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F1.6.3.1	Faeces	64	8	16	>8	>256	<2/38	16	2	8	8	2	0.5	2	>1	2	<0.5	<0.5	>32
	F2.3.1	Faeces	64	8	16	>8	>256	<2/38	16	4	16	8	2	1	4	>1	>2	1	1	>32
	F2.7.1	Faeces	64	8	8	>8	>256	<2/38	16	2	32	8	2	0.5	4	>1	>2	<0.5	<0.5	>32
	F2.8.1	Faeces	32	4	16	8	>256	<2/38	8	8	16	4	1	0.5	2	>1	2	<0.5	1	>32
	F2.10.2	Faeces	64	4	8	>8	>256	<2/38	8	4	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F2.12.1.1	Faeces	32	2	<4	8	>256	<2/38	2	32	16	2	0.5	0.5	2	>1	2	<0.5	1	32
	F2.12.1.2	Faeces	64	8	8	>8	>256	<2/38	16	4	16	8	4	0.5	4	>1	>2	<0.5	<0.5	>32
	F2.12.2.3	Faeces	64	8	16	>8	>256	<2/38	16	4	16	4	4	1	4	>1	>2	<0.5	<0.5	>32
	F2.13.2	Faeces	64	4	8	>8	>256	<2/38	16	2	16	4	2	0.5	2	>1	>2	<0.5	<0.5	>32
Letaba	W3.4	Water	64	2	8	8	>256	<2/38	4	32	32	2	0.5	0.5	4	>1	2	1	2	>32
	W4.1	Water	>64	8	>32	>8	>256	<2/38	2	4	16	2	2	1	2	0.5	0.5	<0.5	1	>32
	W4.2	Water	64	8	32	>8	>256	<2/38	2	8	32	2	1	0.5	4	>1	1	<0.5	1	>32
	W4.4	Water	64	8	32	>8	>256	<2/38	2	8	16	2	1	0.5	4	>1	2	1	1	>32
	F3.3.2	Faeces	32	4	16	>8	>256	<2/38	8	32	32	2	1	1	4	>1	2	1	2	>32
	F4.3.1.2	Faeces	64	8	16	>8	>256	<2/38	16	4	16	8	2	0.5	4	>1	>2	<0.5	<0.5	>32
	F4.4.1	Faeces	>64	8	16	>8	>256	<2/38	16	8	16	8	4	1	4	>1	>2	<0.5	<0.5	>32
	F4.8.2	Faeces	64	4	8	4	>256	<2/38	16	4	16	8	4	1	2	>1	>2	1	1	>32
	F4.9.1.2	Faeces	32	4	8	8	>256	<2/38	8	16	32	4	0.5	0.5	4	>1	2	1	2	>32
	F4.10.1.1	Faeces	32	2	8	>8	>256	<2/38	8	16	32	2	1	0.5	4	>1	1	1	2	>32
	F4.11.1.2	Faeces	64	4	16	>8	>256	<2/38	16	8	32	4	2	0.5	2	>1	2	1	2	>32
	F4.12.1.1	Faeces	64	4	16	>8	>256	<2/38	16	8	16	8	2	0.5	2	>1	>2	<0.5	1	>32
	F4.14.1	Faeces	64	8	16	>8	>256	<2/38	8	8	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *E. faecalis* (continued)			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Sand	F5.3.2.2	Faeces	32	4	16	>8	>256	<2/38	4	16	16	2	2	1	2	>1	2	1	1	>32
	F5.5.2.1	Faeces	64	8	16	>8	>256	<2/38	16	4	16	8	2	1	2	>1	>2	<0.5	<0.5	>32
	F5.8.3.1	Faeces	32	<1	8	>8	>256	<2/38	8	16	32	2	1	0.5	4	>1	2	<0.5	1	>32
Sabie	W9.1	Water	64	8	16	>8	>256	<2/38	8	4	16	8	4	1	2	>1	2	<0.5	<0.5	>32
	W10.2	Water	64	8	32	>8	>256	<2/38	16	8	32	4	2	0.5	4	1	1	1	1	>32
	F6.1.2	Faeces	64	4	8	>8	>256	<2/38	16	8	16	8	2	1	4	>1	>2	1	1	>32
	F6.2.1.1	Faeces	32	2	8	8	>256	<2/38	8	16	16	4	0.5	0.5	4	>1	2	<0.5	2	>32
	F6.3.2	Faeces	64	4	8	>8	>256	<2/38	16	8	16	8	4	1	4	>1	>2	<0.5	1	>32
	F6.6.1.1	Faeces	64	4	32	>8	>256	<2/38	16	4	16	8	4	1	2	>1	>2	<0.5	<0.5	>32
	F6.7.2	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	2	0.5	4	>1	>2	<0.5	<0.5	>32
	F6.9.2	Faeces	64	4	8	>8	>256	<2/38	16	4	16	8	4	1	4	>1	>2	<0.5	<0.5	>32
	F6.10.1.1	Faeces	64	8	8	>8	>256	<2/38	16	2	16	8	2	1	4	>1	>2	<0.5	<0.5	>32
	F6.14.1	Faeces	64	8	16	>8	>256	<2/38	16	4	8	4	2	1	2	>1	>2	<0.5	<0.5	>32
	F9.3.1	Faeces	32	<1	<4	4	>256	<2/38	8	32	32	2	0.5	0.5	4	>1	2	1	2	>32
	F9.4.2	Faeces	32	2	16	>8	>256	<2/38	8	64	32	4	1	1	4	>1	2	1	2	>32
	F9.6.3	Faeces	16	2	8	>8	>256	<2/38	8	32	16	2	1	0.5	2	>1	2	1	2	>32
	F9.8.1	Faeces	16	2	8	4	>256	<2/38	2	32	32	2	0.5	0.5	2	>1	1	<0.5	1	32
	F9.8.2	Faeces	64	2	8	>8	>256	<2/38	8	16	16	2	0.5	0.5	2	>1	2	<0.5	1	>32
	F10.2.2	Faeces	32	2	8	>8	>256	<2/38	2	16	32	2	1	0.5	4	>1	2	<0.5	1	>32
	F10.5.1	Faeces	16	2	<4	>8	>256	<2/38	4	8	8	2	1	1	2	>1	1	<0.5	1	>32
	F10.10.1	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *E. faecalis* (continued)			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Crocodile	W11.1	Water	64	8	16	>8	>256	<2/38	8	4	16	4	2	1	2	>1	>2	<0.5	<0.5	>32
	F7.2.3	Faeces	64	8	16	>8	>256	<2/38	16	2	8	4	2	1	2	>1	>2	<0.5	<0.5	>32
	F7.6.1	Faeces	32	2	<4	4	>256	<2/38	2	32	<4	<0.5	0.5	0.5	4	>1	2	<0.5	1	>32
	F7.8.1	Faeces	32	4	8	>8	>256	<2/38	16	2	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F7.10.1	Faeces	32	2	<4	8	>256	<2/38	<0.25	32	32	2	1	0.5	4	>1	1	1	2	1
	F7.13.1	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F8.1.2	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F8.3.2	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	2	1	2	>1	>2	<0.5	<0.5	>32
	F8.9.1	Faeces	64	4	8	>8	>256	<2/38	16	2	16	4	2	0.5	2	>1	>2	<0.5	<0.5	>32
	F8.12.1.2	Faeces	64	8	8	>8	>256	<2/38	16	2	16	8	2	1	4	>1	>2	<0.5	<0.5	>32
	F8.13.2	Faeces	64	8	16	>8	>256	<2/38	16	2	16	4	2	0.5	4	>1	>2	<0.5	<0.5	>32
	F8.14.3	Faeces	64	4	16	>8	>256	<2/38	8	16	32	2	1	1	4	>1	2	1	2	32
	F11.10.1	Faeces	64	8	16	>8	>256	<2/38	16	2	8	8	2	0.5	2	>1	>2	<0.5	<0.5	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

MICs for *Enterococcus faecium*			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Sand	F5.8.3.2	Faeces	32	2	8	>8	>256	<2/38	8	8	16	2	1	1	2	>1	1	1	1	>32
	F5.13.2.2	Faeces	32	<1	<4	8	>256	<2/38	2	32	32	2	0.5	0.5	4	>1	2	<0.5	1	>32
Crocodile	F11.12.1	Faeces	32	2	<4	8	>256	<2/38	4	16	32	2	0.5	0.5	4	>1	2	<0.5	1	>32
	F11.12.3	Faeces	64	8	32	>8	>256	<2/38	16	2	16	8	2	0.5	4	>1	>2	<0.5	<0.5	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *Enterococcus durans*			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Oliphants	W1.2	Water	64	8	>32	>8	>256	<2/38	16	4	16	8	2	1	2	>1	>2	<0.5	<0.5	>32
	W1.3	Water	64	8	16	>8	>256	<2/38	16	8	32	8	2	1	4	>1	>2	<0.5	1	>32
	W1.4	Water	64	8	>32	>8	>256	<2/38	16	8	32	8	4	1	4	>1	>2	1	1	>32
	W2.2	Water	64	8	32	>8	>256	<2/38	16	8	16	8	4	2	4	>1	2	<0.5	<0.5	>32
	W2.3	Water	64	16	16	>8	>256	<2/38	16	8	16	8	4	1	4	>1	2	<0.5	1	>32
	W2.4	Water	64	8	16	>8	>256	<2/38	16	8	16	8	4	1	4	>1	2	<0.5	1	>32
	F1.13.2	Faeces	32	2	8	>8	>256	<2/38	16	8	8	1	1	1	2	>1	1	<0.5	1	>32
	F2.1.3	Faeces	16	2	8	>8	>256	<2/38	8	8	16	2	1	0.5	2	>1	1	<0.5	1	>32
	F2.6.2	Faeces	64	2	<4	>8	>256	<2/38	16	4	16	4	2	1	2	>1	1	1	1	>32
	F2.9.3	Faeces	64	2	<4	>8	>256	<2/38	8	4	16	4	2	1	2	>1	2	<0.5	1	>32
	F2.14.2	Faeces	64	4	8	>8	>256	<2/38	2	2	8	2	2	0.5	2	0.5	1	<0.5	1	>32
	F2.14.3.2	Faeces	16	<1	<4	8	>256	<2/38	4	32	16	2	0.5	0.5	2	>1	2	<0.5	1	>32
	F2.15.1	Faeces	16	<1	<4	8	>256	<2/38	4	64	32	4	0.5	0.5	2	>1	2	<0.5	2	>32
Letaba	W3.1	Water	64	4	16	>8	>256	<2/38	1	2	16	4	0.25	<0.25	2	>1	1	<0.5	<0.5	>32
	W3.2	Water	32	2	8	>8	>256	<2/38	4	32	32	2	0.5	0.5	4	>1	2	1	2	>32
	W3.3	Water	32	2	8	8	>256	<2/38	8	64	32	2	0.5	0.5	4	>1	2	<0.5	1	>32
	W4.3	Water	64	4	8	>8	>256	<2/38	4	4	8	4	4	1	4	1	1	1	2	>32
	F3.4.1	Faeces	64	16	>32	>8	>256	<2/38	16	8	16	2	4	1	4	1	1	<0.5	1	>32
	F3.6.3	Faeces	64	4	16	>8	>256	>2/38	8	8	32	4	2	0.5	4	1	1	<0.5	1	>32
	F3.8.2	Faeces	16	2	8	8	>256	<2/38	8	32	32	2	0.5	0.5	2	>1	2	<0.5	2	>32
	F3.11.1	Faeces	>64	4	<4	>8	>256	<2/38	8	2	16	2	1	0.5	4	1	1	<0.5	1	>32
	F3.13.2	Faeces	64	2	<4	>8	>256	<2/38	8	4	16	2	2	1	2	>1	1	1	2	>32
	F4.1.1	Faeces	32	4	16	>8	>256	<2/38	8	32	32	2	1	1	4	>1	2	1	2	>32
	F4.2.2	Faeces	32	4	16	>8	>256	<2/38	8	32	32	2	2	1	4	>1	2	1	2	>32
	F4.5.1	Faeces	32	2	<4	8	>256	<2/38	4	32	32	2	0.5	0.5	4	>1	2	<0.5	1	>32
	F4.14.2	Faeces	16	2	8	2	>256	<2/38	2	16	16	2	0.5	0.5	2	>1	1	<0.5	1	8

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *E. durans*(continued)			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Sand	W5.1	Water	64	4	8	1	>256	<2/38	16	4	8	8	2	0.5	2	>1	>2	<0.5	<0.5	>32
	W5.2	Water	64	2	<4	>8	>256	<2/38	16	4	16	8	1	0.5	4	>1	>2	<0.5	<0.5	>32
	F5.15.2.2	Faeces	32	<1	<4	>8	>256	<2/38	2	32	32	2	0.5	0.5	4	>1	2	<0.5	1	>32
Sabie	W6.1	Water	64	8	16	>8	>256	<2/38	16	4	16	4	2	0.5	2	>1	>2	1	1	>32
	W6.2	Water	64	8	16	>8	>256	<2/38	16	4	32	4	4	1	2	>1	>2	1	2	>32
	W6.3	Water	64	8	32	>8	>256	<2/38	16	4	32	2	2	0.5	2	>1	>2	<0.5	1	>32
	W9.2	Water	>64	8	>32	>8	>256	<2/38	16	4	16	4	2	1	2	1	0.5	1	1	>32
	W10.3	Water	>64	16	>32	>8	>256	<2/38	8	2	16	2	2	1	2	0.5	0.5	<0.5	<0.5	>32
	F6.4.1	Faeces	64	8	8	>8	>256	<2/38	8	2	16	2	2	1	2	0.5	0.5	<0.5	<0.5	>32
	F6.9.3.1	Faeces	64	8	>32	>8	>256	<2/38	16	8	16	2	2	1	4	>1	1	<0.5	1	>32
	F6.13.1.1	Faeces	32	<1	<4	<0.25	>256	>2/38	8	<1	<4	16	1	0.5	2	>1	0.5	1	1	>32
	F9.1.2	Faeces	64	2	<4	>8	>256	<2/38	8	4	16	4	1	0.5	2	1	0.5	1	1	>32
	F9.7.2	Faeces	32	2	8	4	>256	<2/38	4	16	32	2	0.5	<0.25	2	>1	2	1	2	>32
	F9.12.2	Faeces	64	2	<4	>8	>256	<2/38	8	2	8	2	1	0.5	2	1	1	<0.5	1	>32
	F10.4.2	Faeces	64	16	32	>8	>256	<2/38	4	2	16	2	0.5	<0.25	2	1	1	<0.5	<0.5	>32
	F10.7.2	Faeces	64	16	>32	>8	>256	<2/38	16	16	16	2	4	1	4	>1	1	1	1	>32
	F10.7.3	Faeces	64	8	>32	>8	>256	<2/38	16	16	16	2	4	0.5	2	>1	1	<0.5	1	>32
	F10.9.3	Faeces	>64	16	32	>8	>256	<2/38	4	4	16	2	1	0.5	2	1	1	<0.5	<0.5	>32
	F10.12.3	Faeces	64	2	<4	>8	>256	<2/38	4	2	8	4	1	0.5	2	1	0.5	1	1	>32
	F10.15.1	Faeces	64	8	32	>8	>256	<2/38	16	4	16	4	2	1	2	0.5	0.5	<0.5	<0.5	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX A: continued

MICs for *E. durans*(continued)			Antimicrobial Drugs (µg/mℓ)																
RIVER	**ISOLATE**	**SOURCE**	**SPE**	**GEN**	**NEO**	**TIO**	**SDM**	**SXT**	**CLI**	**TUL**	**TIL**	**TYLT**	**PEN**	**AMP**	**FFN**	**DANO**	**ENRO**	**CTET**	**OXY**	**TIA**
Crocodile	W7.1	Water	64	>16	16	>8	>256	<2/38	8	4	16	4	2	0.5	8	>1	>2	1	2	>32
	W7.2	Water	64	8	16	>8	>256	<2/38	8	8	16	4	4	1	2	>1	1	>8	>8	>32
	W7.3	Water	64	8	16	>8	>256	<2/38	4	8	8	4	4	1	2	>1	1	>8	>8	>32
	W7.4	Water	64	8	16	>8	>256	<2/38	8	8	64	2	2	0.5	2	>1	>2	<0.5	1	>32
	W8.2	Water	64	8	16	>8	>256	<2/38	8	8	16	4	2	0.5	4	1	1	0.5	1	>32
	W8.3	Water	64	4	16	>8	>256	<2/38	4	8	16	4	2	0.5	4	>1	1	<0.5	1	>32
	F8.6.1	Faeces	64	8	16	>8	>256	<2/38	16	2	16	8	4	1	2	>1	>2	<0.5	<0.5	>32
	F8.9.3	Faeces	32	2	<4	>8	>256	<2/38	8	64	32	2	1	0.5	4	>1	2	1	2	>32
	F8.15.2	Faeces	64	2	<4	>8	>256	<2/38	8	2	8	2	2	1	2	1	1	<0.5	1	>32
	F11.1.1	Faeces	64	8	16	>8	>256	<2/38	16	4	16	2	2	0.5	2	0.5	0.5	<0.5	<0.5	>32
	F11.4.3	Faeces	64	16	16	>8	>256	<2/38	8	4	8	2	1	1	2	1	1	<0.5	1	>32
	F11.5.3	Faeces	64	8	32	>8	>256	<2/38	8	2	16	2	1	0.5	2	1	0.5	<0.5	<0.5	>32
	F11.7.1	Faeces	64	8	>32	>8	>256	<2/38	16	16	16	2	4	1	4	>1	1	1	1	>32
	F11.11.2	Faeces	32	4	8	>8	>256	<2/38	8	32	32	2	1	0.5	4	>1	2	1	2	>32
	F11.11.3	Faeces	64	16	>32	>8	>256	<2/38	8	8	16	2	2	1	4	>1	1	1	1	>32
	F11.13.1	Faeces	64	16	>32	>8	>256	<2/38	8	2	16	2	2	1	2	0.5	0.5	<0.5	<0.5	>32
	F11.14.2	Faeces	16	2	<4	>8	>256	<2/38	4	32	32	4	0.5	0.5	4	>1	2	1	1	>32
	F11.15.2	Faeces	32	2	8	>8	>256	<2/38	4	32	32	4	1	0.5	4	>1	2	1	1	>32

SPE: Spectinomycin, GEN: Gentamicin, NEO: Neomycin, TIO: Ceftiofur, SDM: Sulphadimethoxine, SXT: Trimethoprim/Sulfamethoxazole, CLI: Clindamycin, TUL: Tulathromycin, TIL: Tilmicosin, TYLT: Tylosin tartrate, PEN: Penicillin, AMP: Ampicillin, FFN: Florfenicol, DANO: Danofloxacin, ENRO: Enrofloxacin, CTET: Chlortetracycline, OXY: Oxytetracycline, TIA: Tiamulin

APPENDIX B: Distribution of MICs of *Escherichia coli*, *Enterobacter cloacae*, *Pantoea* species, *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans* isolated from impala faeces and their water sources in KNP

Table A: Distribution of MICs for all *E. coli* isolates (n=12)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	0.0								75.0	25.0				
Gentamicin	0.0				100.0									
Neomycin	0.0						100.0							
Ceftiofur	0.0		41.7	50.0	8.3									
Sulphadimethoxine	16.7												83.3	16.7
Trimethoprim/Sulfa[2]	16.7					83.3	16.7							
Clindamycin	100.0									100.0				
Tulathromycin	0.0						8.3	58.3	25.0	8.3				
Tilmicosin	100.0									8.3	50.0	41.7		
Tylosin tartrate	100.0										100.0			
Penicillin	100.0								100.0					
Ampicillin	8.3					33.3	41.7	16.7		8.3				
Florfenicol	16.7					16.7	66.7	8.3	8.3					
Danofloxacin	8.3	91.7				8.3								
Enrofloxacin	8.3	91.7					8.3							
Chlortetracycline	41.7					25.0	25.0	8.3	41.7					
Oxytetracycline	41.7				16.7	25.0	16.7		41.7					
Tiamulin	100.0										100.0			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table B: Distribution of MICs for *E. coli* isolated from Olifants (Faeces (F): n=2, Water (W): n=1), Letaba (F: n=4), Crocodile (F: n=1) and Sabie-Sand Rivers (F: n=4)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	Olifants	W	0.0									100.0				
	Olifants	F	0.0								50.0	50.0				
	Letaba	F	0.0								75.0	25.0				
	Crocodile	F	0.0								100.0					
	Sabie-Sand	F	0.0								100.0					
Gentamicin	Olifants	W	0.0				100.0									
	Olifants	F	0.0				100.0									
	Letaba	F	0.0				100.0									
	Crocodile	F	0.0				100.0									
	Sabie-Sand	F	0.0				100.0									
Neomycin	Olifants	W	0.0						100.0							
	Olifants	F	0.0						100.0							
	Letaba	F	0.0						100.0							
	Crocodile	F	0.0						100.0							
	Sabie-Sand	F	0.0						100.0							
Ceftiofur	Olifants	W	0.0		100.0											
	Olifants	F	0.0		100.0											
	Letaba	F	0.0		25.0	75.0										
	Crocodile	F	0.0		100.0											
	Sabie-Sand	F	0.0		50.0	25.0	25.0									
Sulphadimethoxine	Olifants	W	100.0													100.0
	Olifants	F	0.0												100.0	
	Letaba	F	25.0												75.0	25.0
	Crocodile	F	0.0												100.0	
	Sabie-Sand	F	0.0												100.0	
Trimethoprim/ Sulfamethoxazole	Olifants	W	100.0						100.0							
	Olifants	F	0.0					100.0								
	Letaba	F	25.0					75.0	25.0							
	Crocodile	F	0.0					100.0								
	Sabie-Sand	F	0.0					100.0								

APPENDIX B: continued

Table B: Distribution of MICs for *E. coli* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)													
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Clindamycin	Olifants	W	100.0									100.0				
	Olifants	F	100.0									100.0				
	Letaba	F	100.0									100.0				
	Crocodile	F	100.0									100.0				
	Sabie-Sand	F	100.0									100.0				
Tulathromycin	Olifants	W	0.0							100.0						
	Olifants	F	0.0							50.0	50.0					
	Letaba	F	0.0							50.0	25.0	25.0				
	Crocodile	F	0.0							100.0						
	Sabie-Sand	F	0.0						25.0	50.0	25.0					
Tilmicosin	Olifants	W	100.0										100.0			
	Olifants	F	100.0										100.0			
	Letaba	F	100.0										25.0	75.0		
	Crocodile	F	100.0											100.0		
	Sabie-Sand	F	100.0									25.0	50.0	25.0		
Tylosin tartrate	Olifants	W	100.0										100.0			
	Olifants	F	100.0										100.0			
	Letaba	F	100.0										100.0			
	Crocodile	F	100.0										100.0			
	Sabie-Sand	F	100.0										100.0			
Penicillin	Olifants	W	100.0								100.0					
	Olifants	F	100.0								100.0					
	Letaba	F	100.0								100.0					
	Crocodile	F	100.0								100.0					
	Sabie-Sand	F	100.0								100.0					
Ampicillin	Olifants	W	100.0									100.0				
	Olifants	F	0.0						100.0							
	Letaba	F	0.0					25.0	50.0	25.0						
	Crocodile	F	0.0						100.0							
	Sabie-Sand	F	0.0					75.0		25.0						

APPENDIX B: continued

Table B: Distribution of MICs for *E. coli* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Florfenicol	Olifants	W	0.0					100.0								
	Olifants	F	0.0						100.0							
	Letaba	F	25.0						75.0	25.0						
	Crocodile	F	0.0						100.0							
	Sabie-Sand	F	25.0					25.0	50.0		25.0					
Danofloxacin	Olifants	W	100.0					100.0								
	Olifants	F	0.0	100.0												
	Letaba	F	0.0	100.0												
	Crocodile	F	0.0	100.0												
	Sabie-Sand	F	0.0	100.0												
Enrofloxacin	Olifants	W	100.0						100.0							
	Olifants	F	0.0	100.0												
	Letaba	F	0.0	100.0												
	Crocodile	F	0.0	100.0												
	Sabie-Sand	F	0.0	100.0												
Chlortetracycline	Olifants	W	100.0								100.0					
	Olifants	F	0.0					50.0	50.0							
	Letaba	F	100.0								100.0					
	Crocodile	F	0.0						100.0							
	Sabie-Sand	F	0.0					50.0	25.0	25.0						
Oxytetracycline	Olifants	W	100.0								100.0					
	Olifants	F	0.0				50.0	50.0								
	Letaba	F	100.0								100.0					
	Crocodile	F	0.0					100.0								
	Sabie-Sand	F	0.0				25.0	25.0	50.0							
Tiamulin	Olifants	W	100.0										100.0			
	Olifants	F	100.0										100.0			
	Letaba	F	100.0										100.0			
	Crocodile	F	100.0										100.0			
	Sabie-Sand	F	100.0										100.0			

APPENDIX B: continued

Table C: Distribution of MICs for all *Enterobacter cloacae* isolates (n=49)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	0.0								34.7	63.3	2.0			
Gentamicin	0.0				100.0									
Neomycin	0.0						100.0							
Ceftiofur	0.0			26.5	71.4	2.0								
Sulphadimethoxine	49.0												51.0	49.0
Trimethoprim/Sulfa[2]	4.1					95.9	4.1							
Clindamycin	100.0									100.0				
Tulathromycin	34.7							2.0	2.0	61.2	28.6	6.1		
Tilmicosin	100.0											100.0		
Tylosin tartrate	100.0										100.0			
Penicillin	100.0								100.0					
Ampicillin	75.5						4.1	20.4	16.3	59.2				
Florfenicol	30.6						67.3	30.6						
Danofloxacin	0.0	100.0												
Enrofloxacin	0.0	100.0												
Chlortetracycline	4.1					6.1	65.3	24.5	4.1					
Oxytetracycline	4.1					20.4	75.5		4.1					
Tiamulin	100.0										100.0			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table D: Distribution of MICs for *Enterobacter cloacae* isolated from Olifants (Faeces (F): n=6), Letaba (F: n=7), Crocodile (F: n=12) and Sabie-Sand Rivers (F: n=24)

Antimicrobial drugs	River	% Resistance	Distribution (%) of MICs (µg/ml)												
			0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	Olifants	0.0								33.3	66.7				
	Letaba	0.0								42.9	57.1				
	Crocodile	0.0								41.7	50.0	8.3			
	Sabie-Sand	0.0								29.2	70.8				
Gentamicin	Olifants	0.0				100.0									
	Letaba	0.0				100.0									
	Crocodile	0.0				100.0									
	Sabie-Sand	0.0				100.0									
Neomycin	Olifants	0.0						100.0							
	Letaba	0.0						100.0							
	Crocodile	0.0						100.0							
	Sabie-Sand	0.0						100.0							
Ceftiofur	Olifants	0.0			16.7	83.3									
	Letaba	0.0			42.9	57.1									
	Crocodile	0.0			33.3	58.3	8.3								
	Sabie-Sand	0.0			20.8	79.2									
Sulphadimethoxine	Olifants	33.3												66.7	33.3
	Letaba	14.3												85.7	14.3
	Crocodile	91.7												8.3	91.7
	Sabie-Sand	41.7												58.3	41.7
Trimethoprim/ Sulfamethoxazole	Olifants	0.0					100.0								
	Letaba	0.0					100.0								
	Crocodile	16.7					83.3	16.7							
	Sabie-Sand	0.0					100.0								
Clindamycin	Olifants	100.0									100.0				
	Letaba	100.0									100.0				
	Crocodile	100.0									100.0				
	Sabie-Sand	100.0									100.0				

APPENDIX B: continued

Table D: Distribution of MICs for *Enterobacter cloacae* (continued)

Antimicrobial drugs	River	% Resistance	Distribution (%) of MICs (µg/ml)												
			0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Tulathromycin	Olifants	66.7									33.3	66.7			
	Letaba	28.6									71.4	14.3	14.3		
	Crocodile	25.0							8.3		66.7	8.3	16.7		
	Sabie-Sand	33.3								4.2	62.5	33.3			
Tilmicosin	Olifants	100.0											100.0		
	Letaba	100.0											100.0		
	Crocodile	100.0											100.0		
	Sabie-Sand	100.0											100.0		
Tylosin tartrate	Olifants	100.0										100.0			
	Letaba	100.0										100.0			
	Crocodile	100.0										100.0			
	Sabie-Sand	100.0										100.0			
Penicillin	Olifants	100.0								100.0					
	Letaba	100.0								100.0					
	Crocodile	100.0								100.0					
	Sabie-Sand	100.0								100.0					
Ampicillin	Olifants	100.0								16.7	83.3				
	Letaba	85.7							14.3	28.6	57.1				
	Crocodile	50.0						8.3	41.7		50.0				
	Sabie-Sand	79.2						4.2	16.7	20.8	58.3				
Florfenicol	Olifants	33.3						66.7	33.3						
	Letaba	14.3						85.7	14.3						
	Crocodile	25.0				8.3		66.7	25.0						
	Sabie-Sand	37.5						62.5	37.5						
Danofloxacin	Olifants	0.0	100.0												
	Letaba	0.0	100.0												
	Crocodile	0.0	100.0												
	Sabie-Sand	0.0	100.0												

APPENDIX B: continued

Table D: Distribution of MICs for *Enterobacter cloacae* (continued)

Antimicrobial drugs	**River**	**% Resistance**	**Distribution (%) of MICs (µg/ml)**												
			0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**>256**
Enrofloxacin	Olifants	0.0	100.0												
	Letaba	0.0	100.0												
	Crocodile	0.0	100.0												
	Sabie-Sand	0.0	100.0												
Chlortetracycline	Olifants	0.0						33.3	66.7						
	Letaba	0.0					14.3	71.4	14.3						
	Crocodile	16.7						83.3		16.7					
	Sabie-Sand	0.0					8.3	62.5	29.2						
Oxytetracycline	Olifants	0.0					16.7	83.3							
	Letaba	0.0					28.6	71.4							
	Crocodile	16.7					16.7	66.7		16.7					
	Sabie-Sand	0.0					20.8	79.2							
Tiamulin	Olifants	100.0										100.0			
	Letaba	100.0										100.0			
	Crocodile	100.0										100.0			
	Sabie-Sand	100.0										100.0			

APPENDIX B: continued

Table E: Distribution of MICs for all *Pantoea* species isolated from the Crocodile River (Faeces: n=9)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	0.0								100.0					
Gentamicin	0.0				100.0									
Neomycin	0.0						100.0							
Ceftiofur	0.0			100.0										
Sulphadimethoxine	100.0													100.0
Trimethoprim/Sulfa[2]	100.0						100.0							
Clindamycin	100.0									100.0				
Tulathromycin	0.0						22.2	77.8						
Tilmicosin	100.0										100.0			
Tylosin tartrate	100.0										100.0			
Penicillin	100.0								100.0					
Ampicillin	0.0					77.8	22.2							
Florfenicol	0.0				55.6	44.4								
Danofloxacin	0.0	100.0												
Enrofloxacin	0.0	100.0												
Chlortetracycline	100.0								100.0					
Oxytetracycline	100.0								100.0					
Tiamulin	100.0										100.0			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table F: Distribution of MICs for all *Enterococcus faecalis* isolates (n=59)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	5.1								5.1	23.7	66.1	5.1		
Gentamicin	0.0				3.4	20.3	27.1	49.2						
Neomycin	1.7						8.5	35.6	47.5	6.8	1.7			
Ceftiofur	93.2						6.8	10.2	83.1					
Sulphadimethoxine	100.0													100.0
Trimethoprim/Sulfa[2]	0.0					100.0								
Clindamycin	86.4		1.7			11.9	5.1	25.4	55.9					
Tulathromycin	1.7					28.8	23.7	18.6	13.6	13.6	1.7			
Tilmicosin	25.4						1.7	11.9	61.0	25.4				
Tylosin tartrate	0.0			1.7		28.8	23.7	45.8						
Penicillin	0.0			13.6	20.3	52.5	13.6							
Ampicillin	0.0			62.7	37.3		0.0							
Florfenicol	0.0					50.8	49.2							
Danofloxacin	96.6			1.7	1.7	96.6								
Enrofloxacin	88.1			1.7	10.2	32.2	55.9							
Chlortetracycline	0.0			71.2	28.8									
Oxytetracycline	0.0			50.8	30.5	18.6								
Tiamulin	98.3				1.7					5.1	93.2			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table G: Distribution of MICs for *Enterococcus faecalis* isolated from Olifants (Faeces (F): n=12, Water (W): n=0), Letaba (F: n=9, W: n=4), Crocodile (F: n=12, W: n=1) and Sabie-Sand Rivers (F: n=19, W: n=2)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	Olifants	F	8.3									16.7	75.0	8.3		
	Letaba	F	11.1									33.3	55.6	11.1		
	Letaba	W	25.0										75.0	25.0		
	Crocodile	F	0.0									25.0	75.0			
	Crocodile	W	0.0										100.0			
	Sabie-Sand	F	0.0								15.8	31.6	52.6			
	Sabie-Sand	W	0.0										100.0			
Gentamicin	Olifants	F	0.0					8.3	25.0	66.7						
	Letaba	F	0.0					11.1	55.6	33.3						
	Letaba	W	0.0					25.0		75.0						
	Crocodile	F	0.0					16.7	25.0	58.3						
	Crocodile	W	0.0							100.0						
	Sabie-Sand	F	0.0				10.5	36.8	26.3	26.3						
	Sabie-Sand	W	0.0							100.0						
Neomycin	Olifants	F	0.0						8.3	33.3	58.3					
	Letaba	F	0.0							33.3	66.7					
	Letaba	W	25.0							25.0		50.0	25.0			
	Crocodile	F	0.0						16.7	25.0	58.3					
	Crocodile	W	0.0								100.0					
	Sabie-Sand	F	0.0						10.5	52.6	31.6	5.3				
	Sabie-Sand	W	0.0								50.0	50.0				
Ceftiofur	Olifants	F	100.0							16.7	83.3					
	Letaba	F	88.9						11.1	11.1	77.8					
	Letaba	W	100.0							25.0	75.0					
	Crocodile	F	91.7						8.3	8.3	83.3					
	Crocodile	W	100.0								100.0					
	Sabie-Sand	F	89.5						10.5	5.3	84.2					
	Sabie-Sand	W	100.0								100.0					

APPENDIX B: continued

Table G: Distribution of MICs for *Enterococcus faecalis* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Sulphadimethoxine	Olifants	F	100.0													100.0
	Letaba	F	100.0													100.0
	Letaba	W	100.0													100.0
	Crocodile	F	100.0													100.0
	Crocodile	W	100.0													100.0
	Sabie-Sand	F	100.0													100.0
	Sabie-Sand	W	100.0													100.0
Trimethoprim/ sulfamethoxazole	Olifants	F	0.0					100.0								
	Letaba	F	0.0					100.0								
	Letaba	W	0.0					100.0								
	Crocodile	F	0.0					100.0								
	Crocodile	W	0.0					100.0								
	Sabie-Sand	F	0.0					100.0								
	Sabie-Sand	W	0.0					100.0								
Clindamycin	Olifants	F	91.7					8.3		16.7	75.0					
	Letaba	F	100.0							44.4	55.6					
	Letaba	W	25.0					75.0	25.0							
	Crocodile	F	83.3		8.3			8.3		8.3	75.0					
	Crocodile	W	100.0							100.0						
	Sabie-Sand	F	89.5					10.5	10.5	31.6	47.4					
	Sabie-Sand	W	100.0							50.0	50.0					
Tulathromycin	Olifants	F	0.0					41.7	41.7	8.3		8.3				
	Letaba	F	0.0						22.2	44.4	22.2	11.1				
	Letaba	W	0.0						25.0	50.0		25.0				
	Crocodile	F	0.0					75.0			8.3	16.7				
	Crocodile	W	0.0						100.0							
	Sabie-Sand	F	5.3					15.8	21.1	15.8	26.3	15.8	5.3			
	Sabie-Sand	W	0.0						50.0	50.0						

APPENDIX B: continued

Table G: Distribution of MICs for *Enterococcus faecalis* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Tilmicosin	Olifants	F	8.3							25.0	66.7	8.3				
	Letaba	F	44.4								55.6	44.4				
	Letaba	W	50.0								50.0	50.0				
	Crocodile	F	16.7						8.3	16.7	58.3	16.7				
	Crocodile	W	0.0								100.0					
	Sabie-Sand	F	26.3							10.5	63.2	26.3				
	Sabie-Sand	W	50.0								50.0	50.0				
Tylosin tartrate	Olifants	F	0.0					8.3	33.3	58.3						
	Letaba	F	0.0					22.2	22.2	55.6						
	Letaba	W	0.0					100.0								
	Crocodile	F	0.0			8.3		16.7	25.0	50.0						
	Crocodile	W	0.0						100.0							
	Sabie-Sand	F	0.0					42.1	15.8	42.1						
	Sabie-Sand	W	0.0						50.0	50.0						
Penicillin	Olifants	F	0.0			8.3	8.3	66.7	16.7							
	Letaba	F	0.0			11.1	22.2	44.4	22.2							
	Letaba	W	0.0			25.0	50.0	25.0								
	Crocodile	F	0.0			8.3	16.7	75.0								
	Crocodile	W	0.0					100.0								
	Sabie-Sand	F	0.0			21.1	26.3	36.8	15.8							
	Sabie-Sand	W	0.0					50.0	50.0							
Ampicillin	Olifants	F	0.0			83.3	16.7									
	Letaba	F	0.0			66.7	33.3									
	Letaba	W	0.0			75.0	25.0									
	Crocodile	F	0.0			66.7	33.3									
	Crocodile	W	0.0				100.0									
	Sabie-Sand	F	0.0			47.4	52.6									
	Sabie-Sand	W	0.0			50.0	50.0									

APPENDIX B: continued

Table G: Distribution of MICs for *Enterococcus faecalis* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Florfenicol	Olifants	F	0.0					58.3	41.7							
	Letaba	F	0.0					44.4	55.6							
	Letaba	W	0.0					25.0	75.0							
	Crocodile	F	0.0					58.3	41.7							
	Crocodile	W	0.0					100.0								
	Sabie-Sand	F	0.0					47.4	52.6							
	Sabie-Sand	W	0.0					50.0	50.0							
Danofloxacin	Olifants	F	100.0					100.0								
	Letaba	F	100.0					100.0								
	Letaba	W	75.0			25.0		75.0								
	Crocodile	F	100.0					100.0								
	Crocodile	W	100.0					100.0								
	Sabie-Sand	F	100.0					100.0								
	Sabie-Sand	W	50.0				50.0	50.0								
Enrofloxacin	Olifants	F	100.0					25.0	75.0							
	Letaba	F	88.9				11.1	33.3	55.6							
	Letaba	W	50.0			25.0	25.0	50.0								
	Crocodile	F	91.7				8.3	16.7	75.0							
	Crocodile	W	100.0						100.0							
	Sabie-Sand	F	89.5				10.5	42.1	47.4							
	Sabie-Sand	W	50.0				50.0	50.0								
Chlortetracycline	Olifants	F	0.0			83.3	16.7									
	Letaba	F	0.0			44.4	55.6									
	Letaba	W	0.0			50.0	50.0									
	Crocodile	F	0.0			83.3	16.7									
	Crocodile	W	0.0			100.0										
	Sabie-Sand	F	0.0			73.7	26.3									
	Sabie-Sand	W	0.0			50.0	50.0									

APPENDIX B: continued

Table G: Distribution of MICs for *Enterococcus faecalis* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Oxytetracycline	Olifants	F	0.0			75.0	25.0									
	Letaba	F	0.0			33.3	22.2	44.4								
	Letaba	W	0.0				75.0	25.0								
	Crocodile	F	0.0			75.0	8.3	16.7								
	Crocodile	W	0.0			100.0										
	Sabie-Sand	F	0.0			36.8	42.1	21.1								
	Sabie-Sand	W	0.0			50.0	50.0									
Tiamulin	Olifants	F	100.0									8.3	91.7			
	Letaba	F	100.0										100.0			
	Letaba	W	100.0										100.0			
	Crocodile	F	91.7				8.3					8.3	83.3			
	Crocodile	W	100.0										100.0			
	Sabie-Sand	F	100.0									5.3	94.7			
	Sabie-Sand	W	100.0										100.0			

APPENDIX B: continued

Table H: Distribution of MICs for all *Enterococcus faecium* isolates (n=4)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	0.0									75.0	25.0			
Gentamicin	0.0				25.0	50.0		25.0						
Neomycin	0.0						50.0	25.0		25.0				
Ceftiofur	100.0							50.0	50.0					
Sulphadimethoxine	100.0													100.0
Trimethoprim/Sulfa[2]	0.0					100.0								
Clindamycin	75.0					25.0	25.0	25.0	25.0					
Tulathromycin	0.0					25.0		25.0	25.0	25.0				
Tilmicosin	50.0								50.0	50.0				
Tylosin tartrate	0.0					75.0		25.0						
Penicillin	0.0			50.0	25.0	25.0								
Ampicillin	0.0			75.0	25.0									
Florfenicol	0.0					25.0	75.0							
Danofloxacin	100.0					100.0								
Enrofloxacin	75.0				25.0	50.0	25.0							
Chlortetracycline	0.0			75.0	25.0									
Oxytetracycline	0.0			25.0	75.0									
Tiamulin	100.0										100.0			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table I: Distribution of MICs for *Enterococcus faecium* isolated from Crocodile (Faeces (F): n=2) and Sabie-Sand Rivers (F: n=2)

Antimicrobial drugs	River	% Resistance	Distribution (%) of MICs (µg/ml)												
			0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	Crocodile	0.0									50.0	50.0			
	Sabie-Sand	0.0									100.0				
Gentamicin	Crocodile	0.0					50.0		50.0						
	Sabie-Sand	0.0				50.0	50.0								
Neomycin	Crocodile	0.0						50.0			50.0				
	Sabie-Sand	0.0						50.0	50.0						
Ceftiofur	Crocodile	100.0							50.0	50.0					
	Sabie-Sand	100.0							50.0	50.0					
Sulphadimethoxine	Crocodile	100.0													100.0
	Sabie-Sand	100.0													100.0
Trimethoprim/ sulfamethoxazole	Crocodile	0.0					100.0								
	Sabie-Sand	0.0					100.0								
Clindamycin	Crocodile	100.0						50.0		50.0					
	Sabie-Sand	50.0					50.0		50.0						
Tulathromycin	Crocodile	0.0					50.0			50.0					
	Sabie-Sand	0.0							50.0		50.0				
Tilmicosin	Crocodile	50.0								50.0	50.0				
	Sabie-Sand	50.0								50.0	50.0				
Tylosin tartrate	Crocodile	0.0					50.0		50.0						
	Sabie-Sand	0.0					100.0								
Penicillin	Crocodile	0.0			50.0		50.0								
	Sabie-Sand	0.0			50.0	50.0									
Ampicillin	Crocodile	0.0			100.0										
	Sabie-Sand	0.0			50.0	50.0									
Florfenicol	Crocodile	0.0						100.0							
	Sabie-Sand	0.0					50.0	50.0							
Danofloxacin	Crocodile	100.0					100.0								
	Sabie-Sand	100.0					100.0								
Enrofloxacin	Crocodile	100.0					50.0	50.0							
	Sabie-Sand	50.0				50.0	50.0								

APPENDIX B: continued

Table I: Distribution of MICs for *Enterococcus faecium* (continued)

Antimicrobial drugs	**River**	**% Resistance**	**Distribution (%) of MICs (µg/ml)**												
			0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**>256**
Chlortetracycline	Crocodile	0.0			100.0										
	Sabie-Sand	0.0			50.0	50.0									
Oxytetracycline	Crocodile	0.0			50.0	50.0									
	Sabie-Sand	0.0				100.0									
Tiamulin	Crocodile	100.0										100.0			
	Sabie-Sand	100.0										100.0			

APPENDIX B: continued

Table J: Distribution of MICs for all *Enterococcus durans* isolates (n=64)

Antimicrobial drugs	% Resistance	Distribution (%) of MICs (µg/ml)[1]												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	6.3								9.4	18.8	65.6	6.3		
Gentamicin	15.6				6.3	29.7	15.6	32.8	14.1	1.6				
Neomycin	17.2						25.0	20.3	28.1	9.4	17.2			
Ceftiofur	93.8		1.6		1.6	1.6	1.6	7.8	85.9					
Sulphadimethoxine	100.0													100.0
Trimethoprim/Sulfa[2]	3.1					96.9	3.1							
Clindamycin	93.8				1.6	4.7	20.3	39.1	34.4					
Tulathromycin	4.7				1.6	18.8	26.6	25.0	7.8	15.6	4.7			
Tilmicosin	29.7						1.6	14.1	54.7	28.1	1.6			
Tylosin tartrate	0.0				1.6	53.1	29.7	14.1	1.6					
Penicillin	0.0		1.6	17.2	23.4	37.5	20.3							
Ampicillin	0.0		4.7	50.0	43.8	1.6								
Florfenicol	1.6					57.8	40.6	1.6						
Danofloxacin	68.8			9.4	21.9	68.8								
Enrofloxacin	45.3			15.6	39.1	28.1	17.2							
Chlortetracycline	3.1			62.5	34.4				3.1					
Oxytetracycline	3.1			21.9	56.3	18.8			3.1					
Tiamulin	98.4							1.6			98.4			

[1] Bold vertical line indicates breakpoint for resistance. Coloured fields denote range of dilutions tested for each antimicrobial drug. MICs above the range are given as the concentration closest to the range. MICs equal or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of trimethoprim/sulfamethoxazole given, was tested in a concentration ratio of 2/38.

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* isolated from Olifants (Faeces (F): n=7, Water (W): n=6), Letaba (F: n=9, W: n=4), Crocodile (F: n=12, W: n=6) and Sabie-Sand Rivers (F: n=13, W: n=7)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Spectinomycin	Olifants	F	0.0								42.9	14.3	42.9			
	Olifants	W	0.0										100.0			
	Letaba	F	11.1								22.2	33.3	33.3	11.1		
	Letaba	W	0.0									50.0	50.0			
	Crocodile	F	0.0								8.3	25.0	66.7			
	Crocodile	W	0.0										100.0			
	Sabie-Sand	F	7.7									23.1	69.2	7.7		
	Sabie-Sand	W	28.6										71.4	28.6		
Gentamicin	Olifants	F	0.0				28.6	57.1	14.3							
	Olifants	W	16.7							83.3	16.7					
	Letaba	F	11.1					44.4	44.4		11.1					
	Letaba	W	0.0					50.0	50.0							
	Crocodile	F	25.0					33.3	8.3	33.3	25.0					
	Crocodile	W	16.7						16.7	66.7		16.7				
	Sabie-Sand	F	23.1				15.4	30.8		30.8	23.1					
	Sabie-Sand	W	14.3					14.3	14.3	57.1	14.3					
Neomycin	Olifants	F	0.0						57.1	42.9						
	Olifants	W	33.3								50.0	16.7	33.3			
	Letaba	F	11.1						33.3	22.2	33.3		11.1			
	Letaba	W	0.0							75.0	25.0					
	Crocodile	F	25.0						25.0	16.7	25.0	8.3	25.0			
	Crocodile	W	0.0								100.0					
	Sabie-Sand	F	23.1						38.5	15.4		23.1	23.1			
	Sabie-Sand	W	28.6						14.3	14.3	28.6	14.3	28.6			

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Ceftiofur	Olifants	F	100.0							28.6	71.4					
	Olifants	W	100.0								100.0					
	Letaba	F	88.9					11.1		22.2	66.7					
	Letaba	W	100.0							25.0	75.0					
	Crocodile	F	100.0								100.0					
	Crocodile	W	100.0								100.0					
	Sabie-Sand	F	84.6		7.7				7.7		84.6					
	Sabie-Sand	W	85.7				14.3				85.7					
Sulphadimethoxine	Olifants	F	100.0													100.0
	Olifants	W	100.0													100.0
	Letaba	F	100.0													100.0
	Letaba	W	100.0													100.0
	Crocodile	F	100.0													100.0
	Crocodile	W	100.0													100.0
	Sabie-Sand	F	100.0													100.0
	Sabie-Sand	W	100.0													100.0
Trimethoprim/ sulfamethoxazole	Olifants	F	0.0					100.0								
	Olifants	W	0.0					100.0								
	Letaba	F	11.1					88.9	11.1							
	Letaba	W	0.0					100.0								
	Crocodile	F	0.0					100.0								
	Crocodile	W	0.0					100.0								
	Sabie-Sand	F	7.7					92.3	7.7							
	Sabie-Sand	W	0.0					100.0								

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Clindamycin	Olifants	F	85.7					14.3	28.6	28.6	28.6					
	Olifants	W	100.0								100.0					
	Letaba	F	88.9					11.1	11.1	66.7	11.1					
	Letaba	W	75.0				25.0		50.0	25.0						
	Crocodile	F	100.0						16.7	58.3	25.0					
	Crocodile	W	100.0						33.3	66.7						
	Sabie-Sand	F	92.3					7.7	30.8	30.8	30.8					
	Sabie-Sand	W	100.0							14.3	85.7					
Tulathromycin	Olifants	F	14.3					14.3	28.6	28.6		14.3	14.3			
	Olifants	W	0.0						16.7	83.3						
	Letaba	F	0.0					11.1	11.1	22.2	11.1	44.4				
	Letaba	W	25.0					25.0	25.0			25.0	25.0			
	Crocodile	F	8.3					33.3	16.7	8.3	8.3	25.0	8.3			
	Crocodile	W	0.0						16.7	83.3						
	Sabie-Sand	F	0.0				7.7	30.8	23.1	7.7	23.1	7.7				
	Sabie-Sand	W	0.0					14.3	85.7							
Tilmicosin	Olifants	F	14.3							28.6	57.1	14.3				
	Olifants	W	33.3								66.7	33.3				
	Letaba	F	55.6								44.4	55.6				
	Letaba	W	50.0							25.0	25.0	50.0				
	Crocodile	F	33.3							16.7	50.0	33.3				
	Crocodile	W	16.7							16.7	66.7		16.7			
	Sabie-Sand	F	15.4						7.7	15.4	61.5	15.4				
	Sabie-Sand	W	28.6							14.3	57.1	28.6				

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Tylosin tartrate	Olifants	F	0.0				14.3	42.9	42.9							
	Olifants	W	0.0							100.0						
	Letaba	F	0.0					88.9	11.1							
	Letaba	W	0.0					50.0	50.0							
	Crocodile	F	0.0					75.0	16.7	8.3						
	Crocodile	W	0.0					16.7	83.3							
	Sabie-Sand	F	0.0					69.2	23.1		7.7					
	Sabie-Sand	W	0.0					28.6	42.9	28.6						
Penicillin	Olifants	F	0.0			28.6	28.6	42.9								
	Olifants	W	0.0					33.3	66.7							
	Letaba	F	0.0			33.3	22.2	33.3	11.1							
	Letaba	W	0.0		25.0	50.0			25.0							
	Crocodile	F	0.0			8.3	41.7	33.3	16.7							
	Crocodile	W	0.0					66.7	33.3							
	Sabie-Sand	F	0.0			23.1	38.5	23.1	15.4							
	Sabie-Sand	W	0.0				14.3	71.4	14.3							
Ampicillin	Olifants	F	0.0			57.1	42.9									
	Olifants	W	0.0				83.3	16.7								
	Letaba	F	0.0			55.6	44.4									
	Letaba	W	0.0		25.0	50.0	25.0									
	Crocodile	F	0.0			50.0	50.0									
	Crocodile	W	0.0			66.7	33.3									
	Sabie-Sand	F	0.0		15.4	53.8	30.8									
	Sabie-Sand	W	0.0			57.1	42.9									

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Florfenicol	Olifants	F	0.0					100.0								
	Olifants	W	0.0					16.7	83.3							
	Letaba	F	0.0					33.3	66.7							
	Letaba	W	0.0					25.0	75.0							
	Crocodile	F	0.0					50.0	50.0							
	Crocodile	W	16.7					50.0	33.3	16.7						
	Sabie-Sand	F	0.0					76.9	23.1							
	Sabie-Sand	W	0.0					85.7	14.3							
Danofloxacin	Olifants	F	85.7			14.3		85.7								
	Olifants	W	100.0					100.0								
	Letaba	F	66.7				33.3	66.7								
	Letaba	W	75.0				25.0	75.0								
	Crocodile	F	58.3			16.7	25.0	58.3								
	Crocodile	W	83.3				16.7	83.3								
	Sabie-Sand	F	46.2			15.4	38.5	46.2								
	Sabie-Sand	W	71.4			14.3	14.3	71.4								
Enrofloxacin	Olifants	F	42.9				57.1	42.9								
	Olifants	W	100.0					50.0	50.0							
	Letaba	F	44.4				55.6	44.4								
	Letaba	W	50.0				50.0	50.0								
	Crocodile	F	41.7			25.0	33.3	33.3	8.3							
	Crocodile	W	33.3				66.7		33.3							
	Sabie-Sand	F	15.4			38.5	46.2	15.4								
	Sabie-Sand	W	71.4			28.6			71.4							

APPENDIX B: continued

Table K: Distribution of MICs for *Enterococcus durans* (continued)

Antimicrobial drugs	River	Sample	% Resistance	Distribution (%) of MICs (µg/ml)												
				0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Chlortetracycline	Olifants	F	0.0			85.7	14.3									
	Olifants	W	0.0			83.3	16.7									
	Letaba	F	0.0			66.7	33.3									
	Letaba	W	0.0			50.0	50.0									
	Crocodile	F	0.0			50.0	50.0									
	Crocodile	W	33.3			50.0	16.7				33.3					
	Sabie-Sand	F	0.0			61.5	38.5									
	Sabie-Sand	W	0.0			57.1	42.9									
Oxytetracycline	Olifants	F	0.0				85.7	14.3								
	Olifants	W	0.0			33.3	66.7									
	Letaba	F	0.0				55.6	44.4								
	Letaba	W	0.0			25.0	25.0	50.0								
	Crocodile	F	0.0			33.3	50.0	16.7								
	Crocodile	W	33.3				50.0	16.7			33.3					
	Sabie-Sand	F	0.0			30.8	61.5	7.7								
	Sabie-Sand	W	0.0			42.9	42.9	14.3								
Tiamulin	Olifants	F	100.0										100.0			
	Olifants	W	100.0										100.0			
	Letaba	F	88.9							11.1			88.9			
	Letaba	W	100.0										100.0			
	Crocodile	F	100.0										100.0			
	Crocodile	W	100.0										100.0			
	Sabie-Sand	F	100.0										100.0			
	Sabie-Sand	W	100.0										100.0			