ANTIMICROBIAL RESISTANCE PROFILE AND CHARACTERIZATION OF BETA-LACTAMASE-PRODUCING *ESCHERICHIA COLI* IN CAMELS REARED FROM LAIKIPIA COUNTY

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Antimicrobial Resistance Profile and Characterization of Beta-lactamase-producing *Escherichia coli* in Camels Reared from Laikipia County

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to the One Health Research Group at the Institute of Primate Research, Mpala Research Centre, the pastoral communities of Laikipia County, and lastly, the students and staff from the School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology, and ITROMID at the Kenya Medical Research Institute.

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ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial resistance
ARGs	Antibiotic Resistance Genes
ASALs	Arid and Semi-Arid Lands
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
Bla	Beta-lactamase gene
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CTX-M	CefoTaXimases ‘Munich’
DDST	Double Disk Synergy Test
DNA	Deoxyribonucleic Acid
E. coli	Escherichia Coli
ESBLs	Extended Spectrum Beta-Lactamase
FAO	Food and Agriculture Organization
GLASS	Global Antimicrobial Surveillance System
MBLS	Metallo-beta-lactamases
MDR	Multi-drug Resistance

MRSA	Methicillin-resistant Staphylococcus Aureus
OXA	Oxacillinase Enzymes
PCR	Polymerase Chain Reaction
SHV	Sulfhydrl Variable Enzymes
Spp	Species
TEM	Temoneira Enzymes
WHO	World Health Organization
WOAH	World Organization for Animal Health

ABSTRACT

The abuse and misuse of antimicrobials in clinical and agricultural setups have led to an increased interaction of these agents with human, livestock, and environmental microbes. Camel husbandry management practices are hypothesized to be facilitating exposure to antibiotics, development of antimicrobial resistance (AMR), and spread of zoonotic microbes in Kenya's Arid and Semi-Arid Lands (ASALs). Disruption in the ecology of microbial communities by antimicrobials in the camel population promotes the spread of antibiotic resistance genes. With increase in camel population by tenfold in the last decade, driven largely by land use changes and widespread use of camel products for nutritional, therapeutic, and cosmetic value, raises possibilities of Extended Spectrum Beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*), in camels reared in animal production system. This present study aimed at assessing the antimicrobial susceptibility profile, detecting, and characterizing beta-lactamase producing *E. coli* recovered from camels. Fecal swabs were aseptically collected from 304 camels reared by pastoral communities (extensive production system), (n=137), and ranches (intensive production system), (n=167), and stored in Cary Blair transport media for processing. One hundred and twenty-three (123), *E. coli* isolates were isolated from fecal swabs of camels reared in the intensive and extensive livestock production. Among the *E. coli* (n=123), recovered, the highest resistance was observed in Cefaclor 35(28.46%), followed by Cefotaxime 20(16.26%), and Ampicillin12(9.76%). Four (4) ESBL-producing *E. coli* with multi-drug resistance phenotype harbored $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}27}$ genes were identified from these isolates and associated to phylogenetic group B1, B2, and D. Multiple variants of non-ESBL bla_{TEM} gene variants were identified, majority of which were the $bla_{TEM\text{-}1}$ and $bla_{TEM\text{-}116}$ genes. In terms of AMR and beta-lactamase gene prevalence, there was no statistically significant difference between intensive and extensive camel rearing production systems (p-value = 0.61, 95% CI). This study's findings provide insight on the diverse variants of ESBL and non-ESBL producing genes in *E. coli* from camels in the farming practices. Furthermore, advocates for One Health approach in camel production in ASALs to understand antimicrobial resistance transmission mechanism, risk factors, and proper antimicrobial management practices in camel production systems within ASALs.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Antimicrobial resistance (AMR) is a critical and escalating global health threat in clinical and veterinary medicine. Recent studies indicate that nearly 5 million human deaths in 2019 were associated with bacterial AMR, including over 1.27 million deaths directly attributable to it (Murray et al., 2022). Factors that promote the spread of AMR are often multifaceted, with abuse or misuse of antibiotics being the primary driver of AMR emergence (Vikesland et al., 2019). Resistance resulting from poor antibiotic stewardship represents a significant threat to global health, livestock systems and food security (Wall et al., 2016; WHO, 2020). A study estimated that by 2050, per cent gross domestic product (GDP) loss as it relates to antibiotic resistance will reach up to 5–10% in Sub-Saharan Africa (Taylor et al., 2014). Thus, one health approach in the surveillance and stewardship system for humans, animals, and the environment is advocated in addressing antimicrobial resistance.

One-humped camels (*Camelus dromedarius*), in contrast to other livestock in Kenya, have gained greater reverence for their crucial contribution to the country's arid and semi-arid lands' (ASAL), food security (Oselu et al., 2022a). Kenya has the world's fifth-largest camel herd, with an estimated 4.66 million(FAOSTAT, 2022). The significance of camel keeping in Kenya and other regions is increasingly recognized due to the wide range of uses for camel products, which are valued for their nutritional, cosmetic, and therapeutic properties(Oselu et al., 2022a; Swelum et al., 2021). In some communities, for example, camel milk, meat, and offals like liver are consumed raw as they are believed to have medicinal properties for curing ailments(Abrhaley & Leta, 2018). Given the extensive utilization of camels and their byproducts, there is a pressing need to address concerns of the contribution in zoonotic diseases and antimicrobial resistance through prompt action.

Modifications in livestock management practices, along with heightened interactions between livestock and wildlife, are increasingly recognized as significant contributors to the dissemination of zoonotic pathogens and antimicrobial resistance (Bartlett et al., 2022; Graham et al., 2019). In Laikipia North County, Kenya, camels are reared under two primary production systems: the extensive systems managed by pastoral communities and the intensive or semi-intensive production systems practiced within ranches and conservancies(Noor et al., 2013; Oselu et al., 2022b). The pastoral production system is characterized by a limited number of mobile herds that headers can manage grazing on vast rangeland pasture resources. Furthermore, pastoralists resort to over-the-counter medication and rely on unskilled animal handlers for livestock management (Lamuka et al., 2017). In contrast, intensive farming, which includes specialized ranching, diverse ranching, and zero-range ranching, has emerged as a response to land-use changes and the commercialization of agriculture, particularly aimed at large-scale production of dairy and meat products (Oselu et al., 2022a; Phelps & Kaplan, 2017). Practices such as stocking large numbers of animals, selection, and breeding to increase productivity promote the frequency of physical contact among the animals. This provides a conducive environment for "wild" microbes to invade and multiply or existing microbes to evolve into pathogenic strains within the host(Gilbert et al., 2021; Liverani et al., 2013). Irrespective of the rearing system, camels are likely to acquire antimicrobials and antimicrobial resistance genes (ARGs), through different sources or routes of exposure. Mobile elements such as plasmids and transposons act like a vehicle for mobilizing and transferring ARGs between gene locations and bacterial hosts in such environments (Roca et al., 2015; Yuan et al., 2019).

The emergence of antimicrobial resistance to a broad spectrum of beta-lactams has led to the synthesis of beta-lactamases with a broad spectrum, such as extended-spectrum beta-lactamases (ESBL), ampC beta-lactamases, and metallo-beta-lactamases (MBLs),(Smet et al., 2010). ESBLs are plasmid-encoded enzymes that degrade the beta-lactam ring of extended-spectrum cephalosporins, including Cefotaxime, Ceftriaxone, Ceftazidime, or Cefepime and Aztreonam (Gonçalves et al., 2010; Leigue et al., 2013).

These enzymes, such as TEM and CTX-M beta-lactamases are as a result of point mutations at particular loci within the beta-lactamase-encoding gene, resulting in single or multiple amino acid substitutions (Bradford, 2001). There has been an increase in broad-spectrum beta-lactamase-producing Enterobacteriaceae, primarily *Escherichia coli* (*E. coli*), associated with intra-abdominal and urinary infections in food-producing animals(Fashae et al., 2021; Langata et al., 2019; Okoko et al., 2020; Ramadan et al., 2019). *E. coli* is one of the clinically significant Enterobacteriaceae commonly found in the gastrointestinal tracts of animals and humans as a commensal. However, certain strains that carry resistance genes and toxins pose numerous challenges to clinical therapies (Lupindu, 2017). These strains typically belong to one of eight phylogenetic groups (A, B1, B2, C, D, E, F, and Escherichia clade I), which differ with respect to genetic variation, degree of pathogenicity, and ecological niche(Clermont et al., 2013). ESBL-producing Enterobacteriaceae have been prioritized within the field of novel therapeutic strategy development due to their impact on both human and animal health(WHO, 2023). Beta-lactamase-producing Enterobacteriaceae have been isolated from the gut of both healthy camels and camels experiencing clinical diarrhea (Alonso et al., 2016; Bessalah et al., 2016; Carvalho et al., 2020).

Given the growing camel population within Kenya, the potential for widespread dissemination of these bacterial strains and their beta-lactamase genes represents an important public health threat. Different studies have been conducted in Kenya to determine AMR profiles in food-producing animals reared in different animal production settings(Aliwa et al., 2019; Ngaywa et al., 2019). Nonetheless, there is little information on the diversity of emerging beta-lactamase genes found in bacterial isolates from camels in Kenya, as well as comparative studies assessing the effect of different camel production systems on AMR development and dissemination. This study determined the antimicrobial resistance profile and diversity of beta-lactamase genes found in camels reared under intensive and extensive livestock production systems in Laikipia North County, using *E. coli* as an indicator species from the Enterobacteriaceae family.

1.2 Problem Statement

Camels are susceptible to a wide array of infectious microorganisms. They serve as reservoirs for zoonotic pathogens including *Coxiella burnetii*, Hepatitis E virus, Coronaviruses, Shiga toxin-producing *E. coli*, ESBL-encoding *E. coli,* as well as their associated antimicrobial resistance genes, Concurrently, there is a concerning increase in antimicrobial resistance among food-producing animals. These animals are often subjected to antimicrobial agents for therapeutic purposes, disease prevention, control measures, and growth enhancement. Such exposure to antimicrobials often leads to the selection and proliferation of resistant genes, which can be transmitted within bacterial populations through vertical and horizontal gene transfer among food-producing animals. Beta-lactam antibiotics, which are frequently misused both in clinical settings and livestock production, are a major concern as they represent a class of antibiotics critical for human medicine. The misuse of beta-lactams and anthropogenic activities in camel livestock production may led to an uptick in resistance cases. However, there is a lack of comprehensive data on the antimicrobial resistance profiles and the diversity of beta-lactamase genes from camels reared under intensive and extensive livestock production systems in Kenya. This gap in knowledge hinders the development of effective measures to curb antibiotic misuse in camels. Moreover, there is a need for improved antimicrobial stewardship in veterinary medicine, including the adoption of good husbandry practices that limit the spread of beta-lactamase genes among camels and potentially to humans.

1.3 Justification

Camel husbandry management practices are hypothesized to contribute to increased exposure to antimicrobials and ARGs, facilitating the emergence and spread of antimicrobial resistant microbes. Despite the public health importance, there is a notable lack of comprehensive data on the antimicrobial resistance genes present in the camel population across different animal production systems in Kenya. A few studies have been conducted that shed light on the phenotypic antimicrobial resistance patterns in

camels and their products. For instance, research by Aliwa et al. (2019), identified *Clostridium perfringens* isolates with antibiotic resistance patterns to ampicillin and sulfamethoxazole in camel milk from Isiolo. Similarly, Gitao et al. (2014) found the presence of antimicrobial resistance in camel milk in the northeastern region, specifically in Garissa and Wajir. Mutua et al. (2017) identified antimicrobial resistance in bacterial isolates from the nasal cavity of camels in Isiolo, Nakuru, and Samburu. Furthermore, Nüesch-Inderbinen et al. (2020), reported a single ESBL-producing *E. coli* isolate in fecal samples from camels in Laikipia County.

These studies indicate the occurrence of antimicrobial resistance patterns in camels, but they do not provide exhaustive information on antimicrobial resistance genes in the camel population, especially when analyzing different animal production systems that could have clinical significance for human health. Laikipia County has extensive ranches and lands occupied by pastoralists who practice different livestock production systems. Thus, there is a pressing need for the generation of data to establish a baseline picture of beta-lactamase-producing *E. coli* and beta-lactamase-encoding genes in camels from various production systems. The diversity of beta-lactamase variants, including ESBLs and AmpC beta-lactamases, exhibits varying levels of resistance to cephalosporins, underscoring the dynamic nature of the resistance landscape. Constant surveillance and research efforts are required to monitor these evolving resistance patterns. The collected data will be instrumental in guiding researchers and policymakers in developing appropriate strategies to minimize AMR transmission to humans, either directly or indirectly. Moreover, the findings from such studies will enable farmers to devise strategies focused on changing rearing and handling practices that minimize the evolution and spread of AMR in camel populations.

1.4 Research Questions

1. What is the antimicrobial resistance profile of *E. coli* in fecal swabs from camels reared under intensive and extensive production systems in Laikipia County, Kenya?

2. Which beta-lactamase genes and variants are expressed by *E. coli* in fecal swabs from camels raised in Laikipia County under intensive and extensive production systems?
3. How do the beta-lactamase gene variants expressed by *E. coli* from camels reared under different production systems in Laikipia County, Kenya, relate phylogenetically?

1.5 Objectives

1.5.1 General Objective

To determine the antimicrobial resistance profile and molecular characteristics of beta-lactamase in *E. coli* from camels reared under intensive and extensive production systems in Laikipia County.

1.5.2 Specific Objectives

1. To determine the antimicrobial resistance profile of *E. coli* in fecal swabs from camels reared under intensive and extensive production systems in Laikipia County, Kenya.
2. To screen and characterize beta-lactamase genes and gene variants expressed by antibiotic-resistant *E. coli* in fecal swabs from camels reared under intensive and extensive production systems in Laikipia County, Kenya.
3. To conduct phylogenetic analysis of beta-lactamase gene variants expressed by *E. coli* in fecal swabs from camels reared under intensive and extensive production systems in Laikipia County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to Antimicrobial Agents and Antimicrobial Resistance

In the 19th century, the importance of veterinary medicine was known since there was an association between infections in animals and humans. A large number of diseases resulted because of these infectious diseases, and microorganisms were known to be the causative agents (Aarestrup, 2006; Saga & Yamaguchi, 2009). To mention but a few, in 1874, Fleming proved that tuberculosis could be transmitted between animals; Koch went further to identify the causative agent of Anthrax; and in 1855, August Gaertner isolated the bacterium enteritidis from a cow with diarrhea. It has since been proven that bacteria can spread from animals to people and result in disease. Since then, it has been established that bacteria can be transmitted from animals to humans and cause diseases (Aarestrup, 2006; Murray et al., 2022). Later in the 20th century, there was the discovery of antibiotics that led to the sophisticated targeting and treatment of infections. The first antibiotic discovered was against syphilis by Ehrlich and Fleming, followed by the discovery of penicillin in 1928(Aarestrup, 2006). More antibiotics were discovered, laying the foundation for many medical advances that revolutionized the healthcare system. Thus, according to the WHO, ‘antibiotic’ or ‘antimicrobial’ refer to medicines used to inhibit and treat bacterial infections (WHO, 2017).

Unfortunately, the discovery of antibiotics that were regarded as lifesaving has caused a threat that poses a threat to many lives so far. The threat of "antimicrobial resistance" is the capacity of microorganisms (such as bacteria, viruses, and some parasites) to thwart the effects of an antimicrobial (such as antibiotics, antivirals, and antimalarials) (WHO, 2017). Therefore, microorganisms that cause infection can continue to spread unrelentingly due to ineffective standard treatments. This resistance mechanism in microorganisms occurs as a result of new mutations (insertion, deletion, or point mutation) and the transfer of genetic information that encodes resistance in

microorganisms through horizontal gene transfer(Davies & Davies, 2010). The authors further emphasize on antibiotic selective pressure on microorganisms creates more dominantly resistant organisms as a result of long exposure to antimicrobials while phasing out their susceptible counterparts. The first antimicrobial resistance was noted a year later, after the discovery of penicillin. Penicillin was once effective against *Staphylococcus aureus*; however, the introduction of penicillinase-producing resistant bacteria led to a rise in penicillinase-stabilized methicillin(Powers, 2004). Thereafter, a lot of antibiotic resistance was noted in different parts of the world. This resistance to antibiotics is attributed to bacteria and other pathogens that evolve with the aim of surviving by resisting the new drugs used to fight them. In addition, the pace at which novel antibiotics are being discovered is drastically slowing down, while antibiotic use is rising(Kaushik et al., 2014; WHO, 2020). Thus, AMR has since become a public health concern worldwide complicating the progress of curbing infection, which has heightened hospitalization and mortality cases(Allcock et al., 2017).

2.2 Global burden of Antimicrobial Resistance

There are numerous challenges when it comes to estimating the load of AMR globally. The main factors limiting the estimation of AMR burden include partial and variable data of recent and past studies about circulation and prevalence of AMR and or ARGs including its health implications (Hay et al., 2018). Conversely, to mitigate this setback, WHO launched a Global Antimicrobial Surveillance System (GLASS), aimed at providing a standardized approach and central platform to every country for collection, analysis and sharing of AMR data (Pessoa-Silva, 2018; WHO, 2020). Infections brought on by resistant microorganisms are currently estimated to be the cause of 700,000 deaths worldwide each year, and by 2050, that number is expected to rise to 10 million(Europe, 2014; O'Neill, 2014). This review further highlighted the AMR related mortality forecast to cause a hit to global domestic product of 2% to 3.5%, which amounts to between 60 and 100 billion US dollars.

2.3 Camels

2.3.1 Classification of Camels

Camel is the common name for large, humped, long-necked, even-toed ungulates belonging to the genus Camelus of the Camelidae family; order Artiodactyles and suborder Tylopoda (animals with padded feet). Although camels are ruminants, there is a significant distinction between them and other ruminants in the bovinae family(Kadim, 2012) . The Camelidae family contains two varieties of camelids. They consist of both large and miniature camelids belonging to the genera Camelus, Lama, and Vicugna shown in Figure 2.1. Small camelids such as the lama, alpaca, guanaco, and vicuna are native to the Andes Mountains of South America (Kadim, 2012). The one-humped camel (*Camelus dromedarius)*, also known as Arabian camels, were domesticated in the Arabian region approximately five to six thousand years ago (Almathen, 2016; Trinks et al., 2012). They inhabit the hot, arid lands of North Africa and Eastern Asia, while the two-humped camel (Bactrian camel), inhabits the frigid steppes and deserts of Central Asia (Faye, 2014)**.**

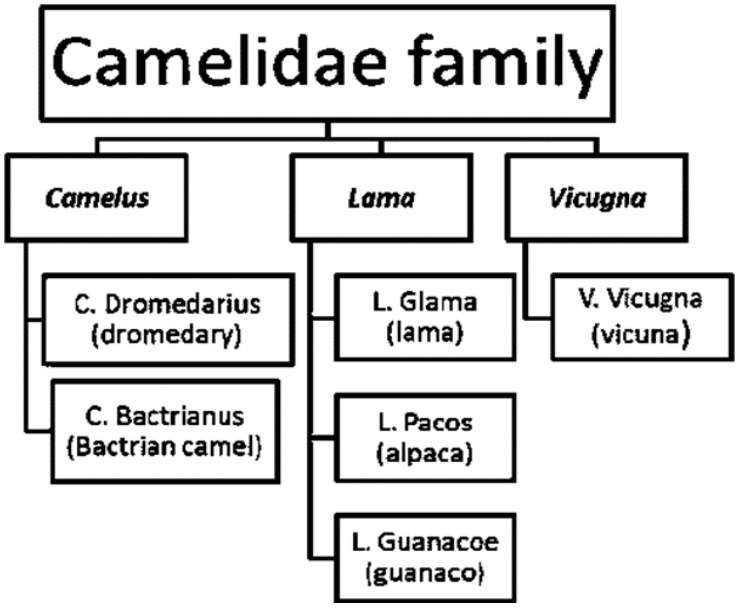

Figure 2.1: Classification of Camelidae Family

2.3.2 The Camel Population and Distribution

The exact head count and distribution of camels vary from one region to the next. Camel is an animal kept by pastoral communities who, by their nature of lifestyle, regularly shift from one place to another and are vaccinated optionally depending on the community; their exact number becomes difficult to estimate(Faye, 2016). The camel population has been steadily increasing parallel to annual growth(A et al., 2019). In comparison to other food-producing animals around the world, camel population has immensely outnumbered most of species like horse, cattle, and sheep except goat population with a slight margin (Oselu et al., 2022a). Africa, especially the Horn of Africa was a home of most of the camel population compared to other continents. Somalia, Sudan, Ethiopia, Niger, Mauritania, Chad, Kenya, Mali, and Pakistan are among the significant countries whose economies benefit from having more than one

million camel heads (FAOSTAT, 2022). Countries in the Far East, such as Afghanistan, Oman, Saudi Arabia, China, Mongolia, India, the United Arab Emirates, and Yemen, are also known for their camel farming(Faye, 2014).

2.3.3 The Camel, a Multipurpose Animal

The economic and cultural significance of camels bred in Kenya's arid and semiarid regions and beyond the country's borders is diverse. These camels are maintained for the sale or personal consumption of milk and meat, the provision of transportation, the assistance in agricultural work, therapeutic and cosmetic purposes, sports derbies, and as a symbol of wealth, respect, and social practices in some pastoral communities, as well as for beauty contests. The primary objective of camel breeding is the production of milk and meat.

2.3.3.1 Importance of Camel in Dairy Production

Milk is one of the significant products from camel production which serves great importance not only for the local populations in arid lands but also for emerging peri-urban markets. There are 3 major reasons for camel milk production. They include: (i). Contribution to food security for communities in the arid and semi-arid lands, (ii). Growing awareness for benefits of camel milk from the urban population and a call for market opportunity, (iii). Inclinations in the advancement of dairy camel intensive system which could be profitable for settled farmers(Faye, 2015). In the worldwide market, camel milk has not been fully integrated though countries like Kenya, Saudi Arabia and Mauritania, camel milk is widely used. FAO statistics showed an increment of 2.45% annually in the camel milk production more than the camels' population(Faye, 2014). Although camels thrive well in areas experiencing harsh weather conditions, they can produce enough quantities of milk to feed and support the livelihood of communities and private ranchers living in these arid areas. The number of camels is steadily rising in neighboring places like Laikipia, Samburu, Pokot, Kajiado, Narok, and many other areas of Kenya because of pastoral communities and other local investors realizing the value

of camels. Pastoral communities have connected these favorable health effects to the milk composition attributed to desert plants that free-roaming camels graze on(Faye, 2016).

2.3.3.2 Contribution of Camel in Meat Production

Estimates of the global production of camel meat are based on the mean carcass weight of the animals and the known number of slain animals. Since many camels are killed in unofficial routes, their numbers are not included in the total statistics(Faye, 2014). The camel is restricted to dry regions, thus the nations where it is located evaluate its contribution. According to data supplied by(Kadim, 2012), 67.7% and 27.6% of camel meat is produced in Africa and Asia, respectively. Small camelids are the source of meat in South America. Camel meat's contribution varies by location, but health concerns are driving demand. Camel carcasses had lower fat (1.2–1.8%) and higher water (4–8%) than beef (A et al., 2019). Camel steak is healthier than beef steak since it has 50 mg/100 g cholesterol compared to 65 mg(Abrhaley & Leta, 2018). Pastoral and other Kenyans like camel meat, making it increasingly popular. Guliye and his colleagues found butcher shops selling camel meat in Nairobi, Mombasa, Kisumu, and other cities. Camel meat sales in arid and semi-arid regions support the local economy and pastoralists. Kenya exports butcher camels to the underserved Arabian Peninsula(Noor et al., 2013).

2.4 Camel Rearing in Kenya

Apart from other climatic conditions that support various economic activities, Kenya is also characterized by desert climate, ASALs.70-88% of total Kenyan lands are ASALs (Ogutu et al., 2016). These lands are known to support livestock rearing since crop production is not well supported by such conditions. Kenya is ranked the fifth country in the world to have the largest camel population(FAOSTAT, 2022; Robinson et al., 2014). Due to climate change and land usage, the camel population has been growing in comparison to the population of 0.8 million from the 1999 census(FAOSTAT, 2022, Oselu et al., 2022a). Their biological and physiological characteristics have enabled

them to withstand the severe environmental conditions in the ASAL(Noor et al., 2013). Furthermore, they consume less amount of water and have body mechanism that ensures water is retained in the body for many days, extraordinary tolerance of dehydration and normal volume of blood(Kagunyu & Wanjohi, 2014). As a result, the camel production system is an essential source of income for communities living in Kenya's ASALs. The management of livestock is thought to be contributing to the spread of zoonotic pathogens and the rise in antibiotic resistance. The animal production system is classified into three categories: Intensive production system (zero-grazing/ranching), Extensive production system (pastoralism) and semi-intensive production system. These categories of the livestock production system are based on socioeconomic factors and land use.

2.4.1 Extensive Production System- Pastoralism

Pastoralism is described as active herding of domestic herd animals characterized by the use of shared rangelands for subsistence, trade and or exchange(Phelps & Kaplan, 2017). Pastoralism is further categorized into:

1. Specialized pastoralism- A pastoralism system that depends entirely on herding of livestock by nomadic communities for livelihood and socio-cultural practices.
2. Diversified pastoralism- A mixed system that depends on animal production, partial nomadism ranging from transhumant to nearly sedentary and direct or indirect feeding.

In Kenya, the camels have been traditionally kept mainly by Cushitic ethnic groups largely the Somali, Rendille, and Gabra. These camels were reared under extensive production systems (pastoralism), characterized by low numbers of herd managed per household mobility and low production input(Phelps & Kaplan, 2017). As the pastoral communities wander from place to place, mobile herds graze on the abundant rangeland pasture resources(Mohamed Noor et al., 2012).

2.4.2 Intensive Production System-Ranching

Recently, there has been an occurrence of the peri-urban animal production system (intensive farming system). This intensive farming is either specialized ranching, diverse ranching or zero-ranging ranching that has been brought about due to land-use change and commercialization (Phelps & Kaplan, 2017). In this system, an individual or group of individuals stock a large number of camels than among pastoralists as per the size of land owned(Phelps & Kaplan, 2017). Firstly, progressive sedentary lifestyle by some pastoral communities contributed to the shift to camel intensification. A few ranchers in Laikipia (e.g. OlMaisor and Kisima), adopted a system of keeping less camels for bush control and milk supply to their workers. Thereafter, an occurrence of the peri-urban camel production system emerged in Laikipia and Isiolo districts. This system is characterized by ranches stocking camels from a significant number to hundreds close to urban market outlets for meat and milk(Mohamed Noor et al., 2012; Noor et al., 2013). The outcome of intensification has been hypothesized to bring about less disease transmission, but studies have revealed that intensification increases disease spread. Practices such as stocking large numbers of animals, selection, and breeding to increase productivity, promote frequency of physical contact among the animals (Gilbert et al., 2021).

2.5 Antimicrobial Resistance in Bacteria

Antibiotic-producing bacteria produce AMR genes as a defense mechanism against a wide range of compounds, including antimicrobials, non-antimicrobials, and herbicides (Martinez & Baquero, 2009). Some of these genes are expressed against antimicrobial agents whose mechanism of action either interferes with cell wall synthesis (beta-lactams and glycopeptides), inhibits protein synthesis (aminoglycosides and chloramphenicol), inhibits nucleic acid synthesis (fluoroquinolones) or interferes with metabolic pathways (sulfonamides). These AMR genes can either occur naturally (intrinsic resistance mechanism), or through acquired resistance mechanism.

2.5.1 Natural (Intrinsic) Resistance

This type of trait is expressed naturally in bacteria of the same genus or species autonomous of prior exposure to antibiotics(C Reygaert, 2018; Wall et al., 2016). This chromosomal gene mediated mechanism broadens the widely known concept of antibiotic use as the only driver of bacterial resistance. Mutational changes in the gene results to a “superior” bacterial population that survives in the occurrence of an antimicrobial agent (Hoffman, 2016). Some of the mechanisms playing a role in the intrinsic resistance include i. Natural activation of the efflux pump activities, ii. Low drug uptake as a result of altering permeability of the outer membrane protein channel(C Reygaert, 2018; Tenover, 2006).

2.5.2 Acquired Resistance

This type of resistance occurs when AMR genes are acquired by susceptible bacteria from other resistant bacteria independent of a reproductive event(Wall et al., 2016). Acquisition of resistance genetic material can either occur between strains of the same species or different genera of bacteria through horizontal gene transfer (HGT), (Peterson & Kaur, 2018). HGT is a genetic exchange mechanism that takes place through the following strategies: conjugation, transformation, and transduction. Conjugation has been established as the proficient mobile genetic element (MGE), gene transfer between adjacent bacteria via pilus structures(C Reygaert, 2018; Wall et al., 2016). Plasmids and transposons are crucial MGEs involved in the dissemination of clinically important resistance genes and development of AMR. Transformation on the other hand involves uptake and incorporation of “naked” DNA segment released in the environment while transduction entails use of bacteriophage in mobilizing of bacterial DNA from one bacterium to the next(Weiss et al., 2018). Mobile gene cassettes are gene structures which allow efficient transferring of stockpiled resistance genes embedded in integrons that can be inserted in transposons which are contained in plasmid (Cloeckaert et al., 2017). This genetic mechanism is another form of acquiring resistance genes in a

bacterial population. beta-lactamase genes are localized in MGEs such as chromosomes and or integrons that facilitates their dissemination between bacteria (Hoffman, 2016).

2.6 Mechanism of Antimicrobial Resistance

There are different mechanisms used by bacteria to obtain a particular type of resistance (intrinsic or acquired resistance). These mechanisms include: limited influx of antimicrobials through altering the permeability of the outer membrane, destruction and chemical change of the antimicrobials through enzymatic action, resistance attributable to beta-lactams rely on degradation of compounds targeting them by use of enzymes thus rendering them ineffective (Wall et al., 2016). Modification of the antibiotic targets either through point gene mutation, ribosomal methylation by enzymes and or complete modification of the target site(Cloeckaert et al., 2017). Acquisition of enzymes conferring resistance that bypass the usual metabolic pathway are used by antibiotic to digest bacteria resulting to thrive of these resistant bacteria(C Reygaert, 2018; Wall et al., 2016).

2.7 Clinical Significance of Beta-lactamases in Veterinary Medicine

Beta-lactamase-encoding genes can be found on chromosomes or plasmids. According to Magiorakos et al. (2012), these plasmid-mediated enzymes efficiently catalyze the irreversible hydrolysis of the bonds creating the beta-lactam ring of the majority class of beta-lactam antibiotics, which range from first to third generation cephalosporin and monobactams. The WHO has classified these beta-lactam antibiotics as a highly significant class of antimicrobials that demands urgent attention in terms of AMR(WHO, 2023). Currently, the main hindrance to the efficacy of beta-lactam antibiotics therapy is the genetic fluidity within a microbial community. This genomic fluidity promotes not only single-point mutation occurrence but also spread of beta-lactamase-coding genes increasing new multiple substrate profile of enzymes(Bush & Jacoby, 2010). Basing on Ambler molecular classification, beta-lactamase genes are classified into various classes that have originated from parent enzymes as a result of point mutation on the genes

coding the active sites of beta-lactamases(Magiorakos et al., 2012; Paterson & Bonomo, 2005). Beta-lactamases are categorized into four classes, Class A to Class D. Ambler Class A contains most of the beta-lactamase that have the ability to degrade penicillin, cephalosporin and few cases of serine carbapenems. Some of the classical examples include TEM-1, TEM-3, SHV-1and CTX-M. Ambler Class B contains the most potent enzymes that hydrolyze metallo-carbapenems like IMP, VIM, and NDM. Amp C beta-lactamases belong to Class C. This class differs from class A by the fact there have been no cases of degradation by beta-lactamase inhibitor, clavulanic acid. Class D has OXA enzymes that can break down 3rd generation cephalosporin. Numerous beta-lactamase gene variants have been identified to date and are divided into nine distinct structural and evolutionary sub-groups that differ in their amino acid sequence. According to Bajpai et al. (2017), Bubpamala et al. (2018), and Kiiru et al. (2012), the enzyme include OXA, TEM, SHV, CTX-M, PER, VEB, BES, TLA, and GES.

Beta-lactamases are considered a public health concern worldwide since AMR-related infections caused by their counterpart bacteria are linked to high mortality rate, high hospitalization cases and consequently strained economy(Abbassi, 2017; Fashae et al., 2021; H-P Dhillon & Clark, 2012). These enzymes are of public health importance because of their unprecedented increase in global prevalence rates in resistant Enterobacteriaceae and the continual challenge in discovery pipeline of novel antibiotics(H-P Dhillon & Clark, 2012). Third-generation cephalosporin were utilized in human medicine because they had potent action against infection caused by beta-lactamase mediated bacteria. Examples of these bacteria include *E. coli* and *K. pneumonia* carrying ampicillin hydrolyzing TEM-1 and SHV-1 beta-lactamases. The advantage of this class of antibiotics over aminoglycosides and polymyxins relies on less effects of nephrotoxicity. *Klebsiella ozaenae* was the first documented bacterial species to be resistant against extended-spectrum cephalosporin due to SHV-2 beta-lactamase gene carried in the bacteria's plasmid(Knothe et al., 1983). Enterobacteriaceae have been listed by the WHO as the common antimicrobial resistant pathogens. They are well known to harbor many resistant genes including ESBLs. Other species that contribute to

the transmission of these genes include *Citrobacter*, *Enterobacter, Morganella, Proteus, Salmonella, Serratia*, and *Pseudomonas*, in addition to *Klebsiella spp*. and *Escherichia spp* (Arlet & Philippon, 1991). Subsequently, there has been an unprecedented rise in ESBL variants from different regions indicating geographic diversity in the amino acid sequence(Winokur et al., 2001). These ESBL variants have been reported both in clinical and non-clinical environments. The emergence and colonization of both pathogenic and commensal bacteria inhabiting food-producing animals (livestock) is quite worrisome because of reported cases of transmission to humans (Nüesch-Inderbinen & Stephan, 2016).

Beta-lactam resistance in a clinical environment has been the subject of numerous studies in Kenya, however data on livestock and/or food items with an animal origin are scarce. The limited investigations that have been done identify the genes that code for the enzymes and the ESBL-producing bacteria. For instance, *E. coli* and *Klebsiella spp*. isolated from camel's milk included ESBL-encoding genes (SHV, CTX-M-3, and CTX-M-14), (Njage et al., 2012). The ESBL-encoding gene $bla_{CTXM\text{-}15}$ was discovered in a recent work that was similar but carried out in camels (Nüesch-Inderbinen et al., 2020). There haven't been many investigations of the camels' ESBL-producing bacteria in Africa. In a Tunisian investigation, the CTX-M-1 gene was discovered, and *E. coli* was isolated from dromedary camels(Sallem et al., 2012). Humans, animals, and the environment are interconnected (One health concept). To assess the AMR threats posed by camels to people and their contribution to antimicrobial resistance, it is crucial to be aware of these genes in domesticated animals. AMR must also be evaluated in terms of how different livestock production systems' husbandry techniques affect it. This will aid in creating appropriate management strategies, should they be required, to stop the spread of disease and intensify the fight against antibiotic resistance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was conducted in Laikipia North County, Kenya. Laikipia County encompasses 9,462 km^2 of land area, of which 21% has high and medium altitudes appropriate for dairy and crop production and 79% has low altitudes suitable for wildlife conservation, extensive and intensive agro-pastoral systems, and pastoralism production systems. Individuals owning private ranches or Maasai families on group ranches and rangelands are responsible for land ownership and management (Sundaresan & Riginos, 2010). Laikipia North County, specifically, had an average population of 36,184 people and 7,827 camel population as of the 2019 census(KNBS, 2019). There are two main types of camel farming in Laikipia North County. The extensive systems, which are managed by the pastoral communities. The pastoral production system is characterized by a limited number of mobile herds that headers can manage grazing on vast rangeland pasture resources. Furthermore, after 1990, most veterinarians in Kenya privatized their services which became costly for livestock keepers thus encouraging self-medication practices and use of unskilled animal handlers. Camels drink water from rivers, ponds or wells shared by large numbers of livestock from different communities, humans, and wild animals. On the other hand, intensive or semi-intensive production systems practiced within ranches and conservancies. These camels graze within fenced lands. However, since most are kept in conservancies they shared the ecosystem with wild animals. The map (Figure 3.1) displays the study sites where camel sampling occurred in Laikipia County. The orange location pin indicates the study site Ilmotiok (Ext. A), the blue location pin denotes the study site Mpala (Int. B), the green location pin signifies the study site Loisaba (Int. C).

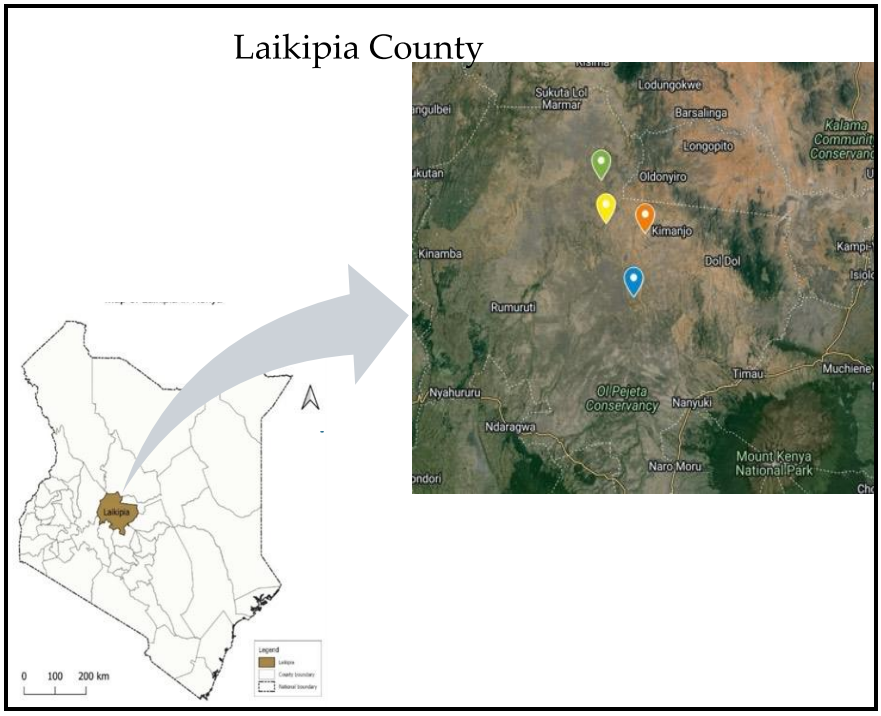

Figure 3.1: Study Sites in Laikipia County, Kenya.

The orange location pin indicates the study site Ilmotiok (Ext. A), the blue location pin denotes the study site Mpala (Int. B), the green location pin signifies the study site Loisaba (Int. C).

3.2 Study Design and Study Animal

This cross-sectional study was conducted in 2020 in North Laikipia County, Kenya, involving three ranches (Mpala, Loisaba, and Suyian), and pastoral village communities from Ilmotiok. These sites were selected based on their engagement in different livestock production systems and the geographical distances between them, aiming to encompass a broad study area. Mpala is centrally located among all the sampling sites, while Loisaba Conservancy lies slightly north, approximately 40 to 50 kilometers away. Suyian Ranch is situated in the northwest, about 50 to 70 kilometers from Mpala. Ilmotiok, located further from these ranches, represents remote pastoral communities. To

capture the diversity of the region, camels from 10 homesteads in Ilmotiok were sampled.

3.3 Sample Size Calculation.

A probability sampling calculation was used to guide the entire sampling procedure. The sample size was calculated using the Fisher formula (Charan & Biswas, 2013).

- $n = \frac{Z^2 \times P \times (1-P)}{d^2}$

  n is the sample size,

  Z is the Z-score associated with your desired confidence level (1.96 for 95% confidence),

  P is the estimated prevalence of the condition in the population (0.124 for 12.4%),

  d is the margin of error (0.05 for 5%).

Using the specific prevalence rate of 12.4% for antimicrobial-resistant *E. coli* in camels from Nüesch-Inderbinen et al. (2020), the sample size for this study on antibiotic resistance of beta-lactamase producing *E. coli* in camels in Kenya was determined. Assuming a 95% confidence level and a 5% margin of error, the formula for sample size calculation is:

$$n = \frac{1.96^2 \times o.124 \times (1-0.124)}{0.05}$$

n= 172

Thus, the minimum sample size of 172 camels from the ranch and pastoral communities was intended to be used.

However, we opted for a large sample size of 304 camels. This is because our study aimed to conduct subgroup analyses for the different livestock production systems, a larger overall sample size was necessary to maintain adequate power within each subgroup. Additionally, with more data, the confidence interval around the prevalence estimate was narrower, offering a more accurate understanding of the resistance rates as compared to previous studies.

3.4 Approval of the Study

The sample collection was conducted in accordance with Institute of Primate Research guidelines as well as international regulations, including those of WHO, NIH, Pven and Helsinki Convention on the humane treatment of animals for scientific purposes and general laboratory practices. The protocol for this study was approved by the Institutional Ethical Review Committee (reference number: ISERC/10/2020), (Appendix I).

3.5 Inclusion and Exclusion Criteria

Thorough health assessment on the camels was conducted by qualified veterinarians before enrollment in the study. The camels had to meet the following required criteria:

3.5.1 Inclusion Criteria

All apparently healthy camels not undergoing antibiotic treatment were eligible for inclusion in the study. Additionally, camels across various age groups, including juveniles, sub-adults, and adults, were included in the study sample.

3.5.2 Exclusion Criteria

All camels that were either sick, undergoing antimicrobial treatment, or within the withdrawal period following treatment were excluded from the study. Additionally,

camels exhibiting unique conditions such as lactation, gestation, any signs of recent illness or compromised health status were also excluded from the study.

3.6 Sampling Method

Completely randomized sampling technique was used (Lavrakas, 2008). With aid from the available veterinarians, fecal samples were obtained directly from the rectal of every third camel aseptically. This was achieved through rectal palpation by a veterinarian using one glove per animal. For the extensive group, sampling was random though based on the ease of availability. 304 samples were collected over a period of 7 days.

3.7 Sample Collection, Handling, and Storage

Using a sterile cotton swab, each fecal matter was swabbed and placed in cryovials containing Cary blair transport media (Himedia Lab Ltd, Mumbai, India). The samples were immediately placed in cooler boxes and later transported to the laboratory where they were stored at -4˚C awaiting processing.

3.8 Culturing of Fecal Samples

The Camel fecal samples were directly streaked onto ESBL CHROMagar (CHROMagar, Paris, France), and MacConkey agar with salts plates (Himedia Lab Ltd., India), to detect lactose fermenters. ESBL CHROMagar served as an internal validation control to confirm the presence of ESBL-producing E. coli. The plates were incubated for 18 to 24 hours at 37°C. Colonies exhibiting red or pink, non-mucoid morphology on MacConkey agar, and pink or red colonies on CHROMagar characteristics typical of E. coli were identified and selected for sub-culturing. All inoculations were performed in a safety hood cabinet, which was sanitized with 70% ethanol before and after each use.

3.8.1 Antibiotic Susceptibility Testing (AST) of *E. coli* Isolates

Antimicrobial Susceptibility Testing was performed on Mueller-Hinton agar (Himedia Lab Ltd., India), using the Kirby-Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI), guidelines (CLSI, 2020; Dolinsky, 2020). The first plate included the following antibiotics: Ampicillin (AMP), 10 µg, Ceftriaxone (CRO), 30 µg, Cefotaxime (CTX), 30 µg, Cefuroxime (CXM), 30 µg, Cefepime (FEP), 30 µg, and Ceftazidime (CAZ), 30 µg. To confirm ESBL-producing *E. coli*, Amoxicillin/Clavulanic acid (AMC), 20/10 µg was centrally placed on the plate, 20 mm away from each beta-lactam antibiotic. The second plate contained the remaining antibiotics: Chloramphenicol (CHL), 30 µg, Tetracycline (TCY), 30 µg, Gentamicin (GEN), 10 µg, Streptomycin (STR), 10 µg, Trimethoprim/Sulfamethoxazole (SXT), 25 µg, Norfloxacin (NOR), 10 µg, Ciprofloxacin (CIP), 5 µg, and Cefaclor (CEC), 30 µg. The plates were incubated at 37°C for 24 hours. The presence or absence of growth around the discs indicated the antimicrobial agent's efficacy against *E. coli* growth. *E. coli* strain ATCC 25922 was used as the quality control reference strain for the procedure. The inhibition zone diameters of the isolates were interpreted as susceptible, intermediate, or resistant, Following The CLSI Guidelines (CLSI, 2020).

3.9 Sub-Culture of *E. Coli* Isolates

Carefully selected colonies from the initial culture plate were subcultured on Nutrient agar (Himedia Lab Ltd., Mumbai, India), and then incubated for 24 hours at 37°C. To determine whether an isolate was gram-positive or gram-negative, a single isolate from the pure culture was subjected to Gram staining, as outlined in Appendix II (Tripathi & Sapra, 2021). The IMViC tests (Indole, Methyl Red, Voges-Proskauer, and Citrate), were employed to confirm the gram-negative colonies, following the methodology described in (Cheesbrough & Cheesbrough, 2009).

3.10 Confirmatory ESBLs Testing (Double Disk Synergy Testing)

Isolates that exhibited synergy zones around any of the third-generation cephalosporin discs (Ceftazidime or Cefotaxime), and the disc containing Amoxicillin/Clavulanic acid were identified as ESBL-producing *E. coli*. According to Drieux et al. (2008), the observation of ghost zones or a keyhole appearance at the edge of the third-generation cephalosporin disc zones towards the AMC disc was considered indicative of ESBL production. Ghost zones refer to faint areas of inhibition that appear around the antibiotic disc, particularly in the vicinity of a disc containing a beta-lactamase inhibitor like clavulanic acid. These zones are less distinct than the clear zones of inhibition typically seen in susceptibility testing(Drieux et al., 2008).

3.11 DNA Extraction and Amplification of Resistant Beta-Lactam *E. Coli* Isolates

56 isolates were selected for PCR to detect beta-lactamase encoding genes based on their antimicrobial sensitivity tests against beta-lactam antibiotics and phenotypic confirmation of ESBL-producing *E. coli*. Cultures were grown in tryptic soy broth (Himedia Lab Ltd., India) at 37 degrees Celsius for 18 to 24 hours. DNA was extracted using the heat treatment procedure described by Dashti et al. (2009) and stored at -20°C for subsequent analysis. PCR was employed to amplify the TEM, SHV, CTX-M, CMY and OXA genes, as previously detailed by Kiiru et al. (2012), and Saisi et al. (2019). The primers used are summarized in Table 3.1.

Table 3.1: Primers and PCR Condition for Detection of Beta-lactamase Genes

Primers	Oligonucleotide sequence (5'-3')	Annealing Temperature	Product Size (bp)
TEM	F-ATGAGTATTCAACATTTCCG	55˚C	
	R-CTGACAGTTACCAATGCTTA		840 (Kiiru et al., 2012)
SHV	F-GGTTATGCGTTATATTCGCC	50˚C	
	R-TTAGCGTTGCCAGTGCTC		854 (Kiiru et al., 2012)
CTX-M	F-ATGTGCAGYACCAGTAARGT	60˚C	
	R-TGGGTRAARTARGTSACCAGA		593 (Kiiru et al., 2012)
CMY	F- ATGATGAAAAAATCGTTATGC	55˚C	1200 (Saisi et al., 2019)
	R- TTGCAGCTTTTCAAGAATGCGC		
OXA	F-TCAACTTTCAAGATCGCA	62˚C	820(Saisi et al., 2019)
	R-GTGTGTTTAGAATGGTGA		

Table 3.1. lists the primers used for the amplification of various β-lactamase genes, including TEM, SHV, CTX-M, CMY, and OXA. The oligonucleotide sequences for both forward (F) and reverse (R) primers are provided along with their respective annealing temperatures and expected product sizes in base pairs (bp).

The PCR reaction for the identified ESBL-encoding genes was conducted in a 25 µl volume containing 12.5µl of 1X Go Taq Green (Promega Corp., USA), master mix, 1 µl of each primer, 2 µl of DNA template, and nuclease-free water to volume. The PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, annealing temperature depending on the beta-lactamase gene in Table 3.2 for 30 seconds to 1 minute, and an extension step at 72°C for 1 minute. A final extension was set at 72°C for 10 minutes for shorter fragments and 20 minutes for longer fragments. The amplified PCR products were analyzed by electrophoresis on a 1.0% agarose gel in 1× TBE buffer, stained with SYBR Green dye (Invitrogen), and

visualized under a UV trans-illuminator. *E. coli* strains KEN0463 and 25922 were used as positive and negative control strains, respectively. Additionally, all beta-lactamase-producing *E. coli* were characterized for phylogenetic classification for the presence of chuA, yjaA, TspE4.C2, and arpA according to the method described by Clermont et al. (2013).

3.12 DNA Sequencing of Beta-Lactamase Genes

Sanger sequencing of the beta-lactamase genes was outsourced to University of Nairobi Institute of Tropical and Infectious Diseases (UNITID). Positive amplicon templates were purified using ExoSAP-IT™ PCR Product Clean-up Reagent (Applied Biosystems, CA, USA), and incubated at 37°C for 15 minutes followed by 80°C for 15 minutes in a Veriti thermocycler. The sequencing reaction was purified with Big Dye X Terminator purification reagent by adding 10 µl of X Terminator and 45 µl of SAM solution to the cycle sequencing products. The reaction plate was then vortexed at 1,800 rpm for thirty minutes. Subsequently, the plate was centrifuged at 1,800g for two minutes at room temperature. A volume of 30 µl of the purified cycle sequencing products was loaded and analyzed using an ABI 3730 genetic analyzer (Applied Biosystems, CA, USA), as described by Chen et al. (2014). Both forward and reverse primers were utilized for sequencing each sample(Chen et al., 2014).

3.13 Phylogenetic Analysis of Beta-Lactamase Genes

Nucleotide sequences and predicted proteins were analyzed using the BLAST and ClustalW programs from the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute, as previously described by Kiiru et al. (2012). The alignment of beta-lactamase gene sequences and reference TEM and CTX-M gene sequences (e.g. MW930862.1 and ON221405.1), was performed using BioEdit software. For the phylogenetic analysis of our beta-lactamase gene variants and their evolutionary relationships with selected similar sequences in the NCBI database, we utilized MEGA software version 5, employing the maximum likelihood method. The evolutionary model

applied was a gamma distribution (G) with six rate categories, as determined by the nucleotide substitution type selected by the Tamura-Nei Model Test program (Dhara et al., 2013). To assess the stability and reliability of the generated phylogenetic tree, 1000 bootstrap replicates were used.

3.14 Data Management and Statistical Analysis

Data on the demographic factors of the camels, including age, gender, location, and livestock production system, along with test samples, were recorded in Microsoft Excel. Each sample and location were assigned unique identifiers prior to analysis. All analyses were conducted in R version 4.0.5. The Fisher's Exact Test for count data was utilized to assess the significant differences in the prevalence of antibiotic resistance and the occurrence of ESBL genes between intensive and extensive camel production systems (Carvalho et al., 2016). Results were interpreted using a 95% confidence interval (CI), considering a p-value of <0.05 as statistically significant.

CHAPTER FOUR

RESULTS

4.1 Socio-Demographics Profileo Study Camels

A total of 304 camels from both intensive and extensive livestock production systems were enrolled in the study. The majority of the recruited camels, 167 (54.9%), originated from ranches, distributed as follows: 80 (47.9%), from Mpala (Intensive B), 55 (32.9%), from Loisaba (Intensive C), and 32 (19.2%), from Suyian (Intensive D). Additionally, 137 (45.1%), camels were obtained from Ilmotiok village, representing the extensive livestock production system, as outlined in Table 4.1. This distribution reflects the frequent movement of pastoralists in search of fresh pasture and water sources for their animals. Regardless of the livestock production system, we observed that female camels outnumbered male camels, aligning with farmers' preference for females due to their reproductive utility. Consequently, a higher proportion of female camels were sampled in our study.

Table 4.1: Socio-Demographic Profile of Camels from Extensive and Intensive Livestock Production

	Count	**Age Group**			**Gender**		***Recovered E. coli***
LPS	**n**	**Adult**	**Juvenile**	**Sub-adult**	**Female**	**Male**	**LPS**
Extensive	137(45.1%)	105(43.9%)	9 (26.5%)	23(74.2%)	110(50.7%)	27(31.0%)	49(39.8%)
Intensive	167(54.9%)	134 (56.1%)	25(73.5%)	8(25.8%)	107(49.3%)	60(69%)	74(60.2%)
Total	304(100%)	239 (100%)	34(100%)	31(100%)	217(100%)	87(100%)	123(100%)

Key: LPS: Livestock Production System, n: number of camels. The percentages were based on the cumulative in each column category.

4.2 Identification of the *Escherichia coli* Isolates from Camels

Out of the 304 fecal swab samples obtained from camels across the two livestock production systems, 123 samples (40.7%) tested positive for the presence of *E. coli* isolates following culture, isolation, and biochemical testing procedures. The numbers were lower than expected and owed to the omission of an initial bacterial enrichment stage within non-differential broth media likely contributed to a lower rate of *E. coli* recovery. Forty nine (39.8%), *E. coli* isolates were recovered from fecal samples of camels reared in pastoralist communities, denoted as Extensive A. Additionally, 74 (60.2%), *E. coli* isolates were recovered from fecal samples of camels reared in ranches, with Intensive C Ranch contributing 23(18.7%), *E. coli* isolates Intensive B Ranch 37(30.1%), *E. coli* isolates and Intensive D Ranch 14 (11.4%), *E. coli* isolates as depicted in Figure 4.1. Fisher's exact test indicated no significant association between the type of livestock production system (extensive or intensive), and the recovery rate of *E. coli* (60.2% vs. 39.8%; p-value = 0.15, 95% confidence interval).

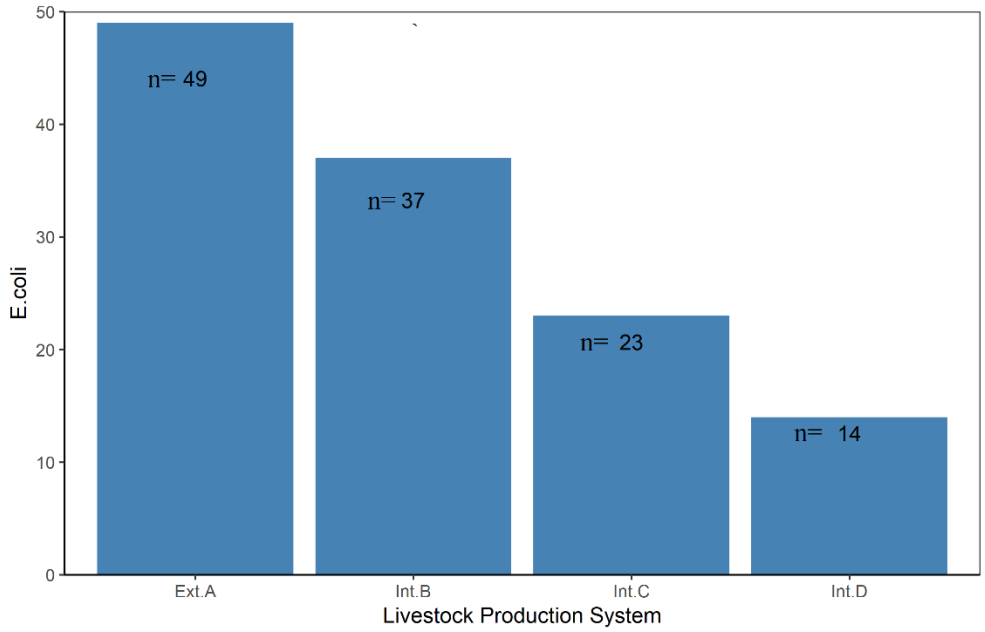

Figure 4.1: Percentage Distribution of *E. Coli* Recovered from Camels in the Extensive and Intensive Livestock Production Systems. Key: Ext. A represents the extensive livestock production system, while Int. B, Int. C, and Int. D represent intensive livestock production systems. "n" indicates the number of *E. coli* recovered from camels reared in each livestock production system.

E. coli isolates were recovered from fecal samples of camels reared in ranches, with Intensive C Ranch contributing 23 isolates (18.7%), Intensive B Ranch contributing 37 isolates (30.1%), Intensive D Ranch contributing 14 isolates (11.4%), and Extensive A contributing 49 isolates (39.8%).

4.3 Antimicrobial Resistance profile of *Escherichia coli* Isolates

Following antimicrobial susceptibility testing (AST) with 15 antibiotics, antimicrobial resistance was detected in 59 (47.9%). of the *E. coli* isolates. All *E. coli* isolates, with the exception of those resistant to Amoxicillin-clavulanic acid, Chloramphenicol, and Norfloxacin, exhibited resistance to at least one antibiotic. 35 (28.5%) *E. coli* isolates showed resistance to Cefaclor, 20 (16.3%), to Cefotaxime, 12 (9.8%), to Ampicillin, 10

(8.1%), to Ceftazidime, 6 (4.9%), to Tetracycline, and 4 (3.3%), to Sulfamethoxazole. Beta-lactam antibiotics encountered the highest levels of resistance. Resistance rates for other antibiotics varied between 1% and 3% as outlined in Appendix III. Intermediate resistance profiles in *E. coli* were most frequently observed with Cefaclor 6 (49.6%), and Ciprofloxacin 45 (36.6%), as shown in Figure 4.2. The double-disk synergy test revealed ESBL phenotype in four (3.3%) of the isolates. They all displayed MDR traits and were resistant to at least one third generation cephalosporin as a result of comparing the diameter of zone of inhibition to the CLSI guidelines in Table 4.2. The antimicrobial profiles for the 123 *E. coli* isolates generally revealed that 64 (52.0%) isolates were sensitive to all drugs, 37 (30.1%) were resistant to at least one antibiotic, 12 (9.7%) were resistant to two antibiotics, and 4 (3.3%) were MDR isolates (Figure 4.2).

Table 4.2: Average Zones of Inhibition Diameter (mm) of one of the ESBL-Producing E. coli Isolates

Antibiotics	**Average zones of inhibition diameter (mm) of *E. coli* isolates**	**Zones of Inhibition diameter (mm)- CLSI**	**Interpretation**
Amoxicillin/clavulanic (AMC)	10.6	≤ 13	Resistant
Cefuroxime (CXM).	6.8	≤ 14	Resistant
Ceftazidime (CAZ)	8.2	≤ 14	Resistant

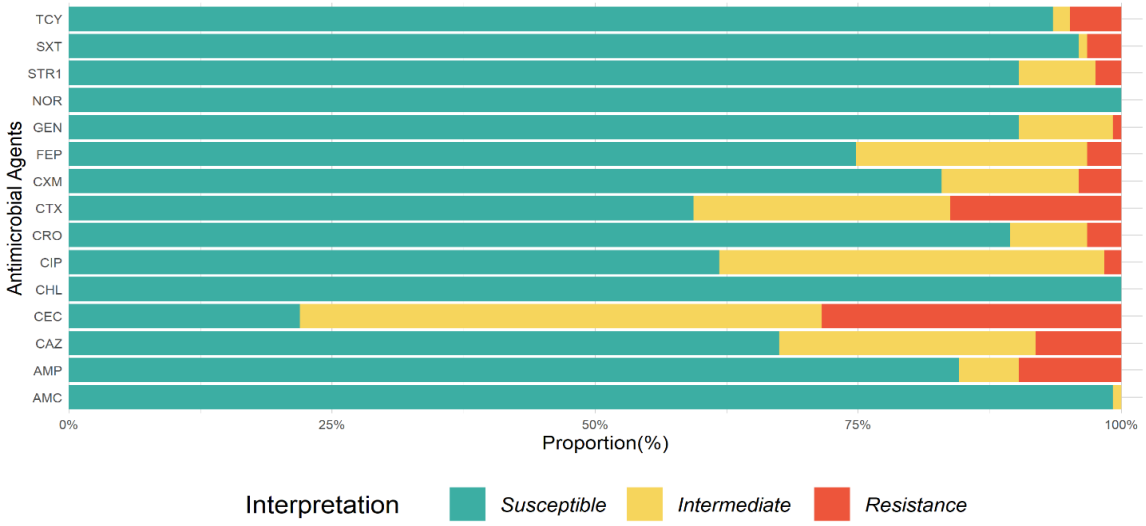

Figure 4.2: Antimicrobial Resistance Profile of 123 *E. coli* Isolated from 304 Fecal Samples of *Camelus Dromedarius*

Key: Amoxicillin-Clavulanate (AMC), Ampicillin (AMP), Ceftazidime (CAZ), Cefaclor (CEC), Chloramphenicol (CHL), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Cefuroxime (CXM), Cefepime (FEP), Gentamycin (GEN), Norfloxacin (NOR, Streptomycin (STR1), Trimethoprim-Sulfamethoxazole (SXT), and Tetracycline (TCY). n is the number of camels. Green indicates susceptibility, yellow indicates intermediate, and red indicates resistance.

4.4 Rates of Antimicrobial Resistance in Intensive and Extensive Camel Production System

Among the 49 *E. coli* isolates recovered from camels reared in the pastoral livestock production system, the highest frequency of antimicrobial resistance was observed for Cefaclor at 16 (32.6%), followed by Cefotaxime with 8 (16.2%), Ceftazidime with 5 (10.2%), and Ampicillin with 4 (8.1%), as shown in Figure 4.3 and Table 4.3. The average resistance rates for the remaining antibiotics were as low as 0.81% (Table 4.3). In the intensive livestock production system, of the 74 *E. coli* isolates, Cefaclor also

exhibited the highest resistance frequency at 19 (25.7%), followed by Cefotaxime 12 (16.2%), and Ampicillin 8 (10.8%). Intermediate resistance was observed in Tetracycline 6 (8.1%) and Ceftazidime 5 (6.8%), with the lowest observed antibiotic resistance ranging between 2% and 5% as outlined in Table 4.3. The most significant resistance patterns in most antibiotics were recorded at Intensive B Ranch compared to other ranches as detailed in *Appendix III*. At Intensive B Ranch, Cefaclor recorded the highest resistance with 13 (26.5%), isolates, followed by 3 (6.1%), at Intensive D Ranch, and 2 (4.1%), at Intensive C Ranch. Statistical analysis revealed no significant association between the camel rearing production system and the occurrence of antimicrobial resistance (p-value = 0.61, 95% confidence interval)

Table 4.3: Frequencies of Antimicrobial Resistance in *E. coli* Isolates Recovered in Camels Reared under Extensive and Intensive Production Systems

Antimicrobial Agents	Livestock Production System (p-value = 0.61, 95% CI.)	
	Extensive, N = 49	Intensive, N = 74
AMC	0 (0%)	0 (0%)
AMP	4 (8.2%)	8 (10.8%)
CAZ	5 (10.2%)	5 (6.8%)
CTX	8 (16.3%)	12 (16.2%)
CRO	1 (2.0%)	3 (4.1%)
CXM	1 (2.0%)	4 (5.4%)
FEP	1 (2.0%)	3 (4.1%)
CEC	16 (32.6%)	19 (25.7%)
TCY	0 (0%)	6 (8.1%)
GEN	1 (2.0%)	0 (0%)
STR1	1 (2.0%)	2 (2.7%)
CHL	0 (0%)	0 (0%)
CIP	0 (0%)	2 (2.7%)
SXT	1 (2.0%)	3 (4.1%)
NOR	0 (0%)	0 (0%)

Key: Ampicillin (AMP), Amoxicillin–Clavulanate (AMC), Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Cefuroxime (CXM), Cefepime (FEP), Cefaclor (CEC), Tetracycline (TCY), Gentamycin (GEN), Streptomycin (STR1), Chloramphenicol (CHL), Ciprofloxacin (CIP), Trimethoprim–Sulfamethoxazole (SXT) and Norfloxacin (NOR). N is the number of *Escherichia coli* recovered from camels reared under each livestock reproduction system.

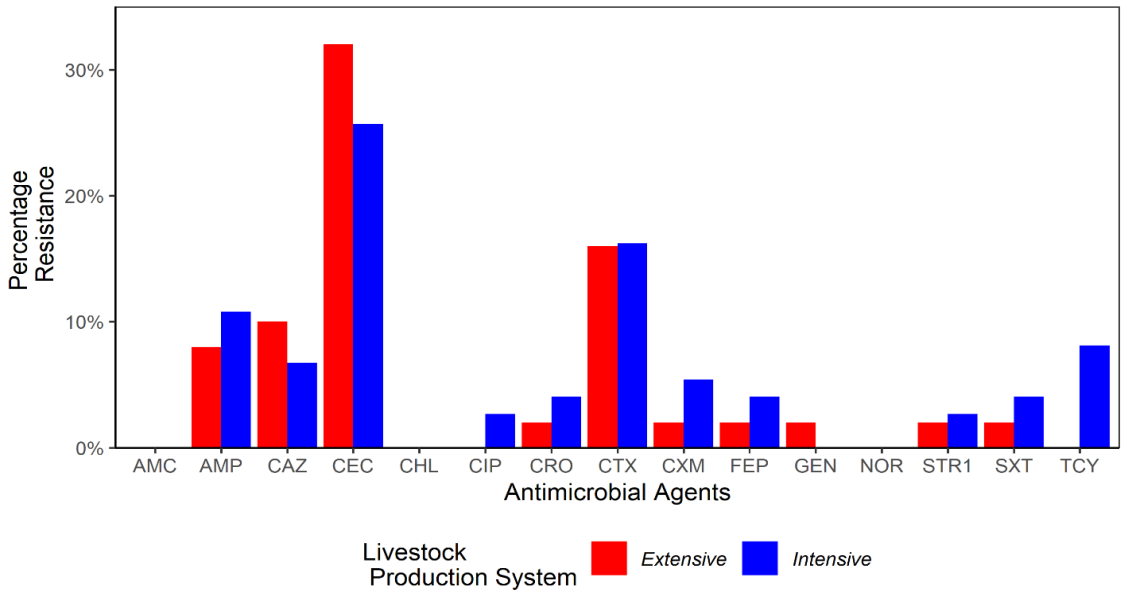

Figure 4.3: Frequencies of Antimicrobial Resistance in *E. coli* Isolates Recovered in Camels Reared under Extensive and Intensive Production System

Key: Amoxicillin-Clavulanate (AMC), Ampicillin (AMP), Ceftazidime (CAZ), Cefaclor (CEC), Chloramphenicol (CHL), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Cefuroxime (CXM), Cefepime (FEP), Gentamycin (GEN), Norfloxacin (NOR, Streptomycin (STR1), Trimethoprim-Sulfamethoxazole (SXT), and Tetracycline (TCY). Red represents extensive livestock production system and blue represents intensive livestock production system.

4.5 Molecular Detection, Phylogenetic Grouping and Characterization of Beta-Lactamase-Producing *E. Coli*

Molecular analyses using polymerase chain reaction (PCR) detected 22 beta-lactamase genes in 56 *E. coli* isolates that exhibited phenotypic resistance to beta-lactam antibiotics, as confirmed by antimicrobial susceptibility testing (AST). The presence of beta-lactamase encoding genes (bla_{TEM} and $bla_{CTX\text{-}M}$) was confirmed in 22 (39.29%) of these 56 phenotypically resistant isolates using gel electrophoresis and a UV Transilluminator as shown in Figure 4.4. Specifically, all 22 (17.87%) of the isolates

contained the *bla*$_{TEM}$ gene, and 4 (3.25%) of these 22 isolates, additionally harbored the *bla*$_{CTX-M}$ gene. These 22 *E. coli* isolates, which tested positive for beta-lactamases, were further categorized based on their phylogenetic relationships. In the distribution of phylogenetic groups among the isolates with bla genes, group B1 (59.09%) emerged as the most common, followed by group D (18.18%), A (9.09%), and B2 (4.55%). Two isolates were classified as unknown due to the need for further analysis to determine their phylogenetic group.

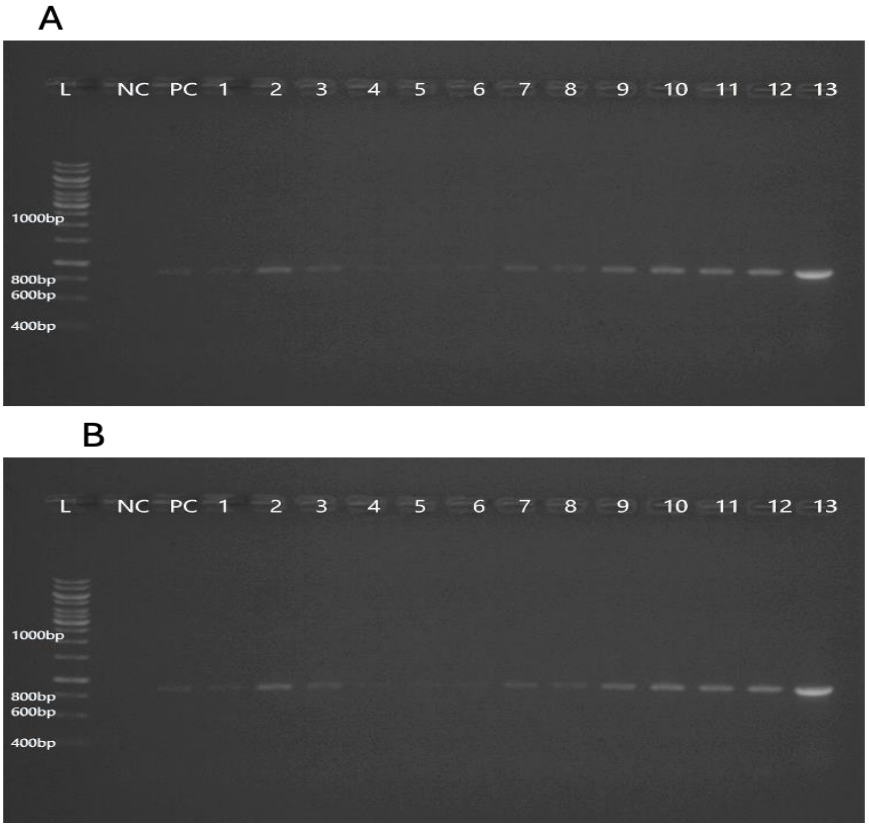

Figure 4.4: Electrophoresis Gel Images for *bla*$_{CTX-M}$ and *bla*$_{TEM}$ Genes

Key: A represents electrophoresis gel of *bla*$_{CTX-M}$ gene (593bp); B represents electrophoresis gel of *bla*$_{TEM}$ gene (840bp); L-Molecular Ladder; NC- Negative Control; PC: Positive Control; bp- base pairs. Note: Letters and Numbers at the top of plate A and B respectively represent random DNA numbers of the *E. coli* isolates.

Sequencing of the PCR-amplified *bla$_{TEM}$* genes from 22 isolates revealed the presence of various subtypes: *bla$_{TEM-116}$* in 15 isolates, *bla$_{TEM-243}$* in 4 isolates, *bla$_{TEM-1}$* in 1 isolate, *bla$_{TEM-104}$* in 1 isolate, and *bla$_{TEM-214}$* in 1 isolate. Analysis of the PCR-amplified *bla$_{CTX-M}$* genes from 4 isolates identified *bla$_{CTX-M-27}$* in 1 isolate and *bla$_{CTX-M-15}$* in 3 isolates. Notably, a co-existence of beta-lactamase genes was observed in a few isolates: 2 harbored both *bla$_{CTX-M-15}$* and *bla$_{TEM-243}$*, and 1 contained both *bla$_{CTX-M-15}$* and *bla$_{TEM-1}$*. All detected *bla$_{TEM}$* genes were categorized as broad-spectrum enzyme types. While ESBL-type *bla$_{TEM}$* variants were not found, ESBL-type *bla$_{CTX-M-15}$* variants were present. The phylogroups B1, D, and A included isolates with multiple broad-spectrum bla alleles. Remarkably, two isolates, one from phylogroup D and another from B2, carried the ESBL-type bla gene.

4.6 Distribution of Beta-Lactamase Genes in Intensive and Extensive Camel Production Systems

Beta-lactamase genes detected were more or less similar in terms of diversity across the intensive and extensive livestock production systems (Figure 4.5). The bla genes detected from extensive farming included bla$_{TEM-116}$ (n = 6 isolates), bla$_{TEM\ 243}$ (n = 1), bla$_{TEM-1}$ (n = 1), and bla$_{CTX-M\ -27}$ (n = 1). The bla genes detected from intensive farming included bla$_{TEM-1}$ (n = 2 isolates), bla$_{TEM-104}$ (n = 1), bla$_{TEM-116}$ (n = 8), bla$_{TEM\ 243}$ (n = 3), and bla$_{CTX-M-15}$ (n = 3). There was no evidence of an association between beta-lactamase gene type occurrence and the production systems studied (p-value = 0.71, 95% Cl). All beta-lactamase-carrying *E. coli* isolates were resistant to at least one beta-lactam antibiotic. All four ESBL-producing isolates containing the *bla$_{CTX-M-15}$* gene displayed an MDR profile (Table 4.4).

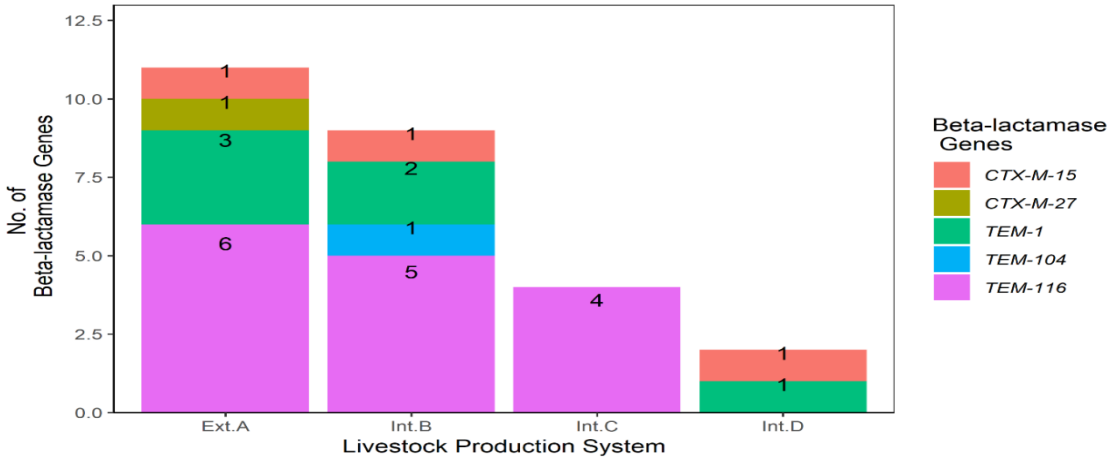

Figure 4.5: Distribution of Beta-Lactamase Genes in Intensive and Extensive Camel Production Systems. Key: Ext. A: pastoralist community. Int. B: Ranch B, Int. C: Ranch C, and Int. D: Ranch D. *p-value = 0.71.

Table 4.4: Antimicrobial Resistance Profile and Beta-Lactamase Genes among 22 *E. coli* Isolates from Camels in Extensive and Intensive Production Systems

Phylo-Groups	Beta-lactamase genes	Broad-Spectrum/ ESBL	Resistance Profile	Isolate Count (%)
D	CTX-M-27	ESBL	AMP, CAZ, CTX, CRO, CXM, FEP, CEC, SXT	1 (1.8%)
B1	TEM-104	Broad-spectrum	AMP, TCY	1 (1.8%)
A, B1, D	TEM-116	Broad-spectrum	AMP, CAZ, CTX, CRO, CXM, FEP, CEC, TCY	15 (26.8%)
A, B1, B2	TEM-1	Broad-spectrum	AMP, CAZ, CTX, CEC, TCY, STR1, SXT	3 (5.4%)
D, B1	TEM-1; CTX-M-15	Broad-spectrum ESBL	AMP, CAZ, CTX, CRO, CXM, FEP, CEC, TCY, CIP, SXT	3 (5.4%)

Key: Amoxicillin-Clavulanate (AMC), Ampicillin (AMP), Ceftazidime (CAZ), Cefaclor (CEC), Chloramphenicol (CHL), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Cefuroxime (CXM), Cefepime (FEP), Gentamycin (GEN), Norfloxacin (NOR, Streptomycin (STR1), Trimethoprim-Sulfamethoxazole (SXT), and Tetracycline (TCY).

Figures 4.6 and 4.7 depict phylogenetic trees illustrating the evolutionary relationship between the *bla*$_{TEM}$ and *bla*$_{CTX-M}$ sequences from this investigation and reference sequences obtained from the NCBI database. The phylogenetic trees indicate that the *bla*$_{TEM}$ and *bla*$_{CTX-M-15}$ genes have minimal levels of genetic diversity. The three *bla*$_{CTX-M-15}$ sequences cluster together, irrespective of whether the isolates originated from pastoralist or ranch production systems. Similarly, all of the *bla*$_{TEM}$ genes obtained in this study clustered together, regardless of production system category. The boostrap consensus tree inferred from 1000 replicates was deemed representative of the evolutionary history of the taxa that were analyzed.

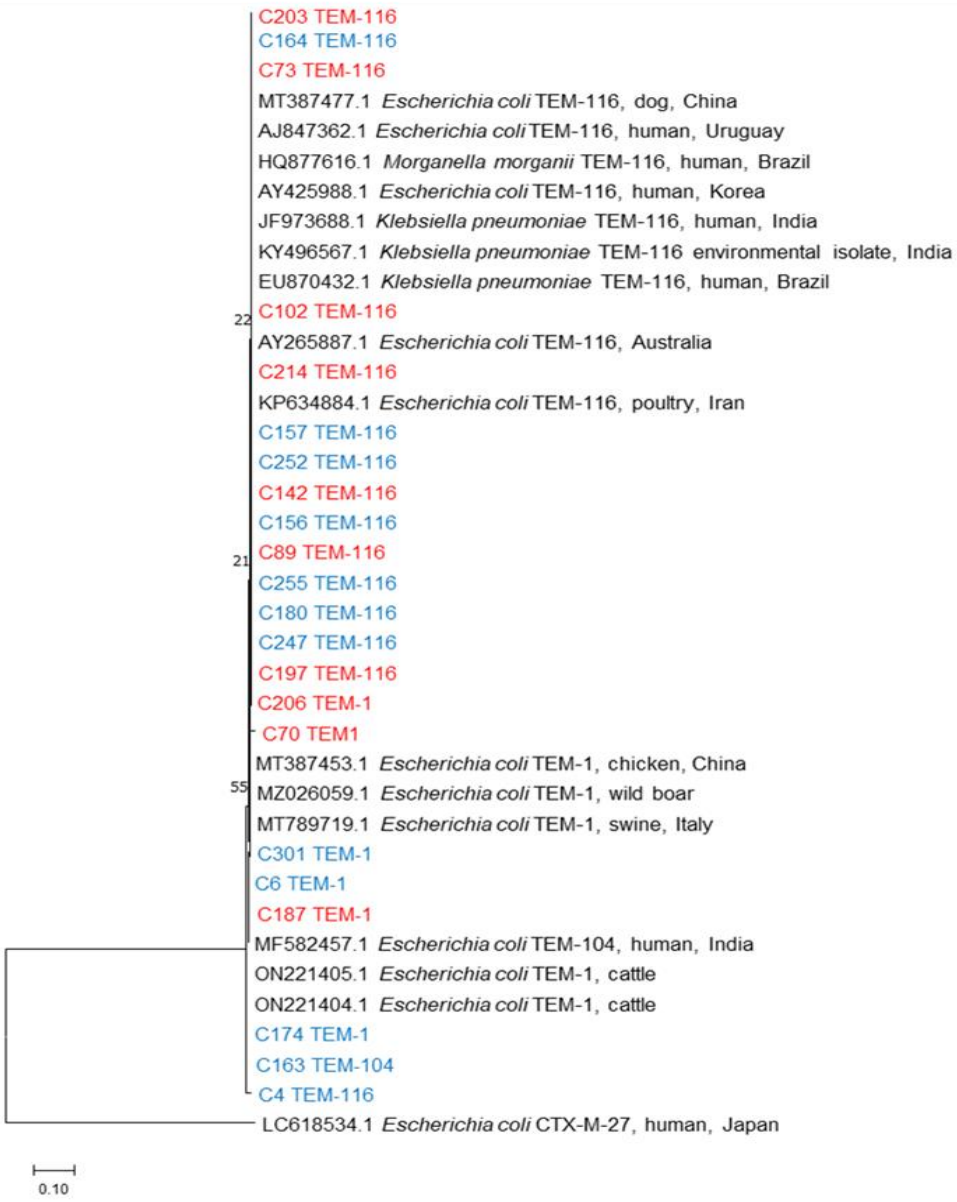

Figure 4.6: Phylogenetic Tree Showing Evolutionary Relationship of *bla$_{TEM}$*

Caption: Red represents *bla$_{TEM}$* gene sequences obtained from extensive production system *E. coli* isolates, blue represents *bla$_{TEM}$* gene sequences obtained from intensive production system *E. coli* isolates and black represents sequences obtained from the NCBI database.

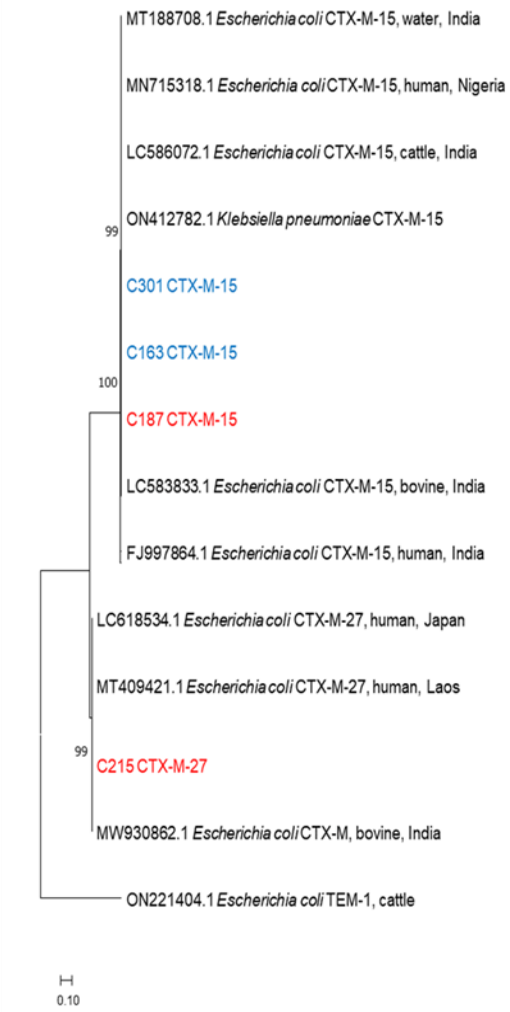

Figure 4.7: Phylogenetic Tree Showing Evolutionary Relationship of *bla$_{CTX-M}$*

Caption: Beta-lactamase gene *bla$_{CTX-M}$* variants in *E. coli* recovered from pastoralism and ranching production systems alongside selected sequences from the NCBI database. Red isolates were from camels reared under an extensive livestock production system and blue isolates were from camels reared under intensive livestock production system. Black represents sequences obtained from the NCBI database.

CHAPTER FIVE

DISCUSSION

5.1 Antimicrobial Susceptibility Profile of *E. Coli* Isolates from Camels in Extensive and Intensive Livestock Production Systems.

Our study aimed to investigate the antibiotic resistance profile and genetic diversity of beta-lactamase genes in *E. coli* isolates recovered from camels in both extensive and intensive livestock production systems. This novel study compared camels raised in intensive (ranches) and extensive (pastoralists) livestock production systems, providing insights into the prevalence of antibiotic resistance and the characterization of beta-lactamase genes in each setting. Our analysis of 123 *E. coli* isolates recovered from fecal samples of 304 one-humped Camelus dromedarius revealed concerning levels of resistance to beta-lactam antibiotics. Notably, resistance was observed against various beta-lactam antibiotics, including Cefaclor (28.46%), Cefotaxime (16.26%), Ampicillin (9.76%), and Ceftazidime (8.13%). These findings are consistent with global trends indicating a rise in antimicrobial resistance, particularly in bacterial pathogens of veterinary significance (Alhababi et al., 2020; Bessalah et al., 2016; Nüesch-Inderbinen et al., 2020).

Of particular concern is the emergence of resistance to second-, third-, and fourth generation cephalosporins among *E. coli* isolates recovered from camels' fecal samples. These findings contrast with other studies concerning AMR in camels within Africa and the Middle East, which reported low or zero resistance for most second-, third-, and fourth generation cephalosporins (Alhababi et al., 2020; Bessalah et al., 2016). Of note, Nüesch-Inderbinen et al. (2020) studied AMR in *E. coli* isolates from camel fecal samples collected from a single ranch in Laikipia County in 2017. In this previous study, low resistance rates for various cephalosporin generations were reported, such as cefazolin (0%), cefotaxime (0.6%) and cefepime (0%). These results are in sharp contrast to the current study, which reports resistance rates above 5% for second- and

third generation cephalosporins within *E. coli* isolates from camels within the same region. Resistance in camel populations, particularly in remote regions such as Laikipia North County, several factors may contribute to the observed shift in beta-lactam resistance among *E. coli* isolates recovered from camels. These factors include the non-regulated prescription of antibiotics over the counter by farmers and pastoralists, inappropriate use of biocides leading to water pollution, and the preference for herbal remedies over antibiotics in livestock and human therapy (Caudell et al., 2017; Lamuka et al., 2017; Vadhana et al., 2015). Additionally, high wildlife-livestock interaction may serve as a conduit for the dissemination of antimicrobial-resistant organisms and resistance genes, further exacerbating the problem of antimicrobial resistance in camel populations (Kamau et al., 2021; Muloi et al., 2019). The presence of beta-lactamase genes, which are associated with mobile genetic elements such as transposons, plasmids, and insertion sequences, highlights the potential for horizontal gene transmission mechanisms to facilitate the spread of antibiotic resistance among bacterial populations(Hoffman, 2016). This resistance pattern has significant implications for both animal and human health, as cephalosporins are widely used in veterinary medicine and are critical for treating bacterial infections in both humans and animals. This discrepancy underscores the need for further investigation into the factors driving antimicrobial resistance.

Moreover, our study observed a shift in susceptibility to Ampicillin among *E. coli* isolates recovered from camels, contrary to findings in similar studies (Alhababi et al., 2020; Bessalah et al., 2016; Nüesch-Inderbinen et al., 2020) . Certain theories could be speculative to the regained susceptibility to Ampicillin among the *E. coli* isolates warranting further investigation. They include 1. injudicious use of second-and third generation cephalosporins, in treatment practice of common respiratory, enteric, and urinary infections among the pastoral communities; 2. One of the farms that participated in the study reported a preference for herbs to antibiotics in treating common enteric and respiratory infections affecting camels. Hence, the resistance profile of beta-lactam antibiotics tested was generally low in comparison to other ranches as outlined in

Appendix III. Therefore, with continuous decreased use of Ampicillin, there is less selection pressure on resistant mutants leading to loss of plasmid resistance mechanism responsible for Ampicillin resistance, thus re-establishing its sensitivity (Choudhary et al., 2013; Kaushik et al., 2014). Importantly, our analysis revealed no significant association between antimicrobial resistance occurrence and the two livestock production systems (p=0.61, CI 95%). However, intensive camel farming demonstrated an upward trend in resistance, highlighting the potential impact of intensification practices on antimicrobial resistance in camel populations (Gilbert et al., 2021).The prevalence of resistance to beta-lactam antibiotics, including cephalosporins among *E. coli* isolates from camels highlights the widespread nature of AMR in veterinary settings. These findings are consistent with previous studies documenting increasing rates of resistance in bacterial pathogens isolated from food-producing animals (Alhababi et al., 2020; Bessalah et al., 2016; Nüesch-Inderbinen et al., 2020; Saidani et al., 2019) .

5.2 Molecular Detection, Phylogenetic Grouping and Characterization of Beta-Lactamase-Producing *E. Coli* from Camels Reared Under the Intensive and Extensive Production Systems

Our study demonstrated an increase in beta-lactamase-producing *E. coli* isolates that exhibited MDR phenotype in camels found in Kenya. This phenotypic occurrence confirmed findings from prior research showing ESBL-producing Enterobacteriaceae frequently exhibit MDR phenotype against non-beta-lactams as a result of their plasmids containing numerous resistance genes (Carvalho et al., 2020; Musila et al., 2021; Saidani et al., 2019). These results are consistent with earlier research on the global prevalence of Enterobacteriaceae producing ESBL in livestock(Braun et al., 2016; Kimera et al., 2020). There are concerns of transmission of these genes from livestock to humans either through product consumption, direct contact or through indirect transmission such as environmental elements(Caudell et al., 2020; Madec et al., 2017). Given the potential risk to public health and financial losses associated with livestock

production, molecular surveillance of beta-lactamase-producing E. coli in animals used for food production is still essential.

Our findings revealed 17.89% bla_{TEM} genes present in isolates sampled and variants namely $bla_{TEM-116}$, $bla_{TEM-104}$, *and* bla_{TEM-1}, were identified This study is the first to document extensive bla_{TEM} gene diversity in Kenyan camel-recovered *E. coli*. From the amino acid sequences alignment and phylogenetic analysis, all the bla_{TEM} variants were different from one another by a single or few nucleotides substitution indicating their high level of similarity irrespective of whether obtained from the intensive or extensive camel rearing production. The finding that most beta-lactam resistant isolates harbored bla_{TEM} genes highlights the extensive role of non-ESBL bla_{TEM} genes in conferring resistance. This is owing to this gene product's enzymatic ability to hydrolyse most penicillin drugs, early cephalosporins and modern beta-lactams(Bush & Jacoby, 2010; Nagshetty et al., 2021). Amino acid sequence alignment and phylogenetic tree analyses revealed that bla_{TEM} variants had low levels of genetic diversity. The observed lack of genetic diversity among the isolates, irrespective of the type of production system the camels were reared in, may be attributed to resources that are shared by camels within the collective ecosystem.

We reported 4 (3.25%) ESBL-producing *E. coli* from the entire 304 healthy camel population tested. These findings show an increase in the number of ESBL-producing carriage from the previous study on camels done in Kenya (Nüesch-Inderbinen et al., 2020). In this study, $bla_{CTX-M-15}$ (n=3) was dominant and one (1) $bla_{CTX-M-27}$ allele. Our results support prior research that indicated the most often found putative ESBL in camels and other livestock in Africa is $bla_{CTX-M-15,}$ which is a member of the CTX-M-1 group (Alonso et al., 2016; Carvalho et al., 2020). All of the isolates of *E. coli* that contained $bla_{CTX-M-15}$ gene were identified in camels that were raised on ranches. Interestingly, two isolates that expressed $bla_{CTX-M-15}$ genes and had MDR phenotypes also co-expressed the bla_{TEM-1} genes. The finding is consistent with research showing that ESBL-producing Enterobacteriaceae frequently display MDR phenotypes when exposed

to non-beta-lactam antibiotics(Carvalho et al., 2020; Musila et al., 2021; Saidani et al., 2019). Our study reports *bla*$_{CTX\text{-}M\text{-}27}$ gene expressed by one of the isolates recovered from a camel reared pastoralist. According to our knowledge, this is the first report of camel-derived *bla*$_{CTX\text{-}M\text{-}27}$ beta-lactamase. The *bla*$_{CTX\text{-}M\text{-}27}$, differs with *bla*$_{CTX\text{-}M\text{-}14}$ by a single nucleotide substitution and its presence is increasing exponentially being frequently reported in Europe and Asia as a result of human-animal interactions(Bevan et al., 2017). In Africa, *bla*$_{CTX\text{-}M\text{-}27}$ has been found in food-producing animals, specifically in chicken(Ayeni et al., 2020), and cattle (Ball et al., 2019). The majority of the CTX-M enzymes have greater hydrolytic activity against ceftriaxone and cefotaxime than ceftazidime (Zhao & Hu, 2013). Interestingly, in addition to ceftriaxone and cefotaxime, *bla*$_{CTX\text{-}M}$ expressing isolates conferred resistance to ceftazidime. Studies have shown the heightened level of resistance to ceftazidime is brought about by Asp240Gly substitution at the β3 strand of the bla$_{CTX\text{-}M\text{-}15}$ and bla$_{CTX\text{-}M\text{-}27}$ enzymes (Bonnet, 2003; Poirel et al., 2002). Seemingly, emergence of these mutants of *bla*$_{CTX\text{-}M\text{-}15}$ enzyme that efficiently catalyzes ceftazidime are increasing at an alarming rate suggesting ceftazidime selection pressure could be one of the reasons behind the evolving enzyme.

The ESBL-producing *E. coli* in this investigation belonged to the phylogroups D, B1, and B2, with B1 being a commensal and B2 and D being associated with virulent extraintestinal strains(Riley, 2014). This suggests the occurrence of beta-lactamase spread through both commensal and pathogenic strains of *E. coli*. Another aspect of this study was to observe differences in resistance patterns between the two livestock production systems. In general, similar frequencies were observed for both AMR and antimicrobial resistance genes among the two production systems. This is in agreement with other studies Gerzova et al. (2015) and Semedo-Lemsaddek et al. (2021) that have revealed no significant difference in the frequencies of resistant isolates or the abundance of resistance genes between free range and conventional farming systems. Nevertheless, the ranch livestock production systems in this study showed increased levels of resistance for many tested antibiotics, warranting further investigation.

5.3 Limitation

While this study offers valuable insights into the antimicrobial resistance patterns and antimicrobial resistance genes (ARGs) circulating among camels reared under different production systems, it acknowledges several limitations that constrain the comprehensiveness of its findings for Laikipia North County. One significant limitation is the relatively small sample size of camel herds from pastoralist production systems, which may restrict the analysis's scope and potentially affect the generalizability of the results to broader populations. Additionally, the omission of an initial bacterial enrichment stage within non-differential broth media likely contributed to a lower rate of *E. coli* recovery from camel samples. These limitations underscore the necessity for media enrichment and call for larger-scale studies with more comprehensive sampling strategies to bolster the robustness and applicability of future research findings in this area. Addressing these shortcomings is crucial for advancing our understanding of antimicrobial resistance dynamics in camel populations and for developing targeted intervention strategies.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Antimicrobial Susceptibility Profile of *E. Coli* Isolates from Camels in Extensive and Intensive Livestock Production Systems.

1. Analysis of fecal swabs indicated increased resistance levels to cephalosporin antibiotics among *E. coli* isolates from camels in both intensive and extensive livestock production systems. Despite differences in management practices between these systems, our findings showed no significant difference in the occurrence of antimicrobial resistance (Fisher Exact Test 95% CI; p-value=0.61). Resistance was notably higher in *E. coli* isolates from camels reared under intensive production systems, underscoring the necessity for targeted intervention strategies in these environments.
2. The study observed a concerning emergence of resistance to second-, third-, and fourth-generation cephalosporins among *E. coli* isolates from camels. This resistance pattern contrasts with other studies in Africa and the Middle East, which reported low or zero resistance for these antibiotics. The findings suggest that factors such as non-regulated antibiotic use, inappropriate use of biocides, and high wildlife-livestock interaction may contribute to the observed resistance.
3. Our study highlighted the critical role of camels within pastoralist communities and the broader agricultural landscape, emphasizing the need for robust husbandry practices to minimize the increase of antimicrobial resistance.

6.1.2 Molecular Detection, Phylogenetic Grouping and Characterization of Beta-Lactamase-Producing *E. Coli* from Camels Reared Under the Intensive and Extensive Production Systems

1. Our research identified a significant prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* isolates harboring $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}27}$ genes, along with multiple variants of non-ESBL bla_{TEM} genes. These genetic markers, predominantly found in phylogenetic groups B1, B2, and D, suggest camels as potential reservoirs for clinically significant resistance determinants.
2. Co-expression of blaCTX-M-15 and blaTEM-1 genes was observed in some isolates, associated with multidrug-resistant phenotypes, indicating the potential for horizontal gene transfer and the accumulation of resistance mechanisms.
3. The lack of a significant difference in the occurrence of antimicrobial resistance genes between intensive and extensive production systems (Fisher Exact Test 95% CI; p-value=0.71) indicates a complex interplay of factors affecting resistance dynamics. Camels may acquire resistance genes from various sources, including other domestic and wild animals or the environment, potentially facilitating transmission to humans through fecal-oral routes.

6.2 Recommendations

6.2.1 Antimicrobial Susceptibility Profile of *E. Coli* Isolates from Camels in Extensive and Intensive Livestock Production Systems.

1. Conduct longitudinal studies to monitor changes in antimicrobial resistance patterns over time in both production systems. This will help identify trends and factors contributing to resistance development.
2. Investigate the specific management practices in intensive camel farming that may be contributing to the observed upward trend in resistance. This could include examining antibiotic usage patterns, feed composition, and environmental factors.

3. Expand the study to include a larger sample size and more diverse geographical regions to validate the findings and assess if the resistance patterns are consistent across different camel populations.
4. Evaluate the impact of non-regulated antibiotic use and inappropriate biocide use on resistance development. This could involve surveying farmers and pastoralists about their antibiotic usage practices and correlating this with resistance patterns.

6.2.2 Molecular Detection, Phylogenetic Grouping and Characterization Beta-Lactamase-Producing *E. Coli* from Camels Reared Under the Intensive and Extensive Production Systems

1. Conduct whole genome sequencing of ESBL-producing isolates to better understand the genetic context of resistance genes and potential virulence factors associated with these strains.
2. Investigate the prevalence and diversity of other antimicrobial resistance genes beyond beta-lactamases in camel *E. coli* isolates to get a more complete picture of the resistance landscape.
3. Conduct functional studies on the newly detected $bla_{CTX\text{-}M\text{-}27}$ gene in camel-derived *E. coli* to understand its origin, spread, and impact on antibiotic resistance.
4. Develop and validate rapid molecular diagnostic tools for the detection of ESBL-producing *E. coli* in field settings, enabling more efficient surveillance and targeted interventions.
5. Investigate the mechanisms behind the spread of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}27}$ genes in camel populations, focusing on potential transmission routes and risk factors.
6. Perform comparative studies between camel isolates and those from other livestock species and humans in the same region to assess potential interspecies transmission of resistant strains or genes.

7. Explore the ecological and environmental factors that may contribute to the spread of resistance genes, particularly focusing on the wildlife-livestock interface in the study region.

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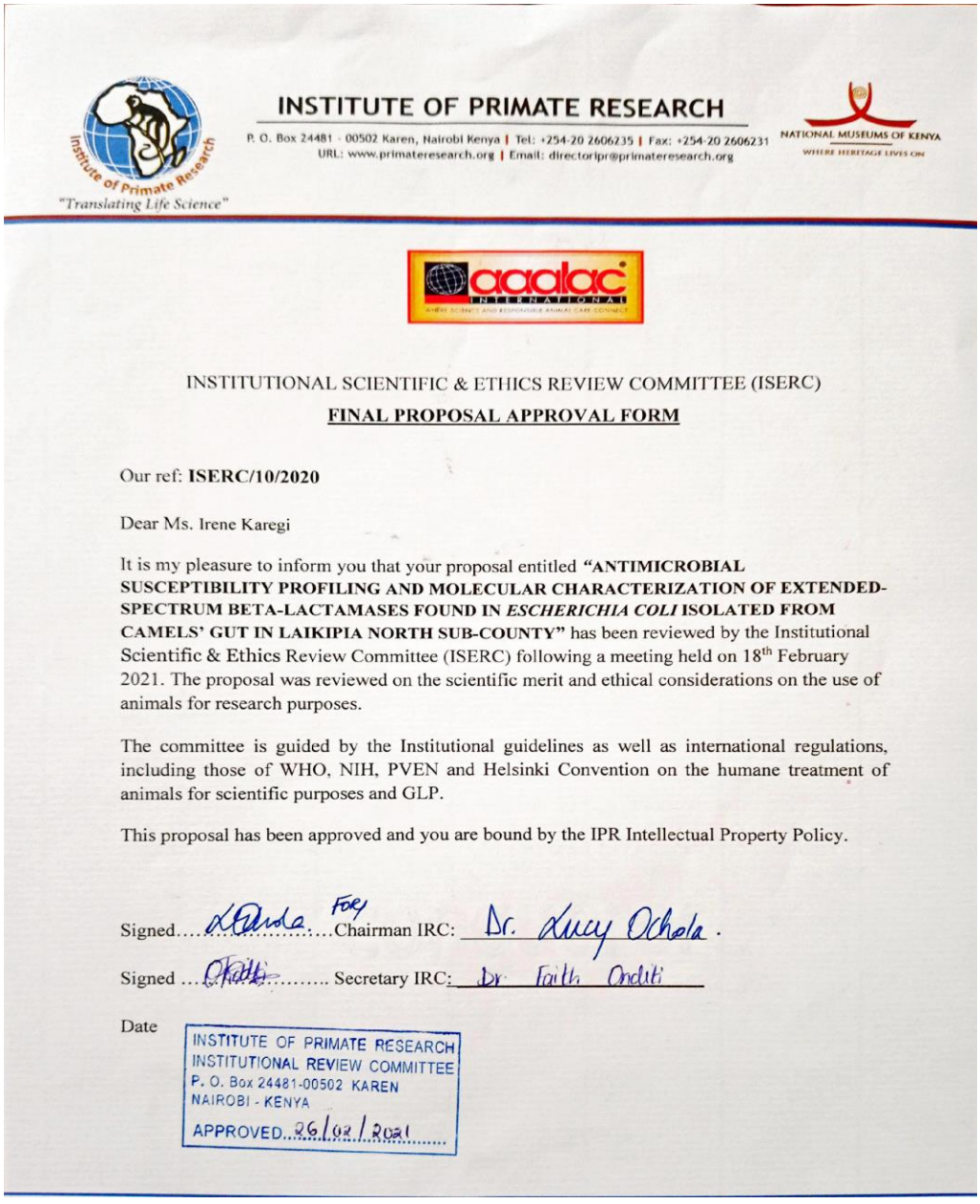
APPENDICES

Appendix I: The Institutional Ethical Review Committee Approval Certificate (Reference Number: ISERC/10/2020)



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INSTITUTIONAL SCIENTIFIC & ETHICS REVIEW COMMITTEE (ISERC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **ISERC/10/2020**

Dear Ms. Irene Karegi

It is my pleasure to inform you that your proposal entitled **"ANTIMICROBIAL SUSCEPTIBILITY PROFILING AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASES FOUND IN *ESCHERICHIA COLI* ISOLATED FROM CAMELS' GUT IN LAIKIPIA NORTH SUB-COUNTY"** has been reviewed by the Institutional Scientific & Ethics Review Committee (ISERC) following a meeting held on 18th February 2021. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes.

The committee is guided by the Institutional guidelines as well as international regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed.....FOR.....Chairman IRC: Dr. Lucy Ochola.

Signed .....Secretary IRC: Dr. Faith Onduti

Date

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED 26/02/2021

Appendix II: Gram Staining Procedure

1. A sterile wire loop is used to transfer a drop of suspended culture to the microscope slide.
2. The culture is spread with an inoculation loop to make a thin smear.
3. The slide is either air-dried by passing it over a gentle flame.
4. Crystal violet stain is added over the fixed culture.
5. After 1minute, the stain is poured off, and the excess stain is rinsed with water.
6. The glass slide is covered with iodine solution for 1 minute.
7. The iodine solution is poured off, and the slide is rinsed with running water.
8. A few drops of acetone is added to the slide and rinsed immediately with water in 5 seconds.
9. The smear is counterstained with basic fuchsin solution for 1 minute. The fuchsin solution is washed off with water, and excess water is blotted with the tissue paper.
10. The slide is air-dried and ready to undergo an examination under a microscope under oil immersion.

Appendix III: Antimicrobial Susceptibility Profile in Counts and Percentages of *E. Coli* Recovered from Camels Reared under Intensive and Extensive Livestock Production Systems.

Antibiotics	Ext.A			Int.B				Int.C		Int.D			Overall		
	I	R	S	I	R	S	I	R	S	I	R	S	ALL.I	ALL.R	ALL.S
AMC	0% (0)	0% (0)	100% (50)	3% (1)	0% (0)	97% (36)	0% (0)	0% (0)	100% (23)	0% (0)	0% (0)	100% (14)	1% (1)	0% (0)	99% (122)
AMP	8% (4)	8% (4)	84% (42)	8% (3)	19% (7)	73% (27)	0% (0)	0% (0)	100% (23)	0% (0)	7% (1)	93% (13)	6% (7)	10% (12)	85% (105)
CAZ	36% (18)	10% (5)	54% (27)	22% (8)	8% (3)	70% (26)	13% (3)	4% (1)	83% (19)	14% (2)	7% (1)	79% (11)	25% (31)	8% (10)	67% (83)
CTX	30% (15)	16% (8)	54% (27)	16% (6)	14% (5)	70% (26)	22% (5)	26% (6)	52% (12)	36% (5)	7% (1)	57% (8)	25% (31)	16% (20)	59% (73)
CRO	8% (4)	2% (1)	90% (45)	8% (3)	5% (2)	86% (32)	4% (1)	0% (0)	96% (22)	7% (1)	7% (1)	86% (12)	7% (9)	3% (4)	90% (111)
CXM	14% (7)	2% (1)	84% (42)	16% (6)	3% (1)	81% (30)	13% (3)	9% (2)	78% (18)	0% (0)	7% (1)	93% (13)	13% (16)	4% (5)	83% (103)
FEP	20% (10)	2% (1)	78% (39)	24% (9)	3% (1)	73% (27)	26% (6)	4% (1)	70% (16)	14% (2)	7% (1)	79% (11)	22% (27)	3% (4)	75% (93)
CEC	48% (24)	32% (16)	20% (10)	49% (18)	38% (14)	14% (5)	52% (12)	9% (2)	39% (9)	57% (8)	21% (3)	21% (3)	50% (62)	28% (35)	22% (27)
TCY	2% (1)	0% (0)	98% (49)	0% (0)	16% (6)	84% (31)	0% (0)	0% (0)	100% (23)	7% (1)	0% (0)	93% (13)	2% (2)	5% (6)	94% (116)
GEN	10% (5)	2% (1)	88% (44)	16% (6)	0% (0)	84% (31)	0% (0)	0% (0)	100% (23)	0% (0)	0% (0)	100% (14)	9% (11)	1% (1)	90% (112)
STR1	6% (3)	2% (1)	92% (46)	11% (4)	5% (2)	84% (31)	0% (0)	0% (0)	100% (23)	14% (2)	0% (0)	86% (12)	7% (9)	2% (3)	90% (112)
CHL	0% (0)	0% (0)	100% (50)	0% (0)	0% (0)	100% (37)	0% (0)	0% (0)	100% (23)	0% (0)	0% (0)	100% (14)	0% (0)	0% (0)	100% (124)
CIP	34% (17)	0% (0)	66% (33)	43% (16)	5% (2)	51% (19)	26% (6)	0% (0)	74% (17)	43% (6)	0% (0)	57% (8)	36% (45)	2% (2)	62% (77)
SXT	0% (0)	2% (1)	98% (49)	0% (0)	8% (3)	92% (34)	0% (0)	0% (0)	100% (23)	7% (1)	0% (0)	93% (13)	1% (1)	3% (4)	96% (119)
NOR	0% (0)	0% (0)	100% (50)	0% (0)	0% (0)	100% (37)	0% (0)	0% (0)	100% (23)	0% (0)	0% (0)	100% (14)	0% (0)	0% (0)	100% (124)

Key: Ext. A: extensive livestock production, Int. B/Int. C/Int. D: intensive livestock production system.I – Intermediate, R - Resistance, S -Susceptible, ALL.I - Overall Intermediate ALL.R - Overall Resistance and ALL.S - Overall Susceptible.

Appendix IV: Fisher's Test on Association between the Livestock Productions and Occurrence of Antimicrobial Resistance

Is there an association between the two livestock productions in terms of resistance only?

H0: There is no association/relationship between the two livestock production systems when it comes to antimicrobial Resistance.

H1: There is an association/relationship between the two livestock production systems when it comes to antimicrobial Resistance.

If the p-value is less than the significance level, we can reject the null hypothesis.

Using the Fishers test for count data at 95% confidence level

Fail to Reject the null hypothesis

This is because the p-value is greater than the significant level of 5%/0.05

```
1) ## ##  Pearson's Chi-squared test with Yates' continuity correction
2) ## ## data:  ## X-squared = 8.5086e-29, df = 1, p-value = 1
3) ## ##  Fisher's Exact Test for Count Data
4)  ## ## data:  ## p-value = 0.6098
5) ## alternative hypothesis: two. sided
```