Contamination levels and transferability of antimicrobial resistance by *Escherichia coli* isolated from raw retail chicken meats in Nairobi, Kenya.

Joyce Arua Odwar

A thesis submitted in partial fulfilment for the degree of Master of Science in Medical Microbiology in the Jomo Kenyatta University of Agriculture and Technology.

2015

DECLARATION

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

Signature: _____ Date: _____

Joyce Arua Odwar

This thesis has been submitted for examination with our approval as University supervisors.

Signature: _____ Date: _____

Prof. Samuel Kariuki

KEMRI, Kenya.

Signature: _____ Date: _____

Dr. Gideon Kikuvi

JKUAT, Kenya.

DEDICATION

I would like to dedicate this thesis to my husband, my dearest parents Mr. and Mrs. George Odwar and my two siblings who mean the world to me. Their unconditional love, encouragement, patience and support motivated me to complete my project through difficult and challenging times.

ACKNOWLEDGMENTS

While it is not possible to allocate to each one the credit that is due to them, the following deserve special attention for making it possible for me to carry out the project smoothly and complete it successfully; Above all I acknowledge and give glory to the Almighty God, the giver of life, health, intellect, wisdom and strength. Special gratitude goes to my supervisors Prof. Samuel Kariuki and Dr. Gideon Kikuvi for their words of advice, encouragement and dedicating their time to direct me compile my thesis. To Dr. John Kiiru who assisted by providing his expertise in the field. To all laboratory staff who tirelessly assisted me with my work both directly and indirectly. To Mr. Kariuki Ngummo who sacrificed his time and resources in assisting me in my data analysis.

TABLE OF CONTENTS

DECLARATION ..... **II**

DEDICATION ..... **III**

ACKNOWLEDGMENTS ..... **IV**

LIST OF TABLES ..... **IX**

LIST OF FIGURES ..... **X**

LIST OF PLATES ..... **XI**

LIST OF APPENDICES ..... **XII**

LIST OF ABBREVIATIONS ..... **XIII**

ABSTRACT ..... **XVI**

CHAPTER ONE **1**

1.0 INTRODUCTION **1**

1.1 Background information 1

1.2 Statement of the problem 3

1.3 Justification of the study 3

1.4 Research questions 4

1.5 Objectives 5

1.5.1 General objective 5

1.5.2 Specific objectives 5

CHAPTER TWO **6**

2.0 LITERATURE REVIEW **6**

2.1 Poultry production 6

2.1.1 Global poultry production 6
2.1.2 Poultry production and consumption in Kenya 6
2.2 Biology of *E. coli* 7
2.2.1 Role of E. coli in causing disease 8
2.2.2 Sources of contamination by *E. coli* in chicken meat 9
2.2.3 Laboratory diagnosis and treatment of *E. coli* infection 10
2.2.4 Prevention of *E. coli* contamination in food 11
2.3 Antimicrobial consumption in food producing animals 12
2.3.1 Global scenario of antimicrobial consumption in food producing animals 12
2.3.2 The effects of antibiotics use in food animals on human health 13
2.4 Antimicrobial resistance 15
2.4.1 Food animals as a source of Antimicrobial resistance 15
2.4.2 Antimicrobial resistance in *E. coli* isolated from poultry 16
2.4.3 Transfer of Antibiotic resistance 18

CHAPTER THREE 20

3.0 MATERIALS AND METHODS 20

3.1 Study design 20
3.2 Study site 20
3.3 Study samples 22
3.4 Sample size calculation 22
3.5 Sampling procedure 22
3.6 Laboratory Procedures 23
3.6.1 Enumeration of *E. coli* and total coliform bacteria from retail chicken meat 23
3.6.2 Isolation and presumptive identification of *E. coli* 24
3.6.3 Confirmative identification of *E. coli* 24
3.6.4 Antimicrobial Susceptibility testing 25

3.6.5 Template DNA preparation 26
3.6.6 Primers and PCR amplification 27
3.6.7 Agarose gel electrophoresis 28
3.6.8 Bacterial in-vitro conjugation tests 29
3.6.9 Plasmid DNA extraction 30
3.7 Data management and analysis 31
3.8 Ethical considerations 31

CHAPTER FOUR 32

4.0 RESULTS 32

4.1 Contamination rate and microbial count determined from the raw retail chicken 32
4.2 Prevalence of antimicrobial resistance among *E. coli* isolates 35
4.3 Antibiotic resistance profiles among *E. coli* isolates. 35
4.4 Distribution of antimicrobial resistance in *E. coli* from chicken meat from the 5 classes of retail outlets 36
4.5 Prevalence of *E. coli* isolates with virulence genes 37
4.6 Prevalence of antimicrobial resistance among virulent and non-virulent *E. coli*. 40
4.7 Conjugative transfer of resistance from resistant *E. coli* to non-resistant recipient 42
4.8 Plasmid DNA profiles in Donors and Trans-conjugants 43

CHAPTER FIVE 45

5.0 DISCUSSION 45

5.1 Contamination levels in raw retail chicken by *E. coli* and *E. coli*/coliform count 45
5.2 Prevalence of antimicrobial resistant *E. coli* isolated from raw retail chicken 51
5.4 Association between antimicrobial resistance and retail outlet classification 54

5.5 *E. coli* isolates harbouring virulence markers 55
5.6 Association between antibiotic resistance and virulence genes in *E. coli* isolated 56
5.7 Co-transfer of antimicrobial resistance 59
5.8 CONCLUSIONS 62
5.9 RECOMMENDATIONS 62
5.10 LIMITATIONS OF THE STUDY 64
REFERENCES 65

LIST OF TABLES

Table 3-1: Control strains of diarrheagenic *E. coli* 27

Table 4-1: Comparison of mean microbial counts among the 5 groups of retail outlets 35

Table 4-2: Antibiotic susceptibility profiles among *E. coli* isolates in retail chicken meats 36

Table 4-3: Relationship between retail outlet classification and antibiotic resistance 37

Table 4-4: Classification of pathogenic *E. coli* isolated from raw retail chicken meats 38

Table 4-5: Relationship between virulence of *E. coli* isolated and antimicrobial resistance 40

Table 4-6: Antibiotic resistance profiles exhibited by *E. coli* donors and trans-conjugants 43

LIST OF FIGURES

Figure 3-1: Map of Nairobi showing the administrative divisions and locations..21

Figure 4-1: Box plot representing distribution of *E. coli* count among the 5 classifications of retail outlets.....33

Figure 4-2: Box plot representing distribution of coliform count among the 5 classifications of retail outlets.....34

Figure 4-3: Drug resistance phenotypes identified in pathogenic and non-pathogenic *E. coli* isolated from raw retail chicken..... 41

LIST OF PLATES

Plate 4-1: Gel photograph showing bands representing virulence genes for some diarrheagenic *E. coli* 39

Plate 4-2: Electro-phoretic plasmid profiles of representative donors and trans-conjugants 44

LIST OF APPENDICES

Appendix I: Locations from where samples were purchased 86
Appendix II: Interpretation guide for results observed on 3M Petri films 86
Appendix III: Procedure for preparation of Eosin Methylene Blue agar 88
Appendix IV: Simplified chart for the identification of family Enterobacteriacae 90
Appendix V: Procedure for preparation of Triple Sugar Iron Agar 91
Appendix VI: Procedure for preparation of Sulphide Indole Motility agar 92
Appendix VII: Procedure for preparation of Simmon's citrate agar 92
Appendix VIII: Procedure for preparation of Mueller Hinton agar 93
Appendix IX: Preparation of normal saline water 94
Appendix X: Zone diameter interpretative standards for antimicrobial resistance .. 96
Appendix XI: Procedure for preparation of MacConkey agar 96
Appendix XII: Procedure for preparation of 1% agarose gel for electrophoresis 97
Appendix XIII: Procedure for preparation of 0.5x Tris-Acetate (TAE) electrophoresis buffer 98
Appendix XIV: Procedure for preparation of Tryptic Soy Broth 99
Appendix XV: Procedure for preparation of drug plates for conjugation experiments 100
Appendix XVI: KEMRI Ethical Review Committee approval letter 102

LIST OF ABBREVIATIONS

µg: Micrograms.

AMC : Augmentin.

AMP : Ampicillin.

AMR : Anti-Microbial Resistance.

AOAC: Association of Analytical Communities international.

APEC: Avian Pathogenic *Escherichia coli.*

ATCC: American Type Culture Collection.

Bp: Base pair

C: Chloramphenicol.

CAZ: Ceftazidime.

CFU: Colony Forming Units.

CIP: Ciprofloxacin.

CN: Gentamicin.

Conc: Concentration

CRO: Ceftriaxone.

DAEC: Diffusely Adherent *Escherichia coli*.

DANMAP: Danish Programme for surveillance of antimicrobial resistance in bacteria from livestock, foods, and humans.

DNA: Deoxyribonucleic Acid.

EC: *E. coli* / Coliform Count

E. COLI: *Escherichia coli.*

EAEC: Entero-aggregative *Escherichia coli*.

EHEC: Entero- Haemorrhagic *Escherichia coli*.

EIEC: Entero-Invasive *Escherichia coli.*

EMB: Eosin Methylene Blue agar.

EPEC: Entero-Pathogenic *Escherichia coli*.

ESBL: Extended Spectrum Beta Lactamases.

ETEC: Entero-Toxigenic *Escherichia coli*.

FAO: Food and Agriculture Organization of the United Nations.

FDA: Food and Drug Administration.

GDP: Gross Domestic Product.

HSD: Honestly Significant Difference.

HUS: Haemolytic Uremic Syndrome.

ICE: Integrative and Conjugative Elements.

ICMSF: International Commission on Microbiological specifications of Food.

ICMSF: International Commission on Microbiological Specifications of Foods.

KN: Kanamycin.

LTD: Limited

MALDM: Ministry of Agriculture and Livestock Development and Marketing.

MDR: Multi Drug Resistant.

Mm: Micro molar

MoLD: Ministry of Livestock and Development.

MT: Metric Tonnes.

NA: Nalidixic Acid.

ONPG: Ortho-Nitro Phenyl β Galactosidase

PCR: Polymerase Chain Reaction.

SPSS: Statistical Package for the Social Sciences

STM: Streptomycin.

STEC: Shiga Toxin producing *Escherichia coli*.

SXT: Trimethoprin/Sulphamethoxazole.

TE: Tetracycline.

TMP/SMZ: Trimethoprin/Sulphamethoxazole.

TNTC: Too Numerous To Count

TSI: Triple Sugar Iron test.

USA: United States of America.

USDA: United States Department of Agriculture.

UTI: Urinary Tract Infections.

VRB: Violet Red Bile.

VRE: Vancomycin Resistant *Escherichia coli*.

WHO: World Health Organization.

XLD: Xylose Lysine Deoxycholate agar.

ABSTRACT

Chicken is a rich source of meat protein and is increasingly being consumed in urban Kenya. However, under poor hygienic conditions, raw chicken meat can be an efficient medium for the spread of infectious agents including bacteria such as *Escherichia coli* and other coliforms which indicate the potential presence of other pathogenic bacteria. In addition, bacterial contaminants in the meat may contain antimicrobial resistance genes that can be transferred to other bacteria and to the human population resulting in food borne infections with multidrug resistant pathogens. This study assessed the microbiological quality and safety of consumption of raw retail chicken meats sold in Nairobi, Kenya. Focus was laid on determining the *E. coli*/Coliform contamination levels, the antimicrobial resistance profiles and virulence of the *E. coli* isolates. A cross sectional study design was used. Sample collection was done from August 2011 to February 2012. Two hundred raw chicken meat samples were randomly purchased from across five different classes of meat outlets around Nairobi city, namely; high income area butcheries, high-middle income area butcheries, low income area butcheries, low-middle income area butcheries and supermarkets. Enumeration of *E. coli* and coliform bacteria was done using 3M petrifilm *E. coli*/Coliform count plates. Isolation and identification of *E. coli* was done by standard cultural and biochemical testing. Isolated *E. coli* were subjected to antimicrobial susceptibility testing using 12 commonly prescribed antimicrobials by means of Kirby Bauer disc diffusion method. Susceptibility data was interpreted according to criteria set by the Clinical and Laboratory Standards Institute (2012). Polymerase chain reaction assays were used to determine presence of virulence genes in the isolated *E. coli*. Isolates resistant to 3 or more antibiotics were subjected to *in-vitro* conjugation and plasmid DNA content analysis to test for transferability of resistance genes. Graphs and tables were used for data presentation and statistical tests were done by means of Statistical Package for Social Sciences (SPSS).

The overall *E. coli* contamination rate was 78% while coliform contamination rate was 97%. The average *E. coli* and coliform counts for all samples were above the acceptable microbial count limit (>100 cfu/ml). There was a significant difference in the *E. coli*/coliform counts ($p < 0.001$) among the 5 classifications of retail outlets. Samples from supermarkets had lower *E. coli* and coliform counts compared to the rest. Seventy five per cent of the isolates exhibited resistance to at least one of the 12 antibiotics tested. Resistance to tetracycline was the highest at 60.3%. *E. coli* isolates that tested positive for the presence of at least one of 10 virulence genes tested were 40.4 %. Fifty five percent of the isolates successfully transferred resistance by conjugation and together with 17 trans-conjugants, all contained plasmids of molecular weights varying between 3.0-53.7Kb. In conclusion, our data showed high levels of contamination in raw retail chicken meat by *E. coli* and other coliform bacteria in various retail outlets in Nairobi, Kenya. Raw chicken meat was observed to carry virulent strains of *E. coli* with ability to transfer their resistance via conjugation to other bacteria. Prevalence of antimicrobial resistance among the *E. coli* isolates was considerably high especially for commonly available antimicrobials such as tetracycline. These data will be useful for risk assessment and risk management for implementation of an effective food safety management system in Nairobi, Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Ensuring safe food supply has been one of the major challenges and concerns for producers, consumers and public health officials in both developing and developed countries. This is because foods excessively contaminated with pathogenic and spoilage micro-organism are undesirable and can cause food borne illnesses (Lindqvist *et al*., 2000; Su *et al.,* 2005; Lynch *et al.,* 2006). Such illnesses cost billions of dollars in medical care and sometimes even result to death (Fratamico, Bhunia & Smith, 2005). Several epidemiological reports have implicated foods from animal origin as major vehicles associated with illnesses caused by food borne pathogens (Busani et al., 2006; Petersen & James, 1998; Todd, 1997).

Coliform bacteria, especially fecal coliforms, are good microbial indicators of the potential presence of disease causing bacteria and also show the general sanitary quality of the food. Food contamination with *E. coli* is closely associated with fecal contamination. This is because *E. coli* are the most prevalent commensal enteric bacteria in animals and humans and are also important zoonotic agents that can be implicated in animal and human infectious diseases (Costa *et al*., 2008).

Raw or undercooked chicken meat is particularly prone to contamination. The microbiological quality of chicken meat as purchased by consumers depends mostly on; the slaughter process, sanitation during processing and packaging, maintenance of adequate cold chain storage from the processing to the retail level and to the consumer and finally sanitation during handling at the retail end (Belluck, 1997; Borche & Arinder, 2002; Andrea, 2012). Chicken meat can also act as a reservoir of drug resistant bacteria since the use of antimicrobial agents throughout the food chain contributes to

growth of resistant *E. coli* (Apata, 2009). Antimicrobial resistance among *E. coli* in food animals such as chicken is of increasing concern due to the potential for transfer of these resistant pathogens to the human population (Ojeniyi, 1998; Obrien, 2002; Angulo et al., 2004; Molbak, 2004).

In urban areas such as Nairobi, marketing of chicken products is generally undertaken in retail outlets such as supermarkets, local butcheries located in different geo-socio-economic status and even from street vendors in some low income settings. Public health research in the United States of America focusing on food qualities demonstrated that stores in low socio-economic status populations, because of a higher prevalence of food safety violations, were shown to be consistently exposed to food that is of lower microbial quality. Because of this access pattern, such populations are placed at increased risk of food-borne illnesses [Algert *et al*., 2006; Moore & Diez-Roux, 2006].

In Kenya, there is a paucity of data on coliform contamination and antimicrobial resistant *E. coli* in raw retail chicken supplied to retail outlets in the city. Studies related to antimicrobial resistant *E. coli* have been done on isolates from farm animals and chicken carcass samples from slaughter (Kikuvi *et al.,* 2006) but not from raw retail chicken which is made available to consumers. Furthermore, limited studies are available on the contamination levels in chicken meat available to populations living in different socio-economic status yet, these data are essential for performing risk assessment and risk management for food safety. This study reports on the microbiological quality and safety of chicken meat available to populations who purchase from different retail outlets (supermarkets, retail outlets in high income areas, retail outlets in middle income areas and retail outlets in low income areas) in the city of Nairobi.

1.2 Statement of the problem

Raw retail chicken meat presents an ideal substrate supporting the growth of bacteria pathogenic to humans such as *E. coli.* When consumed, these contaminated meats constitute a major source of food-borne illnesses in humans. Chicken meat can also act as a reservoir of drug resistant bacteria. Antimicrobial resistance among *E. coli* in food animals such as chicken is of increasing global concern due to the potential for transfer of these resistant pathogens to the human population. This has been documented and could pose a threat to public health (O'Brien, 2002; Angulo *et al*., 2004; Molbak, 2004). Because of their resistance to commonly used antimicrobial agents, these bacteria may cause infections for which limited therapeutic options are available. This results in use of alternative, more expensive and less readily available antibiotics. Furthermore, commensal *E. coli* of animal origin may act as a donor of antimicrobial resistance genes for other pathogenic bacteria.

1.3 Justification of the study

Chicken is fast rising to be a popular meat in Kenya. According to the Ministry of Livestock and Fisheries Development (MoLFD) Annual Report, 75% of rural families in Kenya keep chicken for trade and food security and therefore impact significantly on national food security. For instance, an estimated 14,021,399 chicken were slaughtered for consumption in only 1 year (Nyaga, 2008). Food safety is of utmost importance to all stakeholders including livestock producers, animal health experts, the medical community, consumers, regulatory agencies, the government and the food industry.

E. coli are the most prevalent commensal enteric bacteria in animals across species and humans and are also important zoonotic agents which can be implicated in animal and human infectious diseases (Costa *et al*., 2008). For this reason, they are considered significant in causing a variety of food-borne illnesses in humans. Furthermore, the level of antibiotic resistance in commensal *E. coli* is considered to be a good indicator of the

selection pressure exerted by antibiotic use and also for the resistance problems to be expected in other pathogenic bacteria in humans (Van den Bogaard and Stobberingh, 2000).

Contamination of retail chicken meat by bacteria such as *E. coli* can occur at slaughter, during packaging, during transportation to retail meat outlets, hotels and supermarkets or via butcher's hands and contaminated surfaces at these various retail outlets before distribution to consumers. If the problem is not checked, these resistant *E. coli* may colonize the human intestinal tract and may contribute resistant genes to human endogenous flora as well as other pathogenic micro-organisms thus causing infections with limited therapeutic options. It was critical therefore, to carry out a science based assessment of the risk to consumers arising from exposure to retail chicken meat with *E. coli* and other coliform bacteria that apart from causing food borne illnesses can also contribute to the antimicrobial resistance load that already exists.

In Kenya, very few prevalence and resistance surveillance studies with limited number of *E. coli* isolates from chicken have been published (Kariuki *et al*., 2013). There is limited information showing contamination rate by coliforms and *E. coli* and their antimicrobial resistance profiles in retail chicken meat made available to consumers. Yet these data are essential for performing risk assessment and risk management. This study was therefore important as the results will constitute a source of information for hazard assessment during application of food safety management system in Kenya to reduce the risk of transmission of coliforms and antimicrobial resistant *E. coli.*

1.4 Research questions

1. What are the levels of *E. coli* and other coliform contamination in raw chicken meat purchased in meat outlets in Nairobi, Kenya?

2. What are the antimicrobial resistance patterns of *E. coli* isolated from raw chicken meat purchased in meat outlets in Nairobi, Kenya?
3. Does *E. coli* isolated from raw chicken meat purchased in meat outlets in Nairobi, Kenya have virulent genes?
4. Does resistant *E. coli* isolated from raw chicken meat purchased in meat outlets in Nairobi, Kenya have the ability to transfer resistance genes to other bacteria by means of plasmids?

1.5 Objectives

1.5.1 General objective

To determine the *E. coli*/coliform contamination levels, antimicrobial susceptibility patterns, presence of virulence genes and ability to transfer resistance genes through plasmids by *E. coli* isolated from raw chicken meat in selected retail outlets in Nairobi, Kenya.

1.5.2 Specific objectives

1. To determine the contamination levels by *E. coli* and other coliform bacteria in raw retail chicken meats from selected outlets in Nairobi, Kenya.
2. To determine the antimicrobial susceptibility patterns of *E. coli* isolated from raw retail chicken meats in Nairobi, Kenya.
3. To detect the presence of virulence genes in *E. coli* isolated from raw retail chicken meats in Nairobi, Kenya.
4. To determine the transferability of antimicrobial resistance genes and presence of plasmids from the isolated resistant *E. coli* to other bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Poultry production

2.1.1 Global poultry production

Since the 1960s, the global production of poultry meat has been growing faster than that of any other meat in both developed and developing countries (Chang, 2004). This growth pattern was expected to continue because of the inherent efficiency in feed conversion and the lower production costs associated with intensive poultry production (Taha, 2003). Globally, production of poultry meat approached 70 million metric tons in 2000 with an average annual growth rate of 5.3% during the previous four decades.

Worldwide, chicken continues to be the most popular poultry meat, representing about 85% of the total poultry meat output with the US leading the world in chicken production of 14 million metric tons (Bilgili, 1999). World poultry meat production in developing countries has exceeded that of developed countries during the last decade, with a per annum expansion rate of 7.4% during the last four decades. Broiler meat is popular because it is cheaper, more versatile, and is perceived to give more health benefits than red meat (Shane, 2004).

2.1.2 Poultry production and consumption in Kenya

In Kenya the poultry population is estimated to be about 25-30 million, 80% of which comprises local chicken and the rest, improved breeds. With the ever-increasing prices of red meat, local chicken has become the main source of animal protein in the form of meat and eggs for both the urban and rural population (International research network, 2005). According to figures by the Ministry of Agriculture, livestock, development and marketing (MALDM), Rift valley province has the highest population of poultry with an

estimated poultry population of 6.4 million while North eastern has the least population of poultry estimated at 143,500 (MALDM, 2005/2006).

Traditionally, poultry plays an important role in Kenya. The chickens have been and still are a major source of protein in the form of eggs and meat. In addition, poultry production improves nutrition of the rural people and provides income to the families. Nationally, the poultry industry contributes to the Gross Domestic Product (GDP). Of the 25% annual GDP, 4% is from poultry sub-sector. The industry also contributes to wealth generation in the country. It is estimated that poultry along with other sub-sectors contribute 43% of the total labour force in the agricultural sector (Kingori *et al*., 2010).

Increasing human population in the pastoral areas has led to a decline in grazing land, resource degradation and an overall drop in livestock holdings per household (Lesorogol, 1998). This has led to a dependence on poultry farming in pastoral areas as well since chicken production is a landless enterprise and can provide the much-needed source of protein for the vulnerable groups in pastoral households and at the same time generate income from sale of surplus birds and eggs (Tuitoek *et al*., 1998). Local poultry farmers are now adding value to their chicken by doing some processing and packaging. It is now possible to find local chicken packaged and labelled in some leading supermarkets in Nairobi. This has improved the prices by about 50% (Kingori *et al*., 2010). Therefore chicken consumption is high in the country.

2.2 Biology of *E. coli*

E. coli is a member of the large bacterial family, Enterobacteriacae. It is a Gram-negative, non-spore forming, rod-shaped micro-organism that is often motile by means of flagella or may be non-motile, and can grow with or without oxygen. It is catalase positive, oxidase negative, fermentative, reduces nitrate to nitrite and is β-galactosidase positive. Approximately 95 % of *E. coli* strains are Indole and methyl red positive, but

are Voges-Proskauer and citrate negative. It is characterized by; lactose fermentation with gas production and Indole production from tryptophan when incubated for 48 ± 2 hours at 44°C to separate *E. coli* from other organisms of the faecal coliform group (Todar, 2005; Fratamico and Smith, 2006).

E. coli inhabits the intestinal tract of humans and animals and most of the strains live as commensal organisms where they are harmless to hosts and are useful for producing sources of 'B' and 'K' vitamins. They are also found inhabiting the environment, water and food. *E. coli* are classified as coliform bacteria. The presence of *E. coli* in food or water is an indication of faecal contamination due to uncleanliness and careless handling. It also implies that other enteric pathogens may be present (Fratamico and Smith, 2006). *E. coli* is sero-typed on the basis of the somatic (O), flagella (H) and capsular (K) antigens. More than 170 different O antigen sero-groups are currently recognized. The combination of O and H antigens define sero-types of an isolate. For example *E. coli* O157:H7 is a sero-type of a virulent strain of *E. coli* associated with haemorrhagic colitis and haemolytic uremic syndrome (HUS) (Washington *et al*., 2006).

2.2.1 Role of *E. coli* in causing disease

Although existing mostly as commensals, *E. coli* can become pathogenic upon acquisition of virulence attributes, such as entero-toxins and adhesion or invasion factors (Garcia, 2002) and result in enteric/diarrheal disease, urinary tract infection, and sepsis/meningitis (Hammerum and Heuer, 2009). Until the 1950's the organism was more or less regarded as a normal non-pathogenic cohabitant of the enteric tract of warm-blooded animals and humans (Wasteson, 2001).

However, during the last few decades, a tremendous amount of research has established *E. coli* among the important etiological agents of enteritis and several extra intestinal diseases such as uro-genital infections, wound infections, mastitis, septicaemia, Gram

negative pneumonia and meningitis (Coia, 1998). The *E. coli* strains causing extra intestinal infections have been collectively called extra-intestinal pathogenic *E. coli* (ExPEC). These include two major pathotypes; uro-pathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) (Rodriguez –Siek *et al*., 2005).

At-least six classes of diarrheagenic *E. coli* are known based on specific virulent factors and phenotypic traits. These include; Entero-Toxigenic *E. coli* (ETEC), Entero-Invasive *E. coli* (EIEC), Entero-Haemorrhagic *E. coli* (EHEC) also known as *Shigella* Toxin producing *E. coli* (STEC), Entero-Pathogenic *E. coli* (EPEC), Entero-Aggregative *E. coli* (EAEC) and Diffusely Adherent *E. coli* (DAEC) (Nataro and Kaper, 1998; Washington *et al*., 2006). ETEC are an important cause of diarrhoea in infants and travellers in under-developed countries or regions of poor sanitation. EIEC closely resemble *Shigella* in their pathogenic mechanisms and the clinical syndrome is identical to *Shigella* dysentery and includes dysentery-like diarrhoea with fever. EHEC are represented by the strain O157:H7, which causes a diarrheal syndrome that shows a copious bloody discharge with no fever. EPEC induce watery diarrhoea similar to ETEC and it usually occurs in infants. EAEC resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhoea without invading or causing inflammation.

2.2.2 Sources of contamination by *E. coli* in chicken meat

In living animals the muscles are virtually sterile but other parts of the animal like the skin or gut contains an enormous amount of bacteria. Among them there is a large number of *E. coli* which are often excreted or shed in the environment (Brill, 2007). They can survive and replicate outside its host environment i.e. in water, soil, sediments, sand and algae (Byappanhalli *et al.,* 2003; Anderson *et al.,* 2005). Contamination of meat can occur during carcass processing if the rumen is accidentally cut. In the case of poultry, the intestine may rupture when the carcass is gutted during processing, releasing

its contents into the thoracic and abdominal cavities (Woolcock, 1991). The gut contents of chickens, especially of the caeca, are considered to be peak environments for bacterial colonisation.

Depending on the slaughter hygiene a smaller or higher number of the bacteria can be found in meat during the slaughterhouse operations in particular during skinning, scalding, evisceration, dressing, transport or meat cutting. Contaminated retail meat can also be a result of unhygienic conditions via butchers' hands, tools, contact with the equipment and/or through water or air (Belluck, 1997; Moldlab, 2003). Bacterial contamination in meat is quite normal and not totally avoidable. However, the policy is to keep the amount of bacteria as low as possible. Since *E. coli* is as an indicator for sanitary quality, the organizations such as International Commission on Microbiological Specifications of Foods (ICMSF) and Codex Alimentarius have already established microbiological meat standards and report that the acceptable level for *E. coli* in fresh meat should not be higher than 100 colony forming units/gram or /ml (Meng, 2001).

2.2.3 Laboratory diagnosis and treatment of *E. coli* infection

In general, *E. coli* infections are majorly diagnosed by culturing the bacteria from the site of infection. Samples of blood, stool, urine, sputum, CSF or other clinical material are sent to the laboratory for culture. Microscopy will normally show Gram negative rods, with no particular cell arrangement. The Gram stain is not useful when it comes to stool samples as stool carries many other Gram negative bacteria. The sample is then cultured either in Xylose lysine deoxycholate agar (XLD), MacConkey agar or Eosin Methylene blue (EMB) agar followed by a series of biochemical tests which include; Indole production, methyl red test, voges-proskauer, triple sugar iron test (TSI), β-galactosidase (ONPG) among others (Paton and Paton, 1998; Todar, 2005; Washington *et al*., 2006).

Meningitis requires antibiotics, such as third generation cephalosporins. Pneumonia requires respiratory support, adequate oxygenation and antibiotics such as third generation cephalosporins or fluoroquinolones. *E. coli*-associated UTI's can often be treated with a single dose of antibiotic such as those found in the class of fluoroquinolones, beta lactams, trimethoprin-sulfamethoxazole, tetracyclines and aminoglycosides. In most individuals infected with diarrheal *E. coli*, symptoms of infection last about a week and resolve without any long-term problems. Diarrhoea may require fluid replacement with electrolyte solutions. Antibiotics do not improve the illness. Therefore, apart from good supportive care such as close attention to hydration and nutrition, there is no specific therapy to halt *E. coli* diarrhoea symptoms. Antimicrobials known to be useful in cases of traveller's diarrhoea include doxycycline, trimethoprim-Sulfamethoxazole, fluoroquinolones, and rifaxan. Although traveller's diarrhoea is self-limiting, antibiotics may shorten the duration of diarrhoea (Washington *et al*., 2006).

2.2.4 Prevention of *E. coli* contamination in food

The United States Department of Agriculture (USDA) and the Food Safety and Inspection Service, formulates policies in all kinds of meat harvesting facilities to minimize food borne contamination risks. These procedures include strict sanitation of all environment, utensils and tools used, proper product handling and preservation, carcass sanitizing, employee hygiene, testing of outgoing product and thorough cooking of meat at high temperatures to destroy any organisms. Mandatory regulations enforced by USDA and voluntary efforts by the beef industry have reduced the incidence of *E. coli* O157:H7 in ground beef to less than 1 percent (Payne and Sparks, 2009).

Such preventative measures are effective in reducing *E. coli* infection from food both in and out of the home. Efforts are also encouraged on monitoring and surveillance of food borne infections in food premises by the food and health departments so as to identify

possible sources of infection, which are thoroughly investigated and a closure order issued for establishments that pose an immediate health hazards. Surveillance projects in slaughterhouses are also encouraged in which samples from food animal carcasses are regularly collected to assess and monitor hygienic practices and sanitation in slaughterhouses. Training of slaughterhouse staff on hygiene measures and precautions to be taken during slaughter are particularly encouraged. Health education activities on prevention and control of contamination of meats are also practiced on personnel of the food industry and the general public (Heymann, 2004).

2.3 Antimicrobial consumption in food producing animals

In food producing animals, antibiotics are dispensed to animals for a number of reasons such as therapeutic treatment, disease prophylaxis and growth promotion (Bager, 2000). The administration of antibiotics for treatment or prophylaxis exposes bacterial populations to the selection of resistant forms of bacteria and such forms of bacteria can spread from one organism to another.

2.3.1 Global scenario of antimicrobial consumption in food producing animals

Reliable data on antibiotic consumption for both animals and humans is not widely available and has been reported on in only a few countries such as Denmark (Bager, 2000). Surveillance carried out in Denmark in 1995 to monitor consumption of antimicrobial agents and occurrence of antimicrobial resistance in food animals, food and humans gave unofficial figures. These indicated that although there is a declining trend in the consumption of antimicrobial agents, consumption was still high. About 22,000 kg (80%) of those being consumed were aminoglycosides, macrolides, tetracyclines and penicillin thus resulting in high resistance to these antibiotics from several bacterial species from different animals. Penicillins had the highest consumption. Driven by a concern for human health, in 1995, Denmark initiated a process to end the use of antibiotics as growth-promoters in livestock production. This process involved

both voluntary and legislative elements and led to the situation that virtually no antimicrobial growth-promoters are used in Denmark since the end of 1999 (WHO, 2002).

According to Mellon and colleagues (2001), the yearly overall production of antibiotics in the United States was 17.5 million kilos. From this, 12.5 million kilos were used for non-therapeutic purposes in livestock production and only 1.5 million kilos for human medical therapy. WHO (1998) also indicated that a number of quinolones were licensed for use in food animals in Asia, Latin America and South Africa. The report did not, however, specify the magnitude of use of these antibiotics in the different regions. The report contained an estimate that annual quinolone consumption in animals in China was in the range of 470 tons. A Kenyan study contained reliable quantitative information about antimicrobial consumption in food animals in Kenya. In the five-year period from 1995 to 1999, the annual mean antimicrobial consumption was 14.6 tons. Tetracyclines and trimethoprim-sulphonamides accounted for nearly 78% of this use, nitrofurans and aminoglycosides each accounted for 6-7% of the total use and the other classes of antimicrobials did not exceed 1% of the use (Mitema *et al*., 2001).

2.3.2 The effects of antibiotics use in food animals on human health

There is broad scientific consensus that the use of antibiotics in food animals, on some occasions, has detrimental effects on human health (O'Brien, 2002; Angulo *et al*., 2004; Molbak, 2004). According to Collignon (2003) the use of the antibiotic avoparcin as a growth promoter in food animals in Europe resulted in the development and amplification of Vancomycin Resistant Enterococci (VRE) and subsequent colonization of a significant percentage of the human population via the food chain (between 2 and 17%). A subsequent ban on the use of avoparcin in food animals in the European Union resulted in a marked reduction of the percentage of the general population carrying VRE in their bowel. Vancomycin resistance is a cause for concern because vancomycin is

widely used in intensive care units as a last-line antibiotic for some hospital-acquired infections of *Enterococci* and *Staphylococci* that have become resistant to the more commonly used antibiotics (Rivera & Boucher, 2011).

Collignon (2003) also mentions that the use of the antibiotic enrofloxacin had been approved for use in food production animals in many countries. The use of this antibiotic in food animals resulted in the development of ciprofloxacin-resistant strains of *Salmonella* and *Campylobacter*. These resistant bacteria subsequently caused human infections. According to another study in Canada, the use of animal feed supplemented with the antibiotic tylosin had led to the development of erythromycin-resistant *Streptococci* and *Staphylococci* not only in the animals but also in their caretakers (Khachatourians, 1998).

The Food and Drug Administration (FDA) in the USA in 2001 stated that since the approval of fluoroquinolones for use in food-producing animals, reports had identified a relationship between the approval of fluoroquinolones for therapeutic use in food producing animals and the development of fluoroquinolone resistance in *Campylobacter* in animals and humans. In addition, the approval of these drugs in food-producing animals in the Netherlands, Spain and the United States preceded increases in resistance in *Campylobacter* isolates from treated animals and ill humans. Smith and colleagues (1999) were cited as one of the sources for this claim. A case of domestically acquired ceftriaxone-resistant *Salmonella* was reported in a 12-year old child from Nebraska who acquired the resistant bacteria from the veterinarian father who had been treating several cattle herds with ceftiofur antibiotic for outbreaks of *Salmonella* infection (Fey *et al*., 2000).

2.4 Antimicrobial resistance

2.4.1 Food animals as a source of Antimicrobial resistance

Food animals exposed to additives such as the antibiotics used for growth promotion may serve as a reservoir of resistant bacteria and resistance genes that may spread to the human population through the food chain, thereby limiting the effectiveness of antimicrobial drugs (Aarestrup *et al*., 2001). Contaminated meat and meat products are important sources of antibiotic-resistant bacteria. For instance a study in Minneapolis, USA found that these products were found to be contaminated with resistant *E. coli* and extra-intestinal pathogenic *E. coli* carrying additional virulent factors (Bester and Essack, 2010). Bester and Essack determined that these findings corresponded closely with the prevalence of contamination found for animal carcasses exposed to immediate contamination from the intestines, as well as for processed meat further along the production line. This form of meat contamination was also found in Addis Ababa, Ethiopia, where 8% of the beef and chicken samples collected were contaminated with resistant *E. coli* O157:H7 (Hiko *et al*., 2008). In a number of farms, food animal production usually incorporates some form of antibiotic usage, either therapeutic or prophylactic.

According to reviews by Kelly *et al*., (2009a; 2009b) the transfer of genetic entities, including those carrying antibiotic-resistance qualities, becomes all the more probable in environments with high bacterial loads. A recent study in Iceland revealed that out of 419 *E. coli* isolated from pigs, broiler chicken and humans, 170 (40%) were resistant to one or more antimicrobial agents. In the pig isolates, over 50 % were resistant to one or more antimicrobial agents and 22% were multi resistant (Thorsteinsdottir *et al*., 2010a). A Kenyan study by Ole Mapenai to investigate the occurrence of antimicrobial resistance in *E. coli* in food animals showed that approximately 38% of all *E. coli* isolates from food animals were antibiotic resistant with 26% of them being multidrug

resistant with resistance to ampicillin, tetracycline and sulphonamides being most frequent (Ole Mapenai *et al*., 2006).

2.4.2 Antimicrobial resistance in *E. coli* isolated from poultry

Antibiotics are used to prevent or treat a number of avian diseases including avian pathogenic *E. coli* (APEC) and collibacillosis as well as enhance feed conversion efficiency and improve growth rates (Aarestrup *et al*., 2001). Resistance of these bacterial strains to antimicrobials is therefore important in the selection of agents for treatment. *E. coli* is considered to be the organism of choice as a model to studying resistance levels in bacteria as *E. coli* strains efficiently exchange genetic material not only with each other but also other enteric pathogens. In northern Georgia, USA Zhao *et al*., (2005) tested 95 APEC isolates and found 92% of the isolates to be resistant to three or more antimicrobials.

Yang *et al*., (2004) from China noted that 80% *E. coli* isolated from the livers of chickens that had died on 10 poultry farms were resistant to eight or more antibiotics. Similarly, high levels of Antimicrobial Resistance (AMR) were observed elsewhere in China where 70 *E. coli* isolates recovered from diseased chickens between 2004 and 2005 were examined (Li *et al*., 2007). The isolates showed frequencies of resistance of 100% to tetracycline and trimethoprim-sulphonamide and 79–83% to chloramphenicol, ampicillin, ciprofloxacin and enrofloxacin. Vertical transmission of a pathogenic O45 strain of *E. coli* that was resistant to fluoroquinolones was described in one Denmark study (Petersen *et al*., 2006). Petersen and his colleagues showed that a single clone was responsible and the bacterium recovered from five broiler chicks could be traced back to the broiler parents.

Commensal microbiota play an important role as reservoir of resistance determinants (Blanc *et al*., 2006; Scott, 2002). Antimicrobial resistance in commensal strains of *E.*

coli may play an important role in the ecology of resistance and infectious diseases. Moniri & Dastehgoli (2005) noted resistance to nalidixic acid in 100% *E. coli* from broilers while resistance to ciprofloxacin was detected in 42% of the isolates. In addition to fluoroquinolone resistance, there is considerable interest in resistance to beta-lactams, especially extended spectrum beta-lactams. In one study in Belgium, 295 ceftiofur resistant *E. coli* were recovered from cloacal swabs of 489 broiler chickens from five farms (Smet *et al*., 2008). In the US, 73% of 194 *E. coli* recovered from shell eggs were susceptible to all seven antimicrobials that were tested. Isolates that were resistant were mostly resistant to tetracycline (Musgrove *et al*., 2006).

A prevalence of 42% was reported in Lithuania in one study aimed to estimate the prevalence and the antimicrobial resistance of *E. coli* isolated from chicken liver sold in retail markets (Modestas *et al*., 2010). The isolates demonstrated frequent resistance to the most frequently used antimicrobials including aminopenicillins, quinolones and tetracyclines. Multidrug resistance to the 3rd generation cephalosporins that are exclusively important in human medicine by isolates from retail chicken meat was also recorded in Japan (Ashraf *et al*., 2009). The most commonly reported resistance phenotypes were against ampicillin, streptomycin, spectinomycin, kanamycin, SXT, nalidixic acid, ciprofloxacin and cephalosporins.

In Kenya, Kikuvi and colleagues showed that resistance was highest in chicken *E. coli* isolates as compared to isolates from cattle and pigs (74%) (Kikuvi *et al*., 2006). The chicken isolates also had significantly high multidrug resistance. Extended Spectrum Beta Lactamase (ESBL) producing *E. coli* have been encountered in chicken. With the extensive use of beta lactam antibiotics in poultry such as amoxicillin and cephalosporins and especially extended spectrum cephalosporins, ESBL mediated resistance in Gram negative bacilli has become increasingly critical and therapeutic options for such infections are becoming limited (Hu *et al*., 2005).

Li and colleagues (2009) showed that 60.8% of the isolates tested were ESBL-producing *E. coli.* A survey in 2007 showed that the prevalence of ESBL producing animal-associated bacteria in China was 30% (Liu *et al*., 2007). It is important to detect ESBL producers in order to know the ESBL prevalence in animal-associated bacteria and to limit the spread of these MDR organisms in veterinary settings.

2.4.3 Transfer of Antibiotic resistance

Acquired antibiotic resistance is attributed to both direct and indirect pathways (Zhang *et al*., 2011). Direct pathways, also known as primary pathways are mutations in the gene encoding resistance against the mechanism of particular antibiotics. Indirect or secondary pathways are the gaining of smaller fragments of DNA coding for resistance. Indirect pathways are classified into three categories: Transformation, Transduction and Conjugation (Dessen *et al*., 2001).

Transformation is where a dying bacterium cell may release its plasmids, or short fragments of its DNA into the environment, which allows for the possibility for a healthy bacterium cell, called a recipient, to acquire this material and use it for its own benefit directly through the cell wall (Kelly *et al*., 2009a).

Conjugation allows transmissible plasmids and chromosomal DNA of very large sizes to be transferred from cell to cell, either within or between species, mediating the transmitted genetic material through various specified enzyme activities. The conjugational pathway does not select DNA material simply from the environment, as in the case of transformation, but instead has a direct cell-to-cell exchange of genetic material, where one cell is the donor of the genetic material and the other the new host or recipient (Catry *et al*., 2003).

Transduction is the virus-like injection of genetic material into a host cell after attachment. Transduction is a feature mainly of bacteriophages commonly acting as bacterial viruses (Maurelli, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This study utilized a cross-sectional descriptive study design.

3.2 Study site

The study was conducted in Nairobi, Kenya. Nairobi City is the administrative and commercial capital in Kenya and main town in Nairobi County. It is home to thousands of businesses including the retail chicken business. Nairobi is administratively divided into nine divisions (Mathare, Central, Westlands, Starehe, Dagoretti, Lang'ata, Makadara, Kamkunji and Embakasi) (Figure 3.1). Each division is divided into a number of locations. Within these locations are found a number of retail meat outlets some of which deal with selling of raw chicken meat. There is a huge disparity in income levels and population densities in Nairobi. The people living in the western suburbs are generally the more affluent while the lower and middle-income populations dominate the eastern suburbs (Afullo and Odhiambo, 2009).

Study sites for obtaining the samples were 200 randomly selected retail chicken outlets and supermarkets in twenty eight locations around Nairobi (Appendix 1). Samples were then processed at the Kenya Medical Research Institute, Centre for Microbiology Research laboratories, Kenyatta hospital compound.

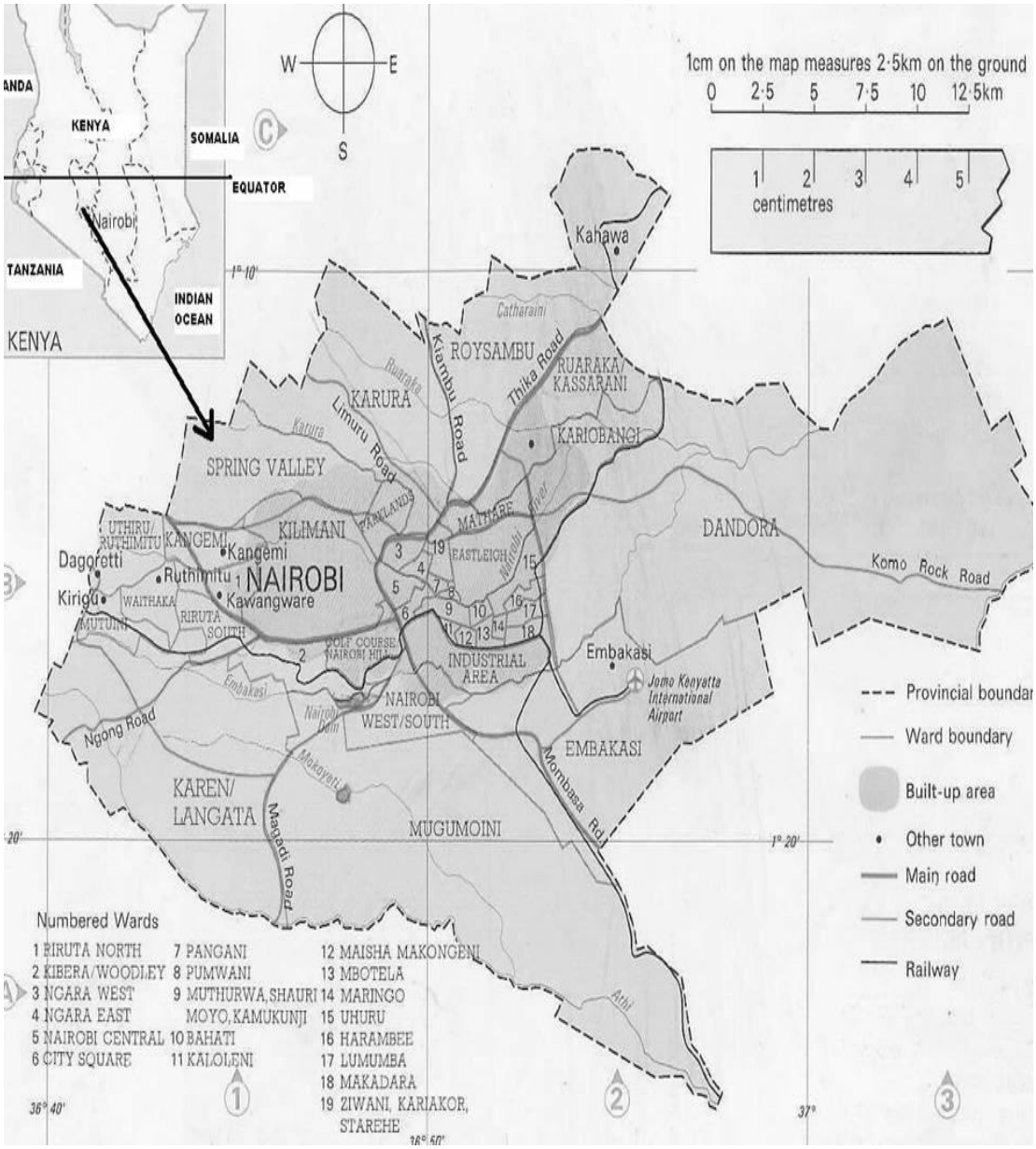


Figure 3-1 Map of Nairobi showing the administrative divisions and locations

Source: (Afullo and Odhiambo, 2009)

3.3 Study samples

The study samples were raw retail chicken meat that were purchased from 200 retail meat outlets in 28 locations around Nairobi in the period between August 2011 to February 2012. The samples included both the chicken skin and chicken flesh for study.

3.4 Sample size calculation

Kariuki and colleagues conducted a study in Kenya on food safety and antimicrobial resistance that tried to show the general food safety overview in meat value chains, including raw retail chicken, but the prevalence of *E.coli*/coliform contaminants was not given (Kariuki *et al.,* 2013). Apart from this, there is no other published study in Kenya giving this prevalence in raw retail chicken. Therefore, the prevalence that was used to calculate the sample size was from a similar study in a developing country recently conducted in Osogbo, Nigeria in which the prevalence of *E. coli* isolated from raw retail chicken was found to be 16% (Adesiji *et al*., 2011). Using the Fisher's formula, (Fishers, 1995), and taking 16% to be the working prevalence rate (P), 1.96 the standard error from the mean (Z) and 5% the absolute degree of precision (D), the sample size (N) was calculated as follows:

$$N = \frac{Z^2 P(1-P)}{d^2} = \frac{1.96^2 X 0.16 x 0.84}{0.05^2} = 206 \text{ samples}$$

3.5 Sampling procedure

A random cross-sectional sampling procedure was followed where by samples were randomly purchased from a pre-existing list of retail outlets within different locations in Nairobi (Appendix 1). The distribution of samples from each location was not equal in number since the number of samples purchased per location depended on availability of chicken retail outlets in each location. Therefore, some locations which had fewer retail outlets as representatives had fewer samples than others.

In order to take into account compounding factors of socio-economic status in the contamination rate and antimicrobial resistance patterns in the *E. coli* under study within Nairobi, the retail outlets where samples were purchased were classified into Supermarkets, outlets from high end locations (low densely populated, up-market residential suburbs), outlets from middle end locations (middle densely populated areas further classified into high middle and low middle income areas) and outlets from low end locations (densely populated slums and informal settings). Classification of locations into these groups was done based on a recent study on residential segregation in Nairobi (K'Akumu and Olima, 2007). Random sampling was done until a desired sample size was reached. For each sample, test sites logged the store name, store location, the purchase date and lab processing date. Freshly packaged chicken samples were immediately transported at 4°C conditions to the laboratory for bacterial isolation.

3.6 Laboratory Procedures

3.6.1 Enumeration of *E. coli* and total coliform bacteria from retail chicken meat

Enumeration of *E. coli* and total coliform bacteria from the chicken samples was performed as described in the Association of Analytical Communities international, official methods of analysis using 3M petrifilm *E. coli*/Coliform count plates (AOAC, 2006) with slight modification. The samples were aseptically removed from the package and 100g pieces of chicken was weighed and placed in 100 ml sterile distilled water. The container was shaken manually for 3 min. 3M petrifilm plates were labeled according to individual sample laboratory numbers and placed on a leveled surface and the top of the plate lifted.

Using a pipette, 1ml of rinse water from the sample was drawn and in a perpendicular position placed onto the center of the bottom film. The top of the film was rolled back down carefully avoiding entrapment of air bubbles and a spreader was placed on the

surface of the top film and gently pressed to distribute inoculum over the circular area before gel was formed. After the gel solidified, the plates from all samples were incubated for 24± 2h at 35°C. Colony counts for each petrifilm were then done the following day and the microbial count results converted to base 10 logarithm of the number of colony forming units per ml (cfu/ml) rinse water obtained from the samples. *E. coli* colonies growing on the petrifilm plate appeared as blue colonies with gas while coliforms were counted as red and blue colonies with gas. An interpretation guide was referred to when interpreting results (Appendix 2).

3.6.2 Isolation and presumptive identification of *E. coli*

Isolation of *E. coli* strains from the chicken samples was performed as described by Andrew and Hammack, (2003) with modifications. 200 µl of rinse water was streaked on Eosin Methylene Blue (EMB) agar (Appendix 3) and incubated overnight at 37°C. The plates were then visually examined to presumptively identify *E. coli* colonies. *E. coli* colonies appeared to be very dark, with a metallic green sheen. Before proceeding, a Gram stain was performed to determine morphology of the growing colonies and a pure culture was obtained by sub-culturing a well isolated *E. coli* colony on another EMB agar plate and incubating at 37^{o}C for 24 h. After incubation, the plates were examined and a single colony selected for confirmative identification of *E. coli* through a series of biochemical tests.

3.6.3 Confirmative identification of *E. coli*

Biochemical tests were used to confirm the identity of selected *E. coli* isolates (Macfaddin, 2000). A simplified chart for identification of *E. coli* is shown in Appendix 4.

In the Triple sugar iron agar test (BBL), the selected isolate was streaked on the surface of a TSI agar slant and then stabbed. The tube was incubated at 37^{o}C for 24 h after

which the agar surface was observed to determine visible color change on both the butt and slant from red to yellow together with gas bubbles in the agar. *E. coli* ATCC 25922 was used as the positive control while *P. aeruginosa* ATCC 9027 was used as the negative control.

In Sulfur, Indole, motility agar test, the isolate to be tested was inoculated in Sulfur Indole Motility (SIM) agar and incubated overnight at 37°C. Following incubation, few drops of Kovac's reagent were added. Formation of a red or pink colored ring at the top with spread of cloudiness in the media/ movement from stabbing point of the inoculant due to motility and lack of blackness in the media was taken as positive for *E. coli*. *E. coli* ATCC 25922 was used as positive control while *Klebsiella pneumonia* was used as negative control.

In Citrate utilization test, selected colonies were inoculated into the slope of Simmon's citrate agar and incubated overnight at 37°C. Presence or absence of *E. coli* in the medium was determined by a color change from green to blue i.e. No color change meant presence of *E. coli*. *E. coli* ATCC 25922 was used as negative control while *Enterobacter aerogenes* ATCC 13048 was used as Positive control.

All positive isolates were stored at -80°C in trypticase soy broth for further analysis (Appendix 5, 6 and 7).

3.6.4 Antimicrobial Susceptibility testing

The Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) was used to test susceptibility of the isolated *E. coli* to antibiotics commonly used in animal and human health. Antimicrobial sensitivity test was done on Mueller-Hinton agar (Appendix 8) according to the recommendations reported by the Clinical and Laboratory Standards Institute (CLSI, 2012). The organisms were tested for their susceptibility to 12 commonly used antimicrobials on disks containing; Ampicillin (AMP 10µg),

Amoxycillin-clavulanic acid (AMC, 30µg), Tetracycline (TE 30µg), sulphamethoxazole-trimethoprin (SXT 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CRO 30µg), Ceftazidime (CAZ 30µg), Kanamycin (K 30µg), Streptomycin (S 10µg), Gentamicin (CN 10µg), Nalidixic acid (NA 30µg) and Chloramphenicol (C 30µg).

E. coli strain 25922 was used to control for bacterial growth and potency of antibiotic disks. Each *E. coli* isolate was inoculated in normal saline water (Appendix 9) and the turbidity adjusted to that of *Barium chloride* 0.5 McFarland standard. Adjusted bacterial inocula was swabbed onto the MH agar plates using sterile swabs and allowed to dry. Using multi disk dispensers, the antimicrobial disks were carefully dispensed onto the surface of the inoculated plates and incubated at 37°C for 24h. Using a ruler, the susceptibility zones were measured and interpreted according to criteria set by the Clinical and Laboratory Standards Institute document M100-S17 (CLSI, 2012). (Appendix 10**)**

3.6.5 Template DNA preparation

DNA from the isolated *E. coli* as well as from control strains was extracted following the procedures described by Ehrt and Schnappinger, (2003) with slight modifications. A total of 5 controls were used (Table 3-1). Pure *E. coli* isolates and control strains were grown in MacConkey agar (Appendix 11) and subsequently sub-cultured in Mueller-Hinton agar and incubated overnight at 37°C. A loop full of overnight bacterial culture was suspended in 1000 μl of sterile distilled water and then boiled for 10 min at 95°C in a heating block (Grant QBT1). The resulting suspension was placed in a centrifuge (Denville scientific Inc) and centrifuged for 5 min at 14,000 rpm. The supernatant was stored at -20 °C and used as the DNA template for PCR amplification.

Table 3-1 Control strains of diarrheagenic *E. coli*

Strain	Target gene
STEC	*stx1, stx2, eaeA*
ETEC	*st, sth, stp, astA, lt*
EPEC	*eaeA*
EIEC	*InvE*
EaggEC	*aggR, astA*

STEC- Shigella toxin producing *E. coli;* ETEC- Entero-toxigenic *E. coli*; EPEC-Entero-Pathogenic *E. coli*; EIEC- Entero-Invasive *E. coli*; EAggEC- Entero-aggregative *E. coli*. *Source:* Oxoid (Unipath Ltd, Basingstoke, UK).

3.6.6 Primers and PCR amplification

Ten PCR primer pairs to detect the target genes enumerating toxins in pathogenic *E. coli* were purchased (Sigma Aldrich Company Ltd, United Kingdom) and are listed in table 3-2. A multiplex PCR system was optimized by the progressive incorporation of primers corresponding to the different genes and several combinations of melting temperatures, primer concentrations and DNA template concentration (Protocol Online, 2003). The multiplex reactions was done in PuRe Taq ready to go PCR beads (GE Health care) containing Potassium chloride (50 Mm), Magnesium chloride (1.5 mM), dNTP,s (200µM in 10mM Tris-HCl, Ph 9.0), and 2.5 units pure Taq DNA polymerase.

The total reaction volume in each PCR tube was 25µl containing 3µl template DNA, 2µl primer (0.2µl of each primer) and 20µ sterile PCR water. Amplification was done by means of a thermo cycler (DYAD) under the following conditions; initial denaturation at 94^0 C for 5 minutes. This was followed by 35 cycles of denaturation at 94^0 C for 1

minute, annealing at 56^0 C for 30s and extension at 72^0 C for 1 min. The final step was amplification at 72^0 C for 10 min. Amplicons were then subjected to agarose gel electrophoresis.

Table 3-2 List of primers used to establish virulence in isolated *E. coli*

Gene name	Primers	DNA sequence 5^1-3^1	Amplicon size (bp)	Primer conc. (Mm)	Reference
stx1	stx1-F	AGTTAATGTGGTGGCGAA	817	25	Fujioka *et al*., 2009
	stx1-R	GACTCTTCCATCTGCCG			
stx2	stx2-F	TTCGGTATCCTATTCCCG	474	25	"
	stx2-R	TCTCTGGTCATTGTATTA			
*eae*A	eaeA-F	AAACAGGTGAAACTGTTGCC	454	25	"
	eaeA-R	CTCTGCAGATTAACCTCTGC			
invE	invE-F	ATACTCTATTTCCAATCGCGT	382	25	"
	InvE-R	GATGGCGAGAAATTATATCCCG			
aggR	aggR-F	GTATACACAAAAGAAGGAAGC	254	25	"
	aggR-R	ACAGAATCGTCAGCATCAGC			
ST	ST-F	TTTATTTCTGTATTGTCTTT	171	50	"
	ST-R	ATTACAACACAGTTCACAG			
STp	STp-F	TCTGTATTATCTTTCCCCTC	186	50	"
	STp-R	ATAACATCCAGCACAGGC			
STh	STh-F	CCCTCAGGATGCTAAACCAG	166	25	"
	STh-R	TTAATAGCACCCGGTACAAGC			
LT	LT-F	AGCAGGTTTCCCACCGGATCACCA	130	25	"
	LT-R	GTGCTCAGATTCTGGGTCTC			
*ast*A	astA-F	GCCATCAACACAGTATATCC	106	25	"
	astA-R	GAGTGACGGCTTTGTAGTCC			

Source: (Sigma Aldrich Company Ltd, United Kingdom)

3.6.7 Agarose gel electrophoresis

Amplification products were subjected to agarose gel electrophoresis to analyze and determine presence of virulence genes. The procedure described by protocol online was used (Protocol online, 2001). One percent agarose gel was prepared (Appendix 12) and 5µl ethidium bromide cautiously added to the solution. After cooling, the solution was poured in a sealed casting tray containing gel combs, the gel allowed to set and the

combs carefully removed. The gel was mounted in an electrophoresis tank (Thermo E.C) and TAE electrophoresis buffer added (Appendix 13).

The amount of 10µl DNA sample was mixed with 3µl loading buffer and each sample was carefully loaded into individual wells in the gel using a micro pipette. The gel was allowed to run in the electrophoresis tank for 2 h at 126 V with periodic checks. Sigma molecular weight ladder (Sigma-Aldrich life sciences) of known size (100 base pairs (bps) was used as the molecular weight standard. After electrophoresis, the gel was illuminated with an ultraviolet lamp to view the DNA band profiles for virulence genotypes. The gel was then photographed with a digital camera and the molecular weight of each band profile was determined.

3.6.8 Bacterial *in-vitro* conjugation tests

Conjugation experiments were performed for all *E. coli* isolates that were found to be resistant to more than 3 antibiotics and acted as the donors (resistant to ampicillin and susceptible to Sodium azide). *E. coli* strain J58 was used as the recipient strain (resistant to Sodium azide and susceptible to ampicillin). Conjugation procedures were performed in 5 days as described by Shohayeb and Sonbol (1994) with slight modifications. On the first day, all donors and the recipient bacteria were revived in MacConkey agar and incubated at 37°C overnight. On the second day, all donors and the recipient bacteria were subsequently sub-cultured in Mueller-Hinton agar to obtain pure cultures and incubated at 37°C overnight.

On the third day, pure *E. coli* isolates (each donor and the recipient) were inoculated in clearly labeled tubes containing normal saline water and the turbidity of each adjusted to that of *Barium chloride* 0.5 McFarland standard. Two hundred micro litres of individual donor broth from the prepared standards was added to labeled bijou bottles containing the conjugation broth (tryptone soy broth) (Appendix 14) followed by 200µl of recipient

in all bottles. The mixture of donor and recipient was inverted thoroughly several times and incubated overnight at 37°C.

On the fourth day, using sterile tips and spreaders, 100µl from the incubated conjugation broth was spread on the following; a set of MacConkey plates to select for trans-conjugants (containing 100µg/ml Sodium azide and 10µg/ml ampicillin), a set of plates to select for recipient strain (containing only 100µg/ml Sodium azide), a set of plates to select for only donor strains (containing only 10µg/ml ampicillin) and a set of plates to select for both donors and recipient bacteria (plates without any of the anti-bacterial agents). The plates were then incubated overnight at 37°C (Appendix 15).

On the final day, all plates were removed from the incubator and observed for growth and colony counts of trans-conjugants. Trans-conjugants were then subjected to Antimicrobial susceptibility tests following the same procedures used for the donor *E. coli*. All positive trans-conjugants were stored at -20 ^{0}C in tryptic soy broth stocking media for further analysis.

3.6.9 Plasmid DNA extraction

The *E. coli* donors and successful trans-conjugants were subjected to plasmid DNA extraction and subsequent analysis in agarose gel electrophoresis. Plasmid DNA was extracted using alkaline lysis technique by means of the plasmid mini extraction kit (Qiagen, West Sussex, United Kingdom) according to manufacturer's instructions. Five ml of overnight bacterial culture was centrifuged at 8,000 rpm for 3 min and the pellet re-suspended in RNase containing suspension buffer. The suspension was lysed by means of an alkaline lysate buffer and after neutralization it was centrifuged for 10 min at 13,000 rpm. Using a pipette the supernatant was applied to a QIA prep spin column and centrifuged for 1 min. After discarding the flow through, the bound DNA remaining in the spin column was precipitated and washed twice using ethanol and after

centrifugation the flow through discarded. The plasmid DNA remaining in the spin column was eluted in elution buffer (10Mm Tris-Cl, p.H 8.5). Plasmids were separated by electrophoresis on horizontal 0.8% agarose gels at 80 volts for 3 h. Plasmid sizes were determined by co-electrophoresis with plasmids of known sizes from *E. coli* strain V157 (NCTC 50193; 53.7, 7.2, 5.6, 3.9, 3.0, 2.7, 2.1Kb). Plasmid DNA bands were visualized with an ultraviolet trans-illuminator after staining with 5µl ethidium bromide.

3.7 Data management and analysis

Data was entered in excel spread sheets and stored in the computer hard drive as well as flash disks. The Statistical software package used for data analysis was Statistical Package for Social Sciences (SPSS) version 17.0. Descriptive statistics was presented through use of graphs and frequency tables. One way ANOVA was used in determining statistical significant difference in mean microbial counts among the different classifications of retail outlets. The chi-square test was used to asses any statistical significant association between antimicrobial resistances in the *E. coli* isolated with regard to retail outlet classification from where the raw chicken was purchased and presence of virulence genes in the isolated *E. coli.*

3.8 Ethical considerations

Retail chicken meats were the study samples and therefore no ethical requirements such as respect for life was considered. However, societal benefits that have resulted from the study were considered that will lead to knowledge and improvements in the protection of the Public health and well-being of both humans and the live animals. The study was approved by the Jomo Kenyatta University of Agriculture and Technology, Board of Post-graduate Studies and the Kenya Medical Research Institute, Ethical Review Committee in accordance with local regulatory requirements (Appendix 16).

CHAPTER FOUR

RESULTS

4.1 Contamination rate and microbial count determined from the raw retail chicken

One hundred and fifty six of the chicken samples examined (78%) were positive for *E. coli* contamination. Contamination level of chicken samples by total coliform bacteria was found to be 97%. The average *E.coli* and coliform count for all samples were 3.911 and 5.2061 Log_{10}cfu/ml respectively.

Retail outlets from where the raw chicken samples were purchased were classified into 5 major groups and the differences in the microbial loads among these groups were presented through graphical box plots. According to observation of the positions of the median line as well as the lower whiskers in figure 4-1, it is evident that the *E. coli* counts for High income, High-middle, low-middle and low income areas are negatively skewed and the bulk of counts are concentrated on the high end of the scale. The *E. coli* counts for samples from supermarkets are evenly distributed. Two extreme outliers are observed in the case of *E. coli* counts from high income areas.

The size of the boxes for high- middle income, low-middle income and low income areas indicate that the middle 50% samples for these groups have their *E. coli* counts spread and vary in number while for high class butcheries and supermarkets the box sizes indicate that the middle 50% of the counts are clumped together. Box plots for supermarkets and high income area butcheries are observed to be much lower in position than the other three box plots.

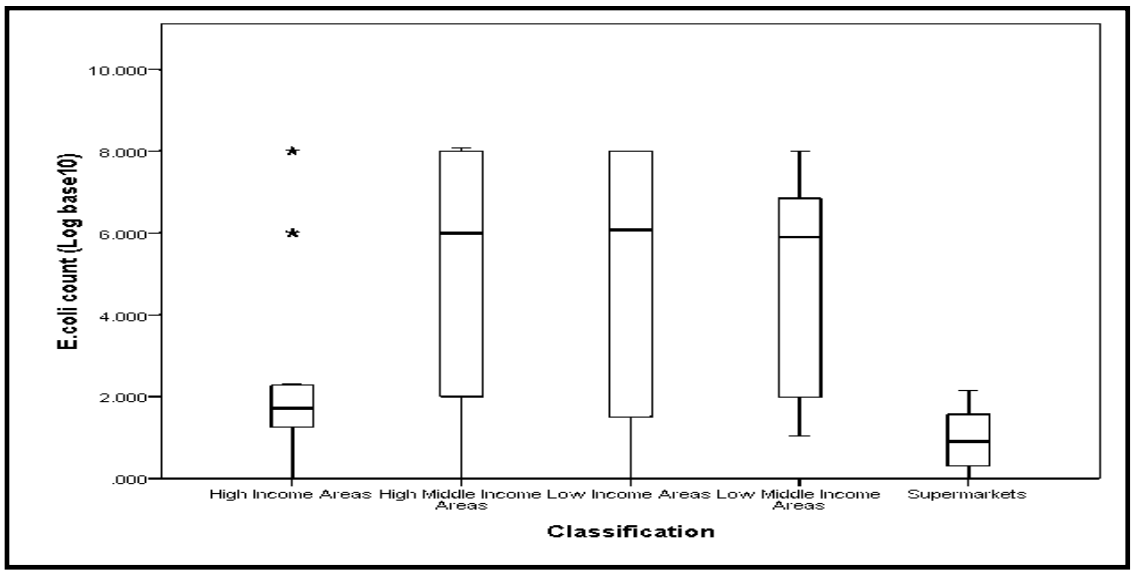


Figure 4-1 Box plot representing distribution of *E. coli* count among the 5 classifications of retail outlets

Figure 4-2 which represents coliform count distribution shows that apart from supermarkets, the other 4 retail outlet classifications have bulk of the count concentrated on the high end of the scale while supermarkets have an even distribution of coliform counts. Outliers were observed for High middle income areas and low income areas on the opposite side of the skew and an extreme outlier is also observed for supermarkets indicating that some samples from the former two groups had coliform counts which were low and high in the case of supermarkets thus these counts are seen to deviate markedly from the rest of the counts.

Values for coliform counts do not vary much in high middle income butcheries, low income butcheries and supermarkets while they are spread out for high income and low middle income area butcheries. The mean Log_{10} *E. coli*/coliform counts for supermarkets, high income, high-middle income, low-middle income and low income

areas were; 0.9375/1.7237, 2.6510/5.0769, 4.9990/5.2953, 4.8084/6.0111 and 4.9882/6.1855 Log_{10} cfu/ml respectively.

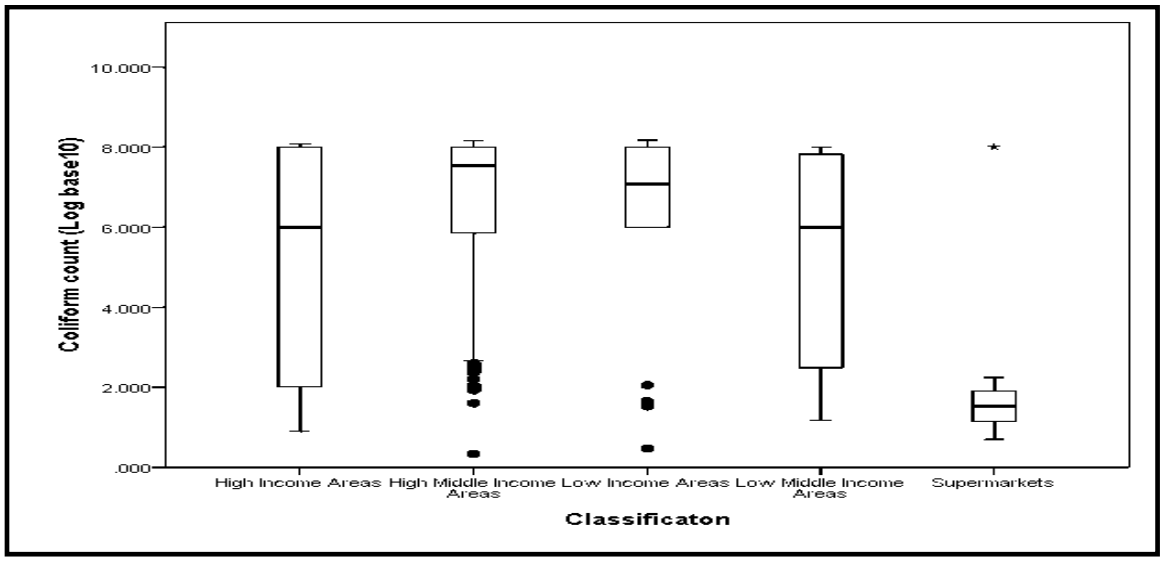


Figure 4-2 Box plot representing distribution of coliform count among the 5 classifications of retail outlets

Analysis of variance was used to determine if there was indeed a significant difference in mean microbial counts among the 5 classifications of retail outlets. There was significant difference in the *E. coli* count ($F(4,175) = 16.676$; MSE=106.576; $p < 0.001$) and coliform counts ($F(4,179) = 18.37$; MSE=105.097; $p < 0.001$) among the groups. According to post-hoc tukeys' Honestly Significant Difference (HSD) test (Table 4-1), samples purchased from supermarkets had a significantly lower *E. coli* and coliform count than those from 3 other retail outlet classifications (outlets in high-middle, low-middle and low income areas) but had no significant difference with samples from outlets in high income areas ($p > 0.05$). The samples from outlets in high income areas had significant lower *E. coli* count than those from high-middle, low-middle and low income areas but showed no significant difference in coliform count.

Table 4-1 Comparison of mean microbial counts among the 5 groups of retail outlets

Group	Difference between means (*E. coli* count)	Tukeys' HSD ŧ value	Difference between means (Coliform count)	Tukeys' HSD value
A-B	1.7135	2.05	3.3532	2.09
A-C	4.0615		4.4618	
A-D	3.8709		3.5716	
A-E	4.0507		4.2874	
B-C	2.348		1.1086	
B-D	2.1574		0.2184	
B-E	2.3372		0.9342	
C-D	0.1906		0.8902	
C-E	0.0108		0.1744	
D-E	0.1798		0.7158	

ŧ- When the difference between means of 2 groups is greater than the tukeys' HSD value then there is a significant difference between these two groups

*****A-Supermarkets
B-High income areas
C-High-middle income areas
D-Low-middle income areas
E-Low income areas

4.2 Prevalence of antimicrobial resistance among *E. coli* isolates.

Antibiotic susceptibility profiles to 12 commonly used antibiotics were done for all the 156 *E. coli* isolates. Overall, 75% (117) of the isolates exhibited resistance to at least one of the 12 antibiotics while the rest of the isolates were fully susceptible.

4.3 Antibiotic resistance profiles among *E. coli* isolates.

Prevalence of antimicrobial resistance by *E. coli* isolates was highest for tetracycline (TE) (60.3%) followed by sulphamethoxazole-trimethoprim (SXT) (49.4 %), ampicillin (AMP) (34.0 %) and streptomycin (STM) 30.1 % (Table 4-2). In general isolates showed highest levels of resistance to Tetracyclines (TE) 60.3%, followed by

Sulphonamides (SXT) 49.4%, Quinolones/Fluoroquinolones (NA and CIP) 19.2%, Phenicols (C) 13.5%, Beta-lactams/Cephalosporins (AMP, CAZ, CRO and AMC) 12% and the lowest resistance in Aminoglycosides (STM, KN and CN) 11.9%

Table 4-2 Antibiotic susceptibility profiles among *E. coli* isolates in raw retail chicken meats

	Disc potency	Sensitive isolates		Intermediate isolates		Resistant isolates		Total
Antibiotic	**µg/disc**	**Frequency**	%	**Frequency**	%	**Frequency**	%	
AMC	30	142	91	10	6.4	4	2.6	156
SXT	30	78	50	1	1.6	77	49.4	156
TE	30	61	39.1	1	0.6	94	60.3	156
AMP	10	100	64.1	3	1.9	53	34	156
CIP	5	148	94.9	1	0.6	7	4.5	156
CAZ	30	155	99.4	1	0.6	0	0	156
KN	30	139	89.1	9	5.8	8	5.1	156
STM	10	103	66	6	3.8	47	30.1	156
CRO	30	138	88.5	0	0	18	11.5	156
CN	10	154	98.7	1	0.6	1	0.6	156
NA	30	133	85.3	0	0	23	14.7	156
C	30	135	86.5	0	0	21	13.5	156

Out of all the 156 isolates, 60.8% exhibited multiple drug resistance traits. 24.5 % of the isolates were fully susceptible while 14.7 % showed resistance to only one antibiotic.

4.4 Distribution of antimicrobial resistance in *E. coli* from chicken meat from the 5 classes of retail outlets

There was no significant difference ($p > 0.05$) in the prevalence of antimicrobial resistance among the 5 classifications of retail outlets (χ^2n-1= 4.178; d.f= 4; p= 0.382) i.e. the prevalence of antimicrobial resistance among *E.coli* isolates from chicken samples was not associated with the classification of retail outlet from where the samples were purchased. However, percentage frequencies indicated that the isolates

from samples in supermarkets had a higher prevalence (84.6%) while isolates from samples in low income area outlets had the lowest prevalence of antimicrobial resistance (62.5%). (Table 4-3)

Table 4-3 Relationship between retail outlet classification and antibiotic resistance

		Number of isolates with resistance phenotype	
		Number	%
Classification of retail outlet	High income areas	22	78.6%
	High-middle income areas	48	70.6%
	Low income areas	10	62.5%
	Low-middle income areas	15	83.3%
	Supermarkets	22	84.6%
Total		117	75.0%

4.5 Prevalence of *E. coli* isolates with virulence genes

Among the 156 *E. coli* isolates recovered from the raw retail chicken, 63 (40.4%) tested positive for the presence of at least one of 10 virulence genes; *stx1, stx2, stp, sth, st, astA, invE, lt, aggR, eaeA* while 93 (59.6%) tested negative for the presence of any of these genes and these were considered as non-pathogenic. ETEC were found to be the most prevalent Among the *E. coli* that were positive for these virulence genes (60.3%) (Table 4-4). A gel picture of representative diarrheagenic *E. coli* carrying virulence genes is shown (Plate 4-1)

Table 4-4 Classification of pathogenic *E. coli* isolated from raw retail chicken meats

Classification of Pathogenic *E. coli*	Frequency of isolates	%
EPEC	13	20.6
ETEC	38	60.3
EIEC	4	6.3
EAEC	3	4.8
STEC	5	7.9

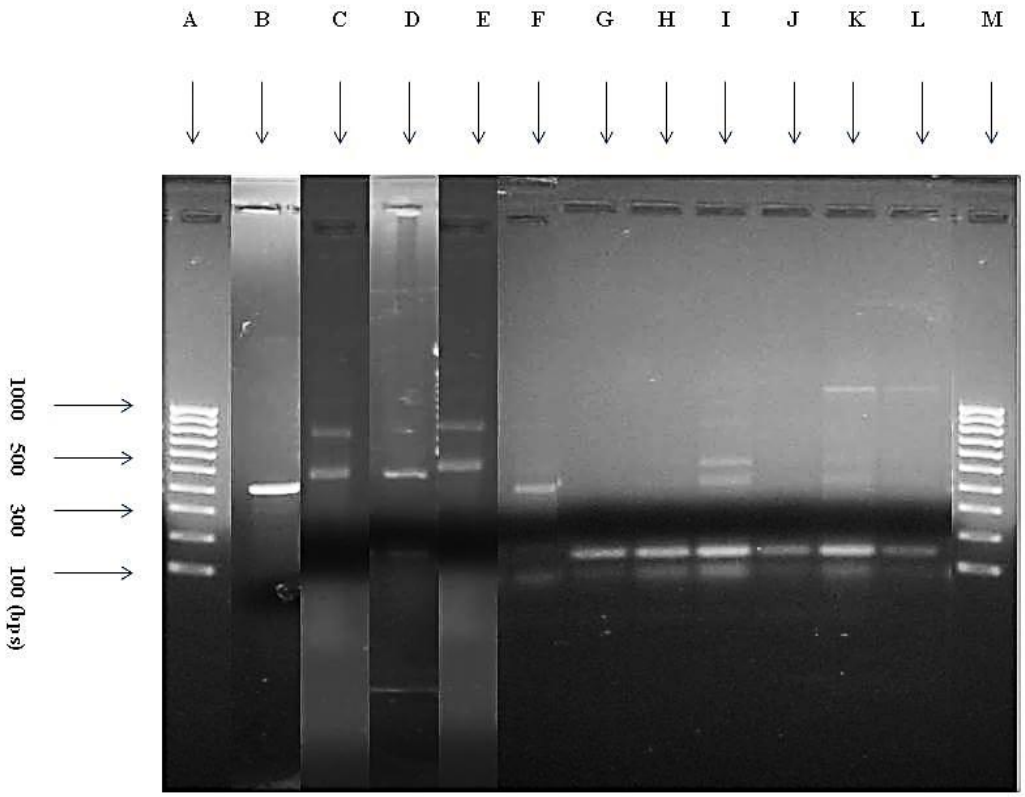


Plate 4-1 Gel photograph showing bands representing virulence genes for some diarrheagenic *E. coli*

Lanes A and M have the 100 bp molecular marker used. Lanes B, D and F have the eaeA gene (454 bp) specific for EPEC. Lanes C and E have the stx2 gene (474 bp) and stx1 gene (817bp) specific for STEC. Lanes G to L have the LT gene (130 bp) specific for ETEC.

4.6 Prevalence of antimicrobial resistance among virulent and non-virulent *E. coli*

There was no significant difference ($p > 0.05$) in the prevalence of antimicrobial resistance between the virulent and non-virulent strains of *E. coli* (χ^2n-1= 1.018; d.f= 4; p= 0.313), therefore the resistance among isolates was not associated with presence or absence of virulence genes. However, the non-virulent *E. coli* strains generally showed a higher percentage of resistance (78.5%) as compared to 71.4% in virulent strains (Table 4-5). This is also well illustrated in figure 4-3. Isolates that were resistant to more than 7 antibiotics were all found negative for virulent genes, while most isolates that were resistant to fewer antibiotics were found to carry virulent genes (Appendix 17).

Table 4-5 Relationship between virulence of *E. coli* isolated and antimicrobial resistance

		Resistance Phenotype		
		No	Yes	Total
Classification of *E. coli*	Virulent *E. coli*	18	45	63
		28.6%	71.4%	100%
	Non-virulent *E. coli*	21	72	93
		21.5%	78.5%	100%
Total		39	117	156
		25%	75%	100%

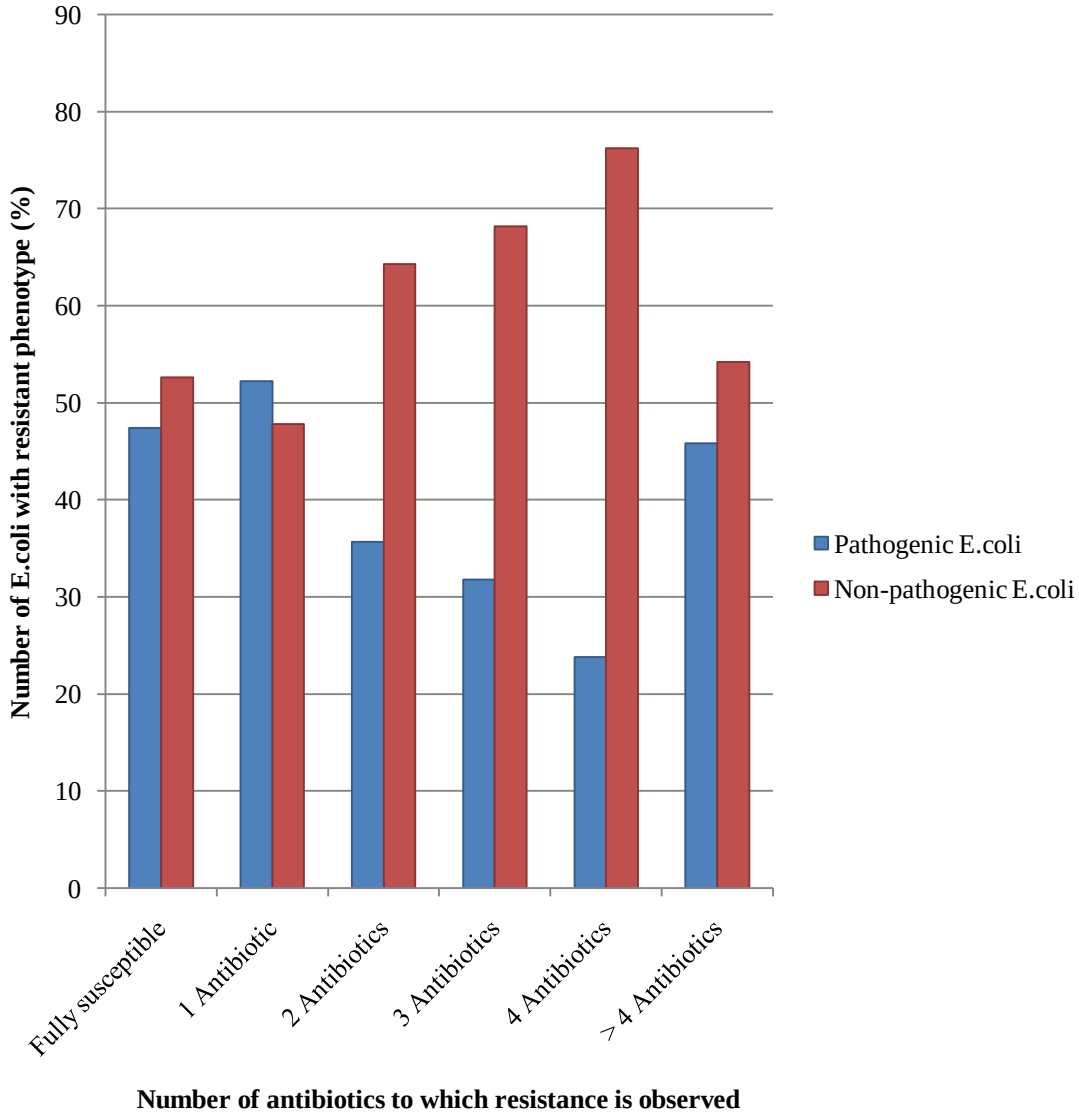


Figure 4-3 Drug resistance phenotypes identified in pathogenic and non-pathogenic *E. coli* isolated from raw retail chicken

4.7 Conjugative transfer of resistance from resistant *E. coli* to non-resistant recipient

A total of 56 *E. coli* isolates that were resistant to 3 or more antibiotics were subjected to *in-vitro* conjugation using *E. coli* J53 strain as the recipient. Fifty five percent (31) of the isolates successfully transferred resistance to at least one antibiotic while 25 were unsuccessful. The 31 successful trans-conjugants were then subjected to antibiotic susceptibility tests using the same antibiotics that had previously been used for the donors. The resistance profiles exhibited by the donors and the trans-conjugants after successful conjugation are shown in table 4-6. Seventeen of the 31 trans-conjugants (58%) received resistance determinants to at-least 1 antibiotic. Fourteen (42%) trans-conjugants received the full antibiotic resistance profile from the donors.

Table 4-6 Antibiotic resistance profiles exhibited by *E. coli* donors and trans-conjug

Donor	*Antibiotic resistance profile*	Trans-conjugant	*Antibiotic resistance profile*
3083	SXT, TE, AMP,	3083/J53	AMP
3084	SXT, TE, AMP, K, S, C	3084/J53	SXT, TE
3160	AMP, S, CRO, NA, C	3160/J53	AMP, S, CRO, NA, C
3259	SXT, TE, AMP	3259/J53	SXT, TE, AMP,
3260	SXT, TE, AMP	3260/J53	SXT[1], TE, AMP,
3261	SXT, TE, AMP, S	3261/J53	SXT, TE, AMP, S
3264	SXT, TE, AMP, S	3264/J53	SXT, TE, AMP, S
3309	TE, AMP, CRO, C, K	3309/J53	AMP
3456	AMC, AMP, S, CRO, NA, C	3456/J53	AMP, S, CRO, NA, C
3586	SXT, TE, AMP	3586/J53	SXT, TE, AMP
3590	SXT, TE, K, S	3590/J53	SXT, TE, K, S,
3690	SXT, TE, AMP, S	3690/J53	SXT, AMP
3831	SXT, TE, CIP, NA	3831/J53	SXT, TE, CIP, NA
4029	AMC, AMP, CAZ, CRO	4029/J53	AMC, AMP, CAZ, CRO
4050	AMC, SXT, TE, AMP, CIP, NA, C	4050/J53	SXT, TE, AMP, NA
4078	AMC, SXT, TE, AMP, S, CRO, C	4078/J53	AMC, SXT, TE, AMP, S, CRO,C
4080	SXT, TE, AMP, S	4080/J53	AMP
4147	AMC, SXT, TE, AMP	4147/J53	SXT, TE, AMP
4152	AMP, S, CRO, C	4152/J53	TE, AMP, CRO, C
4176	SXT, TE, S, NA, C	4176/J53	SXT, TE, S, NA, C
4256	AMC, TE, AMP	4256/J53	AMC, TE, AMP
4258	TE, AMP, K, S, CRO, C	4258/J53	AMP, CRO, C
4259	TE,AMP, K, S, CRO	4259/J53	AMP, CRO, C
4343	SXT, TE, K, S	4343/J53	SXT, TE, K, S,
4479	AMC, SXT, TE,AMP, CIP,K, S,CRO,NA, C	4479/J53	AMP, CRO, C
4484	AMC,SXT, TE, AMP, CIP, S, NA, C	4484/J53	AMC, SXT, TE, AMP, CIP, S,NA, C
4549	SXT, TE, AMP, S, CRO, C	4549/J53	AMP, C
4554	AMP, S, CRO, NA, C	4554/J53	AMP, CRO, C
4639	AMP, S, CRO, C	4639/J53	AMP, CRO, C
4759	SXT, TE,AMP, S	4759/J53	TE,AMP
4763	TE ,AMP ,S, CRO, C	4763/J53	TE, AMP, CRO, C

4.8 Plasmid DNA profiles in Donors and Trans-conjugants

Plasmid DNA was extracted from both donors and trans-conjugants. All 56 multidrug resistant donors and 17 out of 31 trans-conjugants were found to contain at least one or more plasmids of varying molecular weights ranging between 3.0-53.7Kbs. Fourteen

trans-conjugants did not receive any plasmids. Plate 4-2 below gives a representation of results obtained.

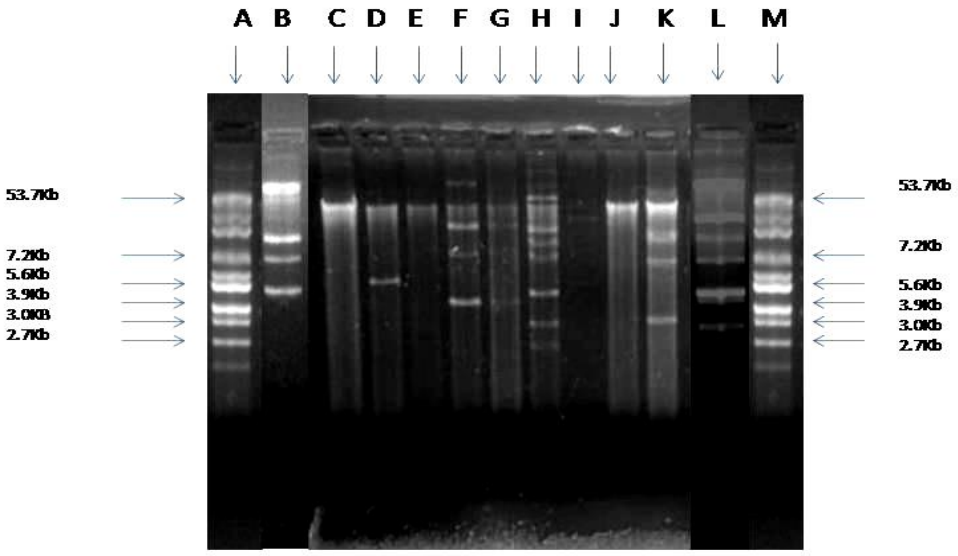


Plate 4-2 Electro-phoretic plasmid profiles of representative donors and trans-conjugants

Lanes A and M represent the *E. coli* VI57 reference plasmid which was used as a marker and positive control. Lanes B, F, H, K and L show the plasmid profile of some of the donors with plasmids of varying molecular sizes. Lanes C, D and J show some trans-conjugants that received one plasmid each from corresponding donors. Lane E and G are examples of trans-conjugants that did not receive any plasmid. Lane I represent the recipient bacteria *E. coli* J53 that contains no plasmid.

CHAPTER FIVE

DISCUSSION

5.1 Contamination levels in raw retail chicken by *E. coli* and *E. coli*/coliform count

It has been reported in many studies that contamination of raw retail chicken by *E. coli* is a major public health concern worldwide and it is an indication of faecal contamination along the value chain (Ruzauskas *et al*., 2010; Hammerum and Heuer, 2009; Nzouankeu *et al*., 2010).

In the current study, the overall *E. coli* contamination level was 78%. The contamination level observed in this study was lower than observed in studies conducted in other developing countries. For instance, contamination levels of 98% in the north east part of India, 100% in Cameroon, 100% in Vietnam (Saikia and Joshi, 2010; Nzouankeu *et al*., 2010; Thangh Huong *et al*., 2009) but much higher than others; 43% in Morocco, 41.7% in Lithuania and 16% in Nigeria (Cohen *et al*., 2007; Modestas *et al*., 2010; Adesiji *et al*., 2011).

Similarly, high levels have been observed in studies from developed countries. Contamination levels as high as 100% were found in a study conducted in Japan, 89% in the state of Minnesota, U.S.A, 80% in four other states in the U.S.A and 90% in two states in Australia (Ashraf *et al*., 2009; Johnson *et al*., 2003; Zhao *et al*., 2012; Pointon *et al*., 2008). These contamination levels prove that there is high microbial cross contamination during production of retail chicken meats in slaughter and post slaughter processing in most countries, both developed and developing including Kenya and thus extra attention needs to be paid in these areas.

The mean *E. coli* and coliform counts for all samples were beyond the acceptable range counts as set by the Hazard Analysis and Critical Control Point system (HACCP),

developed by Food and Agriculture Organization (FAO) and adopted by the Codex Alimentarius Commission in order to identify specific hazards and measures to ensure safety of food. According to this system, the acceptable food safety range for raw meats is 100 cfu/ml or less (2.000 Log_{10} cfu/ml or less), the marginal or intermediate range is over 100 cfu/ml but not above 1000 cfu/ml (over 2.000 but not above 3.000 Log_{10} cfu/ml) and the unacceptable range is above 1000 cfu/ml (above 3.000 Log_{10} cfu/ml) (Meng *et al.,* 2001; Food Safety and Inspection Service, 1996). Only 80 (40%) out of 200 retail chicken samples that were purchased fell under the acceptable range results for *E. coli* counts while 60% of the samples fell under the marginal and unacceptable ranges. 76% of samples fell under the unacceptable range results for coliform counts.

Similar observations were seen in studies in most developing countries. For example a study in Hanoi, Vietnam found that the percentage of retail chicken samples which were unacceptable for each microbial criterion was 72.09% for Coliform counts and 59.3%, for *E. coli* count (Thangh Huong *et al.,* 2009). Two other studies in Morocco recorded 22.4% and 62% chicken samples falling within unacceptable range, (Cohen *et al*., 2007; Amara *et al*., 1995). The current study merely discusses un-investigated factors within this study that could explain the contamination levels observed and the presence of a large number of retail chicken meats with unacceptable ranges of *E. coli*/coliform count. The factors discussed by the current study require more detailed studies in future to conclusively determine the main factors responsible for the results obtained.

Unhygienic practices during slaughter and processing is a possible major cause of contamination. In Nairobi, there are few private chicken slaughter houses such as Kenchic Limited, Alpha fine foods Ltd and Gourmet food products Ltd and only a few municipal chicken slaughter houses which are strictly guided by government laws and regulations and where stringent bio security measures are strictly observed. Such slaughter houses are provided with compulsory hygienic measures to be followed during

the various processing steps in the manufacture of chicken and their products thus minimizing microbial contamination. These hygienic measures include but are not limited to; restricting the entrance to slaughter establishments only to authorized individuals, adopting an all-in all-out single age group principle in the establishment, control entry of rodents into the establishment, regular cleaning and disinfection of the establishment as well as machines and equipments used, adequate personal hygiene of the personnel and regular disinfection of the water delivery system (Nasser *et al.*, 2006; Nyaga, 2007).

Furthermore, most of these private companies such as Kenchic limited own laboratories used to monitor vaccination efficacy in the flocks, test environmental samples from the houses during cleaning and carry out routine disease diagnostic services. Unfortunately, there are large numbers of chicken slaughtered in homes of small scale poultry producers whose hygienic standards cannot be strictly controlled and are thus considered potential risks for contamination due to bio-security flaws (Andrea, 2010).

A second possible factor could be unfavourable cold chain temperature conditions during transport of chicken carcasses from the slaughter house to the retail outlets. A lot of meat products from the small scale farmers do not necessarily undergo cold storage after slaughter nor during transportation to markets often far away in urban centres. The production of such poor quality meat predisposes it to early spoilage as well as posing a threat to the health of the consumer. An unpublished study by a student in the University Of Chiang Mai Vietnam in the year 2007 showed that meat products that are transported in ambient temperatures have a higher contamination rate compared to meat products that are cooled during transport (Van Tuat, 2007).

Another common observation in Nairobi in the transport and packaging of local butchery chicken meats is that instead of individual packaging of each chicken carcass,

the carcasses tend to be lumped together in one large container or sack as they are transported thus exposing them to the open air in which the hygienic standards cannot be met. This can also lead to transfer of contaminants from one carcass whose gut contents might have spilled on the carcass surface to the surface of other clean carcasses and subsequent microbial multiplication. Studies in Iran and Switzerland confirm this fact as they were able to show that there were significant differences in contamination rates between packed and unpacked chicken meat samples (Vaskas *et al*., 2012; Regula *et al*., 2003). Suppliers for supermarkets and mega stores on the other hand use cold chain and a more hygienic approach and appropriately deliver the meat and its related products in sterilized packages (Popkin, 2006).

A third possible factor could be the display and storage practices of the raw chicken carcass at the local retail outlets. Lack of use of proper storage methods such as freezing was observed at some retail outlets irrespective of any prevailing general practice of hygiene. This could have contributed to microbial contamination. Most retail outlets where the chicken samples were purchased also displayed their retail products in glass display cabinets that lacked sufficient cooling temperatures. It was observed that most raw chicken on display for sale were displayed together with other types of meat such as beef, mutton, fish and offals such as the liver, kidneys and even the intestines. The degree of physical contact between these different kinds of meat on display could have led to an exchange and transfer of *E. coli* and other coliform contaminants to the retail chicken meats. The equipment for handling the raw chicken meat in the butcheries such as knives, weighing scale and chopping surfaces were the same equipment used to handle other kinds of meat thus a potential contributor to contamination on the raw chicken (FAO, 2008).

Mean *E. coli*/coliform counts in samples from supermarkets were within acceptable food safety ranges (0.9375/1.7237 Log_{10} cfu/ml) while samples from the rest of the retail

outlets had marginal and unacceptable counts of above 2.000 Log_{10} cfu/ml. ANOVA analysis revealed that there were significant differences in the *E. coli* counts (F (4,175) =16.676; MSE=106.576; p< 0.001) and coliform counts (F (4,179) =18.37; MSE=105.097; p< 0.001) among the groups with supermarkets having the lowest *E. coli* and coliform counts compared to the rest. Butcheries from high income areas followed closely with less *E. coli* counts but high coliform counts. The other three groups (High-middle income, Low-middle income and low income area butcheries) had higher *E. coli*/coliform counts.

These results corresponded closely to those obtained from a study in Philadelphia that sought to determine differences in microbial quality of foods available to populations of differing socio-economic status (Koro *et al*., 2010). The study found that raw poultry, available in markets in low-socio economic status had higher microbial indicator counts compared to markets in high-socio economic status. There could be a number of reasons as to the observations made and this study suggests the following.

This observation could be attributed to the differences in source of the chicken for each market. As indicated on the packaging of the samples, all samples from supermarkets and a majority of samples from high income butcheries were products of government approved private chicken slaughter houses (mainly observed were Kenchic Ltd and Alpha fine foods Ltd) which are governed by laws and regulations and where stringent bio security measures are strictly observed from production to marketing of chicken and their products (Nyaga, 2007). On the other hand, majority of samples from the other retail categories were supplied by small scale poultry producers who may not abide by such regulations and as such, gaps may exists in the bio-security measures along the production line.

Another possible factor that could be attributed to this difference is a disparity in hygienic measures among the classifications that were studied. General observation showed that hygienic conditions in supermarkets and butcheries from high income areas were better compared to the hygienic conditions in butcheries from middle and low income areas. Furthermore, as indicated before, samples from supermarkets were wrapped individually from the slaughter area and subjected to immediate freezing temperatures and maintained at adequate refrigeration from the processing to the retail level.

Inappropriate handling, especially in retail butcheries of middle to low socio-economic status may have an impact on cross contamination of bacteria in raw chicken products. It is notable that few samples from high middle income and low income areas showed acceptable levels of *E. coli* and coliform counts (as low as 1 cfu/ml). This was attributed to the use of freezing conditions for storage in these outlets. Thus, freezing of retail chicken is considered an important factor in reducing microbial contamination and multiplication (Jakabi *et al*., 2005). However, it was also observed that at least four samples from supermarkets had *E. coli* and coliform counts beyond the acceptable range of food safety even though they were displayed under refrigeration. This shows that the spread of contamination is not only confined to seemingly unhygienic environments (Sackey *et al*., 2001).

In contrast to the findings in this study, Adu-Gyamfi and colleagues, (2012) from Accra, Ghana, in the effort to determine the microbiological quality of chicken sold at different retail outlets (Supermarkets, local markets and farms), found that even though hygienic conditions in supermarkets were generally better than those in local markets and farms, there was no significant difference ($P>0.05$) between the mean total viable count, total coliform counts and *Staphylococcus aureus* counts for these retail groups. The different retail outlets all had low microbial counts (Adu-Gyamfi *et al*., 2012). The study

attributed this observation to the efforts in improving the hygienic procedures in the processing of poultry over the years. Such efforts included de-contaminating chicken meat by addition of chemicals to the processing water, irradiation by exposing the chicken to ionising radiations as well as use of improved storage methods such as freezing in most of these retail outlets in addition to the prevailing hygienic conditions that were already observed.

5.2 Prevalence of antimicrobial resistant *E. coli* isolated from raw retail chicken

In this study, 75% of the *E. coli* isolated exhibited resistance to at least one of the 12 antibiotics that were tested. Similar prevalence of antibiotic resistance in *E. coli* isolated from retail chicken have been observed in other studies ranging from 40.6% in Japan, 52% in Iceland, 84.6% in Minneapolis, U.S.A, 83.8% in Vietnam and even 100% isolates in Senegal being resistant to one or more antibiotics (Ashraf *et al*., 2009; Thorsteinsdottir *et al*., 2010a; Van *et al*., 2007; Johnson *et al*., 2005; Fofana *et al.*, 2006). Such prevalences are alarming given the evidence of possible transmission of antibiotic resistant food borne bacteria to consumers and food preparers (Bester and Essack, 2010).

Presence of antimicrobial resistant *E. coli* in retail chicken meat is just one aspect of the much wider public health problems associated with antibiotic resistance in bacteria. In an effort to determine whether there was a difference in antimicrobial resistance between different types of food samples, Johnson *et al*., (2005) found poultry having the highest prevalence of resistant *E. coli* compared to the rest of the food groups. This shows the important role that chicken plays as a reservoir of resistant bacteria that may spread to the human population.

The current study demonstrated higher prevalence of resistance to certain antimicrobial agents such as tetracycline (60.3%), SXT (49.4%), streptomycin (30.1%) and ampicillin

(34.0%). Other studies in Japan, Vietnam, Saudi Arabia and Slovakia on *E. coli* in retail chicken have also shown high prevalences of resistance to these commonly available antimicrobials (Ashraf *et al*., 2009; Al-Ghamdi *et al*., 1999; Ruzauskas *et al*., 2010; Thangh Huong *et al.*, 2009). These resistance phenotypes observed in *E. coli* are of great clinical significance since these antimicrobials are considered to be among the frontline therapeutic drugs for treatment of most bacterial infections in humans (Todar, 2012). In Kenya, where alternative choices for treatment are either too expensive or unavailable, this poses a major public health challenge.

One reason that could explain the high prevalence of resistance in *E. coli* to these antimicrobials could be because of their frequent use in live chicken for therapeutic purposes as well as enhanced growth promotion. Mitema and associates (2001) made an assessment of antimicrobial consumption in food producing animals in Kenya and were able to show that tetracyclines, sulphonamides and trimethoprins, nitrofurans, aminoglycosides, beta lactams and the quinolones were the most commonly used drugs in food producing animals. Tetracyclines and sulphonamides/trimethoprin topped the list in popularity and accounted for nearly 78% of the use. Nonga and colleagues (2009) in Tanzania showed similar results.

Such use has been shown to result in the development of resistant bacteria which can then reach heavily exposed individuals such as slaughter house workers, food handlers and farmers who feed the animals with the antimicrobials (Hammerum and Heuer, 2009). These resistant bacteria can then easily contaminate the carcasses of food animals along the production line to the retail outlets. Hammerum and Heuer (2009) also reviewed findings from other studies regarding *E. coli* from the animal reservoir that carry resistance to antimicrobial agents and revealed that since the 1990's, Extended Spectrum Beta Lactamase producing *E. coli* (ESBL's) have been detected in retail meat and production animals in Europe, Asia, Africa and the United states and attributed this

observation to the use of 3rd generation cephalosporins in food animals. They also showed that fluoroquinolone resistance could arise in the intestines of poultry from susceptible *E. coli* ancestors and subsequently be transmitted to humans via the food chain.

In contrast to the current study, Ashraf and colleagues in Japan observed a high resistance involving antibiotics which are considered to be critically important for human health care following failure of first line antimicrobials. The study showed much higher resistances towards ampicillin (73.9%), the aminoglycosides (streptomycin (66.6%), Kanamycin (55.1%), Gentamicin (7.2%)), the Quinolones and fluoroquinolones (Nalidixic acid (47.8%) and ciprofloxacin (30.4%)), the beta-lactamase inhibitor Augmentin (14.5%) and even the 3rd generation cephalosporin, Ceftazidime (13%) (Ashraf *et al*., 2009). It has been documented (Turnidge, 2003) that there is an indiscriminate use of antibiotics in Japan in both food animals and humans and this could be the reason for this difference.

It is interesting to note that studies in Japan, China, Slovakia and Lithuania all characterising antimicrobial resistance on *E. coli* isolated in live and raw retail chicken show that a high number of *E. coli* (>50%) have a high resistance to fluoroquinolones and this has been associated to widespread use of quinolones and fluoroquinolones especially as feed additives (Ashraf *et al*., 2009; Dai *et al*., 2008; KmeŤ and KmeŤova, 2010; Lei *et al*., 2010; Ružauskas *et al*., 2010; Thangh Huong *et al*., 2009; Yang *et al*., 2004). This study however, observed low resistance levels towards fluoroquinolones as compared to the rest (14.7% for Nalidixic acid and 4.5% for Ciprofloxacin). This might be due to the fact documented by Mitema and colleagues (2001) in a study in Kenya that showed use of quinolones and cephalosporins is not common in animal food production.

5.4 Association between antimicrobial resistance and retail outlet classification

There was no significant difference in distribution of the resistance phenotype among the 5 classifications of retail outlets (χ^2n-1= 4.178; d.f= 4; p= 0.382). However, *E. coli* isolated from samples from supermarkets showed a high frequency of antimicrobial resistance (84.6%) while isolates from samples from low income areas had the least frequency of resistance (62.5%). This might be hypothetically attributed to the critical link that exists between antimicrobial consumption in food animals and subsequent antimicrobial resistance in bacteria isolated. Two studies, one from Uganda and the other from Iceland (Majalija *et al*., 2010; Thorsteinsdottir *et al*., 2010b), on antimicrobial resistance in *E. coli* isolated from food animals have shown that broiler chicken production has been associated with a high prevalence of antimicrobial resistance in isolates from these chickens due to consumption of antimicrobials.

Even though numbers were not considered, the current study observed that most of the samples from supermarkets were improved breeds of chicken/broilers while most samples from low income areas were from local/indigenous chicken breeds. It is a common practice for broiler chicken producers in Kenya to include additives in the commercial feeds of broiler chicken (Mitema *et al*., 2001).

Several classes of antibiotics are included as additives for growth promotion and prevention of infectious diseases (Butaye *et al*., 2003; Singer and Hofacre, 2006). These antibiotics improve feed conversion and body weight gain, presumably by altering the composition and activities of micro flora, such that a one day old bird is able to grow fast and be marketed within a period of less than 2 months (Knarreborg *et al*., 2002). This practice may modify the intestinal flora and create a selective pressure in favor of resistant bacteria (Diarra *et al*., 2007).

On the other hand, indigenous chicken production in most African countries including Kenya are traditionally based on free range breeding systems where birds scavenge for feed around the home environment and at times they may even be given home food left-overs. If the farmer can afford it, they are provided with supplementary feed like maize grains, cassava, sweet potatoes or commercial feed (Nyaga, 2007). As estimated by Upton (2004) they reach a mature and marketable age by 225 days or almost 8 months. Therefore, indigenous chicken breeds are less exposed to undue antimicrobial consumption as compared to improved breeds.

The current study suggests that this may explain why *E. coli* in samples from most low income area butcheries that were a market for only indigenous chicken showed least tendency to antimicrobial resistance. A more comprehensive study is required to establish this hypothesis.

5.5 *E. coli* isolates harbouring virulence markers

Chicken meat contaminated with pathogenic bacteria is a big health concern as poultry contribute substantially to the human diet as an important source of animal protein thus has a direct impact on human health. Pathogenic *E. coli* that contaminates chicken results in significant numbers of diarrheagenic food poisoning cases as well as extra intestinal diseases (Hazell, 2012; Rane, 2011). The current study recorded 40.4% of the *E. coli* contaminants that carried at least one of the 10 virulence genes tested and specific for 5 known diarrheagenic *E. coli.* Of the *E. coli* positive for virulence genes in this study, ETEC was the most common pathogenic *E. coli* (61%) followed by EPEC (21%), STEC (8%), EIEC (6%) and EAEC (5%).

Similar studies also showed presence of *E. coli* contaminants harbouring virulence genes in fresh meat. These include studies done in Burkina Faso with 43% pathogenic *E. coli*, Korea (14%) and Lebanon (14%) (Kagambega *et al*., 2012; Lee *et al*., 2009; El-Rami *et*

al., 2012). Results from the studies mentioned above were also similar in showing that ETEC, EPEC and STEC were the most common diarrheagenic *E. coli* detected among poultry *E. coli* contaminants.

A reason for these observations could be because ETEC, EPEC and STEC are much frequently implicated in various food and water borne diseases and they are known contaminants of meat and meat products (CDC, 2012; Feng *et al*., 2011). A Canadian study also showed that live chicken and other food animals are known reservoirs of these pathogenic *E. coli* therefore contamination could actually be from the animal during evisceration or even from water used during their processing (Bergeron *et al*., 2012).

On the other hand EIEC and EAEC are not implicated much in food and water borne illnesses and there are no known animal reservoirs for these pathogens hence any primary source of contamination appears to be infected humans (Feng *et al*., 2011; Smith *et al*., 2004). Another reason for this observation is that ETEC had several virulent gene markers and thus could be easily detected more than the others. In any case this observation might indicate poor sanitation at any stage of production and processing of the retail chicken which needs to be thoroughly investigated and addressed.

5.6 Association between antibiotic resistance and virulence genes in *E. coli* isolated

The interplay between virulence and antibiotic resistance in *E. coli* is an area that still deserves much study. Findings from research published to date indicate there is a strong link between resistance and virulence (Johnson and Nolan, 2009). The majority of virulence associated plasmids in *E. coli* belong to the F incompatibility group that performs transfer functions and often carry antimicrobial resistance determinants as well and this may explain co-transfer of virulence and resistance factors when gut flora including *E. coli* are under antimicrobial selective pressure (Villa *et al*., 2010). If

virulence genetic determinants are located on the same genetic platform as antimicrobial resistance genes such as plasmids, they may be co-mobilized. Chicken and pork *E. coli* isolates have been shown to exhibit a higher degree of resistance and more virulence genes than any other meat. Some multi-resistant strains have even been shown to carry from 6-9 virulence genes (Ewers *et al*., 2009; Hao-Van *et al*., 2008).

A good example is EHEC (*E. coli* O157:H7), which is an *E. coli* pathotype, that has known virulent strains. Due to its virulence potential, it is one of the most threatening serotypes and yet it is known to exhibit a high degree of antibiotic resistance (De Silva and Mendonca, 2012). Several studies from Asia, Europe and North America have shown that *E. coli* O157 co-expresses both resistance genes and virulence determinants but little is known on the genetic mechanism facilitating the acquisition and transmission of both traits at the same time (Ateba & Bezuidenhout, 2008; Galland *et al*., 2001; Mora *et al*., 2005; You *et al*., 2006).

In the current study however, there was no significant association between antibiotic resistance and presence of virulence genes (χ^2n-1= 1.018; d.f= 4; p= 0.313). On the contrary *E. coli* isolates that did not carry any virulence genes had more resistance (78.5%) compared to the isolates that had virulence genes (71.4%) but the difference was not significant. Conflicting data such as the one in this study on the association of virulence and antibiotic resistance have also been published and have shown that at least in certain strains, a co-relation between these two factors is not significant. For example Orden and colleagues in a study from Argentina in STEC, isolated from diarrheic dairy calves, were shown to exhibit very low degrees of multidrug resistance. They also showed that Necro-Toxigenic *E. coli* (NTEC) with low multidrug resistance has been shown to concurrently express many virulent determinants (Orden *et al*., 2000).

Conversely, another strain which showed very high level of multidrug resistance was detected among low frequency virulent strains suggesting that low frequency of virulence factors may be associated with a high multiple resistance to antibiotics (Cid *et al*., 1996). Johnson and colleagues (2006) compared similarities between human and chicken *E. coli* isolates in relation to ciprofloxacin resistance and found that antibiotic susceptible human *E. coli* isolates contained more virulence associated genes and more frequently expressed virulence-associated O antigens than did resistant human or chicken *E. coli* isolates. A study by Blaquez and associates (1999) concluded that in bacteria, increasing the number of virulence factors will increase the antimicrobial resistance until a certain limit and then the resistance decreases and sensitivity increases.

This was also confirmed by Marwa *et al.*, (2012) who that showed antibiotic resistance was increased by increasing the detected virulence genes until a certain cut-off limit was reached from which the bacteria then starts to become totally susceptible. Where isolates had none, 1 or 2 virulence genes, they showed a high degree of antibiotic resistance. However, this had a cut-off of 2 virulence genes as isolates with 3 virulence genes were more sensitive and those with 4 virulence genes were totally susceptible to all tested antibiotics. The study went on to explain that when bacteria acquire either virulence and/or resistance genes, it leads to bacterial fitness. But if the bacteria continue to acquire these resistances, it may lead to a lethal amount of mutations or genetic re-arrangements that reduces the bacterial fitness and affect the bacterial virulence by loss of genes (Anderson and Hughes, 1996). The current study showed similar patterns as all isolates that were resistant to more than 7 antibiotics were all found negative for virulent genes, while most isolates that were resistant to fewer antibiotics were found to carry virulent genes.

Although this current data can be viewed as re-assuring since it shows no association between virulence and resistance, it is still evident that virulent and resistant *E. coli* are

being disseminated via the food chain by means of contaminated raw retail chicken meat. This can be hazardous because these bacteria combine pathogenic potential with resistance to first line therapeutic agents. It is also evident that non-virulent/commensal *E. coli* have a high potential of disseminating resistance genes to the same first-line therapeutic agents.

5.7 Co-transfer of antimicrobial resistance

In the current study, 55% of donor *E. coli* that exhibited resistances to at least 3 antibiotics were able to transfer antimicrobial resistance to sensitive recipient *E. coli* bacteria. This has also been described in other studies which have shown the same antimicrobial genes detected in isolates from both animal and/or meat are detected in human isolates thus suggesting horizontal transfer (Schjorring and Krogfelt, 2010; Corpet, 1993). Studies in Germany and by researchers in California University on horizontal gene transfer showed that ampicillin and kanamycin resistances were especially highly transferrable and common in Gram negative bacteria such as *E. coli* (Smalla *et al*., 1993; Barlow, 2009). A thesis by Naliaka from Kenyatta University to determine the possibility of transfer of resistance genes from resistant *E. coli* in broiler chicken, also showed that at least 38% of isolates underwent successful conjugation when subjected to such experiment and transferred their resistance to non-resistant recipient bacteria (Naliaka, 2011).

Using animal models, researchers have also shown that the intestines are especially a hot spot for the transfer of resistance genes between *E. coli*. For example, transfer of resistance from *E. coli* of animal source to *E. coli* of human source in the intestines of chicken and mice has been well demonstrated (Hart *et al*., 2006). Furthermore, transfer of resistance between *E. coli* has even been demonstrated in the intestines of humans whereby gene transfer is detected a few days after ingestion of the donor strain by human volunteers (Smith, 1969; Trobus *et al*., 2008). These studies all demonstrate a

high probability of transfer of resistance genes between *E. coli* of animal/ chicken origin and human origin in the intestine.

Plasmid content analysis of donors via gel electrophoresis showed that all 56 multi-drug resistant donors used in the conjugation experiments contained more than one plasmid varying in molecular sizes of 3.0-53.7 Kb. The donors that transferred their resistance phenotypes to trans-conjugants can be said to have contained transferrable/conjugative plasmids and were able to transfer their resistance determinants via conjugation using these plasmids. Studies have shown the presence of transferrable plasmids and high transfer potential in multi-drug resistant bacteria, including *E. coli* as evidenced by similarities in antimicrobial resistance patterns observed between bacteria of the same and even different species. For example, retail chicken wings sold in Halifax, Canada contained *E. coli* isolates carrying CMY-2 bearing plasmids responsible for resistance towards cephalosporins as well as other resistance genes that can be transferred to other pathogens (Forward *et al*., 2004).

Similarities were found between the CMY-2 plasmids in these *E. coli* and the CMY-2 plasmids in *Salmonella* isolates suggesting that the CMY-2 plasmids were transmitted between the 2 genera of bacteria. Another study revealed *E. coli* isolates carrying varying amounts of plasmid numbers, of up to 8 plasmids, with each plasmid containing antibiotic resistance genes to various antibiotics (Nariman *et al*., 1998). The study by Nariman interestingly noted that majority of the *E. coli* strains isolated that carried a low number of plasmids were resistant to most of the antibiotics tested while strains that carried high number of plasmids were susceptible to most of the antibiotics tested. This observation was also seen in the current study.

Plasmid were observed in some but not all trans-conjugants even though the antimicrobial resistance profiles showed the trans-conjugants had indeed received

resistance phenotypes to antibiotics from the donors. Out of 31 trans-conjugants, only 17 showed at least one plasmid band each when observed in the gel after gel electrophoresis. Results such as these, whereby plasmids can be observed in donors but cannot be observed in successive trans-conjugants even though resistant determinants were transferred, could be explained by a couple of factors.

The trans-conjugant might have received limited copy number plasmids/stringently controlled plasmids thus interfering with efficiency of replication. Having a low copy number plasmid also makes it difficult to purify the plasmid away from the chromosomal DNA or to observe it on the gel (Watve *et al*., 2010; Del Solar and Espinosa, 2000).

In addition, other mobile genetic elements known as integrative and conjugative elements (ICE's) that also mediate horizontal gene transfer and disseminate resistance genes via conjugation could be responsible for the resistance gene transfers. These elements are self-transmissible (promote their own mobilization) and are increasingly recognized to contribute to lateral gene flow in prokaryotes. ICE's integrate into the donor/host bacterial genome but have intricate regulatory systems that control excision of the ICE from the chromosome and then like conjugative plasmids, disseminate by conjugative transfer to new host transferring both antimicrobial resistance genes and even virulence factors (Burrus and Waldore, 2004; Wozniak and Waldor, 2010).

Several entities discovered more than a decade ago that had previously been classified as plasmids or conjugative transposons have now been defined as ICE's (Te Poele *et al*., 2008). Therefore, presence of the resistant phenotype in these trans-conjugants from the donor bacteria could be as a result of these Integrative and Conjugative Elements which could not be seen in the gel. This is an interesting area that deserves special attention to determine which of the two elements plays a major role in gene transfer through

conjugation. Finally, the plasmids might have been super coiled or too small to be detected in the gel. (Actis *et al*., 2000).

5.8 Conclusions

I. The study found that raw chicken meats sold in retail meat outlets in Nairobi, Kenya have high contamination levels by *E. coli* and other coliform bacteria and therefore are prone to quick spoilage and are a significant source for the acquisition of pathogenic bacteria. Even though the sources of contamination were not conclusively determined, it is speculated that cross contamination could have occurred at any point between the farms to the retail continuum. The study demonstrated that in terms of contamination levels, supermarkets are the best retail source of purchasing raw chicken meat as their samples had lower microbial loads (which fell under acceptable ranges) compared to the rest of the retail outlets.

II. The study also concluded that there exists high antimicrobial resistance among *E. coli* isolates in the chicken meat sold in various retail outlets in Nairobi and that meat from supermarkets, regardless of their low microbial counts, can be hypothetically implicated to have isolates with the highest prevalence in antimicrobial resistance.

III. Chicken meat is one of the highest but under recognized risk-associated food source in terms of contamination by both antibiotic resistant and virulent *E. coli* that are able to disseminate these elements to other bacteria and subsequently result to an increase to the already high antimicrobial resistance burden in the population.

5.9 Recommendations

I. Since contamination of retail chicken meats can occur at any stage of production, the study recommends that each sector in chicken production should develop and

apply efficient hygiene programmes which will be specifically adapted to their relevant range of production. This includes measures that will guide in prevention of microbial contamination from the beginning of production (slaughter hygiene), minimizing growth in the middle of production (meat cutting and transportation) and reducing or eliminating contamination at the final processing stage (hygiene in the retail market).

II. The government should put extra effort in making sure that the bio-safety rules that encompass all municipal and privately owned slaughter houses to be strictly followed and adhered to by individuals who carry out home slaughter.

III. Prudent use of antimicrobials in food animals should be prioritized in order to preserve these valuable drugs for use in both animals and human health.

IV. Continued and more rigorous surveillance of antimicrobial resistance in raw meats such as chicken is urgently needed to establish trends of antimicrobial resistance in Nairobi and the country at large to prevent the progressive carriage of these resistance genes and minimize the likelihood of horizontal gene transfer to other bacteria.

V. More studies should be carried out in ICE's to determine how big a role they play in the transfer of genes from one bacteria to another.

VI. More studies in Kenya with comprehensive and substantial collections of chicken samples and more investigations on risk factors urgently need to be done and obtained data well documented in order to establish the situation on the overall burden of contamination and antimicrobial resistance among *E. coli* from raw retail chicken for risk assessment purposes as these bacteria together with their mobile genetic elements carrying resistance and even virulence genes are able to be transferred to the human population through such food products.

VII. The dynamics of the association between antimicrobial drug resistance and presence of virulence genes is a complex area that still requires much research.

There is still a gap in understanding the genetic background of antibiotic resistance and virulence of enteric bacteria from chicken or any other food. Therefore the study recommends that this area be paid attention to.

5.10 Limitations of the study

Due to time and financial constraints, the exact source/sources of contamination by *E. coli* and other coliform bacteria were not clearly defined in this study.

REFERENCES

Aarestrup, F. M., Seyforth, A. M., Emborg, H. D., Pedersen, K., Hendriksen, R., and Bager F. (2001). Effects of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in faecal *enterococci* from foods in Denmark. *Antimicrobial agents and chemotherapy*, **45**, 2054-2059.

Actis, L., Tolmasky, M., and Crosa, J. (2000). Bacterial plasmids: Replication of extra chromosomal genetic elements encoding resistance to antimicrobial compounds. *Frontiers in Bioscience*, **4**, 43-62.

Adesiji, Y. O., Alli, O. T., Adekanle, M. A., and Jolayemi J. B. (2011). Prevalence of *Arcobacter, Escherichia coli, Staphylococcus aureus* and *Salmonella* species in Retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research*, **3**, 8-12.

Adu-Gyamfi, A., Torgby-Tetteh, W., and Appiah, V. (2012). Microbiological quality of chicken sold in Accra and determination of D10-value of *E. coli*. *Journal of Food and Nutrition Sciences*, **3**, 693-698.

Afullo, A., and Odhiambo, F. (2009). The primary solid waste storage gaps experienced by Nairobi households. *Ethiopian Journal of Environmental Studies and Management*, **2**, 3

Algert, S. J., Agrawal, A., and Lewis D. S (2006). Disparities in access to fresh produce in low income neighborhoods in Los Angeles. *American Journal of Preventative Medicine*, **30**, 365-370.

Al-**Ghamdi, S. A., El-Morsy, F., Al-Mustafa, Z. H., Al-Ramadhan, M., and Hanif, M. (1999).** Antibiotic resistance of *Escherichia Coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. *Journal of Tropical Medicine and International Health*, **4**(4), 278-283.

Amara, A., Ziani, Z., and Bouzoubaa K. (1995). Antibiotic resistance of *Escherichia coli* strains isolated in Morocco from chickens with coccilobacillosis. *Veterinary Microbiology*, **43**, 325–330.

Anderson, D. I. D., and Hughes. (January 23rd 1996). Muller's ratchet decreases fitness of DNA based microbes. *Proceedings of the National Academy of Sciences*, U.S.A. **93**, 906-907.

Anderson, K. L., Whitlook, J.E., and Harwood, V.J. (2005). Persistence and differential survival of faecal indicator bacteria in subtropical water and sediments. *Applied and Environmental Microbiology*, **71**(6), 3041-3048.

Andrea, W. (July 25th 2010). Mobile slaughter houses will help small farmers. *News observer*, p 3.

Andrew, W. H., and Hammack, T. S. (2003). *Food sampling and preparation of sample homogenate: Bacteriological Analytical Manual (8th Ed) Revision A*. Retrieved June 20th 2011 from the Food and Drug Administration (FAO) website: http://www.fda.gov/food/scienceresearch.

Angulo, F. J., Nargund, V. N., and Chiller T. C, (2004). Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of Veterinary Medicine*, **51**, 374–379.

AOAC, (2006). *Official methods 966.24 for enumeration of Escherichia coli and Coliform bacteria in raw meats*. Retrieved on January 1st 2012 from Journal of AOAC international website: http://www.3M.com/microbiology

Apata, D. F. (2009). Antibiotic resistance in poultry. *International Journal of Poultry Science*, **8**, 404-408.

Ashraf, M. A., Hirofumi, S., and Tadashi, S. (2009). Isolation and molecular characterization of multidrug resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in Japan. *Journal of food science*, **74**, 7.

Ateba, C. N., and Bezuidenhout, C. C. (2008). Characterization of *Escherichia coli* O157 strains from humans, cattle and pigs in North-west province, South Africa. *International Journal of Food Microbiology*, **128**,181-188.

Bager, F. (2000). DANMAP: Monitoring antimicrobial resistance in Denmark. *International Journal of Antimicrobial Agents*, **14**(4), 271-274

Barlow, M. (2009). What antimicrobial resistance has taught us about horizontal gene transfer. *Methods in Molecular Biology*, **532**, 397-411.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turk, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**, 493–496.

Belluck, P. (1997). Federal inspectors seek source of *Escherichia coli* contamination. *Newyork times*, p 1.

Bergeron, C. R., Prussing, C., Boerlin, P., Daignault, D., Dutil, L., Reid-Smith, R., and other aouthors (2012). Chicken as reservoir for extra-intestinal pathogenic *Escherichia coli* in humans, Canada. *Emerging Infectious Disease Journal*, **18,** 3.

Bester, L. A., and Essack, S. Y. (2010). Antibiotic resistance via the food chain: Fact or fiction? *South African Journal of Science*, **106**(9/10), 1-5.

Bilgili, S. F. (1999). Assuring the safety of poultry products in a global market: The US perspective. *14th European symposium on quality of poultry meat*, 14, 513-519.

Blanc, V., Mesa, R., Saco, M., Lavilla, S., Prats, G., Miro, E., Navarro, F., Cortes, P., and Llagostera, M. (2006). ESBL and plasmidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Veterinary Microbiology*, **118**, 299–304.

Blaquez, R., Menasalvas, A., Carpena, I., Ramirez, C., Guerrero, C., and Moreno, S. (1999). Invasive diseases caused by Ciprofloxacin resistant Uro-pathogenic *Escherichia coli*. *European Journal of Clinical Microbiology and infectious Disease*, **18**, 503-505.

Borche E, Arinder P (2002). Bacteriological safety issues in beef and ready to eat meat products as well as control measures. *Meat Science*, **62**(3), 381-390.

Brill, R. (2007). Tiny bacteria loom large over processes of life. *Honolulu Star Bulletin*, **12,** 77.

Burrus, V., and Waldor, M.K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology*, **155** (5), 376-386.

Busani, L., Scavia, G., Luzzi, I., and Caprioli, A. (2006). Laboratory surveillance for prevention and control of food-borne zoonoses. *Annual 1st super Sanita*, **42**, 401-404.

Butaye, P., Devriese, L. A., and Haesebrouck, F. (2003). Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *Journal of Clinical Microbiology*, a review, **16**, 175-188.

Byappanhalli, M., Flowler, M., Shively, D., and Whitman, R. (2003). Ubiquity and persistence of *Escherichia coli* in a Midwestern costal stream. *Applied Environmental Microbiology*, **69** (8), 4549-4555.

Catry, B., Laevens, H., Devriese, L., Opsomer, G., and De Kruif, A. (2003). Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapy*, **26**, 81–93.

Chang, H. S. (2004). Overview of the world broiler industry: implication for the Philippines. *Asian Journal of Agriculture and Development*, **4**, 2.

CDC, (2012). *Escherichia coli*: General information. Retrieved from http://www.cdc.gov/e.coli/general

Cid, D., Piriz, S., Ruiz-Santa-Quiteria, J. A., Valle, J., Vadillo, S., and Dela Fuento, R. (1996). Invitro susceptibility of *Escherichia coli* strains isolated from diarrheagenic lambs and goat kids to 14 antimicrobial agents. *Journal of veterinary Pharmacology and therapy*, **19**, 397-401.

Clinical Laboratory Standards Institute, CLSI. (2012). Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. *CLSI document M100-S22* [Electronic]. Wayne Pennsylvania: USA.

Cohen, N., Ennaji, H., Bouchrif, B., Hassar, M., and Karib, H. (2007). Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca, Morocco. *Poultry Science Association*, **16**, 502-504.

Coia, J. (1998). Clinical, microbiological and epidemiological aspects of *Escherichia coli* O157. *FEMS: Immunology and Medical Microbiology,* **20**(1), 1-9.

Collignon, P., Wegener, H. C., Braam, P., and Butler, C. D. (2003). The Routine Use of Antibiotics to Promote Animal Growth Does Little to Benefit Protein Under nutrition in the Developing World. *Clinical Infectious Diseases,* **41** (7), 1007-1013.

Corpet, D. E. (1993). An evaluation of methods to assess the effect of antimicrobial residues on the human gut flora. *Veterinary Microbiology*, **35**, 199-212.

Costa, D., Poeta, P., Saenz, Y., Coelho, A. C., Matos, M., and Vinue´, L. (2008). Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Veterinary Microbiology*, **127**, 97–105.

Dai, L., Lu, L. M., Wu, C. M., Li, B. B., Huang, S. Y., Wang S. C., Qi, Y. H., and Shen, J. Z. (2008). Characterization of antimicrobial resistance among *Escherichia coli* isolates from chickens in China between 2001 and 2006. *FEMS Microbiology letters*, **286,** 178-183.

De Silva, G. J., and Mendonca, N., (2012). Association between antimicrobial resistance and virulence in *Escherichia coli*. *Landes Biosciences*, **3**(1), 18-28.

Del Solar, G., and Espinosa, M. (2000). Plasmid copy number control: an ever growing story. *Molecular Biology*, **37**, 492-500.

Dessen, A., Di Guilmi, A. M., Vernet, T., and Dideberg, O. (2001). Molecular mechanisms of antibiotic resistance in Gram-positive pathogens. *Current Drug Targets and Infectious Disorders*, **1**(1), 63.

Diarra, M. S., Silversides, F. G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., and other authors (2007). Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium pefringens* and *Enterococcus* counts and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. *Applied and Environmental Microbiology*, **73**(20), 6566-6576.

Ehrt, S., and Schnappinger, D. (2003). Isolation of plasmids from *E. coli* by alkaline lysis: Methods in molecular biology. *Springer protocols*, **235**, 75-78.

El-Rami, F. E., Rahal, E. A., Sleiman, F. T., and Abdolnoor, A. M. (2012). Identification of virulence genes among antibacterial resistant *Escherichia coli* isolated from poultry. *Advanced Studies in Biology*. **4**(8), 385-396.

Ewers, C., Antao E. M., Diehl, I., Phillips, H. C., and Wieler, L. H. (2009). Intestines and environment of the chicken as reservoirs for extra-intestinal pathogenic *Escherichia coli* strains with zoonotic potential. *Journal of Applied and Environmental Microbiology*, **75**, 184-192.

FAO, (2008). *Principles of meat processing hygiene and regulatory practices* [data file]. Retrieved from: http://faostat.org/docrep.

FDA, (2001). *The Human Health Impact of Fluoroquinolone Resistant Campylobacter Attributed to the Consumption of Chicken*. Retrieved from *http://www.fda.gov*.

Feng, P., Stephen, D. W. and Jinneman, K. (2011). *Diarrheagenic Escherichia coli: Bacteriological analytical manual*. 8th Ed, Review A. Retrieved from: *http://www.fda.gov*

Fey, P. D., Safranek, T. J., Rupp, M. E., Dunne, E. F., Ribot, E., Iwen, P. C., Bradford, P. A., Angulo, F. J., and Hinrichs, S. H. (2000). Ceftriaxone-

Resistant *Salmonella* Infection Acquired by a Child from Cattle. *New England Journal of Medicine*, **342** (17), 1242.

Fishers, R. A. and Bennet, J. H. (Eds) (1995). *Statistical methods, experimental design and scientific inference*. 14th Ed. *Oxford university press*, New York. 42-45.

Fofana, A., Bada, A. R., Seydi, M., and Akakpo, A. J. (2006). Antibiotic resistance of *Escherichia Coli* strains isolated from raw meat in Senegal. *Dakar Medical*, **51**(3), 145-150.

Food Safety and Inspection Service, (1996). Guidelines for *Escherichia coli* testing for process control verification in poultry slaughter establishment: HACCP guidelines and policies.Retrieved November 3rd 2012 from the Food Safety and Inspection Service website: http://www.fsis.usda.gov.

Forward, K. R., Matheson, K. M., Hiltz, M., Musgrave, H., and Poppe, C. (2004). Recovery of cephalosporin-resistant *Escherichia coli* and *Salmonella* from pork, beef and chicken marketed in Nova Scotia. *Canadian Journal of Infectious Diseases and Medical Microbiology*, **15**(4), 226-231.

Fratamico, P. M., and Smith, J. L. (2006). *Escherichia coli* infections In Riemann in Cliver, D.O (Ed) *Food-borne infections and intoxications*, 3rd ed Florida: *Elsevier Inc. Academic Press*.

Fujioka, M., Kasai, K., Miura, T., Sato, T., and Otomo, Y. (2009). Rapid diagnostic method for the detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Japan Journal of Infectious Disease*, **62**, 476-480.

Galland, J. C., Hyatt, D.R., Cropper, S. S., and Acheson, D.W. (2001). Prevalence, antibiotic susceptibility and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. *Journal of Applied and Environmental Microbiology*, **67**, 1619-1627.

Garcia, E. A. (2002). Animal health and food borne pathogenic *entero*-haemorrhagic O157:H7 strains and other pathogenic *Escherichia coli* viro-types (EPEC, ETEC, EIEC, EHEC). *Polish Journal of Veterinary Sciences*, **5**, 103-115.

Hammerum, A. M., and Heuer, O. E. (2009). Human health hazards from antimicrobial resistant *Escherichia coli* of animal origin. *Journal of Clinical and Infectious Diseases*, **48**, 916-921.

Hao-Van, T. T., Chin, J., Chapman, T., Tran, L. T., and Coloe, P. J. (2008). Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International journal of Food Microbiology*, **124**, 217-223.

Hart, W. S., Heuzenroeder, M. W., and Barton, M. D. (2006). A study of the transfer of tetracycline resistance genes between *Escherichia coli* in the intestinal tract of a mouse and chicken model. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*. **53**, 333-340.

Hazell, K. (2012). One in five chickens is contaminated with food poisoning bacteria. *Huffington post*, p 6.

Heymann, D. L (2004). Diarrhea caused by *Escherichia coli*. In, *Control of Communicable Diseases Manual*. (**18th ed**) (pp 160-162).

Hiko, A., Asrat, D., and Zewde, G. (2008). Occurence of *Esherichia coli* O157:H7 in retail raw meat products in Ethiopia. *Journal of Infections in Developing Countries*, **2**(5), 389-393.

Holt, J. G (1994). Identification of family Enterobacteriaceae. In, Sneath, P.H., Noel, R.K and Holt J. G (Eds): *Bergeys manual of determinative bacteriology*. (**9th ed**) (pp 154-155). Newyork: Lippincott, Williams & Wilkins

Hu, G. Z., Zhang, C. H., Yuan, L., Yang, Y. R., and Liang, J. (2005). Detection of b-lactamase and extended-spectrum b-lactamase of pathogens isolated from pig

and chicken and their antibiotic susceptibility test. *Agriculture and Science China*, **4**, 877–882.

International research network Kenya, (2005). Meat production in Kenya. Retrieved from *http://www.epzkenya.com*.

Jakabi, M., Gelli, D. S., Ristiri, C. A., De Paula, A. M. R., Sakuma, H., and Lopez, G. I. S. (2005). Presence of *Salmonella* species and *Escherichia coli* O157:H7 in raw meat in Sao Paolo city, Brazil and evaluation of low temperature (refrigeration and freezing) in resistance of these bacteria. *International Atomic Energy*, **16**, 35-42.

Johnson, J. R., Murray, A. C., Gajewski, A., Sullivan, M., Snippes P., Kuswoski M. A., and Smith K. E. (2003). Isolation and molecular characterization of Nalidixic acid resistant extra intestinal pathogen *Escherichia coli* from retail chicken products. *Antimicrobial agents and Chemotherapy*, **7**, 2161-2168.

Johnson, J. R., Kuskowski, M. A., Smith, K., O'Bryan, T. T., and Tatini, S. (2005). Antimicrobial resistant and extra intestinal pathogenic *Escherichia coli* in retail foods. *Journal of Infectious Disease*, **191**, 1040-1049.

Johnson, J. R., Kuskowski, M. A., Menard, M., Gajewski, A., Xercavins, M., and Garau, J. (2006). Similarity between chicken and human *Escherichia coli* isolates in relation to Ciprofloxacin resistance status. *Journal of Infectious Diseases*, **194**, 71-78.

Johnson, T. J., and Nolan, L. K. (2009). Patho-genomics of the virulence plasmids of *Escherichia coli*. *Journal of Microbiology and Molecular Biology*, **73**, 750-774.

K'Akumu, O. A., and Olima, W. H. A. (2007). The dynamics and implications of residential segregation in Nairobi. *Habitat International*, **31**, 87-99

Kariuki, S., Onsare, R., Mwituria, J., Ngetich, R., Nafula, C., Karimi, K., and other authors (2013). Improving food safety in meat value chains in Kenya. General interest paper: FAO/WHO project report. *Food protection trend*, **5**, 172-179

Kagambega, A., Martikainen, O., Lienemann, T., Siitonen, A., Traore, A. S., Barro, N., and Haukka, K. (2012). Diarrheagenic *Escherichia coli* detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. *International Journal of Food Microbiology*, **153**(1-2), 154-158.

Kelly, B. G., Vespermann, A., and Bolton, D. J. (2009b). The role of horizontal gene transfer in the evolution of selected food borne bacterial pathogens. *Food and Chemical Toxicology*. **47**, 951–968.

Kelly, B. G., Vespermann, A., and Bolton D. J. (2009a). Horizontal gene transfer of virulence determinants in selected bacterial food-borne pathogens. *Food and Chemical Toxicology*, **47**, 969–977.

Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association journal*, **159**(9), 1129–1136.

Kikuvi, G. M., Ole-Mapenay, I. M., Mitema, E. S., and Ombui, J.N. (2006). Antimicrobial resistance in *Escherichia coli* isolates from faeces and carcass samples of slaughter cattle, swine and chickens in Kenya. *Israel Journal of Veterinary Medicine*, **61**(3-4), 82- 88.

Kingori, A. M., Wachira, A. M., and Tuitoek, J. K. (2010). Indigenous chicken production in Kenya: A review. *International Journal of Poultry Science*, **9** (4), 309-316.

KmeŤ, V., and KmeŤova, M. (2010). High level of quinolones resistance in *Escherichia coli* from healthy chicken broilers. *Folia Microbiology*, **55**(1), 79-82.

Knarreborg, A., Simon, M. A., Engberg, R. M., Jensen, B. B., and Tannock, G. W. (2002). Effects of dietary fat source and sub therapeutic levels of antibiotics on the bacterial community in the ileum of broiler chickens at various ages. *Journal of Applied and Environmental Microbiology*, **68**, 5918-5924.

Koro, M. E., Anandan, S., and Quinlan, J. J. (2010). Microbial quality of food available to populations of differing socio economic status. *American Journal of Preventive Medicine: Elsevier Inc*, **20**(10), 30.

Lee, G. Y., Jang, H. I., Hwang, Z. G., and Rhee, M. S. (2009). Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry and pork in Korea. *International Journal of Food Microbiology*, **134**(3), 196-200.

Lei, T., Tian, W., He, L., Huang, X.H., Sun Y.X., Deng, Y.T., and other authors (2010). Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. *Veterinary Microbiology*, **146**, 85-89.

Lesorogol, C. K. (1998). *Life on the margins: Perspectives on Pastoralism and development in Kenya*. A paper submitted to USAID/Kenya.

Li, X. S., Wang, G. Q., Du, X. D., Cui, B. A., Zhang, S. M., and Shen, J. Z (2007). Antimicrobial susceptibility and molecular detection of chloramphenicol and florfenicol resistance among *Escherichia coli* isolates from diseased chickens. *Journal of Veterinary Science*, **8**, 243–247.

Lindqvist, R., Anderson, Y., De Jong, B., and Norberg, P (2000). A summary of reported food borne disease incidents in Sweden, 1992 to 1997. *Journal of Food Protection*, **63**, 1315-1320.

Liu, J. H., Wei, S. Y., Ma, J. Y., Zeng, Z. L., Lu, D. H., Yang, G. X., and Chen, Z. L. (2007). Detection and characterisation of CTX-M and CMY-2 β-Lactamases among *Escherichia coli*isolates from farm animals in Guangdong Province of China. *International Journal of Antimicrobial Agents*, **29**, 576–581.

Lynch, M., Painter, J., Woodruff, R., and Braden, C (2006). Surveillance of food borne disease outbreaks in United States, 1998 to 2002. *Morbidity and mortality weekly report, surveillance summaries/ CDC*, **55**, 1-34.

MacFaddin, J. F. (2000). *Biochemical Tests for Identification of Medical Bacteria*. (**3rd *ed***) (363-36). Philadelphia: Lippincott Williams and Wilkins.

Majalija, S., Oweka, F., Wito, G. S., Lubowa, M., Vudriko, P., and Nakamaya, F. M. (2010). Antibiotic susceptibility profiles of faecal *Escherichia coli* isolates from dip-litter broiler chickens in Northern and Central Uganda. *Journal of Veterinary Research*, **3**(4), 75-80.

Marwa, E. A., Essam, T. M., and Anin, M. A. (2012). Involvement of virulence genes and antibiotic resistance in clinical and food borne diarrheagenic *Escherichia coli* isolates from Egypt. *World Journal of Medical Sciences*, **7**(4), 276-284.

Maurelli, A. (2006). Bacterial-epithelial cell cross-talk molecular mechanisms in pathogenesis in McCormick, B.(Ed), *Evolution of bacterial pathogens.* (pp 30–56). Cambridge: Cambridge University Press.

Mellon, M., Benbrook, C., and Benbrook K. L. (2001). Hogging It. Estimates of Antimicrobial Abuse in Livestock. Retrieved from Union of Concerned Scientist website *Cambridge:* http://www.ucsusa.org/foodandenvironment/antibioticsandfood/margaret-mellon-on-hogging-it.html.

Meng, J., Doyle, M. P., Zhao, T., and Zhao, S. (2001). Enterohemorrhagic *Escherichia coli*.In Doyle M.P., and Montville, T.J (Eds) *Food microbiology – Fundamentals and Frontiers*. 2nd ed (pp 193-213). Washington D.C: American Society for Microbiology.

Ministry of Agriculture, Livestock and Development-MALDM (2005/2006). Third livestock development project. Nairobi: Government printer.

Mitema, E. S., Kikuvi, G. M., Wegener, H. C., and Stohr, K. (2001). An assessment of antimicrobial consumption in food producing animals in Kenya. *Journal of Veterinary Pharmacology & Therapeutics*, **24**(6), 385-390.

Modestas, R., Rita, S., Ricardas, K., Marius, V., and Dainius, Z. (2010). Prevalence and antimicrobial resistance of *E. coli* isolated from chicken liver sold in retail markets. *Veterinarija Ir zootechnika*, **52**(74), 1392-2130.

Molbak, K. (2004). Spread of resistant bacteria and resistance genes from animals to humans – the public health consequences. *Journal of Veterinary Medicine*, **51**, 364–369.

Moldlab, (2003). Sewage \ Black-water: Contamination and *Escherichia coli*. Retrieved from: *http://www.moldlab.com/sewage*

Moniri, R., and Dastehgoli, K. (2005). Fluoroquinolone-resistant *Escherichia coli* isolated from healthy broilers with previous exposure to fluoroquinolones: is there a link? *Microbial Ecology in Health and Disease,* **17**, 69–74.

Moore, L.V., and Diez-Roux, A.V (2006). Associations of neighborhood characteristics with the location and type of food store. *American Journal of Public Health,* **96**, 325-331.

Mora, A., Blanco, J. E., Blanco, M., Alonso, M. P, Dhabi, G., and Ekheita, A. (2005). Antimicrobial resistance of shiga toxin (vero-toxin) producing *Escherichia coli* O157:H7 and non O157 strains isolated from humans, cattle, sheep and Food in Spain. *Research in Microbiology*, **156**, 793-806.

Musgrove, M. T., Jones, D. R., Northcutt, J. K., Cox, N. A., Harrison, M. A., Fedorka-Cray, P. J., and Ladely, S. R. (2006). Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. *Poultry Science,* **85**, 1665–1669.

Naliaka, A. (2011). *A comparative study of antimicrobial resistance profiles among enteric bacteria in broilers and traditional chicken from selected farms in Kericho, Kenya*. Unpublished MSc thesis, Kenyatta University, Kenya.

Nariman, A. H. A., Tharwat, A., and El-Baz, W. F. (1998). Plasmid- mediated antibiotic resistance in *E. coli*. *Arab Journal of Biotechnology*, **1**(1), 17-27.

Nasser, A. A., Haddad, A. A., Bahouh, M. A., and Mashally, M. (2006). *Principles of poultry bio-security program*. (pp 76-95). Kuwait: Kuwait institute for scientific research

Nataro, J., and Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, **11**, 142-201.

Nonga, H. E., Mariki, M., Karmurbo, E. D., and Mdegela, R. H. (2009). Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro municipality, Tanzania. *Pakistan journal of Nutrition*, **8**(3), 203-207.

Nyaga, P. (2007). Good bio-security practices in small scale commercial and scavenging production systems in Kenya. Retrieved from http://www.fao.org/docrep

Nyaga, P. (2008). The structure, marketing and importance of the commercial and village poultry industry: An analysis of the poultry sector in Kenya. Poultry sector country review. Retrieved from the Food and Agriculture Organization of the United Nations website: http://www.fao.org/avianflu/en/farmingsystems.html

Nzouankeu, A., Ngandjia, A., Ejenguele, G., Njine, T., and Wouafo, M. H. (2010). Multiple contamination of chickens with *Campylobacter*, *Escherichia coli* and *Salmonella* in Yaoundé, Cameroon. *Journals of Infections in Developing Countries*, **4**(9), 583-586.

O'Brien, T. F. (2002). Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Journal of Clinical and Infectious Diseases*, **34**(3), 78–84.

Ojeniyi, A. A. (1989). Direct transmission of *Escherichia coli* from poultry to humans. *Epidemiology information*, **103**, 513-522.

Ole Mapenai, L. M., Mitema, E., and Ombui, J. N. (2006). Studies of antimicrobials and occurrence of antimicrobial resistance in *Escherichia coli* in food animals in

Kenya and public health implication. Unpublished MSc. thesis. University of Nairobi, Kenya.

Orden, J. A., Ruiz-Santa-Quiteria, J. A.,Garcia, S., Cid, D., and De la Fuente, R. (2000). In-vitro susceptibility of *Escherichia coli* strains isolated from diarrheagenic dairy calves to 15 antimicrobial agents. *Journal of veterinary of veterinary medicine*, **47**, 329-335.

Paton, J. C., and Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical Microbiology Reviews*, **11** (3), 450–79.

Payne, J., and Sparks, D. (2009). *E. coli*: An overview. Stillwater, Oklahoma: Oklahoma state University. Retrieved from http://www.worldcat.org/title/e.coli-an-overview.

Pertersen, KE., and James, W.O (1998). Agents, vehicles and causal inference in bacterial food borne disease outbreaks, 1986 to 1995. *Journal of Antimicrobials and Veterinary Medicine Association*, **212**, 1874-1881.

Petersen, A., Christensen, J. P., Kuhnert, P., Bisgaard, M., and Olsen, J. E (2006). Vertical transmission of a Fluoroquinolone-resistant *Escherichia coli* within an integrated broiler operation. *Veterinary Microbiology*, **116**, 120–128.

Pointon, A., Sexton, M., Dowsett, P., Saputra, T., Kiermeier, A., Lorimer, M., and other authors (2008). A baseline survey of the microbiological quality of chicken portions and carcasses at retail in two Australian States, 2005 to 2006. *Journal of Food Protection*, **71**(6), 1123-1134.

Popkin, B. M. (2006). Technology, transport, globalization and the nutrition transition food poultry. *Journal of Food Poultry*, **10**, 1016.

Protocol online, (2001). Agarose gel electrophoresis - Basic method. Retrieved 8th June 2011 from the protocol online website: http://www.protocolonline.org/prot

Protocol online, (2003). Protocol for PCR using Taq DNA polymerase. Retrieved from: http://www.protocolonline.org/cgi-bin

Rane, S. (2011). Street vended food in developing world: hazard analysis. *India Journal of Microbiology*, **51**(1), 100-106.

Regula, G., Ursula, L., Roger, S., and Danuser, J. (2003). Risk factors for antibiotic resistance in *campylobacter* species isolated from raw retail poultry in Switzerland. *BioMed Central: Public Health*, **3**, 39.

Rivera, A. M., and Boucher, H. W (2011). Current concepts in antimicrobial therapy against select gram-positive organisms: Methicillin-Resistant-*Staphylococcus Aureus*, Penicillin-Resistant *Pneumococci* and Vancomycin Resistant *Enterococci*. *Mayo Clinic Proceedings*, **86**(12), 1230-1243

Rodriguez-Siek, K. E., Giddings, C. W., Doetkott, C., Johnson, T. J., Fakhr, M. K., and Nolan, L. K. (2005). Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology*, **151**, 2097-2110.

Ruzauskas, M., Šiugždinienė, R., Šeputienė, V.,Sužiedėlienė, V., Virgailis, M., Daugelavičius., and other authors (2010). The situation of antimicrobial resistance of enteric bacteria isolated from animal origin to quinolones and fluoroquinolones. *Veterinarija ir zootechnika*, **50**, 73-80.

Sackey, B. A., Mensah, P., Collison, E., and Dawson, E. S. (2001). *Campylobacter,Salmonella, Shigella* and *Escherichia coli* in live dressed poultry from metropolitan Accra. *International Journal of Food Microbiology*, **71**, 2128.

Saikia, P., and Joshi, S. R. (2010). Retail market poultry meats of North-East India: A microbiological survey for pathogenic contaminants. *Research Journal of Microbiology*, **5**(1), 36-43.

Scott, K. P. (2002). The role of conjugative transposons in spreading antibiotic resistance between bacteria that inhabit the gastrointestinal tract. *Cellular and Molecular Life Sciences*, **59**, 2071–2082.

Schjorring, S., and Krogfelt, K. (2010). Assesment of bacterial antibiotic resistance transfer in the gut. *International Journal of Microbiology*, **2011,** 1-10.

Shane, S. (2004). The challenges, successes and issues facing today's industry. *World Poultry,* **20** (2), 16-19.

Singer, R. S., and Hofacre, C. L. (2006). Potential impacts of antibiotic use in poultry production. *Journal of Avian Diseases*, **50**, 161-177.

Smalla, K., Overbeek, L. V., Pulrall, J. P., and Van-Elsas, J. D. (1993). Prevalence of nptII and Tn5 in Kanamycin resistant bacteria from different environments. *FEMS Microbial Ecology*, **13**, 47-58.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Catry, B., and other authors (2008). Diversity of extended-spectrum beta-lactamases and class C beta lactamases among cloacal *Escherichia coli* Isolates in Belgian broiler farms. *Antimicrobial Agents and Chemotherapy*, **52**, 1238–1243.

Smith, H. W. (1969). Transfer of antibiotic resistance from animal and human strains of *Escherichia coli* to resident *E. coli* in the alimentary tract of man. *Lancet,* **1**,1174–1176.

Smith, K. E., Besser, J. M., Hedberg, C. W., Leano, F. T., Bender, J. B., Wickland, J. H., and other authors *(*1999). Quinolone resistant *Campylobacter jejuni* infections in Minnesota. *The new England Journal of medicine*, **340** (20), 1525.

Smith, H., Willshaw, G., and Cheasty, T., (2004). *E. coli* as a cause of out breaks of diarrhoeal disease in the U.K. *MicrobiologyToday,* **31**,117-118.

Shohayeb, M., and Sonbol, F. (1994). Conjugal transfer of antibiotic resistance of *Shigella* isolated in Tanta. *Alexandria Journal of Pharmaceutical Sciences*, **8**, 177-180.

Su, H.P., Chu, S.I., Tsai, J.L., Lee, C.L., and Pan, T.M (2005). Bacterial food borne illness outbreaks in Northern Taiwan, 1995 to 2001. *Journal of Infection and Chemistry*, **11**, 146-151.

Taha, F. A. (2003). The Poultry Sector in Middle-income Countries and its Feed Requirements: The Case of Egypt. Outlook Report No. WRS03-02. Retrieved from: http://www.ers.usda.gov/publications.

Te Poele, E. M., Bolhuis, H., and Dijkhuizen, L. (2008). Actinomycte Integrative and Conjugative Elements. *Antoine Van Leeuwenhoek*, **94**, 127-143.

Thangh Huong, C. T., Hai Duong, N. T., and Thu Hien, N. T. (2009). Contamination of some bacteria isolated from chicken meat in retail markets in Hanoi and examination of the antibiotic resistance ability of *Salmonella* and *E. coli* strains isolated. *Journal of Science and Development*, **7**(2), 181-186.

Thorsteinsdottir, T. R., Haraldsson, G., Fridriksdottir, V., Kristinsson, K. G., and Gunnarsson, E. (2010a). Prevalence and genetic relatedness of antimicrobial resistant *Escherichia coli* isolated from food animals, food and humans in Iceland. *Zooneses and public health*, **57**, 189-196.

Thorsteinsdottir, T. R., Haraldsson, G., Fridriksdottir, V., Kristinsson, K. G., and Gunnarsson, E. (2010b). Broiler chickens as source of human fluoroquinolone resistant *Escherichia coli*, Iceland. *Journal of Emerging Infectious Diseases*, **16**(1), 133-135.

Todar, K. (2005). Ken Todar's Microbial World: *Escherichia coli*. Retrieved from: http://www.bact.wisc.edu/themicrobialworld/*Escherichia coli.*

Todar, K. (2012). Bacterial resistance to antibiotics: In Todars' online textbook of bacteriology. Retrieved from: http://textbookofbacteriology.net, **3**, 4

Todd, A. (1997). Epidemiology of food borne diseases. *World health statistics*, **50**, 1-2.

Trobus, M., Lester, C. H., Olsen, J. E., Frimodt-Moller, N., and Hammerum, A. M. (2008). Natural transfer of sulphonamide and ampicillin resistance between

Escherichia coli residing in the human intestines. *Journal of Antimicrobial Chemotherapy*, **63**, 80-86.

Tuitoek, J. W., Chemjor, J. M., and Ottaro, J. M. (1998). *Morphological characteristics and protein requirements of Kenyan indigenous chicken In Agronam Services Ltd*:*Proceeding of the 6th KARI Scientific Conference*, KARI Headquarters, Nairobi, Kenya.

Turnidge, J. (2003). Antibiotic use in animals-prejudices, perceptions and realities. *Journal of Antimicrobial Therapy*, **53**, 26-27.

Upton M (2004). The role of livestock in economic development and poverty reduction. Retrieved from http://www.fao.org/ag/againfo/projects

Van den Bogaard, A. E., and Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics links between animals and humans. *International Journal of Antimicrobial Agents*, **14**, 327–335.

Van Tuat, C. (2007). A study of antimicrobial resistance of *Escherichia coli* isolated from retail fresh pork in Hanoi, Vietnam. Unpublished MSc. thesis, Chiang Mai University, Vietnam and Freie University, Berlin.

Van, T. T. H., George, M., Istivan, T., Tran, L., and Cole, P. J. (2007). Antibiotic resistance in food-borne bacteria contaminants in Vietnam. *Applied and Environmental Microbiology*, **73**(12), 7906-7911.

Vaskas, T. A., Dahesht, E. A., Seifi, S., Rahmani, M., Motaghifar, A., and Safanavaee, R. (2012). Study and comparison of the bacterial contamination outbreak of chicken meat consumed in some cities of Mazandaran province, Iran. *African Journal of Microbiology Research*, **6** (33), 6286-6290.

Villa, L., Garcia-Fernandez, A., Fortini, D., and Carattoli, A. (2010). Replicon sequence typing of the IncF plasmids carrying virulence and resistance determinants. *Journal of Antimicrobial Chemotherapy*, **65**, 2518-2529.

Washington, C., Stephen, D., William, M., Elmer, W., Gary, W., Paul, C., and Gail, L. (2006). The Enterobacteriacea: Tribe *Escherichiae*. In *Koneman's color atlas and textbook of diagnostic microbiology* 6th ed (pp 212-248). New York: *Lippincott Williams and Wilkins*

Wasteson, Y. (2001). Zoonotic *Escherichia coli*. *Acta veterinaria Scandinavica*, **95**, 79-84.

Watve, M. M., Dahanukar, N., and Watve, M. G. (2010). Socio-biological control of plasmid copy number in bacteria. *PLos ONE*, **5**(2), 1-8.

WHO, (1998). Use of quinolones in food animals and potential impact on human health in Geneva. Retrieved from :*http://www.who.org*

WHO, (2002). Impacts of antimicrobial growth promoter termination in Denmark. Retrieved from: *http://www.who.org*

Woolcock, J.B (1991). World animal science: Microbiology of animals and animal products, Amsterdam. *Elsevier*, **32,** 4.

Wozniak, R. A., and Waldor, M. K. (2010). Integrative and Conjugative Elements enabling dynamic lateral gene flow. *Nature reviews Microbiology*, **8**, 552-563.

Yang. H., Chen, S., White, D.G., Zhao, S., McDermott, P., Walker, R., and Meng, J. (2004). Characterization of multiple-antimicrobial resistant *Escherichia coli* isolates from diseased chickens and swine in China. *Journal of Clinical Microbiology*, **42**, 3483–3489.

You, J. Y., Moon, B. M., Oh, I. G., Baek, B. K., Li, L. G., and Kim, B. S. (2006). Antimicrobial resistance of *Escherichia coli* O157 from cattle in Korea. *International Journal of Food Microbiology*. **106**, 74-78

Zhang, L., Kinkelaar, D., Huang, Y., Li, Y., Li, X., and Wang, H. H. (2011). Acquired antibiotic resistance: Are we born with it. *Applied and Environmental Microbiology*. **77**(20), 7134-7141.

Zhao, S., Blickenstaff, K., Bodeis-Jones, S., Gaines, S. A., Tong, E., and McDermott, P. F. (2012). Comparison of the prevalence and antimicrobial resistances of *Escherichia coli* isolates from different retail meats in the United States, 2002 t0 2008. *American society of Microbiology*, **78**, 23.

Zhao, S., Maurer, J. J., Hubert, S., De Villena, J. F., McDermott, P. F., Meng, J., and other authors (2005). Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. *Veterinary Microbiology*, **107**, 215–224.

APPENDICES

Appendix I: Locations from where samples were purchased and number of samples purchased

Location	Total number of samples purchased
Savannah	3
Donholm	11
Buruburu	16
Umoja 1	8
Umoja 2	6
Embakasi	6
Kayole	8
Dandora	5
Uhuru	4
Makadara	4
Eastleigh	1
Tena	6
Nairobi West	5
South B	7
South C	5
Langata/Langata Road	10
Ongata Rongai	11
Ngong Road	3
Kilimani	4
Woodley	4
Ngumo	4
Hurlingham	6
Gigiri	6
Muthaiga	4
Kariobangi	2
Westlands	9
Githurai	3
Central Business District (CBD)	38
TOTAL=28 LOCATIONS	**TOTAL=200 SAMPLES**

Appendix II: Interpretation guide for results observed on 3M Petri films

Petrifilm *E. coli*/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator

that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting Coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter). AOAC INTERNATIONAL and United States FDA Bacteriological Analytical Manual define Coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid which causes the pH indicator to make the gel color darker red. Gas trapped around red coliform colonies indicates confirmed Coliforms. The following were some of the guides used for interpretation.

a) When there is no visible color change in the gel it means there is no growth.
b) *E. coli* colonies will appear as blue colonies with gas while Coliforms will be the blue plus red colonies with gas.
c) As the *E. coli* or coliform count increases the color of the gel turns to dark red (if Coliforms are more) or purple-blue (if *E. coli* is more).
d) Background bubbles are a characteristic of the gel and are not a result of *E. coli* or coliform growth.
e) Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the media.
f) The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.
g) Petrifilm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a

deepening of the gel color from red to purple-blue. The actual count for this observation is given as 10^6.

h) High concentrations of *E. coli* may cause the growth area to turn purple-blue. The actual count for such an observation is given as 10^8.

i) When high levels of Coliforms are present ($>10^8$) and the gel turns dark red, some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli*. Pick blue colonies without gas and confirm if necessary.

j) When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

k) Food particles are irregularly shaped and are not associated with gas bubbles.

l) Bubble patterns may vary. Gas may disrupt the colony so that the colony "outlines" the bubble. Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony.

Appendix III: Procedure for preparation of Eosin Methylene Blue agar

Materials:

- Sterile plates
- Conical flask
- Distilled water
- EMB media powder (Oxoid)
- Spatula
- Weighing balance
- Foil paper
- Autoclave

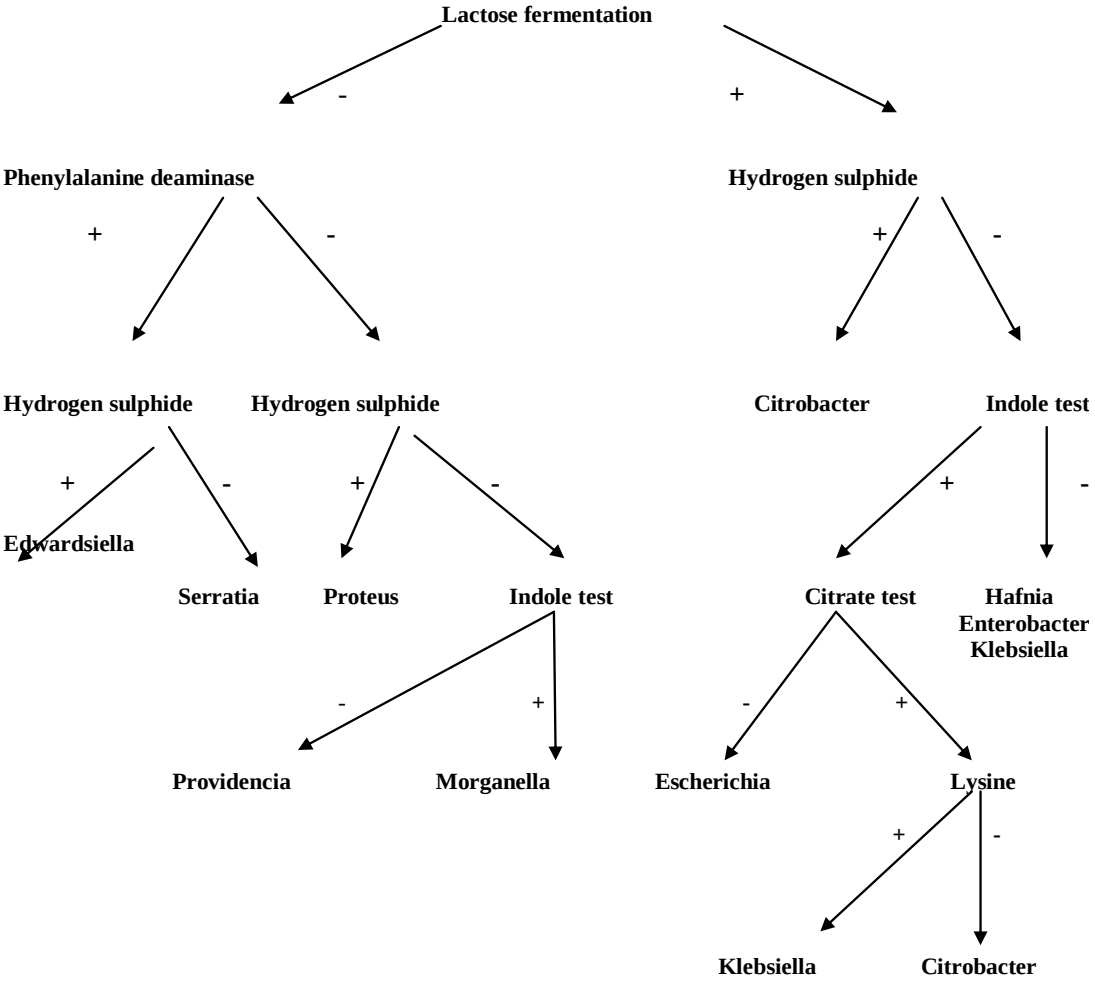
- Water bath
- Control strain *E. coli* ATCC 25922

Procedure:

The media was purchased from Oxoid manufacturers and the procedures followed were as described on the product label;

1. Using a weighing balance, 38 grams of Eosin Methylene blue agar powder was weighed on a piece of foil paper and dissolved in 1 litre of distilled water in a conical flask.
2. The media was autoclaved at121°C for 15 minutes.
3. The media was removed from the autoclave and cooled in a water bath to a temperature of 50°C and held at that temperature.
4. The media was mixed well in order to oxidize the Methylene blue and suspend any precipitate.
5. 20 ml of the agar was then poured in sterile plates and allowed to set.
6. Prepared culture plates were then stored in plastic bags at 4°C.
7. Quality control of culture plates to demonstrate expected performance was carried out using *E. coli* ATCC 25922.

Appendix IV: Simplified chart for the identification of family Enterobacteriacae



These are key tests for identification, but NOT the only differences among the organisms. These test reactions represent the predominant reaction. An organism may not run true to its reaction, or that a species within a genus may be different. It is important to identify what additional tests to run to differentiate among species within a genus (Holt, 1994).

Appendix V: Procedure for preparation of Triple Sugar Iron Agar

Materials:

- Clean glass tubes
- Clean Beaker
- Distilled water
- TSI media
- Spatula
- Autoclave
- Foil paper
- Heating coil
- Control strains *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027
- Weighing balance

Procedure:

The media was purchased from Acumedia manufacturers and the preparation procedures followed were as described on the product label;

1. Using a weighing balance 60 grams of the media was weighed on a piece of foil paper and suspended in 1 litre of distilled water
2. The solution was heated with frequent agitation and boiled for one minute to completely dissolve the medium.
3. The media was dispensed into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, the media was allowed to solidify in a slanted position.
5. Prepared slants were then stored in a rack at 4°C.
6. Quality control of the prepared slants was carried out using control strains *E.coli* ATCC 25922 (acid butt and slant with gas) and *Pseudomonas aeruginosa* ATCC 9027 (Alkaline butt and slant with no gas).

Appendix VI: Procedure for preparation of Sulphide Indole Motility agar

Materials:

- Bijou bottles
- Beaker
- SIM agar media
- Distilled water
- Autoclave
- Spatula
- Autoclave
- Weighing balance
- Controls *E.coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883

Procedure

The media was purchased from Oxoid and the preparation procedures that were followed were as described on the product label;

1. Using a weighing balance, 30 grams of powdered media was weighed on a small piece of foil paper and suspended in 1 litre of distilled water.
2. The mixture was boiled to dissolve the media completely.
3. The media was dispensed into clean bijou bottles (5ml each).
4. The media was sterilized by autoclaving for 15 minutes at 121°C.
5. Prepared media was allowed to cool and stored in a refrigerator at 4°C.
6. Quality control on the media was done using controls *E.coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883

Appendix VII: Procedure for preparation of Simmon's citrate agar

Materials:

- Clean glass tubes

- Beaker
- Simmon's citrate powdered media
- Spatula
- Weighing balance
- Foil paper
- Distilled water
- Heating coil
- Autoclave
- Control strains *E.coli* ATCC 25922 and *Enterobacter aerogenes* ATCC 13048

Procedure:

The agar was purchased from Oxoid manufacturers and preparation procedures that were followed were as described on the product label;

1. Using a weighing balance 24.3 grams of the powdered media was weighed on a piece of foil paper and suspended in 1 litre of distilled water.
2. The liquid agar was mixed well and dissolved by heating with frequent agitation.
3. The liquid agar was dispensed into tubes and autoclaved at 121℃ for 15 minutes.
4. The liquid agar was then allowed to cool in slanted positions in order to obtain short butts of 1.5 cm. depth.
5. The prepared media was then stored at 4℃.
6. Quality control on the citrate slants was carried out using control strains *E.coli* ATCC 25922 (growth inhibited and no change in media color) and *Enterobacter aerogenes* ATCC 13048 (good growth and change of media color from green to blue).

Appendix VIII: Procedure for preparation of Mueller Hinton agar

Material:

- Sterile plates
- Distilled water
- Powdered MH media
- Spatula
- Weighing balance
- Foil paper
- Autoclave
- Conical flask
- Control strains *E.coli* ATCC 25922

Procedure:

The media was purchased from Oxoid and the preparation procedures that were followed were as described on the product label;

1. Using a weighing balance, 38 grams of the powdered media was suspended in 1 litre of distilled water.
2. The mixture was brought to the boil to dissolve the medium completely.
3. The media was then sterilized by autoclaving for 15 minutes at 121°C.
4. The media was removed from the autoclave and cooled in a water bath to a temperature of 50°C and held at that temperature.
5. 20 ml of the agar was then poured in sterile plates and allowed to set.
6. Prepared culture plates were then stored in plastic bags at 4°C.
7. Quality control of culture plates to demonstrate expected performance was carried out using *E.coli* ATCC 25922.

Appendix IX: Preparation of normal saline water

Materials:

- Sodium chloride salt
- Distilled water

- Glass tubes
- Beaker
- Spatula
- Weighing balance
- Foil paper
- Autoclave
- Droppers

Procedure:

1. Using a weighing balance, 0.85 grams of sodium chloride was weighed on a piece of foil paper and dissolved in a beaker containing 100 ml of distilled water.
2. The solution was mixed well and dissolved by heating with frequent agitation.
3. Using sterile droppers, 3ml of the normal saline was dispensed into clean tubes and autoclaved at 121°C for 15 minutes. The normal saline was allowed to cool and stored at room temperature in sterile environment.

Appendix X: Zone diameter interpretative standards for antimicrobial resistance

Test group	Antimicrobial Agent	Disc content (µg)	Zone diameter nearest whole mm		
			R	I	S
PENICILLINS					
	Ampicillin	10	≤ 13	14-16	≥17
β-LACTAMASE INHIBITOR COMBINATION					
	Amoxicillin-Clavulanic acid	20/10	≤ 13	14-17	≥ 18
CEPHEMS (PARENTERAL)					
	Ceftriaxone	30	≤ 13	14-20	≥ 21
	Ceftazidime	30	≤ 14		≥ 18
AMINOGLYOCOSIDES					
	Gentamicin	10	≤ 12	13-14	≥ 15
	Kanamycin	30	≤ 13	14-17	≥ 18
	Streptomycin	10	≤ 11	12-14	≥ 15
FLUOROQUINOLONES					
	Ciprofloxacin	5	≤ 15	16-20	≥ 21
QUINOLONES					
	Nalidixic acid	30	≤ 13	14-18	≥ 19
TETRACYCLINES					
	Tetracycline	30	≤ 11	12-14	≥ 15
FOLATE PATHWAYS INHIBITORS					
	Trimethoprin/Sulphamethoxazole	1.25/23.75	≤ 10	11-15	≥ 16
PHENICOLS					
	Chloramphenicol	30	≤ 12	13-17	≥ 18

Appendix XI: Procedure for preparation of MacConkey agar

Materials:

- Sterile plates
- Powdered MacConkey media
- Spatula
- Weighing balance
- Foil paper
- Distilled water
- Conical flask

- Autoclave

Procedure

The medium was purchased from Oxoid and preparation procedures that were followed were as described on the product label;

1. Using a weighing machine, 47 grams of powdered media was weighed on a foil paper and suspended in 1 litre of distilled water.
2. The mixture was brought to the boil to dissolve the medium completely.
3. The media was then sterilized by autoclaving for 15 minutes at 121°C.
4. The media was removed from the autoclave and cooled in a water bath to a temperature of 50°C and held at that temperature.
5. 20 ml of the agar was then poured in sterile plates and allowed to set.
6. Prepared culture plates were then stored in plastic bags at 4°C.
7. Quality control of culture plates to demonstrate expected performance was carried out using *E.coli* ATCC 25922.

Appendix XII: Procedure for preparation of 1% agarose gel for electrophoresis

Materials:

- Agarose powder
- X 0.5 TAE electrophoresis buffer
- Weighing balance
- Foil paper
- spatula
- Microwave
- Ethidium bromide

Procedure:

1. 1 Gram of agarose powder was weighed into a beaker.
2. 100 ml of X 0.5 TAE buffer was added to the beaker.

3. The mixture was dissolved by boiling in a microwave for 2 minutes.
4. 5µl of ethidium bromide was carefully added into the agarose gel.
5. The gel was then carefully poured into the electrophoresis tank avoiding formation of bubbles.
6. The well combs were then placed on the gel and the gel allowed to cool

Appendix XIII: Procedure for preparation of 0.5x Tris-Acetate (TAE) electrophoresis buffer

Materials:

- Clean beakers
- Weighing balance
- Foil paper
- 48.4 grams Tris base (tris (hydroxymethyl) amino methane
- 11.4ml 1.0 M glacial acetic acid
- 3.7 grams EDTA, disodium salt
- Deionized water

Procedure:

- Using a weighing balance, 48.4 grams of tris base was weighed on a piece of foil paper and dissolved into 800ml of deionized water.
- 11.4ml of acetic acid and 3.7 grams of EDTA disodium salt was added to the solution and mixed thoroughly.
- The solution was diluted to a final volume of 1 litre by adding deionized water to make 10 X TAE stock solution and stored at room temperature.
- In order to make 0.5 X TAE buffer, 50 ml of 10 X stock solution was diluted into 950 ml of deionized water.

Appendix XIV: Procedure for preparation of Tryptic Soy Broth

Materials:

- Beaker
- Clean bijou bottles
- 5ml pipettes
- Pipette aid
- Powdered Tryptic soy media
- Weighing balance
- Foil paper
- Spatula
- Distilled water
- Autoclave

Procedure:

1. Using a weighing balance, 30 grams of the powdered media was weighed on a piece of foil paper and dissolved in one litre of distilled water.
2. The broth was mixed thoroughly and using sterile pipettes, 5ml was dispensed into clean bijou bottles.
3. The dispensed broth was then autoclaved at 121℃ for 15 minutes.
4. The broth was removed from the autoclaved and allowed to cool and stored at 4℃.
5. Quality control on the Tryptic soy broth was carried out using control strains *E.coli* ATCC 25922.

Appendix XV: Procedure for preparation of drug plates for conjugation experiments

Materials:

- Sterile plates
- MacConkey powdered agar
- Distilled water
- Conical flasks
- Sodium azide powder (Assay potency 90µg/mg)
- Ampicillin antibiotic powder (Assay potency 98µg/mg)
- Weighing balance
- Spatula
- Foil paper
- Autoclave
- Water bath

Procedure:

1. 3 sets of media were prepared by weighing 38 grams MacConkey powder into a foil paper and suspending in a 3 conical flasks each containing 1 litre of distilled water.
2. The prepared media was autoclaved at 121°C for 15 minutes.
3. The media was removed from the autoclave and cooled in a water bath to a temperature of 50°C and held at that temperature.
4. To determine the amount of each drug to use for preparing the drug plates, the following formula for drug calculation was used;

$$\text{Volume (ml) of media} = \frac{\text{Weight of drug (mg)} \times \text{Assay potency/ purity of drug (µg/mg)}}{\text{Desired concentration (µg/ml)}}$$

5. Using the above formula the amount of drugs required were calculated and weighed carefully on a weighing balance and mixed with the cooled media. The following sets of drug plates were prepared:
    - MacConkey agar containing 30 µg/ml of ampicillin and 300 µg/ml of sodium azide to select for transconjugants.
    - MacConkey agar containing 30 µg/ml of ampicillin as control for only the donor organism to grow in.
    - MacConkey agar containing 300 µg/ml of sodium azide as control for only recipient organism (J53) to grow in.
6. The liquid agar containing the drugs was mixed well in order to have uniform distribution of the drugs within the media.
7. 20 ml of the agar was then poured in sterile plates and allowed to set.
8. Prepared drug plates were then stored in plastic bags at 4°C.
9. Quality control of drug plates to demonstrate expected performance was carried out using sodium azide resistant recipient *E.coli* J53 and ampicillin resistant donors.

Appendix XVI: KEMRI Ethical Review Committee approval letter

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **July 20, 2011**

TO: **JOYCE ARUA ODWAR,**
STUDENT PROPOSAL, TM 302/0198-2009
PRINCIPAL INVESTIGATOR

THRO': **DR. SAMUEL KARIUKI,** Forwarded 21/7/11
THE DIRECTOR, CMR,
NAIROBI

RE: **SSC PROTOCOL NO. 2036 (*RE-SUBMISSION*): CONTAMINATION RATE AND GENETIC VARIANTS OF ANTIMICROBIAL RESISTANCE IN *E. COLI* ISOLATED FROM CHICKEN CARCASSES AND RAW RETAIL CHICKEN MEAT IN NAIROBI**

Reference is made to your letter dated 30 June 2011 and received on July 5, 2011.

The Committee is satisfied that the issues raised at the initial review are adequately addressed. The study is granted approval for implementation effective this **20th day of July 2011**. Please note that authorization to conduct this study will automatically expire on **19th July 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **20th May 2012**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC.
You are also required to submit any proposed changes to this protocol to the ERC to initiation and advise the ERC when the study is completed or discontinued.
You may embark on the study.

Sincerely,

Caroline Kithinji,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE