Assessment of antibiotic resistance phenotypic pattern in some commensal bacteria isolated from meat and dairy products.

BY

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Declaration

I, *Jeremia Ntambama Moyane,* hereby declare that the mini-dissertation for the *Master of Science in Food Science and Technology* (MScFST) degree at the University of Venda, South Africa, hereby submitted by me has not previously been submitted at this or any other university, and that it is my own work in design and in execution and that all referenced material contained therein has been duly acknowledged.

Signature: .....

Date: 23/02/2014

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Firstly, I thank God for his wisdom and guidance throughout my life and studies.

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Dedication

Dedicated to my wonderful family; my brothers; Alfred and Zacharia, my sisters; Sindile, Thandi and Thobile and most important my parents Ms Roseline Papane and Mr Simon Moyane.

Antibiotic names and abbreviation codes

Antibiotics	codes
Imipenem	IMP10 /IM
Meropenem	MEM10 /ME
Doripenem	DOR10 /DO
Cephalothin	KF30 /KF
Cefotaxime	CTX5 /CT
Ceftazidime	CAZ30 /CA
Amikacin	AK30 /AK
Gentamicin	CN10 /CN
Kanamycin	K30 / K3
Tobramycin	TOB10 /TO
Ampicillin	AP2 / AP
Penicillin G	PG10 /PG
Oxytetracycline	OT30 /OT
Tetracycline	T30 /T3
Doxycycline	DO30 /DO3
Minocycline	MH30 / MH
Nalidixic acid	NA30 /NA
Norfloxacin	NOR10/NO
Ciprofloxacin	CIP5 / CI
Levofloxacin	LEV5 /LE
Erythromycin	E15 / E1
Tylosin	TY30 /TY
Clindamycin	DA10 /DA
Lincomycin	L10 /L1
Vancomycin	VA30 /VA
Co-trimoxazole	TS25 /TS
Nitrofurantoin	NI300 /NI
Chloramphenicol	C30 / C3

General Abstract

This study investigated the pattern of antibiotic resistance in some commensal bacteria isolated from meat and dairy products. This study is important because, antibiotics resistance has become a serious public health concern with economic and social implications throughout the world. The use of antibiotics in animal husbandry has promoted the development and abundance of antibiotic resistance in farm environments. This can cause a potential health problem since resistance genes of pathogenic microorganisms can be transmitted from foodstuffs such as dairy and meat products to human. Therefore, the objective of this study was to assess the antibiotic resistance pattern of commensal bacteria isolated from meat and dairy products using phenotypic antibiotic susceptibility tests. Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Clinical Laboratory Standards Institute (2007) standards. A total of twenty eight (28) antibiotics were used to determine the antibiotic susceptibility of commensals which include eight (8) selected *Acinetobacter* isolates, twenty (20) *Staphylococcus* isolates and sixteen *Morganella morganii* isolates. There was multidrug resistance observed among in all three groups of isolates. Moreover, this study provided information of antibiotic usage in food-producing animals in South Africa and the implication and impact in food chain. Study of antibiotic resistance in developing countries such as South Africa is important as the information could enhance prudent use of antibiotics in food production by detecting transfer of resistant bacteria or resistance genes from food animals to humans.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

Worldwide, there is growing concern about the increased prevalence of antibiotic resistance among bacterial commensals. The use, underuse, overuses and misuse of antimicrobial agents in any environment creates selection pressures that favour the survival of antibiotic-resistant pathogens (White *et al.*, 2001; Davies and Davies, 2010). The report released by WHO in 2000 on infectious-disease stated that such organisms have become increasingly prevalent worldwide (White *et al.*, 2001). Mostly, the routine practice of giving antimicrobial agents to domestic livestock (i.e. preventing and treating diseases, as well as promoting growth) is found to be an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans through the food chain/or foodstuffs (Tollefson *et al.*, 1997; Perreten *et al.*, 1998; Witte, 1998; van den Bogaard and Stobberingh, 2000; Schlegelova *et al.*, 2004). Dutch scientists recently went as far as estimating that between a third and one half of resistance in human infections in the Netherlands originated from a similar resistance in farm animals (Levitt, 2011).

Molecular analyses of antibiotic resistance genes and antibiotic-resistant mobile elements has also shown that identical elements were found in bacteria that colonize both animals and humans, suggesting a role for raw foods in the dissemination of resistant bacteria and resistance genes to humans via the food chain (Teuber, 2001; Van *et al.*, 2007). However, a large amount of information on the transfer of antibiotic resistance genes, including the prevalence of antibiotic resistance in food animals, the possible correlation to human health, subsequent antibiotic management strategies and on-going surveillance programmes that have often led to new

legislation and regulations has been derived from developed countries (Bester and Essack, 2010).

Antibiotic usage urges bacteria sensitive to antibiotics to become resistant, in order to survive (Tenover, 2006; Schjørring and Krogfelt, 2011). Because antibiotic-sensitive strains are suppressed or eliminated, resistant strains are amplified and made more available to recombinant events (O'Brien, 2002). These antibiotic-resistant bacteria can easily transfer their resistance traits to unrelated bacteria once inside the human body (Shoemaker *et al.*, 2001). The survival mechanisms used by antibiotic-resistant bacteria include the acquisition of antibiotic resistance genes from other bacteria/phages (horizontal gene transfer or transduction), mutations in specific genes, and alteration of the bacterial surface. The coexistence of resistance genes with mobile elements such as plasmids, transposons, and integrons facilitates the rapid spread of antibiotic resistance genes among bacteria (Sunde and Nordstrom, 2006). Schjørring and Krogfelt, (2011) states that resistance genes from both Gram-positive and Gram-negative pathogenic bacteria have revealed almost identical sequences, suggesting that transfer of antibiotic resistance genes across genera has occurred and that, transfer events have occurred recently and are evolutionary recent events due to high sequence identity.

It is also suggested that a gene flux occurs in nature from Gram-positive cocci, (*Enterococci/Streptococci*) to Gram-negative bacteria with genes coding for streptogramins being described as examples (Courvalin, 2008). Concerns among the Gram-negative organisms include multidrug resistant *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Acinetobacter baumannii* and members of the

Enterobacteriaceae with extended-spectrum β-lactamases (Aliero and Ibrahim, 2012). Many investigators have speculated that commensal bacteria may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens, and the main threat associated with these bacteria is that they can transfer resistance genes to pathogenic bacteria (Mathur and Singh, 2005). Most importantly, the development of resistance in all types of bacteria is of concern, regardless of whether those bacteria themselves cause disease. Therefore, the presence of the resistant genes among the commensals, like those in the genera *Staphylococcus* and *Acinetobacter*, and also in the *S. maltophilia,* might have serious epidemiological effect as they reside on both human and animal skin or nasal passage (Patil and Chopade, 2001; Adegoke and Komolafe, 2009) and may cause infections that might be difficult to treat (Komolafe and Adegoke, 2008).

Smith (2004) reported that the resistant organisms causing concern among Gram-positive organisms at present are methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *staphylococci*, glycopeptides intermediate sensitivity S. *aureus* (GISA), vancomycin-resistant *Enterococcus* (VRE) species and penicillin-resistant *Streptococcus pneumoniae.* Genes conferring resistance to tetracycline, erythromycin and vancomycin have been detected and characterized in *Lactococcus lactis*, *Enterococci* and in *Lactobacillus* species isolated from fermented meat and milk products (Mathur and Singh, 2005). Milk and meat may contain resistant microorganisms such as *S. aureus* posing a potential risk to consumers (Ombui *et al.*, 2000). This microorganism is usually present on the skin of 40% of all humans and almost 100% of all animals (Synder and Poland, 1990). Members of the genus *Acinetobacter* have clearly emerged as opportunistic nosocomial pathogens

(Forster *et al.*, 1998; Peleg *et al.*, 2008). *A. baumannii* is very important human pathogen that is gradually gaining more attention as a major global public health problem due to its genetic potential to carry and transfer diverse antibiotic resistance determinants (Prashanth *et al.*, 2012), making it one of the organisms threatening the current antibiotic era (Peleg *et al.*, 2008). Drug resistant diarrhoea and nosocomial infections caused by some *Acinetobacter* species have posed serious therapeutic challenges, especially in developing countries (Doughari *et al.*, 2012). Berg *et al.* (1999) tested the antimicrobial resistance for *S. maltophilia* isolates of environmental and clinical origin. They concluded that the isolates were multidrug resistant, irrespective of their origin and 20% or more of the isolates were resistant to 16 out of 19 tested antibiotics. Hoffman and co-workers isolated *S. maltophilia* on ostrich carcasses from the cold rooms and de-boning areas of commercial abattoir in South Africa and they were able to grow and contaminate the meat in these temperature controlled areas (Hoffman *et al.*, 2010).

1.2. Problem Statement

The global emergence of multidrug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). There are numerous factors which influence the development of antibiotic resistance, misuse being the obvious factor (Schjørring and Krogfelt, 2011). The usage of antibiotics in animal husbandry has in turn promoted the development and abundance of antibiotic resistance in farm environments (Heuer *et al.*, 2011). Most results show that after the introduction of an antibiotic in veterinary practise, the resistance in pathogenic bacteria and/or the faecal flora increases, also in human medicine (van den Bogaard and Stobberingh,

2000). Again, manure from pigs was shown to carry a high load of bacteria with antibiotic resistance genes that often reside on mobile genetic elements (Smalla *et al.*, 2000; Heuer *et al.*, 2002).There is an increased use of fermented food products and probiotics, as food supplements and health promoting products and these contain massive amounts of bacteria. These bacteria act as either donors and/or recipients of antibiotic resistance genes in the human gastro intestinal (GI) tract, which also contributes to the emergence of antibiotic-resistant strains (Schjørring and Krogfelt, 2011). Therefore, this can cause potential health problems since this means that resistance genes of pathogenic microorganisms will be transmitted from foodstuffs such as meat and dairy products to human.

1.3. Motivation of the study

Information on the phenotypes and genotypes of antimicrobial resistance in food-borne microorganisms, including the prevalence of antibiotic resistance in food animals is largely restricted to first-world countries, and there is a paucity of information on what is happening in developing countries (Van *et al.*, 2007; Bester and Essack, 2010). Moreover, in those few reported, rates of resistance to antibiotics of bacteria originating from food, such as meat were high in developing countries. Monitoring the prevalence of resistance in indicator bacteria, in different populations makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa (van den Bogaard and Stobberingh, 2000; van Duijkeren *et al.*, 2003). Therefore, a study of antibiotic resistance in developing countries, such as South Africa is important as the information could enhance prudent use of antibiotics in food production. A number of antibiotic resistance studies on samples collected on environments,

hospital patients/areas and other surfaces, such as food processing area have been conducted in South Africa (Essack, 2006; WoseKinge *et al.*, 2010). However, as far as we are aware, there has been very little published about the occurrence of antibiotic-resistant bacteria in meat and dairy products in South Africa and even less about the phenotypic characteristics of these antibiotic-resistant bacteria. This study was to address some of these issues and to provide a current baseline of information on phenotypic characteristics of antibiotic resistance of few commensal microflora residents on farm animals isolated from meat and dairy products in South Africa.

1.4. Research aim and objectives

1.4.1. Broad Aim

To evaluate the antibiotic resistance in some commensal bacteria isolated from dairy and meat products at Irene, Pretoria in the Republic of South Africa.

1.4.2. Specific Objectives

i. To isolate, characterize and identify some commensal bacteria from meat and dairy products.

ii. To determine the phenotypic antibiotic susceptibility of the identified microorganisms

1.5. Research questions

i. Are there problems of multiple antibiotic resistances in commensal bacteria isolated from raw meat and dairy products in Irene, Pretoria?

ii. How prevalent is antibiotic resistant microorganisms in meat and dairy products?

1.6. Hypotheses

i. It is expected that one can obtain multiple antibiotic-resistant isolates from meat and dairy products, as current studies have indicated a growing number of microorganisms that are capable of resistance to two or more antibiotics.

ii. The least processed (heat treated), raw meat and dairy products together with products from the intestine of pork/beef may exert the highest impact of antibiotic resistance.

CHAPTER 2: LITERATURE REVIEW

2.1. Antibiotic resistance

Antibiotics are defined as chemical substances which have the capacity to inhibit growth, and even destroy microorganisms in a dilute solution (ICON, 2003). Antibiotic resistance in bacteria can be intrinsic or acquired. In the case of intrinsic resistance, bacterial strains are inherently resistant to a certain compound and the resistance cannot be transferred horizontally (Fajardo *et al.*, 2008). Acquired resistance occurs by mutation and/or horizontal gene transfer events (Van Meervenne *et al.*, 2012). The ability of bacteria to acquire antibiotic resistance genes and subsequently spread them to many different bacterial species is well known (Hall, 1997). Acquired resistance genes may enable a bacterium to produce enzymes that destroy an antibacterial drug, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug's target site, or to produce an alternative metabolic pathway that bypasses the action of the drug (Tenover, 2006).

Integrons, one such mobile DNA element, have been associated with the transfer of resistance and often contain one or more linked antimicrobial resistance genes (Hall and Stokes, 1993). Integrons are therefore particularly important, since a strong selection pressure exerted by antibiotics can potentially result in the mobilisation and dissemination of linked multidrug-resistance phenotypes (White *et al.*, 2001). Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids (Tenover, 2006). A recent

study by Van Meervenne *et al.* (2012) demonstrated that an environmental plasmid can be transferred to foodborne pathogens (*Salmonella* spp. and *E. coli* O157:H7) under laboratory conditions. Not only does this transfer occur at rather high transfer ratios (order of magnitude 10^{-2}), but the acquisition of the plasmid also made the pathogens resistant to multiple antibiotics. Tetracycline resistance in most bacteria is due to the acquisition of new genes often associated with mobile elements, and these genes are usually associated with plasmids and/or transposons and are often conjugative (Dzidic *et al.*, 2008). Resistance to antimicrobial agents typically occurs as a result of four main mechanisms, namely, enzymatic inactivation of the drug (Davies, 1994), alteration of target sites (Spratt, 1994), reduced cellular uptake (Smith, 2004) and extrusion by efflux (Nakaido, 1994). An increasing number of bacterial isolates is resistant to practically all available therapeutic agents. Multidrug resistance has been demonstrated in *S. aureus*, *Acinetobacter baumannii,* and *Stenotrophomonas maltophilia* (Vladimír, 1994; Berg *et al.*, 1999; Smith, 2004; Doughari *et al.*, 2012). Thus, the emergence of antibiotic resistance in bacterial populations is a relevant field of study in molecular and evolutionary biology as well as in medical practice (Dzidic *et al.*, 2008).

2.2. Commensal bacteria and their characteristic

Commensal bacteria are mostly defined as bacteria which are naturally residents or live in or co-exist mutually with the host without causing disease (Andremont, 2003; EFSA, 2012). Moreover, commensal bacteria are believed to co-evolve with their hosts, however, under specific conditions they are able to overcome protective host responses and exert pathologic effects (Tlaskalová-Hogenová *et al.*, 2004). As such, many commensals can cause disease if they enter body sites that are normally

sterile or when the host's immune defence is weakened (Sharp, 1999). It is estimated that a human adult has about 10^{12} bacteria on their skin, 10^{10} in the mouth and as many as 10^{14} in their gastrointestinal tract (Todar, 2006). The most abundant microflora is present in the inner parts of the gut and the majority of these intestinal bacteria are Gram-negative anaerobes, and the numbers can be up to 10^{14} as mentionedabove (Tlaskalová-Hogenová *et al.*, 2004). Among those Gram-negative anaerobes, *E. coli* is the most studied commensal bacterium and it believed to be the best understood of them all (Lee and Lee, 2003). The immunological unresponsiveness of the host towards the resident commensal microflora is thought to permit their successful colonisation and co-existence within the host gut (Kelly *et al.*, 2005). *S. epidermidis* is a Gram-positive commensal bacterium of the human skin, and *S. epidermidis* and other coagulase-negative staphylococci emerge also as common nosocomial pathogens mainly due to their antibiotic resistance abilities (Ziebuhr *et al.*, 2006). Therefore, as such, most of commensals either Gram-negative or positive can be an opportunistic secondary attacker that takes advantage of a compromised immune system. However, on a positive note, commensal bacteria play an important role in the digestion of food, and are responsible for the extraction and synthesis of nutrients and other metabolites that are essential for the maintenance of the host's health (Brestoff and Artis, 2013). Many of these nutrients and metabolites derived from commensal bacteria have been implicated in the development, homeostasis and function of the immune system, suggesting that commensal bacteria may influence host immunity via nutrient- and metabolite-dependent mechanisms (Brestoff and Artis, 2013).

In food quality point of view, commensal bacteria that contaminate food can harbour transferable resistance genes, and during the passage through the intestine, these bacteria may transfer their resistance genes to host-adapted bacteria or to pathogens (EFSA, 2012). Transfer of either antibiotic resistant bacterium to humans or of their antibiotic resistance genes to pathogens, via the food chain has already been reported (Simeoni *et al.*, 2008; Amador *et al.*, 2009). Moreover, it has been indicated that, resistance increases first in the commensal bacteria (Andremont, 2003), due to several factors which include, (i) the fact that commensal genetic pool is so large, it encompasses many more potential means for conferring resistance, including not only single-nucleotide mutations but also complex resistance mechanisms, (ii) resistant commensal bacteria may be selected each time an antibiotic is administered irrespective of the health status of the host whereas resistant pathogens are subject to selective pressure only when particular host actually are infected with those bacteria (Andremont, 2003). Therefore, as already mentioned, because antibiotic sensitive strains are suppressed or eliminated, resistant strains are amplified and made more available to recombinant events (O'Brien, 2002). One thing that must be noted is if a pathogen causing illness acquires an antibiotic resistance gene from commensal bacteria, that pathogen also becomes resistant to that particular antibiotic. Acquired antibiotic resistance is particularly problematic and can limit treatment options, contributing to more pain and increased deaths (Amador *et al.*, 2009).

Contamination of food either by spoilage bacteria or pathogens from food processing environment remains a challenge for the food industry (Møretrø *et al.*, 2013). Bacteria, including commensals present a challenge because they are becoming

resistant even to disinfectants used in food processing plants. Møretrø *et al.* (2013) have found that *Aerococcus*, *Acinetobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus* species demonstrated resistance to commercial disinfectants on stainless steel surface. Another thing associated with resistance bacteria is their ability to form biofilms. Biofilms are composed of adherent bacterial communities and certain organic byproducts (Oliveira *et al.*, 2010). So in this case many bacteria are able to attach to surfaces, grow and form biofilms. Bacteria such as *Acinetobacter* spp. and *Staphylococcus* spp. have been reported to form these biofilms on many surfaces (Ziebuhr *et al.*, 2006; Vela *et al.*, 2012;Møretrø *et al.*, 2013).

2.2.1. *Staphylococcus* spp.

The genus *Staphylococcus* is in the bacterial family *Staphylococcaceae* (Ludwig *et al.*, 2009). *Staphylococci* are Gram-positive spherical bacteria that occur in clusters resembling grapes due to its perpendicular division planes where cells remains attached to one another following each successive division (de Souza *et al.*, 2012). The genotypic standards for assigning an organism to the genus *Staphylococcus* include determination of guanine plus cytosine (G+C) content of 30-39 mol% and phylogenetic trees constructed by comparison of 16S rRNA or 23R rRNA sequences (Takahashi *et al.*, 1999). The phenotypic criteria is based on the ultrastructure and chemical composition of the cell wall, typically form Gram positive bacteria and catalase reaction positive for all species, except for *S. aureus* subsp *anaerobius* and *S. saccharolyticus*, which are strictly anaerobic (de Souza *et al.*, 2012).

S. aureus is a major pathogen of a cow's mammary gland (Watts and Salmon 1997). Unpasteurized milk may become contaminated with enterotoxigenic coagulase-

positive *Staphylococcus* spp. (Carmo *et al.*, 2002) either through contact with the cow's udder during milking or by cross-contamination during processing (Ramesh *et al.*, 2002). *Staphylococcus* spp. can rapidly acquire resistance to a broad range of antimicrobials, thereby posing a major concern in the treatment of *staphylococcal* infections (Bozdogan *et al.*, 2004).

The study by Turutoglu *et al.* (2009) of methicillin and amino-glycoside resistance in *S. aureus* isolates from bovine mastitis and sequence analysis of their *mecA* genes found that all three *mecA* genes of these isolates were identical to that found in human MRSA strains, except a one-base substitution at nucleotide position 757. And from their data, they concluded that MRSA isolated from bovine mastitis may be originated from human beings. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin, erythromycin, tetracycline and other penicillin-like antibiotics (Ubukata *et al.*, 1989).

2.2.2. *Acinetobacter* spp.

Acinetobacter is a Gram-negative *coccobacillus* that is strictly aerobic, non-motile, catalase positive and oxidase negative. It is ubiquitous in nature, being found in soil and water (Prashanth *et al.*, 2012). Members of the genus *Acinetobacter* have now clearly emerged as opportunistic nosocomial pathogens (Forster *et al.*, 1998). Clinically important species include *Acinetobacter baumannii*, *Acinetobacter johnsonnii*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, and *Acinetobacter* genomo-species 3, and 13 (Doughari *et al.*, 2012). *A. baumannii* is very important human pathogen that is gradually gaining more attention as a major global public health problem due to its genetic potential to carry and transfer diverse antibiotic

resistance determinants (Peleg *et al.*, 2008; Prashanth *et al.*, 2012). *A. baumannii* has multi-resistant phenotypes, including resistance to broad spectrum β-lactams, fluoroquinolones, aminoglycosides and carbapenems and therefore treatment of this pathogen is complicated (Coelho *et al.*, 2004; Jeon *et al.*, 2005; Naas *et al.*, 2005; Vahaboglu *et al.*, 2006). The general mechanisms of resistance are enzyme-mediated resistance, genetic adaption, efflux pumps and changes in the structure of outer membrane components (Cloete, 2003). Changes in the structure of the outer membrane and its components e.g. porins and alterations in the penicillin binding proteins (PBP's), allows for the cells to develop resistance to antimicrobials on the basis of exclusion because the drugs are no longer able to penetrate the cells and therefore the drugs cannot reach their intended site of action in the cell (Cloete, 2003).

As a result of the widespread and inappropriate use of antibiotics, antibiotic resistance emerges more frequently (Yurdakul *et al.*, 2013). Among *Acinetobacter* spp., *A. baumannii* is the most frequently implicated in nosocomial infections, in particular in intensive care units. It was initially thought that multidrug resistance (MDR) in this species was due mainly to horizontal acquisition of resistance genes. However, it has recently become obvious that increased expression of chromosomal genes for efflux systems plays a major role in MDR (Coyne *et al.*, 2011). *A.baumannii-calcoaceticus* complex possess a variety of intrinsic resistance mechanisms that can be expressed constitutively or in response to antimicrobial pressure (Blossom and Srinivasan, 2008). Changes in outer membrane proteins can reduce the access of antimicrobial agents to penicillin-binding proteins in the cell membrane of the bacteria. Mutations in topoisomerase genes can provide

fluoroquinolone resistance. Efflux pumps can expel various classes of antimicrobial agents including β-lactams, quinolones, tetracyclines, and aminoglycosides (Blossom and Srinivasan, 2008).

A study by Güngör and Gökoğlu (2010) showed that microbial counts in personnel hands had significant correlations with the counts of the samples taken from processing stages. A recent study has also showed that bulk milk tank can be a source of *Acinetobacter* spp. milk contamination (Gurung *et al.*, 2013). *Acinetobacter* spp. has been recovered on processing equipment and surfaces even after cleaning and disinfections (Bagge-Ravn *et al.*, 2003; Guðbjörnsdóttir *et al.*, 2005; Srey *et al.*, 2013). Attachment of pathogens and other bacteria to food equipment surfaces can lead to product contamination, spoilages and surface deterioration (Guðbjörnsdóttir *et al.*, 2005). Some *Acinetobacter* spp. are capable of forming biofilms on processing equipment in food industries. These biofilms have major implications in the food industry where they cause a persistent source of contamination (van Houdt and Michiels, 2010). Moreover, evidence indicates that biofilm formation conveys a selective advantage to certain pathogens by increasing their ability to persist under diverse environmental conditions (Hall-Stoodley and Stoodley, 2005).

2.2.3 *Stenotrophomonas maltophilia*

S. maltophilia is a non-fermentative gram-negative environmental species that can cause nosocomial infections and that is characterized by intrinsic resistance to several antibiotics (Denton *et al.*, 1998). The majority of strains of *S. maltophilia* are characterized by their resistance to many currently available broad-spectrum

antimicrobial agents, including those of the *carbapenem* class (Denton and Kerr, 1998).

Although environmental sources such as ice-making machines have been implicated in outbreaks of nosocomial *S. maltophilia* sepsis, the source of infection in other outbreaks and in sporadic cases often remains unidentified. (Qureshi *et al.*, 2005) The importance of *S. maltophilia* in ready-to-eat salads, which are marketed in a manner that assumes the product does not need washing before consumption, is unknown; nevertheless the presence of the bacterium in these products serves to highlight recommendations that these items should be avoided by severely immune-compromised persons, especially those with neutropenia (Heard, 2000). Recently, Apisarnthanarak *et al.* (2003), in a prospective study of hospitalized oncology patients, identified intestinal colonization with *S. maltophilia* in 4 (9.5%) of 41 patients, which emphasizes that foodstuffs may be a potential source of this bacterium for some patients.

The antimicrobial resistance of *S. maltophilia* is attributed to low outer membrane permeability (Cullmann,1991; Garcıa-Rodrıguez *et al.*, 1991) and the unusual production of multiple chromosomally mediated, broad-spectrum β-lactamases, among which are L1 and L2 (Akova *et al.*, 1991; Garcıa-Rodrıguez *et al.*, 1991). L1 and related β-lactamases are group 3 metallopenicillinases and carbapenemases, while L2 and related β-lactamases are group 2e cephalosporinases capable of hydrolyzing penicillins and monobactams. All β-lactamases are induced synchronously, indicating an apparent overlap of regulatory systems (Akova *et al.*, 1991; Payne *et al.*, 1994). In vitro susceptibility testing of *S. maltophilia* is

problematic, and results obtained by such tests should be interpreted with caution (Akova *et al.*, 1991). The National Committee for Clinical Laboratory Standards (NCCLS) (1997) recommends broth or agar dilution testing as the method of choice for susceptibility testing for this organism.

2.3. Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance (See Appendix IV, Journal paper 1)

The intensive use of antimicrobial (antibiotic) agents in industrial animal husbandry have spread into developing countries, and the negative impact on human health and food safety have often followed (Garcés, 2002). The impact may vary considerably between countries and regions, influenced by the interaction between human populations, land use, contaminated water sources, animal demography, national policies (production, trade, food security, animal health, etc.), and national and international trade (WHO, 2012). Hence, antibiotic resistance is a major global societal problem (Mellon *et al.*, 2001; Davies and Davies, 2010; WHO, 2012), involving many different sectors e.g. medicine, veterinary medicine, animal husbandry, agriculture, environment and trade (EC 2011). Epidemiological studies have demonstrated a correlation between antibiotic use and antimicrobial resistance (Goossens *et al.*, 2005; Beerepoot *et al.*, 2011, 2012; den Heijer *et al.*, 2012). Goossens *et al.* (2005) showed that there were higher rates of antibiotic resistance in high consuming countries, probably related to the higher consumption/usage of antibiotics in Southern and Eastern Europe than in Northern Europe. There is a higher report of use of antibiotics in developed countries than in developing countries both for prophylaxis and therapy but, higher therapeutic use than prophylactic use in developing countries (Mitema *et al.*, 2001; Byarugaba *et al.*, 2011). Reports point

that the international travel and trade in animals and animal products increase the risks of antibiotic resistance world-wide (Acar and Röstel, 2001; Mellon *et al.*, 2001; EC, 2011; Byarugaba *et al.*, 2011; WHO, 2012).

Furthermore, antibiotic resistance has been given a low priority in most developing and many developed countries. Most important, developing countries such as South Africa have received much limited attention regarding this problem (Okeke *et al.*, 2007). Globally, only a few developed countries such as Sweden, Denmark, the United Kingdom, and Netherlands have managed to reduce antibiotic consumption in the community which have sometimes resulted, but not always, in a decrease in resistance (Cogliani *et al.* 2011; Mackie, 2011; Carlet *et al.*, 2012).Unfortunately, there is no single or simple solution to the problem of bacterial antibiotic resistance because there are many diverse factors that contribute to irrational use of antibiotics including knowledge, perceptions, attitudes and behaviour of policy-makers, prescribers, manufacturers, dispensers and consumers (WHO, 2012). However, the use of antibiotics in livestock production is suspected to be significantly contributing to the antibiotic resistance in species of bacteria which are common to humans and animals (Acar and Röstel, 2001). Mostly, the routine practice of giving antibiotic agents to domestic livestock (i.e. preventing and treating diseases, as well as promoting growth) is found to be an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to humans through the food chain/or foodstuffs (Perreten *et al.*, 1998; van den Bogaard and Stobberingh, 2000; Schlegelova *et al.*, 2004; Byarugaba *et al.*, 2011).

The increase in antibiotics resistance has been reported in both commensal and pathogenic bacteria, this raises an emerging threat to public health and the environment (Marshall *et al.*, 2009; Thaller *et al.*, 2010; Aiyegoro *et al*, 2011; Byarugaba *et al.*, 2011; Carlet *et al.*, 2012). This high resistance challenge results from two combined factors (Carlet *et al.*, 2012). First, microorganisms are becoming extremely resistant to existing antibiotics, in particular Gram-negative rods (e.g., *E. coli, Salmonella* spp., *Klebsiella* spp., *Acinetobacter* spp.), which are resistant to almost all currently available antibiotics in some settings. Secondly, new antibiotics have become every difficulty to make and/or discovered (Hughes, 2011). Several new powerful compounds active against Gram-positive cocci have been made available in the last few years, but this is not the case for Gram-negative bacteria and almost no new antibiotic class active against multi-resistant Gram-negative rods can be anticipated in the near future (Carlet *et al.*, 2012). Therefore, this work summarizes the problem and the impact of antibiotic resistance in relation to antibiotics usage in farm animal husbandry with the consequences on consumer's health. The work also tried to look at some of the efforts which are being done to contain antibiotic resistance which include alternatives to antibiotic usage.

2.3.1. Antibiotic use in agricultural sector: food-producing animals

Treatment, prophylaxis and growth promoters are the common uses of antibiotics in food-producing animals (Table 1) and is essential for a sustainable and economically sustainable animal industry (Acar and Röstel, 2001; Eagar, 2008). However, the application of these antibiotic drugs in animals, particularly in food animals, may lead to a selection of resistant strains of bacteria, which in turn may proceed to infect both animals and human (Mellon *et al.*, 2001). Molecular analysis of antibiotic resistance

genes and antibiotic-resistant mobile elements has shown that identical elements were found in bacteria that colonize both animals and humans, suggesting a role for raw foods in the spread of resistant bacteria and resistance genes to humans via the food chain (Teuber, 2001; Van *et al.*, 2007). For example, the use of fluoroquinolones (e.g. enrofloxacin) in food-producing animals has resulted in the development of ciprofloxacin-resistant *Salmonella, Campylobacter and E. coli*, which have caused human infections that proved difficult to treat (WHO, 2011).

Data on the volume of antibiotics used in livestock production are scarce in South Africa, and information is lacking about the patterns of antibiotic consumption in food animals (Henton *et al.*, 2011). Moreover, considering the lack of information on the total quantity of antibiotics produced, it is not surprising that information on quantities used for specific purposes in agriculture and human medicine is also limited. Of all the available antibiotics used in livestock production in South Africa about 29% reported (Eagar, 2008) are in the form of premixes, and represents a large percentage of all the registered antimicrobials.

Picard and Sinthumule, (2002) together with Eagar, (2008) reported that the most frequent uses of antibiotics by weight (as measured by sales) were those for treating and preventing diseases in poultry and pigs, and as growth promoters. In a survey by Henton *et al.* (2011) found that tylosin, one of four (4) growth promoters banned in Europe, was the most extensively sold antibiotic in South Africa followed by tetracyclines, sulphonamides and penicillins, respectively. Extensive usage of tylosin in food-producing animals was initially reported by Eagar (2008). In that study it was also found that the mean antibiotic sales for three (3) years period (Table 2) from

8companies were 1.5 million kilograms active ingredient. Where, in terms of total volumes of sales (kg), the macrolides, lincosamides and pleuromutilins represented 42.4% of the antibiotics sold. In the last four years there have been annual increases in unit sales from 25.3 to 29.8 million (Table 3) of broad-spectrum penicillins, fluoroquinolones, carbapenems and penems, carbacephems and glycopeptides in South Africa (Essack *et al.*, 2011).

Moreover, counterfeiting of pharmaceuticals is highly problematic in South Africa, with an estimated one in five medicines sold believed to be counterfeit (BMI, 2010). It is reported that the majority of counterfeit medicines have been imported from India and Pakistan and reach pharmacies through illegal means and the South African Medicines and Medical Devices Regulatory Authority (SAMMDRA) have not been successful in combating this problem (Essack *et al.*, 2011). Therefore, sales data may provide a misleading and inaccurate measure of the use of antibiotics because of counterfeiting. Hence, industry data on antimicrobial use in livestock production almost certainly underestimate usage and are far too general to help scientists explore the linkages between various types of farm use and the emergence and spread of resistance (Union of Concerned Scientists, 2001, www document;http://www.ucsusa.org/assets/documents/food_and_agriculture/hog_chaps.pdf Accessed 04/Sep/2012).

Table 1.Some of commonly used antibiotics in food-producing animals.

Antibiotics	Treatment objective	Food animals	References
Lincomycin	Feed efficiency, growth promoter and disease control	Swine, poultry	Regassa *et al.* (2008) Kuchta, (2008)
Tylosin*	Feed efficiency and growth promoter	Poultry, cattle	Henton *et al.* (2011) Jackson *et al.* (2004) Eagar, (2008)
Penicillin	Feed efficiency, growth promoter and disease control	Swine, poultry	Regassa *et al.* (2008) Doyle, (2006)
Virginiamycin*	Feed efficiency, growth promoter and disease control	Swine, poultry, cattle	Regassa *et al.* (2008) Donabedian *et al.* (2003) Cogliani *et al.* (2011)
Tetracyclines (chlrotetracyclineoxy tetracyline, tetracycline)	Feed efficiency, growth promoter and disease control	Swine, poultry, cattle	Oguttu *et al.* (2012) Regassa *et al.* (2008)
Erythromycin	Disease control	Swine, poultry, cattle, sheep	Jeong *et al.* (2006)

*Banned in European Union

Table 2. Volumes (kg) of antibiotics used during 2002-2004* as sourced from veterinary pharmaceutical companies.

Class of antibiotic	Volume (kg)			Total (kg) over 3yrs
	2002	2003	2004	
Penicillins	49 465	55 677	59 688	165 717*
Cephalosporins	5 470	3 321	3 316	12 107
Tetracyclines	58 342	71 842	58 974	257 755*
Aminoglycosides	3	242	268	1 048*
Macrolides, lincosamides and pleuromutilins	204 325	221 275	223 412	651 690*
Quinolones	582	582	1 082	3 094*
Quinoxalines	30 043	26 468	30 448	86 959
Sulphonamides	35 041	72 277	75 098	190 676*
Polipeptides	27 011	26 985	42 191	69 820
Lonophores	14 736	5 582	43 674	69 820*
Glycolipids	370	425	432	3 936*
Total	**425 388**	**484 676**	**538 583**	**1 538 443***

(Eagar, 2008) * Two of the eight veterinary pharmaceutical companies that provided data were only able to access their data for the whole three year period and not for each year individually.

Table 3. Antibiotic utilisation in units, 2008 – 2011.

Antibiotic	Sum of MAT units, 2008	2009	2010	2011	Count of antibiotics in each class
J1A0 Tetracyclines + combs	327 379	325 061	327 557	327 701	44
J1B0 Chloramphenicols + combs	6 964	6 114	4 527	2 483	8
J1C1 Broad-spect. penicill. oral	10 683 704	11 441 888	11 962 722	12 305 433	277
J1C2 Broad-spect. penicill. inj.	551 335	1 251 442	1 133 503	1 463 327	45
J1D1 Cephalosporins oral	1 797 546	1 813 314	1 934 859	1 874 156	95
J1D2 Cephalosporins inj.	1 674 479	1 758 407	1 663 164	1 697 551	116
J1E0 Trimethoprim combs	3 261 544	4 021 542	3 300 302	3 316 420	124
J1F0 Macrolides + similar type	2 039 968	2 293 495	2 530 404	2 596 281	96
J1G1 Oral fluoroquinolones	3 242 849	3 617 302	3 635 646	3 832 065	95
J1G2 Inj. fluoroquinolones	479 409	554 631	565 952	584 255	21
J1H1 Plain med.-/narrow-spect. penicillins	419 243	386 095	485 923	435 640	42
J1K0 Aminoglycosides	80 624	87 089	83 880	80 349	41
J1P1 Monobactams	4 843	4 674	7 584	5 679	1
J1P2 Penems +carbapenems	679 147	809 668	916 184	1 019 767	8
J1P3 Carbacephems	7 652	15 512	23 191	69 908	3
J1X1 Glycopeptide antibact.	122 156	134 738	162 038	158 674	20
J1X9 All other antibacterials	15 132	14 361	15 849	16 229	10
Grand total	**25 393 974**	**28 535 333**	**28 753 285**	**29 785 918**	**1 046**

Essack *et al.* (2011), MAT = Moving Annual Turnover, *Number of drug formulation

A study by Eagar *et al.* (2012) found that the majority of consumed antibiotics in animals were from the macrolide and pleuromutilin classes, followed by the tetracycline, the sulphonamide and lastly the penicillin class. Their survey results showed that 68.5% of the antibiotics were administered as in-feed medications. About 17.5% of the total volume of antibiotics utilised were parenteral antibiotics, whereas antibiotics for water medication constituted 12% of the total and other dosage forms (topical and aural dosage) constituted 1.5% of the total.

It is not surprising that many chicken farms widely use antibiotics as a prophylactic and a growth stimulant (Medeiros *et al.*, 2011). However, this is particularly problematic because antibiotic for growth promoters are used without veterinary prescriptions or administered for long periods of time at sub-therapeutic concentrations to entire groups or herds of animals (Carlet *et al.*, 2012). These farmers have come to believe that relatively low concentrations of antibiotics in feed and water can help avoid disease-driven losses in livestock with the belief that they increase profit margins despite the lack of well-understood mechanisms (Mellon *et al.*, 2001). However, if the quality of industrial animal farming is improved there would be far less of the problem of disease control and prevention, and hence antibiotic resistance (Garcés, 2002). This is because in most cases overcrowded and unhygienic conditions of industrial animal farming result in the spread and emergence of microbes. Therefore, if conditions were improved, the prophylactic use of antibiotics would not be necessary.

2.3.2. Antibiotic use in farm animals and impact on humans

Certain antimicrobial drugs have been reported to stay in the meat and/or milk of food animals for extended periods of time (Nisha, 2008; McDermid, 2012; Lozano and Trujillo 2012). For example, chicken being fed antibiotics which can never be fully broken down and excreted from its body before it is slaughtered may result in very concentrated by-products and residues in chicken meat (Compassion in World Farming South Africa (CIWFSA) 2012, (www document,http://www.animal-voice.org/antibiotic-residue-is-in-our-chickens/ Accessed 14/Nov/2012). And it is said that South Africa does not have a regulated process of antibiotic residue testing in meat (McDermid, 2012). Data released by the Compassion in World Farming South Africa in September 2009, showed that every single chicken purchased at supermarkets tested positive for tetracycline residue which is one of the most depended upon antibiotics in human health. Part of the Chicken sample displayed a residue of 55% over the legal limit in terms of South African Law (CIWFSA, 2012). Furthermore, the study found that 10 freshly dead carcasses from the Phillippi cull outlet showed 100% antibiotic resistant bacteria on the skin surface including *staphylococci* and *enterobacteriaciae* (CIWFSA, 2012). The antibiotics to which the bacteria were 100% resistant were penicillin and ampicillin, both of which are used for a broad spectrum of human illnesses (Schrag *et al.*, 2002; CIWFSA, 2012).

Concerns about use of penicillins and other antibiotics is the withdrawal period (Eagar *et al.*, 2012). The high content of antibiotic residues such as that of tetracycline (and penicillins, etc.) in food animal products is of great concern since it has been established that these compounds also remain chemically detectable even after cooking, as cooking only decrease its amounts (Javadi, 2011). A comparative

study in Nigeria showed penicillin (14%) was the drug with the highest rate of occurrence in meat samples followed by tetracycline (8%) and streptomycin (4%). These antibiotic traces have harmful effects on consumer's health, such as allergic reactions, liver damage, yellowing of teeth and gastrointestinal disturbance (FAO/WHO, 2002; Jing *et al.*, 2009). Another fact is that over time, through continued consumption of meat products containing these antibiotic residues (indirect consumption of antibiotic residues), people's body will start to develop resistance to that antibiotic molecule which can impact their lives (Andrew, 2003; Nisha, 2008; McDermid, 2012; Lozano and Trujillo 2012). These residues may produce potential threat with direct toxic in human; secondly, the low levels of antibiotic exposure will result in alteration of microflora, causing disease and the possible development of resistant strains which cause failure of antibiotic treatments (Nisha, 2008).

On the processing point of view, Kjeldgaard *et al.* (2012) examined how acceptable levels of antibiotics in meat influence fermentation. Their results show that commonly used bacterial starter cultures were sensitive to residual antibiotics at or near statutorily tolerable levels, and as a result, processed sausages may contain high levels of pathogens. Furthermore, their findings provided a possible explanation for outbreaks and disease cases associated with consumption of fermented sausages and offered yet another argument for limiting the use of antimicrobials in farm animals. With no doubt, there is a need to investigate the role of the availability of antibiotics over the counter for use in animals in South Africa in the development of resistance among humans (Oguttu *et al.*, 2012).

2.3.3. Role of food in disease transmission: Resistance and disease

There is limited information of disease caused by antibiotic resistant bacteria in South Africa, due to the fact that causes of illnesses and deaths are not well counted, as is often the case in low-resource countries (Crowther-Gibson *et al.*, 2011). According to a report from the International Federation for Animal Health (IFAH, 2011), it is estimated that of the 1,500 diseases that affect people, almost two-thirds can pass between animals and humans. The transfer of *S. aureus* isolates between humans and animals, especially in the case of livestock-associated Methicillin-resistant *S. aureus* (MRSA) ST398, has recently gained particular attention (Smith and Pearson 2011). The ST398, which is the swine-associated MRSA, and ST398 human infections, has been recognized in several countries (NIAA, 2011). It is suggested that livestock associated MRSA originally were methicillin-susceptible commensal strains in pigs, whose spread was facilitated by the abundant use of antibiotics in pig and cattle farming (Voss *et al.*, 2005). *S. aureus* is a major human pathogen, a relevant pathogen in veterinary medicine, and a major cause of food poisoning (Sobral *et al.*, 2012). A joint ECDC/EFSA/EMA (2009) scientific report demonstrated that pigs are a major reservoir of a new emerging type of MRSA and concluded that the extensive use of antibiotics for prevention of disease appears to be an important risk factor for the spread of MRSA. With South Africa having a high burden of infectious diseases, including a large portion that are of bacterial origin (Crowther-Gibson *et al.*, 2011), these resistant microorganisms pose a serious health concern in the country where there is a high rate of HIV/AIDS and TB infection. Bacterial infections are quite frequent in HIV-infected patients (Carrega *et al.*, 1997). This is because HIV-induced immune suppression amplifies the risk of bacterial infections, TB and non-tuberculosis, often

involving antibiotic-resistant strains, with severe and / or recurrent potential (Stoian, 2013). For example, infections such as respiratory failure in HIV infected patients are bacterial *pneumonias* which have been reported to be caused by *Pseudomonas*, *S. aureus* and other bacteria (Bajwa and Kulshrestha, 2013). In 2009 an estimated 29% (over 5.5 million) of people were infected with the HIV virus (Crowther-Gibson *et al.*, 2011). Moreover, some evidence indicate that antibiotic resistance rate to nosocomial pathogens are generally high in South Africa (Nyasulu *et al.*, 2012).

Poultry meat has been reported as an important vehicle in foodborne diseases and some studies have suggested that chicken can be a source of antibiotic resistant *Salmonella* spp. in humans (Medeiros *et al.*, 2011). In their study Medeiros *et al.* (2011) found that the prevalence of *Salmonella* spp. was relatively low. However, there were a high proportion of multidrug-resistant strains, including third generation cephalosporins used to treat invasive salmonellosis. Test results from randomly selected spent hens; sold live to residents in *Khayelitsha*, a community near Cape Town revealed that the hens were contaminated by a range of disease-causing bacteria (Garcés, 2002). The concern is that the study showed that the bacteria in both the hens and study community were 100% resistant to most common (oxacillin, vancomycin and methicillin) antibiotics. Therefore, this entails that certain antibiotics would be ineffective in the treatment of people who get infected by eating such hens. Moreover, resistance shown to vancomycin is particularly worrying. As it is a front-line antibiotic used to treat all sorts of infections in humans including chest and heart muscle infections (Nierenberg and Garcés, 2005). Most of the concern about human health consequences of antibiotics use has focused on growth promotion (which boosts the utilisation of the genetic potential for growth of pigs and poultry, improve

feed conversion and reduce waste product output from intensive livestock production) rather than disease prevention (WHO, 1997). The rationale is that the benefits of growth promotion are purely economic and often compensate for and encourage unsanitary conditions (Mellon *et al.*, 2001).

2.3.4. Cases of antimicrobial resistance in South Africa

Only a few relatively recent surveys and reports on antibiotic resistance in isolates from food animals in South Africa have been conducted and they are very few and bunched in Gauteng province (Gelband and Duse, 2011). A number of clinical and environmental data suggest that the rate of antimicrobial resistance is high in South Africa. A current systematic review of published literature (Nyasulu *et al.*, 2012) of antimicrobial resistance surveillance among nosocomial pathogens revealed resistance to commonly used antimicrobial drugs in population: for *S. aureus*, resistance to cloxacillin was 29% and to erythromycin 38%; for *Klebsiella pneumoniae,* resistance to ciprofloxacillin was 35% and to ampicillin 99%; and for *Pseudomonas aeruginosa,* the mean resistance to ciprofloxacillin was 43% and to amikacin 35%. Ateba and his co-authors have also reported antibiotic resistance in dairy and poultry products (Ateba *et al.*, 2010). It is reported that penicillin resistance in South Africa remains mainly intermediate in level, with a low prevalence of fully resistant isolates (Crowther-Gibson *et al.*, 2011).

2.3.5. Current efforts to contain/reverse antibiotic resistance

South Africa is part of the four countries (including India, Vietnam and Kenya) forming the Global Antibiotic Resistance Partnership (GARP). GARP-South Africa was launched on 8 February 2010 at a meeting attended by 40 experts (Suleman and Meyer, 2012). It aims to address the antimicrobial resistance through the situational analysis of antimicrobial resistance in South Africa and collaborating countries. The situational analysis was published as a special supplement to the South African Medical Journal, (SAMJ, 2011). Thereby, the data obtained was said to be used to inform and develop policy and advocacy for antimicrobial resistance-related issues in each of the collaborating countries (Gelband and Duse, 2011). Therefore, GARP is to recognise those issues and recommend policy alternatives that are right for the time and place. Despite poor health status, South Africa has had the most active surveillance for antibiotic resistance of any African country (Gelband and Duse, 2011). However, it has not yet fully translated available antimicrobial resistance surveillance data into policy (Suleman and Meyer, 2012). Hence, there is no evidence of any on-going antimicrobial resistance surveillance for pathogens in South Africa (Nyasulu *et al.*, 2012).

Data from studies indicate that South Africa is using large amount of antibiotic in food-producing animals, this includes a number of antibiotics that have been banned for use in other countries. It is evident that there is a real growing problem of antibiotic resistance in South Africa and with high burden of infectious diseases, including a large portion of bacterial origin, as well as HIV/AIDS epidemic and tuberculosis this put people's life at high risk. Therefore, more effort is needed if

South Africa is determined to overcome this global problem of antibiotic resistance among pathogens.

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CHAPTER 3: Antimicrobial resistance of *Acinetobacter* spp. isolated from raw meat and dairy products

Abstract

The aim of this section was to study the antibiotic susceptibility *Acinetobacter* spp. from raw meat samples collected from abattoir and dairy products purchased from farm shops, in Irene. Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Clinical Laboratory Standards Institute (2007) standards. A total of twenty eight (28) antibiotics were used to determine the antibiotic susceptibility of eight (8) selected *Acinetobacter* isolates. The results showed a complete resistance (100%) to cefotaxime, chloramphenicol, cotrimoxazole, cephalothin, tylosin, penicillin G, lincomycin, ampicillin and vancomycin. However, isolates were susceptible to ceftazidime, levofloxacin, tobramycin and kanamycin, as none of these isolates have shown resistance to these antibiotics. Moreover, the high multidrug resistance against antibiotics tested in this study suggests that bacteria of food animal origin can also be a significant reservoir of resistant genes. The insensitivity of the commensal bacteria to antibiotics is of public health concern, and therefore farmers and food industries must consider the importance of their presence in food products.

3.1. Introduction

The primary objective for all food processing industries is to provide safe, wholesome and acceptable food to the consumer (Bagge-Ravn *et al.*, 2003). However, processing, handling, and preparation of food may lead to cross-contamination of food and surface areas with microorganisms from contaminated

surfaces (Kumar and Anand, 1998). The occurrence of *Acinetobacter* spp. in food processing environments is well documented and it has also been isolated from spoiled food products (Langsrud *et al.*, 2006; Ercolini *et al.*, 2009; Habimana *et al.*, 2010). *Acinetobacter* is a Gram-negative *coccobacillus* that is strictly aerobic, nonmotile, catalase positive and oxidase negative. It is ubiquitous in nature, being found in soil and water (Prashanth *et al.*, 2012). Therefore, the presence of *Acinetobacter* spp. in abattoir raw meat and dairy products could also be due to contamination, since these are ubiquitous organisms and widely distributed in nature (Prashanth *et al.*, 2012; Suelam *et al.*, 2012). Immediately after cutting, the microflora from the carcasses is composed mainly of different species of the genera *Micrococcus* (45-65%), *Pseudomonas* (30-50%), *Bacillus* (10-12%), *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Corynebacterium*, and different Enterobacteriaceae (Vasut and Robeci, 2009).

Acinetobacter spp. is usually among the dominant microflora of the carcasses in stores at refrigeration temperature storage; this is primarily due to greater opportunities to multiply at low temperatures for these species (Vasut and Robeci, 2009). Not only that they can survive in moist environments, but *Acinetobacter* spp. such as *A. baumannii-calcoaceticus* complex can also survive for weeks on dry surfaces for an average of 20 days at a relative humidity of 31% (Blossom and Srinivasan, 2008). As such, raw materials are considered as primary contamination sources in food processing plants, whereas personnel hands and equipment are found to be secondary contamination sources. Although most food processing may have footbaths, the results from the survey by Langsrud *et al.* (2006) indicated that

disinfecting footbaths containing chlorine may act as contamination sources in food factories and should not be used without regular hygienic monitoring.

Monitoring the prevalence of resistance in indicator bacteria, in different populations makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa (van den Bogaard and Stobberingh, 2000; van Duijkeren *et al.*, 2003). Therefore, a study of antibiotic resistance in developing countries, such as South Africa is important as the information could enhance prudent use of antibiotics in food production. A number of clinical antibiotic susceptibility studies of *Acinetobacter* spp. and other pathogen resistance to antibiotics have been conducted (Essack, 2006; WoseKinge *et al.*, 2010). However, as far as we are aware, there has been very little published about the occurrence of antibiotic resistant *Acinetobacter* spp. in raw meat and dairy products in South Africa. Therefore, this work was to study the antibiotic susceptibility of raw meat samples collected from abattoir and dairy products purchased at Irene farm shop.

3.2. Materials and Method

3.2.1. Samples collection, microbial isolation and identification

Meat samples (beef and mutton) were collected from the abattoir at Agricultural Research Council - Animal Production Institute in Irene (Gauteng province). The dairy samples (yogurt, cottage and Italian cheese, *amasi*) were purchased from the shop inside the Institute. A total of thirty six (18 meat and 18 dairy) samples were collected which means 6 (3 meat and 3 dairy) samples at a time for six times. Samples were transported to the laboratory in a cooler box with ice packs. Once at the laboratory, 1 g (solid sample) or 1 ml (liquid) of each sample were homogenized in 9 ml of sterile bacteriological peptone (Oxoid, Hampshire, England) and then incubated at 37°C for 1 to 3 hours (Akoachere *et al.*, 2009). One ml of each sample was serial diluted (10^{-1} to 10^{-6}) and plated on CHROMagar plates incubated at 37°C for 24 hours. After 24 hour 3 or 5 colonies from each plate were picked based on colour and morphology and were then cultured on trypticase soy agar for purification. The isolates were subjected to some biochemical tests, the gram staining and oxidase test. Analytical Profile Index (API) kits were used for further characterization.

3.2.2. Antimicrobial susceptibility test of isolates

Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (formerly National Committee of Clinical Laboratory Standards, NCCLS) standards. A total of twenty eight (28) antibiotic paper discs were used. The inoculum was prepared as follows: a saline suspension was made from a bacterial colony at a turbidity equivalent to a 0.5 McFarland standard. A sterile cotton swab was placed in the bacterial suspension and excess fluid was removed by pressing and rotating the

cotton against the inside of the tube. Each swab was surface spread uniformly onto Mueller Hinton agar (Oxoid, England) plate to yield uniform growth. Antimicrobial paper disks were then applied to the surface of the plate. Multidrug resistant index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate will be resistant by the total number of antibiotics to which the isolate was exposed.

3.2.3. Data analysis

Isolates with MDRI values of more than 0.2 or 20% were considered as highly resistant, MDRI (%) = $\frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotic used}}$ x 100 (Chandran *et al.*, 2008; Doughari *et al.*, 2012). All data were captured (means of triplicates) were calculated and analysed with Microsoft Office Excel (2010) using the general linear model procedure.

3.3. Results and Discussion

The present section evaluated the microbial antibiotic susceptibility of raw meat samples obtained from abattoir and dairy products from local made tuck-shop. *Acinetobacter* spp. count results from both raw meat and dairy products were between 10^4 and 10^3 cfu/ml respectively. Moreover, meat samples indicated high predominance than in dairy samples. A total number of twenty two (22) isolates of *Acinetobacter* sp. were isolated from raw meat and dairy product samples. The API 20NE identification results (Table 3.1) showed that the isolates belonged to *A. baumannii/calcoaceticus*, identification level ranged from 82.7% to 99.9%. From the 22 isolates, 8 were used for antibiotic susceptibility test.

From the results a total resistance (100%) of the isolates was observed to cefotaxime, chloramphenicol, cotrimoxazole, cephalothin, tylosin, penicillin G, lincomycin, ampicillin and vancomycin (Table 3.2). Resistance was also observed to gentamicin (75%), tetracycline (37.5%), amikacin (12.5%), clindamycin (50%), Nalidixic acid (25%), erythromycin (75%) and oxytetracycline (37.5%). However, isolates were susceptible to ceftazidime, levofloxacin, tobramycin and kanamycin, as none of these isolates have shown resistance to these antibiotics.

Table 3.1.*Acinetobacter* spp. identification results

Significant taxa	% ID	Next taxon	Profile#	Comment*
A. baumannii/calcoaceticus	82.7	*Ralstonia pickettii* (12.7%)	0 4 4 0 4 5 1	Acceptable identification
A. baumannii/calcoaceticus	93.4	*R pickettii* (6.0%)	0 4 4 0 4 7 1	Good identification
A. baumannii/calcoaceticus	99.9	*R pickettii* (0.1%)	4 0 4 1 0 6 3	Very good identification
A. baumannii/calcoaceticus	99.4	*R pickettii* (0.5%)	0 0 4 1 4 7 3	Very good identification
A. baumannii/calcoaceticus	99.9	*Pseudomonas putida* (0.1%)	0 0 4 1 0 5 3	Excellent identification
A. baumannii/calcoaceticus	99.4	*R pickettii* (0.5%)	0 0 4 1 4 7 3	Very good identification
A. baumannii/calcoaceticus	99.4	*Achromobacter xylosoxidans* (0.3%)	0 4 4 0 4 7 3	Very good identification

#Profile = value obtained from API 20NE tests and *comment, as described by API 20NE V7.0 (Biomerieux,(*apiweb*™) France)

Generally, meat samples had high prevalence of *Acinetobacter* counts than those of dairy product samples. Moreover, the high prevalence against most antibiotics tested observed in this study suggests that bacteria of food animal origin can be a significant reservoir of resistant bacteria as has been suggested in other studies (Harada *et al.*, 2007; Young *et al.*, 2009). The presence of *Acinetobacter* in dairy products was not expected because most of tested samples were fermented products which mean they had low pH concentrations. However, other studies have also recovered *Acinetobacter* in bulk tank milk and spoiled milk (Langsrud *et al.*, 2006; Gurung *et al.*, 2013). Therefore, we can speculate that the dairy products may have been initially contaminated from the raw milk and during processing. The study by Gurung *et al.* (2013) confirmed the importance of examining bulk tank milk not only for foodborne pathogens but also for *Acinetobacter* spp. which could be of public health concern. Moreover, one has to take into consideration that in South Africa results indicate that most of the ready-to-eat foods do not meet bacteriological quality standards (Nyenje *et al.*, 2012). Therefore addressing the issue of resistance in commensal bacteria will to limit the chances contamination of food by these resistant microorganisms.

Table 3.2. Antibiotic resistance for *Acinetobacter* spp. isolated from raw meat and dairy products.

Antibiotic	code	Resistance (%)	Antibiotic	code	Resistance (%)
Carbapenems*			**Fluoroquinolones***		
Imipenem	IMP10	0	*Nalidixic acid*	NA30	25
Meropenem	MEM10	0	*Norfloxacin*	NOR10	0
Doripenem	DOR10	0	*Ciprofloxacin*	CIP5	0
Cephalosporins*			*Levofloxacin*	LEV5	0
Cephalothin	KF30	100	**Macrolides***		
Cefotaxime	CTX5	100	*Erythromycin*	E15	75
Ceftazidime	CAZ30	0	*Tylosin*	TY30	100
Aminoglycosides*			**Lincosamides***		
Amikacin	AK30	12.5	*Clindamycin*	DA10	50
Gentamicin	CN10	75	*Lincomycin*	L10	100
Kanamycin	K30	0	**Glycopeptide***		
Tobramycin	TOB10	0	*Vancomycin*	VA30	100
Penicillins*			**Sulphonamide***		
Ampicillin	AP2	100	*Co-trimoxazole*	TS25	100
Penicillin G	PG10	100	**Nitrofurantoins***		
Tetracyclines*			*Nitrofurantoin*	NI300	50
Oxytetracycline	OT30	37.5	**Phenicols***		
Tetracycline	T30	37.5	*Chloramphenicol*	C30	100
Doxycycline	DO30	0			
Minocycline	MH30	0			

*Antibiotic group/class

The results of the study indicated resistant to cephalothin and cefotaxime, the 1st and 3rd generation cephalosporins respectively. The decrease susceptibility of gram-negative isolates towards the 3rd and 4th generation cephalosporins has been reported being caused by the Extended Spectrum β-Lactamase (ESBL) or AmpC β-lactamase production (Mishra *et al.*, 2013). These enzymes i.e. ESBLs and AmpC β-lactamases can be carried by bacteria and make them resistant to the 3rd generation cephaloporins and to nearly all other β-lactam antibiotics such as penicillin. Therefore, it must be noted that the β-lactams are by far the most used type of antibiotic in human medicine. It is also noteworthy to highlight the fact that *Acinetobacter* strains are naturally resistant to cephalosporins due to the production of a cephalosporinase (AmpC) that is known to hydrolyse amino-penicillins and the 1st, 2nd and 3rd generation cephalosporins (Hamouda *et al.*, 2011). Interestingly, the results of this study also indicated 100% sensitivity to ceftazidime for all isolates. This sensitivity to ceftazidime is reported to be due to the fact that *Acinetobacter* strains have a low catalytic activity against ceftazidime (Hamouda *et al.*, 2011).

Of particular concern, farmers may be increasingly using these modern and more potent antibiotics such as the 3rd and 4th generation cephalosporins whose use should be strictly limited due to their critical importance for human medicine (FAO/WHO/OIE, 2008). Reports point that 3rd generation cephalosporins are mostly used by farmers for the treatment of bacterial infections in cattle and pigs, control of infection and mortality in day-old chickens (CWF, 2011). Moreover, billions of chickens are reported to receive 3rd generation caphalosporins for treating *E. coli* infection, a practice that has resulted in large reservoirs of resistant bacteria (Zhang, 2013). Bacteria in food-producing animal resistant to 3rd and 4th generation

cephalosporins are considered as a food safety hazard and may contribute to horizontal spread of resistance genes (CMPV, 2009). Clinically, illness and death among humans resulting from bloodstream infections caused by 3rd generation cephalosporin-resistant bacteria such as *E. coli* have been reported in Europe (de Kraker *et al.*, 2011).

The first generation nalidixic acid was the only fluoroquinolone which the isolates were resistance to. Fluoroquinolones have replaced other quinolones over the last two decades, and they are now widely used in clinical medicine as broad-spectrum antimicrobial (Mishra *et al.*, 2013). Some studies have reported a high increase in resistant to fluoroquinolones (Mishra *et al.*, 2013). In contrast, results presented here have showed *Acinetobacter* to be sensitive to levofloxacin, ciprofloxacin and norfloxacin the 3rd, 2nd and 2nd generation fluoroquinolones respectively. However, as mentioned above the isolates were only resistant to the 1st generation, nalidixic acid. Resistant to quinolones can develop because of alterations in bacterial permeability and the development of efflux pumps (King *et al.*, 2000). A major source of human exposure to fluoroquinolone resistance via food appears to be poultry (EFSA, 2008). Samples used in this study were from raw meat (beef and mutton) and dairy products, therefore maybe this could be the reason for the isolates to be more sensitive to this class of antibiotics. Nevertheless, food production systems which involve the use of this class of antibiotics require particular attention to prevent the spread of such resistance of bacteria from these sources (EFSA, 2008).

All the isolates were resistant to chloramphenicol. Resistance to this antibiotic suggest that it may have been used in raising the farm animals either as a growth

promoter, prophylactically or for treatment (Hamouda *et al.*, 2011). Resistance to chloramphenicol diminish the hope of potential reuse of old antibiotics compounds as previous reported (Falagas *et al.*, 2008). The use of antibiotics, whether for prophylaxis or chemotherapy, does not only affect the pathogenic bacteria but also the commensal bacteria. This maintains a pool of resistant bacteria with a pool of resistant genes in the population which further contributes to the general increase and dissemination of bacterial resistance and can be a source of resistance genes for pathogens (Abatih *et al.*, 2009; Blake *et al.*, 2003).

In contrast to the isolates of this study, the clinical *Acinetobacter* isolates from human samples have showed high resistance against fluoroquinolones, aminoglycoside, caphasporin and carbapenem (Lee *et al.*, 2011; Mishra *et al.*, 2013). The results of this study (Table 3.2) showed no resistance in one or more of the following antibiotic classes, carbapenem, fluoroquinolone, cephalosporin, tetracycline and aminoglycoside. However, resistance to tretracycline, sulphonamides, trimethoprim, erythromycin and ciprofloxacin has been reported among *Acinetobacter* spp. from aquatic environments (Petersen *et al.*, 2002; Agerso and Petersen, 2007). Due to the fact that new antibiotics active against gram negative are scarce especially *Acinetobacter* spp. it is necessary to have improved hygiene procedures and optimal antibiotic usage to limit the selection and dissemination of multidrug resistance microorganisms (Coyne *et al.*, 2011). A number of reports show that there are relatively few antibiotics that are active against *Acinetobacter* spp. especially the *A. baumannii* (Neonakis *et al.*, 2011; Giamerellou, 2010; Coyne *et al.*, 2011). The only antibiotics to which *Acinetobacter* is usually

sensitive include meropenem, Amikacin, Rifampin, minocycline and tigecycline (Fishbain and Peleg, 2010; Garnacho-Montero and Amaya-Villar, 2010).

Two macrolides (erythromycin and tylosin) were used in this study and results showed complete resistance in all isolates. One can argue that this resistance could be implicated to over-usage of these antibiotics in farm animals in South Africa. A study by Eagar *et al.* (2012) found that the majority of consumed antibiotics in animals were from the macrolide and pleuromutilin classes, followed by the tetracycline, the sulphonamide and lastly the penicillin class. Moreover, in particular, tylosin one of 4 growth promoters banned in Europe was found to be the most extensively sold antibiotic in South Africa. These antibiotics also fall under the critically important antimicrobials which are the sole, or one of limited available therapy, to treat serious human disease (WHO, 2012).

From multidrug resistance analysis it was observed that *Acinetobacter* isolates were multidrug resistant (Figure 3.1 and Table 3.3). Isolates were resistant to eight (8) or more antibiotics. Moreover, the isolates showed 4 different multidrug resistance patterns (Table 3.3). By definition, multidrug-resistant *Acinetobacter* are isolates resistant to at least 3 classes of antibiotics (Magiorakos *et al.*, 2012). Therefore, on this view the results indicate that the isolated *Acinetobacter* were MDR because they were resistant to more than 3 classes of antibiotics. Additionally, these microbes were able to grow on CHROMagar medium supplemented with supplement *ref CR102* for specific screening of MDR *Acinetobacter* (CHROMagar, France).

Table 3.3. Multidrug resistance among *Acinetobacter* isolates (n =8) from raw meat and dairy product samples.

Phenotype resistance pattern	Frequency (%)	No. of drugs to which isolates were resistant	MDRI %
T-P-K-Ch-E-L-A-TS-C	3 (37.5)	9	32.14
T-P-K-Ch-E-L-O-A-TS-C	2 (25)	10	35.71
T-P-K-Ch-E-L-O-A-TS-T3-C	1 (12.5)	11	39.29
T-P-K-Ch-L-A-TS-C	2 (25)	8	28.57

T = tylosin, P= penicillin, K = Cephalothin, Ch = Chloramphenicol, E= erythromycin, L = lincomycin, O = oxytetracycline, A = ampicillin, TS = cotrimoxazole, T3 = Tetracycline, C = cefotaxime, MDRI = Multidrug Resistant Index

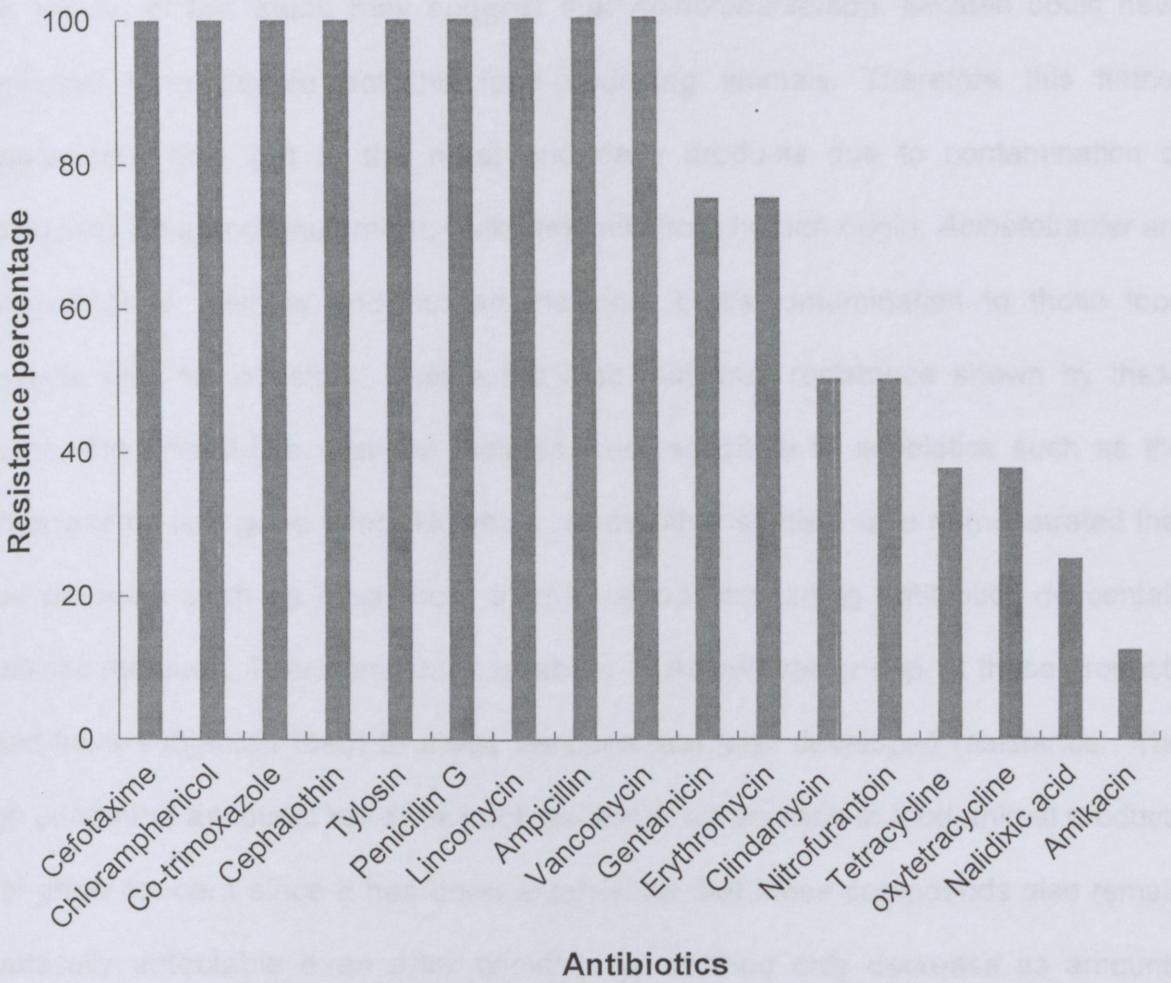

Figure 3.1: Level and pattern of resistance among *Acinetobacter* isolated from raw meat and dairy product samples

However, in contrast Hamouda *et al.* (2011) reported that animal isolates were not MDR and lacked significant antibiotic resistance features such as RIs, class 1 intergrons and ISAb1. But on the other hand, isolates recovered from pig faecal samples harboured one type of resistant bla_{OXA-51} –like gene which has already been reported in human clinical *Acinetobacter* isolates (Hamouda *et al.*, 2011). Another, study by Garung *et al.* (2013) did not detect MDR *Acinetobacter*, that study determined the prevalence and antibiotic susceptibility of *Acinetobacter* from raw bulk tank milk.

The results of this study may suggest that *Acinetobacter*spp. isolated could have originated from people not the food-producing animals. Therefore this further suggest that they got to the meat and dairy products due to contamination of processing area and equipment, bulk tank milk from human origin. *Acinetobacter* are commensal to animals and human therefore cross-contamination to those food products may be possible. Beside the high multidrug resistance shown by these results, the knowledge that the isolates were sensitive to antibiotics such as the carbapenems is a good thing. However, since other studies have demonstrated that food products such as meat from animal raised consuming antibiotics do contain antibiotic residues. Therefore, the availability of *Acinetobacter* spp. in these products could have subjected them to these residues and later developed resistance. The high content of antibiotic residues such as that of tetracycline in food animal products is of great concern since it has been established that these compounds also remain chemically detectable even after cooking, as cooking only decrease its amounts (Javadi, 2011). A comparative study in Nigeria showed penicillin (14%) was the drug with the highest rate of occurrence in meat samples followed by tetracycline (8%) and streptomycin (4%). These antibiotic traces have harmful effects on consumer's

health, such as allergic reactions, liver damage, yellowing of teeth and gastrointestinal disturbance (FAO/WHO, 2002; Jing *et al.*, 2009).

3.4. Conclusion

This study has shown high resistance of *Acinetobacter* spp. isolated from raw meat and dairy product samples therefore, this call for prudent use and alternatives of antibiotics in food production animals. Moreover, could suggest that food processers don't have to focus only to pathogens as food safety indicator, but they must consider other commensal bacteria because these microbes may form resistant biofilms which could also help the growth of other pathogenic bacteria in processing environment.

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CHAPTER 4: Antibiotic resistance pattern of *Staphylococcus* spp. Isolated from raw meat and dairy products

Abstract

The aim of this section was to study the antibiotic susceptibility of *Staphylococcus spp.* from raw meat samples collected from abattoir and dairy products purchased farm from farm shops, in Irene. Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Clinical Laboratory Standards Institute (2007) standards. A total of twenty six (26) antibiotics were used to determine the antibiotic susceptibility of twenty (20) selected *Staphylococcus* isolates. The results in Table 4.1 showed that *S. xylosus* was the predominant isolate, the next was *S. epidermis* followed by *S, aureus* and *S. simulans* and lastly *S. lentus* with 30, 25, 15, 15 and 10 percent respectively. The isolates were resistant to ceftazidime, gentamycin, nalidixic acid, nitrafuration, ampicillin, penicillin, oxytetracycline, tetracycline, doxycycline, clindamycin and lincomycin. The insensitivity of the commensal bacteria to antibiotics is of public health concern, and therefore farmers and food industries must consider the importance of their availability in food products.

4.1. Introduction

Food items such as dairy products, ground beef and poultry are most likely to be associated with antibiotic resistant pathogens (DeWaal and Grooters, 2013). A white paper published by the Centre for Science in the Public Interest showed that these 3 food categories were implicated in more than half (56%) of the reported 55 foodborne outbreaks (DeWaal and Grooters, 2013). Since most *Staphylococcus* spp. normally resident in human and animals their presence in raw meat and dairy

products might have resulted from cross-contamination. Therefore this emphasizes the need to improve the hygiene conditions in processing areas. Moreover, evaluation of the antibiotic resistant phenotypes of *Staphylococcus* spp. could serve as a tool for determining the hygiene standards in the processing of food products (Daka *et al.*, 2012). *Staphylococcus* spp. can rapidly acquire resistance to a broad range of antimicrobials, thereby posing a major concern in the treatment of staphylococcal infections (Bozdogan *et al.*, 2004). Although species such as *S. aureus* is a commensal of several mammalian species, until recently animals were considered of negligible significance as reservoirs for human *S. aureus* infections (Smith *et al.*, 2013). However, since 2004 multiple reports of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals worldwide, and apparent animal-to-human transmission, have heightened concerns about the risks of animal populations as potential reservoirs of zoonotic MRSA infections (Cuny *et al.*, 2010; Weese, 2010). Therefore, this work was to study the antibiotic susceptibility of raw meat samples collected from abattoir and dairy products purchased at Irene farm shop.

4.2. Materials and Method

4.2.1. Samples collection, microbial isolation and identification

Meat samples (beef and mutton) were collected from the abattoir at Agricultural Research Council - Animal Production Institute in Irene (Gauteng province). The dairy samples (yogurt, cottage and Italian cheese, *amasi*) were purchased from the shop inside the Institute. A total of thirty six (18 meat and 18 dairy) samples were collected which means 6 (3 meat and 3 dairy) samples at a time for six times. Samples were transported to the laboratory in a cooler box with ice packs. Once at

the laboratory, 1 g (solid sample) or 1 ml (liquid) of each sample were homogenized in 9 ml of sterile bacteriological peptone (Oxoid, Hampshire, England) and then incubated at 37°C for 1 to 3 hours (Akoachere *et al.*, 2009). One ml of each sample was serial diluted (10^{-1} to 10^{-6}) and plated on Mannitol salt agar for *Staphylococcus* spp., plates incubated at 37°C for 24 hours. After 24 hour 3 or 5 colonies from each plate were picked based on colour and morphology and were then cultured on trypticase soy agar for purification. The isolates were subjected to some biochemical tests, the gram staining and oxidase test. Analytical Profile Index (API) kits were used according to manufacturer's instructions for further characterization (Biomerieux, France).

4.2.2. Antimicrobial susceptibility test of isolates

Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (formerly National Committee of Clinical Laboratory Standards, NCCLS) standards. A total of twenty eight (28) antibiotic paper discs were used. The inoculum was prepared as follows: a saline suspension was made from a bacterial colony at a turbidity equivalent to a 0.5 McFarland standard. A sterile cotton swab was placed in the bacterial suspension and excess fluid was removed by pressing and rotating the cotton against the inside of the tube. Each swab was surface spread uniformly onto Mueller Hinton agar (Oxoid, England) plate to yield uniform growth. Antimicrobial paper disks were then applied to the surface of the plate. Multidrug resistant index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate will be resistant by the total number of antibiotics to which the isolate was exposed to.

4.2.3. Data analysis

Isolates with MDRI values of more than 0.2 or 20% were considered as highly resistant, MDRI (%) = $\frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotic used}}$ x 100 (Chandran *et al.*, 2008; Doughari *et al.*, 2012). All data were captured (means of triplicates) were calculated and analysed with Microsoft Office Excel (2010) using the general linear model procedure.

4.3. Results and Discussion

This section evaluated the microbial antibiotic susceptibility of *Staphylococcus* spp. isolated from raw meat samples obtained from abattoir and dairy products from local made tuck-shop. The results in Table 4.1 showed that *S. xylosus* was the predominant isolate, the next was *S. epidermis* followed by *S.aureus* and *S. simulans* and lastly *S. lentus* with 30, 25, 15, 15 and 10 percent respectively. In contrast to the results obtained here, Goja *et al.* (2013) isolated *Staph* spp. in fresh beef; *S. epidermis* was the highest of their isolates by 13.8% and followed by *S. aureus* (12%). *S. xylosus* counted for 3.4% and *S.lentus* was 1.7%, however *S. simulas* was not isolated from that study. Twenty (20) *Staphylococcus* spp. were isolated, results are shown on Table 4.1. The isolates were resistant to ceftazidime, gentamycin, nalidixic acid, nitrafuration, ampicillin, penicillin, oxytetracycline, tetracycline, doxycycline, clindamycin and lincomycin (Figure 4.1). Similar results were obtained by Vela *et al.* (2013), *S.* xylosus isolates were resistant to nalidixic acid (86.9%), penicillin (70.2%), lincomycin (46.4%), ampicillin (27.4%), tetracycline (21.4%) and erythromycin (11.9%).

The resistance to tetracycline, lincomycin, erythromycin and β-lactam antibiotics are associated to the *tet*K, *lin*A, *ern*B and *bla*Z genes resistance (Vela *et al.*, 2012). Similar observation of resistance to *S. xylosus* was previously observed in another study by Vele *et al.* (2012), where the isolates were resistant to nalidixic acid (86.9%), novobiocin (85.7%), penicillin (70.2%), lincomycin (46.4%), oxacillin (42.9%), ampicillin (27.4%), tetracycline (21.4%), erythromycin (11.9%), bacitracin (10.7%), and streptomycin (2.4%). Moreover, these species are associated with biofilm formation which can be a source of food contamination in food processing areas (Planchon *et al.*, 2006; Ziebuhr *et al.*, 2006; Vela *et al.*, 2012). Furthermore, it is well articulated that the ability of coagulase-negative staphylococci to form biofilm can play an essential role in the colonization of biotic and abiotic surfaces, increasing the likelihood of bacterial survival and persistence (Otto, 2004). This can also suggest that biofilm formation might be an essential factor mediating the long-term colonization capability of *S. xylosus*, together with other biofilm forming bacteria and could in part explain the abundance of biofilm-producing strains of this bacterium in farm animals production such as inside broiler barns (Vela *et al.*, 2012).Therefore, resistance and biofilm formation of these microorganisms raises a serious concern in food quality and hence to public health.

Table 4.1.*Staphylococcus* spp. isolated from raw meat and dairy products (n=20).

Staph. species	Total no. of isolates	% of individual *Staph.* spp. to the total No. of isolates
S. xylosus	6	30
S. epidermidis	5	25
S. aureus	3	15
S. simulans	3	15
S. lentus	2	10

Results show that isolates were resistant to Ampicillin and Penicillin G, which having high percentage of 86.36% and 81.82% respectively. Similar high resistance to Penicillin G. was also reported by Daka *et al.* (2012) where the *S. aureus* isolates were 67.9% and 70.9% resistant. Resistance of penicillin G could be used to assess the susceptibility of *S. aureus* isolates against other β-lactam antibiotics (Pace and Yang, 2006). Moreover, resistant to ampicillin and other β-lactam antibiotics shown in this study is of great concern to consumers. The study of antibiotic resistance in foodborne pathogens has shown resistant to ampicillin in 70.9% and as high as 85.4% (47 of 55) to tetracycline foodborne outbreaks (DeWaal and Grooters, 2013).

Ampicillin and tetracycline a commonly used antibiotic in food animal producing are regarded as critically important and highly important in human medicine by the WHO respectively. An antibiotic is ranked critically important when; (i) it is the sole or of the few options for treatment of human infections, and (ii) it is used to treat diseases caused by organisms that may be transmitted via non-human sources. Whereas those that meet just one of the above criteria are ranked as highly important (WHO, 2012). Table 4.2 shows that most isolates were low multidrug resistance as most had MDRI as 11.5% to 19.2% which was less than 20%, suggesting that there not

multidrug resistant. However, a number of them had a relative high MDRI which range of 23.1% to 30.8% respectively. Isolates that have a MDRI > 0.2 (above 20%) may have originated from an environment where several antibiotics are being used (Tambekar *et al.*, 2006). Moreover, in respect to the isolates which had a low MDRI, one can argue that since some of those isolates were resistant to at least three classes of antibiotics they can be considered multidrug resistant.

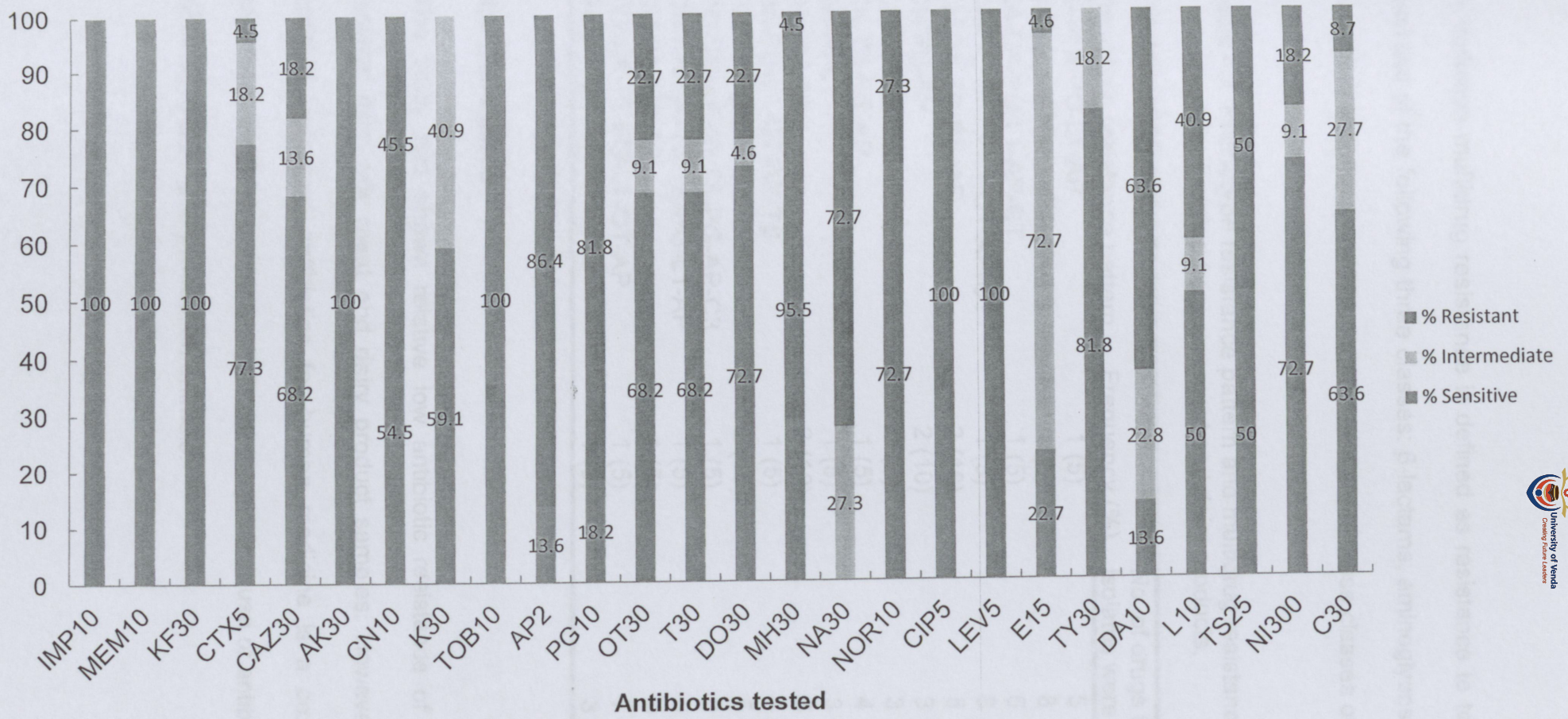

Figure 4.1. Results for antibiotic susceptibility for *Staphylococcus* spp. isolated from raw meat and dairy products.

By definition multidrug resistance is defined as resistance to tested antibiotics in at least two of the following three classes: β-lactams, aminoglycosides and quinolones. So by that isolates were found to be resistant to those classes of antibiotics.

Table 4.2. Phenotype resistance pattern and multidrug resistance of *Staphylococcus* spp. isolated from raw meat and dairy products.

Phenotype resistance pattern	Frequency (%)	No. of drugs to which isolates were resistant	MDRI (%)
CA-CN-PG-L1-AP	1 (5)	5	19.2
CA-DA-NI-T3-L1-OT-AP-TS	1 (5)	8	30.8
CA-DA-PG-L1-AP-CT	1 (5)	6	23.1
CA-DO-NI-T3-L1-OT-AP-CT	1 (5)	8	30.8
CN-DA-TS-PG-AP	2 (10)	5	19.2
CN-PG-AP	2 (10)	3	11.5
CN-TS-PG	1 (5)	3	11.5
CN-TS-PG-AP	1 (5)	4	15.4
DA-NI-L1	1 (5)	3	11.5
DA-PG-AP	2 (10)	3	11.5
DA-PG-L1-OT-AP-TS	1 (5)	6	23.1
DA-TS-PG-AP-C3	2 (10)	5	19.2
DO-CN-DA-NI-TS-PG-AP-C3	1 (5)	8	30.8
DO-CN-DA-T3-TS-PG-L1-AP	1 (5)	8	30.8
DO-CN-DA-TS	1 (5)	4	15.4
DO-DA-T3-PG-L1-OT-AP	1 (5)	7	26.9
PG-AP-CT	1 (5)	3	11.5

4.4. Conclusion

This study has shown relative low antibiotic resistance of *Staphylococcus spp.* isolated from raw meat and dairy product samples. However, their resistance to critically important antibiotics for human medicine is a concern. Therefore this resistance to those antibiotics calls for the prudent use of antibiotics and alternatives of antibiotics in food production animals.

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CHAPTER 5: Antimicrobial resistance of *Morganella morganii* isolated from raw meat and dairy products

Abstract

The aim of this section was to study the antibiotic susceptibility of *Morganella morganii* from raw meat samples collected from abattoir and dairy products purchased from farm shops, in Irene. Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Clinical Laboratory Standards Institute (CLSI, 2007) standards. A total of twenty eight (28) antibiotics were used to determine the antibiotic susceptibility of sixteen (16) selected *M. morganii* isolates. Results showed sensitivity to carbapenems (93.7%), cephalosporins (12.5 -87.5%), aminoglycosides (25 – 87.5%), fluoroquinolones (50 – 100%) and co-trimoxazole (56.2%). However, isolates were resistant to chloramphenicol 25%, nalidixic acid 43.7%, cephalothin 75%, tetracyclines (37.5%), macrolides (87.5 – 93.7%) and lincosamides (75 – 100%). Although most *M. morganii* strains are known to rarely cause diseases, but their intrinsic resistance to numerous antibiotics make this bacterium a public health concern, more especially that it is known to colonize human and animal gut. Moreover, to our knowledge, this is the first report in South Africa of the presence and antibiotic susceptibility of *M. morganii,* in raw meat and dairy products.

5.1. Introduction

The extensive use of antibiotics in animal production has resulted in antibiotic resistant zoonotic of bacteria that can be transmitted to humans through the food chain (ECDC/EFSA/EMA 2009;Walsh and Fanning, 2008). Like most commensal

bacteria, *Morganella morganii* is a bacterium which naturally resides or lives in or co-exists mutually with the host without causing disease (Andremont, 2003; EFSA, 2012). *M. morganii* is naturally/usually present in the human gut, specifically in the colon and also in environment settings(O'Hara *et al.*, 2000). It has been reported to have the ability to enter the urethra, whereby it can cause urinary tract infections(O'Hara *et al.*, 2000; Singla *et al.*, 2010; Yazıcı *et al.*, 2013). *M. morganii* is a gram-negative, rod-shaped bacterium. It is a motile, aerobic, facultative anaerobic, and a member of the *Enterobactiaceae*. This bacterium is the only species in the genus *Morganella*, which belongs to the tribe *Proteeae* of the family *Enterobacteriaceae* (O'Hara *et al.*, 2000; Manos and Belas, 2006; Zhu *et al.*, 2010; Chen *et al.*, 2012). *M. morganii* is considered an uncommon cause of nosocomial infections (Singla *et al.*, 2010). However, it is difficult to predict when a *Morganella* infection will occur (O'Hara *et al.*, 2000). Furthermore, even though it is only a rare opportunistic pathogen (mostly in immune system deficiency patients), the important fact to be considered is that this bacterium can cause serious diseases (Yazıcı *et al.*, 2013). These microorganisms may pose a serious health concern in a country like South Africa where there is a high rate of HIV/AIDS and TB infection. Moreover, bacterial infections are quite frequent in HIV-infected patients (Carrega *et al.*, 1997). This is because HIV-induced immune suppression amplifies the risk of bacterial infections, TB and non-tuberculosis, often involving antibiotic-resistant strains, with severe and / or recurrent potential (Stoian, 2013).

As mentioned *M. morganii* widely distributed in nature and therefore it may contaminate food products such meats and dairy products during processing through contact with contaminated surfaces (O'Hara *et al.*, 2000; Amador *et al.*, 2009).

Hence, there are very few studies on this bacterium especially those from food isolates. Although the reports of *M. morganii* associated with foods (raw meat and dairy products) is limited, *M. morganii* have been isolated in cheese (Amador *et al.*, 2009), pork (Singh and Virdi, 1999), and ground beef and hamburgers (Durlu-Ozkaya *et al.*, 2001). Moreover, *M. morganii* is the most prolific histamine former and plays the major role in histamine accumulation during storage of fish (Kim *et al.*, 2001). Histamine is the main causative agent of scombroid poisoning, a foodborne chemical intoxication (FDA, 1998). This scombriod poisoning is considered one of the prevalent illnesses associated mostly with seafood consumption and it is usual accompanied by a variety of symptoms, such as rash, nausea, diarrhea, flushing, sweating and headache (Kim *et al.*, 2003).

Like other members of the *Enterobacteriaceae, Morganella* species are capable of producing inducible chromosomal AmpC β-lactamases making them resistant to action of primary and extended spectrum penicillins and cephalosporins (O'Hara *et al.*, 2000). Therefore, *M. morganii* strains are known to be naturally resistant to penicillin, ampicillin, ampicillin/sulbactam, oxacillin, 1st-generation and 2nd-generation cephalosporins, erythromycin, colistin, and polymyxin B (Miller, 2012). However, most strains are sensitive to cefepime, imipenem, meropenem, piperacillin, aminoglycosides, floroquinolones and chloramphenicol (Miller, 2012; Hakyemez *et al.*, 2012; Yazıcı *et al.*, 2013).

The surveillance for antibiotic susceptibility in *Enterobacteriaceae* is crucial because species of this family are among the most significant and prevalent human pathogens (Karlowsky *et al.*, 2003). As mentioned above, there is limited information

about the presence and antibiotic susceptibility of *M. morganii* in raw meat and dairy products in South Africa. Therefore, this work was to study the antibiotic susceptibility of dairy products and raw meat samples collected from abattoir.

5.2. Materials and Method

5.2.1. Samples collection, microbial isolation and identification

Meat samples (beef and mutton) were collected, transported and stored as described in Chapter 3, page 41.Once at the laboratory, 1 g (solid sample) or 1 ml (liquid) of each sample were homogenized in 9 ml of sterile bacteriological peptone (Oxoid, Hampshire, England) and then incubated at 37°C for 1 to 3 hours (Akoachere *et al.*, 2009). One ml of each sample was serial diluted (10^{-1} to 10^{-6}) and plated on Mannitol Salt Agar (MSA) (supplemented with 0.1% of imipenem) plates incubated at 37°C for 24 hours. After 24 hour 3 or 5 colonies from each plate were picked based on colour and morphology and were then cultured on trypticase soy agar for purification. The isolates were subjected to some biochemical tests, the gram staining and oxidase test. Analytical Profile Index (API) kits were used for further characterization. Isolates were further sent to Inqababiotec (Pretoria, South Africa) for molecular identification.

5.2.2. Antimicrobial susceptibility test of isolates

Antibiotic susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (formerly National Committee of Clinical Laboratory Standards, NCCLS) standards. A total of twenty eight (28) antibiotic paper discs were used. The inoculum was prepared as follows: a saline suspension was made from a bacterial colony at a turbidity

equivalent to a 0.5 McFarland standard. A sterile cotton swab was placed in the bacterial suspension and excess fluid was removed by pressing and rotating the cotton against the inside of the tube. Each swab was surface spread uniformly onto Mueller Hinton agar (Oxoid, England) plate to yield uniform growth. Antimicrobial paper disks were then applied to the surface of the plate. Multidrug resistant index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate will be resistant by the total number of antibiotics to which the isolate was exposed to.

5.2.3. Data analysis

Isolates with MDRI values of more than 0.2 or 20% were considered as highly resistant, MDRI (%) = $\frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotic used}}$ x 100 (Chandran *et al.*, 2008; Doughari *et al.*, 2012). All data were captured (means of triplicates) were calculated and analysed with Microsoft Office Excel (2010) using the general linear model procedure.

5.3. Result and Discussion

This section evaluated the microbial antibiotic susceptibility of *Morganella morganii* isolated from raw meat samples obtained from abattoir and dairy products from local made tuck-shop. The results from antibiotic susceptibility determination for the 27 antibiotics (Figure 5.1), showed that isolates were sensitive to carbapenems (93.7%), ceftazidime and cefotaxime (87.5%), aminoglycosides i.e. amikacin, gentamycin, tobramycin at 85.7, 62.5 and 81.3% respectively. Isolates were also sensitive to tetracyclines i.e. oxytetracycline, tetracycline, doxycycline, minocycline at 50, 56.2, 62.5 and 56.2% respectively and they were also sensitive to

fluoroquinolones with an exception for nalidixic acid. Results also showed some sensitivity to co-trimoxazole 56.2%, nitrofurantoin 43.7% and 31.3% to chloramphenicol, however isolates were also exhibited intermediate susceptibility for these antibiotics. In general, *M. morganii* is considered to be susceptible to most of the above antibiotics (Miller, 2012; Hakyemez *et al.*, 2012). But contrary to reported susceptibility profiles of the organisms (Stock and Wiedemann, 1998; Miller, 2012; Hakyemez et al., 2012); the isolates were susceptible to cefotaxime (87.5%) a 2nd generation cephalosporin.

Only 6.3% of the isolates were resistant to carbapenems, 75% was resistant to cephalothin a 1st generation cephalosporin, 81.3% was resistance to ampicillin and penicillin G, while isolates showed relatively low resistance to tetracyclines at average of 37.5%. The isolates showed 56.2% resistance and 43.7% intermediate susceptibility to nalidixic acid and as anticipated resistance was high to erythromycin (93.7%) and tylosin (87.5%). Resistance was also observed in Lincosamides (clindamycin 75% and lincomycin 100%) and vancomycin 100%; however the concern was the resistance to co-trimoxazole (31.3%), and chloramphenicol (25%) *because M. morganii* isolates are known to be be sensitive to these antibiotics (Stock and Wiedemann, 1998; Miller, 2012; Hakyemez *et al.*, 2012; Yazıcı *et al.*, 2013).

As observed in Figure 5.1, tetracyclines had relative high resistance which was around 37% of the isolates. In most gram-negative species, tetracycline resistance is due to the acquisition of an operon which consists of an efflux gene *tet*(A) and a repressor gene *tet*(R) that are divergently transcribed from overlapping operator regions (Sawant *et al.*, 2007). About nine types of efflux genes consisting of the

above operon have been described so far for gram-negative bacteria, *tet*(A) to *tet*(E) and *tet*(G) to *tet*(J) (Schnabel and Jones, 1999). Resistance to these antibiotics is of concern since they continue to be one of the most widely used antibiotics in human medicine and animal agriculture, as they are relatively inexpensive and have relatively few side effects.

Results for the prevalence phenotype resistance pattern is shown in Table 5.1, DA-NA-TY-KF-PG-E1-L1-AP-VA, DA-TY-PG-E1-L1-AP-VA and DO-C3-DA-NA-T3-TY-YS-KF-PG-E1-L1-OT-AP-VA-MH were the most prevalent pattern observed from this study. The isolates have showed to be resistant to 4 or more of the antibiotics used, whereby isolates were resistant to as much as 16 antibiotics. The isolates had a relatively high multidrug index with values reaching 57.1%. Moreover, majority of the isolates had MDR Index above 20%, therefore by definition this indicated that the *M. morganii* strains isolated in this study were multidrug resistant. It is considered that the emergence of highly resistant strains of *M. morganii* have been associated with use of 3rd-generation cephalosporins (Miller, 2012).

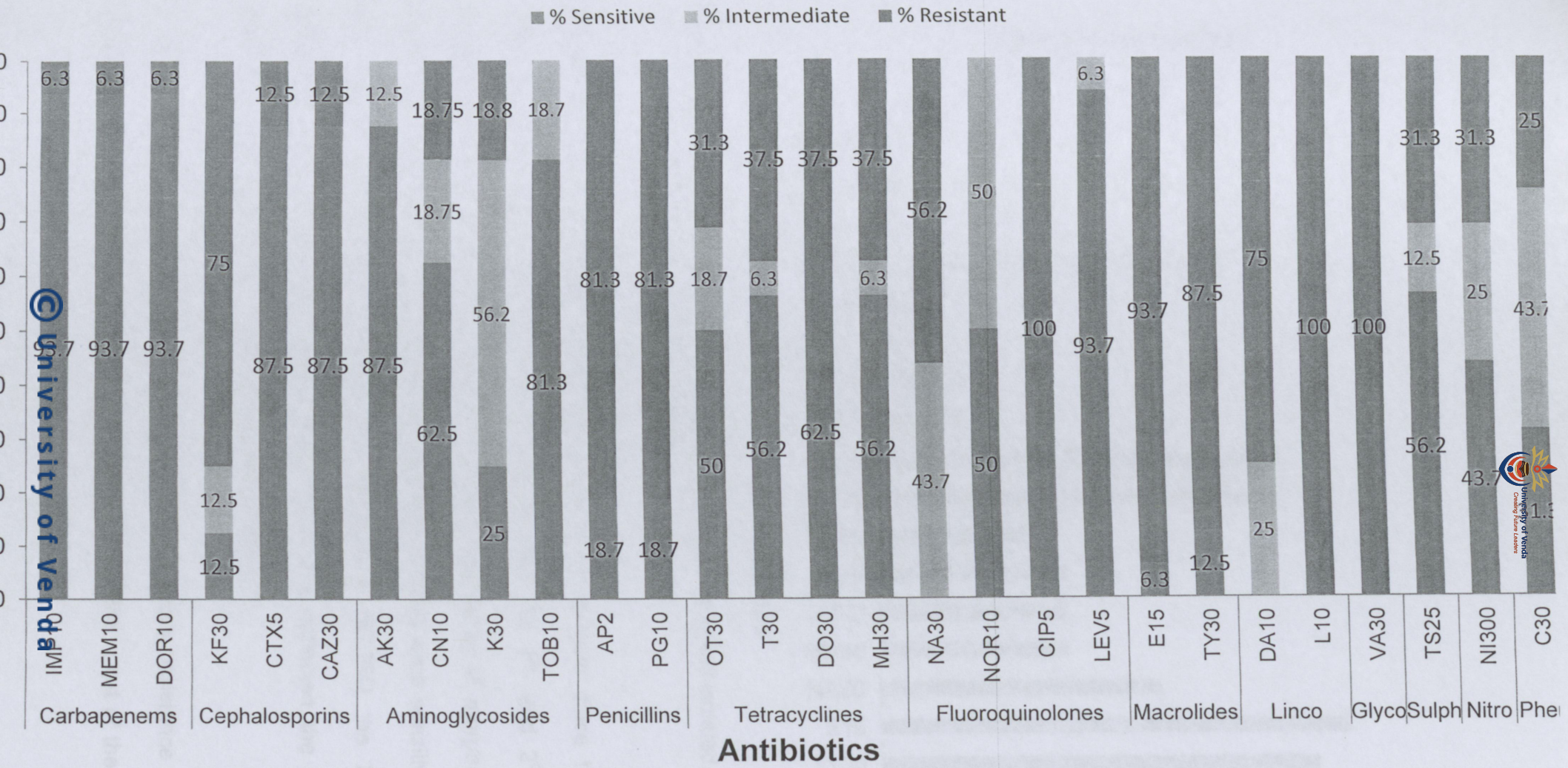

Figure 5.1. Results for antibiotic susceptibility for *M. morganii* isolated from raw meat and dairy products.

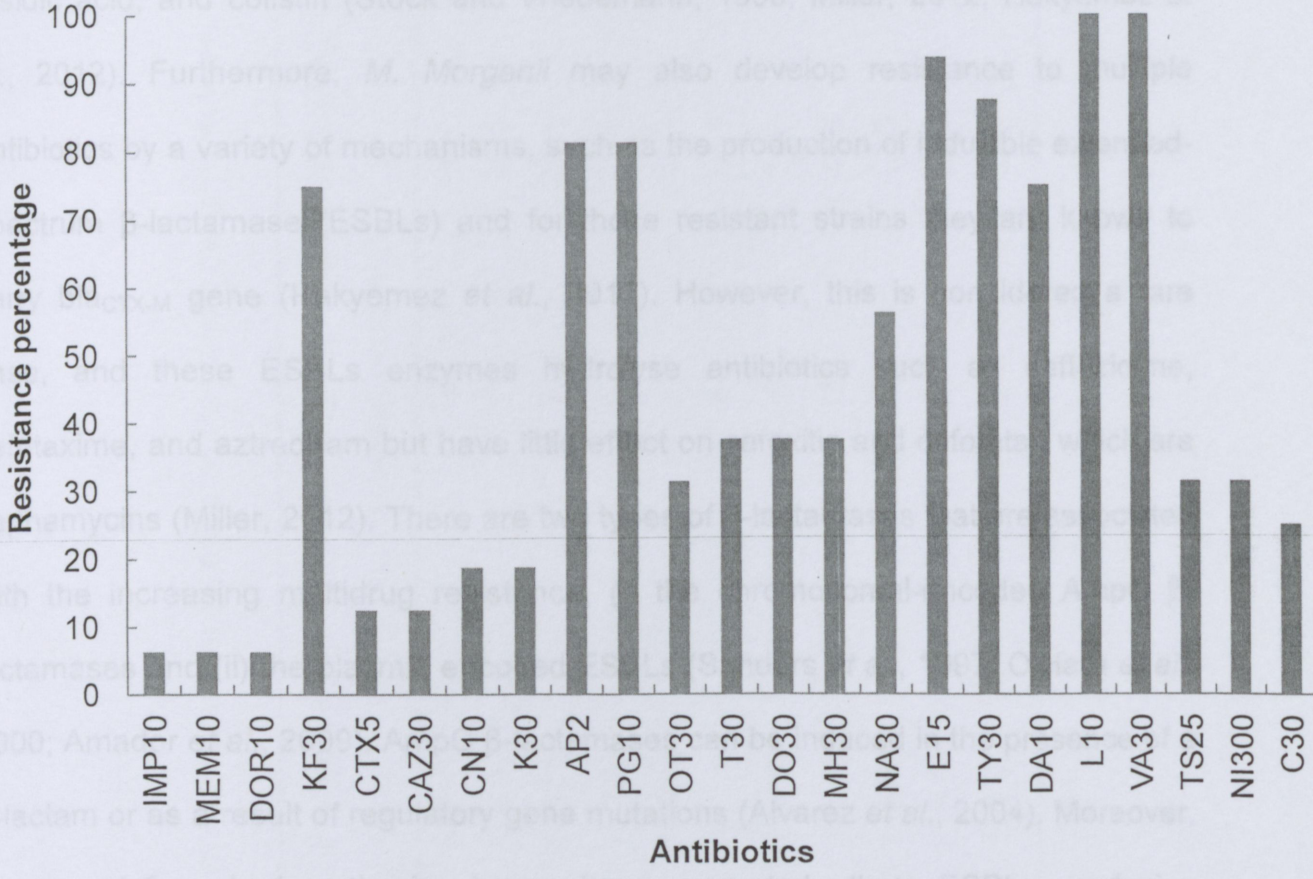

Figure 5.2. Level and pattern of resistance among *M. morganii* isolated from raw meat and dairy product samples

Resistance to 3rd-generation cephalosporins is of concern since these are the preferred and more effective cephalosporins than the 1st- and 2nd -generation cephalosporins in empirical treatment of diseases caused by *M. morganii* (Hakyemez *et al.*, 2012). In this study however, *M. morganii* isolates were sensitive (Figure 5.1 and 5.2) to cefotaxime (87.5%) and ceftazidime (87.5%) the 2nd- and 3rd-generations' caphalosporins respectively. As it was expected the isolates were resistant to cephalothin a 1st- generation cephalosporin.

It must also be noted that *M. morganii* have intrinsic resistance to numerous antibiotics which includes, oxacillin, ampicillin, amoxicillin, most of the 1st- and 2nd -

generation cephalosporins, macrolides, lincosamides, glycopeptides, fosfomycin, fusidic acid, and colistin (Stock and Wiedemann, 1998; Miller, 2012; Hakyemez *et al.*, 2012). Furthermore, *M. Morganii* may also develop resistance to multiple antibiotics by a variety of mechanisms, such as the production of inducible extended-spectrum β-lactamase (ESBLs) and for those resistant strains they are known to carry bla_{CTX-M} gene (Hakyemez *et al.*, 2012). However, this is considered a rare case, and these ESBLs enzymes hydrolyse antibiotics such as ceftazidime, cefotaxime, and aztreonam but have little effect on cefoxitin and cefotetan which are cephamycins (Miller, 2012). There are two types of β-lactamases that are associated with the increasing multidrug resistance, (i) the chromosomal-encoded AmpC β-lactamases and (ii) the plasmid encoded ESBLs (Sanders *et al.*, 1997; O'Hara *et al.*, 2000; Amador *et al.*, 2009). AmpC β-lactamases can be induced in the presence of a β-lactam or as a result of regulatory gene mutations (Alvarez *et al.*, 2004). Moreover, some reinforced hypothesis have been reported that ESBL producing *Enterobacteriaceae* could be transmitted to humans by the food supply, particularly by the consumption of ready-to-eat food that are eaten without being cooked, such as cheese (Amador *et al.*, 2009).

Table 5.1. Prevalence of resistance phenotypes to antibiotic and multidrug resistance index for *Morganella morganii* isolates.

Phenotype resistance pattern	Frequency (%)	No. of drugs to which isolates were resistant	MDRI (%)
CA-CT-IM-ME-DO-DA-KF-PG-E1-L1-AP-VA	1 (6.3)	12	42.9
CA-CT-L1-VA	1 (6.3)	4	14.3
DA-NA-TY-KF-PG-E1-L1-AP-VA	2 (12.5)	9	32.1
DA-NI-TY-E1-L1-VA	1 (6.3)	6	21.4
DA-TY-PG-E1-L1-AP-VA	2(12.5)	7	25
DO-C3-DA-NA-T3-TY-YS-KF-PG-E1-L1-OT-AP-VA-MH	2(12.5)	5	17.9
DO-C3-NA-NI-T3-TY-TS-KF-PG-E1-L1-OT-OT-AP-VA-MH	1 (6.3)	16	57.1
DO-CN-DA-NA-NI-T3-TY-KF-PG-E1-L1-OT-AP-VA-MH	1 (6.3)	15	53.8
DO-NA-T3-TY-TS-KF-PG-E1-L1-AP-VA-MH	1 (6.3)	12	42.9
K3-CN-DA-T3-KF-PG-E1-L1-AP-VA	1 (6.3)	10	35.7
K3-DA-TY-KF-PG-E1-L1-AP-VA	1 (6.3)	9	32.1

5.4. Conclusion

The results indicated that *Morganella morganii*, is endogenous to intestinal tract of human and mammals. However, *M. morganii* has been recognized as a relatively unimportant human pathogen and has not been reported in farm/domestic animals. Perhaps, this has also led to situation of it not being considered important in food processing plants. But the results of this study have shown its presence in meat and dairy products and its resistance to multidrug. This emphasizes the importance of sanitation in the processing plant to prevent cross-contamination. To our knowledge, this is the first report in South Africa of the presence and antibiotic susceptibility of *M. morganii*, in raw meat and dairy products. Therefore, this suggest that more work need to be done to assess the impact of these and more other similar commensal bacteria on transferring antibiotic resistance and their implication on food safety and quality.

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CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATION

In this study the prevalence and antibiotic resistance in some commensal bacteria from dairy and meat products at Irene, Pretoria in South Africa were evaluated. The study of antibiotic resistance in developing countries such as South Africa is important as the information could enhance prudent use of antibiotics in food production by detecting transfer of resistant bacteria or resistance genes from food animals to humans. South Africa has had the most active surveillance for antibiotic resistance of any African country. But, the concern is that it has not yet fully translated to available antimicrobial resistance surveillance data into policy. Moreover, a national surveillance programme of antibiotics usage on the food-producing animals and antibiotic resistance is required to help to mitigate the problem of lack of availability of information. This study has shown high resistance to antibiotic in *Acinetobacter* spp. isolated from raw meat and dairy products. This therefore calls for prudent use and finding alternatives to the use of antibiotics in food animals. The phenotypic resistance pattern of isolates observed in this study raises concerns; therefore it is recommended that further investigation on the impacts and dynamics of genetic antibiotic determinants should be conducted through molecular methods. The main obstacle for researchers working to identify solutions to antibiotic resistance is a lack of data especially data on antibiotic usage (Dewaal and Grooters, 2013). Information like these required in order to tackle the issue of resistance in our communities and food industries. The growth of antibiotic resistance level especially in developing countries like South Africa could have a real negative impact since these countries do not have better access to more effective medications. Evaluation of the antibiotic resistant phenotypes could serve as a tool for determining the hygiene standards in the processing of food products (Daka *et*

al., 2012). Data obtained could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the consumption of contaminated products.

Crude plant extracts have been a promising alternative and potential resistance modifying agents in fight against antibiotic resistance (Sibanda and Okoh, 2007; Aiyegoro *et al.*, 2009; Savoia 2012). For example, Aiyegoro *et al.* (2009, 2011) proposed that extracts of the leaves of *Helichrysum pedunculatum* and *Afzelia africana* stem bark could be of relevance in combination therapy and as a source of resistance modifying principles that could be useful as treatment for microbial infections. Therefore, these breakthroughs are the promising signs that in the next years some different molecules discovered by ingenious screening programs and obtained from different plant oils and extracts will become useful therapeutic tools (Savoia, 2012).

More work on a national surveillance programme of antibiotics usage on the food-producing animals and the surveillance programme on antibiotic resistance in bacteria must be established in South Africa. These programmes should collect a well arranged data on usage, such as the usage per animal species (drugs type, daily doses) or usage on farm level. It should also include the testing (quantitative susceptibility tests and molecular analysis of resistant genes) of a wide range of bacteria from animals and food products. This information will help to mitigate the problem of lack of availability of information on the amount of antibiotics which are currently being used in livestock production in South Africa. Moreover, a consumer risk perception study on the use of antibiotic in livestock should be conducted, as it is

an important factor because it reflects the subjective assessment that people make on the use of antibiotics in food-producing animals as it affect the food they consumes.

To summarize, the following is recommended as ways to reduce the increased number of antibiotic resistance bacteria in foods and our community in general:

- More work on molecular analyses of antibiotic resistance genes and antibiotic-resistant mobile elements of commensal bacteria is needed.
- National surveillance programme of antibiotics usage in food-producing animals is needed to enable and increase the availability of information for researchers and the policy makers.
- Research study to assess consumer awareness of antibiotic usage in farm animals and to get their perception on this subject.
- More work to find ways as alternative to antibiotic usage in food-producing animals, for example the use of plant extracts to treat sick animals.

Appendix I. Conference paper 1

Poster Acceptance Letter

REF NO: EFFO2013_0160 (Please quote in all correspondence)

27 June 2013

Email: moyanej@arc.agric.za

Dear Prof. J.N. Moyane,

Thank you for submitting an abstract to present at 2013 EFFoST Annual Meeting. On behalf of the Organising Committee I am delighted to inform you that your abstract entitled "Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance" has been accepted for **poster presentation** at the Conference.

Title:	Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance
Authors:	J.N. Moyane, A.I.O. Jideani, O.A. Aiyegoro
Presenting Author:	J.N. Moyane

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It is a condition of abstract acceptance that you or a nominated presenting co-author registers for the conference by the **author registration deadline of 20 September 2013. The abstracts of all unregistered presenters will be removed from the poster programme after this date.** Should the addressee above not be the nominated presenter, please inform me of the name and email address of the presenter immediately: Content-EFFoST2013@elsevier.com

Please register online to attend the conference by following this personal link: http://conferences.elsevier.com/EFFO2013?email=moyanej@arc.agric.za&abstracts=0157,0160

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Standard rate student	€350
Early bird academic	€750
Standard rate academic	€825
Standard rate exhibitor	€1650
Conference Buffet Dinner	€40

All authors will be responsible for the payment of their own registration fees, travel and accommodation expenses. Unfortunately the conference organisers do not have funds available to support the attendance of individual delegates.

Poster Acceptance Letter

REF NO: EFFO2013_0157 (Please quote in all correspondence)

27 June 2013

Email: moyanej@arc.agric.za

Dear Prof. J.N. Moyane,

Thank you for submitting an abstract to present at 2013 EFFoST Annual Meeting. On behalf of the Organising Committee I am delighted to inform you that your abstract entitled "Microbial contamination of meat and the potential danger of antibiotic resistance from commensal bacteria isolates" has been accepted for **poster presentation** at the Conference.

Title:	Microbial contamination of meat and the potential danger of antibiotic resistance from commensal bacteria isolates
Authors:	J.N. Moyane, A.I.O. Jideani, O.A. Aiyegoro
Presenting Author:	J.N. Moyane

Please check the above details of your presentation carefully, as all conference material will be printed with this information. If there are any corrections please inform us as soon as possible by email to: Content-EFFoST2013@elsevier.com

It is a condition of abstract acceptance that you or a nominated presenting co-author registers for the conference by the author registration deadline of 20 September 2013. **The abstracts of all unregistered presenters will be removed from the poster programme after this date.** Should the addressee above not be the nominated presenter, please inform me of the name and email address of the presenter immediately: Content:-EFFoST2013@elsevier.com

Please register online to attend the conference by following this personal link: http://conferences.elsevier.com/EFFO2013?email=moyanej@arc.agric.za&abstracts=0157,0160

Registration is available online using a credit card. Registration rates are as follows:

Early bird student	€300
Standard rate student	€350
Early bird academic	€750
Standard rate academic	€825
Standard rate exhibitor	€1650
Conference Buffet Dinner	€40

All authors will be responsible for the payment of their own registration fees, travel and accommodation expenses. Unfortunately the conference organisers do not have funds available to support the attendance of individual delegates.

Poster Presentations

Acceptance Letter: Invitation to Present at The 18th Biennial South African Society for Microbiology (SASM 2013)

15 September 2013

Mr. Jeremia Moyane
Agricultural Research Council
South Africa

Dear Mr. Jeremia Moyane

We are pleased to inform you that your Poster Presentation has been accepted for inclusion in the scientific programme.

Please expect to receive further information concerning this Poster Presentation.

If you wish to query anything, please contact the conference organisers shelley@soafrica.com and quote the details below.

Document ID # : 30
Presentation Type: Poster Presentation
Research Area : Food Microbiology
Title : Microbial contamination of abattoir meat and the potential antibiotic resistance of commensal bacteria isolates
Contributor : Mr. Jeremia Moyane

Kind regards,
The 18th Biennial South African Society for Microbiology (SASM 2013)

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African Journal of Microbiology Research

Review

Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance

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Accepted 3 June, 2013

The widespread and intensive use of antibiotic agents in modern food production systems is believed to be contributing significantly to antibiotic resistance among bacteria. Antibiotic usage in food-producing animals tend to be increasing and data show that even those that have been banned in other countries such as growth promoters are still being used in South Africa. Moreover, very few relatively recent surveys and reports on antibiotic resistance isolates from food animals in South Africa have been carried out and are crowded in Gauteng province. However, despite poor health status which include a large portion of bacterial infections, as well as HIV/AIDS epidemic and tuberculosis, South Africa has had the most active surveillance for antibiotic resistance of any African country. But, the concern is that it has not yet fully translated available antimicrobial resistance surveillance data into policy. Moreover, a national surveillance programme of antibiotics usage on the food-producing animals and antibiotic resistance is required to help to mitigate the problem of lack of availability of information.

Key words: Antibiotic, food-producing animals, bacteria, resistance, surveillance.

INTRODUCTION

The intensive use of antimicrobial (antibiotic) agents in industrial animal husbandry have spread into developing countries, and the negative impact on human health and food safety have often followed (Garcés, 2002). The impact may vary considerably between countries and regions, influenced by the interaction between human populations, land use, contaminated water sources, animal demography, national policies (production, trade, food security, animal health, etc.), and national and international trade (WHO, 2012). Hence, antibiotic resistance is a major global societal problem (Mellon et al., 2001; Davies and Davies, 2010; WHO, 2012), involving many different sectors example medicine, veterinary medicine, animal husbandry, agriculture, environment and trade (EC 2011). Epidemiological studies have demonstrated a correlation between antibiotic use and antimicrobial resistance (Goossens et al., 2005; Beerepoot et al., 2011, 2012; den Heijer et al., 2012). Goossens et al. (2005) showed that there were higher rates of antibiotic resistance in high consuming countries, probably related to the higher consumption/usage of antibiotics in southern and eastern Europe than in northern Europe. There is a higher report of use of antibiotics in developed countries than in developing countries both for prophylaxis and therapy but, higher therapeutic use than prophylactic use in developing countries

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(Mitema et al., 2001; Byarugaba et al., 2011). Reports point that the international travel and trade in animals and animal products increase the risks of antibiotic resistance world-wide (Acar and Röstel, 2001; Mellon et al., 2001; EC, 2011; Byarugaba et al., 2011; WHO, 2012).

Furthermore, antibiotic resistance has been given a low priority in most developing and many developed countries. Most important, developing countries such as South Africa have received much limited attention regarding this problem (Okeke et al., 2007). Globally, only a few developed countries such as Sweden, Denmark, the United Kingdom, and Netherlands have managed to reduce antibiotic consumption in the community which have sometimes resulted, but not always, in a decrease in resistance (Cogliani et al. 2011; Mackie, 2011; Carlet et al., 2012). Unfortunately, there is no single or simple solution to the problem of bacterial antibiotic resistance because there are many diverse factors that contribute to irrational use of antibiotics including knowledge, perceptions, attitudes and behaviour of policy-makers, prescribers, manufacturers, dispensers and consumers (WHO, 2012). However, the use of antibiotics in livestock production is suspected to be significantly contributing to the antibiotic resistance in species of bacteria which are common to humans and animals (Acar and Röstel, 2001). Mostly, the routine practice of giving antibiotic agents to domestic livestock (that is, preventing and treating diseases, as well as promoting growth) is found to be an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to humans through the food chain/or foodstuffs (Perreten et al., 1998; van den Bogaard and Stobberingh, 2000; Schlegelova et al., 2004; Byarugaba et al., 2011).

The increase in antibiotics resistance has been reported in both commensal and pathogenic bacteria, this raises an emerging threat to public health and the environment (Marshall et al., 2009; Thaller et al., 2010; Aiyegoro et al, 2011; Byarugaba et al., 2011; Carlet et al., 2012). This high resistance challenge results from two combined factors (Carlet et al., 2012). First, microorganisms are becoming extremely resistant to existing antibiotics, in particular Gram-negative rods (example, *Escherichia coli, Salmonella spp, Klebsiella* spp., *Acinetobacter* spp.), which are resistant to almost all currently available antibiotics in some settings. Second, the availability of new antibiotics has become extremely dry (Hughes, 2011). Several new powerful compounds active against Gram-positive cocci have been made available in the last few years, but this is not the case for Gram-negative bacteria and almost no new antibiotic class active against multi-resistant Gram-negative rods can be anticipated in the near future (Carlet et al., 2012). Therefore, this work summarizes the problem and the impact of antibiotic resistance in relation to antibiotics usage in farm animal husbandry with the consequences on consumer's health. The work also tried to look at some of the efforts which are being done to contain antibiotic resistance which include alternatives to antibiotic usage.

ANTIBIOTIC USE IN AGRICULTURAL SECTOR: FOOD-PRODUCING ANIMALS

Treatment, prophylaxis and growth promoters are the commonly uses of antibiotics in food-producing animals (Table 1) and is essential for a sustainable and economically sustainable animal industry (Acar and Röstel, 2001; Eagar, 2008). However, the application of these antibiotic drugs in animals, particularly in food animals, may lead to a selection of resistant strains of bacteria, which in turn may proceed to infect both animals and human (Mellon et al., 2001). Molecular analysis of antibiotic resistance genes and antibiotic-resistant mobile elements has shown that identical elements were found in bacteria that colonize both animals and humans, suggesting a role for raw foods in the spread of resistant bacteria and resistance genes to humans via the food chain (Teuber, 2001; Van et al., 2007). For example, the use of fluoroquinolones (example enrofloxacin) in food-producing animals has resulted in the development of ciprofloxacin-resistant *Salmonella, Campylobacter* and *E. coli*, which have caused human infections that proved difficult to treat (WHO, 2011).

Data on the volume of antibiotics used in livestock production are scarce in South Africa, and information is lacking about the patterns of antibiotic consumption in food animals (Henton et al., 2011). Moreover, considering the lack of information on the total quantity of antibiotics produced, it is not surprising that information on quantities used for specific purposes in agriculture and human medicine is also limited. Of all the available antibiotics used in livestock production in South Africa about 29% reported (Eagar, 2008) are in the form of premixes, and represents a large percentage of all the registered antimicrobials. Picard and Sinthumule (2002) together with Eagar (2008) reported that the most frequent uses of antibiotics by weight (as measured by sales) were those for treating and preventing diseases in poultry and pigs, and as growth promoters. In a survey Henton et al. (2011) found that tylosin, one of 4 growth promoters banned in Europe, was the most extensively sold antibiotic in South Africa followed by tetracyclines, sulphonamides and penicillins, respectively. Extensive usage of tylosin in food-producing animals was initially reported by Eagar (2008). In that study it was also found that the mean antibiotic sales for 3 years period (Table 2) from 8 companies were 1.5 million kilograms active ingredient. Where, in terms of total volumes of sales (kg), the macrolides, lincosamides and pleuromutilins represented 42.4% of the antibiotics sold. In the last four years there have been annual increases in unit sales from 25.3 to 29.8 million (Table 3) of broad-spectrum penicillins, fluoroquinolones, carbapenems and penems, carbacephems

Table 1. Some of commonly used antibiotics in food-producing animals.

Antibiotic	Treatment objective	Food animal	Reference
lincomycin	Feed efficiency, growth promoter and disease control	Swine, poultry	Regassa et al. (2008) Kuchta, (2008)
Tylosin*	Feed efficiency and growth promoter	Poultry, cattle	Henton et al. (2011) Jackson et al. (2004) Eagar, (2008)
Penicillin	Feed efficiency, growth promoter and disease control	Swine, poultry	Regassa et al. (2008) Doyle, (2006)
Virginiamycin*	Feed efficiency, growth promoter and disease control	Swine, poultry, cattle	Regassa et al. (2008) Donabedian et al. (2003) Cogliani et al. (2011)
Tetracyclines (chlrotetracyclineoxytetracyline, tetracycline)	Feed efficiency, growth promoter and disease control	Swine, poultry, cattle	Oguttu et al. (2012) Regassa et al. (2008)
'Erythromycin	Disease control	Swine, poultry, cattle, sheep	Jeong et al. (2006)

*Banned in European Union

Table 2. Volumes (kg) of antibiotics used during 2002-2004* as sourced from veterinary pharmaceutical companies.

Class of antibiotic	Volume (kg)			Total (kg) over 3 years
	2002	2003	2004	
Penicillins	49 465	55 677	59 688	165 717*
Cephalosporins	5 470	3 321	3 316	12 107
Tetracyclines	58 342	71 842	58 974	257 755*
Aminoglycosides	3	242	268	1 048*
Macrolides, lincosamides and pleuromutilins	204 325	221 275	223 412	651 690*
Quinolones	582	582	1 082	3 094*
Quinoxalines	30 043	26 468	30 448	86 959
Sulphonamides	35 041	72 277	75 098	190 676*
Polipeptides	27 011	26 985	42 191	69 820
Ionophores	14 736	5 582	43 674	69 820*
Glycolipids	370	425	432	3 936*
Total	**425 388**	**484 676**	**538 583**	**1 538 443***

(Eagar, 2008). * Two of the eight veterinary pharmaceutical companies that provided data were only able to access their data for the whole three year period and not for each year individually.

and glycopeptides in South Africa (Essack et al., 2011). Moreover, counterfeiting of pharmaceuticals is highly problematic in South Africa, with an estimated one in five medicines sold believed to be counterfeit (BMI, 2010). It is reported that the majority of counterfeit medicines have been imported from India and Pakistan and reach pharmacies through illegal means and the South African Medicines and Medical Devices Regulatory Authority (SAMMDRA) have not been successful in combating this problem (Essack et al., 2011). Therefore, sales data may

Table 3. Antibiotic utilisation in units, 2008 – 2011.

Antibiotic	Year		Sum of MAT units,		Count of antibiotics in each class*
	2008	2009	2010	2011	
J1A0 Tetracyclines + combs	327 379	325 061	327 557	327 701	44
J1B0 Chloramphenicols + combs	6 964	6 114	4 527	2 483	8
J1C1 Broad-spect. penicill. oral	10 683 704	11 441 888	11 962 722	12 305 433	277
J1C2 Broad-spect. penicill. inj.	551 335	1 251 442	1 133 503	1 463 327	45
J1D1 Cephalosporins oral	1 797 546	1 813 314	1 934 859	1 874 156	95
J1D2 Cephalosporins inj.	1 674 479	1 758 407	1 663 164	1 697 551	116
J1E0 Trimethoprim combs	3 261 544	4 021 542	3 300 302	3 316 420	124
J1F0 Macrolides + similar type	2 039 968	2 293 495	2 530 404	2 596 281	96
J1G1 Oral fluoroquinolones	3 242 849	3 617 302	3 635 646	3 832 065	95
J1G2 Inj. fluoroquinolones	479 409	554 631	565 952	584 255	21
J1H1 Plain med.-/narrow-spect. penicillins	419 243	386 095	485 923	435 640	42
J1K0 Aminoglycosides	80 624	87 089	83 880	80 349	41
J1P1 Monobactams	4 843	4 674	7 584	5 679	1
J1P2 Penems and carbapenems	679 147	809 668	916 184	1 019 767	8
J1P3 Carbacephems	7 652	15 512	23 191	69 908	3
J1X1 Glycopeptideantibact.	122 156	134 738	162 038	158 674	20
J1X9 All other antibacterials	15 132	14 361	15 849	16 229	10
Grand total	**25 393 974**	**28 535 333**	**28 753 285**	**29 785 918**	**1 046**

Essack et al. (2011), MAT = Moving Annual Turnover, *Number of drug formulation.

provide a misleading and inaccurate measure of the use of antibiotics because of counterfeiting. Hence, industry data on antimicrobial use in livestock production almost certainly underestimate usage and are far too general to help scientists explore the linkages between various types of farm use and the emergence and spread of resistance (Union of Concerned Scientists, 2001, (www document;http://www.ucsusa.org/assets/documents/food_and_agriculture/hog_chaps.pdf Accessed 04/Sep/2012).

A study by Eagar et al. (2012) found that the majority of consumed antibiotics in animals were from the macrolide and pleuromutilin classes, followed by the tetracycline, the sulphonamide and lastly the penicillin class. Their survey results showed that 68.5% of the antibiotics were administered as in-feed medications. About 17.5% of the total volume of antibiotics utilised were parenteral antibiotics, whereas antibiotics for water medication constituted 12% of the total and other dosage forms (topical and aural dosage) constituted 1.5% of the total. From the figures above, it is not surprising that many chicken farms widely use antibiotics as a prophylactic and a growth stimulant (Medeiros et al., 2011). However, this is particularly problematic because antibiotic for growth promoters are used without veterinary prescriptions or administered for long periods of time at sub-therapeutic concentrations to entire groups or herds of animals (Carlet et al., 2012). These farmers have come to believe that relatively low concentrations of antibiotics in feed and water can help avoid disease-driven losses in livestock with the belief that they increase profit margins despite the lack of well-understood mechanisms (Mellon et al., 2001). However, if the quality of industrial animal farming is improved there would be far less of the problem of disease control and prevention, and hence antibiotic resistance (Garcés, 2002). This is because in most cases overcrowded and unhygienic conditions of industrial animal farming result in the spread and emergence of microbes. Therefore, if conditions were improved, the prophylactic use of antibiotics would not be necessary.

ANTIBIOTIC USE IN FARM ANIMALS AND IMPACT ON HUMANS

Certain antimicrobial drugs have been reported to stay in the meat and/or milk of food animals for extended periods of time (Nisha, 2008; McDermid, 2012; Lozano and Trujillo, 2012). For example, chicken are being fed with antibiotics and in some cases hormones which can never be fully broken down and excreted from its body before it is slaughtered, this may result in very concentrated by-products and residues in chicken meat (Compassion in World Farming South Africa (CIWFSA) 2012, (www document,http://www.animal-voice.org/antibiotic-residue-is-in-our-chickens/ Accessed 14/Nov/2012). And it is said that South Africa does not have a regulated process of

antibiotic residue testing in meat (McDermid, 2012). Data released by the Compassion in World Farming South Africa in September 2009, showed that every single chicken purchased at supermarkets tested positive for tetracycline residue which is one of the most depended upon antibiotics in human health. Part of the Chicken sample displayed a residue of 55% over the legal limit in terms of South African Law (CIWFSA, 2012). Furthermore, the study found that 10 freshly dead carcasses from the Phillippi cull outlet showed 100% antibiotic resistant bacteria on the skin surface including *staphylococci* and *enterobacteriaciae* (CIWFSA, 2012). The antibiotics to which the bacteria were 100% resistant were penicillin and ampicillin, both of which are used for a broad spectrum of human illnesses (Schrag et al., 2002; CIWFSA, 2012).

Concerns about use of penicillins and other antibiotics is the withdrawal period (Eagar et al., 2012). The high content of antibiotic residues such as that of tetracycline (and penicillins, etc.) in food animal products is of great concern since it has been established that these compounds also remain chemically detectable even after cooking, as cooking only decrease its amounts (Javadi, 2011). A comparative study in Nigeria showed penicillin (14%) was the drug with the highest rate of occurrence in meat samples followed by tetracycline (8%) and streptomycin (4%). These antibiotic traces have harmful effects on consumer's health, such as allergic reactions, liver damage, yellowing of teeth and gastrointestinal disturbance (FAO/WHO, 2002; Jing et al., 2009). Another fact is that over time, through continued consumption of meat products containing these antibiotic residues (indirect consumption of antibiotic residues), people's body will start to develop resistance to that antibiotic molecule which can impact their lives (Andrew, 2003; Nisha, 2008; McDermid, 2012; Lozano and Trujillo 2012). Whereby, these residues produces potential threat with direct toxic in human, second the low levels of antibiotic exposure will result in alteration of microflora, causing disease and the possible development of resistant strains which cause failure of antibiotic treatments (Nisha, 2008).

On the processing point of view, Kjeldgaard et al. (2012) examined how acceptable levels of antibiotics in meat influence fermentation. Their results show that commonly used bacterial starter cultures were sensitive to residual antibiotics at or near statutorily tolerable levels, and as a result, processed sausages may contain high levels of pathogens. Furthermore, their findings provided a possible explanation for outbreaks and disease cases associated with consumption of fermented sausages and offered yet another argument for limiting the use of antimicrobials in farm animals. With no doubt, there is a need to investigate the role of the availability of antibiotics over the counter for use in animals in South Africa in the development of resistance among humans (Oguttu et al., 2012).

ROLE OF FOOD IN DISEASE TRANSMISSION: RESISTANCE AND DISEASE

There is limited information of disease caused by antibiotic resistant bacteria in South Africa, due to the fact that causes of illnesses and deaths are not well counted, as is often the case in low-resource countries (Crowther-Gibson et al., 2011). According to a report from the International Federation for Animal Health (IFAH, 2011), it is estimated that of the 1,500 diseases that affect people, almost two-thirds can pass between animals and humans. The transfer of *Staphylococcus aureus* isolates between humans and animals, especially in the case of livestock-associated Methicillin-resistant *S. aureus* (MRSA) ST398, has recently gained particular attention (Smith and Pearson, 2011). The ST398, which is the swine-associated MRSA, and ST398 human infections, has been recognized in several countries (NIAA, 2011). It is suggested that livestock associated MRSA originally were methicillin-susceptible commensal strains in pigs, whose spread was facilitated by the abundant use of antibiotics in pig and cattle farming (Voss et al., 2005). *S. aureus* is a major human pathogen, a relevant pathogen in veterinary medicine, and a major cause of food poisoning (Sobral et al., 2012). A joint ECDC/EFSA/EMA (2009) scientific report demonstrated that pigs are a major reservoir of a new emerging type of MRSA and concluded that the extensive use of antibiotics for prevention of disease appears to be an important risk factor for the spread of MRSA. With South Africa having a high burden of infectious diseases, including a large portion that are of bacterial origin (Crowther-Gibson et al., 2011), these resistant microorganisms pose a serious health concern in the country where there is a high rate of HIV/AIDS and TB infection. Bacterial infections are quite frequent in HIV-infected patients (Carrega et al., 1997). This is because HIV-induced immunosuppression amplifies the risk of bacterial infections, TB and non-tuberculosis, often involving antibiotic-resistant strains, with severe and/or recurrent potential (Stoian, 2013).For example, infections such as respiratory failure in HIV infected patients are bacterial *pneumonias* which have been reported to be caused by *Pseudomonas*, *Staphylococcus aureus* and other bacteria (Bajwa and Kulshrestha, 2013).In 2009 an estimated 29% (over 5.5 million) of people were infected with the HIV virus (Crowther-Gibson et al., 2011). Moreover, some evidence indicate that antibiotic resistance rate to nosocomial pathogens are generally high in South Africa (Nyasulu et al., 2012).

Poultry meat has been reported as an important vehicle in foodborne diseases and some studies have suggested that chicken can be a source of antibiotic resistant *Salmonella* spp. in humans (Medeiros et al., 2011). In their study Medeiros et al. (2011) found that the prevalence of *Salmonella* spp. was relatively low. However,

there were a high proportion of multidrug-resistant strains, including third generation cephalosporins used to treat invasive salmonellosis. Test results from randomly selected spent hens; sold live to residents in Khayelitsha, a community near Cape Town revealed that the hens were contaminated by a range of disease-causing bacteria (Garcés, 2002). The concern is that the study showed that the bacteria in both the hens and study community were 100% resistant to most common (oxacillin, vancomycin and methicillin) antibiotics. Therefore, this entails that certain antibiotics would be ineffective in the treatment of people who get infected by eating such hens. Moreover, resistance shown to vancomycin is particularly worrying. As it is a front-line antibiotic used to treat all sorts of infections in humans including chest and heart muscle infections (Nierenberg and Garcés, 2005).

Most of the concern about human health consequences of antibiotics use has focused on growth promotion (which boosts the utilisation of the genetic potential for growth of pigs and poultry, improve feed conversion and reduce waste product output from intensive livestock production) rather than disease prevention (WHO, 1997). The rationale is that the benefits of growth promotion are purely economic and often compensate for and encourage unsanitary conditions (Mellon et al., 2001).

CASES OF ANTIMICROBIAL RESISTANCE IN SOUTH AFRICA

Only a few relatively recent surveys and reports on antibiotic resistance in isolates from food animals in South Africa have been conducted and they are very few and bunched in Gauteng province (Gelband and Duse, 2011). A number of clinical and environmental data suggest that the rate of antimicrobial resistance is high in South Africa. A current systematic review of published literature (Nyasulu et al., 2012) of antimicrobial resistance surveillance among nosocomial pathogens revealed resistance to commonly used antimicrobial drugs in population: for *S. aureus*, resistance to cloxacillin was 29% and to erythromycin 38%; for *Klebsiella pneumoniae,* resistance to ciprofloxacillin was 35% and to ampicillin 99%; and for *Pseudomonas aeruginosa,* the mean resistance to ciprofloxacillin was 43% and to amikacin 35%. Ateba and his co-authors have also reported antibiotic resistance in dairy and poultry products (Ateba et al., 2010). It is reported that penicillin resistance in South Africa remains mainly intermediate in level, with a low prevalence of fully resistant isolates (Crowther-Gibson et al., 2011).

CURRENT EFFORTS TO CONTAIN/REVERSE ANTIBIOTIC RESISTANCE

South Africa is part of the four countries (including India, Vietnam and Kenya) forming the Global Antibiotic Resistance Partnership (GARP). GARP-South Africa was launched on 8 February 2010 at a meeting attended by 40 experts (Suleman and Meyer, 2012). It aims to address the antimicrobial resistance through the situational analysis of antimicrobial resistance in South Africa and collaborating countries. The situational analysis was published as a special supplement to the South African Medical Journal, (SAMJ, 2011). Thereby, the data obtained was said to be used to inform and develop policy and advocacy for antimicrobial resistance-related issues in each of the collaborating countries (Gelband and Duse, 2011). Therefore, GARP is to recognise those issues and recommend policy alternatives that are right for the time and place. Despite poor health status, South Africa has had the most active surveillance for antibiotic resistance of any African country (Gelband and Duse, 2011). However, it has not yet fully translated available antimicrobial resistance surveillance data into policy (Suleman and Meyer, 2012). Hence, there is no evidence of any on-going antimicrobial resistance surveillance for pathogens in South Africa (Nyasulu et al., 2012).

Crude plant extracts have been a promising alternative and potential resistance modifying agents in fight against antibiotic resistance (Sibanda and Okoh, 2007; Aiyegoro et al., 2009; Savoia 2012). Aiyegoro et al. (2009, 2011) proposed that extracts of the leaves of *Helichrysumpedunculatum* and *Afzeliaafricana* stem barkcould be of relevance in combination therapy and as a source of resistance modifying principles that could be useful as treatment for microbial infections. Therefore, these breakthroughs are the promising signs that in the next years some different molecules discovered by ingenious screening programs and obtained from different plant oils and extracts will become useful therapeutic tools (Savoia, 2012).

RECOMMENDATIONS

More work on a national surveillance programme of antibiotics usage on the food-producing animals and the surveillance programme on antibiotic resistance in bacteria must be established in South Africa. These programmes should collect a well arranged data on usage, such as the usage per animal species (drugs type, daily doses) or usage on farm level. It should also include the testing (quantitative susceptibility tests and molecular analysis of resistant genes) of a wide range of bacteria from animals and food products. This information will help to mitigate the problem of lack of availability of information on the amount of antibiotics which are currently being used in livestock production in South Africa. Moreover, a consumer risk perception study on the use of antibiotic in livestock should be conducted, as it is an important factor because it reflects the subjective assessment that people make on the use of antibiotics in food-producing animals as it affect the food they consumes.

Conclusion

Data from studies indicate that South Africa is using large amount of antibiotic in food-producing animals, this includes a number of antibiotics that have been banned for use in other countries. It is evident that there is a real growing problem of antibiotic resistance in South Africa and with high burden of infectious diseases, including a large portion of bacterial origin, as well as HIV/AIDS epidemic and tuberculosis this put people's life at high risk. Therefore, more effort is needed if South Africa is determined to overcome this global problem of antibiotic resistance among pathogens.

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