PREVALENCE, RISK FACTORS AND ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI PATHOTYPES* ISOLATED FROM LIVESTOCK AND RATS IN SLUMS AREAS OF NAIROBI

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DECLARATION

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

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DEDICATION

To my parents; Mr. Philip Macharia Kiriro and Mrs. Susan Mwelu Macharia, brothers; Richard Kiriro and Stanley Maina, and to my wife Damaris Nyambura , my children Caleb Macharia, Michael- lyam Waweru and Tatiana-Ann Mwelu, relatives , colleagues and all friends

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LIST OF ABBREVIATIONS AND ACRONYMS

AMC	Amoxycillin/clavulinic acid
AMP	Ampicillin
C	Chloramphenicol
CAZ	Ceftazidine
CIP	Ciprofloxacin
CN	Gentamycin
CRO	Ceftriazone
CXM	Cefuroxime
DAEC	Diffuselyadherent *E. coli*
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorragic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC (HL)	Enterotoxigenic *E. coli* HEAT LABILE
ETEC (HS)	Enterotoxigenic *E. coli* HEAT STABLE
I	Intermediate
NA	Naladixic acid
R	Resistant
S	Susceptible
S	Streptomycin
STEC	Shiga toxin *E. coli*
SXT	Trimethoprime/Sulfamethxazole
TE	Tetracycline
VTEC	Verocytogenic *E. coli*

ABSTRACT

E. coli is a facultative aerobic commensal microflora in livestock and human. Its detrimental effects in human and animal has attracted the most research interest. The current study was conducted to determine the antimicrobial resistance, and risk factors for occurrence of pathogenic *E. coli* in livestock and rats. Two slums areas were selected, Korogocho and Viwandani. Two hundred and six households were randomly selected for this study. Fecal samples were aseptically collected from a total of 795 animals and 13 rats from households in the two slums. The samples were cultured for isolation of *E. coli* using standards methods. The isolates were characterized to various pathotypes and tested for antimicrobial resistance phenotypes using disc diffusion method. Risk factors for occurrence of pathogenic *E. coli* pathotype in livestock and rats were assessed using a questionnaire administered to the household heads of the selected 206 households. Data was analyzed using STATA $^{(R)}$ and SPSS$^{(R)}$ for descriptive statistics and logistic regression to estimate the prevalence, risk factors for occurrence of *E. coli* pathotypes and to analyze household attributes in the two regions. Different attributes were analyzed and significant differences compared between the two areas of study at <0.05 level of significance. A total of 1171 *E. coli* were isolated from livestock and 85 from rats.

The prevalence of *E. coli* n=795 was (49%), with chicken recording the highest prevalence of (35%) and doves and rabbits recording the lowest percentage of (1%) each. There was a significantly higher number of *E. coli* isolated from rabbits in Korogocho as compared to Viwandani ($p < 0.05$). Significantly more animals fed close to the dump site in Viwandani (45%) compared to Korogocho (24%) ($p = 0.02$) and more animals feed next to open sewer in Viwandani (48%) compared to Korogocho (26%) ($P = 0.001$). Significantly more animals accessed clean water in Korogocho (67%) than Viwandani (44%) ($p = 0.001$), while more animals in Viwandani (73%) mixed with other animals from other households compared to (30%) Korogocho ($p = 0.00$)

Multiplex PCR was done on 231 *E. coli* pools. One pool represented six *E. coli* isolates from the same sample. Eighteen virulence genes were used to identify virulence characteristics for EHEC, EPEC, EAEC, ETEC, EIEC and DAEC *E. coli* pathotypes. The overall prevalence of *E. coli* pathotypes was n=795 (5.91%). EHEC had a prevalence of (2.4%), EPEC (1.76%), ETEC (HL) (0.75%), ETEC (HS) (0.13%), EAEC (0.50%) and DAEC (0.38%). The prevalence of resistant *E.coli* isolated from livestock (n=1171) was (56%) for tetracycline, followed by Trimethoprime/sulfamethoxazole (53%) and Streptomycin (45%).The lowest prevalence was in ciprofloxacin, amoxicillin and clavulinic acid at (1%) each. The prevalence of resistance of *E.coli* isolated from rats (n=85) was (17%) for tetracycline, Trimethoprime/sulfamethoxazole(9%) and Streptomycin at (13%), ampicillin(9%), chloramphenicol (4%), ceftazidime (1%), ceftriazone (5%) andcefuroxime (2%).The study indicated serious multidrug resistance with 446 isolates being resistant to three(3) or more antibiotics. One isolated showed resistance to ten (10) antibiotics. In rodents five (5) *E. coli* isolates showed resistance to three (3) or more antibiotics with one isolate showing resistance to six (6) antibiotics.

Risk factor analysis for occurrence of *E. coli* pathotypes indicated that goats, and over the counter sale of antibiotics to farmers to be significantly associated with the occurrence of *E. coli* pathotypes (odds ratio >1) and ($p < 0.05$).

This study found the prevalence of *E. coli* pathotypes in livestock to be 5.9% with a diverse antimicrobial–resistant *E. coli* population distributed in various livestock species and rats. This poses a potential risk of transfer of antibiotic-resistant *E. coli pathogens* and antibiotic resistant commensal bacteria into human population during contact. To control transfer of antibiotic-resistant pathogens, the community should be educated on simple concepts like hygiene (hand washing) after handling livestock. Farmers should not buy drugs over the counter but consult a qualified veterinary for animal treatment. Also after handling livestock especially goats, people should wash hands thoroughly. Also due to multidrug resistance demonstrated in this study, the public should be enlightened on the importance of drug withdrawal on meat and milk products after antibiotics are administered to livestock.

Government institutions dealing with medicine both veterinary and human need to come together and create policies that will govern the use of antimicrobial agents. Sensitization of the public on dangers of misuse of antimicrobial agents in both human and animals should be done. The importance of culture and antimicrobial sensitivity testing in the management of bacterial diseases should be stressed and closely monitored in hospitals and veterinary laboratories. Bodies regulating drugs used in agriculture as antimicrobials should be included in policy making. Other methods of reducing infection should be adopted. This includes vaccination, proper hygiene and avoiding overcrowding. This will significantly reduce the need for antibiotic use.

CHAPTER ONE: INTRODUCTION

1.0 Background Information

Antibiotics have been of great help in livestock which produce more milk, meat and eggs and have lower incidence of disease and motality (Tanwar *et al.,* 2014). However antibiotic residues in milk, meat and eggs are of great concern in public health issues. The residues cause development of antibiotic resistance which is a major impediment in treatment of human diseases(Oliver *etal*., 2015). Livestock are often cited as a reservoir for resistant bacteria and antibiotic resistant genes, and these has implication for both animals and human health (Thames *et al*., 2012).Small farms form the majority of farm in the developing world and in many low/middle income countries. They are the major suppliers of meat and milk products to local markets. However, few countries where livestock is kept have proper systems to monitor the quality and safety of food animal products (Sandvang and Aarestrup, 2000). Misuse of antibiotics in animal agriculture is of much importance lately has been of a great importance (Levy and Marshall, 2004). The global challenge on antimicrobial resistance in *E. coli* has raised interests on further research on understanding its dynamics and transmission in human and animal populations and their environment(Houser *et al*., 2008).

Animal have been indicated as carriers of both virulent and non-virulent strains of *E .coli* without showing signs of disease . It is their fore important that strains of *E. coli* from intestines of healthy animals be investigated (Kaesbohrer *et al*., 2012). Normal flora has received a lot of attention because they have been shown to harbor elements of drug resistance and disease pathogens. Research has indicated need for continuous surveillance of commensal bacteria for antibiotic resistance will provide early and important trends on antibiotic resistance (Fleckenstein

et al., 2010). Rodents found in populated Urban areas have been shown to be carriers of important pathogens responsible for disease in human and livestock (Jay-russellet *et al*., 2014).

1.1 Statement of the Problem

E. coli represent one of the most important bacteria that cause disease and death worldwide. Treatment of pathogenic E. coli is being threatened by emergence of multidrug resistance. (Taylor *et al*, 2012). Research over years has indicated *E. coli* of great significance in human and livestock disease and is classified into, commensals, intestinal pathogens, and extra intestinal disease causing *E .coli* strains. *E. coli* disease on visceral organs occur commonly in human population and livestock cause infection of the urinary system, skin and tissue infection, lung infection, gastrointestinal tract infection, brain infection and septicaemia (Maynard *et al*., 2004).

The top health challenges facing the current century is antibiotic resistance (Marshall and Levy, 2011). Antibiotics are used a lot in livestock for disease treatment, prevention and in other countries as a growth promoter(Marshall and Levy, 2011).bacteria with genes responsible for resistance can transfer within animals ,human and the environment.. Also the food chain is very important vehicle for transfer of antimicrobial resistance pathogens to human and livestock due to contamination (Kang *et al*., 2005). There is need for continuous surveillance of antibiotic resistance in healthy livestock populations the, environment and along the food chain. It has been indicated that ingestion of contaminated food supplies plus human coming into contact directly with animals that through feces contaminate the environment with resistant antibiotic resistant pathogens, release bacteria propagate to the exposure and spread of resistant microorganism (Kaesbohrer *et al*., 2012).

Following the use of antibiotics , antibiotic residues are spread through food chain and contamination of the environment though feces (Harms and Ku, 2010). Of major concern is the occurrence of resistance in higher classes of antimicrobials, this make antimicrobial resistance a global affair since the world has become a village when it comes to trade. Continents ,regions and countries should come together and create policies that regulate the use of antimicrobials (Aarestrup, 2005). The use of antimicrobials is not of a concern in livestock alone but also in crop farming. An example is the use of streptomycin in fruit farming Kayode *et al*.,(2008) showed that there is a relationship between, economic status, cultural behaviors in the occurrence of antimicrobial resistance.

Rodents which are associated with polluted environment are important carriers and reservoirs of several pathogens of zoonotic significance (Guenther *et al*., 2012a). Rodents on the other hand act as reservoirs for more than sixty different diseases which are of zoonotic importance (Gratz, 2000). A study by Gakuya *et al*., (2001)has shown that rodents from Nairobi slums are an important reservoirs for drug resistant *E .coli.* Rodents create and maintain an important disease cycle linking the sewage systems and congested human populations in urban areas systems and populated urban environments and are important in transmission of zoonotic diseases (Guenther *et al*., 2012a).

1.2 Justification

E. coli can acquire, maintain and transmit resistant genes from other organism in the environment E coli is an important bacteria in transmitting resistant genes in another organisms and the environment. Due to its omnipresence in humans and animals and its role as a disease

causing organism and normal flora organism, *E. coli* represents a useful indicator of resistance transmission in bacterial ecosystems and of selective pressure imposed by the antimicrobials used in treatment of animals and humans(White *et al*., 2002). Resistance to several drugs and the cost incurred in treatment is of concern to both human and veterinary medicine.. As indicated by the world health organization (WHO) approximately 80% of diseases a in developing countries are related to water and environmental hygiene and livestock act as an important link through fecal contamination by faeces. Although many studies have been done on drug resistance and pathogenic *E. coli* (White *et al.*, 2002). There is insufficient information on the above in urban and peri-urban areas especially in the slums of Nairobi. This study will generate important information for policy making in public health sector.

1.3 Hypotheses

Livestock and rodents are not reservoirs of pathogenic and antimicrobial resistant *E. coli*

1.4 Objectives

1.4.1. General Objectives

To establish the prevalence of pathogenic *E. coli*, risk factors of occurrence in livestock and rodents, and their antimicrobial resistance patterns.

1.4.2Specific Objectives

1. To determine the prevalence of *E. coli* pathotypes isolated from livestock and rodents in Korogocho and Viwandani slums
2. To determine the risk factors for occurrence of *E. coli* pathotypes in livestock and rodents in slum areas in Korogocho and Viwandani slums
3. Determine the antimicrobial resistance patterns among *E. coli* isolated from livestock and rodents in Korogocho and Viwandani slums

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of *Escherichia coli*

Escherichia coli is a mobile gram negative bacilli, of the genus ,tribe Escherichia and family enterobactericae (Nataro, Kaper, 1998). It includes those localize in the gastrointestinal tract and those that affect the other visceral organs (Köhler and Dobrindt, 2011). Those that affect the gastro intestinal tract are broadly classified into enterotoxigenic *Escherichia coli* , enteropathogenic *Escherichia coli* , shiga toxin-producing *Escherichia coli*, enteroinvasive *Escherichia coli*, Enteroaggregative *Escherichia coli* and diffusely adherent *Escherichia coli* (DAEC) (Kuhnert et al., 2000).*E. coli* strain isolated from other visceral organs include , Uropathogenic *Escherichia coli* ,neonatal/infants meningitis-associated *Escherichia coli* and sepsis-causing *Escherichia coli* (Köhler and Dobrindt, 2011). Avian pathogenic *E. coli* (APEC) cause infection of the respiratory system in birds, necrotic *Escherichia coli* (NTEC) that causes infections in animals and human both humans and animals (Köhler and Dobrindt, 2011). An important intriguing of these potential pathotype is an *Escherichia coli* strain that is usually associated with Crohns Disease has been associated with one of the *E. coli* named Adherent invasive *E.coli*(Schwarz *et al*., 2001).

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Escherichia coli, Enteroaggregative *Escherichia coli* and diffusely adherent *Escherichia coli* (DAEC) (Kuhnert et al., 2000).*E. coli* strain isolated from other visceral organs include , Uropathogenic *Escherichia coli* ,neonatal/infants meningitis-associated *Escherichia coli* and sepsis-causing *Escherichia coli* (Köhler and Dobrindt, 2011). Avian pathogenic *E. coli* (APEC) cause infection of the respiratory system in birds, necrotic *Escherichia coli* (NTEC) that causes infections in animals and human both humans and animals(Köhler and Dobrindt, 2011). An important intriguing of these potential pathotype is an *Escherichia coli* strain that is usually associated with Crohns Disease has been associated with one of the *E. coli* named Adherent invasive *E .coli* (Schwarz *et al*., 2001).

2.1.1 Enterotoxigenic *Escherichia coli* (ETEC)

Enterotoxigenic strains identified due to the production of intestinal toxins and possession adhesive fimbriae. Another classification is base on their heat stability into either heat stable or heat labile (Wasteson, 2001). ETEC has been associated with severe form of diarrhea in children living in the developing countries (Kuhnert *et al*., 2000). ETEC has been associated with diarrhea and death in piglets, calves and lambs. ETEC infections has been shown to be rare in rabbits, poultry or horses, but indicated in sheep due to possession of receptors for enterotoxins (Dubreuil and Schifferli, 2016).

Fecal Contamination of the environment , this includes water and food act as a major infection route (Kuhnert *et al*., 2000). An ETEC bacterium after entry with food inhabits the distal jejunum and proximal ileum mucosa via fimbriae projections or other adhesive factors. Some ETEC infections may result in villous atrophy and bacteremia. Studies have shown insignificant

damage to the enterocyte but enterotoxins cause serious damage and alteration in enterocyte function(Debroy and Maddox, 2001). Diarrhea is caused due to the production the toxins ,heat labile and heat stable (Cicuta *et al*., 2000). The toxin strain expressed may be *LT* only, *ST* only one or both an *LT* and *ST* toxins (Debroy and Maddox, 2001). ETEC has filamentous structures called fimbriae which are species specific and are specific to receptors where they adhere to the epithelium of the intestines (Wasteson, 2001) due to it having species-specific binding property.

2.1.2Enteropathogenic *Escherichia coli* (EPEC)

EPEC virulence is mediated by plasmid and chromosomal encoded genes, these together form the bundle forming pili , secreted proteins and intimin virulence factors (Clarke, 2001).EPEC virulence is due to the strains potential to cause lesions on the epithelial cells due to their attaching and effacing properties on cells.. Contamination of water by human faeces and sewage efflux is an important source of EPEC disease (Wasteson, 2001). EPEC pathogen has been classified into two; the typical and atypical EPEC has two broad divisions. The difference is due to EPEC adherence factor plasmid in associated with typical EPEC and not atypical EPEC. This EPEC adherence factor plasmid on cultured epithelial cells encodes localized adherence which is facilitated by a bundle forming pilus gene, *Bfpa* which the atypical EPEC lacks (Vieira *etal*., 2009). The genes responsible for Attaching and Effacing lesions are found on the locus of enterocyte effacement , a pathogenic locus that has genes coding for intimin, a kind of secretion mechanism, and also codes for a number of proteins and translocated intimin receptor (Stephan *et al., 2004*). Enteropathogenic *E. coli* strains is a cause of diarrhea in young children in developing countries (Tennant *et al*., 2009).Cows have been shown in research to harbor the

strain 0157:H45 EnteroPathogenic *E. coli* strains that cause disease in human and regarded as zoonotic(Stephan et al., 2004).A study in a semi-rural community in Ecuador, found apparent source of human infection for atypical enteropthogenic *Escherichia coli* to be pigs, dogs and chicken(Vasco and Graham, 2016).A study in the United States-Mexico border where they do leafy green production, faecal samples from stray dogs indicated zero presence of STEC but isolated atypical EPEC having fourteen serotypes (Jay-russell *et al*., 2014).

.2.1.3Enteroaggregative *E. coli* (EAggEC)

This group of diarrheoagenic *E. coli* was recently discovererd and is associated with diarrhea which is watery, especially in young children of the developing world (Osek, 2003).EAggEC has a characteristic of forming brick like stack on the cell surface on histological viewi(Clarke, 2001), it produces toxins of a number of aminoacids called Enteroaggregaive E.coli heat stable enterotoxin one . This enterotoxin and the one for Heat stable shiga toxin 1 ST1 of enterotoxigenic *E.coli* are genetically and immunologically distinct enterotoxins (Yamamoto and Nakazawa, 1997). The Enteroaggregative *E. coli* produces an aggregative adherence property pattern on human cell lines named HEP-2 and HELa. The aggregative adherence property is linked to a 55-65 MDa plasmid that habors the genes for apparent, virulence factor fimbriae and enterotoxins. Three genes namely ,*aggA* denoted as *(AAF/1)* , *aafA* denoted as (*AAF2)* and *agg3A* denoted as *(AAF/3)* which are fimbrial were correlated with *AA* phenotype (Carvalho *et al*., 2008). The ability of EAEC to adhere to HEP-2 cells is because of the proper aggregative adherence, which its expression is regulated by the *aggR* gene which is localized in a large plasmid *PAA* (Scavia *et al*., 2008).

2.1.4 Enteroinvasive *E. coli* (EIEC)

Studies on EIEC indicate they have similar pathogenic features to those of Shigella spp. Both of these pathogens have been indicated to colonize the colonic epithelium, a characteristic facilitated by plasmid and chromosomal loci. EIEC outbreak has been associated with food, water and also interpersonal transmission(Nataro *et al*., 1998). EIEC has features of colonizing the epithelial cell of the colon, where it multiplies and causes death of the tissues. The genes responsible located in a virulence plasmid *(Pinv)* of 140MDa that has been shown to encodes a type 3 secretion system(Vidal et al., 2005).EIEC colonizes the distal small intestines and the colon and are important cause severe diarrhea in children in less developed countries and food related outbreaks of gastro intestinal disease in mature people in developed nations(Taddei and Fasano, 2005).

2.1.5 Shiga (vero) toxin producing *E. coli* (STEC/VTEC/EHEC)

EHEC 0157 is the most important O serogroups in human when it comes to pathogenicity(Wani *et al*.,2003). EHEC produces toxins (verotoxins) also known as shigela like toxins that causes a cytotoxic activity on vero cells. Due to this activity, EHEC is also termed as verocytogenic *E. coli*(Clarke, 2001). the attaching and effacing (AE) characteristic of EHEC, on intestinal epithelium cell, make it closely related to EPEC, but EHEC produces a potent cytotoxin,shiga toxin (*stx)*(Kaper, 2005). STEC infections of STEC have been widely been demonstrated in both domestic and wild animals. Disease has been demonstrated naturally in calves which causes bloody diarrhea. In pigs that are being weaned it causes edema disease. In dogs especially the grey hounds, EHEC has been shown to cause skin and kidney problems (Apriolia et al., 2005).

After disease with EHEC 0157, pigs have been demonstrated to shed the same bacteria for sixty days, contaminating the environment (Mrion *et al*., 2015).another study in England has indicated pigs as potential carriers of STEC 0157:H7 (Nakazawa *et al*., 1999). STEC infections are rare in human. Serious disease like haemolytic uraemic syndrome and haemorrhagic colitis in young children and the aged (Apriolia *et al*., 2005). EHEC and STEC are related in that the former is a subset of the later. STEC has LEE gene and has attaching and effacing activity. EHEC 0157:H7 strain has been associated the massive disease epidemics in a lot of patients (Blaser., 2001). EHEC Shiga toxins are divided into two, shiga toxin 1*(stx1)* and shiga toxin 2*(stx2).* In addition, Pathogenic STEC does not only produce shiga toxins, but habour other factors like the surface protein intimin,encoded by the gene *eae*. This protein mediates the attaching and effacing(A/E) lesions on the intestinal epithelial cells (Zweifel *et al*.,2005).

2.1.6 Diffusely adherent *E. coli* (DAEC)

Diffusely adherent *E. coli* is characteristic for its unique property to diffuse and adhere on the whole cell evenly(Ha *et al*.,2014). This phenotypic characteristic is encoded by plasmid outer membrane protein of 100 kilo Dalton, referred to as adhesion involved in diffuse adherence (AIDA)(Ha *et al*., 2014).

DAEC has been divided into two, the typical strains and atypical strains which have distinct properties. The strain typical is defined by its ability to posses *Afa* or *Dr* adhesions related genetic organization enabling binding to human Diffuse Adhering factors and enhancing formation DAF clusters. The atypical strains are defined by their ability to harbor *Afa* or *Dr* adhesions or alternative adhesins having the same l genetic profile, the difference is their

inability to bind to human *DAF* (Taddei and Fasano, 2005). DAEC pathotypes phenotypically express the diffuse adherence (*DA*) property to cells of the intestinal epithelium on labo experiment assay on their ability to invade HEP-2 or HeLa cells found in intestinal epithelial cell . In many cases , the Diffuse adherence pattern property of these strains is encoded by *afa* or *dra* or *daa* related gene cluster families that facilitate in the production of adhesins (Servin, 2005).

2.2Isolation and Identification of *E. coli*

Different methods have been used to isolate *E. coli*. In a study done in turkey, rinsed meat was streaked in macConkey agar plates and incubated at 35^{o}c for 24 hours. Those colonies that were lactose positive were then streaked onto eosin-methylene blue agar plates. The colonies that were green or with dark or purple centers were subcultured in tripticase soy broth and incubated for 24 hours at 37^{o}c. Indole posive cultures were confirmed to be *E. coli*(Zhao et al., 2001). Another older study done in 1991 used sorbitol macConkey agar (SMAC) and later incubated positive in trypticase soy broth enrichment media (Downes *et a*l .,1991) current methods are being used like Matrix-associated laser desorption ionization time-of-flight(MALDI-TOF) mass spectrometry and has been shown to accurately identify specific bacteria in clinical situations(Seng *et al*., 2018). In a study done in another study, a rapid method for identification of E. coli was done using an. agar named PGUA agar that allows the identification of bacteria with β-glucuronidase activity in mixed culture (Trepeta and Edberg, 1984). IMVIC tests have been utilized in confirming suspected E. coli isolated. IMVIC is a combination of four tests that include, indole production,methyl red test, the Voges-Proskauer test, and citrate utilization (Trepeta and Edberg, 1984).

2.3 Identification of *E. coli* Pathotypes

One method that has been applied in identification of DAEC gene is immunoblotting analysis. This was demonstrated by SDS-PAGE, and yielded a 107 kDa protein. When sequenced showed 98% of homology with the amino terminal region (Taddei and Fasano, 2005)**.** Phenotypic and genotypic assays have been developed for detection of *E. coli* habouring genes exampleis cytotoxic assays performed with vero cells (Servin, 2005).Polymerase chain reaction (PCR) has been utilized widely for identification of *E. coli* pathotypes, using primers that identify different genes in E. coli isolated (Stewart *et al*., 2011). In some special cases like identification of E. coli 0157:H7, sorbitol macConkey was utilized and a sandwich enzyme –linked immunosorbent assay (ELISA) was used for identification (Zhao et al., 1995).

2.4. *Escherichia coli* as an Indicator Organism

The most prevalent intestinal bacteria in human and animals is *E. coli*. *E. coli* has the capacity to acquire and also preserve resistant genes found in other organisms in the environment and animal population. Resistance to antibiotics in normal flora bacteria animals is a clear pointer to the effect of selective pressure resulting from antibiotic use in target population and later act as reservoir of resistance genes (Varga *et al*., 2008). *E coli* and Enterococci is considered as good models for describing the effects of selective pressure due to misuse antibiotic and challenges expected when treating pathogens (Moyaert *et al*., 2006). *E. coli* has been indicated as an important source of resistance genes to other pathogens of disease importance such as Salmonella (Glenn *et al*., 2012). As a major bacterium in the gastrointestinal tract, it is frequently used as a commensal bacteria for model study of Gram-negative bacteria when

studying antimicrobial resistance in animals (Marchant and Moreno, 2013). *Escherichia coli* is an important bacterium in the science world. It is used for research in biochemical and genetic studies dies for production of recombinant proteins, of all organism it is the most studied (Blaser *et al*., 2001). *E. coli* habours several mobile genetic elements like plasmids and other elements which are associated with disease (Johnson and Nolan, 2009).routine monitoring antimicrobial resistance in bacteria like *Escherichia coli* and Enterococci help to prevent erroneous information on resistance levels. Disease causing organism when used can give false on antimicrobial resistance patterns since they isolated following treatment (Kruif , 2003).

2.5 Molecular Aspects of Antimicrobial Drug Resistance

Multidrug resistance is defined by the ability of an organism to be resistant to three or more antimicrobial antibiotics (Ruiz-garbajosa and Canto, 2011). Multidrug resistance is classified into two, primary or secondary resistance. Primary resistance is mostly mediated by mutation and occurs in organisms that have never been exposed to any antimicrobial agents (Tanwar et al., 2014).

Secondary resistance also termed as acquired resistance mostly occurs when an organism is exposed to a certain antimicrobial agent (Tanwar *et al* ., 2014). A further categorization to Secondary resistance on the ability of organism to be resistant to the common first line of drugs used ,this is referred to as intrinsic resistance has (Tanwar *et al*., 2014).Another commonly used terminology is Extensive resistance, This refers to the ability of a pathogenic bacteria to be resistant to one or two defined most effective antibiotics (Tanwar *et al*., 2014). Co-resistance refers to bacteria acquiring different genes responsible for antibiotic resistance, also

microorganism mutations at different genetic locations that forms resistance to different antibiotics classes. in Cross-resistance, resistant genes acquired or multiple or mutation affect or by acquisition of resistance genes affecting antibiotics of the same class (White *et al*., 2001) Antibiotics work in several mechanisms, this involve prevention of nucleic acid and protein synthesis by microorganisms, inhibition of cell wall formation and disruption of the integrity of the cell (McDermott *et al*.,2003).

2.6 Determinants of Antimicrobial Resistance

In microorganisms several factors are responsible for spread of antibiotic resistance this include, mutation or presence of factors of resistance, transfer of resistance factors either vertical or horizontal, and selection pressure in bacteria populations (Catry*et al*., 2003). It has been noted that antimicrobial resistance can be acquired from new genes from other microorganisms, or from mutations from long existing genes or horizontal gene transfer(Glenn *et al*., 2012).

Mutation is changes in bacterial genome which are transferred from generation to generation. This mutation can occur naturally without any influence, or from a chemical or physical influence.(Sridhar, 2006), it has also been noted that resistant genes movement takes place in two levels; the first level is within the cell. Resistance genes can move within the chromosome, between chromosomes and other genetic elements like plasmids and phages. Two elements facilitate this, transposons and integrons (Maynard *et al.*, 2003). The second level is between cells, also known as horizontal spread of antimicrobial determinants, several mechanisms facilitate this process. Transformation is the first one and is defined as the uptake of naked DNA. The second is transduction and this refers to transfer of bacteriophages having resistance

elements.the last one is conjugation and these involves transfer of antimicrobial resistance by plasmids or other elements involved in conjugation (Boerlin and Reid-smith, 2008).

Studies have also indicated that resistant genes found on plasmids or transposons that are transfererable and can easily be transferred to other microorganism by conjugation. (McDermott *et al*., 2003) .Transposons described as parts of DNA that move freely and can fix themselves into different locations on the bacterial chromosomes, as well as relocate into plasmids and microbe DNA (Fluckey *et al*., 2007). Some transposons or plasmids harbor unique genetic elements known as integrons and enables them to attract external genes. Theirfore a number of genes for resistance may be inserted into a given integron resulting to what is referred to as multiple antimicrobial resistance to antibiotics (Sáenz *et al*., 2004). These genetic elements contain terminal areas are involves with recombination of proteins into specific regions in the genome. This is facilitated by enzymes like (Alekshun and Levy, 2007).

Bacterial plasmids are extrachromosomal DNA that multiply without any external influence and are very important in bacterial niches. Naturally occurring plasmids harbor and facilitate several characteristics, this include resistance to antimicrobials, pathogenicity of microorganism and metabolism of rare substances(Johnson and Nolan, 2009). A number of plasmids have been identified over time, but in *E. coli* one plasmid known as IncF is of great importance. This gene encodes for disease causing traits in *E. coli*(Johnson and Nolan, 2009).

Other factors of important in antimicrobial resistance are gene cassettes. Gene cassettes are important mobile elements, mostly located in specific sites in an integron. They consist of a gene fringed at a combination site refered to as a 59-base element. This gene is identified due an integron-encoded site specific enzyme recombinase (int1) (Recchia and Hall, 1995). Gene

cassettes are free molecules and have a lot of significance important when integrated into an integron (White *et al*., 2001)

2.7 Primary Mechanisms of Antimicrobial Resistance Development

2.7.1 Mechanisms of Antimicrobial Resistance

Typically mechanism of resistance fall into three categories; inactivation of the antimicrobial, efflux or changes in permeability or transport of the antimicrobial and modification or replacement of the antimicrobial agent (Frye and Jackson, 2013). Further as categorized by (Angela *et al*., 2011) bacteria become resistant to antimicrobials through a number of mechanisms. Permeability changes in the bacterial cell wall which restricts antimicrobial access to the target sites, active efflux of the antibiotic from the microbial cell, enzymatic modification of the antibiotic, degradation of the antimicrobial agent, acquisition of alternative metabolic pathways to those inhibited by the drugs, modification of antibiotic targets and overproduction of target enzyme.

2.7.2 Beta Lactams

Beta lactams containing penicillin and cephalosporins kill the bacteria by interfering with cell-wall biosynthesis. The mode of action is through blockade of cross linking enzymes in peptidoglycan layers of cell wall(Walsh, 2000). In enterobacteriacea, resistance to beta lactams is by production of betalactamases that hydrolytically cleave the beta lactams ring thus rendering the antibiotic agent inactive. To counter these, stable beta lactams such as extended-spectrum cephalosporins were developed (Wiegand *et al*., 2007).The beta lactams used in veterinary medicineinclud,penicillins,ampicillin,amoxillin,benzylpenicillin,cloxacillin,hetacillin,nafcillin,pe nethamatehydroiodide) (Lietal.,2007); Penicillin beta lactamase inhibitor combination,

amoxicillin/clavulanate(Li et al., 2007), first generation cephalosporins (cefadroxil,cefapiril,cehpalexin),third generation cephalosporin (cefovecin,cefpodoxime,ceftiofur and fourth generation cephalosporins (Li *et al.*,2007). Beta lactamases are the commonest causes of bacterial resistance to beta lactam antimicrobial agents(Livermore, 1995).This resistance is either encoded chromosomally or on plasmids. Resistance to extended-spectrum beta lacatams has been associated with the production of broad-spectrum beta lactamases such as extended-spectrum beta lactamases (ESBLs) AmpC beta lactamases and metallo beta lactamases (MBLs) (Smet *et al*., 2010).ESBLs are a group of enzymes encoded by genes transcribed on plasmids that are spread among enterobacteriaceae. They have been shown to confer resistance against third generation cephalosporins (Ewers *et al.*, 2012).

2.7.3 Tetracyclines

Tetracyclines belong to a family of broad-spectrum antibiotics. Their efficacy, low cost and lack of side effects make it the most popularly used antibiotic in livestock farming, including aquaculture(Koo and Woo, 2011). Tetracycline's are categorized as first-generation tetracycline, such as tetracycline; chlortetracycline and oxytetracycline and second generation tetracycline's such as minocycline and doxycline (Koo and Woo, 2011). Tetracycline inhibit proteins synthesis by preventing the attaching of aminoacyl tRNA to the 30s ribosomal subunit acceptor site(Roberts and Chopra, 2001). Since their production in the 1950s, tetracyclines have been widely used in human and veterinary medicine, as growth promoter in animal industry and for prophylaxis in plant agriculture and aquaculture. At present resistance to tetracycline's has spread to almost all bacteria genera, and this situation perhaps is the consequence of previous overuse (Aminov and Mackie, 2001). Tetracycline's are broad spectrum agents, exhibiting

activity against a wide range of gram- negative and gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasmas and rickettsiae and protozoan parasites(Roberts and Chopra, 2001). They are also used prophylactically for prevention of malaria caused by mefloquine-resistant plasmodium falciparum. Tetracycline resistance in most bacteria is due to the acquisition of new genes, often associated with mobile elements. These genes are usually associated with plasmids and/or transposons and are often conjugative (Roberts, 2005).

2.7.4 Quinolones and Floroquinolones

Quinolones are broad spectrum antimicrobials agents that have been used widely in clinical medicine and veterinary practice in treatment of infectious diseases caused by enteric bacteria such as *Escherichia coli* (Wang *et al*., 2003). The most frequent mechanism of quinolone resistance in *E. coli* includes alterations in genes that encode subunits of the quinolone targets DNA gyrase (in the gyrA and gyrB genes) and topoisomerase IV (in parC and par E genes) (White *et al*., 2000).Quinolones block the reaction and trap gyrase or topoisomerase IV as a drug-enzyme-DNA complex with subsequent release of lethal double stranded DNA breaks(Jacoby, 2005). Three mechanism of resistance to quinolones are currently recognized : Mutation that alter the drug targets, mutation that reduce drug accumulation, and plasmids that protect cells from the lethal effects of quinolones (Jacoby, 2005). The first generation quinolones represent agents such as naladixic acid and pipemidic acid and have limited activity against Gram-negative bacteria,(Flemming and Reiner, 2001). Second generation quinolones include Ciprofloxacin, sarafloxacin and erofloxacin and these have been indicated for use in veterinary to treat a variety of intestinal and systemic infections in food animals and companion animals (Flemming and Reiner, 2001). In FloroQuinolones resistant isolates, mutational hotspots are localized in defined

regions known as the quinolone resistant determining regions (QRDRS) (Karczmarczyk *et al.*, 2011).

2.7.5 Aminoglycosides

Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome, where they bind to the A-site and disrupt proteins synthesis (Jana, 2006). They are among the most commonly used broad spectrum antibiotics for treating a broad range of life-threatening infections in humans and animals and for bacterial disease control in plants (Kikuvi et al., 2007). Some of the most commonly used aminoglycosides in animal husbandry include Gentamycin and Streptomycin. Others in the group include neomycin,tobramycin,netilmicin and apramycin(Sandvang and Aarestrup, 2000). Resistance occurs by different mechanisms such as prevention of drug entry , active extrusion of drugs, alteration of the target (mutational modification of 16rRNA and mutational modification of ribosomal proteins), and enzymatic inactivation through the expression of enzymes (Jana, 2006). Enzymatic inactivation is normally due to acetyl transferases, nucleotidyltransferases and phosphotransferases (Jana, 2006).

2.7.6 Chloramphenical

The use of chloramphenical in veterinary medicine in the European Union is currently limited to pets and non -food-producing animals. The reason is due to dose-independent aplastic anemia in humans (Schwarz *et al*., 2004).Florfenical which is a flouorinated structural analogue of thiamphenical and chlorampenical is approved by Food and Drug Administration for treatment of bovine respiratory pathogens such as Pasteurellaspp but also against *E .coli* related cattle enteric diseases in the United States(White *et al*., 2000). Florfenical is related to chloramphenical and can select for cross-resistance among bacterial pathogens (White *et al*., 2000). In

prokaryotes chloramphenical is a highly specific and potent inhibitor of bacterial proteins biosynthesis, chloramphenical-dependent inhibition of bacterial protein biosynthesis is mainly due to the prevention of peptide chain elongation (Schwarz et al., 2004). Resistance to chloramphenicol is known to be mediated enzymatically by plasmid-located chloramphenicol acetyltransferases (CATs) or by the non enzymaticchlorampenicol resistance genes CMLA or FLOR that encodes efflux pumps (Kikuvi *et al*., 2007) however, there are reports on other mechanisms of chloramphenicol resistance such as inactivation by phosphotransferases, mutation of the target site and permeability barriers (Schwarz *et al*., 2004)

2.7.7 Sulphonamides and Trimethoprim

Trimethoprim and sulfonamides are synthetic antibacterial agents with a wide antibacterial spectrum for treating pathogens of family enterobacteriacea affecting the respiratory system, skin and urinary tract(Pentii *et al.,* 1995). Sulfonamides compete with the structural analog p-amino-benzoic acid for binding to dihydropteroate synthase (DHPS), a catalytic enzyme in the folic acid biosynthesis pathway thus inhibiting the formation of dihydrofolic acid (Perreten and Boerlin, 2003). Resistance in gram-negative bacilli generally arises from the acquisition of dihydropteroate synthase (DHPS) genes in the integrons that are not inhibited by the drug(Hae *etal*., 2015). There are several mechanism of trimethoprime resistance: development of permeability barriers, efflux pumps, existence of naturally insensitive target dihydrofolate reductase enzymes , mutational and regulation changes in the target enzymes and the acquirement of drug-resistant target enzymes (Hae et al., 2015) .Sulphonamide resistance is often encoded by Sul1,Sul2 and Sul3 genes in Enterobactericea (Hammerum *et al*., 2006).

2.8Prevalence of Antimicrobial Resistance in *E. coli*

2.81Worldwide Situation

A study was done in China in the years between 2007 and 2012 on antimicrobial resistance of *E. coli*. This study focused on 1002 chicken *E. coli* strains isolated from layers and broiler flocks. Antimicrobial resistance prevalence of *E. coli* strains to ampicillin,doxycycline,tetracycline and naladixic acid were consistently kept at 62%-100%. The *E. coli* resistance to naladixic acid and ciprofloxacin had an increasing trend as high as 100% for naladixic acid while the prevalence of resistance to gentamycin had a decreasing trend(Wang *et al.*, 2013). A study done on Irish cattle farms, situations were no better. The most prevalent antimicrobial resistance on *E. coli* isolates was streptomycin (100%), followed by tetracycline (99%) sulfonamides (98%) ampicillin (82%) and neomycin (62%) (Karczmarczyk *et al*., 2011). In Korea a study indicated phenotypic resistance of *E. coli* to be generally high across all groups of antibiotics. This study collected samples along the value chain, pork, beef, fish and fishery products processed foods. Prevalence of resistance was as follows: ampicillin (66.1%) tetracycline (55%), gentamycin (19%), Streptomycin (67.8%), cephalothin(24%), ciprofloxacin(44.6%), chloramphenical(26.4%) trimethoprime/sulfamethoxazole (41.3%) ,chloramphenical (26.4%) (Kang *et al*., 2005).

2.8.2Regional Situation

A study done in Lira Uganda, on feacal broiler *E. coli* isolates indicated high resistance high resistance to ampicillin (87.2%), tetracycline (55%), cotrimazole (41.3%), chloramphenical (13.8%) ciprofloxacin (2.8%) and Gentamycin (0%) (samwel *et al*., 2010). Another study done on *E. coli* resistance to antibiotics in apparently healthy cattle in Tanzania, indicated a prevalence of resistance of (33.1%)for tetracycline,(88%) sulphamethoxazole/trimethoprime and (21.3%)

ampicillin (Madoshi *et al*., 2016). A doctor of philosophy thesis belonging to Abdul at Sokoine University of agriculture , Morogoro Tanzania (2014) on *E. coli* isolated from faecal samples farm animals, pets and wild life, indicated tetracycline having a resistance of (79.4%),sulphadimidine/trimethoprime (77.1%), ampicillin (74.6%) and less resistance to cefataxime (40%) (Katakwebe, 2014). Another study done in Ethiopia on *E. coli* isolates from raw meat samples obtained from abattoirs indicated a resistance pattern of ampicillin 83.60%, penicillin 75.40%, erythromycin 83.60%, doxycycline 100% but tetracycline was 0% which was very unusual(Mohammed*et al*., 2014). A study done in Ibadan, Nigeria on antimicrobial resistance among commensal *E. coli* from cattle faeces and beef had the following pattern of resistance, amoxicillin (97.9%), ampicillin (97.9%), cefuroxime (25.1%), chlorampenical (69.3%), ciprofloxaxin (11.7%), cotrimazole (45.9%), erythromycin (59.4%), gentamycin (36.5%), naladixic acid (21.1%), ofloxacin (14%), streptomycin (78.9%) and tetracycline (33.9%) (Amosun *et al.*, 2012).

2.8.3Local Situation

A study was done in Tigoni, Limuru on broiler chicken;found antimicrobialresistance of *E. coli* to be tetracycline (75.9%) and cotrimaxazole (72.4%) (Adelaide *et al*., 2008). Another study on cattle, swine and goats faeces, reported the highest resistance of *E. coli* in streptomycin, tetracyclinetrimethoprime/sulfamethoxazole (Kikuvi et al., 2004). A study on resistance of *E. coli* isolated from rats collected from Kabete, Kibera and Kawangware indicated the highest resistance in sulphamethoxazole ,ampicillin and streptomycin (refer to figure 2.1 below).

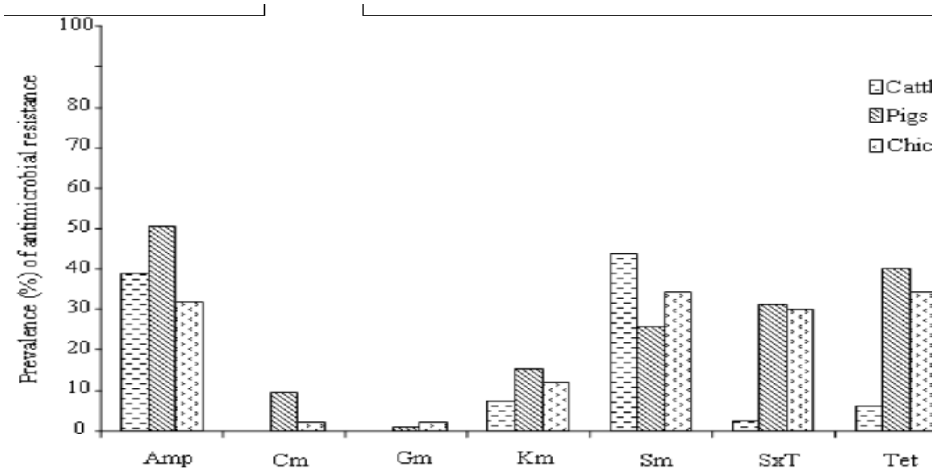

Figure 2.1: Antibiotic resistance in cattle, pigs and chicken in a study done by Kakuvi on Antimicrobial agents: Amp, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; SxT, sulphamethoxazole/trimethoprim; Tet, (Kikuvi *et al*., 2004)

Table 2.1: Antimicrobial susceptibility profile of *E. coli* isolated from rats trapped from Kabete, Kibera and Kawangware Nairobi, Kenya.

Antimocrobial agent	**No(%) of *E. coli* (N=60)**		
	Resistant	**Intermediate resistance**	**susceptible**
Ampicillin	12(20.0)	33(53.3)	16(26.7)
Co-amoxyclav	1(1.7)	5(8.3)	54(90)
Streptomycin	8(13.3)	40(66.7)	12(20)
Sulphamethoxazole	15(25.0)	22(36.7)	23(38.3)
Tetracycline	2.(3.3)	23(38.3)	35(58.3)
Trimethoprim	3(5.0)	7(11.7)	50(83.3)
Cefuroxime	0(0.0)	9(15.0)	51(85.0)
Ceftazidime	0(0.0)	8(13.3)	52(86.7)
Naladixic acid	0(0.0)	7(11.7)	53(88.3)
Gentamycin	0(0.0)	8(13.3)	52(86.0)
Ciprofloxacin	0(0.0)	2(3.3)	58(6.7)

Source: (Gakuya*et al*.,2001)

2.9Detection of antimicrobial resistance through phenotyping and genotyping

2.9.1 Phenotyping

There are a number of methods for antimicrobial phenotypic susceptibility testing of bacteria. The first include dilution method. One of the dilution methods is agar dilution method that involves the incorporation of different concentration of the antimicrobial substance into a nutritious agar media followed by the application of a standardized number of cells to the surface of the agar plate (Kikuvi *et al*., 2007).The other dilution method is broth dilution, often determined in 96 well microtiter plate format. Bacteria are inoculated into a liquid growth medium in the presence of different concentration of an antimicrobial agent. Growth is assessed after incubation for a defined period of time (16-20hrs) and the MIC value is read. The MIC thus is the minimum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth (Chryssanthou, 2006).

The other method is disc diffusion method which is probably the most widely used method for determining antimicrobial resistance because of convenience, efficiency and cost(Lass-flo, 2012). A growth medium, usually Muller-Hinton agar is evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 10^8 colony forming units per ml).Commercially prepared disk, each of which are impregnated with a standard concentration of a particular antibiotic, are evenly dispersed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disk with decreasing concentration further away from the disk. After an

overnight incubation, the bacterial growth around each disk is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disk.

The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition, since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. The zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible intermediate or resistant (Hamasuna *et al*., 2009). Other tests for checking phenotypic resistance include E-test. E. test is a commercially available test that utilizes a plastic test strip impregnated with gradually decreasing concentration of a particular antibiotic. This strip displays a numerical scale that corresponds to the antibiotic concentration contained therein. This method provides a convenient quantitative test of antibiotic resistance of clinical isolate. However a separate strip is needed for each antibiotic and therefore the cost of this method can be high (Ambaye et al., 1997). In E-test, MHA plates are inoculated in the same way as in disk diffusion testing. A maximum of five E-test strips are applied to each MHA plate, and the plates incubated at 35^0C for three days. The minimum inhibitory concentrations are determined in accordance with guidelines provided by the manufacture (Vandenbosscheet *al*., 2002).

The other test includes mechanism-specific tests. Resistance may be established through tests that directly detect the presence of a particular resistance mechanism. For example, beta lactamase detection can be accomplished using an assay such as chromogenic cephalosporin test

and detection for chlorampenicol acetyltransferase (CAT) may utilize commercial colorimetric assays such as CAT reagent kit. Beta lactamases are a group of enzymes capable of hydrolyzing the beta-lactam bond of both penicillins and some cephalosporins thereby causing these antibiotics to become inactive.Chromogenic cephalosporin is a gold standard for beta –lactamase test (Llanes *et al.*, 2003). The above test is a rapid commercially available test for prediction of Extended Spectrum Beta-Lactamases expression in enterobacteriaceae cultured from patients' materials and pigs. The principle of the Bromp^{TM} test is based on the cleavage of the substrate HMRZ-86, a chromogenic cephalosporine (El-jade *et al.*, 2016). Bacterial resistance to chloramphenicol is most commonly conferred by the enzyme chloramphenicol acetyltransferase (CAT) encoded by *cat* gene. A florescent chloramphenicol acetate esterase assay kit has been developed to detect the *cat* gene (Sohaskey, 2004).

2.9.2Genoyping

Molecular investigation is used to determine mechanism for resistance and genes responsible for phenotypic resistance(Guerra *et al.,* 2003).Resistance traits are genetically encoded and test for specific genes that confer antibiotic resistance. Polymerase chain reaction or PCR is one of the most commonly used molecular techniques for detecting certain DNA sequences of interest. This involves several cycles of denaturation of sample DNA ,annealing of specific primers to the target sequence (if present), and the extension of this sequence as facilitated by a thermostable polymerase leading to replication of a duplicate DNA sequence, in an exponential manner, to a point which will be visibly detectable by gel electrophoresis with aid of a DNA-intercalating chemical which fluoresces under UV light (Oliveira and Lencastre, 2002).It was invented 1983 by Dr Kary Mullis (Dieffenbach, 1993). An example is in detection of streptomycin resistance

genes *strA, strB,* and *aadA1* and the chloramphenicol resistance genes *Cat A1,CatA3* and *cmlA*(Kikuvi *et al.,* 2007).

The other method is DNA hybridization which is based on the fact that the DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine; or uracil for RNA). Therefore, a labeled probe with a known specific sequence can pair up with opened or denatured single stranded DNA from the test sample, as long as other sequences complement each other. If this hybridization occurs, the probe labels this with detectable radioactive isotope, or antigenic substrate enzyme or chemiluscent compound. Whereas if no target sequence is present or the isolate does not have a specific gene of interest, no attachment of probe will occur therefore no signals will be detected (Roberts *et al*., 2001). In tetracycline, specific oligonucleotides probes have been designed that hybridize with specific gene of interest but not to related genes. For example , if one is screening for presence of *tet* (m) gene,the oligoneuclotide probes will not hybridize to *tet*(O) or *tet* (S) genes (Roberts *et al*., 2001).

2. 10Risk factors for *E. coli* infection

Studies have been done concurrently in human and animals in the same farm and found to share the same *E .coli* pathotypes. This suggested a transfer of strains between hosts (Madoshi *et al.,* 2016). This has been complicated by emergence of antimicrobial resistance in these zoonotic enteropathotypes. This is attributed to growth promoting and prophylactic use of antimicrobials (Mcewen and Fedorka-cray, 2002). Occurrence of zoonotic pathogens has been linked to environment and also human activities. In a study done in Madagascar, risk of infection was linked to a water source. Individual different pathogens were associated with certain behavior,

including ,use of medication, experienced diarrhea and use of toilets (Bublitz *et al.*, 2014) Seasons have been associated with occurrence of *E. coli* pathotypes. In a study done in Pakistan , high rates of isolation have been noted during floods (Bokhari *et al*., 2013).This was supported by another study that indicated seasons and ages of calves as important factors for bacterial infection (Shahrani and Momtaz, 2014) .

A study done in Northern China indicated that continuous input from animal operation; discharge and field runoff could be a cause of presence of antimicrobial resistant *E. coli* in rivers. This can be attributed to extensive use or partial abuse of veterinary antibiotics in livestock farming areas(Zhang *et al.,* 2014). It is suggested that environmental factors, specifically water, sanitation and hygiene contribute to the development of resistant pathogens (Fletcher , 2015). In a study done in Nigeria on donkey, the type of food given to animals is a potential predictor for intestinal shedding of *E. coli* serotype 0157 (Jedial *et al*., 2015).In another study in Scottish cattle farms, source of water and location of farms were risk factors for the presence of 0157(Halliday *et al*., 2006). In a study done in the United Kingdom, statistical analyses indicated that young animals have higher prevalence of pathogenic organisms (*Campylobacte*r spp and *E. coli*). Furthermore, when waste contained any form of bedding, lowered prevalence and levels of both pathogenic *Listeria* spp and *Campylobacter* spp was recorded.

Waste generated by livestock consuming a diet composed principally of grass was less likely to harbor *E. coli* 0157 or *Salmonella* spp. This study indicated that, age of animal bedding and type of food affect level of bacterial pathogen in faeces (Hutchison *et al*., 2005). A study in Puduchery region recorded that involvement of educated family members in farming practices

can create awareness and improved knowledge toward zoonotic diseases (Shahrani *et al*., 2014).In Central Californian coast, rodents posed a minimal risk as environmental reservoirs of *E. coli* 0157:H7, but they played a role in environmental dissemination of *Salmonella* and *Protozoa* (Kilonzo et al., 2013). Stray dogs are a risk to occurrence of *E. coli.* In a study done in Mexico, faecal samples from stray dogs indicated presence of atypical enteropathogenic *E. coli* strains comprising 14 different serotypes (Jay-russell *et al.,* 2014)

CHAPTER THREE: MATERIALS AND METHODS

3.1Study Design

This was a cross-sectional study

3.2Study Site

The Nairobi Urban Health and Demographic Surveillance System (NUHDSS) run by African Population and Health Research Center covers a Demographic Surveillance Area (DSA) that studies the two slums of Korogocho and Viwandani in Nairobi City, Kenya. NUHDSS was chosen because of the wealth of data they have on households of this two slums. The DSA in Korogocho is divided into seven villages: Highridge, Grogan "A", Grogan "B", Gitathuru "C", Nyayo/Kisumu Ndogo, Korogocho "A" and Korogocho "B". Viwandani slum is located 7 kilometers from the Nairobi city centre and has close proximity to the city's industrial area.The DSA in Viwandani is divided into 5 villages: Paradise, Jamaica, LungaLunga, Donholm and Kingston(Emina et al 2010).

3.3Study Population

There were 29,912 households under the Demographic Surveillance System (DSS) run by APHRC in both Korogocho and Viwandani; 11,095 (37%) in Korogocho and 18,817 (63%) in Viwandani. There were a total of 342 households under DSS that own livestock in both slums, with 93 (27%) in Viwandani and 249 (73%) in Korogocho.

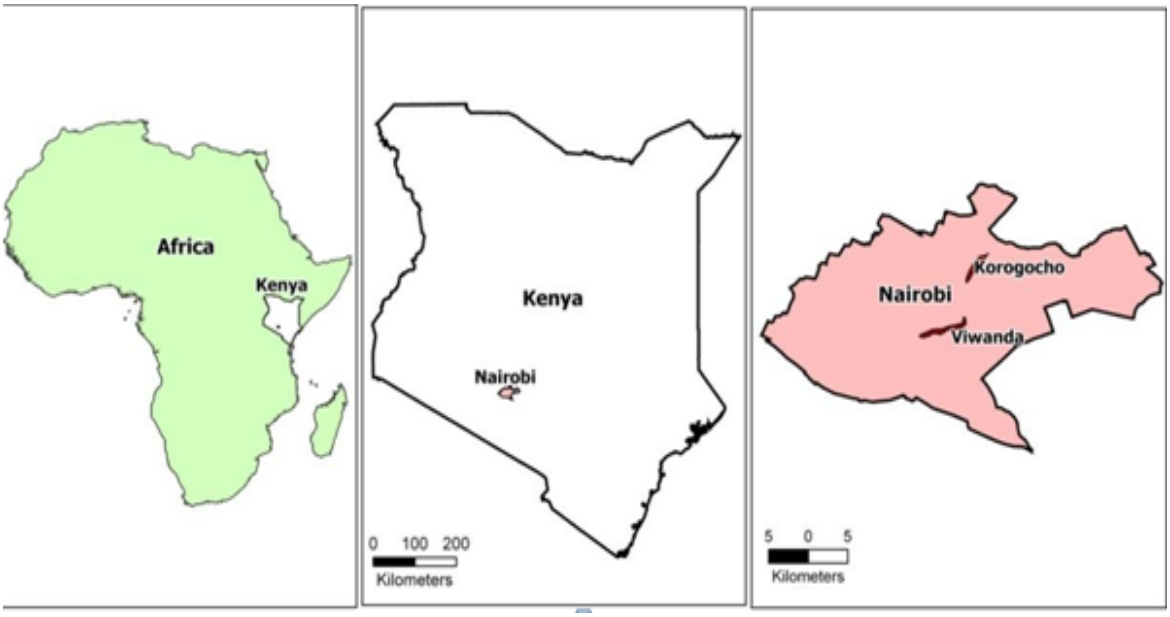

Figure 2.2: Site for sample collection

3.4Sample Size Determination

In order to attain the maximum sample size, a prevalence of 50% *(E. coli*) in animals was used. A design effect of 2 was used to cater for the clustering within the households. The sample size was thus calculated as follows;

$N= Z_{\alpha}^2 p (1-p)/ d^2$

Where N= sample size,

Z = Z statistic for a level of confidence,

P = expected prevalence or proportion

(In proportion of one; 50%, P = 0.5), and

d = precision (in proportion of one; if 5%, d = 0.05).

$N= (1.96^2 * 0.5 * 0.5) / 0.05^2$

N= 385 animals without design effect

N with design effect = 770 animals

Since ducks were not considered in the original study design but were among the poultry owned by farmers in the study areas, 10% of ducks were sampled in every household visited that had ducks.The final sample size was therefore 795.

There were a total of 342 households under DSS that owned various animal species of livestock in both slums, with 93 (27%) households in Viwandani and 249 (73%) in Korogocho. However, a total of 206 households were willing to participate in the study. Proportional allocations of the animals to be sampled are given in the appendices. A total of 795 animals were sampled from the 206 households that kept various animal species.

3.5 Planning for the Study

Formal request was sent to the Ministry of Livestock and Development (MoLD) to visit the study areas. The representative of the Director of Veterinary Services provided a letter allowing the research group to access the study areas and facilitated the contacts of the District Veterinary Officer (DVO) in Charge of Korogocho and Viwandani. The researcher explained the aim of the research and the intentions for data collection. The DVO directed the researcher to the chiefs (Korogocho and Viwandani). The researcher explained the aim of the project and their approaches to data collection. The researcher, with the chiefs' permission arranged for a meeting with the village elders to explain their intentions for data collection and the logistics thereof. The researcher then went onto the livestock owners' households to collect data. Approval was also sought from the Nairobi Museums to allow the research team to trap rodents.

3.6 Random Selection Process

A sampling frame consisting of a list of households in each category was used to randomly select the households to be sampled. The households were allocated serial number. Using excel the households were randomly aligned. These households were proportionally, 27% population of animals in Viwandani, 73% population of animals in Korogocho out of the total number of animals in both areas as explained in appendix 2 on sample determination.

A sample frame was constructed of the animals in the herd by listing their names. If, for any reason, two or more animals had the same name, a further identity by a number (e.g. Daisy 1, Daisy 2 etc) was used. A similar procedure was used to establish and identify certain unnamed animals in a herd by identifying them as the first calf of Emma, the second calf of Flora.

To select the animals to be sampled the name of each animal in the herd was written on a piece of paper, place the name cards in a hat and then draw out one card. The above method was used for households with three, six and ten animals, where one, two and three animals were selected respectively. For households with 20 animals I divided 20/7 which comes to approximately 3. Such a small number of animals were constrained to one corner of the structure then proceeded to select every 3rd animal. Household with 39 animals, every fourth animal was selected since 39/10 is approximately 4. For households with 50 animals, the herd was 5 groups i.e. 50/10 from which two animals were randomly selected from each group. The household with maximum number of animals was 200. This was divided into 10 groups (200/20) with 20 animals each. From each of these groups of animal was randomly selected. Irrespective of how many animals a household owned, a maximum of 10 animals were sampled in a single household.

3.7 Data Collection

Data collection in the study areas was divided into two phases:

3.7.1 Individual Questionnaire Administration to the Livestock Owners.

One person per household (household head) was interviewed i.e. the livestock owner. A total of 206 house hold heads were interviewed .The aim of these interviews was to obtain individual data on value chain used, farm hygiene, disease control and personal perceptions towards several themes such as food safety, antimicrobial use etc. Samples were collected immediately after the questionnaire was fully answered.

3.7.2 Sample Collection

3.7.2.1 Cattle

Calves were restrained manually. A lubricated gloved finger was gently passed through the anus and massaged the rectal wall to stimulate rectal evacuation. Larger cattle, they were restrained using a halter. Using a sterile glove through the anus, faecal material was withdrawn and aseptically put into sterile faecal pots, barcoded and transported to the laboratory at the University of Nairobi in a cool box. A total of 26 cows were sampled

3.7.2.2 Sheep and Goats

The animals were manually restrained and using a sterile glove the index finger was inserted in the rectum to evacuate fecal material. This was labeled using a barcode and placed in a cool box. For kids, a sterile swab was used to swab the rectum. This was labeled, put in peptone water and placed in a cool box. A total of 81 goats were sampled

3.7.2.3 Pigs

Small pigs were restrained manually and large pigs were restrained using a snare. Using a gloved hand, the index finger was used to evacuate feces aseptically from the rectum. These labeled samples were then placed in a cool box. For piglets, a sterile swab was used to swab the rectum. This was then put in peptone water and placed in a cool box after labeling .A total of 45 pigs were sampled

3.7.2.4 Poultry (chickens, ducks, doves)

To obtain the sample with minimal distress to the live bird, one assistant held the bird against their chest with the wings folded. A cloacal faecal swab was taken using a sterile swab. This swab was then placed in peptone water, labelled and then placed in a cool box. A total of 560 chicken, 33 ducks and 28 doves were sampled.

3.7.2.5 Rabbits

Rabbits were restrained and a sterile swab was used to take a swab. The swab was labelled, put in peptone water and placed in a cool box. All the samples were transported to the laboratory at the University of Nairobi for processing. A total of 22 rabbits were sampled

3.7.2.6 Sampling of Rodents

Traps were set in the selected households keeping livestock. Between 2 to 5 traps were set per house. Traps were placed within the households to avoid interference by other animal or theft. Silver cyprinid fish were placed inside the traps to attract rodents. Traps were checked every 12

hours for 24 hours. Traps with rodents were transported to the laboratory. A total of 13 rats were sampled

3.8 Laboratory Analysis

3.8.1 Collection of faecal and intestinal Scrapings from trapped rodents

The live rat was euthanized by placing it in a jar with isofluorane soaked cotton wool. Once it euthanized, the rat was placed in dorsal recumbence on the post mortem table. A photo was taken for identification. The weight, length and sex of the rodent were taken. On its dorsal recumbence, the abdomen was opened aseptically. Faeces and intestinal scrapings were taken.

3.8.2 Isolation of *E. coli* from faeces and rodent Intestinal Scraping

One gram of feces/swab was transferred in buffered peptone water and homogenized. A loop full of the homogenate was cultured in MacConkey agar for 18 to 24 hours at 37^0C.Six pin point colonies characteristic of *E. coli*, were picked, sub-cultured on sterile MacConkey agar and incubated at 37^oC for 18-24 hrs.

3.8.3 Isolation of *E. coli* from cloacal Swabs

For the cloacal swabs already in Buffered peptone water, a loop full was cultured onto MacConkey for 18 to 24 hours at 37^0C. Six colonies characteristic of *E. coli*, were picked and sub cultured in MacConkey overnight. Biochemical tests were done on the six colonies in LIM, TSI, CITRATE, MR.VP and citrate. The colonies that tested positive on biochemical test were stored in skimmed milk at -40^0C overnight then transferred to -80^0C

3.8.4 Biochemical Tests

The following biochemical tests were done on each of the six colonies isolated

3.8.4.1 Lysine –indole-Motility (LIM) test

One colony of suspected *E. coli* form MacConkey was picked with a sterile inoculating wire loop. The medium was stabbed to within one half inch from the bottom of the tube. This was incubated aerobically at 35^{o}C for 18-24 hours. The results were recorded for lysine motility. Growth away from the stab line was recorded positive for motility. A positive test for lysine-decarboxylase was a purple band and a yellow butt. A negative test was a narrow purple band and a yellow butt. Three to four drops of Kovac's reagent were added and an indole reaction observed and results recorded. A color change in the reagent layer from yellow to red or pink after addition of Kovac's reagent was positive for *E. coli.* A negative test was indicated by change in color from yellow to bright yellow.

3.8.4.2 Growth in Triple Sugar Iron Medium Test

Using isolated, pure colony inoculators, the specimen was stabbed and streaked on the agar. Incubation was done aerobically at 35-37^{o}c for 18-24 hours. The medium reaction was examined. For *E. coli* growth was evident, with yellow slat, yellow butt, gas positive and hydrogen sulfide produced.

3.7.4.3 Utilization of Citrate Test

Using a light inoculum, the Simon's citrate medium was inoculated using growth from pure culture in MacConkey. The slant was inoculated by streaking the surface in a serpentine manner.

This was incubated for 24-96 hours at 35^0C. Growth and color change were examined. For *E. coli, a* positive test was evidenced by no growth with the medium remaining green in color.

3.8.4.4 Methyl Red (MR) Test

The Methyl Red-Voges-Proskauer (MR-VP) broth was inoculated with pure culture from MacConkey. This was incubated at 35^0c for 18-24 hours. Five drops of methyl red reagent was added and if it were positive for *E. coli* a red color immediately developed.

3.8.4.5 Voges-Proskauer (VP) Test

The Methyl Red-Voges-Proskauer (MR-VP) broth was inoculated with pure culture from MacConkey. This was incubated at 35^0C for 18-24 hrs. Barrits A and Barrits B reagents were added and for *E. coli* no color change was observed.

The colonies that tested positive on biochemical test were stored in skimmed milk at -40^0C overnight then transferred to -80^0C.

3.8.5 Antimicrobial Sensitivity Testing using Disc Diffusion Method

3.8.5.1 Standardization of Inoculums for *E. coli*

The inoculums were prepared by first reviving the stored *E. coli* pooled isolates. Two to three isolated colonies were picked using a sterile wire loop. This was transferred into a sterile tube containing normal saline (0.85% NaCl). This was then vortexed to emulsify the inoculums. The innoculum was adjusted to 0.5 McFarland ($\sim 1\times 10^8 C.F.U\ Ml^{-1}$). McFarland standards are used as a reference to adjust the turbidity of bacterial suspension so that the number of bacteria will be within a given standard range for antimicrobial testing (Lui *et al*., 2016).

3.8.5.2 Inoculation of Mueller-Hinton plate

Visual examination of Muller Hinton agar plates prior to use was done to make sure it was free of contamination, correct depth of approximately 4mm and were not excessively wet, cracked or dry. A sterile cotton swab was then dipped into the inoculums. This was then rotated several times pressing firmly on the wall of the tube above the fluid level to remove excess innoculum from the swab. The entire surface of Mueller Hinton agar plate was streaked using the swab. Streaking was repeated at angles of 60 degrees at least three times. Inoculation was completed by running the swab around the rim of the agar. Excess moisture on the agar surface was allowed to be absorbed prior to applying the antimicrobial discs.

3.8.5.3 Dispensing Antimicrobial Discs

Using a sterile forceps (by flaming with alcohol), discs (Oxoid) impregnated with drugs were(Table 3.1) dispensed on the agar surface. The discs were pressed gently on the top to make complete contact with the agar surface. Separate agar plates were inoculated using control strain of *E. coli* ATCC 25922 and similar discs dispensed on the agar surface. This agar plates were then incubated at 35 ^{o}C for 16-18 hrs. Diameters of the halos were measured in mm. According to the diameter, *E. coli* isolates were classified into: resistant, intermediate and susceptible (CLSI, 2012). The table below shows the drug concentrations in discs used in antimicrobial susceptibility testing

Table 3.1.: Drug Concentration of Discs used in Antimicrobial Susceptibility Testing;

Drug	CONCENTRATION (µg)	DESCRIPTION
Naladixic acid	30	1st generation fluoroquinolones
Ciprofloxacin	5	2nd generation fluoroquinolones
Cefuroxime	30	2nd generation cephalosporin
Ceftazidime	30	3rd generation cephalosporin
Ceftriaxone	30	3rd generation cephalosporin
Streptomycin	10	amino glycosides
Gentamycin	10	amino glycosides
Amoxicillin/clavulinic acid	20/10	Amino penicillin plus batalactamase
Chloramphenical	30	Phenicols
Ampicillin	10	amino penicillin
Trimethoprime/sulfamethoxazole	1.25/23.75	Sulfonamides + diaminopyrimidines
Tetracycline	30	Tetracycline

(Source :CLSI, 2012b).

3.8.6Pathotyping of *E .coli*

Pooled *E .coli* isolates of the same sample was pathotyped using PCR. The total numbers of pools per sample were 231. These pools represented *E. coli* isolates of a single sample.

3.8.6.1 DNA Extraction

E. coli bacterial strains stored in skimmed milk at -80^{o}C were revived in Tryptone soy agar, at 35^{o}C overnight. Using a wire loop 3 to 5 colonies were suspended in 1000ul of sterile distilled water. The bacteria were lysed by boiling for 10 min in a water bath. The lysate was centrifuged at 15000rpm for 5min and the supernatant was used directly as template.

3.8.6.2 DNA Amplification of Target Genes

Nine genes were targeted; diarrheagenic strains were amplified using oligonucleotide primer pairs listed in Table 3.2. Five point five (5.5) microliter of crude protein preparation was mixed with twelve point five(12.5) microliter of master mix, one(1) microliter of forward primer and one(1) microliter of reverse primer (Table 3.2). Six (6) micro liters of DNA/RNA free water was used to make a final volume of 25 micro liters. Primers which had an original concentration of 2µM were evaluated at different concentration and the 0.2µM final concentration for individual primers was chosen for all reactions. At this concentration no primer dimer were seen. The PCR program used for amplification consisted of denaturationat94 ^{o}C for 2 min, followed by 40 cycles of denaturation at 92 ^{o}C for 30 sec, annealing at 54 ^{o}C for 30 sec, extension at 72 ^{o}C for 30 sec. At the end of 40 cycles, a 5-min extension at 72^{o}C was allowed before samples were ready for electrophoresis.

3.8.6.3 DNA Gel Electrophoresis and Image Recording

DNA amplicons were separated on a 2% agarose gel electrophoresis containing ethidium bromide and using Tris-acetate-EDTA (TEA) as running buffer. Images of DNA separation from gel were viewed through a UV light and captured with a digital camera. Materials found contaminated were disposed according to guidelines by putting the materials in activated charcoal. .Figure 3.2 shows primers used for the various target genes, accession numbers and their product sizes.

Table 3.2: Primers used for the various Target Genes, their Accession Numbers and Product Sizes.

Primer number	Oligo size	Sequence (5' – 3')	Target gene	Product size (bp)	Accession number
1	STXF	5'-GAGCGAAATAATTTATATGTG-3'	VT EHEC	518	AB012102.1
2	STXR	5-TGATGATGGCAATTCAGTAT-3'			
3	AEAF	5'-CTGAACGGCGATTACGCGAA-3'	eae EPEC	918	KP197126.1
4	EAER	5'-CCAGACGATACGATCCAG-3'			
5	BFPAF	5'-AATGGTGCTTGCGCTTGCTGC-3'	bfpA EPEC	324	FM180569.1
6	BFAR	5'-GCCGCTTTATCCAACCTGGTA-3'			
7	AGGF	5'-GTATACACAAAAGAAGGAAGC-3'	AggR EAEC	254	CP011332.1
8	AGGR	5'-ACAGAATCGTCAGCATCAGC-3'			
9	LTF	5'-GCACACGGAGCTCCTCAGTC-3'	eltA ETEC	218	EU113247.1
10	LTR	5'-TCCTTCATCCTTTCAATGGCTTT-3'			
11	STF	5'-GCTAAACCAGTAGAGSTCTTCAAAA-3'	estA ETEC	147	CP006002.1
12	STR	5'CCCGGTACAGRGCAGGATTACAACA-3'			
13	VIRF	5'-AGCTCAGGCAATGAAACTTTGAC-3'	virF EIEC	618	CP007038.1
14	VIRR	5'-TGGGCTTGATATTCCGATAAGTC-3'			
15	IPAHF	*5'-CTCGGCACGTTTTAATAGTCTGG-3'*	*ipaH EIEC*	933	CP011417.1
16	IPAR	5'-GTGGAGAGCTGAAGTTTCTCTGC-3'			
17	AFAF	5'-GCTGGGCAGCAAACTGATAACTCT-3'	*afaB* DAEC	794	KR338833.1
18	AFAR	5'-CATCAAGCTGTTTGTTCGTCCGCCG-3'			

CHAPTER FOUR

4.0 RESULTS

4.1 Characteristics of Households in Korogocho and Viwandani Slums

The information on household characteristics was extracted from the questionnaire that was administered in Korogocho and Viwandani slums. It touched across management practices and other attributes as indicated in the tables 1.1. A total of 206 household heads were interviewed 144(70%), in Korogocho and 62 (30%) in Viwandani. Indicated in the table 4.1 are the attributes that were analyzed and compared between the two areas. Tables 4.1 to 4.8 shows a comparison of the household attributes in the two sampling sites

Table 4.1: Livestock Ownership and Period of Keeping Livestock

Attribute	Korogocho n=144(%)	Viwandani n=62(%)	P value <0.05
Man owning livestock	51(35%)	33(53%)	0.017*
Woman owning livestock	70(49%)	22(35%)	0.082
Both owning livestock	23(16%)	7(11%)	0.382
Keeping livestock between 2010 to 2015	72(50%)	42(68%)	0.019*
Keeping livestock between 2000-2009 (10 years)	40(28%)	10(16%)	0.074
Keeping livestock between 1990-1999(10years)	18(13%)	7(11%)	0.807
Keeping livestock 1989 and below	13(9%)	3(5%)	0.303

The results above indicate that significantly more men owned livestock in Viwandani compared to Korogocho. Significantly higher proportions of households in Viwandani keep livestock than Korogocho between the year 2010 and 2015

Table 4.2: Ownership of the Farm and Level of Education

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Own farm	106 (74%)	36(58%)	0.027*
Rent farm	35(24%)	20(32%)	0.237
Ownership not known	3(2%)	6(10%)	0.014
Primary education	71(49%)	39(63%)	0.073
Secondary education	43(30%)	16(26%)	0.553
Tertiary education	7(5%)	0(0%)	0.077

No significant differences were observed on the level of education between the two areas. Significantly more people own land in Korogocho compared to Viwandani.

Table 4.3: Livestock Species Kept

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Poultry	120(83%)	50(81%)	0.641
Rabbits	9(6%)	13(21%)	0.002*
Dogs	9(6%)	7(11%)	0.215
Cats	11(7.64%)	5(8.06%)	0.917
Cow	12(8%)	8(13%)	0.310
Goats	29(20%)	16(26%)	0.367
Pigs	7(5%)	9(15%)	0.018*
Other livestock (ducks)	5(4%)	5(8%)	0.917

There are significantly more rabbits and pigs in Viwandani compared to Korogocho.

Table 4.4: Source of Water and feed for Livestock in Viwandani and Korogocho

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Feed source scavenging	40 (28%)	33 (53%)	0.00*
Feed source HH left over's	85(59%)	35(56%)	0.577
Feed source Swirl	19(13.19%)	8(12.90%)	0.955
Feed source (forage bought)	34(30%)	13(21%)	0.675
Feed source (agro vet)	94(65%)	46(74%)	0.159
Water source (tap)	132(92%)	55(89%)	0.501
Water source (open sewer)	8(6%)	7(11%)	0.146
Water source (road)	5(4%)	16(23%)	0.000*
Household water	19(13%)	1(1.62%)	0.070

No much difference was seen on the source of feed and water. Significantly more animals' scavange and drink water on roads (open drainages) in Viwandani compared to Korogocho

Table 4.5: Medicine used to treat Livestock and Personnel Treating

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Medicine used in livestock(antibiotics)	39(27%)	16(26%)	0.849
Medicine used in livestock (dewormers)	45(31.25%)	18(29%)	0.757
Medicine source (herbal)	10(6.9%)	7(11.29%)	0.298
No medicine used	60(42%)	21(34%)	0.293
Vaccines	23(16%)	13(21%)	0.386
Animal treatment (owner)	109(76%)	46(74%)	0.819
Animal treatment (government)	4(3%)	0(0%)	0.185
Animal treatment (vet)	12(8%)	4(6%)	0.643
Animal treatment (agro vet)	43(30%)	16(26%)	0.069

No significant differences were observed statistically when it comes to treatment of animals between the two areas

Table 4.6: Person Feeding the Animals and Cleaning the Shed

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Feeding animal (man)	75(52%)	41(66%)	0.062
Feeding animal(woman)	89(62%)	30(48%)	0.074
Feeding animal (employee)	3(2.08%)	1(1.61%)	0.822
Cleaning shed (man)	70(49%)	37(60%)	0.145
Cleaning shed (woman)	81(56%)	26(42%)	0.059
Cleaning shed (employee)	3(2.08%)	1(1.61%0	0.861

The two areas have similar work force structure when it comes to feeding the animals and cleaning the animal shed

Table 4.7: Mode of Cleaning and Knowledge

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Mode of cleaning (remove)	135(93.75%)	58(93.54%)	0.956
Mode of cleaning (water only)	20(14%)	4(6.45%)	0.127
Mode of cleaning (water and soap)	4(3%)	4(6%)	0.211
Knowledge on antibiotic resistance	59(41%)	19(31%)	0.161
Knowledge on antibiotic residues	96(67%)	44(71%)	0.544
Knowledge on drug withdrawal	65(45%)	24(39%)	0.393
Knowledge on zoonosis	73(51%)	26(42%)	0.248
Knowledge on aflatoxin	32(22%)	14(23%)	0.955
Knowledge on pathogen in manure	39(27%)	18(29%)	0.744
No knowledge	37(26%)	23(37%)	0.099

The two areas also have the same modes of cleaning animal shed. Knowledge on different aspects of antimicrobials and pathogen does not vary significantly.

Table 4.8: Environment where Livestock Feed

Attribute	Korogocho n=144	Viwandani n=62	P value <0.05
Feed close to the dumpsite	34(24%)	284(45%)	0.02*
Feed next to open sewer	37(26%)	30(48%)	0.001*
Mix while feeding	43(30%)	45(73%)	0.000*
Had training	7(5%)	4(7%)	0.621
Feed contaminated by rodents	12(8%)	2(3%)	0.182
Livestock faeces contaminate feeds	134(93%)	53(85%)	0.085
Water contaminated (livestock faeces)	129(90%)	52(84%)	0.249
Access to clean water	97(67%)	27(44%)	0.001*
Draining to farms	16(11%)	5(8%)	0.507

Differences were seen on the environment where livestock feed. Significantly more animals feed close to the dumpsites and next to open sewer in Viwandani compared to Korogocho. Significantly more animals in Viwandani mix while feeding. Significantly more livestock access clean water in Korogocho compared to Viwandani.

The attributes that had a significant difference between the two study areas were, animal ownership (pigs, rabbits),scavenging, road as source of water, feeding next to the dumpsite, feeding next to the sewer, mixing while feeding and people who started keeping livestock between 2009 and 2014.

4.2 Prevalence of *E .coli* in livestock

The overall prevalence of *E. coli* was 49% (391/795). The isolation rate of *E. coli* in Korogocho was 50% (268/532) and 46.8% (123/263) in Viwandani. The species that took the biggest proportion was chicken followed by goats and this was proportionally allocated as detailed in

table 4.9. There was a significant difference in the percentage of *E. coli* isolated from rabbits in the two study areas.

Table 4.9 : Isolation of *E.coli* from different animal species in Viwandani and Korogocho informal settlements in Nairobi

Species	**Isolation rate of *E.coli*, [% (n)]**		**P= value**
	Korogocho	Viwandani	
Cattle	10(n=14) (71%)	6(n=12) (50%)	0.072
Goats	19(n=33) (58%)	20(n=43) (47%)	0.666
Pigs =	18(n=27) (67%)	10(n=18) (56%)	0.451
Poultry – Chickens	193(n=392) (49%)	83(n=167) (50%)	0.898
Ducks	9(n=27) (33%)	2(n=6) (33%)	1.000
Rabbits	8(n=12) (67%)	0(n=10)	0.001
Doves	6(n=18) (33%)	0(n=4)	0.172
Rodent	5(n=9)(56%)	2(n=3)67%	0.735

E. coli was isolated from all the livestock species that were sampled in Korogocho. There was no isolation of *E. coli* from doves and rabbits in Viwandani.

4.3 *E. coli* pathotypes Isolated in Livestock

Pathotyping was done on 18 different *E .coli* pathotype genes (table 5.0).Only six genes belonging to different *E. coli* pathotypes were identified. From the 206 households sampled, a total of 47 *E. coli* pathotypes were isolated (23%).The overall prevalence of *E. coli* pathotypes was n=795 (5.9%). EHEC had a prevalence of (2.4%), EPEC (1.76%), ETEC (HL) (0.75%), ETEC (HS) (0.13%), EAEC (0.50%) and DAEC (0.38%) (Table 4.10).

Table 4.10.:Pathotyping of the *E.coli* isolates from different Animal Species in Korogocho and Viwandani Informal Settlements in Nairobi

Site	Species (n)1171	ETEC		EHEC	EPEC	EAEC	DAEC
		Size 218bp(HL)	Size 147bp(HS)	Size 518bp	Size 918bp	Size 254bp	Size 750bp
Korogocho	Chicken n=809	1	1	5	3	1	3
	Cow n=56	0	0	1	0	0	0
	Dove n=29	0	0	0	0	0	0
	Duck n=22	0	0	0	1	0	0
	Goat n=133	0	0	7	3	0	0
	Pigs n=90	0	0	2	2	0	0
	Rabbits n=32	0	0	0	1	0	0
Viwandani	Chicken n=809	3	0	1	2	1	0
	Cow =56	0	0	0	0	1	0
	Dove =29	0	0	0	0	0	0
	Duck n=22	1	0	0	0	0	0
	Goat n=133	0	0	3	2	1	0
	Pigs n=90	1	0	0	0	0	0
	Rabbits n=32	0	0	0	0	0	0
		0.75	0.13%	2.4%	1.75%	0.50%	0.38%

4.4 Distribution of *Escherichia coli* pathotypes across livestock species

The table4.11 indicates the *E. coli* pathotypes isolated from livestock. Chicken recorded the highest proportion of pathotypes at45% (n=47) followed by goats 36 %. Doves and rabbits recorded zero *E. coli* pathotypes. In rodents, no pathotypes were identified**.**

Table 4.11: Distribution of *Escherichia coli* pathotypes across Livestock Species

SPECIES	EHEC	EPEC	ETEC (HL)	ETEC(HS)	EAEC	DAEC	Total
Chicken	6	5	4	1	2	3	21
Cow	1	0	0	0	1	0	2
Doves	0	0	0	0	0	0	0
Goats	10	5	0	0	1	0	17
Pigs	2	2	1	0	0	0	5
Rabbits	0	1	0	0	0	0	0
Ducks	0	1	1	0	0	0	2
Total	**19**	**14**	**6**	**1**	**4**	**3**	**47**

Out of the 47 pathotypes, 40% were EHEC, 30% EPEC, 13% ETEC (HL), 9% EAEC, 6 %DAEC, and 2% ETEC (HS).Only one pathotype of ETEC 147bp was identified and was from Viwandani (Table 4.11). Some results of PCR analysis are shown in figures 4.0 and 4.1

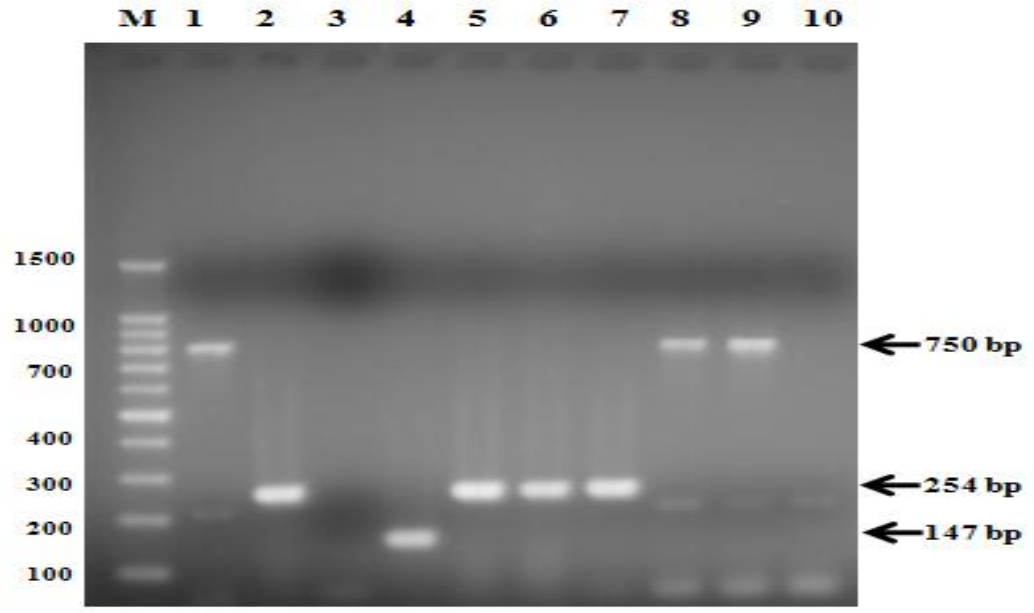

Figure 4.0 : PCR plate for DAEC,EAEC,ETEC HS

Key: M -Molecular marker (1 kb plus); lane 1 positive control DAEC 794 bp;lane 2 positive control EAEC 254 bp; lane 3 negative control; lane 4 ETEC 147 bp;lane 5,6,7 EAEC 254 bp;lane 8 and 9 DAEC 794 bp.

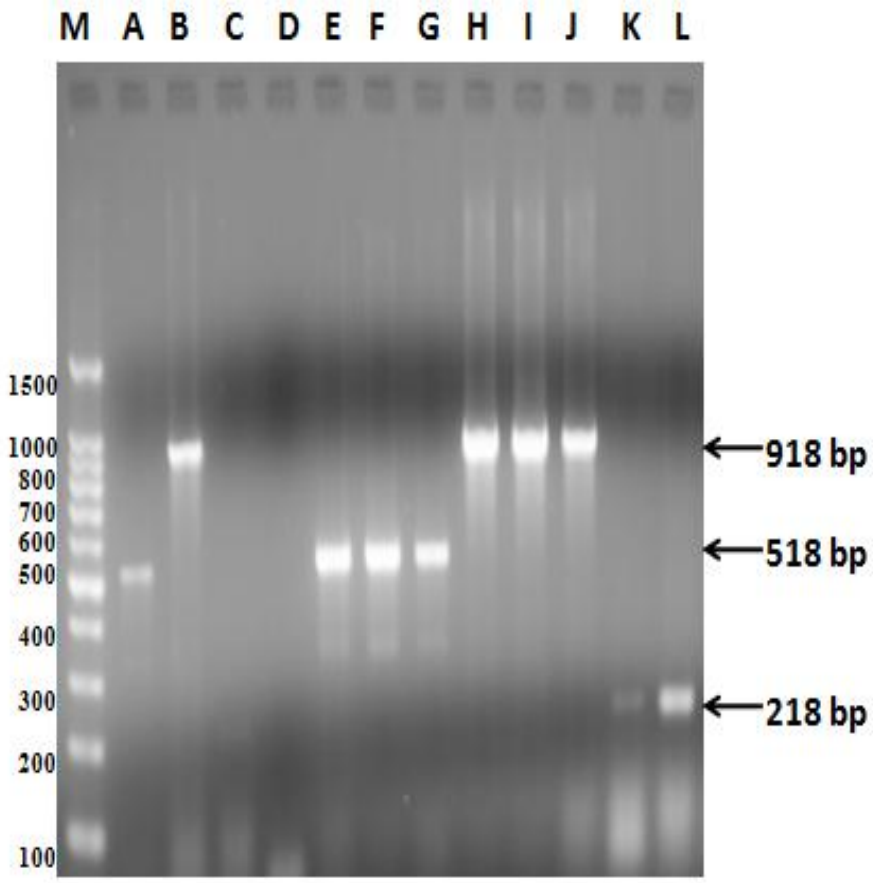

Figure 4.1:PCR plate for EHEC,EPEC,ETEC HL.

Key: M-molecular marker (1 kb plus); lane A positive EHEC 518 bp;lane B positive control EPEC 918 bp ;lane C positive control ETEC HL 218 bp; lane D negative control; lane E,F,G EHEC 518 bp; Lane H,I,J EPEC 918 bp; lane K and ETEC HL 218 bp.

4.4.1 DAEC Pathotypes

A PCR performed to detect the *afaB* genes encoding the DAEC phenotypes yield a specific band corresponding wit 794bp. For the samples analysed ,the presence of the PCR band indicated positive results(Figure 4.0). Two isolates tested positive and all were from chicken in Korogocho slums (figure 4.1)

4.4.2 ETEC Pathotypes

A PCR performed to detect the *estA* genes encoding the heat stable ETEC phenotypes yield a specific band corresponding with147bp and *eltA* gene encoding the heat labile ETEC phenotype yielding a specific band corresponding to 218bp. For the samples analysed,the presence of the PCR band indicated positive results(figure4.0).For ETEC heat stable only one chicken swab sample from Korogocho tested positive.(figure 4.1). For ETEC *eltA* (218 bp). Six isolates tested positive including, one isolate from chicken in Korogocho, 3 from chicken isolates in Viwandani,one isolate from a duck in viwandani, and one isolate from a pig in viwandani (table 4.10).

4..4.3 EAEC Pathotype

A PCR performed to detect the *AggR* genes encoding the EAEC phenotypes yield a specific band corresponding with254bp. For the samples analysed, the presence of the PCR band indicated positive results(Figure 4.0).Four samples tested positive, two from chicken isolates, onefromKorogocho and anotherfromViwandani. The other two were from a cow and a goat in viwandani(Table 4.1).

4.4.4 EHEC Pathotype

A PCR performed to detect the VT genes encoding the EHEC phenotypes yield a specific band corresponding with518bp. For the samples analysed,the presence of the PCR band indicated positive results (Figure 4.1). Nineteen isolates tested positive for *VT* genes, six of which were from chicken, five from different samples in korogocho,and the other from viwandani. Ten goat isolates tested positive, seven from Korogocho and three from Viwandani. In pigs two isolates

were positive and both were from Korogocho. One isolate from a cow in Korogocho tested positive (figure 4.10).

4.4.5 EPEC Pathotype

A PCR performed to detect the *eae* genes encoding the DAEC phenotypes yield a specific band corresponding with918bp and *bfpA* gene corresponding with 324bp specific band. For the samples analysed,the presence of the PCR band indicated positive results (figure 4.1). The EPEC isolated in this study was atypical EPEC lacking the *bfpA* gene and shigatoxin virulence factors, but having eae gene. Fourteen samples tested positive, five from chicken, three chicken samples in Korogocho and two in Viwandani. Five goat samples tested positive, three from Korogocho and two from viwandani. In Korogocho, two pig samples and one duck sample tested positive (Table 4.10 and 4.11).

4.5: Antimicrobial resistance patterns in E. coli isolated from livestock in Korogocho and Viwandani slums

Antimicrobial resistance is of great concern because resistance has spread across entire bacterial populations and ecological niches (Boerlin and Reid-smith, 2008). Antimicrobial resistance was done on single isolates. A total of 1171 isolates were tested for antimicrobial resistance to twelve antimicrobial agents as indicated in table 4.12

Table 4.12: Antimicrobial resistance for *E. coli* isolates from Livestock and Rodents

	Resistance across species for the different antibiotics	Drugs N= 1171 1solates AMC (1%)	AMP(35%	CAZ (3%)	CRO (3%)	CXM (2%)	NA (8%)	TE (56%)	CIP (1%)	SXT (53%)	CN (3%)	S (45%)	C (4%)
KOROGOCHO n=842 isolates	Chicken n=603	8	202	17	20	13	47	388	3	378	18	288	25
	Cow n=31	0	12	2	0	0	2	13	0	11	2	16	1
	Doves n=29	0	8	1	3	0	4	16	0	15	2	16	0
	Ducks n=17	0	4	1	0	1	2	4	0	6	0	6	0
	Goats n=64	0	12	4	1	0	6	17	0	13	1	24	11
	Pigs n=65	0	20	1	2	2	0	28	2	20	0	33	2
	Rabbits n=32	0	3	2	2	1	2	15	1	10	0	12	3
	Rodents n=60	0	7	1	4	1	0	7	0	9	0	12	4
VIWANDANI n=329 isolates	Chicken n=206	0	71	6	5	2	15	129	4	119	5	75	6
	Cow n=25	1	1	0	0	0	1	4	1	5	1	7	1
	Ducks n=5	0	5	0	0	0	0	2	0	4	0	2	0
	Goats n=69	0	18	2	4	6	4	27	0	28	0	34	0
	Pigs n=24	0	13	0	0	0	6	8	5	12	1	16	0
	Rodents n=24	0	1	0	1	1	0	10	0	0	0	1	0

KEY:

AMC=Amoxycillin/Clavulinicacid,AMP=Ampicillin,CAZ=Ceftazidine,CRO=Ceftriazone,CXM =Cefuroxime,NA=Naladixic acid

TE=Tetracycline, CIP=Ciprofloxcin, SXT=Trimethroprime/Sulfamethoxazole, CN=Genatmycin, C=chloramphenical, S=Streptomycin

The highest resistance was in tetracycline 56% followed by trimethoprime/sulfamethozazole at 53 % and Streptomycin 45 %. The lowest resistance was in ciprofloxacin and amoxicillin /clavulinic acid at 1%. All the cephalosporin indicated a consistency in resistance of between 2% and 3%.Tetracycline had the highest resistance of 56%; chicken contributing the highest had prevalence of 44% followed by goats at 3.8%. The lowest resistance was in ducks at 0.5 %. SXT had a resistance of 53%, and the highest herd resistance was recorded in chicken at 42.3%, followed by goats at 3.5% and the lowest in ducks at 0.8%. Sulphonamides had a resistance of 45% with the highest resistance in chicken at 30.8% followed by goats at 5% and ducks with the lowest at 0.6%.

On analyzing resistance across two areas on the three drugs with the highest resistance (TE,SXT,S), in Korogocho, the resistance pattern for TE, SXT and S was 64%,63% and 48 % respectively, while in Viwandani the resistance pattern was 63% ,58% and 34 % respectively. For goats the herd resistance for TE, SXT and S in Korogocho was 27%, 20 % and 38 % respectively. In Viwandani the resistance pattern was 39 %, 41% and 49% respectively. The resistance patterns in chickens was mainly noted on TE, SXT and S which were 1%, 5% 12% respectively. Generally, there was no significant difference between the two study areas on resistance to three antimicrobial agents TE and SXT and S. However, the difference was significant in isolates from goats.

4.6 Multiple drug resistance among *E. coli* isolates in livestock

This study has shown multiple resistance patterns to *E. coli* isolates. Many isolates showed resistance to three and more antibiotics (446) with a number of isolates showing resistance to 6, 7, 8 and 10 isolates as indicated in table 4.13.

Table 4.13.Multiple resistance among *E. coli* isolates

NO OF DRUGS	CHICKEN	COW	DOVES	DUCK	GOATS	PIGS	RABBITS	TOTAL NO OF ISOLATES
0	167	26	10	7	60	28	12	310
1	105	11	2	3	18	15	6	160
2	195	8	1	6	25	16	4	255
3	152	6	5	2	6	9	7	187
4	136	3	9	3	9	18	0	178
5	34	0	1	1	9	4	3	52
6	10	1	0	0	6	0	0	17
7	5	0	1	0	0	0	0	6
8	4	1	0	0	0	0	0	5
10	1	0	0	0	0	0	0	1
TOTALS	809	56	29	22	133	90	32	1171

Table 4.14 shows the multiple resistance patterns among E>coli isolates. The most common resistance pattern was AMP,TE,SXT,S ;TE,SXT;TE,SXT,S ;TE,S with 131,130,72,and 50 isolates respectively.

Table 4.14 Multiple resistance patterns among *E. coli* isolates

RESISTANCE PATTERN	CHICKEN	COW	DOVES	DUCK	GOATS	PIGS	RABBITS	TOTALS
AMP TE SXT S	101	2	5	1	8	13	0	131
TE SXT S	56	4	5	0	0	1	6	72
TE SXT	110	3	0	1	7	6	3	130
AMP TE S	3	1	0	1	0	4	0	9
TE S	39	0	0	0	8	3	0	50
AMP SXT S	12	1	0	1	3	2	0	19
AMP SXT	17	0	4	0	0	0	0	21
SXT S	11	1	0	1	1	3	0	17
AMP TE SXT S C	14	0	0	0	7	3	0	24
AMP TE SXT	63	0	1	0	1	0	0	65
AMP TE	5	1	0	0	0	0	1	7

4.7Prevalence of antimicrobial resistance among *E. coli* isolated from rodents

The highest prevalence of antimicrobial resistance among *E. coli* isolates from rodents was tetracycline (17%) followed by sulphonamide at 13% and AMP at 9%. The lowest resistance pattern was in ceftazidime at 1% and cefuroxime at 2%, amoxicillin/clavulinic acid, naladixic acid, ciprofloxacin, gentamycin showed zero resistance (table 4.15) indicating the resistance patterns in rodents.

Table 4.15: Resistance Patterns in Rodents

DRUGS	I	R	S	TOTAL	% Resistance
AMP	7	8	70	**85**	**9**
AMC	0	0	85	**85**	**0**
CAZ	1	1	83	**85**	**1**
CRO	0	5	80	**85**	**5**
CXM	2	2	81	**85**	**2**
NA	85	0	0	**85**	**0**
TE	0	17	68	**85**	**17**
CIP	0	0	85	**85**	**0**
SXT	0	9	76	**85**	**9**
CN	1	0	84	**85**	**0**
S	49	13	23	**85**	**13**
C	2	4	79	**85**	**4**

Table 4.16: Multiple resistance among *E. coli* isolates in Rodents

No of resistant drugs	No of isolates
0	54
1	23
2	3
3	2
4	1
5	1
6	1

Rodent have been shown in these study to have resistance to more than one antibiotic as shown in table 4.16 above

4.8 Risk factor analysis for *E. coli* pathotypes isolated from livestock

Univariate logistic regression analysis were done on 54variables based on the questionnaire administered (Table 4.16).Those that had a p-value ≤ 0.20(Jean et al., 2000)were included in the multivariate regression analysis table 4.17

Table 4.17: Univariate analysis for *Escherichia coli* Pathotypes

Variable	Odd ratio	Std err	Z	P>|z|	[95% Conf. Interval]
animal owner ship man	1.68097	0.6416	1.36	0,174	0.7956,3.5518
Keeping Poultry	0.40680	0.1767	-2.02	0.038	0.1736 ,0.9531
Keeping Cow	2.6298	1.517	1.68	0.094	0.8459,8.1466
Keeping Goats	2.5238	1.3389	1.75	0.081	0.8922,7.1385
Keeping Pig	3.2709	2.1533	1.80	0.072	0.9001,11.886
animals	0.5529	0.2115	-1.55	0.121	0.261,1.1702
Feeding on leftovers					
Feeding along roads	1.9028	0.7879	1.55	0.120	0.8452,4.2838
Feeding on bought forage	2.5134	1.5930	1.45	0.146	0.7257,8.7045
Feeding on other purchases	0.2976	0.2252	-1.60	0.109	0.6753,1.3115
Cleaning shed by removing	7.4666	4.778	3.14	0.002	2.129,26.177
Cleaning using soap	0.2391	0.2493	-1.37	0.170	0.031 ,1.5742
Treatment from agro vet	0.4313	0.2128	-1.70	0.088	0.1640 ,1.1342
Medicine used dewormer	0.3963	0.1538	-2.39	0.017	0 .1853;0.8478
Medicine used vitamin	0.4608	0.1791	-1.99	0.046	0.2151;0.9871
Floor drain into animal shed	0.3763	0.1522	-2.42	0.016	0.1703;0.8313

On univariate logistic regression some variables had a p-value <0.05. This included keeping poultry, cleaning shed by removing waste, use of de-wormer, multivitamins and floor draining into farm.

Table 4.18: Risk factor analysis for *Escherichia coli* pathotypes (Multivariate regression analysis)

Variable	Odd ratio	Std Err	Z	P>[Z]	(95% Conf. Interval]
animal owner ship man	1.2276	0.5620	0.45	0.654	0.5005;3.0112
Keeping Poultry	0.7002	0.4021	-0.62	0.535	0.2272;2.1579
Keeping Cow	0.9833	0.7204	-0.04	0.969	0.2269;4.1561
Keeping Goats	3.5981	1.9647	2.34	**0.019***	1.2339;10.4921
Keeping Pig	0.9833	0.7357	-0.02	0.982	0.2269;4.2611
animals Feeding on leftovers	0.8630	0.4124	-0.31	0.758	0.3383;2.017
Feeding along roads	1.2284	0.0526	0.24	0.810	0.2291;6.5878
Feeding on bought forage	0.9597	0.4982	-0.08	0.931	0.3469;2.6547
Feeding on other purchases	0.6395	0.2945	-0.97	0.332	0.2594;1.5769
Cleaning shed by removing	1.8524	1.6581	0.69	0.491	0.3205;10.7069
Cleaning using soap	0.1998	0,2050	-1.57	0.117	0.0267;1.4931
Treatment from agro vet	5.8120	4.6208	2.21	**0.027***	1.2234;27.6103
Medicine used dewormer	1.0110	0.5113	0.02	0.983	0.3752;2.7340
Medicine used vitamin	0.6852	0,3913	-0.66	0.508	0.2237;2.0982
Floor drain into animal shed	0.3468	0.3864	-0.95	0.342	0.0391;3.0798
H_2 O contaminated with feces	0.7498	0.4927	-0.44	0.661	0.2068;2.7185

On multivariate analysis a cut off p-value =0.05 was adopted(Jean *et al*., 2000). Only keeping goats and use of over the counter drugs were below <0.05 and had an odds ratio of 3.5981 and 5.8120. It means that goats are a risk in occurrence of *E. coli* pathotype in faeces and might be carriers of the same without causing disease. Having animals treated by agrovet attendants through farmers' explanation of livestock clinical signs and getting drugs over the counter are risk factors for occurrence of *E. coli* pathotypes in faeces

Table 4.19: Final model for Logistic Regression

This indicates the final regression equation

Variable	Odd ratio	Std Err	Z	P>[Z]	(95% Conf. Interval]
Goats	5.0797	2.1181	3.90	0.000	2.2433;11.5020
Agrovet treat	6.1205	4.2168	2.63	0.009	1.5861; 23.6172

The p values still show significance difference with a p value< 0.05. This indicates that the two factors remain risks in the occurrence of *E. coli* in faeces.

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CHAPTER FIVE

5.0 DISCUSION, CONCLUSIONS AND RECOMMENDATIONS

5.1Discussion

Characteristics of households owning livestock

This objective was achieved through the administration of a questionnaire to the household heads. Viwandani had significantly higher numbers of men who owned livestock than Korogocho with a (P = 0.017) (58%, 35%). Seventy four percent of the people who owned livestock owned land in Korogocho compared to 58% in Viwandani ($p < 0.05$). The reason is probably because Viwandani represents a more transient community, which attracts a youthful and highly mobile population, while Korogocho represents a stable poor urban community with a more settled population (Emina *et al*., 2011). This is supported also with the results on when a person started keeping livestock. Table 4.1 shows those who started keeping livestock between 2009 -2014. A significantly higher percentage of households started keeping livestock in Viwandani in more recent years compared to Korogocho. This indicates the importance of livestock in the livelihood of Viwandani residents.

Significantly higher number of animals feed next to a dumpsite and open sewer in Viwandani than Korogocho (P= 0.02 and 0.01respectively).More animals from different farmers mixed while feeding in Viwandani than Korogocho (p=0.000). This is important because environmental attributes like type of bedding, sanitation (which are part of household attributes), affect levels of bacterial pathogens in faeces(Hutchison *et al*., 2005; Fletcher, 2015). In addition, animals in Korogocho accessed clean water compared to Viwandani (67 %, 44% p 0.001). This was supported by the fact that most animals in Viwandani scavenged compared to Korogocho (p=

0.00). As they scavenge at the dump site and along open sewers, they accessed pools of water along the roads. Pigs were significantly higher in number in Viwandani than Korogocho (p= 0.018). This probably was because those people in Viwandani bordering the Ngong River to the south keep a lot of pigs. This is because pigs have a ready market, and require less capital to keep and can scavenge for food.

Occurrence of *E. coli* in livestock

The prevalence of *E .coli* in this study was 49% (391/795) in Korogocho indicating a prevalence of 34% (268/795) and Viwandani 15% (121/795). Prevalence at herd level was 49% (276/560) in chicken, 62% (16/26) in cattle, 29% (8/28 in doves), 33% (11/33) in ducks, 62% (28/45) in pigs and 36 % (8/22) in rabbits. In a study done among working donkey in Nigeria, out of the 326 bacterial isolates,203 (61.7%) were *E .coli* (Jedial *et al.,* 2015*).*This is comparable to this study as cows and pigs had a prevalence of 62%. In Nigeria, maize straws were a positive predictor for occurrence of *E. coli* in donkey faeces. This means that the type of food given to livestock in Korogocho and Viwandani should be examined. In a study on diarrheic calves in Iran, out of the 826 diarrheic faecal samples collected, 76.45% were positive for *E. coli* (Shahrani *et al*., 2014). This was higher compared to the current study probably because in the current study samples were collected from apparently healthy animals.

Adelaide *et a*l., (2008) in a study on broiler chicken slaughtered at Tigoni processing in Limuru reported the prevalence of *E. coli* ranging from 27% to 31%. This was slightly lower compared to the prevalence of *E. coli* in chicken in Korogocho and Viwandani slums which was 49% and 50% respectively. The difference is probably because of the different set ups. Korogocho and

Viwandani is heavily populated slum and chicken in these areas scavenge for food. Broiler set up is more enclosed with biosecurity being of utmost importance. This study showed the potential of contamination of the area by diarrhea causing pathogens from livestock faeces as the level of environmental hygiene is poor.

Escherichia coli pathotypes in livestock

Enterotoxigenic *E. coli*

ETEC is an important and global cause of severe and watery diarrhea in the offspring of some animals species such as new born (suckling) calves and suckling and weaned pigs(Nagy and Fekete, 2005). It is very rare or almost nonexistent in other important farm animals like rabbits, horses and poultry according to (Dubreuil and Schifferli, 2016),but this study has indicated poultry to be an important carrier of ETEC. The overall prevalence of ETEC was found to be (0.88%), ETEC (HL) (0.075%) and ETEC (HS) (0.013%). Out of the seven ETEC isolates, five were from chicken, one from duck and one from pigs. In a study done in pigs in Argentina (Cicuta *et al*., 2000), 31/127 piglets with diarrhea were positive for heat stable (*ST*) and heat liable (*LT*)*E. coli* strains. In this study, only one pig tested positive for ETEC. This could be attributed to the difference in sample size and the fact that all the 45 pigs tested were apparently healthy and not diarrheic as was the case in the study by Cicuta *et al*. (2000). ETEC are responsible for an estimated 300,000-500,000 deaths annually in children under age of five(Fleckenstein *et al*., 2010).

This study was able to detect *E. coli* from livestock using primers adopted from *E. coli* isolated from a human study. It shows livestock especially poultry pose a high risk for occurrence of ETEC outbreaks in human and therefore hygiene should be observed after handling livestock.

Enteropathogenic *E. coli*

In this study, EPEC was analyzed using a primer that encoded for EPEC adherence factor (EAF) that is mediated by bundle forming pilus (*bfpA*) (Typical EPEC). All the positive samples were atypical EPEC. They lacked the (*bfpA*) but had the *eae* gene. None of the isolates was positive for bundle forming pilus (*bfpA*).Typical EPEC have been indicated in studies to be very rarely isolated from cattle (Wani *et al*., 2007).This was similar to the current study where zero typical EPEC pathotypes were isolated. In a study done in Australia, bovine feces were shown to harbortypical EPEC with a prevalence of 14.1%, which is far higher compared to the 2.4% prevalence found in this study. The difference could be because the current study was non selective and collected samples from apparently healthy animals but the study in Australia collected samples from Bovine with gastrointestinal infection. A study done by Wani *et al.* (2003)on diarrhea in calves found all EPEC isolated to be atypical as they lacked EAF plasmid or *bfpA* gene but had *eae* gene. Most studies on EPEC focused on calves and piglets with diarrhea (Dubreuil and Schifferli, 2016), but other species can be a risk of carrying EPEC. In two other studies, one in Ecuador (Vasco and Graham, 2016) and the other in Mexico(Jay-Russell et al., 2014), pigs, dogs and chicken were shown to be the source of human infections for atypical EPEC. In this study, atypical EPEC were shown to occur in chicken, goats, pigs and duck isolates. Out of the 14 EPEC isolates, six were from chicken, five from goats, two from pigs and one from ducks. The findings of a study done in Spain by Fuente *et al*., (2005)support the results of the current study that healthy goats are carriers of EPEC. These authors isolated EPEC from 7.7% of 222 fecal samples drawn from healthy dairy goats. The current study had an isolation rate of 2% (14/795) with a prevalence of 5/81(6.17%) in goats. Birds have also been shown to be a reservoir of atypical enteropathogenic *E. coli* (Kobayashi *et al,* 2009). In a study done in wild

birds in Tokyo Bay. *Stx*- and *eae*- positive strains of *Escherichia coli* were found in only 5% (113/447) of the birds. Their findings do not compare well with those in this study where almost half of the pathotypes isolated were from chicken. EPEC is a recognized cause of diarrhea, particularly in children in less developed countries(Tennant *et al*., 2009). An example is a study where 50 *Escherichia coli* in Libyan hospitals (20 from children with diarrhea and 30 from healthy children) were investigated and nine were positive for EPEC (Malik et *al.*,2006). The results of this study indicate that most livestock species are important carriers of EPEC and people and especially mothers having children less than five years should observe hygiene after handling livestock.

Shiga toxin *E. coli*

Naylor *et al*.(2005) traced the origin of EHEC strains that caused human outbreaks to ruminant gastro-intestinal tract. In this study, out of the 47 *E. coli* pathotypes isolated nineteen (19) were EHEC from healthy animals. Of these, 11 isolates were from goats, 5 from chickens, two from pigs and one from cattle with a prevalence of 5.9%. A study done by Arimi *et al,* (2005) on unpasteurized milk found the prevalence of *E. coli* 0157:H7 to be 0.08%. Kang'ethe *et al* (2007) reported an EHEC 0157:H7 prevalence of 2.5% in milk and 5.2% in pooled cattle faeces. In this study, goats had the highest prevalence of EHEC. This indicates that livestock are important as reservoirs of EHEC strains and contamination of the environment with animal faeces can lead to outbreaks of pathogenic *E. coli* in humans. EHEC rarely cause disease in animals and these are recognized as their main natural reservoir. This observation was supported by the findings of Apriolia *et al.*,(2005) which indicated that chicken are important carriers of STEC.

Enteroaggregative *E .coli* (EAEC)

EAEC is an emerging Enteric pathogen (Dupont, 2004)that causes an acute diarrheal illness among children in both developing and developed regions. In Kenya, no study has been done on EAEC in livestock. However, this study isolated EAEC from 4 samples giving a prevalence of 0.50%. In an experimental study using piglets, Two strains of EAEC of human origin fed to gnotobiotic pigs caused diarrhea and death on a majority of them (Tzipori *et al.,* 1992).This indicates that although not documented in livestock, EAEC can cause gastrointestinal lesions in livestock. Further analysis of *E. coli* isolates from this study is required to determine if they relate to isolates in human. This is because the use of primers adopted from research done on human studies were used in this study to detect *E. coli* pathotypes indicating that human isolates may be related to these livestock isolates and thus livestock can be reservoirs of EAEC which cause infection in human.

DAEC (Diffusely adherent *E. coli*)

Diffuse adherent *E. coli* has been associated with the persistent watery diarrhea in children(Le and Servin, 2006). No previous study has been done on DAEC in livestock or rodents. This study isolated three DAEC isolates from chicken. Further analysis should be done to identify if there is any relationship with human pathotypes and this will determine if livestock is an important reservoir for human DAEC outbreaks.

A study done on children with diarrhea in four hospitals in Kenya namely Malindi,Alupe,New Nyanza and Malindi (Sang *et al.*, 2012) found the prevalenceof *E. coli* pathotypes as follows: Enteroaggregative 8.9%, enterotoxigenic 1.2%, enteroinvasive 0.6%, shigatoxigenic 0.5% and

DAEC 0.38%. Livestock therefore pose a potential risk for human infection. Model experiments should be done in animals to see if DAEC causes any gastrointestinal lesions in animals.

Antimicrobial resistance pattern in livestock

Studies done in Kenya, one at Tigoni poultry processing plant in Limuru Kenya found resistance to tetracycline to be 75.9%,cotrimazole 72.4% and ampicillin 39%(Adelaide *et al.,* 2008). The trend was similar in this study which had 56% resistance to tetracycline, 53% to, trimethoprime/sulphamethoxazole and 32% to ampicillin. The difference in prevalence was probably because, in commercial farms antibiotics are used a lot to control chronic respiratory diseases and coccidiosis. A study in Uganda, on broiler chicken indicated a high resistance of *E. coli* isolates to Ampicillin, tetracycline and cotrimazole (Majalija *et al*., 2010). In Tanzania a study on indigenous chicken indicated high resistance in tetracycline 75%, ampicillin 63.3%, and cotrimazoze 53% (Hamisi *et al*., 2014). In yet another study done on a variety of livestock species in Kenya, resistance was highest in chicken isolates and most resistance was to Ampicillin, tetracycline and sulphamethoxazole/trimethoprime (Kikuvi *et al.,* 2004). The results of these studies indicate that the three drugs have developed high resistance across the three countries. There is need to implement measures which guard against misuse of antimicrobial drugs in livestock production in order to safeguard other classes of antibiotics. It is important to minimize the emergence and dissemination of antibiotic resistant clones to humans, either directly from livestock or indirectly through the environment. Hence, proper hygiene procedures and adherence to correct prescriptions need to be reinforced.

Although the prevalence of cephalosporin's resistance was at 2%-3% in the present study, it is a cause of a concern since cephalosporins are not being used at all in livestock in Korogocho and Viwandani slum. This resistance could be attributed to environmental contamination from their use in humans. The use of these antibiotics in humans could be the contributing factor to the occurrence of resistance to cephalosporin in human pathogens, which are disseminated to animals through human waste due to lack of sufficient toilets and sewage systems.

Occurrence of *E. coli* in Rats

Eighty five *E. coli* were isolated from 10 of the 13 rats examined.The isolates were mainly resistant totetracycline,with aprevalence of 17% followed by Streptomycin at 13% and Ampcillin at 9%. The lowest resistance pattern was in ceftazidimeat 1% and cefuroxime at 2%, amoxicillin/clavulinic acid, naladixic acid, ciprofloxacin, gentamycin showed zero resistance.A studyby Gakuya *et al.* (2001) in Kabete, Kibera and Kawangwarefound the resistance of *E, coli* isolated from rats to be ampicillin (20%), co-amoxyclav (1.9%), streptomycin (13.3%), sulphamethoxazole(25%), tetracycline (3.3%),trimethoprim

(3%),cefuroxime,ceftazidime,nalidixic acid, gentamycin and ciprofloxacin at (0%)

Major differences between the study by Gakuya *et al*. (2001) and this one were seen in ampicillin and tetracycline. This could be due to sample size and environmental differences.. Gakuya*et al* (2001). had a bigger sample size of 60 while the current study managed to trap thirteen rats.In another study done in Berlin Germany(Guenther *et al.*, 2012b), faecal samples from 87 brown rats yielded *E. coli* that showed phenotypic resistance to at least three antimicrobial classes and also produced shiga toxins.. No *E. coli* pathotypes were isolated in these study and these could be due to the small number of rats trapped. In a study done in

Trinidad and Tobago (Nkogwe *et al*., 2011), 204 rats were sampled for isolation of *E. coli* and the prevalence was 83.8%. This was similar to this study having a prevalence of 80%, although the sample size was small. In a study in Germany, fecal samples confirmed urban rats as reservoirs of Extended-Spectrum –β-Lactamase(ESBL) producing *Escherichia coli* (Guenther *et al*., 2013). Resistance was indicated in penicillin and cephalosporins phenotypically, which are associated with ESBL resistance genotypically .Although the sample size was small; this study indicated that rodents are an important vehicle for transmitting genes responsible for resistance to the human population. Therefore, measures should be put in place to control misuse of drugs during management of human and animals diseases that later contaminate the environment.

Risk factor analysis for occurrence of *E. coli* pathotypes in livestock

Logistic regression was done to determine the risk factors associated with occurrence of *E. coli*pathotypes. Many variables (indicated in appendix 3) were included. Only goats and farmers accessing antibiotics from agrovet were significantly associated with pathogenic *E. coli.*

Herd prevalence of *E. coli* pathotype in chicken was 5% (21/393), while goats had a herd prevalence of 42 %(16/38). Goats had an odds ratio of 5.0797 and this was >1, indicating that goats are a risk. In this study, EHEC was the most prevalent pathotype. Ruminants have been shown to be reservoirs of pathogenic *E. coli*. This supports the significant association of goats to *E. coli* pathotypes. In addition, most goats scavenge in dumpsites and open sewer. In Viwandani, samples were taken from a dumpsite to the upper Ngong River. In Korogocho, samples were taken from goats grazing along the roads. There was no significant difference in occurrence of *E.coli* with a p-value of 0.367. The *E. coli* pathotypes identified were probably acquired from these polluted environments as environmental factors contribute to resistance and shedding of

bacterial pathogens in feces (Fletcher, 2015). Therefore, household members owning goats should maintain proper hygiene.

Farmers accessing antibiotics through agrovet shop services had an odd ratio of 6:12 indicating a risk to acquisition of *E. coli* pathotypes. Due to misuse of over the counter drugs there is development of resistance over time. This leads to acquisition of resistant *E. coli*. Studies have been done on risk factors associated with occurrence of *Escherichia coli* in herds of animals, environment and management have been shown to play a major role. In a study done on veal herds on occurrence of VTEC 0157, ventilation and presence of dogs were significantly associated with shedding of these pathogens (Berends*et al*.,2008).

5.2 Conclusions

1. The *E.coli* pathotypes isolated in this study were, EHEC, DAEC, EAEC, ETEC (HL), ETEC (HS) and EPEC with EHEC and EPEC as the predominant pathotypes.
2. Livestock and rats are reservoirs of pathogenic *E. coli* with a potential to transmit resistant *E. coli* pathogens to humans.
3. *E. coli* isolates from livestock had the highest prevalence of resistance to tetracycline (56%), followed by trimethoprim/sulfamethoxazole(53%) and streptomycin (45%) while amoxicillin, clavulinic acid and ciprofloxacin had the lowest resistance of 1% each
4. Tetracycline and streptomycin showed the highest resistance among *E. coli* isolated from rodents
5. Goats and over the counter sale of antibiotics to farmers were risk factors for occurrence of *E. coli* pathotypes.

6. Doves, rabbits, rodents and ducks may be important in the dissemination of antibiotic resistant *E .coli* strains which they pick from the slum contaminated environment.

5.3 Recommendations

1. There is need to create awareness of the importance of prudent use of antibiotics and observation of withdrawal periods following antibiotic use, proper cooking of meat and milk and general handling of livestock, and on proper treatment of sick animals to eliminate *E. coli* from animal population.
2. There is need to compare isolates from this study with those isolated from human to ascertain if they are related in order to determine the role of livestock in human disease.
3. Carry out genotypic resistance studies to check for presence of genes responsible for resistance and create a phylogenetic tree to check for any relationships.
4. Further PCR analysis should be done on EHEC isolates from this study to get the proportions of stx1 and stx2 shiga-toxin producing EHEC strain

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APPENDICES

Appendix 1: Questionnaire

To be filled after explaining the project to the participant and after he or she has signed the consent form for the interview

A. Questionnaire information

1.	ID given by the project	
2.	Area of the farm	
3.	Date of questionnaire	
4.	GIS location	
5.	Name of interviewer/enumerator	

B. characteristics of households

		<25 years	25-29 years	30-40 years	>40 years
6.	What is your age range?				

7. At what is your level of education?

None	☐	Secondary School	☐	University level	☐
Primary level	☐	College level	☐	Other: …..	☐

8. Place/Area of where you live, if different? ☐

9. How many people you support financially and who are not employed (like family)? ☐

10. How many people do you employ And are not family? ☐

11. Do you own or rent your house or Accommodation? ☐

14. How many of the following animals do you have?

	Now	Minimum	Maximum
Dairy cows			
Dairy goats			
Beef			
Sheep			
Other Goats			
Broiler			
Layers			
Indigenous			
Quails			
Duck/geese			
Pigs (sows)			
Pigs (growers)			
Rabbits			
Dogs			
Cats			
Ducks			
Geese			
Other….			

Assessing the risk

26. What are the most common sources of feed for your animals?

	Indicate spp	How much do you buy per week?	Dumping site?	Next to open sewer?	Mix with other animals while eating?
Scavenging					
Households leftovers					
Grazing on the roadsides					
Grazing on a private land					
Swirl from hotels					
Bring forage/hay into farm					
Purchase feed Agro vet					
Purchase feed other (_____)					
Feed from silage					
Other					

27. What are the most common sources of water for your animals?

	Indicate spp	How much do you have to buy per week?	Are there other animals (not yours) drinking from the same source? Spp
Tap water			
River			
Open sewer			
Swamp			
Household waste water (from_____)			
Surface water on roads			
Water vendors			
Borehole			
Rain water store in tanks			
Common communal watering points			
Other: ……..			

34. Who treats your animals?

	Sick animals (indicate spp and location)	Source of medicines	Type
Owner			
Worker / Stockman			
Animal health technician			
Herbalist			
Qualified Veterinary doctor			
An experienced farmer			
Government services			
Private clinics (_____)			
Call Agro vet people			
Other:_____			

Type: Antiobiotic, dewormers, multivitamin, herbal medicine, don't know, other

35. Do you give any vaccines to your animals?

	Indicate spp	Who provide you with it?
None		

36. Do you give any antimicrobial for prevention or production purpose?

	Indicate spp	Who provide you with it?
None		

38. How is the cleaning done for the areas where animal live?

	indicate spp	Frequency (per week)
Remove the slurry/ or sweep floor		
Clean with water only		
Clean with soap and water		
Use disinfectant		
All-in all-out (time ____days)		

39. Do you have any training on?

	Yes/No	Last time?	Who provided?
Food safety			
Animal disease management			
Animal management in general			

40. Have you heard of?

	Yes/No
Antimicrobial resistance	
Antimicrobial residues	
Disease passing from animals to humans	
Pathogens in manure	
Aflatoxins	

Thanks for your participation

If you don't mind we would like to contact you by phone in the future to clarify some questions we may have. We will appreciate if you can give us your telephone contact.

Contact details Tel: _____

APPENDIX 2: Sample Size Determination

Proportional allocation of the animals to be sampled will then be done as follows;

Table 1: **shows the total number of households in each category and their proportions in Korogocho and Viwandani.**

CATEGORIES	H.H VIWA	Proportions	H.H KOCH	Proportions	TOTAL H.H	PROPORTIONS
1-3 cattle	26	0.077381	23	0.10798122	49	0.089416
4-6 cattle	4	0.0119048	7	0.03286385	11	0.020073
7-10 cattle	3	0.0089286	3	0.01408451	6	0.010949
1-3 goats	28	0.0833333	8	0.03755869	36	0.065693
4-10 goats	29	0.0863095	17	0.07981221	46	0.083942
11-20 goats	0	0	3	0.01408451	3	0.005474
21-39 goats	1	0.0029762	0	0	1	0.001825
1-5 chickens	118	0.3511905	25	0.11737089	143	0.260949
6-10 chickens	62	0.1845238	32	0.15023474	94	0.171533
11-50 chickens	14	0.0416667	21	0.09859155	35	0.063869
51-200 chickens	2	0.0059524	2	0.00938967	4	0.007299
1-10 Other	20	0.0595238	8	0.03755869	28	0.051095
11-20 Other	2	0.0059524	4	0.01877934	6	0.010949
20-30 Other	1	0.0029762	0	0	1	0.001825
1-5pigs	10	0.0297619	0	0	10	0.018248
6-15 pigs	13	0.0386905	60	0.28169014	73	0.133212
15-35 pigs	2	0.0059524	0	0	2	0.00365
Total livestock keepers	335		213		548	

Table 2: **shows the distribution the 750 samples.**

No hh	No hh	Animals(multiplying values in last column with values in second column)	categories	Maximum no of animal	Max no of animals ×0.33 to nearest whole number
6.706204	7	7	1-3 cattle	3	1
1.505474	2	4	4-6 cattle	6	2
0.821168	1	3	7-10 cattle	10	3
4.927007	5	5	1-3 goats	3	1
6.29562	6	18	4-10 goats	10	3
0.410584	1	7	11-20 goats	20	7
0.136861	1	10	21-39 goats	39	10
19.57117	20	40	1-5 chickens	5	2
12.86496	13	39	6-10 chickens	10	3
4.790146	5	50	11-50 chickens	50	10
0.547445	1	10	51-200 chickens	200	10
3.832117	4	12	1-10 Other	10	3
0.821168	1	7	11-20 Other	20	7
0.136861	1	10	20-30 Other	30	10
1.368613	1	2	1-5pigs	5	2
9.990876	10	50	6-15 pigs	15	5
0.273723	1	10	15-37 pigs	37	10
Totals 75	80	284			

A total of 750 animals will be sampled, one sample per animal. A minimum of 75 households will be sampled since a maximum of ten and a minimum of one animal will be sampled per household (750/10)

33% of households per category will be sampled but a minimum of 1 and a maximum of 10.

The first column shows the number of household per category. This has been arrived at by multiplying the proportions per each category from data on last column of table one by the total number of households (75).

Column two (2) is the number of households to the nearest whole number.

Column three is the total number of animals per category. This is achieved in two steps. step one by multiplying 33%(0.33) by the maximum number of animals per category ,if the value is more than 10,the value 10 is used for step two. Step two is multiplying the values in step 1 by the number of households per each category.

The number of animals/samples are 284 but the total number of samples needed are 750,these means the extra 466 animals needed (750-284),46.6 households=466/10 , must be distributed based on the proportions of last column of table two (1).

Table 3: Distribution of the extra 46.6 households

Extra HH	No hh	Animals(multiplying values in last column with values in second column)	categories	Max no of animals ×0.33 to nearest whole number
4.166788	4	4	1-3 cattle	1
0.935401	1	2	4-6 cattle	2
0.510219	1	3	7-10 cattle	3
3.061314	3	3	1-3 goats	1
3.911679	4	12	4-10 goats	3
0.255109	1	7	11-20 goats	7
0.085036	0	0	21-39 goats	10
12.16022	12	24	1-5 chickens	2
7.993431	8	24	6-10 chickens	3
2.976277	3	30	11-50 chickens	10
0.340146	1	10	51-200 chickens	10
2.381022	2	6	1-10 Other	3
0.510219	1	7	11-20 Other	7
0.085036	0	0	20-30 Other	10
0.850365	1	2	1-5pigs	2
6.207664	6	30	6-15 pigs	5
0.170073	1	10	15-37 pigs	10
TOTAL	49	174		

Column one shows the distribution of the extra 26 households.

Column two shows the household distribution to each category to the nearest whole number.

Column three is the total number of animals per category. This is achieved in two steps. Step one by multiplying 33 %(0.33) by the maximum number of animals per category, if the value is

more than 10, the value 10 is taken as the maximum. Step two achieved by multiplying the values in step 1 by the number of households per each category.

The number of animals/samples are 174 but the total number of samples needed in table two (2) were 466,these means the extra 292 animals needed (466-292),29 households=292/10 , must be distributed based on the proportions of last column of table one (1).

Table 4**: Distribution of the extra 29 house holds**

Extra HH	No hh	Animals(multiplying values in last column with values in second column)	categories	Max no of animals ×0.33 to nearest whole number
2.610949	3	3	1-3 cattle	1
0.586131	1	2	4-6 cattle	2
0.319708	1	3	7-10 cattle	3
1.918248	2	2	1-3 goats	1
2.451095	2	6	4-10 goats	3
0.159854	1	7	11-20 goats	7
0.053285	0	0	21-39 goats	10
7.619708	8	16	1-5 chickens	2
5.008759	5	15	6-10 chickens	3
1.864964	2	20	11-50 chickens	10
0.213139	1	10	51-200 chickens	10
1.491971	1	3	1-10 Other	3
0.319708	1	7	11-20 Other	7
0.053285	0	0	20-30 Other	10
0.532847	1	2	1-5pigs	2
3.889781	4	20	6-15 pigs	5
0.106569	1	10	15-37 pigs	10
TOTAL	34	126		

Column one shows the distribution of the extra 29 households.

Column two shows the household distribution to each category to the nearest whole number.

Column three is the total number of animals per category. This is achieved in two steps. Step one by multiplying 33 %(0.33) by the maximum number of animals per category, if the value is more than 10, the value 10 is taken as the maximum. Step two achieved by multiplying the values in step 1 by the number of households per each category.

The number of animals/samples are 126 but the total number of samples needed in table three (3) were 292, these means the extra animals needed (292-126), 16.6 households=166/10 , must be distributed based on the proportions of last column of table one (1)

Table 5: Distribution of the extra 16.6 house holds

Extra HH	No hh	Animals(multiplying values in last column with values in second column)	categories	Max no of animals ×0.33 to nearest whole number
1.484307	2	2	1-3 cattle	1
0.333212	1	2	4-6 cattle	2
0.181752	1	3	7-10 cattle	3
1.090511	1	1	1-3 goats	1
1.393431	2	6	4-10 goats	3
0.090876	0	0	11-20 goats	7
0.030292	0	0	21-39 goats	10
4.331752	5	10	1-5 chickens	2
2.847445	3	9	6-10 chickens	3
1.060219	1	10	11-50 chickens	10
0.121168	0	0	51-200 chickens	10
0.848175	1	3	1-10 Other	3
0.181752	1	7	11-20 Other	7
0.181752	0	0	20-30 Other	10
0.30292	1	2	1-5pigs	2
2.211314	3	15	6-15 pigs	5
0.060584	0	0	15-37 pigs	10
TOTAL	22	70		

Column one shows the distribution of the extra 16 households.

Column two shows the household distribution to each category to the nearest whole number.

Column three is the total number of animals per category. This is achieved in two steps. Step one by multiplying 33 %(0.33) by the maximum number of animals per category, if the value is more than 10,the value 10 is taken as the maximum. Step two achieved by multiplying the values in step 1 by the number of households per each category.

The number of animals/samples are 70 but the total number of samples needed in table three (4) were 126 these means the extra animals needed (126-70), 9.6 households=96/10, must be distributed based on the proportions of last column of table one (1).

Table 6: Distribution of the extra final 9.6 households

Extra HH	No hh	Animals(multiplying values in last column with values in second column)	categories	Max no of animals ×0.33 to nearest whole number
0.858394	2	1	1-3 cattle	1
0.192701	1	2	4-6 cattle	2
0.105109	1	3	7-10 cattle	3
0.630657	1	1	1-3 goats	1
0.805839	2	9	4-10 goats	3
0.052555	0	0	11-20 goats	7

0.017518	0	0	21-39 goats	10
2.505109	5	6	1-5 chickens	2
1.646715	3	6	6-10 chickens	3
0.613139	1	40	11-50 chickens	10
0.070073	0	0	51-200 chickens	10
0.490511	1	9	1-10 Other	3
0.105109	1	5	11-20 Other	7
0.017518	0	1	20-30 Other	10
0.175182	1	2	1-5pigs	2
1.278832	3	10	6-15 pigs	5
0.035036	0	0	15-37 pigs	10
TOTAL	22	95		

Table 7: Shows the distribution of households to be sampled in each category at Korogocho and Viwandani.

Total h.h	Categories	Korogocho	No HH	Viwandani
17	1-3 cattle	12.41	12	5
6	4-6 cattle	4.38	4	2
5	7-10 cattle	3.65	4	1
12	1-3 goats	8.76	9	3
17	4-10 goats	12.41	12	5
3	11-20 goats	2.19	2	1
1	21-39 goats	0.73	1	0

48	1-5 chickens	35.04	35	13
31	6-10 chickens	22.63	23	8
15	11-50 chickens	10.95	11	4
3	51-200 chickens	2.19	2	1
11	1-10 Other	8.03	8	3
5	11-20 Other	3.65	4	1
1	20-30 Other	0.73	1	0
5	1-5pigs	3.65	4	1
25	6-15 pigs	18.25	18	7
3	15-37 pigs	2.19	2	1
Totals 208			152	56

The proportion of households in Viwandani and Korogocho is 27% (96H.H) and 73% (249).

Column one (1) shows the total number of households per each category to be sampled.

Column two (2) shows the number of households to be sampled per each category in Korogocho. This has been achieved by multiplying the total households per category in column one (1) by the percentage households (73%).

The last column shows the number of households to be samples In Viwandani. Achieved subtracting total number of households per each category by the number of households in Korogocho

Appendix 3: Univariate Analysis Results

Variable	Odd ratio	Std err	Z	P>|z|	[95% Conf. Interval]
Poultry	.3287671	.1413618	-2.59	0.010	.1415444 , .7636319
Rabbits	.483871	.3713265	-0.95	0.344	.1075253 , 2.177451
Dogs	.718894	.5615188	-0.42	0.673	.1555258 ,3.322977
Cats	1.114286	.7429074	0.16	0.871	.3016411 ,4.116258
Cow	2	1.037425	1.34	0.181	.7236039, 5.527886
Goats	4.764113	1.910256	3.89	0.000	2.171089 ,10.45409
Pigs	1.989796	1.114202	1.23	0.219	.6640054 , 5.962734
Secondary	1.105072	.458982	0.24	0.810	.4896117 ,2.494191
Primary	1.017391	.3886279	0.05	0.964	.4812182 , 2.150968
Ne	1.201299	.6446012	0.34	0.733	.4196694, 3.438703
Removeclean	.5015723	.3098729	-1.12	0.264	.1494372 , 1.683482
Waterclean	.8815592	.5100584	-0.22	0.828	.2836336 ,2.739967
Soapclean	4.15	3.274795	1.80	0.071	.8838099 ,19.48666
Scavenging	.8412698	.3382597	-0.43	0.667	.3825448 ,1.850071
Hhleftovefood	.5976431	.2286778	-1.35	0.179	.2823218 ,1.265142
Grroadfeed	4.524138	3.168767	2.16	0.031	1.146418 ,17.85372
Swirlhotelfeed	.9786535	.5696514	-0.04	0.970	.3127263 ,3.062623
Foragebroughtfeed	2.464286	1.014402	2.19	0.028	1.099769, 5.521801
Agrovetfeed	.7947368	.518666	-0.35	0.725	.2211602,2.855878
Purchaseotherfeed	.4721311	.1836173	-1.93	0.054	.220303 ,1.011824
Doesdrainageotherhh	.2483553	.2593499	-1.33	0.182	.0320767 ,1.922904
Isfeedprorodent	.8494624	.6699025	-0.21	0.836	.1810798 , 3.984907
Iswaterfromclean source	1.300971	.5220084	0.66	0.512	.5925527 2.856328
Fecalcontoffeed	1.722222	1.331375	0.70	0.482	.3784968 ,7.836392
Fecalcontofwater	.4398496	.2170258	-1.66	0.096	.1672298 ,1.156898

Feedstoreclean	.3136819	.2376195	-1.53	0.126	.0710705 ,1.384489
Feedingnexttodumpsite	1.452381	.5809205	0.93	0.351	.6631604,3.180845
Feedingnexttoopensewer	1.475	.5800116	0.99	0.323	.6824574, 3.187928
mixwithotherwhilefeeding	1.283531	.4961928	0.65	0.518	.6016523,2.738214
k0wledge	.6885246	.2753947	-0.93	0.351	.3143819 ,1.507931
Ownermed	.5618467	.2907828	-1.11	0.265	.2037417 ,1.549372
Vetmed	.7791563	.6113029	-0.32	0.750	.1674161 , 3.626203
Farmermed	1.739583	2.036554	0.47	0.636	.1753606 ,17.25673
Ownertreat	.9609375	.4285279	-0.09	0.929	.4009625 ,2.302961
Vettreat	1.655172	1.003826	0.83	0.406	.5042059 ,5.433486
Agrovettreat	7.288889	4.666035	3.10	0.002	2.078541 ,25.56019
Korogochoa	.3774038	.3984324	-0.92	0.356	.0476625 ,2.988381
Korogochob	1.99373	1.231733	1.12	0.264	.594007 ,6.691775
Groganb	1.327586	.7893014	0.48	0.634	.4139906 ,4.257307
Lungalunga	1.164751	.6857356	0.26	0.796	.367364 ,3.692917
Highridge	.6083333	.3919775	-0.77	0.440	.1720572, 2.150851
Sinai	.6328125	.6822711	-0.42	0.671	.0764797 ,5.23605
Donholm	1.154122	.9301789	0.18	0.859	.2378018 ,5.601292
Kingstone	1.788889	1.244517	0.84	0.403	.4575183 ,6.994525

Appendix 4: Resistance across Species

	Chicken	Cow	Doves	Duck	goat	Pig	Rabbit	Total
			AMC					
I	59	3	5	1	7	12	4	91
R	8	1	0	0	0	0	0	9
S	742	52	24	21	126	78	28	1071
			AMP					
I	66	9	10	4	22	8	2	121
R	273	13	8	9	30	33	3	369
S	470	34	11	9	81	49	27	681
			CAZ					
I	46	5	1	1	4	5	2	64
R	23	2	1	1	6	1	2	36
S	740	49	27	20	123	84	28	1071
			CRO					
I	31	2	**1**	1	1	6	1	43
R	25	0	3	0	5	2	2	37
S	753	54	25	21	127	82	29	1091
			CXM					
I	46	4	**8**	0	7	12	2	79
R	15	0	**0**	1	6	2	1	25
S	748	52	**21**	21	120	76	29	1067

			NA					
I	42	8	2	4	8	7	2	79
R	62	3	4	2	10	6	1	25
S	705	45	23	16	115	77	29	1067
			CIP					
I	19	2	4	0	3	3	1	32
R	7	1	0	0	0	7	1	16
S	783	53	25	22	130	80	30	1123
			CN					
I	62	4	4	1	1	9	3	94
R	23	3	2	0	1	1	0	30
S	724	49	23	21	121	80	29	1047
			C					
I	13	0	3	0	2	2	1	21
R	31	2	0	0	11	2	3	49
S	765	56	26	22	120	86	28	1171

	Chicken	**Cow**	**Doves**	**Duck**	**goat**	**Pig**	**Rabbit**	**Total**
			TE					
I	1	0	0	0	1	1	0	3
R	517	17	16	6	44	36	15	651
S	291	39	13	16	88	53	17	517

Total	809	56	29	22	133	90	32	1171
			SXT					
I	4	1	0	0	2	0	0	7
R	497	16	15	10	41	32	10	621
S	308	39	14	12	90	58	22	543
Total	809	56	29	22	133	90	32	1171
			S					
I	339	28	11	11	58	38	15	500
R	**361**	**23**	**16**	**8**	**58**	**48**	**12**	**526**
S	**109**	**5**	**2**	**3**	**17**	**4**	**5**	**145**
Total	**809**	**56**	**29**	**22**	**133**	**90**	**32**	**1171**

Appendix 5: Resistance pattern across *Escherichia coli*pathotypes

	Frequency	Percentage
AMC		
I	11	7.64
R	1	0.69
S	132	91.67
AMP		
I	19	13.19
R	48	33.33
S	77	53.47
CAZ		
I	5	3.47
R	5	3.47
S	134	93.06
CRO		
I	0	0
R	2	1.39
S	142	98.61
CXM		
I	11	7.64
R	2	1.39

S	131	90.90
NA		
I	10	6.94
R	6	4.17
S	128	88.89
CIP		
I	3	2.08
R	0	0
S	141	97.92
CN		
I	15	10.42
R	6	4.17
S	724	85.42
C		
I	3	2.08
R	15	10.42
S	126	87.50

	Frequency	**Percentage**
TE		
I	10	6.94
R	6	4.17
S	128	88.89
SXT		

I	2	1.39
R	64	44.44
S	78	54.17
S		
I	60	41.67
R	67	46.53
S	17	11.81