ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF BACTERIA FROM THE RESPIRATORY TRACT OF CAMELS IN SAMBURU, NAKURU AND ISIOLO COUNTIES, KENYA

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DECLARATION

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This thesis is dedicated to my grandfather Joseph Mutua Ndeti whom I consider a pillar in my education my sisters Faith Ngina, Esther and Dorothy and my brother Joel Mutua.

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List of abbreviations

E. coli - *Escherichia coli*

MDR - Multidrug resistance

FAO - Food and Agriculture Organization

KNBS - Kenya National Bureau of Statistics

GARP - Global Antibiotic Resistance Partnership

ASALs - Arid and Semi-arid areas.

CoNS - Coagulase negative *Staphylococcus*

CoPS - Coagulase positive *Staphylococcus*

PCR - Polymerase chain reaction

ELISA - Enzyme-linked immunosorbent assay

DNA - Deoxyribonucleic acid

H_2O_2 -Hydrogen peroxide

H_2 -Hydrogen gas

TSI -Triple Sugar Iron

CO_2 -Carbon (IV) oxide

ABSTRACT

Camels contribute significantly to livelihoods of local people in the semi-arid and arid areas of Kenya. One main challenge affecting camel production is disease, particularly the pulmonary diseases caused by viruses, bacteria and fungi. To date, very little work has been done on the respiratory system flora of apparently healthy camels. The objective of this study was to identify bacterial species found in the upper respiratory system and lungs of apparently healthy camels; the isolates from nasal cavity were tested for susceptibility to selected antimicrobials. A total of 255 nasal swabs were collected from apparently healthy camels in Isiolo, Samburu and Nakuru counties, Kenya, from which four hundred and four (404) bacterial isolates were identified, following the identification criteria given by Shears *et al*., (1993) and Quinn *et al*., (1994). They included: *Bacillus* 160/404 (39.60%), coagulase negative *Staphylococcus* 121/404(29.95%), *Streptococcus* species other than *Streptococcus agalactiae* 104/404 (25.74%), coagulase positive *Staphylococcus* 16/404 (3.96%) and *Streptococcus agalactiae* 3/404 (0.74%). Additionally, coagulase negative *Staphylococcus* (37.04%), coagulase positive *Staphylococcus* (37.04%), *Streptococcus* species (14.81%) and *E. coli* (11.11%), were also isolated from camel lungs obtained from Athi river camel slaughterhouse, some of which were showing pathological lesions. When the nasal isolates were tested for antimicrobial susceptibility, they were found to be most susceptible to Gentamycin (95.8%), followed by Tetracycline (90.5%), Kanamycin and Chloramphenicol (each at 85.3%), Sulphamethoxazole (84.2%), Co-Trimoxazole (82.1%), Ampicillin (78.9%) and finally Streptomycin (76.8%). Antimicrobial resistance was reported in

ascending order in Gentamycin (4.21%), followed by Tetracycline (9.47%), Kanamycin and Chloramphenicol (14.74%), Sulphamethoxazole (15.79%), Co-Trimoxazole (17.89%), Ampicillin (21.05%) and finally Streptomycin (23.16%).

Multidrug resistance was reported in 30.5% of all isolates subjected to the test antimicrobials. Most of the resistant organisms showed resistance to a combination of two antimicrobials which was 20% of the total number resistant.

This study indicated presence of similar bacteria in both nasal cavity and lungs, thus strongly suggesting the involvement of the otherwise harmless nasal commensals in pulmonary disease causation in camels. These nasal bacteria may find their way to the lungs in cases when the animals are stressed, as a result of the harsh conditions that the animals live in and also in the way they are used for transport and are burdened by humans. The levels of antimicrobial resistance to the antimicrobials used, as demonstrated in this study, indicate that the antimicrobial resistant normal flora (bacteria) harbor resistance genes which are transferable to pathogenic bacteria in the animal, not to mention transfer of resistant bacteria to other animals and humans; compounding the antimicrobial resistance situation. This study identified Gentamycin, Tetracycline, Kanamycin and Chloramphenicol as the most effective antimicrobials that can currently be used for treating respiratory or other infections in camels.

CHAPTER ONE: INTRODUCTION

1.1 Background information

The world camel population is estimated to be 27 million (FAO, 2011) with significant numbers found in the sub-Saharan countries of Ethiopia, Kenya, Djibouti, Eritrea, Ethiopia and Somalia, some regions in Asia and in the Arabian Peninsula (Mirzae, 2012, Tarek *et al.,* 2012).

The camel is a source of livelihood in the communities living in the arid and semi-arid areas of Kenya (Noor *et al*., 2013). Kenya hosts over 6% of all camels found in Africa with a significant increase in the animals reported from 0.8 million heads in the year 1999 to 3 million heads in the year 2009 (Kangunyu and Wanjohi, 2014). This has partially been attributed to some pastoralists engaging in camel keeping to supplement cattle production especially in the dry season (Farm Africa, 2002).

Camels in the arid and semi-arid areas have become a fortress to the pastoral communities, as they are able to produce and survive well despite harsh environmental conditions (Kangunyu and Wanjohi, 2014). In these areas the animals have varied diets which include shrubs and trees (Guliye, 2010). Field, (2005) made an estimate that camels produce six times more milk than the local cattle.

Despite the benefits associated with the animals, policymakers, government and scientists have concentrated their efforts on cattle, goats and sheep, leaving the camel a neglected animal. However, due to the climatic changes pastoralists in the other parts of the country, other than the traditional areas, have shifted their efforts to camel keeping with the aim of subsidizing their farming and ensure food security (Kangunyu and Wanjohi, 2014).

Camels, unlike other livestock, are thought to be less susceptible to many diseases (Dirie and Abdurahman, 2003); perhaps owing to the limited data on major camel health problems in Kenya. Pulmonary diseases are considered to be one of the emerging diseases causing considerable loss of camel productivity and death (Zubair *et al.,* 2004; Abubakar *et al,*.2010). Viruses, bacteria, and fungi have been incriminated as the main causative agents of these respiratory infections in mammals (Warnery and Kadeen, 2002).

Among the common bacteria, *Staphylococcus aureus* has commonly been associated with pneumonia in humans and animals (Alhendi, 1999; Ragle *et al.,* 2010; Rahimi and Alian, 2013). The bacterium is also associated with other disease conditions including mastitis, osteomyelitis, toxic shock syndrome, endocarditis and nosocomial infections in animals and humans (Sousa *et al*., 2005)

Isolation of bacteria from the respiratory system of both diseased and apparently healthy camels has been documented. In India, Arora and Kalra, (1973) reported isolation of *Klebsiella pneumoniae* and *diplococci* from pneumonic lungs. Chauahan *et al,* (1987) also reported eight genera of bacteria from nasal swabs taken from apparently healthy camels. In yet another study, Shigidi (1973) in Somalia, reported isolation of six genera of bacteria in the upper respiratory tract. Al-Doughaym *et al,* (1999) reported 9 genera of bacteria from the respiratory tract of diseased and apparently healthy camels in Saudi Arabia. Azizollah *et al,* (2009) identified different genera of bacteria in an abattoir in Iran from respiratory tract of apparently healthy camels. Other countries where the respiratory bacteria of camels have been studied include: Nigeria (Abubakar *et al.,* 2010), Ethiopia (Awol *et al.,* 2011), Somalia (Mogadishu) (Abdulrahaman, 1987), among others.

Previous research has focused on the respiratory tract of other animals (Azizollah, *et al*., 2009) but in the *Camelidae,* studies are commonly conducted in the lungs. The existence of bacteria in the nasal cavities of apparently healthy camels in Kenya has not previously been documented. The sensitivity of these to commonly used antimicrobials is also not known. This study intended to fill-in these gaps, especially in view of the fact that antimicrobial resistant normal flora can easily transfer antimicrobial resistance to otherwise susceptible pathogens. Antimicrobial resistance poses serious problems in the treatment of camel respiratory infections, in addition to risks of transfer of resistance genes to human pathogens.

1.2 Objectives

1.2.1 General objective

To identify and determine antimicrobial susceptibility profiles of bacteria from the respiratory system of camels in Samburu, Isiolo and Nakuru counties, Kenya

1.2.2 Specific objectives

1. To identify bacteria from nasal cavities of camels in Samburu, Isiolo and Nakuru counties
2. To identify bacteria from lungs of camels from Athi River camel slaughterhouse
3. To determine phenotypic antimicrobial resistance profiles of the nasal isolates

1.3 Justification of study

Although antimicrobial therapy targets the pathogenic microorganisms in a population, a simultaneous selection pressure is also exerted on the commensal bacteria leading to maintenance of antimicrobial resistance in these bacteria (Barbosa and Levy, 2000).

Having antimicrobial resistance in normal flora bacteria should be viewed seriously since they can transfer the resistance to pathogenic bacteria, making them difficult to treat, which will in turn lead to loss of camel productivity and increased treatment costs. There is also a possibility that the resistant nasal normal flora can find their way to the lungs and cause pneumonia which will be difficult to treat, not to mention the fact that some of them are zoonotic and can in turn transfer the resistance to other human pathogens. This study was important since it was geared towards identification of bacteria that are normally found in the Kenyan camel's nasal cavities and lungs. It was also, by design, geared towards establishing the antimicrobial susceptibility profiles of the nasal isolates.

CHAPTER TWO: LITERATURE REVIEW

2.1 Global distribution of camels

Estimation of world's camel population is constrained by two factors; one: due to the fact that camel keepers are in constant movement and two: absence of routine vaccination(s) programs for these animals (Faye, 2015). It is however estimated that the world camel population is around 20- 25 million heads (Bornstein and Younan, 2013).

Environmental, social and cultural factors are major contributors to the distribution of camels in the world. The arid and semi-arid areas of Africa and Asia are known to be the convenient habitats of these animals (Biffa and Chaka, 2002). More than 80% of these animals live in Africa with an estimated 60% in Ethiopia, Kenya, Djibouti, Eritrea, and Somalia (Mirzae, 2012, Tarek *et al.*, 2012).

2.2 Significance of camels

Camels are considered the most important animals in the pastoral communities that ensure food security; to them camel is a source of food in terms of milk and meat production, income in form of cash, transport and for cultural purposes (Guliye *et al.*, 2007).

Camels produce higher amounts of milk compared to other livestock in the same environmental conditions (Ramet, 2001). In dry lands under the normal average management conditions, a lactating camel can produce 1900 liters of milk per lactation. Camel milk has the same nutritive value as that of other livestock. In addition, camel milk is high in vitamin C, an important attribute considering that fresh fruits and vegetables are scarce in the arid and semi-arid areas (Farah, 1993; Ramet, 2001; Farah, 2004).

Compared with other types of red meat in terms of carcass quality and mineral content, camel meat has higher moisture and mineral content and low fat levels which makes it a healthier diet (Herrmann and Fischer, 2004).

In addition to their importance in food production, camels are commonly known as beasts of burden (Hussein, 1993). In Kenya, camels are an important source of transportation of surplus milk to the market and water for other animals; they are also utilized for human consumption. Camels are also used to transport the elderly, sick and young people particularly when the pastoral communities are shifting from one area to another. In addition, camels have a social and cultural importance in payment of dowry and compensation for injured parties in tribal clashes (Hussein, 1993). Besides providing their owners with source of food; camels are also regarded as a banking system and security against drought, disease and other natural calamities that affect other livestock

2.3 Respiratory tract infections in camels

Although camels are thought to be less susceptible to diseases, pulmonary diseases are considered to be one of the emerging diseases causing considerable loss of camel productivity and death (Zubair *et al.,* 2005; Abubakar *et al,*. 2010). Also, although a definitive etiology of camel respiratory problem has not fully been determined, bacteria, viruses, fungi and some parasites have been incriminated as main causes of these problems (Schwartz and Dioli; 1992 Warnery and Kadeen, 2002).

Respiratory tract infection in camels occurs commonly in two forms: acute and chronic. The acute form is the most serious and is characterized by nasal discharge, fever, sneezing and reduced feed intake. The chronic form is characterized by coughing, loss of weight, dullness,

lacrimation and prolonged recumbency. The predisposing factors of respiratory disease in camels are: change of climate, poor nutrition, dust storms, migration and, generally, stress of any kind to the animals (Hansen, 1989; Bekele, 2010).

Although the definitive cause of respiratory infection in camels remains unclear, *Pasteurella* species (Bekele, 1999), *Streptococcus* species (Yigezu *et al.,* 1997), *Mannhemia hemolytica* (Al-Taraz, 2001; Abubakar *et al*., 2008) have been isolated in respiratory diseases of camels; antibodies to *Morbili* virus (*Roger et al*., 2001) have also been detected.

Other bacteria that have also been isolated from cases of camel pneumonia include; *Staphylococcus* species, *Bordetella* species, *Rhodocuccus equi*, *Klebsiella pneumonae*, *Escherichia coli* and also *Neisseria* species (Chauhan *et al.,* 1987; Al-Doughaym *et al*., 1999; Abubakar *et al,* 2008). *Corynebacterium pseudotuberculosis* and *Arcanobacterium pyogenes* have been associated with lung abscesses in both young and adult camels (Abubakar *et al*., 1999).

Adenoviruses, respiratory syncytial virus, *Morbili* virus and *Parainflunza* 3 are some of the viruses associated with respiratory problems in camels (Roger *et al.,* 2003). High antibody titers of Middle East Respiratory Syndrome coronavirus have also been detected in camels (Wareth *et al*., 2014).

2.4 Camel production and its challenges in Kenya

Population of the Kenyan camels (*Camelus dromedaries*) is estimated to be 2.97 million heads (KNBS, 2010). Camels are an important source of livelihood to communities living in the arid and semi-arid lands (ASALs) of Kenya (Noor *et al*., 2013) which cover over 83% of the Kenyan land mass and support about 30% of Kenyan population.

Among all the animals kept in the Northern Kenya the camel is most suited to the harsh conditions in these areas. Camels play an important role in support of the livelihood and culture of the nomadic communities inhabiting the northern parts of Kenya for provision of milk, meat, as a means of transport and source of income from sale of live camels or their products (Noor *et al*., 2013).

In arid areas of Northern Kenya, camels have traditionally been kept under pastoral production systems which are characterized by low production inputs and herd/household mobility which is necessitated by search of pastures, water, mineral licks and community feuds (Noor *et al*., 2013). This enables the pastoral communities utilize rangeland resources more efficiently (Farah *et al*., 2004; Guliye *et al*., 2007). Recently there has been an emerging trend of camel rearing in the peri-urban areas like Isiolo. This has been attributed to availability of markets to the camel products by consumers living in these areas (Noor *et al*., 2013).

Despite all benefits associated with camel rearing in Kenya, camels face challenges in their natural habitat, these include: diseases (Njiru *et al*., 2001), drought (Kaufaman and Binder, 2001) and predation (Onono *et al*., 2010).

Diseases, inadequate veterinary services and little attention by the government agencies are the major factors that hinder the development of camel husbandry. Notably pneumonia is one of the most devastating diseases observed in camel rearing areas (Köhler-Rollefson *et al*., 2001); others include; trypanosomiasis, parasitic and viral infections, camel pox, and contagious skin necrosis (Damake, 1998). Rearing of camels mostly occurs in the arid and semi-arid areas of the country characterized by migration, harsh environmental conditions and poor infrastructure which makes

the animals and the communities inaccessible to research. This limits people's knowledge to the general aspects of these animals (Schwartz and Dioli, 1992).

Promotion of camel production and health is also very poor; this being exacerbated by negligence by the development planners and researchers (Bekele, 2010). Vaccination programs, treatments and control of diseases in cattle and other livestock exist but there are almost none for camels. Treatments are usually done by the camel owners who have little or no knowledge about the diseases they are treating or the drugs they are using, which can also contribute to spread of antibiotic resistance and also pose risks to humans.

Bekele (2010) demonstrated that insufficient labor in terms of feeding and watering camels is a major concern affecting camel herd size and population growth. This is partly due to human mobility, alternative jobs, and increased school enrolments of the pastoral communities' children.

2.5 Camel nasal flora

Bacterial flora in a mammalian host can broadly be classified as resident or transitory (Azizollah *et al.,* 2009). Resident bacteria are constant for a given area in the body at a specific age of the host. Transitory microorganisms remain only for a short period within a host. As each organ in the animal body is unique, creating differing microbial environments, some microorganisms will be favored more than others (Mcfarland, 2000; Soruma and Sunda, 2001).

Previous research has focused on samples collected from diseased domestic and wild animals particularly those with clinical signs of pneumonia, including nasal discharge and cough (Welsh *et al.,* 2004; Katsuda *et al.,* 2006). However various nasal flora have been reported in different apparently healthy animals: in dogs (Smith, 1961), in camels (Chauhan *et al*., 1987), in cattle

(Alhendi, 1989), in poultry (Calneck *et al*., 1995) and in some zoo animals (Saddek *et al*., 1994). Other reports on bacterial isolation from apparently healthy animals have been documented: caprine respiratory tract (Mergra *et al*., 2006), canine upper respiratory system (Ajuwape *et al*., 2006) and sea lion nasal tract (Hernandez-castro *et al*., 2005). Several bacterial species have been isolated, for example: Azizollah *et al*., (2009) isolated *Staphylocccus, Neisseria, Bacillus, Streptococcus and Escherichia coli.* Isolation of *Lancefield group B Streptococcus* from the nasopharynx of apparently healthy camels was also reported by Younan and Bornstein, (2007). Al-Doughaym *et al.*, (1999) isolated *Staphylococcus aureus*, *Corynebacterium pyogenes*, coagulative-negative *Staphylococcus, Bacillus* species, *Streptococcus pyogenes, Diptheroids, E.coli, Klebsiella pneumoninae, Diplococcus pneumonae* and mixed isolates from nasal swabs collected from camels in Sudan. Bacterial flora colonizing the respiratory tract of apparently healthy camels are rare (Mohamed, *et al.*, 2014). Although under normal circumstances, concentration of the resident bacteria is maintained at a particular level, in cases of stress of any kind, the mucocilliary and clearance mechanism of the respiratory system is suppressed, allowing for multiplication of the commensal bacteria; this may then result in an abrupt shift from normal flora to pathogenic bacteria (Brogden *et al*., 1998).

The upper respiratory tract of the camel is adapted to harsh desert conditions (Abdulsalam, 1999; Alhendi, 1999); for example, the nostrils are straight and narrow towards the nasal cavity (Tayeb, 1964). Thus, most of the normal flora in apparently-healthy camels only cause disease when the animal is under poor sanitation, stress due to transportation, sudden change in feed, low herd health status and/or immunosupression (Wareth *et al.*, 2014). However, some of these bacteria are zoonotic and can cause illness in humans (Schlegel *et al*., 2000; Sousa *et al*., 2005)

2.6 Bacterial isolation and identification

Microbial identification is based on microscopy of the specimen and culture of the sample on a solid media (Weiser *et al*., 2012). The conventional methods or culture based methods of detecting bacteria from samples are based on culturing and isolation of colonies for further analysis (Khan *et al.,* 2013). These methods are based on the ability to grow organisms in the laboratory. Some of these methods include: culture and colony counting, Gram staining and morphological characterization among others. Generally, samples are plated on solid enrichment media or differential media to isolate specific organisms. To isolate general microbial population in a sample, the sample can be plated on a general purpose solid medium. These methods are widely used and are advantageous in that they are cheap, they only detect viable bacteria and the isolates can be further studied (Engberg *et al*., 2000; Adzitey and Nurul, 2011). The limitations of these conventional methods are that microorganisms are capable of altering their phenotypic characteristics due to environmental changes or genetic mutations (Hakorvita, 2008). Furthermore these methods are often laborious and time consuming, requiring a minimum of 5 days to isolate and identify an organism (Khan *et al*., 2011).

Molecular methods such as immunological and nucleic acid-based techniques have improved the identification of microorganisms. These methods are more sensitive and quicker than the traditional methods and can also add value at a relatively low cost, for example, in identification of individual strains of organisms (Cai *et al*., 2013). They can also be performed and results interpreted by people with no taxonomical expertise (Weile and Knabbe, 2009). The development of immunoassays has improved detection of organisms since they enable rapid identification and characterization without culturing steps (Weile and Knabbe, 2009). The immunological procedures depend on binding of diagnostic antibodies and antigen determinants

of a specific organism. A good example of these techniques include the enzyme-linked immunosorbent assay (ELISA) which has advantage in that it is able to have high throughput, relative low cost and has the ability to quantify the target pathogen. However these methods lack sensitivity and specificity (Weile and Knabbe, 2009).

Nucleic acid based techniques include; polymerase chain reaction (Wang *et al*., 2000; Cai *et al* 2013), which involves amplification of a target nucleic acid sequence which can be a particular gene, repetitive sequence or arbitrary sequence.DNA sequencing (Newell *et al*., 2000; Cai *et al*., 2013), pulsed field gel electrophoresis (Arbeit, 1999; Trindade *et al*., 2003), multi-locus sequence typing (Urwin and Maiden 2003), random amplified polymorphism deoxyribonucleic acid (Wassenaar and Newell, 2000), plasmid profile analysis (Trindade *et al*., 2003; Frederick *et al*., 2013) and fluorescent *in situ* hybridization technique (Bottari *et al*., 2006).

Other methods include restriction fragment length polymorphism (Kabodjora *et al*., 2002; Babalola, 2003), amplified fragment length polymorphism (Shi *et al*., 2010), Ribotyping (Wassenaar and Newell, 2000; Shi *et al*., 2010), among others.

2.7 Antimicrobials and antimicrobial resistance

2.7.1 General information

Antimicrobials are naturally occurring (antibiotics), synthetic or semi synthetic substances that can be taken orally, parenterally or topically to inhibit microorganisms. They are used to treat human and animal diseases. They can also be used for other purposes like growth promotion in animals (Philips *et al.,* 2004). "Antimicrobial resistance" refers to tolerance of an organism to a compound which the organism was previously susceptible to (Davies and Davies, 2010). The spread of antimicrobial resistance genes can occur between and within bacteria in animal and human populations. It can also occur through zoonotic bacteria along the food chain (Buller *et al*., 2014). The increased antimicrobial resistance within organisms has been viewed as combination of three factors which include; microbial characteristics, antibiotic's selective pressure and social and technical changes that promote spread of resistant microorganisms (Dzidic *et al*., 2008). Research has attributed antimicrobial resistance in bacteria to application of human and veterinary medicine (Feinman, 1998; Barbosa and Levi 2000; Blackman, 2002), agriculture and aquaculture (Angulo *et al*., 2004). The use of antimicrobials as growth promoters and treatment in animals has also greatly contributed to the development of antimicrobial resistance. Evidence has demonstrated the probability of antimicrobial resistant bacteria from poultry, cattle and pigs finding their way into the food chain and ending-up in humans (Perreten *et al*., 1997, Teuber *et al*., 1997; Wegener, 2003) where they colonize the gut and transfer the resistance to the human flora. The use of sub therapeutic doses and poor quality antimicrobials in treatment of diseases has also greatly contributed to the development of antimicrobial resistance.

To survive in the presence of an antimicrobial, bacterial organisms must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial agent. These

disruption mechanisms come about in various ways but the end result is partial or complete loss of susceptibility to the antibiotic(s). The mechanisms involved include microorganism mediated resistance or molecular mediated resistance. Microorganism mediated resistance involves, prevention of the antibiotics from reaching its target by reducing its ability to penetrate into the cell, expulsion of the antimicrobial agents from the cell via general or specific efflux pump, inactivation of antimicrobial agents via modification or degradation and modification of the antimicrobial target within the bacterium (Forbes *et al*., 2002).

Molecular mechanisms of antimicrobial resistance can be intrinsic or acquired. Intrinsic resistance is an inherent bacterial resistance to an antibiotic which is as a result of adaptive processes that are not necessarily associated with a particular class of antibiotics (Bockstael and Aerschot, 2009). It is naturally coded and expressed by all members of a particular species of bacteria. An example of an organism that exhibits this kind of resistance is *Pseudomonas aeruginosa*, which, due to its low permeability, is resistant to many antimicrobials (Yoneyama and Katsumata, 2006). Acquired resistance, on the other hand, occurs as a result of selective pressure that makes an organism develop mechanisms to counter the effects of an antimicrobial the organism was previously susceptible to (Wright, 2005).

Acquired resistance can occur through mutation of genes, acquisition of foreign genes or a combination of the two mechanisms (Dzidic *et al*., 2008). Acquisition of these foreign genetic materials occurs through horizontal gene transfer. Horizontal gene transfer can occur through three mechanisms namely transformation, transduction and conjugation. Transformation involves uptake of ‘naked’ DNA from the environment by a susceptible bacterium and the DNA is incorporated into the recipient’s DNA by recombination. Conjugation refers to transfer of genetic material from one cell to another via sex pili where chromosome or plasmid borne

resistance genes are transferred to the recipient bacterium. Transduction involves transfer of genetic material by a bacteriophage (Forbes *et al*., 2002). The transferred genes can be further incorporated into the recipient organism by recombination. The transferred sequences may contain either single mutation or more serious sequence alteration (Dzidic *et al*., 2008). In most bacteria, resistance to tetracycline, has been attributed to mobile genes (elements) which are either mediated by plasmids and/or transposons. These elements confer the antibiotic resistance by conjugation.

2.7.2 Antimicrobial resistance situation in the world

Since antimicrobials and other related medicines were discovered in the early 20th century, they have greatly reduced the risks and threats posed by infectious diseases especially in the developing countries of the world. However, these benefits are quickly being brought into a halt by the emergence of organisms that are resistant to commonly used antimicrobials (GARP, 2011). Recent reports by the World Economic Forum Global Risks have singled out antimicrobial resistance as one of the greatest risk to human health (Walker and Fowler, 2011). It has been estimated that in Europe, almost 25,000 people die annually due to the menace caused by multidrug-resistant bacteria; this costs the European Union economy €1.5 billion annually (Walker and Fowler, 2011). In the United States of America, a figure of over 2 million of its citizens are thought to be infected with antimicrobial -resistant bacteria annually, with almost 23,000 deaths occurring as a result of these bacteria (World Health Organization, 2014). Sadly, in addition to the resistance being observed towards the existing antimicrobials, there are no new agents being developed.

Kenya is experiencing high levels of antimicrobial resistance with high resistance rates being reported in microorganisms causing respiratory, enteric and hospital-acquired infections, for

example penicillin-resistant *Streptococcus pneumoniae*, vancomycin resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus*, resistant *E. coli* and non-Typhi *Salmonella*; (GARP, 2011). Overuse and misuse of antimicrobials, underuse, inappropriate dosing, poor quality antimicrobials and lack of restriction to selling and distribution of antimicrobials (where sometimes antimicrobials are sold at bus stops by hawkers) have greatly contributed to the development of antimicrobial resistance (Kakai and Wamola, 2002). Difficulty in fixing this menace in the country has been attributed to lack of adequate regulatory authority and inadequate regulatory resources to enforce key policies that have been put in place (GARP, 2011).

In Kenya, Tetracycline is the most commonly used antimicrobial, partly due to its availability and affordability. Half of all the antimicrobials used in the country are Tetracycline followed by Sulphonamides and the rest is shared among Beta-lactams, Macrolides and Quinolones (Mitema *et al*., 2001). About a fifth of antimicrobial consumption is associated with chicken with the rest of consumption being shared between cattle, goats, sheep and pigs. This presents an avenue for the spread of antimicrobial residues to humans as preference of white meat has grown tremendously, leading to spread of antimicrobial resistance. In Kenya, studies have indicated presence of bacteria that are resistance to Tetracycline and Sulphonamides in chicken and swine, Bacteria which are resistant to Ampicillin, Streptomycin, Tetracycline and Chloramphenicol have been reported in pork tissue (GARP, 2011). A study conducted in intensive rearing areas of chicken in Thika showed that significant percentage of isolated *E.coli* were resistant to Tetracycline. However the isolates were also highly sensitive to Neomycin and Apramycin (Kariuki *et al*., 1997). In yet another study, the resistance of *Staphylococcus aureus* between large and small farms was compared. A high proportion of resistance to commonly used antibiotics was reported in bacteria isolated from small farms compared to those isolated from

large farms; report on multi-drug resistant (MDR) strains was also higher in bacteria isolated from small farms as compared to those isolated from large farms. In small farms, isolates were also more likely to demonstrate resistance to Tetracycline and Penicillin than in large farms (Shitandi and Sternesjo, 2004).

2.7.3 Multi drug resistance

The phenomenon of multi-drug resistance in bacteria has been reported to be a great challenge in dealing and management of infectious diseases in the world as there are fewer or no drugs that can cure these kinds of bacteria. Multidrug resistance can be generated through two mechanisms: accumulation of multiple genes with each gene encoding for resistance to one antibiotic within one organism and increase in expression of genes encoding for multidrug efflux pumps which pump a wide variety of drugs (Nikaido, 2009). The phenomenon has been associated with horizontal gene transfer as resistant genes can be found in clusters and transferred together to the recipient bacterium. The transfer of multi-drug resistance genes is enabled by DNA elements with the ability to attract genes specifically encoding for antibiotic resistance, called the intergrons, which can be found located in the chromosomal DNA or broad host range plasmids (Rowe-Magnus and Mazel, 1999; Ploy *et al*., 2000). Intergrons are mobile DNA elements that are able to capture several genes most notably resistance ones (Hall, 1997). Intergrons have been reported in both Gram positive and Gram negative bacteria, but they seem to have a major role in spread of resistance in Gram negative bacteria (Dessen *et al.,* 2001).

2.8 Antimicrobial susceptibility testing

Antimicrobial or antibiotic susceptibility testing (AST) refers to *in vitro* methods that are used to determine the susceptibility or resistance of an organism to an antimicrobial agent/antibiotic (Walker and Fowler, 2011).

The importance of antimicrobial susceptibility testing is to confirm susceptibility of a microorganism to a given antimicrobial or to detect resistance in individual bacterial isolates (Jorgensen and Turnidge, 2007). The testing also helps physicians and veterinarians to determine the proper antimicrobial agent for a particular organism. The methods involved in susceptibility testing involve culturing of a sample to obtain pure isolates and testing using antimicrobials to determine susceptibility or resistance of the particular isolate (Walker, 2007; CLSI, 2013). The testing can be done using phenotypic and/or genotypic procedures.

2.8.1 Phenotypic antimicrobial susceptibility testing.

A number of *in vitro* antimicrobial susceptibility tests exist and these include: the disk diffusion method, the agar diffusion method and broth dilution which measures the minimum inhibitory concentration (MIC) of the antimicrobial (Walker, 2007; CLSI, 2008).

Diffusion method involves diffusion of an antimicrobial of a known concentration from disks, tablets or strips into a solid (agar) medium that has been inoculated with a pure culture of the test bacterium. The method is based on the determination of an inhibition zone proportional to the susceptibility of the bacterium to the antimicrobial present in the disk, tablet or strip. Diffusion of the antimicrobial on the solid medium creates a concentration gradient. A zone of inhibition is created to a point where the antibiotic becomes so dilute that it can no longer inhibit the test organism. The diameter of the zone of inhibition is directly proportional to susceptibility of the organism to the tested antimicrobial (CLSI, 2008).

In the disk diffusion method a paper disk with standard antimicrobial concentration is placed on the surface of agar medium which has been inoculated with a bacterium at a standardized concentration of cells per milliliter (Bauer *et al*., 1966). This method has advantages in that it is easy to perform, cheap and does not require expensive equipment to perform.

The broth dilution technique is based on the bacterial susceptibility testing against varying concentrations of an antimicrobial; usually a serial two-fold dilution of the antimicrobial (Jorgensen and Turnidge, 2007). The method involves serial dilution of an antibiotic in tubes or microtitre plates where a standard concentration of the test organism is added. Results are recorded as MIC in mg/mL, which is interpreted as the lowest concentration of the antibiotic which inhibits or completely kills the organism (showing no signs of growth/turbidity).

Agar dilution method involves the testing of a defined bacterial susceptibility against a varying concentration of an antimicrobial incorporated into an agar medium. This method is often recommended as the method of choice in testing of susceptibility of fastidious organisms such as anaerobes and *Helicobacter* species (CLSI, 2006c).

Other phenotypic antimicrobial susceptibility methods include the gradient strips and the E-test (Walker, 2007).

2.8.2 Genotypic antimicrobial susceptibility testing

The use of genotypic susceptibility testing of resistance in bacteria has been encouraged as it increases rapidity and accuracy of susceptibility testing (Cai *et al.*, 2003).

Nucleic acid amplification techniques (e.g. PCR and DNA sequencing) offer increased sensitivity, specificity and speed in detection of known resistance genes (Cai *et al*., 2003; Perretan *et al*., 2005).

Polymerase Chain Reaction (PCR) procedures are available for the rapid detection of multi-resistance genes of bacteria. Resistance genes that can be detected by use of PCR technique include the *mec*A gene for the detection of methicillin/oxacillin resistance in *Staphylococcus aureus* and coagulase-negative *Staphylococcus*, Vancomycin-resistant *Enterococcus*, and detection of fluoroquinolone resistant mutants (Cai *et al.*, 2003; Perreten *et al.*, 2005).

Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) can be used to detect antimicrobial resistant bacteria. Although the routine use of this method in the detection of antimicrobial resistance is not commonly used as a diagnostic tool, identification of vancomycin resistant enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) has been documented (Wolters, 2011, Griffins, 2012).

CHAPTER THREE; MATERIALS AND METHODS

3.1 Study areas

Samburu, Isiolo and Nakuru counties were selected for the study (Figure 3.1), specifically Naimaralal and Opiroi locations of Samburu, Burat and Isiolo west locations (Isiolo) and Gilgil area of Nakuru. These areas were selected conveniently because they have large numbers of camels, are secure, easily accessible and are in different ecological zones.

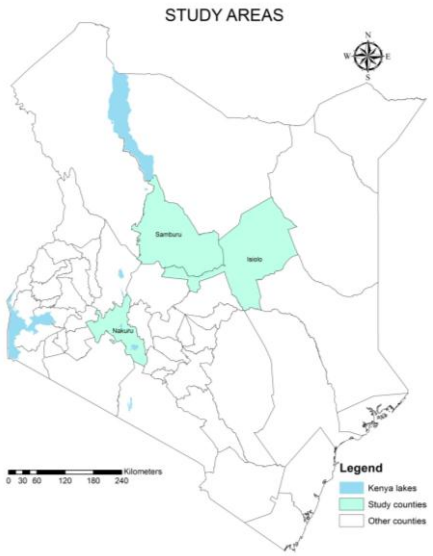

Figure 1: Map of Kenya showing location of Nakuru, Samburu and Isiolo Counties where the study was carried out; Source: ArcGIS version 10.1

Isiolo County lies within the ASALs of Northern Kenya. The County is located between Longitude 36°50' and 39°30' East and Latitudes 0^0 5' and 2° North, and has a total area of 25,605 km^2. The County is mostly flat with low lying plains with altitudes ranging between 180 metres above sea level at Lorian swamp in the northern area and 1000 metres above sea level in the southern area. The agro-climatic zones of the county are divided into three: semi-arid zone which

occupies 5% of the total area, arid zone which occupies 30% and the very arid area which occupies about 66% of the total area of the county (Sombroek *et al*., 1982; Herlocker *et al*., 1993). The county receives two rainy episodes in March to May and October to December a year but the rains are often unpredictable therefore frequent droughts that lead to loss of livestock and also human lives are common occurrences in the county. The county is also characterized by hot temperatures throughout the year, ranging between 24^{o}C and 30^{o}C which cannot support rain fed crops. This necessitates the need to keep hardy animals like camels.

Samburu County is located in the former Rift Valley province of Kenya. The county lies between latitudes 1^{o} 10' and 36^{o} 40' East and has a total area of roughly 21,000km^{2} with a population of about 224,000 people. It stretches North from the Wuoso Ng'iro River to the South from Lake Turkana. The County borders Marsabit to the North and Northeast, Isiolo to the East, Turkana to the West and Northwest and Laikipia and Baringo to the Southwest. Temperature ranges between 25^{O} C and 35^{o}C. The county receives rainfall between 200 mm and 250 mm annually which is unpredictable at times and sometimes the county can receive no rain at all the whole year. Livestock rearing is common in this county with community mainly keeping cattle, camels, sheep and goats.

Nakuru County lies within the Great Rift Valley and borders eight other counties which include; Kericho and Bomet to the West, Baringo and Laikipia to the North, Nyandarua to the East, Narok to the South-west and Kajiado and Kiambu to the South. It lies between Longitude 35^{o} 28` and 35$^{o}$ 36` East and Latitude 0^{o} 13 and 1^{o} 10` South. The county covers an area of 7,495.1 km^{2}. The county has three climatic zones (II, III and IV). The county receives bimodal type of rainfall with short rains falling between October and December and long rains falling between March

and May. Temperature ranges between 12^{o} C to 29^{o}C with Molo and Kuresoi sub-counties being cold while Naivasha, Gilgil and Rongai sub-counties experiencing extreme hot weather.

3.2 Sample size

All the three counties were taken as one population of camels as the animals are normally exposed to almost the same ecological and rearing conditions, therefore a convenient number of 255 nasal swabs were collected from the three counties.

The 255 nasal swabs were proportionally allocated based on the camel population in the counties.

3.3 Study design

A convenient sampling strategy that depended on the availability of camels, ease of access, security and difference in ecological zones of Nakuru, Samburu and Isiolo counties was done. All camels present at the time of farm visit were sampled. A convenient sample of twenty (20) lung tissues was also collected immediately after slaughter of camels at Athi River camel slaughterhouse.

3.4 Nasal swab collection and handling

With the help of the owners, the camels were restrained using well secured ropes. A total of 255 nasal swabs were collected (Figure 2). Following the method described by Mohamed *et al,* (2014), the external nares were carefully and thoroughly cleaned before disinfecting them using 70% alcohol. Then, swabbing was done by introducing sterile cotton swabs directly into the nasal cavities and rubbing them smoothly against the mucosa in a circular motion (Figure 3). The swabs were then placed into bijoux bottles containing Stuart transport media (Oxoid Ltd., Hamppshire England), wrapped with air-tight polythene bags and put in cool boxes. The samples were stored in -20^{0}C freezers in designated Veterinary offices in the study counties. These were

transported to University of Nairobi upper Kabete Veterinary Microbiology laboratory for processing after a week.

Figure 2: Camel restraint by their owners

Figure 3: Swabbing of the nasal mucosa of camel using a sterile cotton swab

3.5 Abattoir survey

A separate sampling was done at the camel slaughterhouse with the aim of comparing the bacterial genera and species isolated from the lungs and those isolated in the upper respiratory system (nasal cavity).

3.5.1 Lung tissue collection

A total of 20 lung samples were collected from Athi River abattoir in Machakos County to compare bacteria isolated from both the nasal cavity and the lungs. Camels included in the study were considered adults and healthy. Physical examination which included observing for abnormal nasal and ocular discharges, coughing and sneezing was used to rule out any respiratory infection in the sampled camels. After slaughter, sterile surgical blades were used to incise small pieces of the lung tissue which were put in sterile zip lock plastic bags. These were then put in a cool box and transported to the laboratory for bacterial isolation on the same day of sampling. Fifteen (15) normal lung tissues and 5 that showed some pathology were included in the study.

3.6 Bacterial isolation and identification

Isolation and identification of bacteria were carried out using standard procedures (Shears *et al*., 1993; Quinn *et al*., 1994). The nasal swabs were streaked onto both Blood Agar containing 5% bovine blood and MacConkey Agar using a sterile inoculating loop. For the lung tissues, the surface of the tissue was first disinfected with 70% alcohol to minimize surface contamination then a sterile surgical blade was used to cut through the tissue after which a sterile inoculating loop was introduced into the cut section, this was rolled gently to get some tissue which was then streaked on Blood and MacConkey agar media.

The agar plates were aerobically incubated at 37^0C for 24 hours. After incubation, each different colony was examined macroscopically to ascertain colonial morphology, presence or absence of hemolysis, and/or pigment production. In cases where no growth occurred after 24 hours, the incubation period was extended up to 72 hours. If still no growth had occurred after the 72 hour incubation period, the sample was considered bacteriologically negative, with respect to aerobic bacteria. Mixed growth cultures were sub-cultured on fresh Blood and MacConkey agar to obtain pure colonies. Primary identification test involved Gram staining, according to procedure described by Forbes *et al*, (2002) and Bebora *et al* ,(2007) to test for reaction, cellular morphology and spore formation. Biochemical tests were additionally done; they included catalase, coagulase, CAMP and gelatin liquefaction tests for Gram positive bacteria. *Staphylococcus* species were identified based on growth on Blood (Figure 8) and MacConkey agar, Gram stain reaction, coagulase test, and catalase test. *Streptococcus species* were differentiated by CAMP test (Figure 9), growth characteristics on Blood agar and MacConkey agar, Gram stain and catalase production. *Bacillus* species were identified based on growth on Blood agar (Figure 6), Gram stain (Figure 7) and gelatin liquefaction tests. Gram negative isolates from lung tissues were further tested using Triple Sugar Iron, IMViC tests (indole, methyl red, Voges-Proskauer and Citrate utilization tests). *Mycoplasma* and anaerobic bacteria were not included in the isolations. Where possible, organisms were identified up to the species level.

3.6 Test procedures

Additional test (biochemical) procedures were done according to standards described by Forbes *et al*, (2002).

3.6.1 Catalase test

Gram positive cocci both in clusters and chains were subjected to catalase test. Catalase positive organisms were considered to be *Staphylococcus* species whereas catalase negative isolates were considered to be *Streptococcus* species.

3.6.2 Coagulase test

Coagulase test was used to differentiate *Staphylococcus aureus* (coagulase positive *Staphylococcus*) and other coagulase negative staphylococci. *Staphylococcus aureus* produces coagulase which normally clots plasma into gel in tube or agglutinate cocci in slide. Beta hemolytic isolates on Blood Agar, Catalase positive, Gram positive cocci in clusters were subjected to coagulase reaction using rabbit plasma. Isolates that caused clumping of rabbit plasma on a glass slide were considered Coagulase Positive *Staphylococcus* (*Staphylococcus aureus*) while those which did not cause any coagulation (non hemolytic on BA) were considered Coagulase negative *Staphylococcus*.

3.6.3 Gelatin liquefaction test

Isolates producing hemolysis on blood agar (Figure 6), catalase positive, Gram positive rods with spores (Figure 7) were inoculated into gelatin bottles; this was then observed for at least 72 hours at room temperature. Isolates with the above characteristics and able to liquefy gelatin from its solid state were considered to be *Bacillus* species.

3.6.4 CAMP test

Isolates confirmed to be *Streptococcus agalactiae* (Lancefield group B) produced a characteristic arrow head shape clear zone of hemolysis around a zone of complete hemolysis by *Staphylococcus* beta hemolysin toxin on blood agar (CAMP test) (Figure 9). Other catalase negative, Gram positive cocci in chains and CAMP negative were considered other *Streptococcus* species.

3.6.5 Indole test

Indole test was done to test the ability of an organism to split tryptophan molecule into Indole which is one of the metabolic products of amino acid tryptophan (Forbes *at al*., 2002). Gram negative short rod (lactose fermenters) isolates were inoculated into peptone water and incubated at 37^{o} C for 18-24 hours. After this, Kovac's reagent was added into the inoculum. Development of a red ring indicated a positive result (Indole positive).

3.6.6 Methyl Red test (MR test)

Glucose Phosphate Peptone water (GPPW) was inoculated with a test organism and incubated at 37^{0}C for 18-24 hours, after which MR reagent was added to the broth. Maintenance of the red colour indicated a positive test.

3.6.7 Citrate Utilization test.

The test organism was cultured in a medium which contained sodium citrate, an ammonium salt and the indicator bromothymol blue. Positive test was indicated by turbidity and a change in colour of the indicator from light green to blue, due to alkaline reaction following citrate utilization. No colour change from light green to blue was considered a negative test.

3.7 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using disk diffusion test using procedure described by Clinical and Laboratory Standards Institute (CLSI, 2006). Isolated bacteria were tested for susceptibility to selected antimicrobials including; Ampicillin, Tetracycline, Streptomycin, Co-trimoxazole, Kanamycin, Gentamycin, Sulphamethoxazole and Chloramphenicol. Isolates were grown on Blood agar for 24 hours; five (5) colonies were picked from each plate and suspended in 5 ml of sterile normal saline which was then adjusted to a density approximately equal to McFarland Opacity Standard No. 0.5. A dry sterile cotton swab was then placed inside the suspension; excess liquid from the swab was expressed against the wall of the tube and the swab

used to spread the bacterial suspension evenly on the surface of Mueller Hinton agar, in order to get confluent growth. Antimicrobial disks were then placed on the surface of the inoculum and incubated for 18-24 hours (Figure 4). Zones of inhibition were measured to the nearest millimeter and interpretation as to whether the bacterium is resistant or susceptible (Appendices 6 and 7) to the particular antimicrobial was done according to standards defined by Clinical and Laboratory Standards Institute (CLSI, 2006).

Figure 4: Antibiotic Susceptibility testing showing the antibiotic disks on the plate.

3.8 Data management and statistical analysis

All data were entered in Microsoft Excel 2010 to create a database after which it was transferred to Stata version 13 for analyses. Descriptive statistics (percentages, proportions, graphs and frequency tables) were generated using the same statistical package. Associations between ages, sex and areas of study and the type of bacterial isolates was carried out using the Pearson Chi square test at 5% level of significance.

CHAPTER FOUR: RESULTS

4.1 Characteristics of sampled animals (nasal swabs)

A total of 255 nasal swabs (62 were from Nakuru, 115 from Samburu and 78 from Isiolo counties) were collected from apparently healthy camels. The camels based on age, were put into two age groups: young (animals <2years old) and adults (animals >2years old). A total of 150 adult (37 were from Nakuru, 70 from Samburu and 43 from Isiolo) and 105 young camels (25 were from Nakuru, 45 from Samburu and 35 from Isiolo) were sampled in this study (Table 1)

Table 1: Distribution of sampled camels by age in Nakuru, Samburu and Isiolo counties

Age groups	**Nakuru**	**Samburu**	**Isiolo**	**Total no. sampled**
Adult	37	70	43	150
Young	25	45	35	105
Total	62	115	78	255

Most (82%; 211/ 255) of the sampled camels were females, of which 24% (50/211) were from Nakuru, 46% (98/ 211) from Samburu and 30% (63/ 211) from Isiolo. Out of the 44 male camels sampled, 12 were from Nakuru, 17 from Samburu and 15 from Isiolo (Table 2).

Table 2: Distribution of camels sampled from the three counties by sex n=255

Sex of animals	Nakuru	Samburu	Isiolo	Total
Male	12	17	15	44
Female	50	98	63	211
Total	62	115	78	255

4.2 Isolation and identification of bacteria from the nasal cavity of camels

Almost all the samples (96%; 245/ 255) showed positive bacterial growth yielding different bacterial genera and species. The 10 samples with no bacterial growth were all from Samburu. The 245 positive samples all yielded Gram positive bacteria. A total of 404 isolates representing different genera and species of bacteria were obtained from the 245 samples whereby in some instances, a sample would yield more than one isolate of bacteria (164 from Samburu, 133 from Nakuru and 107 from Isiolo). The percentages of isolates from each site is summarized in Table 3 and Figure 5.

Table 3: Number of bacterial isolates from Samburu, Nakuru and Isiolo Counties

County	Number of isolates	Percentage of bacterial isolates
Samburu	164	40.59
Nakuru	133	32.92
Isiolo	107	26.49
Total	404	100

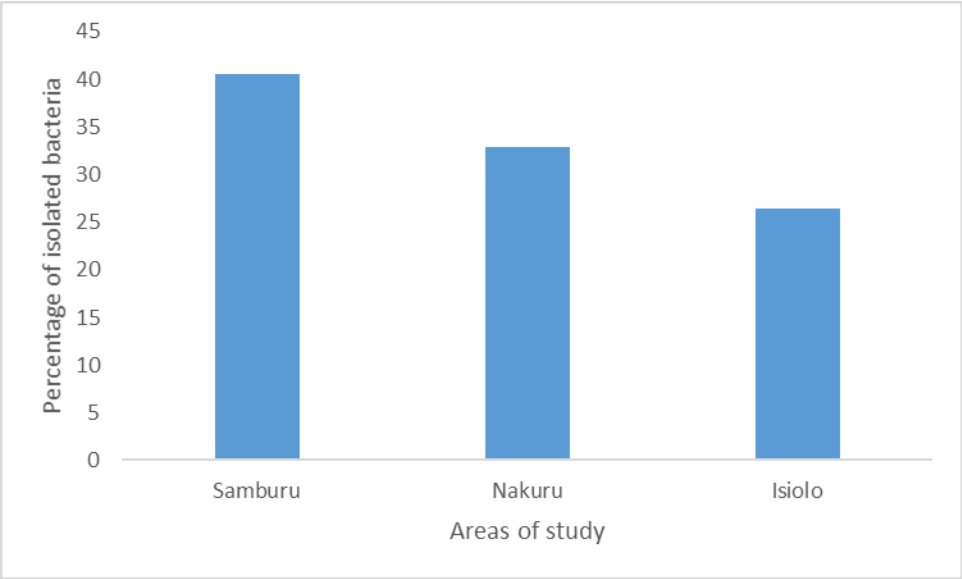

Figure 5: Percentage of isolates identified from nasal cavity of camels in the three counties included in the study

Isolated bacteria included: *Bacillus* at 39.6% (160/404), coagulase negative Staphylococcus at 29.95% (121/404), Streptococcus species other than *Streptococcus agalactiae* at 25.74% (104/404), coagulase positive Staphylococcus at 3.96% (16/404) and *Streptococcus agalactiae* was the least isolated at 0.74% (3/404) (Table 4).

Table 4: Percentage of bacteria isolated from the nasal cavity of camels the three counties included in the study

Bacteria	**Number isolated**	**Percentage of isolated bacteria**
Bacillus	160	39.60
Coagulase negative *Staphylococcus*	121	29.95
Coagulase positive *Staphylococcus*	16	3.96
Streptococcus other than *Streptococcus agalactiae*	104	25.74
Streptococcus agalactiae	3	0.74
Total	404	100

Bacillus species was the most frequently isolated in Isiolo at 56.07% (60/107), followed by Samburu at 40.85% (67/164) and Nakuru at 24.81% (33/133). Coagulase negative *Staphylococcus* was most frequently isolated from Nakuru at 36.84% (49/133), followed by Samburu at 29.27% (48/164) and Isiolo at 22.43% (24/107). *Streptococcus* was highest isolated from Nakuru at 35.34% (47/133), followed by Samburu at 21.95% (36/164) and Isiolo at 19.63% (21/107) Coagulase positive *Staphylococcus* (*Staphylococcus aureus*) was highest isolated from Samburu at 6.1% (10/164), followed by Nakuru at 3% (4/133) and Isiolo at 1.87% (2/107). *Streptococcus agalactiae* was isolated from Samburu at 1.83% (3/164) with no isolation of *Streptococcus agalactiae* from both Nakuru and Isiolo counties (Table 5). Figure 6 shows a case of *Bacillus* species growing on Blood agar, while Figure 7 gives the micro-morphology of the same organism. Figure 8 shows *Staphylococcus* growing on blood agar and Figure 9 shows positive CAMP reaction of *Streptococcus agalactiae.*

Table 5: Percentage of bacteria isolated from Samburu, Isiolo and Nakuru Counties separately

Bacteria	**Samburu N=164**		**Isiolo N=107**		**Nakuru N=133**	
	No. isolated	% of isolates	No. isolated	% of isolates	No. isolated	% of isolates
Bacillus	67	40.85	60	56.07	33	24.81
CoNs	48	29.27	24	22.43	49	36.84
CPS	10	6.1	2	1.87	4	3
Streptococcus agalactiae	3	1.83	0	0	0	0
Other streptococci	36	21.95	21	19.63	47	35.34

The area sampled, namely Nakuru, Isiolo, and Samburu, was significantly associated with the type of bacteria isolated (P=0.000).

Figure 6: Showing *Bacillus* species growing on Blood agar – showing beta hemolysis

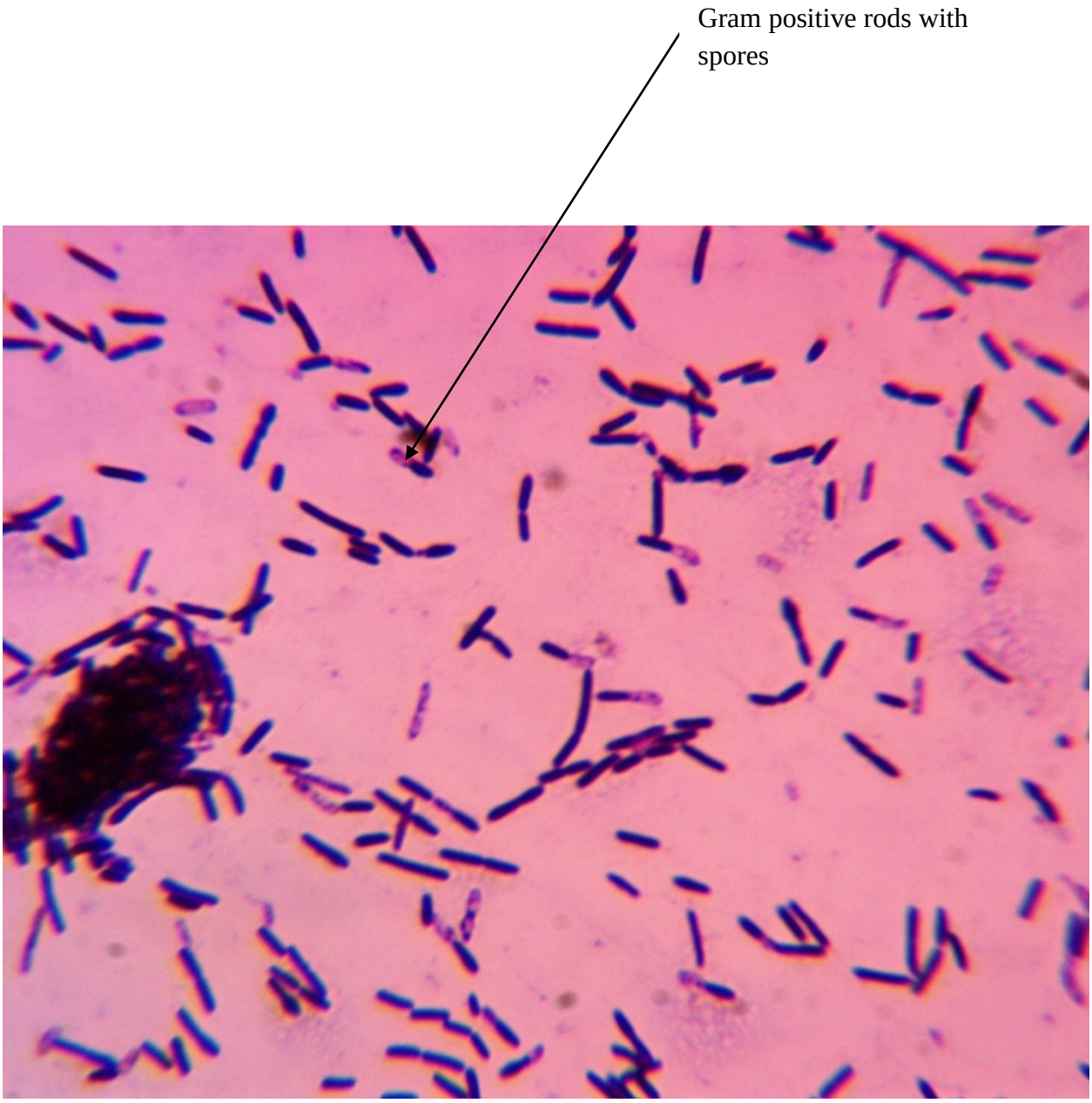

Figure 7: Gram stain: *Bacillus* species showing Gram positive rods with spores (arrow showing the spore in the vegetative cell)

Figure 8: Showing *Staphylococcus* species gowth on Blood agar plate

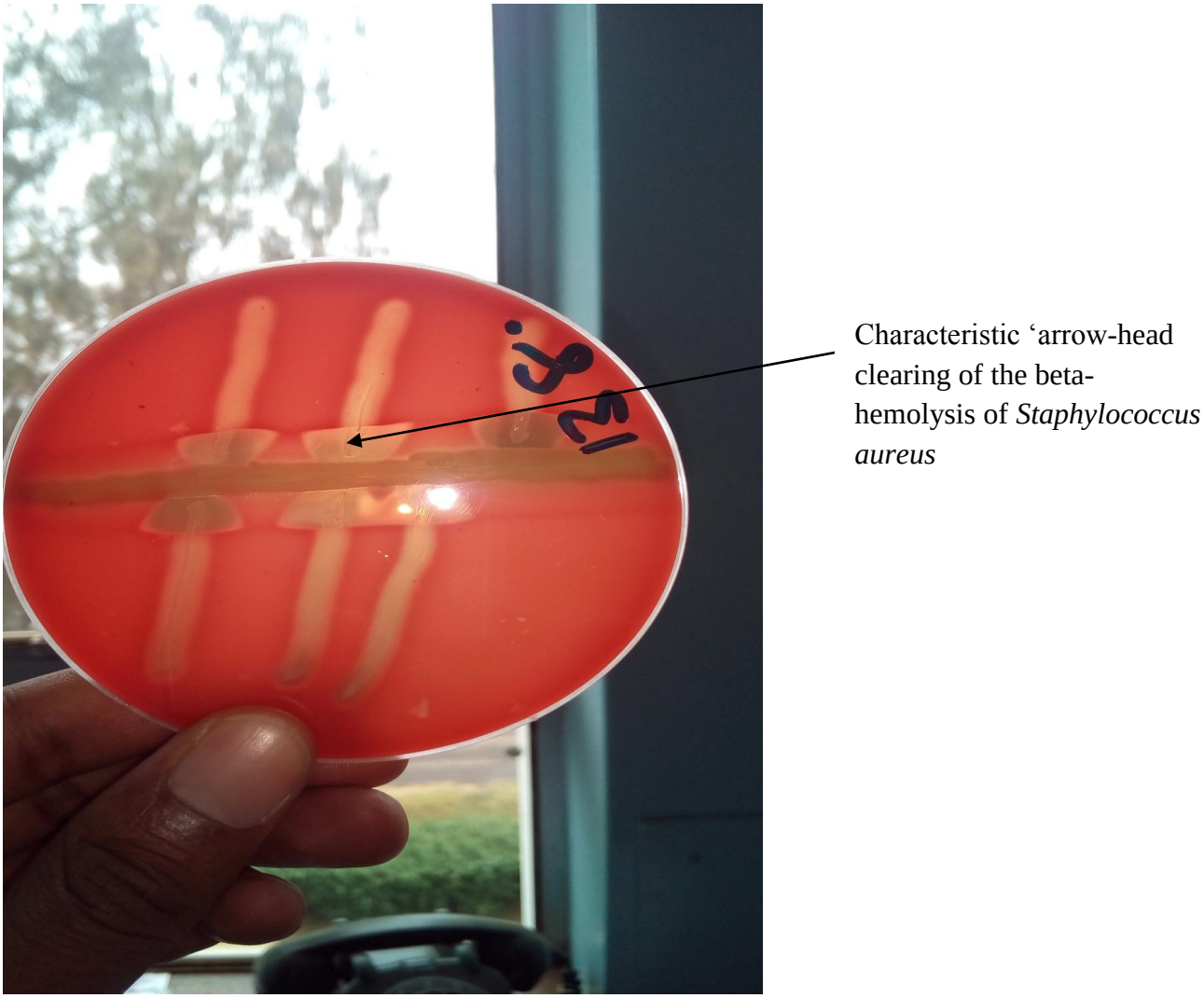

Figure 9: Positive CAMP test (*Streptococcus agalactiae*) arrows indicating the characteristic ‘arrow-head clearing of the beta-hemolysis of *Staphylococcus aureus*

There was similarity in terms of the type of bacteria isolated between the two ages of camels sampled i.e. *Bacillus*, coagulase negative *Staphylococcus*, coagulase positive *Staphylococcus* and *Streptococcus*. The percentage of bacteria isolated was higher in adult camels (animals over 2 years) at 57.68% (233/404), than in the young camels (animals less than 2 years old) at 42.33% (171/404). In adult camels, *Bacillus* was most frequently isolated at 43.35% (101/233) followed

by Coagulase negative *Staphylococcus* at 29.61% (69/233), *Streptococcus*, other than *Streptococcus agalactiae* at 23.18% (54/233), coagulase positive *Staphylococcus* at 2.58% (6/233) and *Streptococcus agalactiae* at 1.29% (3/233). In young camels, the proportion of bacteria isolated was similar to the adults; *Bacillus* species was most frequently isolated at 34.5% (59/171) followed by coagulase negative *Staphylococcus* at 30.41% (52/171), *Streptococcus* other than *Streptococcus agalactiae* at 29.24% (50/171), coagulase positive *Staphylococcus* at 5.85% (10/171), except for *Streptococcus agalactiae* which was not isolated from all the samples collected from young animals. Table 6 represents the percentage of bacteria isolated from the two age groups.

Table 6: Percentage of bacterial isolation from adult and young camels

Bacteria	No. isolated in adult camels n=233	% isolated	No isolated in young camels n=171	% isolated
Bacillus	101	43.35	59	34.50
CoNS	69	29.61	52	30.41
CPS	6	2.58	10	5.85
Streptococcus agalactiae	3	1.29	0	0
Other streptococcus	54	23.18	50	29.24

The percentages in the two age groups did not differ significantly from each other (P=0.076).

Overall, bacteria were more frequently isolated in female camels (80.69%; 326/404) than in male ones (19.31%; 78/404). *Bacillus* was the most frequently isolated organism (40.18% for females; 37.18% for males), followed by coagulase negative *Staphylococcus* (29.14%, for females; 33.33% for males), then streptococci other than *Streptococcus agalactiae* (25.46 for females; 26.92% for males), coagulase positive *Staphylococcus* (4.6% for females; 1.28% for males) and *Streptococcus agalactiae* (0.85% for females; 1.28% for males). The percentages were not significantly different from each other (P=0.335). (Table 7)

Table 7: Percentage of bacteria isolated in females and male camels sampled

Bacteria	No. isolated in females n=326	% isolate	No. isolated in males n=78	% isolate
Bacillus	131	40.18	29	37.18
CoNS	95	29.14	26	33.33
CPS	15	4.6	1	1.28
Streptococcus agalactiae	2	0.85	1	1.28
Other streptococcus	83	25.46	21	26.92

4.3 Antimicrobial susceptibility testing

Thirty two (32) isolates of *Bacillus* were tested against different antimicrobials. All of them were sensitive to Gentamycin and Kanamycin (100% sensitivity), thirty (93.75%) isolates were sensitive to Ampicillin, and 29 (90.6%) were sensitive to Streptomycin. The four antimicrobials that *Bacillus* showed resistance to, in descending order, were Chloramphenicol at 34.4% (11 isolates), Sulphamethoxazole at 21.9% (7 isolates), Co-Trimoxazole at 18.8% (6 isolates) and Tetracycline at 12.5% (4 isolates) (Figure 10).

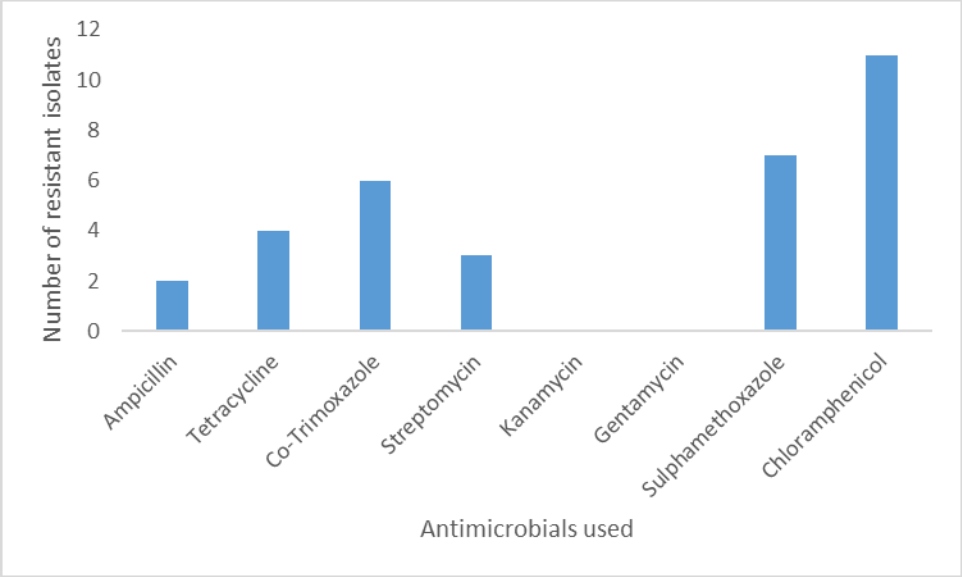

Figure 10: Number of resistant *Bacillus* isolates

Out of the 9 coagulase positive *Staphylococcus* isolates (*Staphylococcus aureus*) tested the sensitivity rates were; Kanamycin, Gentamycin and Chloramphenicol – 100% (all the nine); Tetracycline and Sulphamethoxazole – 88% (eight isolates), Ampicillin – 77% (7 isolates), Co-Trimoxazole and Streptomycin - 66% (6 isolates). Percentage resistance was recorded as follows; Ampicillin at 23% (2 isolates), Tetracycline at 12% (1 isolate), Co-Trimoxazole and Streptomycin at 34% (3 isolates) and Sulphamethoxazole at 12% (1 isolate). (Figure 11).

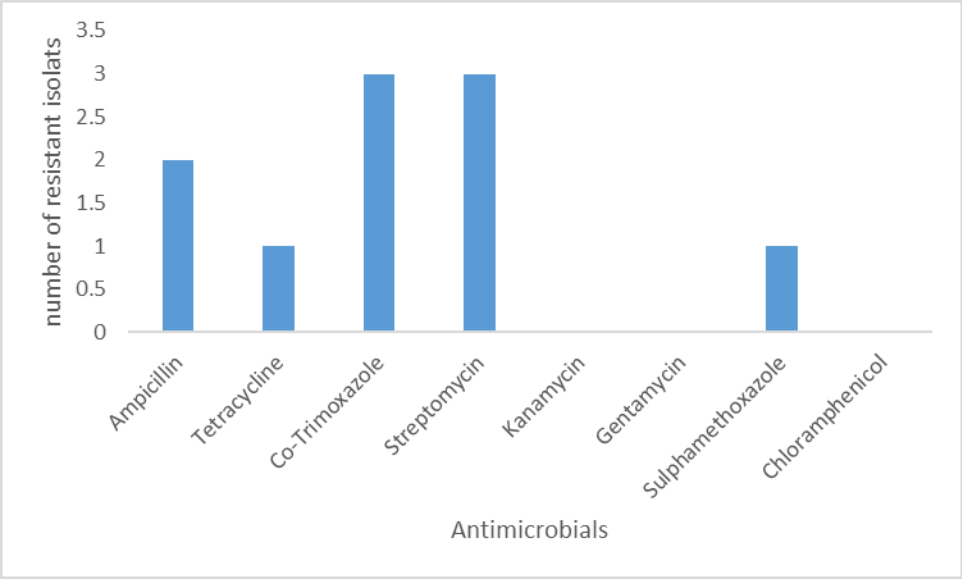

Figure 11: Number of resistant Coagulase positive *Staphylococcus* isolates

The antibiogram of Coagulase negative *Staphylococcus* indicated high susceptibility to Co-trimoxazole and Kanamycin with all 27 isolates (100%) being sensitive, followed by Gentamycin and Chlorampenicol with 26 isolates (96.3%) sensitive, followed by Streptomycin and Sulphamethoxazole with 25 isolates (92.6%) sensitive. This was followed by Tetracycline with 24 isolates (88.9) sensitive and finally Ampicillin with 15 isolates (55.6%) susceptible. The number of resistant isolates recorded was as follows; Ampicillin 12 isolates (44.4%), tetracycline 3 isolates (11.1%), Streptomycin and Sulphamethoxazole 2 isolates (7.4%) and finally Gentamycin and Chloramphenicol 1 isolate (3.7%). (Figure 12).

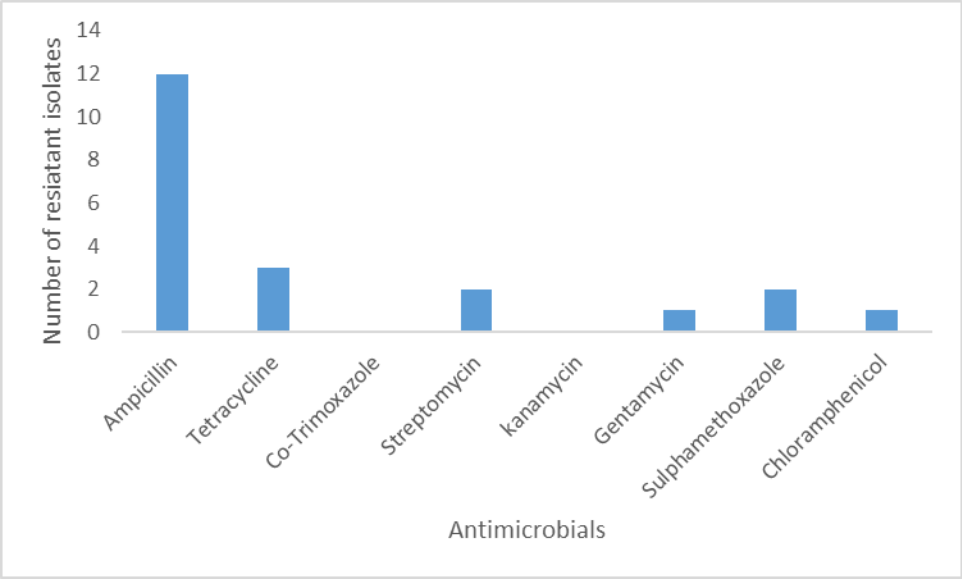

Figure 12: Number of resistant Coagulase negative *Staphylococcus* isolates

All (100%) of the 3 isolates of *Streptococcus agalactiae* tested showed sensitivity to Gentamycin, Sulphamethaxazole and Chloramphenicol. Two isolates (66.7%) were sensitive to Tetracycline and Co-trimoxazole, while only one isolate (33.3%) was sensitive to Ampicillin, Streptomycin and Kanamycin (Figure 13)

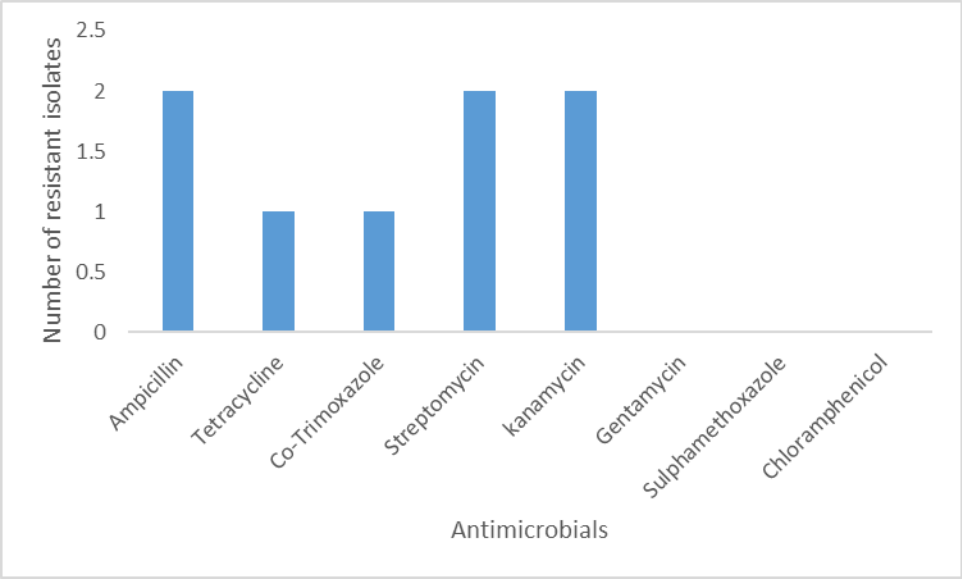

Figure 13: Number of resistant *Streptococcus agalactiae* isolates

Out of 24 isolates of *Streptococcus* species other than *Streptococcus agalactiae* tested for sensitivity, the isolates showed highest sensitivity to Tetracycline with all isolates (100%) being susceptible, 22 isolates (91.7%) were sensitive to Ampicillin, 21 isolates (87.5%) were sensitive to Gentamycin and Chloramphenicol, 19 isolates (79.2) were sensitive to Suphamethoxazole, 17 isolates (70.8%) were sensitive to Co-Trimoxazole. The number of resistant isolates were as follows; Ampicillin 2 isolates, Gentamycin and Chloramphenicol 3 isolates, Sulphamethoxazole 5 isolates, Co-Trimoxazole 7 isolates, Streptomycin and kanamycin 12 isolates each. (Figure14)

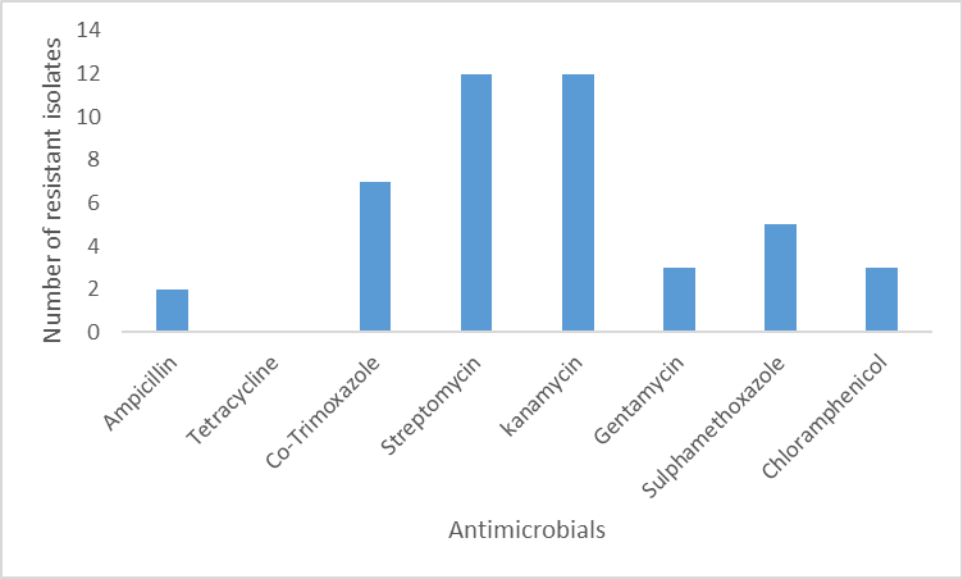

Figure 14: Number of resistant *Streptococcus* isolates

Generally Gentamycin was the most effective against all the isolates tested at 95.8% (91/95), followed by Tetracycline at 90.5% (86/95), followed by Kanamycin and Chloramphenicol at 85.3% (81/95), followed by Sulphamethoxazole 84.2% (80/95), followed by Co-Trimoxazole at 82.1% (78/95), followed by Ampicillin at 78.9% (75/95) and finally Streptomycin at 76.8% (73/95). Resistance levels were recorded being minimal in Gentamycin at 4.21% (4/95), followed by Tetracycline at 9.47% (9/95), Kanamycin and Chloramphenicol at 14.7% (14/95), Suphamethoxazole at 15.8% (15/95), Co-Trimoxazole at 17.9% (17/95), Ampicillin at 21.1% (20/95) and finally Streptomycin at 23.2% (22/95) (Figure 15).

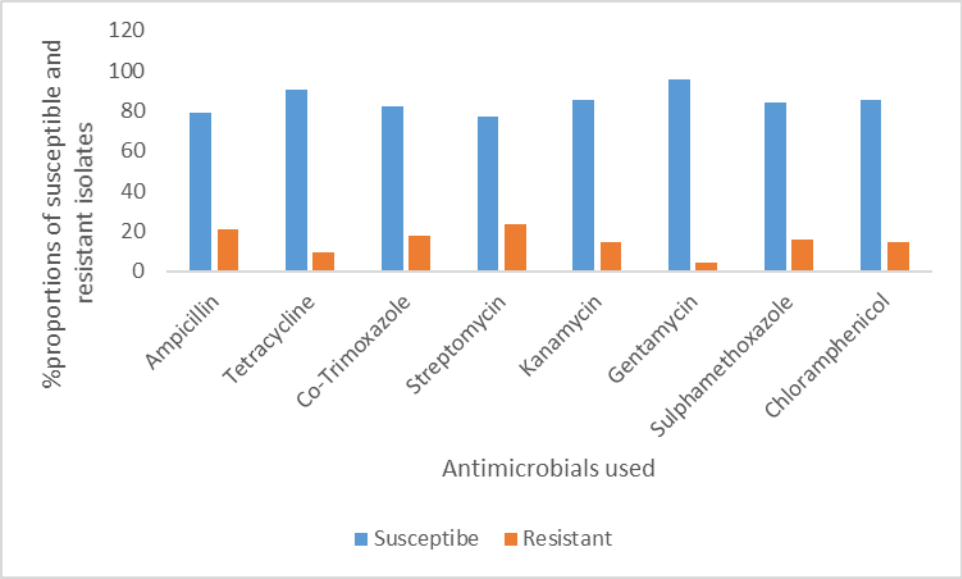

Figure 15: General antimicrobial susceptibility profiles of all tested isolates.

Figure 16 shows two examples of antibiotic susceptibility reactions of *Bacillus* isolate (A) and a Coagulase positive *Staphylococcus* (B) on Muller Hinton agar plates.

A

Figure 16: (A) Antibiotic susceptibility testing reaction of a *Bacillus* isolate on Muller Hinton agar

B

(B) Antibiotic susceptibility testing reaction of a Coagulase positive *Staphylococcus* on Muller Hinton agar

4.4 Multi-drug resistance

Although in this current study most isolates showed resistance to one antimicrobial, some of the bacterial isolates in different genera and species showed multi-drug resistance (resistance to more than one antimicrobial), with respect to the antimicrobials used. Generally, out of the ninety five isolates tested 30.5% (29/95) showed resistance to more than one antimicrobial used in this study. In *Bacillus* species, 31.25% of the 32 isolates (10/32) tested for resistance to 8 different antimicrobials showed resistance to more than 2 antimicrobials.

Coagulase negative *Staphylococcus* also showed similar resistance with most isolates showing resistance to not more than one antimicrobial. However, 14.82% of the isolates (4/27) showed resistance to two or more antimicrobials used for testing.

Out of the 9 coagulase positive *Staphylococcus* bacteria tested for resistance, 33.3% (3/9) showed resistance to 2 of the antimicrobials used.

Out of the 3 *Streptococcus agalactiae* tested for resistance, one isolate (33.3%) showed resistance to more than two antimicrobials.

Out of 24 isolates of *Streptococcus* species other than *Streptococcus agalactiae* tested for resistance, most isolates showed resistance to one antimicrobial. However, 41.67% (10/24) of the isolates showed multiple resistance to two or more antimicrobials used.

Multidrug resistance was reported highly in combination of any two antimicrobials at 20% (19/95) followed by three (3) antimicrobials 6.32% (6/95), four (4) antimicrobials at 2.11% (2/95) followed by six (6) antimicrobials and seven (7) antimicrobials at 1.05% (1/95)

4.5 Bacterial isolates from camel slaughterhouse.

From the 15 normal lung tissues, (tissues with no evidence of pathology e.g. abscesses and any form of inflammation) 8 (53%; 8/15) samples produced different genera and species of bacteria, from which fifteen different genera and species of bacteria were isolated.

Isolated bacteria included; coagulase negative *Staphylococcus* and *Streptococcus* species (40% (6/15), coagulase positive *Staphylococcus* (13.3%; 2/15) and finally *E. coli* (6.7%; 1/15). (Table 8)

Table 8: Description of bacteria isolated from normal lungs from Athi River abattoir, n=15

Bacteria	**Number isolated**	**Percentage isolated**
Coagulase negative *Staphylococcus*	6	40
Coagulase positive *staphylococcus*	2	13.3
Streptococcus species	6	40
E.coli	1	6.7

From the five lung tissues with apparent pathology (abscesses and inflamed tissues), 4 samples produced 12 different isolates representing different bacterial genera and species. Coagulase negative *Staphylococcus* and *Streptococcus* species were the predominant bacteria isolated (33.3%; 4/12) (Table 9). There were no *Bacillus* species isolated from the lung tissues.

Coagulase positive *Staphylococcus*, coagulase negative *Staphylococcus* and *Streptococcus* species were isolated both from the upper and lower respiratory system. In terms of the observed differences, isolation of *Streptococcus agalactiae* and *Bacillus* species were only reported in upper respiratory system with *E. coli* only isolated in the lower respiratory system (lungs) (Tables 4 and 8).

Table 9: Percentage of bacteria isolated from camel lungs with apparent pathology, n=12

Bacteria	**Number isolated**(4 samples produced 12 different isolates)	**% isolated**
Coagulase negative *Staphylococcus*	4	33.3
Streptococcus Species	4	33.3
Coagulase positive *Staphylococcus*	2	16.7
E.coli	2	16.7

Generally, twelve (12) of the 20 lung tissues sampled, yielded 27 different genera and species of bacteria. These included: coagulase negative *Staphylococcus* and *Streptococcus* species at 37.04% (10/27), coagulase positive *Staphylococcus* at 14.81% (4/27) and *E. coli* at 11.11% (3/27).

5.0 DISCUSSION

The current study shows that a variety of bacterial species were found in the nasal cavity of apparently healthy camels. These organisms could have reached the nasal cavity through inhalation, direct or indirect contact or during drinking. However, the normal flora in apparently-healthy camels can be altered by several factors such as bad sanitation, stress due to transportation, sudden change in feed, low herd health status and immunosuppression. This could end-up lowering the resistance of the respiratory system to infection (Muna *et al*., 2015) thus, the existing nasal organisms could end-up finding their way down the system and eventually cause pathology in the respiratory system of the respective camels (Wareth *et al.,* 2014).

Isolation of a variety of bacterial species from the nasal cavity of camels in this study shows that different bacterial species colonize the respiratory system of the camels. This is supported by other authors who demonstrated presence of diverse bacterial species in the nasal tract of the apparently healthy camels (Abdulsalam, 1999), from nose, trachea, tonsils and lungs of apparently healthy camels (Azizollah *et al*., 2009), and from lungs of apparently healthy and diseased lungs (Abubakar *et al*.,2008). Al-Doughaym *et al.*, (1999) isolated *Staphylococcus aureus, Corynebacterium pyogenes,* coagulase negative *Staphylococcus, Bacillus* species, *Streptococcus pyogenes, diptheroids, E.coli, Klebsiella pneumoniae, Diplococcus pneumoniae* and mixed isolates from nasal swabs collected from camels in Sudan.

Only Gram positive bacteria were isolated in this study; similar results were reported by Azizollah *et al*. (2009) who predominantly isolated Gram positive bacteria from nasal cavity, trachea, tonsils and lungs of healthy camels in central Iran.

In the current study, coagulase positive *Staphylococcus* (*Staphylococcus aureus*) was isolated at a proportion of 3.96%. A study by Shigidi, (1973) reported isolation of *Staphylococcus aureus* at 2.6% from the nasal cavity of 64 apparent healthy camels. Chauhan *et al*, (1987), working on bacterial flora from the nasal cavity of healthy camels, reported isolation of *Staphylococcus aureus* at 10.5%. The differences in isolation frequency of this organism in the nasal cavity of apparently healthy camels could be attributed to various factors, including: different ecological zones, sample size and the health status of the camels. Higher prevalence rates have been recorded from diseased camels, for example; Al-Doughaym *et al*, (1999) reported a rate of 19.2% in pneumonic respiratory tract of camels and Wareth *et al*, (2014) reported a rate of 37.4% in pathological lungs in Cairo Egypt. Rana *et al*, (1993) also reported the isolation of *Staphylococcus aureus* from pneumonic lungs of slaughtered camels. Comparing this with isolations from some other animals, Yimer and Asseged, (2006) isolated *Staphylococcus aureus* at 6.3% from nasal tract of apparently healthy sheep, while Ajuwape and Aregbesola, (2002) reported isolation of *Staphylococcus aureus* at 100% in respiratory tract of rabbits. The extremely high prevalence in rabbits could be attributed to their eating habits. Rabbits are coprophagous; camels are mainly browsers. In addition, many pathogenic bacteria, including *Staphylococcus aureus,* were frequently found in pneumonic lungs of camels from slaughter house in Cairo Egypt (Farrag *et al*., 1953). The presence of these organisms in pneumonic lungs suggests that, in cases of stress, these bacteria can cause pathology, even though they are normally carried as normal flora in the nasal cavity of the animals .It has been demonstrated that *Staphylococcus aureus* organisms occur commonly as commensals on the skin and the mucous membranes and also as a common environmental contaminant. They thus commonly reside in the upper respiratory system of animals and cause disease in stressful conditions. The organism

is commonly associated with pneumonia in humans and animals (Alhendi, 1999; Ragle *et al.*, 2010; Rahimi and Alian, 2013); it is also associated with other disease conditions including mastitis, osteomyelitis, toxic shock syndrome, endocarditis and nosocomial infections in animals and humans (Sousa *et al*., 2005).

In camels, *Staphylococcus aureus* has commonly been associated with a number of conditions; most notably mastitis; in Ethiopia the organism was the common reported pathogen in cases of camel mastitis (Regassa *et al*., 2013), in Kenya and Sudan, the organism was reported as the most prevalent organism after *Streptococcus agalactiae* in most cases of udder infections (Obied and Bagadi, 1996; Younan *et al.*, 2001). Other conditions associated with *Staphylococcus aureus* in camels include; eye infections (Yeruh *et al*., 2002), joint infections (Bani Ismail *et al*., 2007), respiratory diseases and subclinical pneumonia (Wareth *et al*., 2014).

Isolation of *Streptococcus* organisms in this study was in agreement with other studies conducted by Shigidi, (1973), Mahmoud *et al*, (1988) and Rana *et al*, (1993) though they were not definitely identified and characterized. The isolation of *Streptococcus* organisms at a proportion of 25.74%, in this study, was higher than what was reported by Awol *et al*, (2011) and El-tigani *et al*, (2004) at 19.3% and 13.9% respectively. It was also higher compared to that reported by Azizollah *et al*, (2009) at 4.5%. Buxton and Fraser, (1977) indicated that, in nature, *Streptococcus* organisms were widely distributed including the respiratory system of many domestic animals. Isolation of *Streptococcus agalactiae* (Lancefield Group B *Streptococcus*) from the nasal cavity, in this study, is in agreement with Younan and Bornstein, (2007) who isolated the same from the nasopharynx of apparently healthy camels in east African camels. *Streptococcus agalactiae* was also isolated at 0.28% by Ahmed and Musa, (2015) from pneumonic camels. This organism has also been isolated from udder infection of camels, camels

with septic arthritis, skin abscesses and secondary respiratory infections (Poyart *et al*., 2002). *Streptococcus agalactiae* is normal flora and an opportunistic organism in camels found in East Africa. In apparently healthy camels the organism is found in the nasopharynx and ordinary lymph nodes while in cases of disease, the organism is found in respiratory infections, skin abscesses, tick bite lesions, abscessed lesions vaginal infections, cases of mastitis, athritis and gum infections (Bornstein and Younan, 2013).

In Kenya the organism has been reported to be a cause of peri-arthricular abscesses characterized by inflammation and pus accumulating around joint in camel calves. Multiple peri-arthricular abscesses in camel calves cause poor locomotion and suckling ability leading to retarded growth and mortalities when the disease advances to chronic state (Younan *et al*., 2007). In humans the organism is a common cause of pneumonia, sepsis, and meningitis in neonates, causing considerable morbidity and mortality (Schlegel *et al*., 2000). Isolation of *Streptococcus*, such as beta hemolytic *Streptococcus*, was documented in active respiratory disease of camels (Thabet, 1994). *Streptococcus* organisms were also considered potential pathogens and some of them are commensals in the gastrointestinal tract, genital, respiratory tract and skin of man and animals (Biberstain and Zee, 1990). *Streptococcus* organisms were frequently demonstrated from exudates of skin necrosis in camels (Edelstein and Pegram, 1974).

Most of the organisms isolated in this study have been associated with pneumonia. *Staphylococccus aureus, Streptococccus* species and *Bacillus* species, *Corynebacterium, Pasteurella* species and *Klebsiella pneumoniae* have been isolated from pulmonary lesions of camels (Zubair *et al*., 2004; Abukar *et al*., 2010; Rana *et al*., 1993; Wareth *et al*., 2014; Ahmed and Musa, 2015). The consistent isolation of some of above mentioned organisms from pneumonic camels i.e. *Staphylococcus, Streptococcus* and *Bacillus* organisms in this study,

might indicate their partial or full role in development of respiratory disease in camels more so when the immune mechanism of the animal has been altered by various factors like transportation, change in weather and environmental conditions, unhygienic conditions, nutritional status and health status of the animal (Wareth *et al*., 2014). Such external stressors will lower the immunity of the respiratory system to infection with an abrupt shift of commensal bacteria in the upper respiratory system to pathogenic bacteria and most probably the existing organisms will have an upper hand in presenting various types of pathologies in the respiratory system (Herthelius *et al*., 1989; Brogden *et al*., 1998).

In this study it was interesting to note that there were higher isolation rates of bacteria in females and older animals than in males and younger animals.

This study also showed that different genera of bacteria reside in the lower respiratory system (lungs) of the camel both in normal and diseased camel lungs. From the normal lungs, *Staphylococcus* species, *Streptococcus* species and *E. coli* were isolated. *Staphylococcus* species were reported by Azizollah *et al*, (2009) in normal lung tissues of camels. However in his study *Streptococcus* and *E. coli* were not isolated from the lungs. The isolation rate of *Staphylococcus* species at 40% in the normal lung tissues was higher compared to what was reported by Abubakar *et al*, 2010 and A-Doughaym *et al*, 1999 at 22.8% and 24.8% respectively. Isolation of *Staphylococcus aureus* in normal lung tissues was however contrary to what was reported by Abubakar *et al*, 2010 where isolation of *Staphylococcus aureus* was never reported. Isolation of *Staphylococcus aureus* in pathological lung tissues at 16.7% was higher compared to what was reported by Abubakar *et al*, 2010 and Shigidi, (1973) at 2.2% and 2.6%, respectively. Results of this study was also to some extent similar to one reported by Abubakar *et al*, (2010) in bacterial flora of normal and diseased lungs in camels where isolation of *Staphylococus* species,

Streptococcus species, *Klebsiella* species, *Bacillus* species among other bacteria were isolated. It was also comparable to the findings of Mohamed *et al*, (2014) who also studied bacterial examination of respiratory system of camels; they isolated *E.coli, Staphylococcus* species among other bacteria. The results were, however, different to those of this study as isolation of *Pseudomonas*, *Bacillus* and *Klebsiella* was reported. There were also other reports of diverse bacteria from different studies in the respiratory system of camels (Ahmed and Musa, 2015; Abukar *et al*., 2010; Al-Doughaym *et al*. 1999; Wareth *et al.*, 2014).

Bacterial genera and species isolated from normal lungs were also comparable to the bacteria isolated from the nasal cavity of camels from Isiolo, Samburu and Nakuru counties. However in the lungs, there was no isolation of *Bacillus* and *Streptococcus agalactiae*. Although generally, isolation frequency of bacteria decreased while moving to the lower respiratory system, there was similarity of bacteria isolated from both normal lungs and diseased lung to those isolated from the normal upper respiratory system. This is quite suggestive of the idea that these bacteria live as commensals in the upper respiratory system and invade the lungs under stress conditions. This is supported by the fact that stress factors tend to weaken the mucocilliary clearance system and generally the respiratory defense system which then allows proliferation of normal flora leading to an abrupt shift from being commensal bacteria to being pathogenic bacteria (Brogden *et al*., 1998).

From this study, *Staphylococcus aureus* had the highest susceptibility to Gentamycin, Chloramphenicol and Kanamycin followed by Tetracycline and Sulphamethoxazole, followed by Ampicillin, followed by Co-trimoxazole and Streptomycin. These results were to some extent agreeable with those of Abdulsalam, (1999); he found *Staphylococcus aureus* to be highly sensitive to Ampicillin, Doxycycline, Streptomycin, Gentamycin and Neomycin. The results of

this study were also in agreement with one reported by Al-Doughaym, (1999). A study by Gitao *et al*, (2014) reported *Staphylococcus aureus* from mastitis milk in camels to be resistant to Ampicillin, Co-Trimoxazole, and Sulphamethoxazole. In yet another study, Al-Thani and Al-Ali, (2014) reported *Staphylococcus* species to be resistant to tetracycline, Penicillin and Ampicillin in different Qatari farms. This was agreeable to this study's results; resistance was recorded highest in Ampicillin and Tetracycline. However Al-Thani and Al-Ali, (2014), reported the organisms to be very susceptible to Cephalothin, norfloxacin and Co-Trimoxazole. A high incidence of resistance in *Staphylococcus* species was also reported by Mártonová *et al*, (2008).

Streptococcus organisms had the highest susceptibility to Tetracycline followed by Ampicillin, they were also sensitive to Chloramphenicol and Gentamycin, followed by Sulphamethoxazole, then Co-Trimoxazole and they were least susceptible to Streptomycin and Kanamycin. This was in agreement to results got by Abdulsalam (1999) where *Streptococcus* isolates were sensitive to Ampicillin and Doxycycline. However Abdulsalam (1999) reported *Streptococcus* organisms to be highly susceptible to Streptomycin at 100%.

All (100%) the 3 isolates of *Streptococcus agalactiae* tested, showed marked sensitivity to Gentamycin, Sulphamethaxazole and Chloramphenicol. The isolates were sensitive to Co-Trimoxazole and Tetracycline at 66.7%, to Ampicillin, Kanamycin and streptomycin at 33.3%. A study by Gitao *et al*. (2014) reported that *Streptococcus agalactiae* to be more resistant to Ampicillin, Co-Trimoxazole, Nitrofurantoin, Streptomycin and Sulphamethoxazole. The organism was reported to be susceptible to Gentamycin and Tetracycline.

Generally most of the isolates were sensitive to the antimicrobials used. The susceptibility percentages of the organisms in the descending order were as follows; Gentamycin at 95.8%

(91/95), Tetracycline at 90.5% (86/95), Kanamycin at 85.3% (81/95), Sulphamethoxazole and Chloramphenicol at 84.2% (80/95), Co-Trimoxazole at 82.1% (78/95), Ampicillin at 78.9% (75/95) and finally Streptomycin at 76.8% (73/95). Gitao *et al*, (2014) reported mastitis isolates from camel milk to be more susceptible to Gentamycin and Tetracycline. They also showed that most organisms had marked susceptibility to Co-Trimoxazole, Sulphamethoxazole and Ampicillin. A variety of studies in the last two decades have shown resistance to be increasing to the commonly used antimicrobials including Co-Trimoxazole, Ampicillin, Tetracycline, and Chloramphenicol (Okeke *et al*., 2000; Iwalokun *et al*., 2001). The studies have also indicated the presence of low levels of resistance to Nalidixic acid and Fluoroquinolones. This is due to the fact that in the sub-Saharan countries the commonly used first line antibiotics include: Ampicillin, Gentamycin, Tetracycline, Penicillin, Co-Trimoxazole and Chloramphenicol with the second line of antibiotics varying with the locality. However, it becomes quite disheartening in the view that in most developing countries there is lack of enough second line antibiotics, making it difficult to manage resistant infections (Fasehun, 1999). Similar results were also reported by Muna *et al,* (2015) where bacteria isolated from camels suffering from pneumonia were susceptible to Gentamycin. Presence of antimicrobial resistance in nonpathogenic bacteria (normal flora) has been reported in non-pathogenic *Escherichia coli* in young children in Kenya; they were resistant to Ciprofloxacin and commonly used antibiotics like Co-Trimoxazole, Tetracycline, Ampicillin and Chloramphenicol (Kariuki, 2009; Kariuki, 2010). In yet another study to determine the antimicrobial resistance of *E. coli* in adults in both urban and rural areas in different countries in healthy individuals showed that *E. coli* had very high resistance to Ampicillin and Tetracycline at 89% and 92% respectively; higher in urban than rural areas (GARP, 2011).

Although most organisms showed marked susceptibility to the antimicrobials used in this study, there were some isolates which showed resistance to them; this is quite important as this shows normal flora/resident bacteria can harbor resistance genes to antibiotic(s). Transfer of antimicrobial resistance in bacteria has been documented to occur between different animal species, within humans, from animals to humans and from humans to animals (Marshall *et al*., 1990). This makes it worth noting that transfer of resistance genes can occur to otherwise susceptible pathogenic bacteria making them difficult to treat, not to mention transfer of resistance to human pathogenic bacteria as a result of the interaction between pastoral communities and camels. The impacts of antimicrobial resistance in both humans and animals cannot be overemphasized, with respect to the great economic and social effects to the concerned communities; including higher numbers of hospitalization cases, longer hospital stay leading to contracting of nosocomial infections, and increase in the cost of disease treatment. This is even worse in the developing countries where the economy is still struggling (Kapil, 2005).

Overuse and improper use of antimicrobials has contributed to development of multidrug resistance in bacteria (Shryock and Richwine, 2010; Ding and He, 2010). In this study a variety of genera and species of bacteria showed resistance to more than one antibiotic out of the eight different antibiotics tested. Generally, out of all the isolates tested, 30.5% (29/95) showed resistance to more than one antimicrobial tested against. Both coagulase negative and coagulase positive *Staphylococcus* showed resistance to multiple drugs. This was also reported in other studies (Normand *et al*., 2000; Authier *et al*., 2006; Al-Thani and Al-Ali, 2012). Bhatt *et al*, (2014) reported isolating a high proportion of multidrug resistant *Staphylococcus* organisms from surgical wounds. This presents a great threat as these organisms are commonly associated

with a good number of animal and human infections. Bhatt *et al,* (2014) also reported 56.4% MDR in Gram positive bacteria and 66.9% in Gram negative bacteria. Similar results were reported by Mulu *et al,* (2006), where a significant percent of isolates were found to have multiple antimicrobial resistances. In yet another study, Raza *et al,* (2013) reported multidrug resistance in Gram positive and Gram negative organisms to be at 47.5% and 88.33%, respectively. Expression of multiple drug resistance indicates that resistant organisms are developing mechanisms to counter effects of different antimicrobials that are in use for treatment of bacterial infections.

Imprudent use of antimicrobials to treat any infections including parasitic and viral diseases by physicians and veterinarians, and in agriculture and aquaculture, has increased the selective pressure of bacteria with no known benefits to their patients and the community (Barbosa and Levi 2000; Feinman, 1998; Blackman, 2002; Angulo *et al*., 2004). Use of sub-therapeutic doses and poor quality antimicrobials has also contributed to selection of resistant strains to antimicrobials and subsequent spread to other bacteria. Other than providing selection pressure to resistant bacteria, studies have reported that humans encourage spread of resistance to antimicrobials by provision of suitable environmental conditions for bacterial multiplication and exchange of genetic elements. Just to mention but a few, poor sanitation, warm and moist environmental conditions; these contribute not only to the multiplication of bacteria but also the spread of resistance to organisms which might not be necessarily pathogenic. These organisms present a threat as they act as reservoirs for antimicrobial resistance genes (Okeke, 2000; Iwalokun *et al*., 2001).

In this study, there was similarity of bacteria isolated from both normal lungs and diseased lung to those isolated from the nasal cavity of apparently healthy camels. This suggests the idea that

these bacteria live as commensals in the upper respiratory system and invade the lungs under stress conditions causing disease. Presence of the antimicrobial resistance is not to be ignored as there is possibility that the antibiotic resistant normal flora (bacteria) may be harboring resistance genes which are transferable to pathogenic bacteria in the animal, not to mention transfer of resistant bacteria to other animals and humans.

6.0 Conclusions

- Different genera and species of bacteria were isolated from the nasal cavity of camels from Nakuru, Samburu and Isiolo counties, Kenya.

- Different bacteria (genera and species) were isolated from both normal lungs and lungs with pathology from Athi river camel slaughterhouse. These bacteria had similarity with those isolated from the nasal cavity. Bacteria isolated from the nasal cavity of apparently healthy camels were also documented in other studies to be involved in active causation of respiratory diseases in camels. This suggests the involvement of normal flora in disease causation during episodes of stress like transportation, adverse climatic change, immunosuppression and change of feed.

- This study recorded resistance in normal flora from the nasal cavities of camels in Nakuru, Samburu and Isiolo counties, Kenya.

- In this study Gentamycin and Tetracycline were found to be the drugs of choice currently in treatment of respiratory or other camel bacterial diseases.

7.0 Recommendations

- Awareness programs need to be carried out to alert camel owners on dangers of misuse use of antimicrobials to avoid emergence and spread of antimicrobial resistance.

- Awareness creation to Veterinary surgeons and veterinary paraprofessionals on prudent use of antimicrobials to prevent emergence and spread of antimicrobial resistance in camels.

- Sensitivity testing of bacteria before treatment should be done to ensure prudent use of antimicrobials

- Efforts should be made by government, researchers and non-governmental organizations in tackling the spread of antimicrobial resistance in camels.

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9.0 Appendices

9.1 Appendix 1: colony characteristic recording sheet.

Colony characteristics on pure culture								
Sample number	**Sample id**	**shape**	**Edge**	**hemolysis**	**size**	**opacity**	**pigmentation**	**Remarks**

9.2 Appendix 2.Staining reaction and cellular morphology recording draft sheet

Sample number	Staining reaction	Shape	Size	Arrangement	Staining	Remarks

9.3 Appendix 3: Primary identification criteria draft sheet

Sample id	**Growth on BA**	**Growth on MacCkonkey**		**Gram reaction**	**arrangement**	**catalase**	**oxidase**	**genera**
		LF	NLF					

9.4 Appendix 4: Main differential characteristics of Gram positive bacteria.

Sample id	**Growth aerobically**	**Hemolysis**	**Gram reaction**	**catalase**	**Coagulase**	**Gelatin liquefaction**	**CAMP test**	**Result**
	+	+/-	Gram positive cocci in clusters	+	+	NA	NA	*Staphylococcus aureus*
	+	+	Gram positive rods with spores	+	NA	+	NA	*Bacillus* spp
	+	+	Gram positive cocci in chains	-	NA	NA	+	*Streptococcus agalactiae*
	+	+/-/partial	Gram positive cocci in chains	-	NA	NA	-	Other *Streptococcus* spp

+=positive, -=negative, NA=not applicable

9.5 Appendix 5: Antibiotics susceptibility testing registration format

Sample id	**Antibiotic disk used**	**Disk code**	**potency**	**Zone of inhibition in mm**	**Interpretation**
					S or R

S=susceptible R=Resistant

9.6 Appendix 6: Zone interpretation criteria for *Staphylococcus* organisms.

Antibiotic used	**Disk content**	**Diameter of zone of inhibition to the nearest mm**	
		Resistant	**Susceptible**
Ampicillin		≤28	≥28
Tetracycline		≤14	≥15
Streptomycin		≤11	≥12
Co-Trimoxazole		≤10	≥11
Kanamycin		≤13	≥14
Gentamycin		≤12	≥13
Sulphamethoxazole		≤10	≥11
Chloramphenicol		≤12	≥13

9.7 Appendix 7: Zone interpretation criteria for *Streptococcus* and *Bacillus* organisms

Antibiotic used	**Disk content**	**Diameter of zone of inhibition to the nearest mm**	
		Resistant	**Susceptible**
Ampicillin		≤21for *Streptococcus* organism ≤10 for *Bacillus*.	≥22 for Streptococcus organisms and ≥11 for *Bacillus*
Tetracycline		≤14	≥15
Streptomycin		≤11	≥12
Co-Trimoxazole		≤10	≥11
Kanamycin		≤13	≥14
Gentamycin		≤12	≥13
Sulphamethoxazole		≤10	≥11
Chloramphenicol		≤12	≥13

9.8 Appendix 8: Primary identification biochemical tests used

Gram's stain

Gram stain is a test commonly used to differentiate bacteria into two broad categories; Gram positive and Gram negative bacteria (Forbes *et al*., 2002)

Procedure: A thin smear of the test colony is made on a glass slide and allowed to dry by air. The smear is then heat fixed by passing through a Bunsen burner flame 3-4 times. The slide is flooded with crystal violet for 1 minute and washed with running tap water. Iodine is added on the slide (mordant) for 1 minute and washed with running tap water. The slide is then decolorized with acetone for 6-7 seconds after which the slide is again washed with running tap water. The slide is then counter stained with safranin for 1 minute and washed with tap water and air dried or blot dried and then observed with a microscope at x100

Catalase test

Catalase test demonstrates the presence of enzyme catalase in an organism. The presence of the enzyme mediates the breakdown of hydrogen peroxide to oxygen and water. Evidence of the

enzyme is indicated when a small inoculum of the test organism is introduced on a slide with 3% hydrogen peroxide and rapid effervescence of oxygen bubbles occurs. Lack of the enzyme in organism is indicated by absence of gas bubbles. The test is commonly used to differentiate *Staphylococcus* from *Streptococcus* species (Forbes *et al*., 2002)

Procedure: one or two colonies are taken from a plate of pure culture (in this case from MacCkonkey agar. Blood agar gives false positive results) and placed on a clean glass slide. A drop of 3% H_2O_2 is added. Presence of effervescence indicates a positive test.

Slide coagulation test

Coagulase test is used to differentiate *Staphylococcus aureus* (coagulase positive *Staphylococcus*) and other coagulase negative *Staphylococcus*. *Staphylococcus aureus* produces coagulase which normally clots plasma into gel in tube or agglutinate cocci in slide. Most strains of *Staphylococcus aureus* produce two forms of coagulase, free and bound coagulase. Bound coagulase is detected in slide coagulase test while free coagulase is detected in tube coagulase test. Slide coagulase test can be used detect isolates of *Staphylococcus aureus* and tube coagulase test can be used for confirmation. The principle behind this test is that, the bound coagulase cross-links the α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual cocci stick to each other and clumping is observed (Forbes *et al*., 2002)

Procedure: On a clean glass slide, the test organism is treated with a drop of rabbit plasma and mixed well. The slide is then rocked back and forth gently for about 10-15 seconds. Clumping of the plasma seen with unaided eye is taken as positive.

Oxidase test

Oxidase test is used to determine the presence of bacterial cytochrome oxidase enzyme using the oxidization of the substrate "tetramethyl-p-phenylenediamine dihydrochloride" to indophenol a dark purple colored end product. A positive test (presence of oxidase) indicated by the development of a dark purple colour. No colour development indicates a negative test and the absence of the enzyme (Forbes *et al*., 2002).

Procedure: sterile distilled water is used to moisten oxidase test strips on a glass slide. The test strip is then streaked with a test organism with a sterile splint. Development of purple colour within 10 seconds indicates a positive reaction.

Gelatin liquefaction test

The test is performed to test for the ability of an organism to produce enzyme gelatinase. Organisms producing gelatinase liquefy gelatin from its solid form to liquid form (Forbes *et al*., 2002)

Procedure: The test organism is stab inoculated in a bottle of solid gelatin and left to stand in room temperature for at least 72 hours. Change of gelatin from its solid state to liquid state is considered positive.

CAMP test.

The hemolytic activity of the beta-hemolysin produced by most strains of *Staphylococcus aureus* is enhanced by extracellular protein produced by group B *Streptococci* (*Streptococcus agalactiae*). Interaction of the beta-hemolysin with this factor causes "synergistic hemolysis," which is easily observed on a blood agar plate (Forbes *et al*., 2002)

Procedure: on the center of a Blood agar plate a straight streak line of beta hemolytic *Staphylococcus aureus* is made, several streaks of beta hemolytic *Streptococcus* are made perpendicular to that of *Staphylococcus aureus* making sure that they do not touch each other. The plates are incubated for 18-24 hours at 37^0C. *Streptococcus agalactiae* (Lancefield group B) produce a characteristic arrow head shape clear zone of hemolysis around a zone of complete hemolysis by *Staphylococcus* beta hemolysin toxin on blood agar (Figure 9).

Indole reaction

Indole test is done to test the ability of an organism to split tryptophan molecule into Indole which is one of the metabolic product of amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of Indole, Pyruvic acid and ammonia. The test is done to differentiate members of *Enterobacteriacea* (Forbes *et al*., 2002).

Procedure: peptone water is inoculated with the test organism and incubated for 18-24b hours at 37^0C. Kovac's reagent is then added to the broth. Development of a red ring indicates a positive reaction.

Methyl red test.

This test determines the ability of an organism to produce and maintain stable acid end products from glucose fermentation and to overcome the buffering capacity of the system. when glucose broth is incubated with the test organism for 72 hours and drops of Methyl red added, a red color indicates a positive test (PH below 6) while a yellow color indicates a negative test (*Forbes et al*., 2002).

Procedure: Glucose Phosphate Peptone water (GPPW) is inoculated with a test organism and incubated at 37^0C for 18-24 hours, after which MR reagent is added to the broth. Development of a red colour indicates a positive test.

Citrate Utilization test.

The test is based on the ability of an organism to use citrate as its only sole source of carbon and ammonia as its only source of nitrogen (Forbes *et al*., 2002).

Procedure: The test organism is cultured in a medium which contains sodium citrate, an ammonium salt and the indicator bromothymol blue. Positive test is indicated by turbidity and a change in colour of the indicator from light green to blue, due to alkaline reaction following citrate utilization. No colour change from light green to blue is considered a negative test.

Triple sugar iron agar test (TSI)

TSI agar is used to determine whether a gram negative rod utilizes glucose and lactose or sucrose fermentatively and forms hydrogen sulphide (H_2S).

The formation of CO_2 and H_2 is indicated by the presence of bubbles or cracks in the agar or by separation of the agar from the sides or bottom of the tube. The production of H_2S is indicated by blackening of the butt of the medium in the tube (Forbes *et al*., 2002).

Procedure: using a straight inoculation loop, the test organism is streaked in the TSI from the butt to the slant and incubated for 18-24 hours. *E. coli* was indicated by yellowing of both the butt and the slant with gas production.

9.9 Appendix 9: Common media used in the study

Blood agar (oxoid, Hampshire England)

Formula in grams per liter: Lab-Lemco powder---10.0; Meat peptone---10.0; Sodium chloride---5.0; bacteriological agar---15.0 & the final PH is 7, 3± 0, 2 at 25^{o}c.

Preparation: 40 grams of the medium was suspended in 1 litre of distilled water. This was gently agitated for 5 minutes, boiled to dissolve, and then sterilized at 121^{o}C for 15 minutes. The medium was then cooled to 40^{o}C. 5% defibrinated bovine blood was then added, swirled gently to avoid hemolysis of RBCs and then poured in to petri dishes and allowed to cool and solidify. One Blood agar plate was then left in the incubator at 37^{o}C for 18-24 hours to test for the sterility of the media.

MacCkonkey agar (oxoid, Hampshire England)

Formula in grams per liter: peptone---20.0; Lactose---10.0; agar---12.0; Bile salts---5.0; Neutral red---0.075 and the final PH is 7, 1± at 25 ^{o}c.

Procedure: 50 grams of the medium was dissolved in 1 litre of sterile distilled water. The mixture was then boiled to dissolve completely and then sterilized at 121^{o}C for 15 minutes. The medium was then allowed to cool to around 40^{o}C after which it was poured in petri dishes.

Muller –Hinton agar (Oxide, Hampshire, England)

Formula in grams per liter: Beef dehydrated infusion from---300.0; Casein hydrolysate---17.5; Starch---1.5; Agar---17.0 and the final PH is 7.3 ± 0.1 at 25^{O}C.

Preparation: 38 gram of the medium was suspended in 1 litre of distilled water. The mixture was dissolved by boiling until a uniform suspension was obtained. It was sterilized using an autoclave at 121^{o}C for 15 minutes. It was then allowed to cool to abou45^{o}C. Finally the medium was poured in to sterile Petri plates and allowed to solidify and then stored at +4^{o}C.