GENETIC DIVERSITY AND ANTIBIOTIC RESISTANCE OF *Escherichia coli* ISOLATED FROM HUMAN, CATTLE, AND BUFFALO IN THE MAASAI MARA ECOSYSTEM, KENYA

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DECLARATION

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

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To my family, I devote this thesis with special thanks to Prof. (Eng.) Abel N. Mayaka, Alex, Franklin, Flavia, Kelvin, Owen, and Ariana.

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ABBREVIATION AND ACRONYMS

16S rRNA	16S ribosomal Ribonucleic Acid
AMR	Antimicrobial Resistance
AR	Antibiotic Resistance
BLAST	Basic Local Alignment Search Tool
β-lactam	Beta-lactam
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
ExPEC	Extraintestinal Pathogenic *Escherichia coli*
EMB	Eosin Methylene Blue
GI	Gastrointestinal
gDNA	Genomic Deoxyribonucleic acid
HGT	Horizontal Gene Transfer
ILRI	International Livestock Research Institute
InPEC	Intestinal Pathogenic *Escherichia coli*
KWS	Kenya Wildlife Service
MHA	Mueller-Hinton Agar
MLST	Multi Locus Sequence Typing
MGEs	Mobile Genetic Elements
MME	Maasai Mara Ecosystem
MMNR	Maasai Mara National Reserve
MR-VP	Methyl-Red Voges-Proskauer
NA	Nutrient Agar
NACOSTI	National Commission for Science, Technology and Innovation

PBPs	Penicillin-Binding Proteins
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
STEC	Shiga toxigenic *Escherichia coli*
SRS	Stress Response System
TSI	Triple Sugar Iron
VP	Voges-Proskauer
ATCC	American Type Culture collection
WHO	World Health Organization

ABSTRACT

Antibiotic resistance is an emerging health crisis globally with a significant impact on human and animal populations. The emergence and spread of antibiotic resistance are attributed to the wrong use of antibiotic products in human and animal health care. This is further amplified and complicated in the human-livestock-wildlife interfaces where there is selective pressure and close interaction. This interface and its impact on antibiotic resistance dynamics are inadequately evaluated in Kenya. The study aimed to establish the genetic diversity and prevalence of antibiotic resistance of *Escherichia coli* isolates from human, cattle, and buffalo populations in the Maasai Mara Ecosystem located in Kenya. *E. coli* was isolated by phenotypic and biochemical methods from stool samples of humans and fresh dung samples of cattle and buffalo collected from the Maasai Mara Ecosystem. Molecular techniques were used to characterize *E. coli* isolates. *E. coli* isolates were tested against tetracycline, gentamicin, ciprofloxacin, ceftriaxone, and amoxicillin-clavulanic acid using the Kirby-Bauer disk diffusion method and then assigned to phylogenetic groups according to chuA, yjaA, and TspE4.C2 genetic markers. *E. coli* isolates exhibiting resistant strains were genetically characterized based on their 16S rRNA gene region of the gDNA. Phylogenetic analysis established that all the four phylogroups (A, B1, B2, and D) were present in the *E. coli* isolates from the sympatric hosts except phylogroup B2 which was absent in buffalo population. *E. coli* isolates from the sympatric hosts were predominant in phylogroup A and B1. Buffalo was predominated by isolates of *E. coli* in phylogroup B1 and D. Further, the genetic sequence of resistant *E. coli* isolates recorded 14 unique haplotypes, with haplotype 4 and haplotype 7 being present in all the three sympatric hosts. Humans recorded the highest resistance to the tested antibiotics at 94% followed by buffalo at 50% and cattle at 45%. Humans, cattle, and buffalo recorded the highest antibiotic resistance to tetracycline at 83%, 45%, and 33%, and lowest antibiotic resistance to amoxicillin-clavulanic acid at 46%, 94%, and 100% respectively. Further, antibiotic resistance was prevalent in isolates of *E. coli* from buffalo with interactions and the resistant pattern exhibited those of humans and cattle against tetracycline 27%, gentamicin 23%, and ciprofloxacin 14% as compared to *E. coli* isolate from isolated buffalo which only recorded resistance to tetracycline antibiotic at 5%. It was concluded that isolates of *E. coli* from humans, cattle, and buffalo were genetically diverse. *E. coli* isolates from the sympatric hosts were resistant to frequently used antibiotics in human medicine and veterinary practices and finally, antibiotic-resistant in buffalo correspond to those antibiotics used in humans and food-producing animals. It was recommended that additional studies using a One-Health approach, are needed to identify the main reservoir of antibiotic resistant strains and to determine the transmission pathway in the multi-host system.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

In the history of antibiotic therapeutics, antibiotic resistance has existed in parallel to the development of antibiotic formulations. In recent years, its magnitude and rapid rate of emergence coupled with the spatial transmission of resistant strains is not just a threat to public health around the world but is a huge setback to socio-economics, food security, and conservation of endangered wild animals (Sayah *et al.*, 2005; Ventola, 2015). Antibiotic resistance's impact on the healthcare system is predicted to be severe following an upsurge in multidrug-resistant bacteria, which is a premonition that the state can slide back to the pre-antibiotic era (Woodford *et al.*, 2011). Antibiotic resistance in food-producing animals is a risk to food security. This is due to a decrease in the successful treatment of the animals, which leads to increased mortality and decreased productivity hence resulting in a global food and animal industry crisis (Bengtsson and Greko, 2014; Van Boeckel *et al.*, 2019).

The emergence and transmission of antibiotic resistance are driven via interwoven aspects that end up in a vicious cycle. For instance, the unregulated usage of antibiotics is among the main factors driving the emergence of bacterial resistance. Even though such usage may be for example intended to maximize livestock production to meet the growing demand for human food, eventually it propagates resistant bacterial strains that defy therapy, increases the cost of production, lowers the yield, and threatens food security (Rushton *et al.*, 2014; Grace, 2015; Van Boeckel *et al.*, 2019).

Similarly in humans, Unnecessary usage of antibiotics to treat irrelevant infections such as mild pain and discomfort like in sore throat leads to the cumulative impact in the proliferation of resistant strains, yet in the end, antibiotics turn out to be ineffective in the treatment of major infections such as pneumonia, tuberculosis, gonorrhea, and salmonellosis, this leads to long hospitals stays, increased cost of healthcare and increased mortality (Lushniak, 2014; Okoth *et al.*, 2018).

Antibiotic resistance is a global phenomenon and Kenya isn't an exception (Kariuki *et al.*, 2011; Christabel *et al.*, 2012). A situational analysis on antibiotic resistance in Kenya showed an increasing trend of resistance to commonly used antibiotics (66%, 85%, 71%, and 43%) to ampicillin, cotrimoxazole, tetracycline's and penicillin, respectively (Bejon *et al.*, 2005; Bii *et al.*, 2005; Kariuki, 2010). It is estimated that antibiotics are excreted in urine and stool by 90% of farm animals, hence afterward are spread via fertilizer, ground water, and surface runoff (Bartlett *et al.*, 2013).

Besides being a public health problem, antibiotic resistance is a 'One Health' challenge. "One Health" is an approach that is used to attain optimal health in humans, animals, and the environment. Close contact between humans, animals, and their environments provides more opportunities for antibiotic resistance strains and diseases to pass between animals and people (Rousham *et al.*, 2018; Graham *et al.*, 2019; White and Hughes, 2019). This is based on the fact that increased overlap in habitat and sharing of landscape resources among humans, livestock, and wildlife, has not only introduced the resistant microbial strains into wildlife populations but creates an opportunity for cross-species transmission of these organisms.

Several research studies have discovered the presence of bacteria strains that are resistant to antibiotics, particularly *Escherichia coli* (*E. coli*) in several wildlife species including reptiles, birds, and mammals (Sayah *et al.*, 2005; Apun *et al.*, 2008; Jobbins and Alexander, 2015; Okullu *et al.*, 2016). The occurrence of resistant bacterial strains in wildlife, regardless of the source, is most worrying because wildlife species occur in high densities, live longer and occur in large invariable habitats hence have the potential to interact with soil microbes, an attribute that makes wildlife hold an important epidemiological role as a host that maintains, amplifies and spreads resistant strains or genes.

The prevalence and diversity of resistant bacterial strains in Kenya are not well described, especially at the human-livestock-wildlife interface. Indeed, antibiotic resistance calls for a 'One Health' approach to understand its dynamics but also to develop multi-sectorial approaches to curb its impact. A fundamental question that guides this study is whether humans, livestock, and wildlife that interact can share resistant bacterial profiles, and if they do, at what proportions? Maasai Mara is one of the ecosystems in Kenya where humans, livestock, and wildlife interact in varying degrees and thereby presents a suitable natural environment to apply a 'One Health' approach to antibiotic-resistant survey. As a result, the primary goal of this study is to determine if *Escherichia coli* from humans, cattle, and African buffalo are susceptible to common antibiotics, as well as to assess the genetic diversity.

1.2 Statement of the problem

It is well known that resistance to antibiotics is a globally recognized therapeutic problem with grave consequences for the health of humans and animals (Chang *et al.*, 2015; Aslam *et al.*, 2018). In addition, it has major implications on socio-economics as it leads to high hospital costs and hefty costs on livestock production. As a consequence, antibiotic resistance is a driver of poverty, especially in rural Africa where livestock farming is a key socio-economic venture (Lushniak, 2014; Friedman *et al.*, 2016). Even though resistant strains of bacteria have been reported in humans and food-producing animals, In Kenya, pertinent questions remain on how these bacteria spread? Do resistant strains in humans flow to animals and vice versa? Understanding how resistant bacterial strains get transmitted is very critical in formulating control measures.

Evidence reveals that resistant bacteria or resistant elements might be passed through the food chain from animals to humans (Sayah *et al.*, 2005; Laxminarayan *et al.*, 2013; Muloi *et al.*, 2019), which means that ecological overlap promotes spill-over and spill-back. There are limited studies that have investigated antibiotic resistant profiles in highly overlapped populations of humans, livestock, and wildlife (Mercat *et al.*, 2016; Iramiot *et al.*, 2020). In Kenya, it is known that the human, livestock, and wildlife interface plays a key epidemiological role in animal diseases and zoonosis. However, there is a knowledge gap as limited studies have looked into the epidemiological side of the interface on antibiotic resistance, especially among pastoral communities. As such, data is needed on the resistance levels against the antibiotics being used by these communities, the prevalence of the resistant bacteria, and whether these resistant bacteria are shared among humans, livestock, and wildlife.

1.3 Justification of the study

Human and animal health are both threatened by antibiotic resistance. The phenomenon has impacted negatively on socioeconomic development globally. Through person-to-person or animal-to-person transmission and vice-versa, these bacteria are further disseminated and can cause multinational outbreaks or even pandemic expansion of resistant pathogens. *Escherichia coli* species is a primary candidate used in research related to antibiotic resistance (AR) because it is enteric commensal with extremely broad distribution (Kubašová *et al.*, 2017; Gousia, 2019) and is exposed to a variety of antibiotics regularly, allowing for the selection of resistant strains that could serve as reservoirs (Roberts, 2018). Furthermore, finding the same resistant *E. coli* strain types in humans, livestock and wildlife will aid in determining the risks of resistant determinant and zoonotic transmission.

Surveillance of AR has been reported in many countries including developing countries (Founou *et al.*, 2016). However, AR data from Kenya are largely limited to infectious pathogens isolated from patients with infections, particularly diarrheal disease. Little is known about the existence of AR among potential pathogens in the commensal flora of humans, livestock, and wildlife. Therefore, the goal of the study was to find out *E. coli* genetic diversity and antibiotic resistance prevalence in humans, cattle, and buffalo at the Maasai Mara Ecosystem. The results and information generated from this study will help scientists and policy makers to understand how to deal with and mitigate antibiotic resistance in multi-host systems. The result will also contribute information in the use of *E. coli* as a tool for determining transmission routes of pathogens and antibiotic resistant strains in multihost system.

1.4 Hypotheses

i) Humans, cattle, and buffalo living in spatial proximity harbor different strains of *E. coli*.

ii) Antibiotic resistance is high in *E. coli* isolates from humans as compared to cattle and buffalo.

iii) Buffalo interacting with humans and cattle have a high prevalence of antibiotic resistant *E. coli* isolates than isolated buffalo

1.5 Objectives

1.5.1 General objective

To determine genetic diversity and antibiotic resistance of *Escherichia coli* isolated from humans, cattle, and buffalo in the Maasai Mara Ecosystem.

1.5.2 Specific objective

i) To profile *E. coli* strains from humans, cattle, and buffalo.

ii) To determine the prevalence and patterns of antibiotic resistance in *E. coli* isolates from humans, cattle, and buffalo.

iii) To identify antibiotic resistance profiles in *E. coli* isolates from buffalo with varying degrees of human and cattle interaction.

1.6 Significance of the study

Knowledge obtained from this investigation is going to be useful in developing effective policies and sustainable tools and measures to regulate the usage of antibacterial drugs and to minimize antibiotic resistance genes from being passed down through the population. The study will make available baseline data in terms of antibiotic resistance in wildlife that is less exposed to antibacterial drugs, the influence of resource sharing, and the flow of antibiotic resistance. Results and observations from this study are essential as they can be used in designing an important biotechnological model in the management of antibiotic resistant epidemiology as well as rationalizing the present resistance management efforts. All these can be adapted and integrated into other disease management efforts**.**

CHAPTER TWO

LITERATURE REVIEW

2.1 Theoretical review

2.1.1 Biology and Ecology of *Escherichia coli*

The bacteria *Escherichia coli* is the most well-known commensal bacteria (Jang *et al.*, 2017) Normally, It lives in warm-blooded animals and humans' gastrointestinal tracts (Köhler and Dobrindt, 2011; Acuff and Dickson, 2017), the environment and a few cold-blooded animals (Marshall *et al.*, 2009; Jang *et al.*, 2017). It consists of diverse strains of bacteria which are pathogenic and non-pathogenic (Thakur, 2017). *E. coli* strains that aren't pathogenic help in vitamin K and B complex synthesis, food digestion, and absorption. Most intestinal and extraintestinal infections are caused by pathogenic *E. coli* strains, which are subdivided into pathotypes according to the host target tissue and disease state (Salyers and Whitt, 2002; Logue *et al.*, 2017). Intestinal Pathogenic *E. coli* (InPEC) and Extraintestinal Pathogenic *E. coli* (ExPEC) are two types of pathogenic *E. coli* (Jang *et al.*, 2017; Logue *et al.*, 2017)

2.1.2 Phenotypic identification of *E. coli*

Escherichia coli belong to the *Enterobacteriaceae* family in the genus *Escherichia.* They are Gram-negative coliforms that are facultatively anaerobic, non-spore forming, and rod-shaped (Fratamico and Smith, 2006). *E. coli* easily grow in different bacteriological media and over different temperature ranges (15-45°C). *E. coli* produce flat dark purple colonies characterized by a distinct green metallic sheen when cultured in Eosin Methylene blue (EMB) agar.

Its ability to utilize amino acid tryptophan to produce indole, and ferment lactose and other sugars to produce gas (ISO, 2005; Talaro, 2005), is the main characteristic that differentiates it from other fecal coliforms.

Indole and methyl red positive, Voges-Proskauer and citrate negative *E. coli* strains account for about 95% of all *E. coli* strains (Fratamico and Smith, 2006). To distinguish *E. coli* from other bacteria, a variety of biochemical tests are used, including:

The Triple sugar iron test: It assesses bacteria's ability to ferment sugars (lactose, glucose, and sucrose) and produce hydrogen sulfide. The production of gas and the change in color of the pH indicator indicate sugar fermentation (Quinn *et al.*, 2011; Hall, 2013).

Citrate utilization test: It determines whether bacteria can use citrate as their only carbon source. The citrate agar slant from Simmons is commonly used. The bacterium which can grow in the media produces citrate permease enzyme, which converts citrate to pyruvate which enters the bacteria metabolic cycle to yield energy (Quinn *et al.*, 2011)**.**

Indole test: This test is done on bacteria to see if they can degrade tryptophan and produce indole. Kovac's or Ehrlich's reagents are used to detect indole (Quinn *et al.*, 2011).

Methyl Red-Voges-Proskauer test: This is a biochemical test that detects bacteria's ability to ferment mixed acids. The products are a complex mixture of lactic, acetic, and formic acids that are visible when a methyl red indicator is added. (Quinn *et al.*, 2011).

2.1.3 Molecular identification of *E. coli*

To gain more understanding of the microbial population epidemiology and microbial diversity, several microbial typing techniques are applied for either genomic, clinical diagnostic, or pathogenesis associated microbiology study (Van Belkum, 2002). For instance, phenotypic methods such as culture and biochemical tests are used to distinguish within microbial species in clinical microbiology studies (O'Hara *et al.*, 2000). Additionally, antibiogram typing is done routinely to determine antimicrobial susceptibility of bacteria as a first-line phenotypic technique to guide in treatment (Boers *et al.*, 2012). Phenotypic techniques may be unsatisfactory in comprehensive studies aimed at molecular evolution of specific bacterial species, as well as their population structure, dynamics, and evolution, hence the determination of a microbial genotype is a necessity (Van Belkum, 2002). Due to this, several techniques for molecular typing have been established to compare microbial genotypes (Boers *et al.*, 2012).

A variety of techniques are employed to study the genetic diversity of *E. coli.* For example, typing with Multilocus sequence typing (MLST) is extensively used in microbes due to its unambiguity and sequence analysis of housekeeping genes even though DNA sequencing is costly (Boers *et al.*, 2012). Whole Genome Sequencing is high resolution genotyping method that can be used to enhance our understanding of bacterial genetics but they are very expensive techniques. Based on the assumption that *E. coli*'s genetic structure is clonal, a typing method was proposed that divides the bacterium into major phylogenetic groups or phylogroups.

The phylogroups are important as they are used to group *E. coli* into pathotypes such as diarrheagenic, non-pathogenic, and ExPECs (Köhler and Dobrindt, 2011). The phylogroup techniques include Triplex Polymerase Chain Reaction (PCR) and Quadruplex PCR methods.

2.1.4 Triplex PCR method

This method was initially described in the year 2000 and since then it has been widely used to classify *E. coli* isolates into phylogenetic groups. It is a low-cost, quick, and simple technique (Clermont *et al.*, 2000), as compared to MLST (Herzer *et al.*, 1990). The triplex PCR method uses a combination of three genetic markers which include chuA (Bonacorsi *et al.*, 2000), yjaA (Blattner *et al.*, 1997), and TSPE.C2 (Gordon *et al.*, 2008). *E. coli* is divided into four phylogroups; A, B1, B2, and D using this technique. The chuA, yjaA, and TSPE4.C2 genes are detected using polymerase chain reaction amplification, which generates 279, 211, and 152 base pair (bp) fragments, respectively. Using a simple dichotomous key method defined by the presence or absence of these three fragments, the strains of *E. coli* are then allocated to a phylogroup as either A (chuA -, TSPE4.C2 -), B1 (chuA -, TSPE4.C2 +), B2 (chuA +, yjaA +) and D (chuA +, yjaA -). (Figure 2.1) (Clermont *et al.*, 2000).

The precision at which the technique allocates *E. coli* to correct MLST-based is rated at 80–85% (Gordon *et al.*, 2008). The method has been used widely by researchers in typing and sub-typing *of E. coli* in both commensal and pathogenic (Tenaillon *et al.*, 2010).

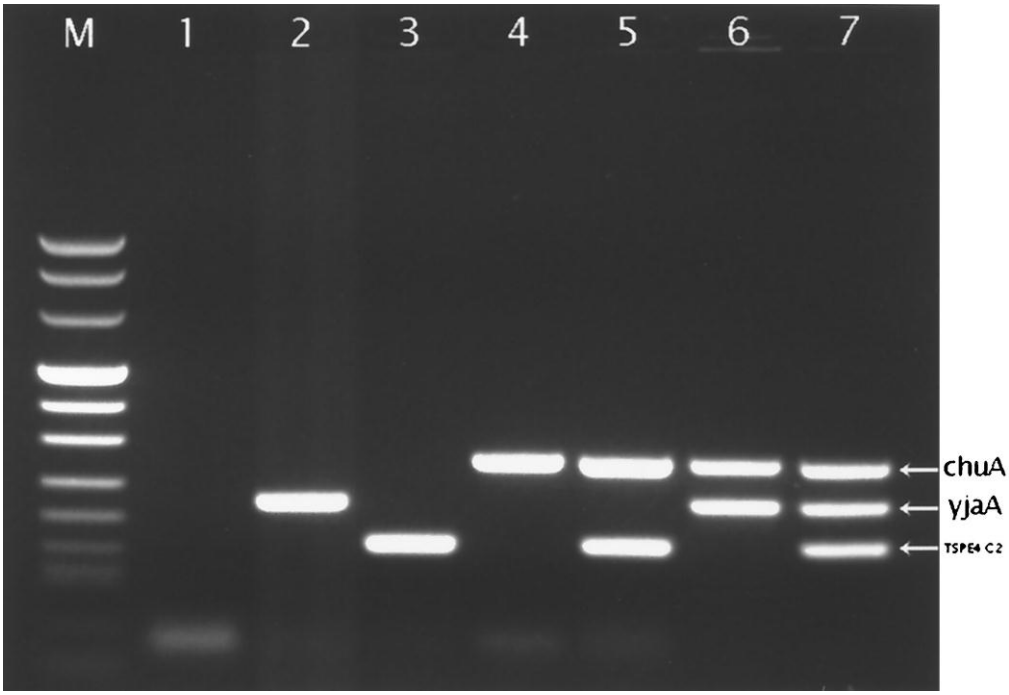

Figure 2.1: Triplex PCR profiles for *E. coli* phylogenetic groups.
Key: A strain's phylogenetic group can be determined by amplification of chuA, yjaA, and TSPE4.C2. For example Lanes 1 and 2, group A; lane 3, group B1; lanes 4 and 5, group D; lanes 6 and 7, group B2. Lane M contains the Ladder marker (Clermont *et al.*, 2000).

2.1.5 The Quadruplex PCR method

The quadruplex PCR method was a modification of the triplex method that involved the addition of a new gene arpA to the original target genes, chuA, YjaA, and TspE4.C2. Modified gene fragment sizes were arpA (400bp), chuA (288bp), yjaA (211bp), and TspE4.C2 (152bp). The method has identified eight main phylogroups, of which seven are *E. coli sensus stricto* (A, B1, B2, C, D, E, and F) while the eighth one is cryptic clade I of *Escherichia*. It has further made it easy to authenticate the efficacy of phylogroup assignment using triplex PCR procedure (Gordon *et al.*, 2008). Gordon *et al.*, (2008), though, noted that a small percentage of triplex PCR genotypes (A0, D1, and D2) were assigned incorrectly. Elsewhere, the strain of 0157:H7 which belongs to phylogroup E, F, and C had remained unassigned (Clermont *et al.*, 2011).

After PCR amplification using appropriate primers, *E. coli* is allocated to one of the eight phylogroups through a complex iterative approach, based on the scoring for the presence or absence of the arpA/chua/yjaA/TspE4.C2 genes in that order. For example; +-+-, indicates arpA +, chua -, yjaA +, and TspE4.C2 (Clermont *et al.*, 2013).

The technique has enabled at least 95% of *E. coli* to be identified, characterized, and classified to a phylogroup. Also, other *E. coli* cryptic clades (II to V) have been acknowledged. In addition, it has been revealed that around 13% of *E. coli* isolates fall in the new phylogroup C, E, F, and clade1 (Clermont *et al.*, 2013).

2.1.6 Distribution of *E. coli* Phylogroups in different hosts

Escherichia coli phylogenetic groups have diverse characteristics which make them differ in the distribution of hosts (Higgins *et al.*, 2007), phenotypic and genotypic characteristics, pathogenicity and virulence, biological niche, and resistant traits (Jang *et al.*, 2017; Raimondi *et al.*, 2019; Touchon *et al.*, 2020). *E. coli* phylogroups can be distributed non-randomly across host species (Carlos *et al.*, 2010). For example, group A is predominately found in humans (Escobar-Páramo *et al.*, 2006; Li *et al.*, 2010) despite the fact that A and B1 predominate in tropical regions (Escobar-Páramo *et al.*, 2004). Group B1 is said to be more prevalent in herbivores although it can be present in all hosts (Higgins *et al.*, 2007; Ishii *et al.*, 2007; Carlos *et al.*, 2010) and can stay in the environment for an extended period (Walk *et al.*, 2007; Jang *et al.*, 2017).

According to studies, strains of B2 and D are more virulent and pathogenic as compared to strains of A and B1 (Chakraborty *et al.*, 2015; Raimondi *et al.*, 2019). Moreover, extraintestinal pathogenic strains dominate groups B2 and D, and *E. coli* (O157:H7) producing verocytotoxin predominate in group D, where cattle serve as the primary reservoir (Carlos *et al.*, 2010). Phylogroup A and B1 are commensal strains that are more prevalent in herbivores. Antibiotic susceptibility testing reveals that phylogroups A and B1 have a higher resistance pattern, whereas B2 and D have a lower resistance pattern. (Chakraborty *et al.*, 2015; Raimondi *et al.*, 2019).

2.1.7 Antibiotics

Antibiotics are extremely important in the prevention and treatment of infectious diseases since the 1940s when they were first discovered (Zaffiri *et al.*, 2012; Penesyan *et al.*, 2015). They have been very useful in the advancement and continuation of modern medicine and hence are considered to be a breakthrough of modern science (Rossolini *et al.*, 2014). Antibiotics are bioactive secondary metabolites (de Lima Procópio *et al.*, 2012) and are classified into different classes which act on Gram-negative and/or Gram-positive bacteria (Coates *et al.*, 2011). The strength of antibacterial agents is mainly determined by their structure and degree of affinity to specific target sites within microbial cells. The nature of antibacterial agents' structure and degree of affinity for specific microbial cell target sites determine their effectiveness. Beta-lactams (β-lactams), aminoglycosides, fluoroquinolone, and tetracycline are some of the classes which are used in the treatment of bacterial infection (Coates *et al.*, 2011).

Beta-lactams contain the β-lactam ring and are grouped into penicillin derivatives, cephalosporins, monobactams, and carbapenems. To increase the spectrum of activity and prevent AR caused by β-lactamase production, penicillin derivatives such as amoxicillin are combined with clavulanic acid which is a β-lactamase inhibitor. The combination forms Amoxicillin-clavulanic acid (Coates *et al.*, 2011). Cephalosporins are broad-spectrum antibiotics that have greater antibacterial activity on gram-negative bacterial than Gram-positive bacteria. They are categorized into different generations based on bacterial activity whereby the newest generation has a better antibacterial activity in comparison to the previous generation (Coates *et al.*, 2011).

Aminoglycosides are therapeutically important antibiotics in the aerobic Gram-negative bacteria treatment in humans and animals. They include gentamicin and streptomycin. Their use is widely limited due to their toxic nature and ability to be released as residues in food animals (Coates *et al.*, 2011). Sulphonamides are amongst the oldest antibiotics in the treatment of bacterial infections, hence it records high resistance. Its combination with trimethoprim to form Sulfamethoxazole-trimethoprim increases the bacterial activity. *E. coli*, *Streptococci*, and *Staphylococci* are all susceptible to this combination. (Coates *et al.*, 2011).

Fluoroquinolones are antibacterial drugs with a broad spectrum of action that are used to treat infections caused by bacteria in humans and animals. Tetracycline is an antibiotic that is used to treat bacterial infections and it consists of oxytetracycline, chlortetracycline, doxycycline, and minocycline (Walsh, 2003; Coates *et al.*, 2011).

2.1.8 Mechanisms of Antibiotic Action

Quite a lot of literature has highlighted the mechanisms of antibiotic action (Hancock, 2005; Dubey, 2014; Ebimieowei and Ibemologi, 2016; Walsh and Wencewicz, 2016; Kapoor *et al.*, 2017). When exposed to the bacterial cell, antibiotics act on specific target sites within the bacterial cell. Understanding the mode of action of antibiotics is very critical in appreciating the development of AR. These mechanisms include the modification of cell membrane function, inhibition of cell walls, nucleic acid, and protein synthesis.

Beta-lactam (β-lactam) drugs act by preventing cell wall synthesis. The antibiotic agents adhere to the Penicillin-binding proteins (PBPs) enzymes involved in transpeptidation reactions on bacteria. Hence, peptidoglycan synthesis is inhibited, blocking the final transpeptidation and autolysis which results in bacterial death. When the β-lactam drug is combined with beta-lactamase inhibitors, it inhibits β-lactam by the production of beta-lactamase enzymes hence restoring the β-lactam drug antibacterial activity against lactamase-secreting bacteria i.e. Amoxicillin-Clavulanic acid (Hancock, 2005; Walsh and Wencewicz, 2016; Kapoor *et al.*, 2017).

Polymyxin act by rupturing the cytoplasmic membrane's functional integrity, allowing cell macromolecules and ions to escape. This damages the cell by altering the functions of the cell membrane (Hancock, 2005; Walsh and Wencewicz, 2016).

Chloramphenicol, Erythromycin, and Clindamycin act on the 50S ribosomal unit. On the other hand, Tetracycline and Aminoglycosides act on the 30S ribosomal subunit. Modification of bacteria’s ribosomal units inhibits protein synthesis.

Sulphonamides and trimethoprim act by competing for the enzyme required by para-aminobenzoic acid (PABA) which is involved in folic acid synthesis needed in nucleic acid synthesis. This inhibits nucleotide synthesis hence acts as antimetabolites.

Fluoroquinolones block DNA gyrase involved in the synthesis of deoxyribonucleic acid (DNA) while Rifampin inhibits Messenger RNA (mRNA) synthesis (Walsh and Wencewicz, 2016; Kapoor *et al.*, 2017).

2.1.9 Antibiotic Resistance

Antibiotic resistance (AR) arises when a bacteria does not respond to one or several antibiotics which are commonly used in the treatment whereas antimicrobial resistance (AMR) is a more general phrase, covering drug resistance in the treatment of infections caused by microorganisms. The antibiotic era has been characterized by several challenges such as a high level of resistance, limited supply of novel classes of antibiotics, and the reduction in discovery and production of new antibiotics by pharmaceutical companies (Aminov, 2010; Coates *et al.*, 2011; Piddock, 2012; Brown and Wright, 2016; Totsika, 2016).

The main selective pressures that influence variations in the occurrence of AR are the widespread antibiotics use in medicine, veterinary practices, and agriculture (Boerlin and Reid-Smith, 2008; Pikkemaat *et al.*, 2016). As articulated by Bessat *et al.*, (2019) inappropriate use of antibiotics, for instance, over-prescription, prophylaxis, wrong prescription, and under-dosage are a few of the contributing factors to the evolution of AR. Other key contributors to bacterial resistance include environmental changes and the disposal of unused therapeutic drugs in the environment. (Coates *et al.*, 2011; Laxminarayan and Heymann, 2012; Ayukekbong *et al.*, 2017).

Antibiotic Resistance emergence is a common part of bacterial evolution. It is a survival tactic in bacteria that relies on their phenotypical traits that are fit for the environment (Hughes and Andersson, 2015; Lukačišinová and Bollenbach, 2017). Emergence represents the conversion of wild-type to resistant phenotype. The emergence of antibiotic resistance is stimulated when bacterial populations are exposed to antibiotics. This imposes a selective pressure allowing just the resistant sub-populations of the bacteria to continue to exist.

Resistance can be either acquired or intrinsic (Rhodes and Schweizer, 2016). Intrinsic resistance is brought about by natural genes present in the genome of bacteria. It can also result from the inheritance of bacterium characteristics that make it resistant to certain antibiotics. (Lozano *et al.*, 2016). This mode of resistance is common to bacteria and is not dependent on the selective pressure from antibiotics (Gillespie, 2001; Cox and Wright, 2013). On the other hand, acquired resistance comes about when a specific bacterium develops resistance against a common antibiotic that it was previously susceptible to (Sandoval-Motta and Aldana, 2016).

As mentioned by Kumar, (2017), acquired resistance traits, as opposed to the intrinsic, are only present in selected strains of bacterial species. Important to note is that there exist two mechanisms by which acquired resistance in bacteria is developed. They include the acquisition of naturally existing resistance genes from neighboring bacteria and spontaneous mutations in chromosomal genes (Alekshun and Levy, 2007).

Horizontal gene transfer can occur over bacterial species boundaries or within a bacterial species (Daubin and Szöllősi, 2016). The transfer is aided through DNA integration in transposons, bacteriophages, mobile genetic elements, and plasmids or naked DNA uptake within a bacterial species. Even if there is no selection, gene transfer elements that are resistant to their new host adapt quickly and are not easily lost. (Bengtsson-Palme *et al.,* 2017). Because a majority of resistant genes are grouped on mobile genetic elements, a single transfer of these genes can influence the development of resistance to a wide range of drugs (Koike *et al.*, 2017). Bacteria employ different mechanisms in resistance development. These include the modification of target site, modification of enzymes, decreased uptake due to shift in antibacterial agent, and by-pass of the metabolic pathway (Tenover, 2006).

2.1.10 Antibiotic Sensitivity/Susceptibility testing

This is a very useful test in clinical practices as it gives general guidelines and knowledge about the sensitivity of antibiotics in bacterial isolates (Reller *et al.*, 2009). It selects the most effective antibiotic and detects the possible drug resistance in bacterial isolates. According to Reller *et al.*, (2009), the test is critical on members of *Enterobacteriaceae, Pseudomonas, Staphylococcus, Enterococcus,* and *Streptococcus pneumonia* that can acquire resistance. Many rapid (broth microdilution, automated instrument) and manual (disc diffusion, gradient diffusions) test methods are available for susceptibility testing (Reller *et al.*, 2009).

The Kirby-Bauer disc diffusion (Biemer, 1973), is widely used due to its flexibility, cost-effectiveness, and simplicity (Hudzicki, 2009; Reller *et al.,* 2009). The method involves the diffusion of the antibiotic disc with fixed concentrations into a solid medium inoculated with a pure bacterial isolate.

A bacterial inoculum is seeded on the Mueller-Hinton Agar (MHA) plate's surface. Antibiotic discs were aseptically set up on the inoculated agar surface and the plates are incubated at 35°C for 16-24 hours. Using a clear ruler and a millimeter scale, each antibiotic disc zone of growth inhibition is measured (mm). The diameter of the zone of inhibition indicates the isolate's susceptibility and the rate of drug diffusion through the agar medium and can be classified as susceptible, intermediate, or resistant following published standards in the Clinical and Laboratory Standards Institute (CLSI) or US Food and Drug Administration (FDA) (Reller *et al.,* 2009).

2.1.11 Impacts of antibiotic resistance

Resistance to antibiotics is an escalating risk for human and animal welfare (Chang *et al.,* 2015). Besides health risks, the rate of development, as well as the spread of antibiotic resistant strains, has negatively impacted the affected populations' socio-economic growth (Gaygısız *et al.,* 2017).

Antibiotic resistance's impact on human health is more severe (Friedman *et al.,* 2016). Currently, an increase in multidrug-resistant bacteria or antibiotic resistance signals a strong prediction to the return of the pre-antibiotic era (Woodford *et al.*, 2011). This is whereby common infections will be frequently untreatable. Reports indicate that in human medicine, the consequences of antibiotic resistance go further than a failure of treatment in individual cases (Laxminarayan *et al.,* 2013).

According to Laxminarayan *et al.*, (2013), it stands hazardous to perform important medical procedures like organ transplantation, major surgery, and cancer chemotherapy without effective antibiotics. It is estimated that by the year 2050, infections resulting from resistant bacteria will cause approximately ten million people to die each year (Kraker *et al.*, 2016).

In wildlife and livestock production, the consequences of antibiotic resistance are comparable to those for humans (Vittecoq *et al.*, 2016). The authors' further state that the resultant effect on animals is increased suffering and mortality. Despite this, currently available antibiotics will continue to be used in veterinary medicine in the future (Aminov, 2010). Reduced successful treatment alternatives for animals in this case is a major cause of reduced productivity of animals that produce food. This could lead to a major crisis in the food and animal industry globally (Bengtsson and Greko, 2014). Further, the affected families have to incur the high cost of treatment of their animals and family members' healthcare (Lushniak, 2014).

2.1.12 The role of commensal *E. coli* in antibiotic resistance

Commensalism is an organism-to-organism relationship in which one benefits from the other without affecting the other. It may occur in bacteria and their hosts, whether they're animals or human hosts in different locations of their body sites such as the skin and the GI tract (Faust *et al.*, 2012). Commensal bacteria according to the definition are harmless to their host, even though in certain circumstances they can turn out to be pathogenic (Marshall *et al.*, 2009; Brown *et al.*, 2012).

During the process of antimicrobial infectious disease treatment, antimicrobial selective pressure is said to be exerted on commensals (Marshall *et al.*, 2009). The commensal population contains a diverse group of bacteria and genes that are important in the AMR emergence by selection and transfer of resistant strains and genes (Andremont, 2003; Courvalin, 2008).

As articulated by Andremont, (2003), it is believed that AMR emerges first in the commensal flora and disseminates via horizontal gene transfer to other microbes. In regards to this, the proportion of commensals with AMR is regarded as a reliable indicator of antimicrobial selection pressure and a predictor of pathogen resistance emergence (van den Bogaard and Stobberingh, 2000). Microbiota in the GI tract remains a major commensal in the body (Andremont, 2003), and an important reservoir for multidrug-resistant bacteria (Wellington *et al.*, 2013). *E. coli* is used as a model organism in the scientific research and development of modern molecular biology. Its fast growth rates and genetic simplicity makes it preferable in laboratories (Idalia and Bernardo, 2017; Jang *et al.*, 2017).

E. coli exceptional ability to gain and spread resistance genes, acquire conjugative plasmids with ease, is an attribute that makes it relevant in human medicine and resistance monitoring programs (Swedres-Svarm, 2016). Furthermore, the bacteria acquire a pool of mobile resistance traits that may be transferred to other bacterias such as *Salmonella*, thus performing a significant role in resistance's spread and persistence (Marshall and Levy, 2011).

According to Kariuki, (2010), the widespread antibiotic resistance phenotypes in commensal bacteria, for instance, *E. coli* in humans, animals, and the wider environment remains a key indicator of antibiotic resistance levels and the transmission of resistant genes to the endogenous bacterial populations.

2.1.13 Veterinary drug use and resistance

Veterinary pharmaceuticals are widely used in food animals for health, nutrition, reproduction, and productivity (Clement *et al.*, 2019). The drugs are extensively used to reduce the burden of infectious diseases which threatens socio-economics by either killing rapidly a large number of animals or instilling fear of the spread of zoonotic diseases (Perry and Grace, 2009; Meseko *et al.*, 2014). Zoonotic diseases in animals account for 60% of all animal diseases, hence the treatment is comparatively important as a control mechanism and a measure to decrease the chances of transmission of zoonotic diseases to humans (Taylor *et al.*, 2001) which can cause epidermics and pandemics.

An estimated 50% of all antimicrobials produced globally are used in veterinary services. The choice of the drug is determined by factors such as efficacy, availability, and cost which are often determined by the manufacturers in developed countries before being imported to developing countries in large quantities hence aimed at frequent and intensive usage (Clement *et al.*, 2019).

Studies have disclosed that routine antibiotic usage and misuse in livestock and poultry have tremendously improved animal production (Kardos, 2015; Clement *et al.*, 2019). On the other hand, frequent antibiotic use in food animals poses a great threat of bacterial resistance transmission into humans via the food supply chain and environment (Landers *et al.*, 2012; Clement *et al.*, 2019). Thousands of tons of antibiotics are assumed to be excreted to the environment annually. Antibiotics used in livestock are estimated to be excreted in urine and stool and spread via fertilizer, ground water, and surface runoff in 90 percent of cases. (Bartlett *et al.*, 2013).

2.1.14 Antibiotic resistance genes: their emergence and spread

The human-animal interface is complicated, with several paths which encourage the spread of pathogens resistant to antibiotics. Bacteria and genes that are resistant to antibiotics can spread within and between different types of bacteria and species of animals, from human to animals and vice-versa. Increased overlap in habitat, sharing of landscape resources among humans, livestock, and wildlife create an opportunity for interchange of bacteria and genes that are resistant (Hassell *et al.*, 2017; Tormoehlen *et al.*, 2019). Phylogenetic or environmental boundaries have no effect on the bacteria or bacteria's genes after resistance acquisition (Smillie *et al.*, 2011).

Antibiotic resistance can spread both horizontally and vertically (Guardabassi and Kruse, 2008). “Spread” in this context denotes the transmission of bacterial resistance genes from one generation to the next, as well as the transfer of host and environmental resistance elements among bacteria. Vertical spreading occurs when a new generation inherits resistance determinants while horizontal spreading results from the sharing or exchanging of resistant genes among bacteria (Partridge *et al.*, 2018).

Bacteria with resistant genes can then disseminate between hosts directly through direct contact with skin, or indirectly through contact with feces or saliva that has been contaminated or exposure to food, feed, soil, air, water, or wildlife that is contaminated (Aminov, 2011; Wellington *et al.*, 2013; Founou *et al.*, 2016; Jang *et al.*, 2017; Muloi *et al.*, 2018), or by occupational exposure (Marshall and Levy, 2011; Landers *et al.*, 2012; Woolhouse *et al.*, 2015).

Introducing antibiotics in the environment allow AR strains of bacteria to multiply in the absence of would be their potential competitors (Jang *et al.*, 2017; Durão *et al.*, 2018). This is hastened by favorable ecological changes which enable the bacteria to adapt to these new environmental conditions (Woolhouse *et al.*, 2015). Bacteria may be stimulated to produce reactive oxygen species when exposed to bactericidal antibiotics like beta-lactams, fluoroquinolones, and aminoglycosides (Kohanski *et al.*, 2007; Dwyer *et al.*, 2009). When reactive oxygen species come into contact with bacterial DNA, they can damage it, leading to the accumulation of mutations.

Hence, even low doses of bactericidal antibiotics may lead to the resurgence of multidrug-resistant mutants (Kohanski *et al.*, 2010). In addition, it has been revealed that exposure to reactive oxygen species can also lead to activation of the stress response system (SRS) (Poole, 2012). DNA damage induces the SRS response by arresting cell division and inducing mutagenesis and DNA repair (Aertsen *et al.*, 2004). This response has been revealed to promote resistant genes transfer through the increase of expression of genes that can be transferred (Van der Veen and Abee, 2011).

The global spread of AMR is influenced by a variety of factors, including animal, food, and human movement (Laxminarayan *et al.*, 2013). Animals are well-known to constitute a vast reservoir of enteric bacteria which they transfer to the environment by organic waste (Venglovsky *et al.*, 2009). In addition, food consumption especially that originating from animals is a vital tool for the spread of resistant bacteria to humans (Xiong *et al.*, 2018).

Once resistant bacteria reach the new host, they may colonize, infect, or exist transiently (Manaia, 2017). In the new host, due to co-resistance or cross-resistance, the resistant bacteria either obtain resistance genes from other bacteria or transmits resistance genes to them (Cantón and Ruiz-Garbajosa, 2011; Bengtsson-Palme *et al.*, 2017; Partridge *et al.*, 2018). Antibiotic use by individuals who share the same environment has proven to enhance the spread of bacteria resistance (O'Brien, 2002; Levy and Marshall, 2004). Antibacterial treatment in the bacteria population reduces the ratio of susceptible to resistant microbes (McEwen and Fedorka-Cray, 2002), and the individual treatment of the residing microbiota. This increases the risk of exposure and colonization of resistant strain from the environment to the treated individual (Willing *et al.*, 2011).

Through evolutionary progress resistance genes can be retained, and be dispersed among bacterial populations (Van Overbeek*et al.,* 2002). As further explained by the authors, genes that confer resistance can be passed from one human pathogen to the next. This makes the commensal bacteria a reservoir for potentially pathogenic bacteria's resistance genes. Antibiotic resistant *E. coli* has a great interest in human medicine because of the clonal spread and shared transfer of resistance. These applications hasten the selection of antibiotic resistance in bacterial populations.

2.1.15 Antibiotic resistance in wildlife

Wildlife is indirectly being subjected to clinically relevant antibiotics (Wang *et al.*, 2017). Nonetheless, AR in wildlife has been progressively reported in several studies similar to the situation in humans and livestock (Woolhouse *et al.*, 2015; Wang *et al.*, 2017). This explains the complication of AR in wildlife, as well as the likelihood of interspecies spread amongst humans, livestock, wildlife, and the environment.

Spillover of antibiotics used in humans and livestock contributes to wildlife resistance (Skurnik *et al.*, 2006). For example, approximately 70% of tetracycline antibiotics are released in an active form through urine and feces in the environment (Daghrir and Drogui, 2013). The consequences of dissemination of genes that cause antibiotic resistance in wildlife (Martinez, 2009) and interspecies transmission (Benavides *et al.*, 2012) are poorly understood especially where wildlife is involved.

The presence of genes that cause antibiotic resistance in natural settings is linked to human activities that contaminate the natural environments. The contamination has been heightened within the wildlife ecosystem due to the sharing of habitants with human settings. This puts constant antibiotics pressure on human activities such as livestock husbandry, clinical settings, and the continuous leakage of these resistant traits to natural settings via manure and wastewater which alter the bacteria populations (Radhouani *et al.*, 2014). Because of this, the wildlife population has been considered to play a role as the reservoir host and biological mediators of transmission of zoonotic pathogens. This is also heightened by the increasing human activity to wildlife populations which is evident by increased human intrusion into wildlife territory, significant habitat fragmentation, and loss of biodiversity.

2.2 Empirical review

For the advancement of solutions to antibiotic resistance, it is vital to understand the variables, methods, and approaches associated with the control and management of antibiotic resistant bacteria, genes, and their hosts. In this respect, researchers have sought, in their works, to study these methods, variables, and approaches. For example;

Mainda *et al.*, (2015), carried out a study that concentrated antibiotic use on small, medium, and commercial-sized dairy farms in Zambia's central region, as well as antibiotic resistance in *E. coli*. They employed a stratified random sampling method to examine 104 farms, which represented roughly 20% of all dairy farms in the region. Animal faecal samples were randomly sampled from each farm and a standardized questionnaire was completed on the usage of antibiotics. They reported having obtained *E. coli* isolates from 98.67 percent of the animals sampled, which were then tested to see if they were resistant to six different classes of antibiotics.

Resistance was estimated to be present in a variety of farming systems as; tetracycline (10.61; 95%), ampicillin (6.02; 95%), sulfamethoxazole/trimethoprim (4.49; 95%), cefpodoxime (1.91; 95%), gentamicin (0.89; 95%) and ciprofloxacin (0%). Uni-variate analyses showed that some diseases, exotic breeds, location, farm size, and management systems are risks for resistance detection, on the other hand, multivariate analyses indicate a link to lumpy skin disease and a protective effect in animals older than 25 months. Mainda *et al.*, (2015),

Study provides novel intuitions into the factors that influence antibiotic use and their relationship with antibiotic resistance in Southern Africa's understudied region. The results of their study were well articulated and they can be used to further research on the control, prevention, and management of antibiotic resistance.

A study was done by Srivani *et al.*, (2017) whose aim was to examine the prevalence, virulence determinants, and antibiotic susceptibility of Shiga toxigenic *Escherichia coli* (STEC) in buffalo calves with diarrhea from the states of Andhra Pradesh and Telangana. There were 375 feces samples in total. STEC were isolated, and a multiplex polymerase chain reaction was used to detect virulence genes. Isolates were tested for antimicrobial resistance by the disk diffusion method. From their study, the researchers reported that *E. coli*-associated diarrhea was found in 85.04 percent of buffalo calves, with STEC accounting for 35.01 percent. They also reported that tetracycline had the highest antimicrobial resistance at 63.21% followed by ampicillin at 48.11%, while antibiotics such as chloramphenicol, gentamycin (96.33%), and imipenem (99.06%) were found to be susceptible.

In diarrheic buffalo calves, multidrug resistance was found in 69.81 percent of STEC isolates. Srivani *et al.*, (2017), concluded that multidrug resistant *E. coli* are becoming more common, hence in cases of buffalo calf with diarrhea, a careful selection of an antimicrobial agent may be required. This research was well carried out and its results are well articulated. However, the study focuses only on the virulent Shiga toxigenic *E. coli* and does not highlight diversity and patterns of antibiotic resistance in commensal *E. coli.*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in the Maasai Mara Ecosystem (MME) in Narok County, at the interface of humans, livestock, and wildlife. The MME encompasses the Maasai Mara National Reserve (MMNR) surrounded by several communities and privately managed conservancies and human settlements (Figure 3.1). MMNR is a conservation protected area that is located on the South-Western part of Kenya (1° 00′ and 2° 00′ S and longitudes 34° 45′ and 36° 00′ E) along the Kenya-Tanzania border occupying an area of approximately 1510km².

The ecosystem experiences short and long rains in the months of November-December and March-June respectively whereas the dry season extends from July to October. The vegetation mainly consists of grassland with shrubs and thorny bushes and rolling hills (Serneels *et al.*, 2001). The rise in temperature levels in the Mara region has widely contributed to the increasing habitat desiccation (Ogutu *et al.*, 2008).

The ecosystem is supported by the Mara and Talek rivers (Walpole, 2003). The ecosystem is further characterized by a huge density and rich diversity of wildlife that includes but are not limited to the famous wildebeest migration, lions, elephants, and the African buffalo (Mduma and Hopcraft, 2008; Ngene *et al.*, 2017).

The Maasai people who mainly inhabit the MME are transhumant pastoralists. They keep large herds of cattle for their socio-economic income. They live in small villages, together with their livestock, surrounded by a fence mostly of *Acacia* thorns called a *Boma*. They are known to be prudent users of antibiotics.

The Maasai people are prudent users of antibiotics especially oxytetracycline to treat and prevent diseases in their animals (Group, 2011; Roulette *et al.*, 2017). The area is served by different health centres which are located on the ecosystem. The health centres include Sekenani Health Centre, EMF Aitong Health centre, Talek Community Health centre, and Naikara Health centre.

The direct competition for resources within the MME has led to the intense pressure of conflict between human and livestock-wildlife which is a major crisis (Mukeka *et al.*, 2018). The conflict is intensified by the rapidly increasing population densities of humans, grazing herds, and resident wildlife, the straying of wildlife outside their designated areas, and changing trends of land use at the reserve boundary (Lamprey and Reid, 2004).

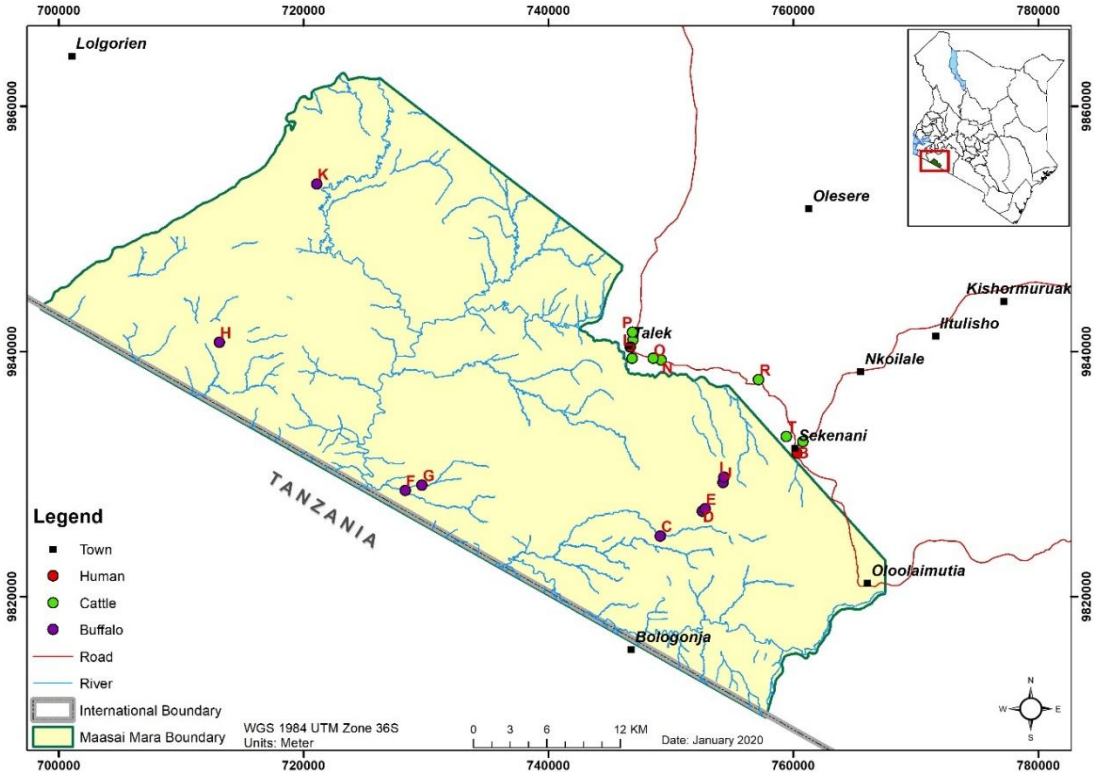

Figure 3.1: A map of Maasai Mara Ecosystem showing sampling points for humans, cattle, and African Buffalo in Kenya

3.2 Study subjects and ethical considerations

Human, cattle (*Bos taurus*), and African buffalo *(Syncerus caffer)* subjects were used in this study. Previous studies have revealed the interaction of humans, livestock, and wildlife at the MME (Bhola *et al.*, 2012). The National Commission for Science, Technology, and Innovation issued a research permit (NACOSTI/P/18/58265/24147) (Appendix 1). The ethics permit for the human component was approved by the Kenyatta University Ethics Review Committee (KU/ERC/APPROVAL/VOL.1 (123) (Appendix 2). Authority for the access and use of wildlife samples was approved by the Kenya Wildlife Service (KWS/BRM/5001) (Appendix 3).

3.3 Sample Size

The sample sizes for testing differences in proportions of *E. coli* isolates that are susceptible to antibiotics using chi-square goodness of fit, a power analysis was determined by G*Power 3.1.9.2 software (Faul *et al.*, 2007). Assuming an intermediate effect size (w=0.3) and power of 0.95 as recommended, then a total sample size of 200 samples comprising of samples from buffalo, cattle, and humans were sufficiently used to achieve the study objectives. The sample size in a human was 50 fecal samples, in cattle was 50 dung samples and in buffalo was 50 dung samples with interaction and 50 dung samples without interaction.

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

Human fecal sampling was done at Sekenani health centre and Talek community health centres, (Health centres within the 20km buffer Zone outside the reserve boundary) after ethical consideration requirements were fulfilled. The sampling involved patients attending the above health centres and presenting with diarrhea. The patients must be at least 18 years of age and must volunteer to take part in the study by signing a consent form. Further, the patients must be residents of MME within a 20km buffer Zone outside the MMNR boundary. Both sexes were equally considered.

Cattle dung sample collection was done from households within the 20km buffer zone outside the reserve boundary. Households were identified as those with livestock (cattle) and those who agree to participate in the study. It was assumed that cattle were healthy and sampling included all age groups.

Buffalo sample collection was carried out at MMNR. African buffalo population naturally occurs as distinct herds which average 10-500 individuals. Buffalo herds were identified and those within a distance gradient in terms of location as being near (<20km) or further (>20km) from the human settlement were noted and sampled. It was assumed that buffalo were healthy and sampling included all age groups.

NB: Since the reserve is not fenced-off, the area <20km inside and >20km outside the reserve boundary represents a shared habitat area for both humans, livestock, and wildlife.

3.4.2 Exclusion criteria

Human sampling excluded patients under the following category; patients under the age of 18 years. Those with diarrhea but already taking antibiotics. Those with severe or unstable mental or physical health. Those who decline to sign the consent forms and those who withdrew from the exercise. Further individuals who identify as living outside the boundaries 20km buffer zone. MME.clinics outside the 20km buffer zone were not sampled, these clinics include EMF Aitong Health centre and Naikara Health centre.

Cattle sampling will exclude those homesteads which decline to give consent for sample collection, homesteads without cattle, and those which lie outside the 20km buffer zone. Buffalo sampling excluded solidarity old males and lone buffalo herds which tend to be violent.

3.5 Sample collection

Dung samples from the African buffalo were collected under the guidance of Kenya Wildlife Services. Purposeful sampling was used from the identified buffalo herds. Herds were identified and approached by a vehicle. The animals were observed at a distance and fresh dung samples were collected within minutes of being voided. This allowed correct identification of the animal species voiding the dung.

For cattle, dung samples were collected either in the cattle boma or in the field. The animals were observed and freshly voided dung was collected. The dung samples from buffalo and cattle were aseptically obtained by gently opening the inner part of the dung bolus and swabbing the inner portion with a sterile swab stick. This was to avoid dung bolus that was in contact with the surface to curb environmental contamination.

Human fecal sampling was hospital-based on gastrointestinal patients. The study employed a convenience sampling technique. The clinician presented the study and consenting forms to eligible patients (Appendix 4). The clinician then provided sterile fecal pots with swabs to the consenting patients who handed over the fresh sample. For confidentiality purposes, the human samples were named with a unique identification code.

The swabs containing the samples from humans, cattle, and buffalo were aseptically dipped into sterilized bijou bottles containing Stuart's transport media (Himedia, India). The bottles containing the swabs were then closed. The bottles were labeled with details of location coordinates, unique sample identifier, and date of collection and then placed in a cooler box. Human and animals samples were collected daily for two months (Plate 3.1-3.3).

Plate 3.1: Brief consultation with Maasai elders and seeking voluntary consent for cattle dung collection in Maasai Mara Ecosystem.

Plate 3.2: Carrying out dung collection in the cattle boma in Maasai Mara Ecosystem

Plate 3.3: Dung collection from a herd of African buffalo within the Maasai Mara National Reserve

3.6 *Escherichia coli* isolation and identification

Escherichia coli isolation was done using Eosin Methylene blue (EMB) agar, a differential, and selective culture medium. Each bijou bottle was aseptically opened and the swab containing the collected human fecal and animal dung samples was removed then inoculated in a separate bijou bottle containing enriched buffered peptone broth (Himedia, India). Incubation was done for 24 hours at 37^{o}C. This was to enable the recovery of all bacteria since buffered peptone broth is a non-selective pre-enrichment.

By use of a sterile inoculation loop, a small portion of the incubated broth was selected and aseptically streaked onto EMB medium (Himedia, India) on agar plates. For 24 hours, the plates were incubated at 37°C while inverted, in line with the guidelines of the Clinical and Laboratory Standards Institute (Wayne, 2012). After incubation, each sample medium was inspected for growth presenting blue-black colonies that had metallic green sheen color. For further purification, the characteristic colonies from each medium were obtained and aseptically streaked on Nutrient agar (NA) (Himedia, India). In the current study, the tip of a 24-hour colony was picked and incubated for 24 hours at 37°C. The isolation was done alongside Standard *E. coli* American Type Culture Collection (ATCC) 25922. The presumptive pure discrete *E. coli* colonies were further confirmed by the standard biochemical tests for *Enterobacteriaceae*. The test includes; triple sugar iron agar test, citrate utilization test, methyl red-Voges Proskauer test, and indole test. The tests were run alongside *E. coli* ATCC 25922.

3.6.1 Triple Sugar Iron (TSI) agar test

A colony of *E. coli* on NA was gently touched by a sterilized straight inoculation loop. This was followed by aseptically inoculating the colony into a TSI medium through the tube's center to the bottom. After stabbing, streaking was then performed on the surface of the agar slant. The tubes were closed loosely using a ball of cotton wool and incubation was done for 24 hours at 37°C. They were inspected for color change and observations made.

3.6.2 Citrate utilization test

In this study, a loop-full of *E. coli* colony was streaked over the slant of Simmon's citrate agar. The contents were then incubated at 37°C with a loose cap for 24 to 48 hours. The slant was inspected and observation recorded.

3.6.3 Methyl Red-Voges-Proskauer (MRVP) test

A loop-full of 24 hours old *E. coli* was inoculated in MRVP broth. The broth containing the bacteria was then incubated at 37^{o}C for a maximum period of 72 hours. After 72 hours of incubation, 1ml of the broth was put in a sterile test tube and 5ml of methyl red was added to test for MR test. Observations were made and recorded.

To test for VP, 1ml of the original broth was aliquoted to a sterile test tube and five drops of the Napthol reagent were added, followed by 5 drops of Potassium Hydroxide. The tube was gently shaken and set aside for 15 minutes. Observations were made and recorded.

3.6.4 Indole test

In this study, a loop-full of a well isolated *E. coli* colony was inoculated into the broth of tryptone and incubated overnight at 37^{o}C. A few drops of Kovacs reagent were added into the inner wall of the tube containing the broth culture. Observations were made and recorded.

3.7 Molecular characterization

The pure *E. coli* isolates were further subjected to molecular characterization to identify and determine their genetic diversity. Molecular characterization involved four steps namely, the extraction of genomic deoxyribonucleic acid (gDNA), Polymerase Chain Reaction (PCR), 16S rRNA gene sequencing, and Clermont quadruplex PCR.

3.7.1 Bacterial DNA extraction

The bacterial isolates obtained from the samples were individually re-suspended in 200µI in 1.5ml eppendorf tubes alongside *E. coli* ATCC 25922. The suspensions were centrifuged for 3 minutes at 10,000 RPM and the supernatant was discarded. PureLink Genomic DNA Mini Kit from Invitrogen was used to extract DNA in line with the manufacturer's protocol. Quantification of DNA was done using NanoDrop$^{TM\ 2000}$ Spectrophotometer. To check for DNA integrity, the DNA was run on a 1.5 percent Agarose gel with 1X Tris-Borate-EDTA (TBE) buffer. The DNA samples were standardized so that all samples used for PCR had a concentration of 25ng/µI (Desjardins and Conklin, 2011).

3.7.2 Molecular amplification of the *E. coli* 16S rRNA gene region

To confirm *Escherichia coli* strains, the 16S rRNA gene region of the gDNA was amplified by conventional PCR with P3F/P5R primers (Tsen *et al.*, 1998). The primer sequence was as follows (Table 3.1). A total volume of 50 ul was achieved as per the protocol. The PCR parameters were as follows; initial denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 3 min. A total of 35 PCR cycles were performed using the GeneAmp 9600 PCR system from Perkin-Elmer (Tsen *et al.*, 1998). Agarose gel (1.5%) was prepared with ethidium bromide and the amplicons were run alongside a PCR ladder (100bp). The gels were photographed using the gel documentation system, which used Ultra-violet (UV) light to visualize them. The amplicons were purified using the QIAquick PCR purification Kit according to the manufacturer's instructions. Sanger sequencing was done at Macrogen Inc., Europe using original primers P3F/P5R

Table 3.1: Primer sequences for amplifying the 16S rRNA gene region of the gDNA of *E. coli* isolates.

Primer	Primer Sequence	Reference
p3F	5’ATTAGATACCCTGGTAGTCC -3’	Tsen *et al.*, (1998)
p5F	5’-GGTTACCTTGTTACGACTTC -3	Tsen *et al.*, (1998)

3.7.3 Molecular amplification by the Clermont quadruplex gene regions of *E. coli*

To classify *E. coli* phylogenetic groups, Clermont Quadruplex PCR method primers were used in the amplification of the targeted gene ArpA, chuA, yjaA, and TspE4.C2 (Clermont *et al.*, 2013). Multiplex PCR was used to amplify the specified primers (Table 3.3) in a reaction volume of 20µl. The reaction volume contained 2µl of 10X buffer (supplied with *Taq* polymerase), 2µM each dNTP, 2 U of *Taq* polymerase, 2 µlof DNA template, and the appropriate primers. The primer concentration was as follows; chuA.1b (20 pmol), chuA.2 (20 pmol), yjaA.1b (20 pmol), yjaA.2b (20 pmol), TspE4C2.1b (20 pmol), TspE4C2.2b (20 pmol), AceK.f (40 pmol), ArpA1.r (40 pmol). PCR reactions were performed under; initial denaturation at 94°C for 4 minutes, followed by 30 cycles (99°C for 5 seconds, 59°C for 20 seconds) and a final extension step (72°C for 5 minutes) (Clermont *et al.*, 2013).

The amplicons were resolved alongside a Gel pilot PCR ladder (100bp) in a 2% agarose gel stained with SYBR® Safe DNA gel stain and run at 100 volts for 30 minutes. The gels images were obtained using a gel documentation system after being exposed to UV light. The bands were used to assign *E. coli* into phylogenetic groups.

Table 3.2: The primer sequences used in the quadruplex phylo-typing method

Target	Primer ID	Primer sequence (‘5-3’)	Reference
ChuA	chuA.1b	ATGGTACCGGACGAACCAAC	Clermont *et al.*, (2013)
	chuA.2	TGCCGCCAGTACCAAAGACA	Clermont *et al.*, (2013)
YjaA	yjaA.1b	CAAACGTGAAGTGTCAGGAG	Clermont *et al.*, (2013)
	yjaA.2b	AATGCGTTCCTCAACCTGTG	Clermont *et al.*, (2013)
ArpA	AceK.f	AACGCTATTCGCCAGCTTGC	Clermont *et al.*, (2013)
	ArpA1.r	TCTCCCCATACCGTACGCTA	Clermont *et al.*, (2013)
TspE4.C2	TspE4C2.1b	CACTATTCGTAAGGTCATCC	Clermont *et al.*, (2013)
	TspE4C2.2b	AGTTTATCGCTGCGGGTCGC	Clermont *et al.*, (2013)

3.8 Antibiotic sensitivity test

The standard Kirby-Bauer disk diffusion method was used to determine the antibiotic sensitivity profiles of *E. coli* in this study (Baur *et al.*, 1966). The isolates were subjected to a panel of five antibiotics (Table 4.3). The antibiotics were selected based on the chemical structures, critically important and commonly used in both human and animal medicine (Organization, 2017; Van *et al.*, 2020; Ma *et al.*, 2021). The sensitivity tests were done using commercial discs (Himedia, India). A sterile swab was used to pick a single pure 24 hours old *E. coli* colony from Nutrient Agar medium and make a suspension corresponding to 0.5 McFarland turbidity standards.

Aseptically the suspension was picked by dipping a fresh new swab which was used to spread the *E. coli* suspension evenly onto the entire dried surface of Mueller-Hinton medium (Oxoid, United Kingdom). Within 15 minutes, an antibiotic-impregnated disc of the selected antibiotics (Table 3.4) was placed individually on top of the agar using sterile forceps. Five antibiotic-impregnated discs were placed per plate against each isolate. The culture was incubated for 18 hours at 35°C.

Antibiotic activity of antibiotics was confirmed by examining the presence or absence of a zone of inhibition. The diameter of the zone of inhibition was read using a ruler in mm and recorded. *E. coli* were classified as resistant, intermediate, or susceptible to the antibiotic based on the diameters per CLSI guidelines (Wayne, 2012) (Appendix 1.0).

Table 3.3: The antibiotics used to test for *E. coli* antibiotic resistance profiles in humans, cattle, and buffalo in the Maasai Mara Ecosystem

Class of antibiotic	**Name and concentration of antibiotic**
Penicillin's (β-lactam)	Amoxicillin-clavulanic acid (AMC, 30µg)
Cephalosporin	Ceftriaxone (CRO, 30µg)
Aminoglycosides	Gentamicin (CN, 10µg),
Fluoroquinolones	Ciprofloxacin (CIP, 5µg)
Tetracycline	Tetracycline (TE, 30µg)

3.9 Preparation of long-term stocks

A single *E. coli* colony was streaked onto Nutrient agar by a sterilized wire loop and then incubated at 37^{o}C for 12 to 18 hours. A significant amount of the overnight culture was obtained with a sterile swab aseptically and emulsified in vials containing double-strength nutrient broth and supplemented with 50% sterile glycerol. After that, the isolates were kept at -20°C (Gorman and Adley, 2004).

3.10 Data analysis

3.10.1 Phylogenetic analysis of 16S rRNA gene of *E. coli* gDNA sequences

SeqTrace analysis software was used to align and edit the chromatograms for forward and reverse sequences, and the low-quality sequences were rejected (Stucky, 2012). Cluster X version 2 was used to align the consensus nucleotide sequences (Thompson *et al.*, 1994), in the Molecular Evolutionary Genetics Analysis (MEGA X) software (Kumar *et al.*, 2016). The consensus nucleotide was blasted using the Basic Local Alignment Search Tool (BLASTn) program (Altschul *et al.*, 1990) to confirm that they were *E. coli*. The confirmed consensus nucleotide was collapsed using FaBoX to obtain the unique sequences herein stated as haplotypes. The haplotypes obtained were compared with similar sequences from National Center for Biotechnology Information (NCBI) GenBank (Benson *et al.*, 2009) using the Basic Local Alignment Search Tool (BLASTn) program (Altschul *et al.*, 1990).

3.10.2 Determination of *Escherichia coli* Phylogenetic groups

To assign *E. coli* into a phylogenetic group, the simple triplex PCR method criteria was used instead of the quadruplex PCR method which was used in the amplification stage. This was because of difficulties experienced during the scoring of phylogroups by the Clermont Quadruplex PCR method whereby most of the results obtained needed further classification by amplifying with specific primers (Clermont *et al.*, 2013).

The simple triplex PCR technique assigns *E. coli* isolates into phylogroups A, B1, B2, and D. The technique involves the PCR detection of chuA, yjaA, and TspE4.C2 genetic markers at different fragments as follows: chuA (297 base pairs), yjaA (211base pairs) and TspE4.C2 (152 base pairs).

A PCR ladder was used to score the presence of different gene products for phylogroup detection. This method uses the presence or absence of a combination of bands to define *E. coli* strains into phylogroups. For instance, phylogroup A (*chuA* -, TspE4.C2 +), B1 (*chuA* -, TspE4.C2 +), B2 (*chuA* +, *yjaA* +) and D (*chuA* +, *yja+A* -) (Clermont *et al.*, 2000; Gordon, 2010).

3.10.3 Statistical Analysis

The prevalence of *E. coli* was determined as a percentage of positive bacterial isolates out of the total samples collected from each host. To test the association between *E. coli* phylogroups and their hosts, a chi-square test of association was performed. When the expected chi-square is less than five, a Monte Carlo approximation to calculate the p-value was used. A similar chi-square test was used to evaluate the probability of a relationship between antibiotic resistance and host.

To test the influence of distance of buffalo herds from human habitation and the antibiotic resistance, a Generalized Linear Model with a binomial error structure using the R software for statistical computing was used. In these analyses, the presence of antibiotic resistance coded as 1 and 0 for a lack of resistance was used as response variable and distance of buffalo host from human habitation as a predictor variable. In addition, to test the effect of distance from human habitation to the number of *E. coli* strains that are resistant to antibiotics in buffalo, a Generalized Linear Model with a Poisson error structure was applied. For these models, the number of antibiotics that a single host isolates was resistant to was taken as response variation and the distance of hosts from human habitation was taken as an independent/predictor variable.

CHAPTER FOUR

RESULTS

4.1 Genetic Profiling of *E. coli* strains isolated from humans, cattle, and buffalo

4.1.1 Isolation and identification of *E. coli* strains from humans, cattle, and buffalo

Each sample was taken to represent one *E. coli* isolate. When cultured on an EMB growth medium, *E. coli* ferments lactose to produce flat dark purple colonies characterized by a distinct green metallic sheen (Plate 4.1).

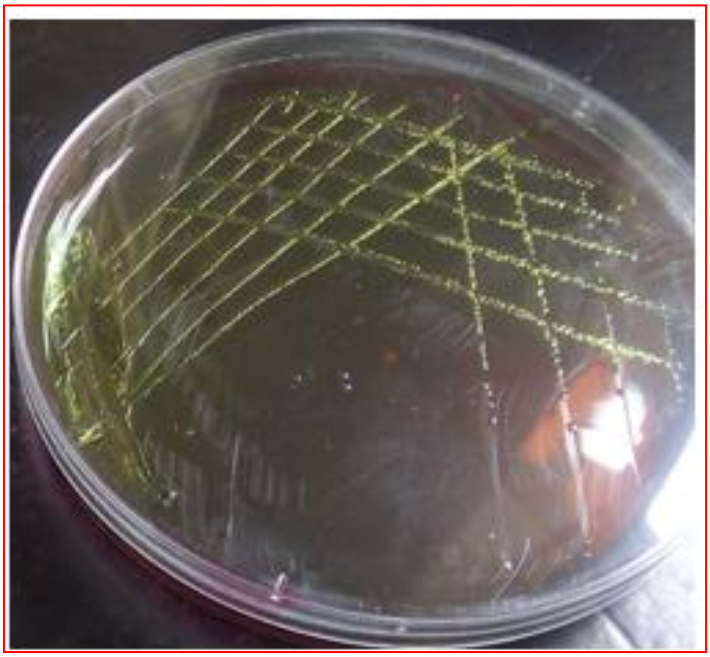

Plate 4.1: An image of bacterial growth on Eosin Methylene Blue medium showing green metallic sheen that is characteristic of an *E. coli* colony.

Based on the isolates' morphological traits on nutrient agar medium, colony shape was circular; elevation was raised; the colonies' margins observed were entire; colonies revealed smooth surfaces; colony sizes were large; the texture of the colonies was either moist or mucoid. When analyzed for translucent and opacity on NA medium, colonies were found to be either opaque or transparent.

Biochemical analysis observations were as follows; the presumed *E. coli* was TSI positive. Both the slant and the butt were yellow (Acid slant/acid butt reaction) indicating fermentation of glucose, lactose, and/or sucrose. There was gas production indicated by cracks on the medium and no production of hydrogen sulfide due to the absence of blackening of the medium at the bottom. The bacteria were negative for the citrate test due to persisting green color after the incubation. The indole test was positive because after adding the Kovac's reagent to the peptone broth and incubating it, a bright red layer formed at the interface. The bacteria also showed positive methyl red test due to the formation of a distinct red and negative Voges-Proskauer test due to persistent yellowish color.

Table 4.1: Biochemical test results for the identification and confirmation of *E. coli* colonies

Test	***Escherichia coli***
TSI	Positive ((Ac S/Ac B/G)
Citrate	Negative
Indole	Positive
Methyl-red	Positive
Voges-proskauer	Negative

Key: Ac S-Acid slant, Ac B-Acid butt, G- gas production

Based on morphological and biochemical characteristics of bacterial colonies from human, cattle, and buffalo isolates, 99/121 (82%) were *E. coli.* Of the positive samples for *E. coli*, 35 belonged to humans, 24 to buffalo, and 40 to cattle (Table 4.2).

Table 4.2: Prevalence of *E. coli* isolates from human, cattle, and buffalo samples in the Maasai Mara Ecosystem

Study species	**Samples collected**	**Samples positive for *E. coli n* (%)**
Human	39	35 (90%)
Cattle	51	40 (78%)
Buffalo	31	24 (77%)
Total	**121**	**99 (82%)**

4.1.2 Molecular analysis of the 16S rRNA gene of *E. coli*

Genomic DNA extracted from the 99 isolates of *E. coli* was positively amplified, however, only 72 amplicons were obtained. The amplified 16S gene region of PCR amplicons showed a definite and appropriately sized band in lanes (bp) (Plate 4.2).

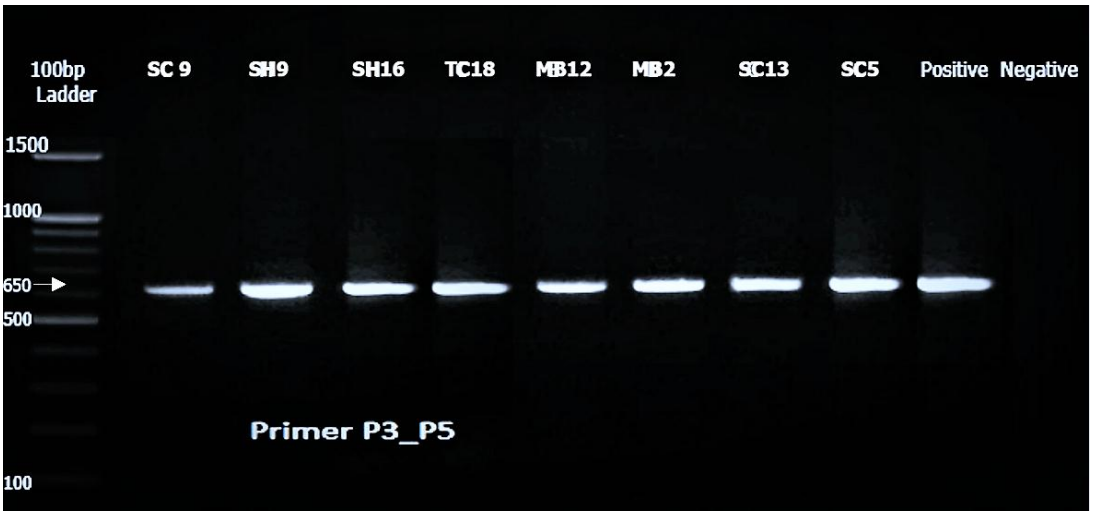

Plate 4.2: PCR amplified product for the 16S rRNA gene region of gDNA from *Escherichia coli* isolates from humans, cattle, and buffalo on an agarose gel.
Key: (100bp-ladder, SC- Sekenani Cattle, SH- Sekenani Human, TC-Talek Cattle, MB- Mara Buffalo) Positive control-*E. Coli* ATCC 25922).

4.1.3 Haplotype diversity of antibiotic resistant *E. coli* isolates from human, cattle, and buffalo in the Maasai Mara Ecosystem

Out of the 72 amplicons, only 50 isolates that had resistance yielded positive amplicons and were sequenced. Resistant isolates included any isolate which was resistant to either one or multiple antibiotics used in this study. Upon editing, 46 sequences of the 16S rRNA were clean, 19 from humans, 16 from cattle, and 11 from buffalo and were used in the analysis.

The genomic sequence revealed 14 unique haplotypes when collapsed by FaBox software (Table 4.3). Haplotypes were assigned identity numbers as H1 to H14. The haplotype was blasted using the NCBI blast Algorithm and all the sequences matched *Escherichia coli* by a 99% similarity index (Table 4.3). Eight different haplotypes were discovered in buffalo *E. coli* isolates while six different haplotypes were discovered in both human and cattle *E. coli* isolates (Table 4.3). Haplotypes 4 and 7 were present in all the three sympatric hosts and were detected at different frequencies (Table 4.3).

Haplotype 4 was detected in buffalo at a frequency of 18.2 %, in cattle at a frequency of 31.3 % and in humans at a frequency of 63.3 % (Table 4.3). Haplotype 7 was detected in cattle, buffalo and human at frequencies of 25 %, 18.2 % and 15.8 % respectively (Table 4.3). Similar haplotypes were detected in two hosts at different frequencies. Haplotype 2 was detected at frequencies of 6.3 % in cattle and 5.3 % in humans while haplotype 9 was detected at frequencies of 9.1 % in buffalo and 25.0 % in cattle. Most of the haplotypes were present in only one host. They included haplotype 1, 3, 5, 6, 8, 10, 11, 12, 13 and 14 (Table 4.3).

Table 4.3: Proportions of haplotypes among isolates of *Escherichia coli* from human, cattle, and buffalo

Haplotype IDs	**Hosts and percent of *E.***			**Percent identity**	**Genbank Accession Number**	**Genbank species ID**
	Human, n=19	Cattle, n=16	Buffalo, n=11			
H1	0	0	1(9.1%)	99.70%	CP020516	*Escherichia coli*
H2	1	1	0	99.85%	CP020516	*Escherichia coli*
H3	1	0	0	99.70%	MG557808	*Escherichia coli*
H4	12	5	2	100.00%	CP020516	*Escherichia coli*
H5	1	0	0	99.85%	CP020516	*Escherichia coli*
H6	0	0	1 (9.1)	99.85%	CP020520	*Escherichia coli*
H7	3	4	2	100.00%	CP020520	*Escherichia coli*
H8	0	0	1	99.56%	MH671432	*Escherichia coli*
H9	0	4	1	100.00%	KX708709	*Escherichia coli*
H10	0	1	0	99.85%	MG557808	*Escherichia coli*
H11	0	1	0	99.70%	MH671432	*Escherichia coli*
H12	0	0	2	99.85%	MF582341	*Escherichia coli*
H13	0	0	1	99.85%	LC389167	*Escherichia coli*
H14	1	0	0	100.00%	KP772060	*Escherichia coli*
Haplotype	6	6	8			

4.1.4 Distribution of *E. coli* phylogroups by the simple triplex PCR method

All the 72 amplicons which were obtained during DNA extraction were subjected to the Clermont Quadruplex PCR method. 22, 33, and 17 isolates of *E. coli* were from humans, cattle, and buffalo respectively. The expected fragment sizes for the positive genes were as follows; Arp (400bp), chuA (297bp), yjaA (211bp), and DNA fragment TspE4.C2 (152bp) (Plate 4.3).

NB: The Arp (400bp) gene was not used in the analysis although the Clermont Quadruplex PCR method was used in the amplification. This was due to the challenges which were encountered since most of the results obtained by Quadruples PCR needed further classification by using other primers to specify the exact phylogroup. Hence, the simple triplex PCR method was used in the analysis.

The presence or absence of chuA, yjaA, and TspE4.C2 amplified genes were scored following the simple triplex PCR method to determine which phylogroups the isolates of *E. coli* from cattle, humans, and buffalo belonged to (Table 4.4, 4.5, &4.6).

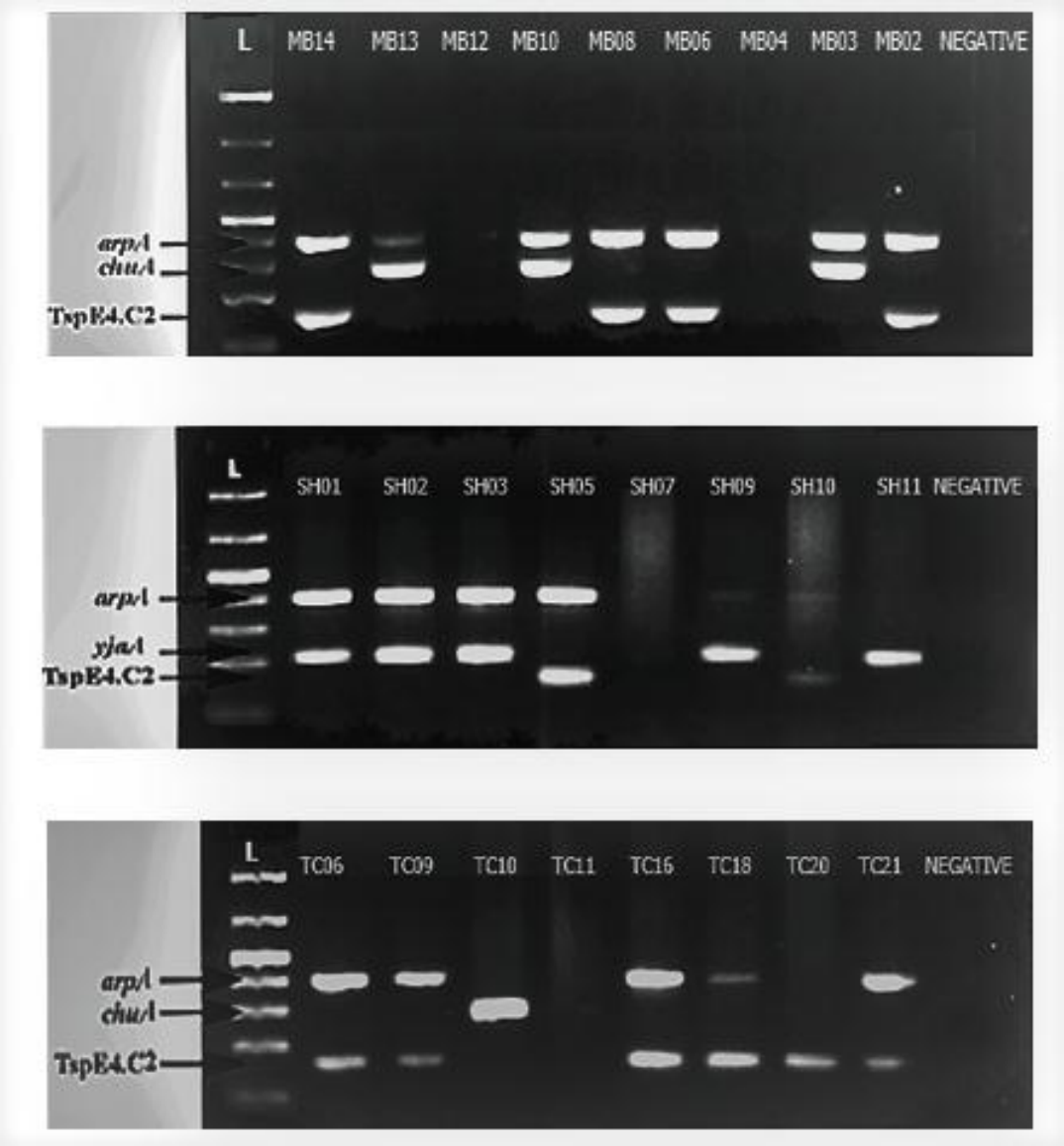

Plate 4.3: Gel Images (top, middle and bottom panels) of amplified fragment size band for Arp, chuA, yjaA, and TspE4.C2 genes of *E. coli* isolates from humans, cattle, and buffalo in the Maasai Mara Ecosystem.
(Key: L-100bp Ladder, SH-Sekenani Human, TH-Talek Human, MB-Mara Buffalo, TC-Talek Cattle).

Table 4.4: Interpretation of a top image of Plate 4.3 (Panel one)

Isolate ID	*E. coli* genes				
	arpA	**chuA**	**yjaA**	**TspE4.C2**	**Phylogroup**
SH01	+	-	+	-	A
SH02	+	-	+	-	A
SH03	+	-	+	-	A
SH05	+	-	-	+	B1
SH07	-	-	-	-	A
SH09	+	-	+	-	A
SH10	+	-	-	+	B1
TH01	-	-	+	-	A

Key: (+) denotes positive gene amplification, (-) denotes gene not amplified

Table 4.5: Interpretation of a middle image of plate 4.3 (Panel two)

Isolate ID	*E. coli* genes				
	arpA	**chuA**	**yjaA**	**TspE4.C2**	**Phylogroup**
MB14	+	-	-	+	B1
MB13	+	+	-	-	D
MB12	+	-	-	-	A
MB10	+	+	-	-	D
MB08	+	-	-	+	B1
MB06	+	-	-	+	B1
MB04	-	-	-	-	A
MB03	+	+	-	-	D
MB02	+	-	-	+	B1

Key: (+) denotes positive gene amplification, (-) denotes gene not amplified

Table 4.6: Interpretation of a bottom image of plate 4.3 (Panel three)

Isolate ID	*E. coli* genes				
	arpA	**chuA**	**yjaA**	**TspE4.C2**	**Phylogroup**
TC06	+	-	-	+	B1
TC09	+	-	-	+	B1
TC10	-	+	-	-	D
TC11	-	-	-	-	A
TC16	+	-	-	+	B1
TC18	+	-	-	+	B1
TC20	-	-	-	+	B1
TC21	+	-	-	+	B1

Key: (+) denotes positive gene amplification, (-) denotes gene not amplified

E. coli isolates from phylogroups A and B1 predominated in MME. Human *E. coli* isolates were predominated by phylogroup A while phylogroup B1 and D predominated in buffalo *E. coli* isolates (Figure 4.1). *E. coli* isolates in phylogroup B2 was observed in both human and cattle and absent in buffalo (Figure 4.1).

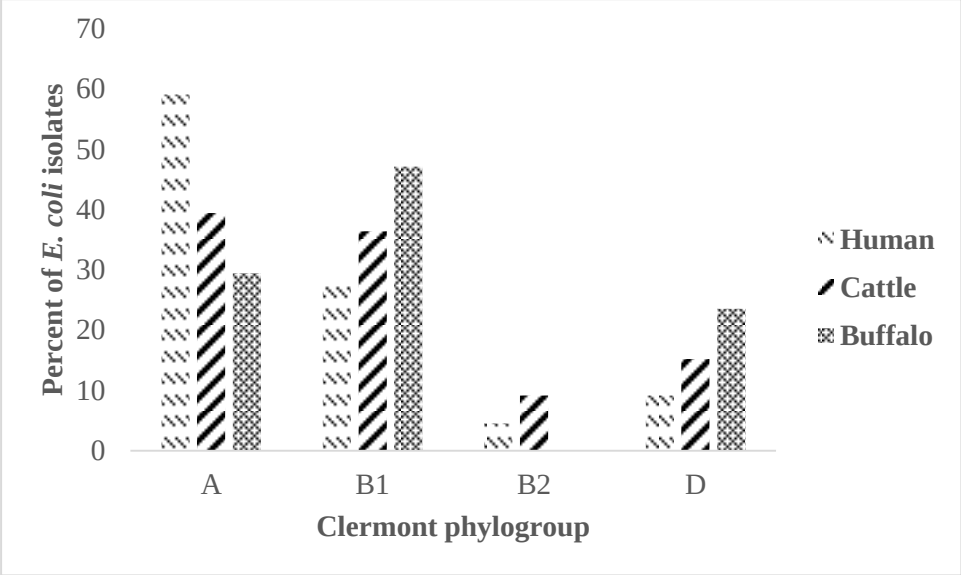

Figure 4.1: Proportion of Phylogroups of *E. coli* isolates from humans, cattle, and buffalo in the Maasai Mara Ecosystem.

4.1.5 The distribution of *E. coli* haplotypes among phylogroups

Haplotypes were variedly distributed across the hosts. Although most of the haplotypes were present in single hosts, some were commonly shared across the three hosts (Table 4.7). Haplotype 4 and 7 were present across the three hosts (Table 4.7). It was also observed that some haplotypes belonged to several different phylogroups. Specifically, haplotype 7 and 9 belonged to phylogroup A, B1, and D whereas haplotype 4 belonged to all the phylogroups (Table 4.7). Most of the *E. coli* isolates in haplotype 4 were grouped in phylogroup A and B1 and a few in phylogroup B2 and D (Table 4.7).

Table 4.7: The distribution of haplotypes and occurrence of haplotypes across phylogroups in *E. coli* isolates from human, cattle, and buffalo

Haplotype IDs	**Number of *E. coli***	**Host**			**Phylogroup**			
		Human	**Cattle**	**Buffalo**	**A**	**B1**	**B2**	**D**
H1	1	0	0	1	1	0	0	0
H2	2	1	1	0	2	0	0	0
H3	1	1	0	0	1	0	0	0
H4	19	12	5	2	9	8	1	1
H5	1	1	0	0	1	0	0	0
H6	1	0	0	1	0	1	0	0
H7	9	3	2	4	4	3	0	2
H8	1	0	0	1	0	1	0	0
H9	5	0	4	1	1	3	0	1
H10	1	0	1	0	0	1	0	0
H11	1	0	1	0	1	0	0	0
H12	2	0	0	2	1	1	0	0
H13	1	0	0	1	0	0	0	1
H14	1	1	0	0	1	0	0	0

To visualize the relationship among *E. coli* 16S haplotypes and to be able to cluster them according to groups representing clusters with minimum changes, a median-joining network implemented in the popart software was used (Leigh and Bryant,2015). Clusters were formed by grouping together connected haplotypes separated by less than 3 mutational steps. Three haplogroups were formed separated by 3 mutational steps; Haplogroup (HG) 1, 2, and 3 (Figure 4.2). Haplogroup 1 was the most detected with 8 haplotypes, this was followed by haplogroup 2 (4 haplotypes) and lastly haplogroup 3 (2 haplotypes) (Figure 4.2).

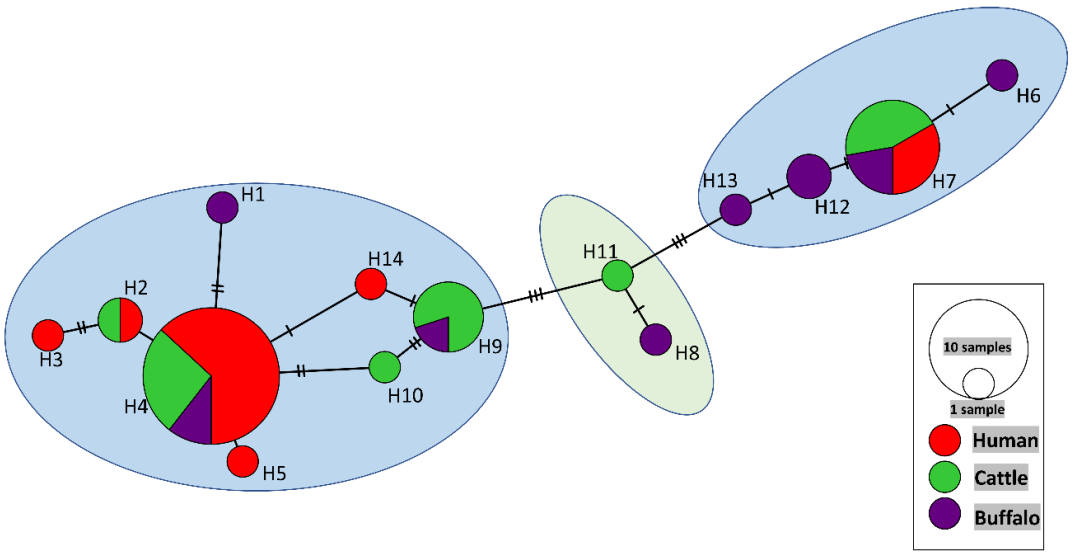

Figure 4.2: Grouping of haplotypes into haplogroups by the median-joining network. Three haplogroups were identified separated by 3 mutational steps.
Key: The larger group with more haplotypes (n= 31) is haplogroup 1; the group with a moderate number of haplotypes (n = 12) is haplogroup 2; the middle group with two haplotypes is haplogroup 3.

In the distribution of the haplogroups across the phylogroup, it was noted that phylogroup A, B1, B2, and D were represented in haplogroup 1. Phylogroup A and B1 dominated across all haplogroups. Phylogroup B2 was only present in haplogroup 1 while phylogroup D was present in haplogroup 1 and 2 (Table 4.8).

Table 4.8: Distribution of *E. coli* phylogroups across the three haplogroups

Haplogroup	**Phylogroup**				
	A	B1	B2	D	**Total**
HG1	16	12	1	2	**31**
HG2	5	5	0	3	**12**
HG3	1	1	0	0	**2**
Total	22	18	1	5	**46**

4.1.6 Genetic Diversity of *Escherichia coli*

When tested for haplotype sharing, mean genetic diversity (theta) based on 16S was higher in buffalo (mean and SE 0.0077 + 0.00214) and cattle (0.00630 + 0.00197) and comparably lower in humans (0.0046 + 0.0014). When tested for phylogroup sharing, there was extensive *E. coli* phylogroup sharing among sympatric cattle, humans, and buffalo, as shown by lack of a statistically significant association between phylogroups and host of *E. coli* isolates using Monte Carlo Chi-square approximation (χ^2= 6.2288, P= 0.3843) or Fisher's exact tests (P = 0.454). However, there was also an association between host and *E. coli* haplotype (Monte Carlo simulation χ^2= 37.192, P = 0.01449; or Fisher, Exact test; P = 0.017) but not between host and haplogroup (Monte Carlo simulation χ^2= 7.6967, P = 0.08746; Fisher Exact test: p = 0.05574).

4.2 Prevalence of antibiotic resistant *E. coli* isolates from human, cattle, and buffalo

Overall, *E. coli* antibiotic resistance to more than one antibiotic was 64% (63/99) in humans, cattle, and buffalo (Table 4.9). Human *E. coli* isolates had the highest antibiotic resistance at 94% (33/35) (Table 4.9). This was followed by *E. coli* isolates from buffalo and cattle at 50% (12/24) and 45% (18/40) respectively (Table 4.9). The *E. coli* isolates showed varied susceptibility patterns when compared across selected antibiotics and the hosts of the isolates. Among the five antibiotics tested, *E. coli* isolates from humans, cattle and buffalo showed the highest resistance to tetracycline at 83%, 45%, and 33% respectively (Table 4.10).

There was an observed unique trend in susceptibility patterns of isolates recovered from buffalo samples (n=24). Across the antibiotics, no isolates were found to be resistant to Amoxicillin-Clavulanic Acid or Ceftriaxone. Across humans, cattle, and buffalo, the resistance of recovered *E. coli* isolates was highest against Tetracycline and lowest against Amoxicillin-Clavulanic Acid (Table 4.10). The susceptibility profile of *E. coli* isolates that were susceptible, intermediate, and resistant to the selected antibiotics is outlined (Appendix 6)

Table 4.9: Antibiotic resistant pattern of *E. coli* isolates from human, cattle, and buffalo to at least one antibiotic

Study species	**Positive *E. coli* isolates**	***E. coli* resistant isolates n (%)**
Human	35	33 (94%)
Cattle	40	18 (45%)
Buffalo	24	12 (50%)
Total	**99**	**63 (64%)**

Table 4.10: Proportions of *Escherichia coli* isolates from humans, cattle, and buffalo that are Resistant to different antibiotics

Antibiotics	Resistant profiles in Percentage n (%)		
	Human, n=35	**Cattle, n=40**	**Buffalo, n=24**
Tetracycline	29 (83%)	18 (45%)	8 (33%)
Gentamicin	18 (52%)	7 (18%)	6 (25%)
Ciprofloxacin	13 (37%)	3 (7.5%)	3 (13%)
Ceftriaxone	12 (34%)	2 (5%)	0 (0%)
Amoxicillin-Clavulanic Acid	8 (23%)	1 (2.5%)	0 (0%)

4.2.1 Individual antibiotic resistance profiles of *E. coli*

E. coli resistance to tetracycline was associated with the host of isolate using Monte Carlo simulation of chi-square test (χ^2 = 19.7, df = NA, p-value = 0.0004998). This antibiotic recorded the highest level of resistance by *E. coli* isolates from all hosts as evidenced by Table 4.10. *E. coli* resistance to Ceftriaxone was associated with the host of *E. coli* isolate using both exact chi-square tests (χ^2 = 18.415, df = 4, p-value = 0.001024) and Monte Carlo simulation of chi-square test (χ^2 = 18.415, df = 4, p-value = 0.001024). Unlike cattle and humans, buffalo had no *E. coli* isolates resistant to ceftriaxone (Table 4.10). *E. coli* resistance to Ciprofloxacin was associated with the host of *E. coli* isolate with both exact chi-square tests (χ^2 = 13.654, df = 4, p-value = 0.008484 (Table 4.10). *E. coli* resistance to Gentamicin was associated with the host of *E. coli* isolate using chi-square tests (χ^2 = 9.8402, df = 4, p-value = 0.04321).

This is the second antibiotic that showed one of the highest resistances by *E. coli* isolates from all hosts (Table 4.10). *E. coli* resistance to amoxicillin-clavulanic acid was associated with the host of *E. coli* isolate with both exact chi-square tests (χ^2 = 35.681, df = 4, p-value = 3.365e-07). Amoxicillin-Clavulanic Acid was effective against 100 percent of buffalo *E. coli* isolates and the majority of cattle *E. coli* isolates. Only humans had high levels of resistance to Amoxicillin-Clavulanic Acid (Table 4.10).

4.2.2 Patterns of multi-drug resistance of *E. coli* in human, cattle, and buffalo

Among the isolates from humans, cattle, and buffalo, different resistance patterns to antibiotics were observed (Table 4.11). Seven (29.2 %) of the isolates recovered from buffalo samples were resistant to only one antibiotic while 5 (20.8%) were resistant to two different antibiotics. However, this was not the trend with isolates recovered from the human sample which showed fluctuating trend on the antibiotics tested (Table 4.11). The multidrug resistance categories of *E. coli* isolates were associated with hosts of *E. coli* isolate using chi-square association tests (χ^2 = 45.654, df = 10, p-value = 1.657e-06). Isolates obtained from human and cattle samples showed multidrug resistance, although it was high in humans as compared to cattle. Of the total isolates from humans, 4 (11.4%), 8 (22.9%), and 3 (8.6%) showed multiple resistance against 3, 4, and 5 different antibiotics respectively (Table 4.11). *E. coli* from buffalo samples did not show any multidrug resistance patterns (Table 4.11). General multidrug resistance to three antibiotics was recorded at 6%, to four antibiotics at 10 % and resistance to all the antibiotics tested was recorded at 3 % (Appendix 7).

Table 4.11: Resistance patterns in human, cattle, and buffalo *Escherichia coli* isolates in the Maasai Mara Ecosystem

Host	Frequency (%) of antibiotic resistance						
	0	**1[a]**	**2[b]**	**3[c]**	**4[c]**	**5[c]**	**N**
Buffalo	50%	29.2%	20.8%	0%	0%	0%	24
Cattle	57.5%	17.5%	17.5%	5.0%	5.0%	0.0%	40
Human	2.9%	48.6%	5.7%	11.4%	22.9%	8.6%	35

[a]Resistance to only one antibiotic, [b]Resistance to only two antibiotics, [c]Multi-drug resistance

4.3 Spatial effect of resistance to antibiotics in *E. coli* isolates from buffaloes with varying degrees of interaction with humans and cattle

There was no influence of distance from human on the presence of antibiotic resistance *E. coli* in buffalo using a logistic regression (beta= -0.00007, z-value= -0.87. df=22, P= 0.384) or on the extent of multidrug resistance evaluated using a Poisson regression (beta= -0.000025, z-value= -0.492, df= 22, p-value = 0.623). Comparison of antibiotic resistance *E. coli* in buffaloes with and without interaction per antibiotic tested was analysed (Table 4.12). According to the results, antibiotic resistance was prevalent in isolates of *E. coli* from buffalo with interactions when tested against Tetracycline, Ciprofloxacin, and Gentamicin as compared to *E. coli* isolates obtained from buffalo without interaction (Table 4.12).

Table 4.12: Prevalence of antibiotic resistance *E. coli* isolates from buffalo with and without interaction in the MME

Antibiotics	**Prevalence of resistant *E. coli* n (%)**	
	Buffalo (Interaction)	**Buffalo (No interaction)**
Tetracycline	6 (27%)	1 (5%)
Gentamicin	5 (23%)	0 %
Ciprofloxacin	3 (14%)	0 %
Ceftriaxone	0 (0%)	0 %
Amoxicillin- Clavulanic Acid	0 (0%)	0 %
Resistant to at least 1	11 (50%)	1(5%)
Multi-Drug Resistance (3+)	0 %	0%

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Isolation and genetic profiles of *E. coli* strains isolated from humans, cattle, and buffalo

5.1.1 Morphological and Biochemical identification of *E. coli*

The bacterium *E. coli* is a commensal that is widely used to assess antibiotic resistance in multi-host systems and thus its correct identification is important. Based on the necessary parameters, *E. coli* were successfully cultured, isolated, and confirmed by both morphological and biochemical indicators. The positive reaction of isolates of *E. coli* with Indole and Methyl red coupled by the absence of reaction on citrate and Voges-Proskauer tests confirmed the isolates were *E. coli* (Fratamico and Smith, 2006).

High rate of recovery of isolates of *E. coli* from fecal samples 82% (99/121) shows that the sampling procedure and preservation in the field were adequate. In addition, it also suggests that humans and animals in Maasai Mara Ecosystem harbor a high prevalence of commensal. Specifically, humans had a higher prevalence of *E. coli* in comparison to the sympatric animals, though cattle and buffalo had comparable prevalence. A similar pattern has been observed in a city ecosystem in Kenya where humans had higher *E. coli* prevalence compared to either bovines or caprines (Hassell *et al.*,2019; Muloi *et al.*, 2019).

Overall, there are only about 22 studies that seem to investigate antibacterial resistant *E. coli* between humans and animals (Muloi *et al.*, 2018) and hence this study will significantly contribute baseline data for comparison. Some of the studies include the one carried out in Tanzania whereby *E. coli* was used to determine whether the practice of co-grazing with cattle and wildlife constitutes a risk of transmission of antibiotic resistant bacteria to wild ungulates. From the study results, it was concluded that there is antibiotic resistant *E. coli* and enterococci in wild animals in the absence of antibiotic pressure (Katakweba *et al.*, 2015).

Klous *et al.*, (2016) carried out another study on antimicrobial resistance in humans, animals, and the wider environment. From the study, it was clear that farm animals and soil microbes act as reservoirs of the resistance gene. Another study on the human/livestock/wildlife interface was carried out in South Africa whereby *E. coli* populations were used to assess the risk of bacterial and antibiotic resistance dissemination between hosts. The findings of the study confirmed that buffalo and cattle share similar phylogroup profiles dominated by B1 (44.5%) and E (29.0%). It also recorded a significant gradient of antibiotic resistance from isolated buffalo to buffalo in contact with cattle populations (Mercat *et al.*, 2016). Hassell *et al.*, (2019) also carried out a study on the interface in Nairobi, Kenya. The study findings indicated that wildlife carries a low prevalence of *E. coli* isolates susceptible to all antibiotics tested and a high prevalence of clinically relevant multidrug resistance which varied between taxa and by foraging traits.

5.1.2 Molecular analysis of the 16S rRNA gene of resistant *E. coli* isolates from human, cattle, and buffalo

According to the findings of the present study, the resistant *E. coli* in the sympatric human, cattle, and buffalo were genetically diverse based on the 16S rRNA gene sequencing and was represented by 14 unique haplotypes which occurred in only three haplogroups. The 16S rRNA gene consists of a conserved and highly variable region hence the reason it was used in species identification (Kolbert and Persing, 1999; Rajendhran and Gunasekaran, 2011; Fuks *et al.*, 2018). The genetic diversity of *E. coli* haplotypes could be due to slight changes in genetic make-up attributed to selection pressure (Ebner *et al.*, 2011) or may be due to a combination of new alleles (De Jong *et al.*, 2011; Johnson *et al.*, 2019). Under selection pressure, the mutation rate and population size of a single clone (haplotype) build up genetic variation in the population, and therefore most of the time a population is not genetically uniform (Kao and Sherlock, 2008; Barrick and Lenski, 2009; De Jong *et al.*, 2011). The genetic variations have been revealed to arise following clonal interference which is environmental dependent (Chattopadhyay *et al.*, 2007).

The distribution of the haplotypes across the hosts was variable. Some haplotypes occurred in a single host while others in multiple hosts which suggests the dominance of particular haplotypes in the ecosystem. This is maybe due to the “Evolution of a single clone” from the genotype parent and progeny, which results in a number of closely related haplotypes (Nejati-Javaremi and Smith, 1996; Janssen *et al.*, 2016).

This may indicate haplotype association and the probability that they evolved from a single locus of genotype parent, even though haplotypes don't always show the same ancestry (Nejati-Javaremi and Smith, 1996; Guan, 2014). For instance, haplotypes 4 and 7 were detected in all hosts but at different frequencies. Further, the occurrence of H4 in humans at the highest frequency suggests that the haplotype is perhaps more diverse and dominant in humans than cattle and buffalo, or reflects reduced opportunities for cross-species transmission.

This pattern in the frequency of occurrence may also depict the directionality, whereby antibiotic resistant strains flow from humans to cattle and buffalo at different rates based on their level of interaction (Klous *et al.*, 2016; Mercat *et al.*, 2016). In contrast, H7 occurred at the highest frequency in cattle followed by buffalo, and last in humans. This pattern presents an alternative directionality where antibiotic resistance originates from animals to humans. This is supported by high selective pressure from usage and misuse of antibiotics in animals whereby antibiotics and elements that are resistant to antibiotics are released to the environment in urine and fecal droppings by livestock which spread through groundwater, surface runoff, and fertilizer (Johnson *et al.*, 2001; Bartlett *et al.*, 2013; Mercat *et al.*, 2016). Resource-scarce communities like pastoralist communities in Maasai Mara, consumption of groundwater from streams, wells, and run-off is a viable transmission source of antibiotic resistance elements that originate from animals.

Although this study provides "clear evidence of commonality" of antibiotic resistant isolates from humans and animals, it does not verify cross-species transmission or exclude the possibility that humans and animals acquire the same antibiotic resistant *E. coli* from external sources without directly exchanging the strains.

Wildlife populations may also serve as a conduit, resistant strains can evolve into more variant forms and when introduced back into humans and the domestic population, they increase the public health risks (Davies and Davies, 2010). The co-occurrence of haplotypes 4 and 7 in all the hosts implies the mutual sharing of these haplotypes within the ecosystem. The co-occurrence of the two major haplotypes may involve frequency-dependent selection, wherein a host, the different haplotypes are detected at different frequencies. The occurrence of haplotypes detected in all the hosts in the highest frequencies as compared to haplotypes detected in only one host can be attributed to the fact that large populations have high levels of mutational input, therefore the frequency of haplotypes with beneficial mutations are thought to increase and escalate (Desai and Fisher, 2007).

5.1.3 Phylogroups of *E. coli* isolates from human, cattle, and buffalo

The concept of classifying *E. coli* into phylogenetic sets is based on clonal structure whereby each host is predominated by only one phylogroup (Tenaillon *et al.*, 2010). According to the findings, phylogroups of *E. coli* isolates were not randomly distributed among humans, cattle, and buffalo but were structured in conformity with the clonal characteristics. *E. coli* isolates in Phylogroup A, B1, B2 and D were present in all the sympatric hosts except phylogroup B2 which was absent in the buffalo host.

Although several host and environmental factors (Gordon and Cowling, 2003; Escobar-Páramo *et al.*, 2004; Escobar-Páramo *et al.*, 2006; Lescat *et al.*, 2013; Chakraborty *et al.*, 2015) are linked to the inter-host diversity of phylogroups.

In the present study, the variation in phylogroups is likely influenced by the interaction of humans, cattle, and buffalo. Contacts network in Maasai Mara occur frequently, this has been shown by a study carried out on animal movement at the ecosystem (Omondi *et al.*, 2021), which may explain the similarity *E. coli* phylogroup profiles from humans and animals.

In some instances, phylogroups may be broadly classified as either 'generalists' (phylogroups A and B1) or 'specialists' (phylogroups B2 and D) depending on the variability of occurrence in diverse hosts (Carlos *et al.*, 2010). In the MME, Phylogroup A was the most common in *E. coli* isolates, followed by B1, D, and B2, which shows the higher frequency of 'generalist' over 'specialists' in this ecosystem. In context to *E. coli* pathotypes, Phylogroups B2 and D are extraintestinal pathogenic whereas A and B1 are commensals (Chakraborty *et al.*, 2015). Commensal predominance has also been described among different animal species. The dominance of phylogroup A and B1 indicated that the majority of human, cattle, and buffalo *E. coli* strains are commensal however, the high prevalence of phylogroup D in buffalo and cattle compared to humans suggests that the *E. coli* population in the African buffalo and cattle, presents a public health risk given that transmission opportunity exists via sharing of ecosystem resources like human consumption of surface and groundwater.

Human *E. coli* isolates in MME were predominated by phylogroup A. This result is consistent with previous findings indicating that *E. coli* phylogroup A is dominant in some human populations (Escobar-Páramo *et al.*, 2006; Li *et al.*, 2010). The exception is observed for people who live in the tropics in whom both A and B1 predominate (Escobar-Páramo *et al.*, 2004), and which is still consistent with findings from this study since Maasai Mara is in the tropics. Other studies, postulate that socio-economics may influence phylogroups in commensals, communities in low socio-economics such as Africa are predominated with phylogroup A whereas those in Europe and America have phylogroup B2 (Tenaillon *et al.*, 2010), this is line with the study since MME is in Africa.

In contrast to humans, animal hosts tend to be predominated by *E. coli* populations in the phylogroups B1 (Higgins *et al.*, 2007; Ishii *et al.*, 2007; Carlos *et al.*, 2010) which are consistent with results from this study. Specifically, *E. coli* isolates from buffalo and cattle were predominated by phylogroup B1.

The genetic structures of commensal *E. coli* populations in animal hosts were similar to the study findings except for phylogroup B2 which was absent in buffalo. Buffalo and cattle in MME were dominated by B1. This may be due to the phylogenetic proximity and the diet overlap between cattle and buffalo hence similar *E. coli* phylogroups' profiles. This is in line with another study on *E. coli* population structure which recorded similar results (Mercat *et al.*, 2016).

Susceptibility to different antibiotics has also been linked with *E. coli* phylogroups. For instance, suggestions reveal that commensal *E. coli* populations in phylogroups A and B1 demonstrate high susceptibility to several tested antibiotics (Walk *et al.*, 2007; Mosquito *et al.*, 2015; Raimondi *et al.*, 2019). Results from this study, however, show that isolates in phylogroups A and B1 had high resistance to tested antibiotics which is supported by earlier studies that showed *E. coli* in phylogroup A and B1 easily develop resistance to third-generation cephalosporins (Deschamps *et al.*, 2009; Mammeri *et al.*, 2009). Overall, it is clear that the *E. coli* population in phylogroup B2 was the least across all the phylogroups. It is suggested that most antibiotic resistance is thought to be lower in *E. coli* phylogroup B2 (Johnson *et al.*, 2001; Johnson *et al.*, 2004), hence the low prevalence of Phylogroup B2 in the ecosystem in cattle and humans where there is frequent antibiotic usage is an indication that resistant strains might be less (Tenaillon *et al.*, 2010).

5.2 Antibiotic resistance patterns in *E. coli* isolates from humans, cattle, and buffalo

5.2.1 Prevalence of antibiotic resistance *E. coli* in humans, cattle, and buffalo

Testing of the isolated *E. coli* for antibiotic resistance showed the presence of *E. coli* strains that are resistant in humans, cattle, and buffalo. This is an indication that both medical and veterinary practices contribute to antibiotic resistance. In Kenya, some of the human practices include over the counter prescription, sale of counterfeit drugs, unqualified drug vendors, and buying under dose by Kenyans instead of full dose (Kariuki *et al.*, 2011; Christabel *et al.*, 2012), hence the population at MME can't be an exception.

Kenyan farmers have promoted antibiotic usage rather than proper hygiene and feeding practices in livestock production. The Maasai communities are semi-nomad pastoralists, a practice that makes them keep large herds of animals. The Maasai people frequently use antibiotics on their livestock to keep the animals healthy and prevent them from infectious diseases such as foot and mouth, east coast fever, brucellosis and to avoid the risk of zoonotic infections such as brucellosis. Mostly they self-administer antibiotics to their livestock without prior consultations from their veterinary officers. This leads to wrong dosages and potential misuse of the drugs. These antibiotics are sourced from nearby veterinary pharmacies without proper prescription depending on availability, cost, and ease of administration. These observations are in line with other studies on veterinary drug practices and antibiotic resistance in food-producing animals (Roderick *et al.*, 2000; Kariuki *et al.*, 2011; Caudell *et al.*, 2017; Roulette *et al.*, 2017; Clement *et al.*, 2019).

E. coli from humans recorded the highest resistance to the tested antibiotics than in cattle and buffalo. Perhaps this is attributable to regular antibiotic use and abuse in the human population as listed by different authors (Boerlin and Reid-Smith, 2008; Kariuki *et al.*, 2011; Christabel *et al.*, 2012; Kardos, 2015; Bessat *et al.*, 2019). In the Maasai community, AR may also be driven by the people's practice of consuming raw animal products such as meat, milk, and blood which are often contaminated with antibiotic traces since the drug withdrawal period may not be properly observed. This is in accordance with a study which was carried out in Tanzania regarding antibiotic resistance's emergence and spread in the Maasai population which observed the prudent use of antibiotics in Maasai livestock and high level of antibiotic resistance *E. coli* in milk (Roulette *et al.*, 2017; Mangesho *et al.*, 2021).

The buffalo under this study feed on natural fodder and grass hence they are not directly exposed to pharmaceutical antibiotics in form of feed additives or growth promoters. And yet, a significant number of isolates that were resistant to drugs were recorded from samples obtained from buffalos having interaction with anthropogenic areas used by humans and their livestock. This may reflect the cross transmission of resistant genes of *E. coli,* the ingestion of drug resistant bacteria as well as antibiotics traces, both from streams that may have resulted in selective pressure with the ultimate development of antibiotic resistance in buffalo (Guardabassi *et al.*, 2004; Wellington *et al.*, 2013; Wang *et al.*, 2017).

Antibiotic resistance profiles identified in buffalo herds corresponded to the most commonly used antibiotics in humans and farm animals. This is an indication that antibiotics used in human and livestock often contributes to their release into the environment through waste leading to environmental antibiotic pollution as proposed in other studies (Venglovsky *et al.*, 2009; Pikkemaat *et al.*, 2016; Manyi-Loh *et al.*, 2018). It may be further supported by the fact that the Maasai people live in proximity with their animals, and that wildlife and domestic animals freely interact in grazing and watering points (Gakuya *et al.*, 2012). Based on these results, the current study hypothesized that human-animal interface might be linked to the transmission of resistant strains in human, cattle, and wildlife.

5.2.2 Susceptibility patterns of different antibiotics to *E. coli* isolates

The results showed that tetracycline and gentamicin antibiotics experienced the highest resistance to *E. coli* isolates across the three sympatric hosts. Isolates from humans had the highest resistance followed by cattle while buffalo had the least for these antibiotics. Tetracycline and gentamicin are the most frequently accessed and used for both medical and veterinary treatments. The ease of access, especially over the counters combined with their relatively lower cost encourages frequent use to treat relevant and irrelevant ailments (Sayah *et al.*, 2005). In contrast, Amoxicillin-Clavulanic Acid showed the least resistance in the three sympatric hosts. The patterns of resistance and susceptibility for these antibiotics were similar across the hosts and suggest a similar pattern in their use and probably emergence and propagation of resistance elements in the ecosystem (Aminu and David,2018; Yassin *et al.*, 2017).

Although in the present study, resistance to most of the antibiotics was highest in people compared to animals, it does not necessarily translate to levels of antibiotics

usage. According to Caudell *et al.*, (2018) culture and ecology are stronger predictors of acquisition, growth, and dissemination of antimicrobial resistant bacteria. Caudell *et al.*, (2018) further imply that practices that lead to greater exposure to bacteria are key drivers of antimicrobial resistance at the household level rather than antimicrobial use in people or animals. The pastoralist Maasai community is known to frequently self-administer antibiotics and particularly oxytetracycline (Roderick *et al.*, 2000; Caudell *et al.*, 2017; Roulette *et al.*, 2017). Similarly, about 93% among the Maasai community do not adhere to withdrawal period for antibiotics. This means that small doses of antibiotics are consumed daily at the household level and increases exposure of antibiotics to bacterial commensals.

In addition, the presence of *E. coli* strains that are resistant to antibiotics in cattle milk coupled with consumption of unboiled milk increases exposure to resistant strains (Caudell *et al.*, 2018). Human exposure to antibiotic resistance elements is likely to be higher for humans compared to animals especially wildlife whose main exposure is contact with contaminated environmental resources. For instance, the area occupied by the pastoralist community is showing high antibiotic contamination of groundwater that is specifically loaded with resistance elements against tetracycline and gentamicin (Wahome *et al.*, 2014). Yet, in pastoralist areas, groundwater is a significant source of water for both animals and humans.

It was significant to highlight that *E. coli* isolates in the human-animal interface in Maasai Mara had developed multi-drug resistance. This was more pronounced in *E. coli* isolates of human samples compared to animals. This pattern seems common and had been recorded by Thorsteinsdottir *et al.,* (2010) who suggest that shared factors drive multi-drug resistance. Multiple resistances among bacterial communities have been linked to integrons because they carry multiple resistant genes at one time and are capable of transferring antimicrobial resistance across the bacterial communities (van Essen-Zandbergen *et al.,* 2009).

5.3 Genetic diversity and antibiotic resistance patterns in *E. coli* isolates from buffalo with varying degrees of interaction with cattle

Wildlife is naturally not exposed to antibiotics and their acquisition of resistance is indirect through exposure to contaminated environmental resources shared by humans and livestock (Cole *et al.,* 2005; Kozak *et al.,* 2009; Pikkemaat *et al.,* 2016; Wang *et al.,* 2017). Results from this study show that AR in isolated buffalo was less compared to AR in buffalo with contact with cattle and human population. This is in line with other studies which show that the proximity of wildlife to human-livestock is a predictor or risk factor to wildlife acquisition of resistance elements (Wahome *et al.,* 2014; Katakweba *et al.,* 2015; Mercat *et al.,* 2016).

Moreover, the buffalo herds close to humans and livestock recorded resistance to tetracycline, gentamicin, and ciprofloxacin. These resistance patterns to antibiotics mirrored those of humans and cattle. The acquisition of resistance elements in buffalo may be attributed to the sharing of resources especially water and grazing areas.

Rivers at the human-livestock-wildlife interface are heavily polluted with wastes from humans and livestock and consumption of such water transfers the resistant bacteria, genes, or elements to the wild hosts (Shobrak and Abo-Amer, 2014; Pikkemaat *et al.*, 2016). Wildlife, including the African buffalo in Maasai Mara Ecosystem, consume water from streams and rivers that criss-cross the human-animal dominated areas.

The Maasai pastoralists are heavy users of antibiotics on their livestock, especially tetracycline and as observed in this study, the resistance from livestock and humans is likely transferred to the sympatric buffalo since tetracycline antibiotic recorded resistance to both isolated and non-isolated buffalo. In Kenya, populations of baboons (*Papio anubis*) that frequent human dwellings harbor antibiotic resistance commensals and genes (Kariuki *et al.*, 2018). Elsewhere, wild birds have been found to harbor resistant *E. coli* including the multi-drug phenotypes probably due to their exposure to human environments, further suggesting that the same birds may play a crucial role in the spread of resistant genes and elements through their fecal droppings (Dolejska *et al.*, 2009; Shobrak and Abo-Amer, 2014).

5.4 Conclusions

i) Humans, cattle, and buffalo harbor genetically diverse *E. coli* isolates. According to this study 16S rRNA, genomic sequences revealed 14 unique haplotypes while the phylogenetic analysis revealed all the four phylogroups (A, B, C, and D).

ii) *E. coli* isolates from the humans recorded the highest resistance to the tested antibiotics as compared to cattle and buffalo. Tetracycline and Gentamicin antibiotics recorded the highest resistance to *E. coli* isolates from sympatric human, cattle, and buffalo

iii) *E. coli* isolates from buffalo with interaction with humans and cattle recorded a high prevalence of antibiotic resistance compared to *E. coli* isolates from isolated buffalo.

5.5 Recommendations

i) The presence of identical *E. coli* haplotypes and phylogroup in humans, cattle, and buffalo can be used to identify pathogen transmission pathways in multi-host systems, though more research with different animal models in the ecosystem is needed to achieve this optimally.

ii) Transmission of antibiotic elements in the sympatric human, cattle, and buffalo should be studied to identify the main reservoirs and the route of transmission. Further, the fate of antibiotics in manure, soil, and water should be studied.

iii) Based on the findings of this study, it’s important to study and understand whether wild animals are carriers of resistant strains and their role in the dissemination of resistant strains to other hosts and the environment

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APPENDICES

Appendix 1: NACOSTI Research Permit

THIS IS TO CERTIFY THAT:
MS. WINFRIDAH BWARI ONYARI
of KENYATTA UNIVERSITY , 0-503
Nairobi,has been permitted to conduct
research in *Narok County*

Permit No : NACOSTI/P/18/58265/24147
Date Of Issue : 15th September,2018
Fee Recieved :Ksh 1000

on the topic: *DIVERSITY AND ANTIBIOTIC RESISTANCE PROFILES OF ESCHERICHIA COLI AT THE HUMAN-LIVESTOCK AND WILDLIFE INTERFACE AT MAASAI MARA ECOSYSTEM*

for the period ending:
13th September,2019

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014.

CONDITIONS

1. The License is valid for the proposed research, location and specified period.
2. The License and any rights thereunder are non-transferable.
3. The Licensee shall inform the County Governor before commencement of the research.
4. Excavation, filming and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
5. The License does not give authority to transfer research materials.
6. NACOSTI may monitor and evaluate the licensed research project.
7. The Licensee shall submit one hard copy and upload a soft copy of their final report within one year of completion of the research.
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TEL: 020 400 7000, 0713 788787, 0735 404245
Email: dg@nacosti.go.ke, registry@nacosti.go.ke
Website: www.nacosti.go.ke

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEARCH LICENSE

Serial No.A 20629

CONDITIONS: see back page

Appendix 2: Kenyatta University Ethics Research Permit

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: **KU/ERC/ APPROVAL/VOL.1** (123) Date: 20th June, 2018

Winfridah Bwari Onyari
P.O Box 15653-00503
Nairobi.

Dear Winfridah,

APPLICATION NUMBER: PKU/749/1817 "DIVERSITY AND ANTIBIOTIC RESISTANCE PROFILES OF *ESCHERICHIA COLI* AT THE HUMAN-LIVESTOCK AND WILDLIFE INTERFACE AT MAASAI MARA ECOSYSTEM"

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "**Diversity and Antibiotic Resistance Profiles of *Escherichia Coli* at the Human-Livestock and Wildlife Interface at Maasai Mara Ecosystem** " received on 13th February, 2018 and discussed 12th June, 2018

2. APPLICANT

Winfridah Bwari Onyari

3. SITE

Maasai Mara Ecosystem

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines and **APPROVED that the research may proceed for a period of ONE year from 20th June , 2018.**

5. **ADVICE/CONDITIONS**

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN 20 JUN 2018 ETHICS REVIEW COMMITTEE P.O. BOX 43844 - 00100 NAIROBI

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I ..Winfadah B. Onyari..... accept the advice given and will fulfill the conditions therein.

Signature ..... Dated this day of..20/June/..... 2018.

cc.
DVC-Research Innovation and Outreach

Appendix 3: KWS Research Permit

KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

KWS/BRM/5001

7 June 2017

Ms Winfrida Swari Onyari
Kenya Wildlife Service
P.O.Box 40241-00100
NAIROBI
e-mail: winnridah@gmail.com

Dear Ms. Onyari,

PERMISSION TO CONDUCT RESEARCH IN MAASAI MARA ECOSYSTEM

We acknowledge receipt of your letter dated 22nd May 2017 requesting for permission to conduct research on a project titled: **'Diversity and anti-biotic resistance profiles of *Escherichia coli* at the human-livestock and wildlife interface at Maasai Mara ecosystem**. The study will generate data and information that will assist in the epidemiology, surveillance and control of parasitic infestation in the human-wildlife interface in Maasai Mara ecosystem.

You have been granted permission to conduct the study from **June – September 2017**. However, you will abide by the set KWS regulations and guidelines regarding the carrying out of research in and outside protected areas. You will also be required to work closely with our Head of Veterinary Services (HVS), whom you will give the progress report on the study.

You will submit a bound copy of your MSc thesis to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours Sincerely,

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:
- Head, Veterinary Services
- Senior Scientist, CRCA
- Senior Warden, Narok Station
- Research Scientist, Maasai Mara Station

Appendix 4: Informed Consent for Patients

STUDY TITLE: GENETIC DIVERSITY AND ANTIBIOTIC RESISTANCE OF *Escherichia coli* FROM HUMAN, CATTLE, AND BUFFALO IN THE MAASAI MARA ECOSYSTEM

Investigator: WINFRIDAH BWARI ONYARI

Instructions to clinicians:

Before issuing sterile fecal pots with swabs, greet the potential participant and read the following statement aloud. Do not ask for or write down the participant's name. Once you have finished reading the statement, indicate whether the patient agrees to participate or not. Then sign the form to show that you asked the participant to give informed consent. Use one copy of this form for each person that you ask permission to take part in the study. As well, give one copy to the participant.

Health facility name----------

"Hello, my name is ……. We are conducting a study "Genetic Diversity and Antibiotic Resistance of *Escherichia coli* in Human, Cattle, and Buffalo at Maasai Mara Ecosystem". This study will form a baseline for efforts aimed at using *E. coli* as a tool for the identification of pathogen transmission pathways in multi-host systems. This will be critical in combating antimicrobial resistant pathogens in disease management efforts. I am asking you to take part in the study by giving your fresh fecal sample that will be used for answering the objectives of this study. I will provide you with a sterile fecal pot with swabs which you will use to collect a fresh fecal sample and hand them over to us. The samples you will provide will be kept private to the extent allowable by the law and will not be shown to anyone else or used for any other purpose other than that stipulated in this study.

Participation in this study is voluntary and you can choose not to participate at any time. If you choose not to participate, there is no risk for you and the care you receive at this facility will not be affected. The study is anonymous; your name will not be documented anywhere or linked to the data we collect from you. There is no direct benefit to you for taking part in this study. There is will be no cost to you for taking part in the study and no compensation for participating. No one except the investigator will have access to your samples".

If you have future concerns about this study please feel free to contact **WINFRIDAH BWARI ONYARI**, the investigator of the study, on, **+254-710-527-955** or by email at winnfridah@gmail.com

AGREEMENT and CONSENT: Do you have any questions? Are you willing to take part in the study?

Circle one of the following options to show if the patients agree to take part in the study:

YES Continue

NO Thank the patient and end the conversation.

Signature of the clinician ……

Date…….

Appendix 5: Antibiotic resistance readings, their interpretation, and overall resistant pattern of *E. coli* isolate.

Key: Antibiotic resistance readings in millimeter (mm) and their interpretation; Resistant (R), Intermediate (I), or Susceptible (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
TH01	10mm (R)	28mm (S)	8mm (R)	16mm (S)	20mm (S)
TH02	10mm R)	32mm (S)	32mm (S)	20mm (S)	28mm (S)
TH03	10mm (R)	28mm (S)	26mm (S)	14mm (I)	28mm (S)
TH04	6mm (R)	26mm (S)	20mm (S)	16mm (S)	22mm(S)
TH05	10mm (R)	30mm (S)	30mm (S)	18mm (S)	24mm(S)
TH27	6mm (R)	24mm (S)	24mm (S)	14mm (I)	14mm (I)
TH51	24mm (S)	26mm (S)	22mm (S)	14mm (I)	16mm (I)
TH52	10mm (R)	36mm (S)	36mm (S)	20mm (S)	24mm (S)
TH53	16mm (S)	28mm (S)	22mm (S)	6mm (R)	14mm (I)
TH54	8mm (R)	12mm (R)	14mm (R)	12mm (R)	26mm (S)
TH55	8mm (R)	8mm (R)	10mm (R)	14mm (I)	16mm (I)
SH56	22mm (S)	24mm (S)	30mm (S)	12mm (R)	18mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
TH58	8mm (R)	8mm (R)	14mm (R)	8mm (R)	20mm (S)
TH59	8mm (R)	24mm (S)	26mm (S)	6mm (R)	6mm (R)
TH60	10mm (R)	18mm (R)	6mm (R)	12mm (R)	12mm (R)
TH69	10mm (R)	10mm (R)	14mm (R)	10mm (R)	16mm (I)
TH89	6mm (R)	20mm (I)	14mm (R)	12mm (R)	14mm (I)
TH94	20mm (S)	16mm(R)	32mm (S)	14mm (I)	20mm (S)
SH01	6mm (R)	30mm (S)	24mm (S)	18mm (S)	24mm (S)
SH02	10mm (R)	30mm (S)	22mm (S)	18mm (S)	18mm (S)
SH03	8mm (R)	28mm (S)	26mm (S)	20mm (S)	16mm (I)
SH05	10mm (R)	32mm (S)	28mm (S)	22mm (S)	18mm (S)
SH07	10mm (R)	28mm (S)	30mm (S)	30mm (S)	16mm (I)
SH09	10mm (R)	30mm (S)	20mm (S)	20mm (S)	16mm (I)
SH10	12mm (I)	26mm (S)	26mm (S)	12mm(R)	18mm (S)
SH16	6mm (R)	8mm (R)	14mm (R)	12mm(R)	16mm (R)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
SH18	8mm (R)	12mm (R)	14mm (R)	12mm (R)	20mm (S)
SH35	8mm (R)	8mm(R)	6mm (R)	6mm (R)	12mm (R)
SH57	8mm (R)	6mm (R)	14mm (R)	12mm (R)	12mm (R)
SH67	10mm R)	10mm (R)	14mm (R)	10mm (R)	16mm (I)
SH92	6mm (R)	26mm (S)	26mm (S)	16mm (S)	10mm (R)
SH93	6mm (R)	20mm (I)	16mm (I)	10mm (R)	8mm (R)
SH120	6mm (R)	24mm (S)	14mm (R)	12mm (R)	10mm (R)
SH121	10mm (R)	6mm (R)	24mm (S)	16mm (R)	6mm (R)
SH139	12mm (I)	26mm (S)	20mm (I)	12mm (R)	18mm (S)
SC02	10mm (R)	28mm (S)	30mm (S)	8mm (R)	18mm (S)
SC05	10mm (R)	26mm (S)	28mm (S)	14mm (I)	20mm(S)
SC06	8mm (R)	28mm (S)	34mm (S)	18mm (S)	20mm (S)
SC07	12mm (I)	30mm (S)	34mm (S)	18mm (S)	24mm (S)
SC08	8mm (R)	26mm (S)	28mm (S)	10mm (R)	24mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
SC09	22mm (S)	32mm (S)	30mm (S)	20mm (S)	22mm (S)
SC10	10mm (R)	30mm (S)	16mm (I)	14mm (I)	20mm (S)
SC11	14mm (I)	30mm (S)	32mm (S)	20mm (S)	22mm (S)
SC12	24mm (S)	30mm (S)	30mm (S)	18mm (S)	20mm (S)
SC13	10mm (R)	30mm (S)	32mm (S)	14mm (I)	22mm (S)
SC14	20mm (S)	32mm (S)	28mm (S)	20mm (S)	22mm (S)
SC15	20mm (S)	32mm (S)	34mm (S)	18mm (S)	18mm (S)
TC01	6mm (R)	26mm (S)	30mm (S)	12mm (R)	26mm (S)
TC02	8mm (R)	20mm (I)	12mm (R)	16mm (S)	22mm (S)
TC04	16mm (S)	28mm (S)	32mm (S)	18mm (S)	18mm (S)
TC05	10mm (R)	24mm (S)	30mm (S)	14mm (I)	20mm (S)
TC06	8mm (R)	18mm (R)	32mm (S)	16mm (S)	20mm (S)
TC07	22mm (S)	26mm (S)	30mm (S)	18mm (S)	20mm (S)
TC08	10mm (R)	24mm (S)	30mm (S)	16mm(S)	22mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
TC09	12mm (I)	26mm (S)	30mm (S)	16mm (S)	24mm (S)
TC10	24mm (S)	30mm (S)	28mm (S)	20mm (S)	20mm (S)
TC11	22mm (S)	30mm (S)	30mm (S)	20mm (S)	28mm (S)
TC16	14mm (I)	24mm (S)	30mm (S)	18mm (S)	18mm (S)
TC17	22mm (S)	30mm (S)	32mm (S)	16mm (S)	24mm (S)
TC18	10mm (R)	26mm (S)	16mm (I)	18mm (S)	18mm (S)
TC19	14mm (I)	30mm (S)	32mm (S)	18mm (S)	20mm (S)
TC20	20mm (S)	30mm (S)	28mm (S)	18mm (S)	30mm (S)
TC21	22mm (S)	30mm (S)	32mm (S)	14mm (I)	20mm (S)
TC23	10mm (R)	28mm (S)	14mm (R)	10mm (R)	22mm (S)
TC24	18mm (S)	26mm (S)	28mm (S)	18mm (S)	22mm (S)
TC25	20mm (S)	24mm (S)	24mm (S)	18mm (S)	22mm (S)
TC28	14mm (I)	28mm (S)	28mm (S)	18mm (S)	24mm (S)
TC29	22mm (S)	32mm (S)	26mm (S)	20mm (S)	20mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
TC30	14mm (I)	26mm (S)	26mm (S)	16mm (S)	20mm (S)
MC13	8mm (R)	30mm (S)	30mm (S)	12mm (R)	22mm (S)
MC17	8mm (R)	12mm (R)	18mm (I)	12mm(R)	8mm (R)
MC19	16mm (S)	22mm (I)	24mm (S)	14mm (I)	24mm (S)
MC29	10mm (R)	24mm (S)	12mm (R)	6mm (R)	16mm (I)
MC198	8mm (R)	20mm (I)	20mm (I)	6mm (R)	20mm (S)
MC200	14mm (I)	24mm (S)	24mm (S)	18mm (S)	22mm (S)
MB01	20mm (S)	30mm (S)	24mm (S)	16mm (S)	22mm (S)
MB02	6mm R)	24mm (S)	16mm (I)	12mm (R)	20mm (S)
MB03	10mm (R)	28mm (S)	12mm (R)	14mm (I)	20mm (S)
MB04	22mm (S)	28mm (S)	28mm (S)	16mm (S)	18mm (S)
MB05	14mm (I)	24mm (S)	14mm (R)	16mm (S)	22mm (S)
MB06	20mm (S)	26mm (S)	32mm (S)	16mm (S)	24mm (S)
MB07	8mm (R)	28mm (S)	28mm (S)	16mm (S)	20mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
MB08	10mm (R)	30mm (S)	32mm (S)	18mm (S)	22mm (S)
MB09	14mm (I)	20mm (I)	20mm (I)	14mm (I)	18mm (S)
MB10	22mm (S)	28mm (S)	30mm (S)	20mm (S)	26mm (S)
MB11	14mm (I)	26mm (S)	26mm (S)	14mm (I)	18mm (S)
MB12	20mm (S)	24mm (S)	24mm (S)	10mm (R)	24mm (S)
MB13	22mm (S)	28mm (S)	26mm (S)	18mm (S)	28mm (S)
MB14	8mm (R)	34mm (S)	12mm (R)	14mm (I)	28mm (S)
MB16	20mm (S)	32mm (S)	30mm (S)	12mm (R)	22mm (S)
MB18	20mm (S)	26mm (S)	28mm (S)	12mm (R)	20mm (S)
MB24	10mm (R)	24mm (S)	16mm (I)	22mm (S)	20mm (S)
MB30	10mm (R)	22mm (I)	18mm (I)	12mm (R)	22mm (S)
MB76	20mm (S)	24mm (S)	24mm (S)	16mm (S)	22mm (S)
MB102	6mm (R)	30mm (S)	34mm (S)	8mm (R)	18mm (S)
MB108	24mm (S)	28mm (S)	26mm (S)	20mm (S)	32mm (S)

Animal ID	Tetracycline	Ceftriaxone	Ciprofloxacin	Gentamicin	Amoxicillin-Clavulanic Acid
MB127	26mm (S)	26mm (S)	24mm (S)	18mm (S)	26mm (S)
MB143	24mm (S)	26mm (S)	22mm (S)	22mm (S)	34mm (S)
MB149	16mm (S)	24mm (S)	24mm (S)	18mm (S)	18mm (S)

Key: Interpretation criteria according to the Clinical and Laboratory Standards Institute guidelines (Wayne, 2012).

Antibiotics	Resistant (R)	Intermediate (I)	Susceptible (S)
Tetracycline	≤ 11	12-14	≥ 15
Ceftriaxone	≤ 19	20-22	≥ 23
Ciprofloxacin	≤ 15	16-20	≥ 21
Gentamicin	≤ 12	13-14	≥ 15
Amoxicillin-Clavulanic Acid	≤ 13	14-16	≥ 17

Appendix 6: Susceptibility profiles of *E. coli* isolates from humans, cattle, and buffalo to different antibiotics in percentage (%)

Antibiotics	Human, n=35			Cattle n=40			Buffalo n=24		
	R	I	S	R	I	S	R	I	S
Tetracycline	83%	6%	11%	45%	20%	35%	33%	13%	54%
Ceftriaxone	34%	23%	43%	5%	8%	87%	0%	13%	88%
Ciprofloxacin	37%	29%	34%	8%	10%	82%	13%	17%	70%
Gentamicin	52%	14%	34%	18%	20%	62%	25%	17%	58%
Amoxicillin-Clavulanic Acid	23%	31%	46%	2.5%	2.5%	95%	0%	0%	100%

Key: R-Resistant, I-Intermediate, S-susceptible

Appendix 7: Patterns of resistance profiles of *E. coli* isolates by antibiotics

Pattern	Antibiotics	Number of resistant isolates
1	TET	24
	CEF	1
	GEN	8
Total		**33 (33 %)**
2	TET GEN	6
	TET CIP	3
	TET CEF	1
	TET AMC	1
Total		**11 (11 %)**
3	TET CIP GEN	4
	TET GEN AMC	1
	TET CEF CIP	1
Total		**6 (6 %)**
4	TET CEF CIP GEN	6
	TET CEF GEN AMC	2
	TET CIP GEN AMC	2
Total		**10 (10 %)**
5	TET CEF CIP GEN AMC	3
Total		**3 (3 %)**

Key: TET; Tetracycline, CIP; ciprofloxacin, CEF; ceftriaxone, GEN; Gentamicin, AMC; Amoxicillin Clavulanic Acid.