

**PREVALENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA*  
SP., *SHIGELLA* SP. AND *ESCHERICHIA COLI* IN FRESH RETAIL CHICKEN  
IN THE ACCRA METROPOLIS**

**BY**

**GUETABA, MOSES YAO**

**(10443365)**

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN  
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF  
MASTER OF PHILOSOPHY DEGREE IN ANIMAL SCIENCE**

**DEPARTMENT OF ANIMAL SCIENCE  
COLLEGE OF BASIC AND APPLIED SCIENCES  
UNIVERSITY OF GHANA**

**JULY, 2015**

**DECLARATION**

I hereby declare that this thesis, which is submitted to the Department of Animal Science, College of Basic and Applied Sciences, University of Ghana, for the award of Master of Philosophy in Animal Science degree, is the result of my own investigation. This thesis has not been submitted or presented for another degree elsewhere, either in part or in whole, except for other people's work which was duly cited and acknowledged.

**STUDENT**

**NAME:** MOSES YAO GUETABA

**SIGNATURE:** .....

**DATE:** .....

**SUPERVISOR**

**NAME:** DR. S. OHENE-ADJEI

**SIGNATURE:** .....

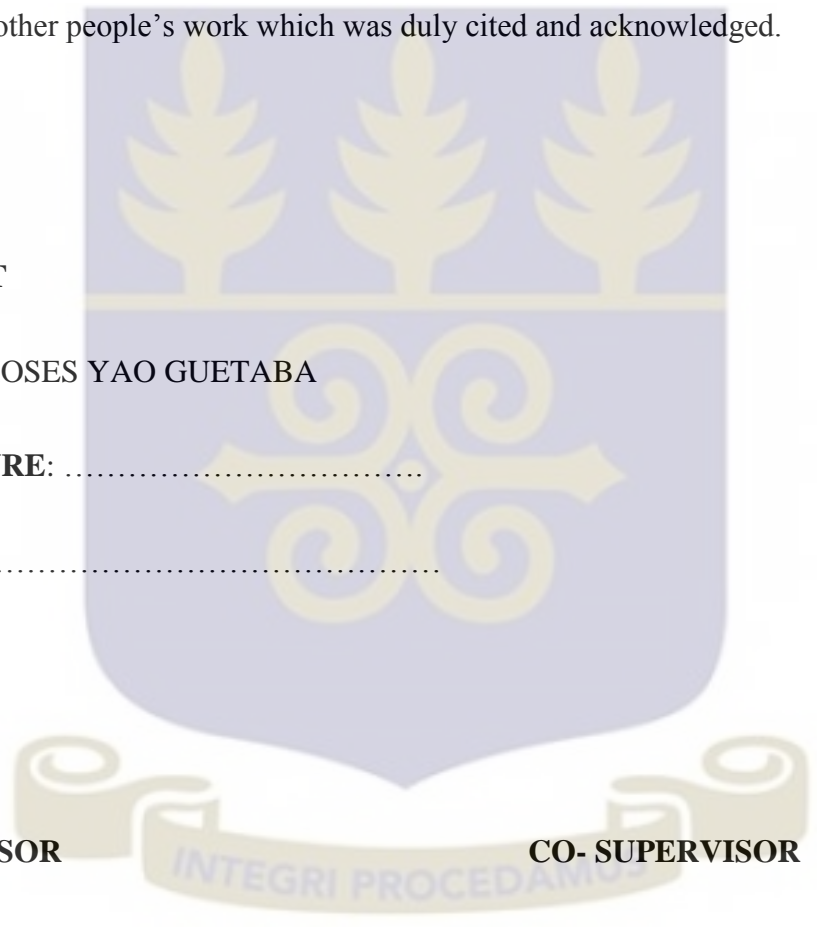
**DATE:** .....

**CO- SUPERVISOR**

**NAME:** PROF ANNA R.BARNES

**SIGNATURE:** .....

**DATE:** .....



## DEDICATION

I dedicate this work to God Almighty for His Mercy and Grace which has brought me this far.



## ABSTRACT

Prevalence and antibiotic resistance (ABR) profiles of selected food borne pathogens in retailed fresh chicken, sold in the Accra Metropolis, were investigated. Fifty (50) fresh whole chicken carcasses, purchased from commercial cold stores, open markets, meat shops and local poultry farms, in the Accra metropolis, were used. Samples for evaluation were swabs from the breast, wing and vent area of the chicken carcasses. Target pathogens, *Salmonella* sp., *Shigella* sp. and *E. coli* were isolated using standard microbiological and biochemical methods. Antimicrobial susceptibility was determined by the Disc diffusion method using 8 commonly used antibiotics: Ampicillin (10µg), Chloramphenicol (30µg), Cefotaxime (30ug), Ceftriaxone (30µg), Gentamicin (10µg), Cefuroxime (30ug), Cotrimoxazole (25µg) and Tetracycline (10µg). A total of 147 confirmed isolates were obtained from 3 anatomical regions: the wing, breast and the vent of the chicken dressed on the farms and those purchased from commercial cold stores. Data was analyzed using Statistical Analysis System version 12. *E. coli* and *Salmonella* sp. isolates were confirmed for both locally dressed and imported chickens. An increased likelihood of occurrence of target isolates was observed in locally dressed chicken (79.1%) compared to imported (28.9%). The number of *Salmonella* sp. isolates from the commercial cold stores (27.52%) was similar to that from the local farms (25.50%). Similarly the number of *E. coli* isolates from farms (25.50%) was not different from those isolated from markets (21.48%). No *Shigella* sp. isolate was confirmed. For *E. coli* (n=70) and *Salmonella* sp. (n=79) isolates respectively, resistance to Chloramphenicol was 9% to 24%; for Cefuroxime was 9.4% to 36.9%, for Cotrimoxazole; 31.5% to 40.9%; for Tetracycline; 31.5% to 47.7% and for Ampicillin; 32% to 48.3%. The Antibiotic resistance associated with *Salmonella* sp. was higher than for *E. coli*. However, there was no significant difference ( $p>0.05$ ) between them.

## ACKNOWLEDGEMENT

I acknowledge God Almighty for granting me sufficient grace to successfully complete this course and dissertation.

My sincere gratitude goes to my Principal Supervisor, Dr. S. Ohene- Adjei, whose expert guidance and contributions brought this work to a successful conclusion completion.

My heart-felt gratitude goes to my co-supervisor Prof. A. Barnes for her relentless dedication to this work. My warmest thanks go to all Senior Members of the Animal Science Department, not forgetting Mr. Jonathan Quaye, a Senior Technologist.

I am grateful to my directors, Mr. Francis K. Nutakor, District Director of Agriculture- South Tongu District and Mrs. Margret Asuman, District Director of Agriculture- Dodowa for their advice and all the support they gave me.

I also thank the Director of CSIR-Animal Research Institute and all the staff of the department of Microbiology especially, Mr. Cornelius Takyi Arthur, a Principal Technologist, Mr. Stephen Ogbetey, Mr. Leonardo Agbormegah, Mrs. Frances Natan Mensah, and all the National Service personal, especially, Mr. Enoch Ackah and Mr. Anthony Koomson for the sacrifices and immense contribution they gave me during my laboratory work.

My final thanks goes to all my colleagues especially Mr. Godwin Agbenyegah, for his support.

## LIST OF ACRONYMS AND ABBREVIATIONS

FDA	Food and Drug Administration
APHIS	Animal and Plant Health Inspection Service
BSAC	British Society for Antimicrobial Chemotherapy
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
EUCAST	European Committee on Antimicrobial Susceptibility Testing
OIE	World Organisation for Animal Health
FSIS	Food Safety and Inspection Service
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Foods
PCR	Polymerase Chain Reaction
SRGA	Swedish Reference Group for Antibiotics
USDA	United States Department of Agriculture

**TABLE OF CONTENTS**

DECLARATION .....	i
DEDICATION .....	ii
ABSTRACT.....	iii
ACKNOWLEDGEMENT .....	iv
LIST OF ACRONYMS AND ABBREVIATIONS .....	v
LIST OF TABLES.....	x
LIST OF FIGURE.....	xi
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1 Background information on infected chicken in Ghana, Africa or world at large.....	1
1.2 Problem Statement .....	4
1.3 Justification .....	5
1.4 Hypotheses .....	5
1.5 Objectives of the study.....	5
CHAPTER TWO .....	6
LITERATURE REVIEW .....	6
2.1 Bacteria and Food Spoilage.....	6
2.2 <i>SALMONELLA</i> SP. ....	7
2.2.1 Incidence of <i>Salmonella</i> sp. contamination in chicken .....	8
2.2.2 Epidemiology of <i>Salmonella</i> sp. in Poultry and Poultry Products .....	10
2.2.3 Prevalence of <i>Salmonella</i> sp. in Poultry Flocks .....	10
2.2.4 Prevalence of <i>Salmonella</i> sp. in Poultry Products at the Processing Plant.....	11
2.2.5 Distribution of <i>Salmonella</i> sp. Serotypes .....	14
2.2.6 Control and Prevention.....	15
2.3 <i>ESCHERICIA COLI</i> .....	18
2.3.1 Biology .....	18
2.3.2 Habitat of <i>E. coli</i> .....	19
2.3.3 <i>E. coli</i> Pathotypes .....	20

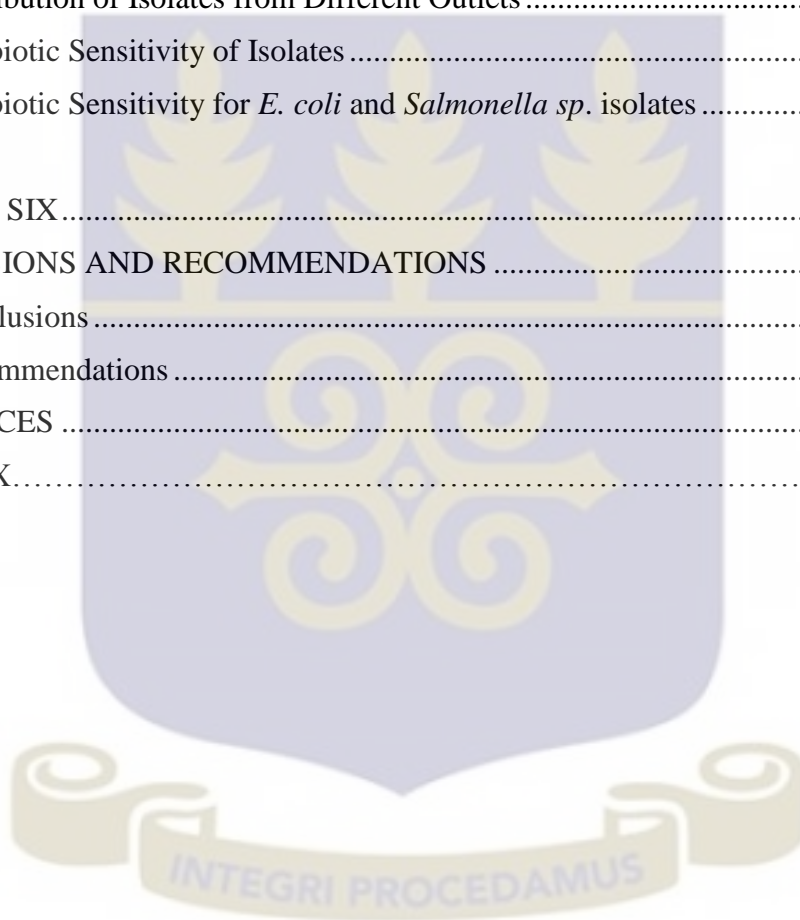


2.3.4.1 Enterotoxigenic <i>E.coli</i> (ETEC).....	20
2.3.4.2 Enteropathogenic <i>E.coli</i> (EPEC).....	21
2.3.4.3 Atypical Enteropathogenic <i>E.coli</i> (A-EPEC).....	23
2.3.4.4 Enterohaemorrhagic <i>E.coli</i> (EHEC).....	24
2.3.4.5 Enteroinvasive <i>E.coli</i> (EIEC).....	26
2.3.4.6 Diffusively Adherent <i>E.coli</i> (DAEC).....	28
2.3.4.7 Enteroggregative <i>E. coli</i> (EAEC).....	28
2.3.4.8 Enterointestinal <i>E. coli</i> .....	30
2.3.5 Epidemiology and Control.....	31
2.3.5.1 Epidemiology of <i>E. coli</i> in Poultry and Poultry Products.....	31
2.3.5.2 Future Prospect for the comprehension of <i>E. coli</i> .....	31
2.3.5.3 Symptoms of <i>E. coli</i> infection.....	32
2.4 <i>SHIGELLA</i> .....	33
2.4.1 Biology .....	33
2.4.2 Epidemiology.....	33
2.4.3 Symptoms .....	34
2.4.4 Control and Prevention of <i>Shigella</i> .....	35
2.5. Antimicrobial Resistance .....	35
2.5.1 Antibiotics and Antimicrobials.....	35
2.5.2 Antibiotic Resistance Strategies .....	37
2.5.2.1 Prevention of the antimicrobial from reaching its target by reducing its ability to penetrate in the cell.....	37
2.5.2.2 Expulsion of the antimicrobial agents from the cell via general or specific efflux pumps.....	38
2.5.2.3 Inactivation of antimicrobial agents via modification or degradation.....	39
2.5.2.4 Modification of the antimicrobial target within the bacteria.....	40
2.5.3 Evidence of antimicrobial resistance.....	41
2.5.4 Molecular mechanisms of resistance .....	41
2.5.5 Biological versus Clinical Resistance.....	42
2.5.6 Acquired Resistance .....	44



2.5.7 Mutation.....	44
2.5.8 Horizontal Gene Transfer .....	45
2.5.9 Detecting antimicrobial resistance.....	46
2.4.10 Laboratory approaches and strategies.....	47
2.4.11 Test Methods in Detecting Antimicrobial Resistance .....	48
CHAPTER THREE .....	50
MATERIALS AND METHODS.....	50
3.1 Sampling Areas .....	50
3.2 Sampling Methods.....	51
3.3 Laboratory Techniques.....	52
3.3.1 Preparation of Samples .....	52
3.3.2 Culture Techniques.....	52
3.3.3 Isolation and Identification .....	53
3.3.4 Culture, Isolation and Identification of <i>Salmonella</i> sp. and <i>Shigella</i> sp.....	53
3.3.5 Culture, isolation and identification of <i>Escherichia coli</i> .....	54
3.4 Antibiotic Susceptibility Test (Stokes Diffusion Diagnostic Technique).....	55
3.4.1 Antibiotic Susceptibility Testing.....	55
3.4.2 Media Preparation.....	56
3.4.3 Purification of selected bacteria .....	56
3.4.4 Preparation of inoculum .....	56
3.4.5 Test Procedure .....	57
3.4.6 Interpretation of Results .....	57
CHAPTER FOUR.....	58
RESULTS .....	58
4.1 Organisms Isolated.....	58
4.2 Isolates by Anatomical Sites .....	59
4.3 Organisms Isolated From Different Sources .....	60
4.4 Organisms Isolated From Different outlets.....	61
4.5 Distribution of antibiotic sensitivity of isolates .....	62
4.6 Antibiotic sensitivity of isolates.....	63

4.7 Distribution of sensitivity of isolates to different antibiotics.....	64
4.8 Distribution of antibiotic sensitivity of imported and locally processed chicken .....	66
CHAPTER FIVE .....	68
DISCUSSION.....	68
5.1 Bacteria Isolated.....	68
5.2 Distribution of Isolates from Different Sources.....	69
5.3 Distribution of Isolates from Different Outlets .....	71
5.4 Antibiotic Sensitivity of Isolates .....	71
5.5 Antibiotic Sensitivity for <i>E. coli</i> and <i>Salmonella sp.</i> isolates .....	72
CHAPTER SIX.....	73
CONCLUSIONS AND RECOMMENDATIONS .....	73
6.1 Conclusions .....	73
6.2 Recommendations .....	73
REFERENCES .....	75
APPENDIX.....	93



**LIST OF TABLES**

Table 1: Frequency (%) Distribution of isolates..... 58

Table 2: Frequency (%) Distribution of isolates by anatomical sites.....59

Table 3: Frequency (%) Distribution of isolates from different sources ..... 60

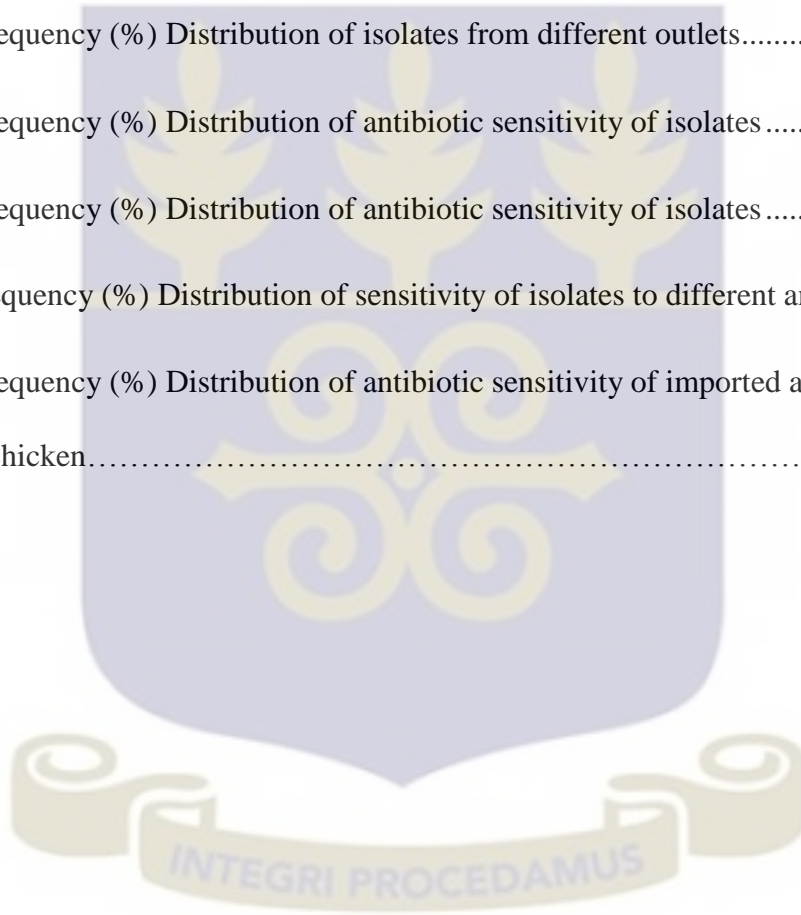
Table 4: Frequency (%) Distribution of isolates from different outlets..... 61

Table 5: Frequency (%) Distribution of antibiotic sensitivity of isolates ..... 62

Table 6: Frequency (%) Distribution of antibiotic sensitivity of isolates ..... 63

Table 7:Frequency (%) Distribution of sensitivity of isolates to different antibiotics ..... 64

Table 8: Frequency (%) Distribution of antibiotic sensitivity of imported and locally processed chicken.....66



**LIST OF FIGURE**

Figure 1: Map of Accra Metropolitan Assembly.....50



## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background information

Poultry meat and eggs are a leading source of animal protein for human consumption in many countries because there is little or no religious and or cultural restriction in the consumption of these products. In Ghana, it is estimated that the per capita consumption of poultry products has increased by 33 percent from 4kg meat in 2010 to 6.6kg in 2012 (Ghana Poultry Report Annual, 2013). Past forecasts, put Ghana's total poultry consumption for mid-year, 2013 at approximately 175,000 MT, up from 167,000 MT in mid-year, 2012. Poultry meat (broiler) imports to Ghana in 2012 accounted for over 90 percent of consumption while the domestic commercial and backyard poultry production provided only about 10 percent, (Ghana Poultry Report Annual, 2013). According to Food and Agriculture Organization (FAO), 2010 report, in Ghana, livestock and poultry meat contributes 40 percent of the national animal protein supply with the rest coming from fish. Gast (2003), reported that, on a global scale, the poultry industry accounts for millions of dollars annually and continues to grow. He also stated that, along with this growth, poultry meat and eggs have been increasingly implicated in food-borne illness. Due to the implementation of greater numbers of monitoring and testing programmes in the poultry industry, isolation of *Salmonella* sp. is reported more often from poultry and poultry products than any other animal source. According to WHO (1983), food-borne contamination and diseases is a global problem. It also reported that, food borne illness is a major international health problem and an important cause of reduced economic growth.

According to Iyer *et al.*, (2013), food-borne pathogens are the leading cause of illness and death in developing countries, killing approximately 1.8 million people annually.

Sackey *et al.* (2001), also had reported that the number of individuals at risk due to food borne diseases would rise due to increase in life expectancy.

Bacteria such as *Salmonella* sp., *Staphylococci aureus* and *Escherichia coli*, which can be conveyed by food, cause food poisoning and other food-borne diseases such as tuberculosis, typhoid fever and cholera (Foskett *et al.*, 2003), dysentery, diarrhea and food poisoning (Macleod and Douglas, 1999) and pneumonia, meningitis, whooping cough, hepatitis and sore throat (Gates, 1987) are caused by bacteria.

Notermans and Hoogenboom-Verdegaal (1992), collected information in different countries that showed that the incidence of some of the food-borne diseases had increased dramatically within few years back but because of under-reporting, the data are of limited value and cannot be compared between countries. The broad spectrum of food-borne infections has changed over time. Well-established pathogens are being controlled, and new ones are emerging. New pathogens may emerge as a result of changing ecology or changing technology that connects a potential pathogen to the food chain. They also can emerge de novo by transfer of mobile virulence factors, often through bacteriophages (Iyer *et al.*, 2013). The number of cases of gastroenteritis associated with food is estimated to be between 68 million and 275 million per year (Naravaneni and Jamil, 2005).

It is also estimated that one in four Americans is affected by a significant food-borne illness each year (Tauxe, 2002). Data indicating trends in food-borne infectious diseases are limited to a few industrialised countries and to even fewer pathogens (Newell *et al.*,



2010) because outbreaks of food-borne illnesses may go underreported (Naravaneni and Jamil, 2005).

Enteropathogenic bacteria such as *Salmonella* sp. *Shigella* sp. *Campylobacter* sp. and enteropathogenic *E. coli* have been isolated from chicken samples in Ghana (Sackey *et al.*, 2001) and elsewhere (Lanata *et al.*, 1992). They have also been implicated in outbreaks of food poisoning (Morton, 1993; Gracey, 1994; Collins and Lyne, 2004). Estimates of the incidence of *Salmonella* sp. in poultry meat and poultry products vary considerably. A United Kingdom wide survey conducted by the country's Food Standards Agency showed an overall frequency of *Salmonella* sp. contamination in retail chicken to be 5.7 % (Mather, 2001). A similar study conducted in Ethiopia showed the incidence of *Salmonella* sp. contamination to be 13.3%. (Todd, 1999). *Campylobacter* and *Salmonella* sp. are reported to be the most important zoonotic pathogens of concern in food borne illnesses. A 2002 report issued by the Centers for Diseases Control and Prevention (CDC) of its ongoing surveillance of food-borne illness, found the highest incidence of food-borne pathogens to be *Campylobacter* followed by *Salmonella* sp., *Shigella*, and *E. coli* O157:H7. Comparable findings had been reported in the United Kingdom by the Food Standards Agency in its 2000 report of cases confirmed by laboratory testing (Food Standards Agency, 2003).

*Salmonella* sp. were isolated from 13 (6.8%) poultry carcasses out of a total of 87 carcasses sampled from open market, supermarket and cold stores and were resistant to erythromycin, cefotiam, penicillin, ampicillin and cefadroxil (Sackey *et al.*, 2001). *Salmonella* sp. had varied susceptibilities to nalidixic acid, chloramphenicol and minocycline (Sackey *et al.*, 2001).



In a research conducted by Sackey *et al.*, (2001), it was revealed that, out of a total of 97 live birds from three selected farms and 87 whole chicken carcasses and chicken parts from two supermarkets, two open markets and one wholesale outlet (cold store) in the Accra metropolis and 6 imported chickens samples from a cold store and two markets were all positive for *Shigella*.

The resistance of bacteria to antibiotics and similar drugs called antimicrobials is considered a major public health threat by the Food and Drug Administration (FDA) and its counterparts around the world (White and Cox, 2013). Antibiotics have transformed health care since they were introduced in the 1940s and have been widely used to fight bacterial infections. However, some infectious organisms have developed resistance to the antibiotics used to treat patients with infections. When bacteria become resistant to an antibiotic, that drug becomes less effective. Medical treatment of people infected with these drug-resistant organisms can become more complicated, leading to longer hospital stays, increased health care costs, and in extreme cases, to untreatable infections (White and Cox, 2013).

## **1.2 Problem Statement**

In Ghana, estimated 420,000 patients are reported to have suffered from food-borne diseases each year, with an annual death rate estimated at 65,000 (Yeboah, 2010).

The resistance of bacteria to antibiotics and similar drugs called antimicrobials is considered a major public health threat by the Food and Drug Administration (FDA) and its counterparts around the world (White and Cox, 2013).

### 1.3 Justification

Information on the prevalence and antibiotic resistance of *Salmonella* sp., *Shigella* sp. and *E. coli* strains isolated from fresh retail chicken in Ghana is necessary to;

1. Establish the existence of the pathogens and potential disease threats to consumers
2. Provide information on the range of antibiotics to which targets organisms are susceptible or resistant to.
- 3. Resistance of the organisms to antibacterial in use

### 1.4 Hypotheses

#### Null hypothesis:

1. There is no *Salmonella* sp., *Shigella* sp. and *Escherichia coli* in local and imported retail chicken carcasses in Accra metropolis.
2. No antibiotic resistance trait is associated with bacteria that will be isolated from fresh chicken.

### 1.5 Objectives of the study

1. To determine the prevalence of *Salmonella* sp., *Shigella* sp. and *Escherichia coli* associated with fresh retail chicken in the Accra metropolis.
2. To determine the antibiotic resistance of the target food-borne organisms.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Bacteria and Food Spoilage

Bacteria such as *Salmonella* species, *Staphylococcus aureus* and *Escherichia coli*, which can be conveyed by food, cause food poisoning and other food-borne diseases such as tuberculosis, typhoid fever and cholera (Foskett *et al.*, 2003), dysentery, diarrhea and food poisoning (Macleod and Douglas, 1999) and pneumonia, meningitis, whooping cough, hepatitis and sore throat (Gates, 1987).

The burden of food-borne diseases remains substantial and it is estimated that one in four Americans is affected by a significant food-borne illness each year (Tauxe, 2002). Data indicating trends in food-borne infectious diseases are limited to a few industrialised countries, and to even fewer pathogens (Newell *et al.*, 2010) because outbreaks of food-borne illnesses may go underreported (Naravaneni *et al.*, 2005).

Enteropathogenic bacteria such as *Salmonella* sp., *Shigella* sp., *Campylobacter* sp. and enteropathogenic *E. coli* have been isolated from chicken samples (Sackey *et al.*, 2001). Bacteria such as *Salmonella* sp., *Shigella* sp., *Campylobacter* sp. and enteropathogenic *E. coli* have also been implicated in outbreaks of food poisoning (Morton, 1993; Collins and Lyne, 2004). Microbial contamination is usually confined to the skin or surface of a carcass. Gram-negative, motile bacteria show greater adherence to such surfaces than do Gram-positive species (Collins and Lyne, 2004). Although deep muscle is usually sterile,

the meat may have a higher pH if the animal has suffered stress and it may be contaminated (Collins and Lyne, 2004).

Most methods of sampling for specimen are destructive, involving scraping off the top 3 mm of a measured area. However, the most practical non-destructive method is swabbing (Collins and Lyne, 2004).

Additionally, large sterile cotton wool pads, wrapped in cotton gauze, can be used and transferred to individual plastic bags. Pads are moistened with a 0.1% peptone water and used to wipe the carcass with it and to prepare the knead (Kitche *et al.*, 1973).

## **2.2 SALMONELLA SP.**

*Salmonella* sp. is a genus of rod-shaped Gram negative enterobacteria that causes typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004). They are found worldwide in both cold-blooded and warm-blooded animals, and in the environment (Ryan and Ray, 2004). *Salmonella* sp. are non-spore-forming, predominantly motile enterobacteria with width of 0.7 to 1.5  $\mu\text{m}$  and length from 2 to 5  $\mu\text{m}$ . They have peritrichous flagella (flagella that are all around the cell body) except *S. Pullorum* and *S. Gallinam*. They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes (Fabrega and Vila, 2013). They produce hydrogen sulfide (Ryan and Ray, 2004).

### 2.2.1 Incidence of *Salmonella* sp. contamination in chicken

*Salmonella* sp. species are responsible for a variety of acute and chronic diseases in both poultry and humans. Contaminated poultry products are among the most important sources for food-borne outbreaks in humans. Isolations of *Salmonella* sp. are reported more often from poultry and poultry products than from any other animal species (Myint, 2004).

A survey conducted earlier on retail poultry revealed *Salmonella* sp. contamination levels of 60% in Portugal (Antunes *et al.*, 2003), 39% in the US (Zhao *et al.*, 2001), 36% in Belgium (Uyttendaele *et al.*, 1999), 36% in Spain (Dominguez *et al.*, 2002) and 25% in England (Jorgensen *et al.*, 2002). A survey conducted in Northern Ireland from 1995 to 2000 found a mean level of 11% of poultry contamination with *Salmonella* sp. (Wilson, 2002). In addition, the latter survey noted that in Northern Ireland there was a decline in incidence over the period studied and levels fell to 6%. In another survey conducted in by Soutos *et al.*, (2003) to determine the presence of *Listeria* and *Salmonella* sp. in retail chicken in Northern Ireland, three samples of 205 retail packs (1.5%) of chicken were positive and yielded three *Salmonella* sp. serovars; *S. infantis*, *S. unknown* (group C1 + C4) and *S. tennessee* in a study conducted by Adeyanju and Olayinks (2014).

*Salmonella* sp. contamination from ninety-nine poultry samples (53 chicken and 46 turkey) obtained from retail markets in Ibadan and Oyo states in Nigeria was 33% [chicken 32.1% (17/53) and turkey 34.8% (16/46)]. Also, twelve (12) *Salmonella* sp. isolates were obtained from a processing plant in Ibadan and Oyo states in Nigeria and prevalence rate calculated as 22.6%. (Adeyanju *et al.*, 2014). Ahmad *et al.*, (2013) reported *Salmonella* sp. contamination on 25% of chicken samples collected from retail outlets in Lohore city. *Salmonella* sp. has frequently been isolated from the abattoir environments and



gastrointestinal tract of all farmed and wild animals, especially poultry (EFSA, 2007; Norrung *et al.*, 2009). Pointon *et al.*, 1989) have also reported high incidence of *Salmonella* sp. from retail chicken in two Australian states (47.7 and 35.5%). A survey conducted by Hassanein *et al.*, (2011) in Egypt revealed that, nine samples out of 825 samples (36.00%) of frozen chicken legs and thirteen samples out of 25 samples (52.00%) of frozen chicken breast fillets were contaminated with *Salmonella* sp.

Chicken products are widely acknowledged to be a significant reservoir for *Salmonella* sp. and have frequently been incriminated as a source of *Salmonella* sp. contamination and consequently thought to be major sources of the pathogen in humans (Baeumler *et al.*, 2000 and Jay, 2000). Furthermore, one of the commonest causes of *Salmonella* sp. infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello *et al.*, 2000).

Varying incidence rates of *Salmonella* sp. in chicken and/or chicken parts have been reported. Plummer *et al.*, (1995) found *Salmonella* sp. in 26.3% of fresh whole chickens, 26.7% of breasts, 14.3% of legs, 0% of drumsticks, 0% of thighs, and 40% of wings. Capita *et al.*, 2003 reported *Salmonella* sp. contamination level of around 40% of wings, legs and giblets of chicken. Moreover, Straver *et al.*, (2007) pointed out that 8.6% chicken breast fillet (chilled raw fillets without skin) collected from five local retail outlets in The Netherlands were contaminated with *Salmonella* sp. Also, poultry meat was extensively contaminated with *Salmonella* sp. (40%) in Sao Paulo, Brazil (Tavechio *et al.*, 2002). Study conducted by Sackey *et al.* (2001) to assess the microbiology of chicken in Accra metropolis, isolated *Salmonella* sp. from 7 (7.2%) gut contents and 13 (6.8%) from carcasses. In a similar research conducted by Adu-Gyamfi *et al.*, (2012) to assess the

microbiological quality of chicken sold in Accra, *Salmonella* sp. was isolated from each of the 9 samples taken from supermarkets and open markets in Accra, showing an overall prevalence of 7.4%.

### **2.2.2 Epidemiology of *Salmonella* sp. in Poultry and Poultry Products**

*Salmonella* sp. can be found in virtually every part of the world and are carried by an extremely wide variety of hosts including humans and other mammals, birds, reptiles, and insects (Kusters *et al.*, 1993; Gast, 1997; Austin *et al.*, 1998; Sato *et al.*, 1999). Knowledge of the incidence and serotype distribution of *Salmonella* sp. responsible for zoonotic diseases transmission in domestic animal populations is essential for understanding the relationships within and between the reservoirs of *Salmonella* sp. in animals and humans. Advances in poultry production practices, changes in consumer lifestyles and preferences, and heightened nutritional awareness have all combined to make poultry products a leading source of protein for much of the world. Thus the incidence of *Salmonella* sp. infection in poultry flocks and associated incidence of *Salmonella* sp. contamination of poultry products are of considerable public health significance.

### **2.2.3 Prevalence of *Salmonella* sp. in Poultry Flocks**

Although *Salmonella* sp. have been isolated in poultry flocks of various species, including both broiler and layer breeds, estimates of the prevalence of *Salmonella* sp. in commercial poultry and their environments have varied considerably. A 1991 survey of poultry in the Netherlands reported that fecal samples from 94% of the meat-type broiler flocks and 47% of the egg-type layer flocks in Netherlands were *Salmonella* sp. positive (Van de Giessen *et al.*, 1991; Edel, 1994). Similarly, in 1994 the environments of 87% of turkey flocks in



Canada were found to be *Salmonella* sp. positive (Irwin *et al.*, 1994) and 53% of flocks tested from either fecal or egg belt sampling in Canada were positive for *Salmonella* sp. (Poppe *et al.*, 1991). In the United States, studies of pooled cecal samples from spent egg-layers in the southern US, detected *Salmonella* sp. in 100% of the flocks (Waltman *et al.*, 1992) and 86% of 406 layer houses from several regions (Ebel *et al.*, 1992).

Strains of *Salmonella* sp. responsible for poultry carcass contamination arrive at the farm when the chicks are purchased. Bailey *et al.*, (1994) reported that up to 98% of samples from the hatchery were contaminated with *Salmonella* sp. and in two trials found a significant association between serotypes found in the hatchery and those found on the final Carcasses (7-36%) (Bailey *et al.*, 2002). *Salmonella* sp. are ubiquitous and reducing the farm-level prevalence requires effective implementation and enforcement of management and biosecurity measures including proper poultry house design, adequate ventilation and reduced environmental humidity (Mallinson *et al.*, 2000; Eriksson De Resende *et al.*, 2001). Production is the beginning of the food supply continuum and failure to control contamination at this stage of production increases the risk of food-borne illness for the consumer and the cost of risk reduction efforts at later stages of production and distribution.

#### **2.2.4 Prevalence of *Salmonella* sp. in Poultry Products at the Processing Plant**

*Salmonella* sp. contamination in poultry products at the processing plant is primarily due to cross contamination by physical contact during carcass processing such as improper cleaning and disinfection of processing lines, improper chilling and storage temperature, poor worker hygiene and infestation with rodents and insects (Lillard, 1990; Trampel,

2000). *Salmonella* sp. have been isolated from water, equipment, and carcasses in processing plants (Trampel, 2000). Lillard (1990), reported a significant increase in *Salmonella* sp. incidence on carcasses exiting the immersion chiller when compared to other processing stages such as pre-scald (at bleed line), post-scald, post-pick and post-evisceration. Venter *et al.* (2000), reported that even in fully automated chicken egg layer management systems, bioaerosols can transmit *Salmonella* sp. to eggs (Venter *et al.*, 2000). *Salmonella* sp. can be transmitted vertically to the progeny and horizontally within and between flocks (Snoeyenbos, *et al.*, 1969; Gast *et al.*, 1990). About 98% of hatchery samples were contaminated with *Salmonella* sp. (Bailey, 1994) Thus, *Salmonella* sp. contamination in poultry products is a result of both infected birds entering the processing plant and contamination at the processing plant or during transportation (Seligmann *et al.*, 1970). Each stage in the production continuum has both direct and indirect effects on the *Salmonella* sp. prevalence in finished poultry products. Carcasses from infected flocks can result in increased *Salmonella* sp. prevalence but an effective Hazard analysis critical control point (HACCP) plan at processing plants can reduce *Salmonella* sp. loads. However, even with a lower *Salmonella* sp. level in the flock, cross contamination during carcass processing with improper cleaning, disinfection, and chilling and storage temperatures can lead to high *Salmonella* sp. loads in the poultry products. Literature does establish that the first step in the poultry product *Salmonella* sp. contamination pathway is from infected flocks. This leads to cross contamination of transportation crates, carcasses, equipment, processing plant personnel, and vehicles and equipment used for retail-outlet processing, transportation and distribution (Seligmann *et al.*, 1970; Poppe *et al.*, 1991). Transmission within the processing plant can however, be reduced by effective

implementation of a hazard analysis critical control point (HACCP) program (USDA, 1995). In 1996, the USDA issued a mandate for implementation at processing plants and abattoirs throughout the United States (USDA, 1996). The national baseline pre-HACCP levels reported by USDA were 20% for carcasses and 44.5% for ground meat in 1996 (USDA, 1994). After implementation of the HACCP guidelines, USDA reported a substantial decline in *Salmonella* sp. prevalence for poultry and other meat products at processing plants (USDA, 1994; USDA, 1996). According to USDA reports, the prevalence of *Salmonella* sp. declined to 11% for carcasses and 16% for ground chicken in 12 processing plants in the US in 2001 (USDA, 1996; Uyttendaele *et al.*, 1998). As a result outbreaks traceable to errors in processing plants are rare and when outbreaks occur they are often associated with changes in processing or packaging technology whose effect is not determined before the product is on market (Fricker, 1987).

While the HACCP program has greatly reduced the level of contamination in poultry meat products at the processor, it has not been able to eradicate *Salmonella* sp. from poultry meat products. Human factors are also important for cross contamination from production until consumption. Historically, most bacterial food poisoning in the US is associated with mishandling, either in the home or in the food service establishment. There is extensive information about what happens at processing, but there is little data on what happens post-processing from arrival at the retail distribution outlet up to the moment of consumption. After processing, the next stages in the production continuum for which data are available are reported incidences of illness and death post consumption. Hence the period of time from processing through transport, distribution, further processing and retail sale is a “gap in the knowledge” related to the farm-to-fork production continuum. There are still

opportunities for bacterial growth and cross contamination of products during the process of transporting, storage, handling and retail distribution of poultry products.

### **2.2.5 Distribution of *Salmonella* sp. Serotypes**

Although more than 2300 serotypes of *Salmonella* sp. have been identified, only about 10% of these serotypes have been isolated from poultry (Gast, 1997). Moreover, an even smaller subset of 14 serotypes accounts for the vast majority of poultry *Salmonella* sp. isolates. The distribution of *Salmonella* sp. serotypes from poultry sources varies geographically and changes over time. Several serotypes are consistently found at a higher incidence. According to the report from the U. S. Department of Agriculture (USDA) National Veterinary Service Laboratory, the most commonly identified species in chickens in the United States were *S. heidelberg*, *S. enteritidis*, *S. hadar*, *S. montevideo*, *S. kentucky* and *S. typhimurium* (Ferris *et al.*, 1991, 1992, 1993). The significance of poultry as a reservoir for human salmonellosis can be illustrated by considering the species commonly isolated from humans. The most often reported to the Centers for Disease Control and Prevention (CDC) from human sources in the United States were *S. typhimurium*, *S. enteritidis*, *S. heidelberg*, *S. hadar*, *S. newport*, and *S. agona* (Bean and Potter, 1992). The specific prevalence of *S. enteritidis* has been a topic of considerable interest in recent years due to the epidemiologic association of salmonellosis and consumption of contaminated eggs. The increasing public health significance of *S. enteritidis* Diseases was shown in a survey of the frequency of reporting of human infections with various *Salmonella* sp. in 21 nations and 10% of these nations reported *S. enteritidis* as their most common species in 1979 to 43% in 1987 (Rodriguez *et al.*, 1990). Murase, *et al* (2001) reported *S. enteriti*, *S. cerro*, *S. montevideo* and *S. mbandaka* were isolated from about 60 % of layer house

environmental positive samples. *Salmonella* sp. isolates obtained from the drain water collected after the washing of the eggs in the egg processing facility were the same serotypes found in the chicken houses. Knowledge of *Salmonella* sp. serotypes is important to identify sources and routes of contamination not only for control and prevention but also for outbreak investigation and vaccine development (Tara, 2003).

### **2.2.6 Control and Prevention**

*Salmonella* sp. can be effectively controlled by coordinated and simultaneous interventions on the problem from several directions. At the farm level, eggs and chicks or poults should only be obtained from *Salmonella* sp.-free breeding flocks. Hatching eggs should be properly disinfected and hatched according to stringent sanitation standards (Gast *et al.*, 1990; Gast *et al.*, 1991; Gast, 1993). Poultry houses should be thoroughly cleaned and disinfected. Rodent and insect control measures should be incorporated into house design and management and verified by periodic testing (USDA, 1996; Luet *et al.*, 2003). Rigidly enforced biosecurity practices should be implemented, restricting entry onto poultry housing premises to only authorized personnel and equipment, preventing horizontal transmission of *Salmonella* sp. between houses (Meyerson and Reaser, 2002; APHIS, 2004). Only pelleted food or feed containing no animal protein should be used, to minimize contamination (Williams and Benson, 1978; Williams, 1981).

Treatments such as medication, competitive exclusion cultures, or vaccination can be applied to reduce *Salmonella* sp. susceptibility (Gast, 1997; Corrier., 1992). Frequent testing of poultry and environmental samples has also reportedly been successful for



*Salmonella* sp. control in the poultry industry. Such coordinated control programs have reportedly been successful in addressing *Salmonella* sp. problems in both chickens and turkeys (McIlloy *et al.*, 1989; Edel, 1994).

At the processing stage, implementation of a Hazard Analysis and Critical Control Points (HACCP) Plan has been effective in reducing *Salmonella* sp. contamination of carcasses. The United States Department of Agriculture (USDA), Food Safety Inspection Services (FSIS) conducts annual surveillance of microbial contamination in processing plants and slaughterhouses to monitor microbial contamination of carcasses. Since implementation of the HACCP program, they have been able to demonstrate that the *Salmonella* sp. prevalence on carcasses in the processing plant was significantly lower than before implementation (Rose *et al.*, 2001). However, state or county departments of health loosely regulate transportation and retail distribution of processed chicken products. Surveillance usually only occurs in response to outbreaks. Effective development and implementation of HACCP and surveillance programs to monitor microbial contaminations at the retail store level are needed to close that final gap in the *Salmonella* sp. control spectrum before the product reaches the consumer.

Other measures, such as gas treated packaging, irradiation, organic acid treatment, and biofilm treatments have proved to effectively lower or inhibit *Salmonella* sp. and other microbial growth in products. Potential pre-harvest interventions to control *Salmonella* sp., or other food-borne hazards, need to be considered in terms of cost, impact and probability of post intervention contamination. The technical feasibility of pre-harvest control of *Salmonella* sp. using microbiological testing and regulation has been demonstrated by the Swedish poultry and swine industries. However, perhaps the most eloquent statement of the

difficulty and cost of implementing the 'Swedish model' for *Salmonella* sp. control is that, despite its apparent success, after 40 years it has not been adopted by any major swine or poultry producing nations because improvement of any single factor only had a limited impact on the level of contamination, and the largest reduction was observed when several factors were improved concurrently (Davies *et al.*, 1997). As one would expect, lack of data was identified as a major limitation to the development of effective control models.

Effective washing and decontamination of hatching eggs at the breeder farm is widely practised for the control of *Salmonella* sp. A wide variety of bacteria can be prevented through the chemical treatment of eggs as soon as possible after laying; this method may also increase egg production and may reduce the number of newly hatched chicks contaminated with *Salmonella* sp. Polyhexamethylene biguanide hydrochloride, hydrogen peroxide and a phenolic compound have been identified as the most effective chemicals to eliminate *Salmonella* sp. from fertile hatching eggs (Cox *et al.*, 1994).

Bacterial cross-contamination is a problem in the hatchery environment: however, a good sanitation programme can significantly reduce *Salmonella* sp. in the hatchery. Sanitised circulating air in the hatching cabinet is effective in reducing the spread of *Salmonella* sp. Sanitising treatments such as ultra violet light, hydrogen peroxide and ozone are effective in reducing Enterobacteriaceae and *Salmonella* sp. in hatching cabinet air samples (Bailey *et al.*, 1996).



## 2.3 *ESCHERICHIA COLI*

### 2.3.1 Biology

*Escherichia coli* (*E. coli*) is a Gram negative facultative bacterium species that ferments glucose and is a member of the Family *Enterobacteriaceae* (Feng and Weagant, 2009). It is widely distributed in the intestine of animals and forms part of the normal intestinal flora that maintains the physiology of a healthy animal (Conway and Macfarlane, 1995). Thus most *E. coli* strains are non-pathogenic but pathogenic strains that cause gastrointestinal illness in humans and opportunistic ones that normally affect immune-compromised patients exists (Nataro and Kaper, 1998). *Escherichia coli* is the most prevalent infecting organism in the family of gram-negative bacteria known as *Enterobacteriaceae* (Eisenstein and Zaleznik, 2000). Cells are typically rod-shaped, and are about 2.0 micrometers ( $\mu\text{m}$ ) long and 0.25–1.0  $\mu\text{m}$  in diameter, with a cell volume of 0.6–0.7  $\mu\text{m}^3$  (Kubitschek, 1990). Optimal growth of *E. coli* occurs at 37 °C (98.6 °F), but some laboratory strains can multiply at temperatures of up to 49 °C (Fotadar, 2005). Growth can be driven by aerobic or anaerobic respiration (Ingledeew and Poole, 1984).

*E. coli* strains can be classified by their O, K and H antigens (Blattner *et al.*, 1997; Perna *et al.*, 2001; Hayashi *et al.*, 2001). The O antigen is defined serologically and determined by the repeating polysaccharide chains that are part of the lipopolysaccharide (LPS) embedded in the outer leaflet of the outer membrane. The H antigen is defined serologically by the antigenic specificity of the bacterial flagellum.

Enterohemorrhagic *Escherichia coli* (EHEC) strains of *E. coli* contain specific virulence properties: lysogenic phage encoding one or more Shiga toxins (with or without a chromosomal pathogenicity island), and often an additional virulence plasmid. These

strains are also referred to as Shiga-toxin producing *E. coli*, or STEC. Most EHEC strains are serotype O157:H7 (Noël and Boedekar, 1997). Among *E. coli* isolates, there is considerable variation and many combinations of somatic (O and K) and flagella (H) antigens. Among pathogenic strains, there are few patterns of these antigens and few phylogenetic groupings. For *E. coli*, there are over 150 antigenically unique O-antigens (Whitfield and Valvano, 1993). K type capsular material occurs in two or four forms on the basis of physical, biochemical and genetic criteria (Whitfield and Roberts, 1999). Over 80 serologically and chemically distinct capsular polysaccharides have been recognised. In addition, a slime layer, colonic acid extracellular polysaccharide, is common to many *E. coli* isolates and can be co-expressed with some K-type capsules. There are 53 H-antigen specificities among *E. coli*.

### **2.3.2 Habitat of *E. coli***

*Escherichia coli* are common inhabitants of the small intestine and large intestine of mammals. They are often the most abundant facultative anaerobes in that environment. The human colon maintains a microbial density approaching  $10^{12}$  organisms per gram of feces, representing a perfectly balanced ecosystem. The commensal microbiota consists of more than 400 species and lives in perfect harmony with the human intestine (Hooper and Gordon, 2001). The presence of *E. coli* in the environment of mammals and birds is usually considered to reflect fecal contamination and not the ability to replicate freely outside the intestine. There is evidence however to suggest that *E. coli* may freely replicate in tropical fresh water (Bermudez and Hazen, 1988).

### **2.3.3 *E. coli* Pathotypes**

Although most *E. coli* are harmless commensals of the human and animal intestine, certain specific, highly-adapted *E. coli* strains are capable of causing a variety of different diseases. Infections due to pathogenic *E. coli* may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infections (Nataro and Kaper, 1998). One of the most notable features of *E. coli* is broad diversity of diseases-causing genotypes. The diseases can encompass different symptoms and gastrointestinal tract pathologies, but there are also diseases at extraintestinal sites. These different genotypes and their disease-causing abilities lead to categories of *E. coli* often referred to as pathotypes. There are seven intestinal and two extraintestinal pathotypes currently recognized.

#### **2.3.4.1 Enterotoxigenic *E. coli* (ETEC)**

ETEC (*enterotoxigenic E. coli*) strains are a major cause of secretory diarrhea in both humans and animals (Bern *et al.*, 1992). ETEC produce toxins which are heat-labile (LT) and/or heat-stable (STa and STb) that are also causing diarrhea. A frequent causal agent of diarrhea in both humans and animals, enterotoxigenic *E. coli* (ETEC) are estimated to cause 600 million cases of human diarrhea and 800,000 deaths worldwide principally in children under the age of five (Spangler, 1992). Economically significant ETEC diarrheal diseases in animals occur in neonatal calves, pigs and lambs. ETEC, cause watery diarrhea that can be mild in nature or, in some instances, can be a severe, cholera-like illness where rapid dehydration can be life-threatening. In endemic areas of ETEC-mediated diarrhea, infants and children under the age of five are the most commonly affected.

One of the principal virulence factors for this pathogen is the heat-labile enterotoxin (LT), which interestingly shares structural and functional similarity to the *Vibrio cholera* cholera toxin (Spangler, 1992). LT toxin has a classic AB toxin subunit holotoxin structure. The B subunits (as a pentamer) bind to host cell surface GM1 and GD1b gangliosides and the A subunit enzymatically ADP-ribosylates the  $\alpha$ - subunit of stimulatory G protein. This G protein regulates host cell adenylate cyclase and LT mediated modification leads to its permanent activation and an increase in intracellular Cyclic adenosine monophosphate levels. This eventually leads to activation of the chloride ion channel of the intoxicated cells, increased chloride ion secretion into the intestinal lumen, and decreased sodium and chloride absorption. The overall result is to reverse the normal intestinal osmotic gradient and cause a net water loss into the gut lumen.

Aside from LT, many ETEC strains also express heat-stable enterotoxins (STs), which also contribute to the watery diarrhea. There are two structurally distinct STs: STa and STb. The STs are small polypeptides that share the common features of heat stability and multiple intramolecular disulfide bonds. The action of STa is well understood. It binds to the extracellular domain of plasma membrane embedded guanylate cyclase. The ETEC toxins are secreted in the terminal small intestine where the ETEC adhere by expression of a complex and diverse group of surface proteins commonly referred to as "colonization factors" (Gaastra and Svennerholm, 1996).

#### **2.3.4.2 Enteropathogenic *E. coli* (EPEC)**

Enteropathogenic *E. coli* (EPEC) are a significant cause of infant diarrhea in developing nations. EPEC were historically recognised on the basis of serotypes such as O55:H6 and

O127:H6. EPEC (an established etiological agent of human infantile diarrhea, is a pathogen that subverts intestinal epithelial cell function to produce distinctive “attaching and effacing” (A/E) lesions. These lesions are characterised by localised destruction (effacement) of brush border microvilli, intimate bacterial attachment to the host-cell membrane and formation of an actin-rich cytoskeletal structure beneath intimately attached bacteria. In developing countries, EPEC is one of the most common pathogens. In Brazil, for example, EPEC can be isolated from stools of over 40% of infants with acute diarrhea and was associated with a mortality of 7% (Fagundes-Neto and Scaletsky, 2000). The pathogenesis of EPEC is in some way unique for enteric bacterial pathogens since it is essentially noninvasive and produces no toxins. EPEC also uses its Type III Secretion System (TTSS) to deliver bacterial effector proteins like EspA and EspB into the host cell to alter the cytoskeleton (Knutton *et al.*, 1998). However, the most fascinating aspect of EPEC pathogenesis is that it inserts, through the type III secretion system, its own receptor into the host cell. Rather than searching for a receptor it provides its own receptor and uses it when needed. Thus, EPEC is able to insert the Tir receptor into the host cell membrane where it serves as the receptor for the bacterial protein intimin after it is phosphorylated on tyrosine by the host cell (Deibel *et al.*, 1998). An EPEC disease is generally the result of growth of EPEC in the small intestine. EPEC cause a watery diarrhea that may contain mucus but typically does not have blood in it. Vomiting, fever, malaise and dehydration are also associated. The symptoms may last for a brief period of several days, although instances of long, chronic EPEC diseases have been noted. Some of the mechanisms of EPEC pathogenesis are well understood. For example, the A/E lesion is the result of a complex system of EPEC proteins that are injected into the host intestinal epithelial cell.



The A/E lesion represents a dramatic rearrangement of the epithelial cytoskeleton where there is an accumulation of actin directly below the attached EPEC cell. This is described as an actin pedestal for the attached bacterial cell. There is a specific pathogenicity island, termed the "locus of enterocyte effacement" (LEE) that encodes the genes responsible for the A/E lesion. The LEE encodes a type III secretion system that provides the intimate adhesion (intimin) its receptor (which is injected into and then presented on the surface of the host cell), and the injected proteins responsible for changes in host cell signaling mechanisms, including actin pedestal formation (Jerse and Tall, 1990). Common to most EPEC strains are plasmids EAF ("EPEC adherence factor"), which encode an adherence factor, the bundle-forming pilus (bfp), (Nataro *et al.*, 1987). Results of human volunteer studies indicate the EAF plasmid is necessary to cause diseases (Levine *et al.*, 1985). Although the A/E characteristic is critical for causing EPEC diseases, probably through destruction of microvilli, the precise mechanism for the diarrhea is not completely understood and may reflect the diversity of EPEC strains.

#### **2.3.4.3 Atypical Enteropathogenic *E. coli* (A-EPEC)**

A-EPEC (atypical enteropathogenic *u7*) is EPEC that have lost the EAF (EPEC adherence factor) plasmid. Some studies (Scotland *et al.*, 1991; Sousa and Dubreuil, 2001) had shown that, probably, A-EPEC is another EPEC category associated with diarrhea of clinical importance. Recent attention has focused on greater understanding of atypical EPEC strains (Trabulsi *et al.*, 2002). These strains more commonly cause diarrhea in industrialised nations than the typical EPEC strains. In addition the atypical EPEC strains have animal and human reservoirs, whereas the typical isolates are almost always associated with

human fecal contamination. The atypical isolates have the ability to cause A/E lesions but lack the EAF plasmids. They often have additional virulence factors not seen among the typical strains. For example, they have significant portions of the pO157 virulence plasmid common to enterohemorrhagic *E. coli* O157:H7 strains and may have a heat stable enterotoxin (EAST-1).

#### **2.3.4.4 Enterohaemorrhagic *E. coli* (EHEC)**

Enterohaemorrhagic *E. coli* (EHEC) strains are implicated in food-borne diseases principally due to ingestion of uncooked minced meat and raw milk. These strains produce shiga like toxin 1 (stx1), shiga-like toxin 2 (stx2) and variants thereof. They are involved in episodes of diarrhea with complications. Serotype O157:H7 is the prototype of increasing importance and is associated with hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (HUS). EHECs typically cause an afebrile bloody colitis and, in about 10% of patients, this infection can be followed by HUS (Pickering *et al.*, 1994). Like EPEC, EHECs elicit an attaching and effacing lesion of the intestinal mucosa, a phenotype that requires a functional *eaeA* chromosomal gene.

These organisms share the ability to cause A/E lesions with EPEC but enterohemorrhagic *E. coli* (EHEC) are set apart from EPEC by possession of Shiga-like toxins and the clinical presentation of their diseases. EHECs cause diseases of the large intestine that may present as simple watery diarrhea and then progress to bloody stools with ulcerations of the bowel. In a small subset of diseased individuals there is onset several days later of severe, life threatening hemolytic-uremic syndrome (HUS). HUS involves a triad of hemolytic anemia, thrombocytopenia and renal failure (Pickering *et al.*, 1994). The transmission of EHEC diseases in humans is through ingestion of contaminated beef or foods contaminated with



cattle feces. In cattle, the EHEC strains are transient members of the intestinal microflora where they do not apparently cause diseases. One of the remarkable features of EHEC is its low infection dose of 10–100 organisms (Pickering *et al.*, 1994). Clearly this microorganism has special acid-tolerance ability when compared to many other enteric bacterial pathogens. Children under the age of five are the major victims of EHEC disease, although the elderly may also exhibit bloody diarrhea and HUS. Epidemiologically in the United States, Japan, and Great Britain, a single serotype O157:H7 is the most common EHEC strain. In other parts of the world, this strain can be observed causing diseases, but other serotypes (e.g., O26 and O111) cause a similar diseases as well. All factors that lead to HUS are unknown except Shiga toxin (sometimes referred to as "Shiga-like toxin" or "verotoxin"), which probably plays an important role in renal injury. Purified Stx-1 injected intravenously in baboons leads to renal diseases with histopathology similar to EHEC-mediated HUS (Tailor *et al.*, 1999). The Shiga toxin inhibits protein synthesis through cleavage of ribosomal RNA. Because EHEC do not cause bacteremia, Shiga toxin is thought to be released while the organism is growing in the large bowel, where it gets disseminated systemically to cause damage to renal endothelial cells and release of inflammatory mediators that eventually damage the kidney (Tailor *et al.*, 1999). There are two evolutionarily related forms of Shiga toxin in *E. coli* (Shiga toxin 1 and Shiga toxin 2). They share approximately 55% amino acid sequence similarity. Shiga toxin 1 is only different from the Shiga toxin of *Shigella dysenteriae* by a single amino acid substitution. There are many Shiga toxin positive *E. coli* strains (STEC) that are not associated with enterohemorrhagic colitis. It is a heterogeneous group that is occasionally associated with HUS, but their general benign nature may be due to their lack of the LEE pathogenicity

island and plasmid virulence factors. The ubiquitous dissemination of the distribution of Shiga toxin genes among *E. coli* strains is due to their transmission as part of lambdoid phages. The EHEC O157:H7 strain likely originated in an O55 EPEC strain where a series of genetic events lead to acquisition of shiga toxin encoding prophages and a large virulence plasmid, pO157 (Reid *et al.*, 2000). The precise role of pO157 in EHEC pathogenesis is unknown but may involve some putative toxin genes and a mucin-specific zinc metalloprotease, StcE (Grys *et al.*, 2005).

#### **2.3.4.5 Enteroinvasive *E. coli* (EIEC)**

EIEC (enteroinvasive *E. coli*) cause a broad spectrum of human diseases. They are biochemically, genetically and pathogenetically closely related to *Shigella* sp. Both characteristically cause an invasive inflammatory colitis, but either may also elicit a watery diarrhea syndrome indistinguishable from that caused by other *E. coli* pathogens. The pathogenesis of diseases caused by EIEC and *Shigella* involves cellular invasion and spread, and requires specific chromosomal and plasmid-borne virulence genes (Nataro and Kaper, 1998).

These organisms are pathogenetically so closely related to *Shigella* species that the nomenclature distinction is questionable. There are a few biochemical traits that can be used to distinguish enteroinvasive *E. coli* (EIEC) from *Shigella*, but the principal virulence genes are shared. The diagnostic confusion between *Shigella* and EIEC is evident in that EIEC isolates are non-motile and 70% are non-lactose fermenters (Silva *et al.*, 1980). In addition, EIEC share with *Shigella* the inability to decarboxylate lysine, a trait common to

other *E. coli*. The traits that EIEC share with *E. coli* but not *Shigella* are the ability to produce gas from glucose and fermentation of xylose.

EIEC cause invasive inflammatory colitis and dysentery with a clinical presentation (blood and mucous stools accompanied by fever and severe cramps) identical to the diseases caused by *Shigella* species. EIEC/*Shigella* invade intestinal epithelium, principally in the large intestine. Once inside the cells, they lyse the phagocytic vesicle and replicate freely in the host cell cytoplasm. The EIEC/*Shigella* cells then spread to neighboring host cells by a motility process whereby actin is nucleated on one pole of the bacillus and subsequent actin polymerisation propels the bacterial cell (Goldberg and Theriot, 1995). Many of genes necessary for cellular invasion and diseases are carried on a large (>200-kb) plasmid found in both EIEC and *Shigella*. A system of type III secretion genes important for delivery of modifiers of host cell signaling and membrane lysis are found on these plasmids. In addition, the plasmid encodes an outer membrane protein (IcsA) that is localised on one pole of the bacterium and directs the actin microfilament polymerisation necessary for spread of bacteria to other host cells. EIEC/*Shigella* rarely invades the bloodstream, but they do invade the lamina propria immediately under the intestinal epithelium, where interaction with macrophages causes the release of pro-inflammatory mediators and even induction of apoptosis (Casalino *et al.*, 2003). Interestingly, the inability to decarboxylate lysine, a trait shared by EIEC and *Shigella*, is the result of mutations and gene rearrangements at the *cadC* gene. The decarboxylation of lysine results in cadaverine, which acts as an inhibitor of inflammation and migration of neutrophils into the lamina propria. The lack of this function is hypothesised to be a pathoadaptive trait that enables EIEC/*Shigella* to cause diseases (Casalino *et al.*, 2003).

#### **2.3.4.6 Diffusely Adherent *E. coli* (DAEC)**

Diffusely adherent *E. coli* (DAEC) strains are defined by the presence of the diffusely adherent pattern in the HEp-2 adherence assay, and cause a watery diarrhea syndrome in adults and children. The pathogenesis of DAEC diarrhea is not as yet elucidated, but several virulence-related characteristics have been identified (Nataro and Kaper, 1998; Servin, 2005). Most DAEC strains express a surface fimbria designated F1845 that may be encoded either by the chromosome or a plasmid. It was shown that DAEC could induce characteristic elongated projections from the surface of epithelial cells in culture.

The epidemiology and pathogenesis of the diffusely adherent *E. coli* (DAEC) are not well understood. DAEC may cause diarrhea in very young children (Scaletsky *et al.*, 2002). They are differentiated from the other diarrhegenic *E. coli* by a distinct adhesion phenotype, again on HEp-2 cells. The adhesion is brought about by F1845 fimbriae, which belong to the Dr family of adhesions (also found in some UPEC strains). The Dr adhesions recognise and bind to host cell surface decay accelerating factor (DAF). DAEC bound to cultured cells elicit a cytopathic phenotype and activation of signal-transduction pathways. The relative significance of DAEC as a pathogen and its mechanisms for causing diseases await further study.

#### **2.3.4.7 Enteroaggregative *E. coli* (EAEC)**

EAEC (enteroaggregative *E. coli*) strains are defined by their distinctive adherence pattern on HEp-2 cells in culture (Nataro and Kaper, 1998). The essential element of the aggregative phenotype is the stacked brick pattern by lying side-by-side with an appreciable distinction of where one bacterium begins and another ends. The EAEC are a

heterogeneous group of bacteria that display a wide array of virulence factors (Sousa and Dubreuil, 2001). EAEC are pathogens associated with persistent diarrhea in the developing world and have been implicated recently in the developed world as causes of both outbreaks and sporadic diarrhea among AIDS patients.

These organisms are defined as *E. coli* that do not possess LT enterotoxin or Shiga toxins but adhere to cultured HEp-2 cells in self-aggregates that are classically referred to as "stacked bricks. Clearly, many *E. coli* strains can mediate the "stacked brick" adhesive phenotype, but there is a subset of these that are bona fide human diarrheal pathogens. Enteroaggregative *E. coli* (EAEC) diseases, as described by human volunteers, is a watery diarrhea that occurs in some cases with abdominal cramps, but no fever. There is no invasion of the bloodstream (Sousa and Dubreuil, 2001). The disease seen in natural EAEC outbreaks is often reported as a persistent, seemingly chronic watery diarrhea. These small epidemics occur in both developing as well as industrialized countries.

There are no common serotypes of EAEC to aid in their recognition in the clinical laboratory. The pathogenesis of EAEC disease is poorly understood, although several potential virulence factors are common to EAEC isolates. EAEC express a fimbrial adhesion called "aggregative adherence fimbriae" ("AAF") (Sousa and Dubreuil, 2001). EAEC isolates often produce a mucinase called "Pic" whose gene has the ability to express from its non-encoding DNA strand a smaller gene that encodes an enterotoxin (*Shigella* enterotoxin [ShET1]) first described in *Shigella* strains (Sousa and Dubreuil, 2001). EAEC strains often produce a heat stable enterotoxin EAST1 that is homologous to the ST1 of ETEC.



#### 2.3.4.8 Extraintestinal *E. coli*

Two pathotypes of *E. coli* are generally recognised causes of extraintestinal human diseases (neonatal septicemia/meningitis *E. coli* and the urinary tract and bloodstream *E. coli*). Some isolates such as *E. coli* O18:K1:H7, are recognised as having the potential to cause both invasive neonatal diseases and urinary tract infections (Johnson *et al.*, 2001). UPEC are a heterogeneous group of clones (Donnenberg and Welch, 1996). Within the uropathogenic *E. coli* (UPEC) grouping are cystitis, pyelonephritis and urosepsis isolates. These strains are the principal causes of morbidity and mortality from either community or hospital-acquired *E. coli* infections. Approximately 60% of adult women will have a UTI in their lifetimes. As much as 90% of all community-acquired UTIs and greater than 30% of the hospital-acquired UTIs are caused by *E. coli*. There have been reports of community-wide outbreaks of UTIs by multidrug resistant UPEC clones (Manges *et al.*, 2001). Uropathogenic *Escherichia coli* (UPEC) strains isolated from women with pyelonephritis, but who have no underlying medical complications, often possess specific O serotypes (O1, O2, O4, O6, O7, O18 and O75 (Orskov and Orskov, 1983). What further suggests that these *E. coli* strains are extraordinary is that they are especially capable of invading the bloodstream (Johnson *et al.*, 1994). Many of the known or putative virulence factors for these strains are not shared with common fecal *E. coli* strains. Examples of such factors are adhesins (e.g., Pap, Sfa, and Dra), hemolysin (Hly), cytotoxic necrotising factor-1(CNF-1), and the aerobactin (Aer) iron sequestration systems.

There are additional factors that are common to all *E. coli* that are critical for pathogenesis of extra intestinal diseases. The principal factors are lipopolysaccharide, capsule production, and type 1 pili. The type 1 pili appear to play a particularly critical role in the

initial colonisation of the bladder (Struve and Krogfelt, 1999). It is suggested that intracellular cellular invasion leads to persistent infections of the urinary tract by successive rounds of intracellular infection, multiplication, release and reinfection of superficial, as well as deeper bladder epithelial layers (Mulvey *et al.*, 2001). Currently no information is available about genes other than those for type 1 pili that are needed for cellular invasion.

### **2.3.5 Epidemiology and Control**

#### **2.3.5.1 Epidemiology of *E. coli* in Poultry and Poultry Products**

*E. coli* has been isolated worldwide from poultry meat (Canton *et al.*, 2008; Adesiji *et al.*, 2011), probably due to the increased usage of antimicrobials (Miranda *et al.*, 2008). Percentage prevalence in poultry meat has been variable depending on method and media used in its isolation. Nineteen percent prevalence was observed in South Africa (Dahal, 2007), 48.4% in Morocco (Cohen *et al.*, 2007) and as high as 98% in India (Saikia and Joshi, 2010). In Nigeria, 16% have been isolated in Osogbo (Adesiji *et al.*, 2011) and 11.1% in Calabar metropolis (Ukut *et al.*, 2010).

Of a total number of 212 chicken samples taken to assess the prevalence of *E. coli* in Greater Washington, D.C., area in 2001, 82 (38.7%) yielded *E. coli*. (Zhao *et al.*, 2001). Contamination rates of *E. coli* was found to be 78% by Odwar *et al.*, (2014) in an experiment to study the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya .



### **2.3.5.2 Future Prospects for the Comprehension of *E. coli***

Knowledge of the pathogenic mechanisms of *E. coli* pathotypes has led to the development of rational interventions for the treatment and prevention of *E. coli* -induced diseases. Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. *E. coli* can respond to environmental signals such as chemicals, pH, temperature, and other stimulants, in a number of very remarkable ways considering it is a single-celled organism. Because of its natural habitat and its ability to subvert, circumvent and/or evade the immune defenses, the survival of these bacteria is safeguarded in nature. The acquisition of different virulence traits, the continuous exchange of genetic elements and the expression of virulence genes generally regulated by environmental factors probably will reveal different strategies shared by *E. coli* strains. Continuous research and investigations into *E. coli* virulence are providing us with useful insights into the origins and evolution of this versatile bacterial pathogen (Sousa, 2006).

### **2.3.5.3 Symptoms of *E. coli* infection in humans**

*E. coli* symptoms change as the infection progresses. Symptoms usually begin two to five days after infection (Griffin and Tauxe, 1991). The initial symptoms include the sudden onset of cramps and abdominal pain, followed by diarrhea within 24 hours. Diarrhea will become increasingly watery, and then noticeably bloody. Also people with *E. coli* infection feel nauseated and experience headaches often. Less common symptoms include fever and chills (Griffin and Tauxe, 1991).

## 2.4 SHIGELLA

### 2.4.1 Biology

*Shigella* sp., of the *Enterobacteriaceae* family, is Gram-negative rod-shaped pathogenic bacteria (Schroeder *et al.*, 2008) and characterized as a facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacteria closely related to *Salmonella* sp..The bacteria are facultative intracellular pathogens that show a high specificity for human or primate hosts. They are non-encapsulated, and do not ferment lactose, or do so slowly. Different serogroups, considered as species, can be differentiated by their biochemical properties, phage or colicin susceptibility, and polyvalent antisera can detect specific polysaccharide antigen (Downes *et al.*, 2001; Kweon, 2008). *S. dysenteriae* is considered the most virulent, and can produce a potent cytotoxin known as shigatoxin (Erqou *et al.*, 2007).

### 2.4.2 Epidemiology

The reported incidence of *Shigella* sp. infections in USA in 2010 was 1,780, which is 3.8 cases per 100,000 population (*S. sonnei* accounts for approximately 78% of all *Shigella* isolates in surveys from the Center for Disease Control and Prevention (CDC, 2009). *S. flexneri* and *S. boydii* accounted for most of the remainder. *S. flexneri* causes 18% of *Shigella* sp. infections in the United States. *S. dysenteriae* is rare in the United States. The highest incidence per 100,000 population for shigellosis (27.77 cases) was among children younger than 5 years (CDC, 2009).

According to the CDC (2009), of 7,746 laboratory confirmed isolates, 687 were identified to have different *Shigella* species level. Distribution by species was similar to previous

years, with *S. sonnei* accounting for the largest percentage of infections (75%), followed by *S. flexneri* (12%), *S. boydii* (0.8%), and *S. dysenteriae* (0.3%). The reporting jurisdictions with the highest incidence rates were Nebraska (13.2 %), New Jersey (7.6%), and Minnesota (7.1%) (CDC, 2009).

The annual number of *Shigella* episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries (with 1.1 million deaths) and 1.5 million in industrialised countries. A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involved children under 5 years of age (Kotloff *et al.*, 1999). The incidence in developing countries may be 20 times greater than that in developed countries. Although the relative importance of various serotypes is not known, an estimated 30% of these infections are caused by *S. dysenteriae* (Kotloff *et al.*, 1999).

### **2.4.3 Symptoms**

Symptoms of shigellosis are sudden onset of severe abdominal cramping, intense fever, emesis, anorexia, and large-volume watery diarrhea. Seizures may be an early manifestation. Abdominal pain, tenesmus, urgency, fecal incontinence, and small-volume mucoid diarrhea with frank blood (fractional stools) may subsequently occur. Elevated temperatures (as high as 106 ° C) are documented in approximately one third of cases, and a generally toxic appearance is noticed. Tachycardia and tachypnea may occur secondary to fever and dehydration. Depending on the degree of dehydration, dry mucous membranes, hypotension, prolonged capillary refill time, and poor skin turgor may be present. Abdominal tenderness is usually central and lower, although it may be generalised.

#### **2.4.4 Control and Prevention of *Shigella***

The spread of *Shigella* from an infected person to other persons can be avoided by frequent and careful hand-washing with soap and hot water (APHA, 2008; Cramer, 2008). Also, *a safe water supply is important for the control of Shigellosis to both human being and animals* (DuPont, 2000). *Furthermore, drinking only treated or boiled water, and eating only cooked hot foods will help prevent Shigella related diseases* (APHA, 2008; CDC, 2009)

### **2.5. Antimicrobial Resistance**

#### **2.5.1 Antibiotics and Antimicrobials**

The introduction of antimicrobials transformed human and animal health systems by revolutionising weaponry in the war against infectious diseases, resulting in improved survivability for both humans and their domestic animals. However, this health triumph was immediately ebbd by the subsequent realisation that bacterial populations could quickly modify themselves to resist antimicrobials, propagate these resistance traits, and even share resistance genes with other contemporary bacteria within their environment (Microbiology model, 2011). Such abilities have seriously compromised the usefulness of antibiotics in the war against microbes and warn of a future when antimicrobials may have very limited usefulness to control bacterial infection.

Antimicrobial resistance is the ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that would normally inhibit or kill this particular kind of organism (Microbiology model, 2011). Antimicrobial resistance is just one of the many

adaptive traits that resilient bacterial subpopulations may possess or acquire, enabling them to out-compete and out-survive their microbial neighbors and overcome host strategies aimed against them (Microbiology model, 2011) . This phenomenon is nearly as old as the discovery of antimicrobials themselves, having been described by pioneers like Ehrlich for trypanosomes (Microbiology model, 2011) and Fleming for *Staphylococci* (Microbiology model, 2011). What is most alarming today is the rate at which antibiotic resistance often develops and how quickly it spreads across the globe and among different species of bacteria. Furthermore, as a result of sequential, cumulative acquisition of resistance traits against different antibiotics, more bacterial pathogens with multiple-drug resistance are being reported worldwide.

Resistance to single antibiotics became prominent in organisms that encountered the first commercially produced antibiotics. The most notable example is resistance to penicillin among *Staphylococci*, specified by an enzyme (penicillinase) that degraded the antibiotic (Alekhshun and Levy, 2007). Over the years, continued selective pressure by different drugs has resulted in organisms bearing additional kinds of resistance mechanisms that led to multidrug resistance (MDR), novel penicillin-binding proteins (PBPs), enzymatic mechanisms of drug modification, mutated drug targets, enhanced efflux pump expression, and altered membrane permeability (Alekhshun and Levy, 2007). Some of the most problematic MDR organisms that are encountered currently include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae* bearing extended-spectrum  $\beta$ -lactamases (ESBL), vancomycin-resistant *enterococci* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant MRSA, and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* (Alekhshun and Levy, 2007).



## 2.5.2 Antibiotic Resistance Strategies

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

The intended modes of action of antibiotics may be counteracted by bacterial organisms via several different means. This may involve preventing antibiotic access into the bacterial cell or perhaps removal or even degradation of the active component of the antimicrobial agent (Microbiology model, 2011). No single mechanism of resistance is considered responsible for the observed resistance in a bacterial organism. In fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent. Four major bacterial resistance strategies are via:

- (a) Prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell
- (b) Expulsion of the antimicrobial agents from the cell via general or specific efflux pumps
- (c) Inactivation of antimicrobial agents via modification or degradation
- (d) Modification of the antimicrobial target within the bacteria

### 2.5.2.1 Prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell

Antimicrobial compounds almost always require access into the bacterial cell to reach their target site where they can interfere with the normal function of the bacterial organism.



Porin channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls. For example, a variety of Gram-negative bacteria reduce the uptake of certain antibiotics, such as amino glycosides and beta lactams, by modifying the cell membrane porin channel frequency, size, and selectivity (Microbiology model, 2011). Prohibiting entry in this manner will prevent these antimicrobials from reaching their intended targets that, for amino glycosides and beta lactams, are the ribosomes and the penicillin-binding proteins (PBPs), respectively (Microbiology model, 2011).

This strategy has been observed in: *Pseudomonas aeruginosa* against imipenem (a beta-lactam antibiotic) *Enterobacter aerogenes* and *Klebsiella* spp. against imipenem. Vancomycin intermediate-resistant *S. aureus* or VISA strains with thickened cell wall trapping vancomycin Many Gram-negative bacteria against aminoglycosides Many Gram-negative bacteria against quinolones (Microbiology model, 2011).

#### **2.5.2.2 Expulsion of the antimicrobial agents from the cell via general or specific efflux pumps**

To be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria possess membrane proteins that act as an export or efflux pump for certain antimicrobials, extruding the antibiotic out of the cell as fast as it can enter (Walsh, 2000). This results in low intracellular concentrations that are insufficient to elicit an effect. Some efflux pumps selectively extrude specific antibiotics such as macrolides, lincosamides, streptogramins and

tetracyclines, whereas others (referred to as multiple drug resistance pumps) expel a variety of structurally diverse anti-infectives with different modes of action (Walsh, 2000). This strategy has been observed in: *E. coli* and other *Enterobacteriaceae* against tetracyclines *Enterobacteriaceae* against chloramphenicol *Staphylococci* against macrolides and streptogramins *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones (Walsh, 2000).

Efflux pumps are variants of membrane pumps possessed by all bacteria, both pathogenic and non-pathogenic, to move lipophilic or amphipathic molecules in and out of the cells. Some are used by antibiotic producers to pump antibiotics out of the cells as fast as they are made, and so constitute an immunity protective mechanism for the bacteria to prevent being killed by their own chemical weapons (Walsh, 2000).

#### **2.5.2.3 Inactivation of antimicrobial agents via modification or degradation**

Another means by which bacteria preserve themselves is by destroying the active component of the antimicrobial agent (Abraham and Chain. 1940). A classic example is the hydrolytic deactivation of the beta-lactam ring in penicillins and cephalosporins by the bacterial enzyme called beta lactamase. The inactivated penicilloic acid will then be ineffective in binding to PBPs (penicillin binding proteins), thereby protecting the process of cell wall synthesis (Abraham and Chain. 1940). This strategy has also been observed in: *Enterobacteriaceae* against chloramphenicol (acetylation) Gram negative and Gram positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation) (Abraham and Chain. 1940).

#### 2.5.2.4 Modification of the antimicrobial target within the bacteria

Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, in spite of the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place (Berger-Bächi, 2002). This strategy has been observed in: *Staphylococci* sp. against methicillin and other beta-lactams (Changes or acquisition of different PBPs that do not sufficiently bind beta-lactams to inhibit cell wall synthesis.) *Enterococci* against vancomycin (alteration in cell wall precursor components to decrease binding of vancomycin) *Mycobacterium* sp. against streptomycin (modification of ribosomal proteins or of 16s rRNA) Mutations in RNA polymerase resulting in resistance to the rifamycins; Mutations in DNA gyrase resulting in resistance to quinolones (Berger-Bächi, 2002).

Some examples of bacterial resistance due to target site modification are the following:

- (a) Alteration in penicillin-binding protein (PBPs) leading to reduced affinity of beta-lactam antibiotics (Methicillin-Resistant *Staphylococcus aureus*, *S. pneumoniae*, *Neisseria gonorrhoeae*, Group A *Streptococci*, *Listeria monocytogenes*) (Berger-Bächi, 2002).
- (b) Changes in peptidoglycan layer and cell wall thickness resulting to reduced activity of vancomycin: Vancomycin-resistant *S. aureus* (Berger-Bächi, 2002).
- (c) Changes in vancomycin precursors reducing activity of vancomycin: *Enterococcus faecium* and *E. faecalis* Alterations in subunits of DNA gyrase reducing activity of fluoroquinolones (Berger-Bächi, 2002).
- (d) Alteration in subunits of topoisomerase IV leading to reduced activity of fluoroquinolones: Many Gram positive bacteria, particularly *S. aureus* and *Streptococcus*

*pneumoniae*. (Berger-Bächi, 2002), changes in RNA polymerase leading to reduced activity of rifampicin: *Mycobacterium tuberculosis* (Berger-Bächi, 2002).

### **2.5.3 Evidence of antimicrobial resistance**

The first antibiotic resistance mechanism described was that of penicillinase. Its presence and activity was first reported by Abraham and Chain in 1940 shortly after its discovery (Abraham and Chain, 1940). Less than 10 years after the clinical introduction of penicillin, penicillin-resistant *Staphylococcus aureus* was observed in a majority of Gram-positive infections in people. The initial response by the pharmaceutical industry was to develop beta-lactam antibiotics that were unaffected by the specific beta-lactamases secreted by *S. aureus*. However, as a result, bacterial strains producing beta-lactamases with different properties began to emerge, as well as those with other resistance mechanisms. This cycle of resistance counteracting resistance continues even today (Bush, 1988).

### **2.5.4 Molecular mechanisms of resistance**

The abilities of bacterial organisms to utilise the various strategies to resist antimicrobial compounds are all genetically encoded (Forbes *et al.*, 1998).

Intrinsic resistance (Forbes *et al.*, 1998) is that type of resistance which is naturally coded and expressed by all (or almost all) strains of that particular bacterial species. An example of intrinsic resistance is the natural resistance of anaerobes to aminoglycosides and Gram-negative bacteria against vancomycin (Forbes *et al.*, 1998). Changes in bacterial genome through mutation or horizontal gene acquisition, on the other hand, may consequently lead

to a change in the nature of proteins expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic (Forbes *et al.*, 1998). This is referred to as acquired resistance, which is limited to selected isolates of that particular species or group of microorganisms. For example, we know that methicillin resistance of *Staphylococcus aureus* is primarily due to changes that occur in the penicillin binding protein (PBP) (Forbes *et al.*, 1998), which is the protein which beta-lactam antibiotics bind and inactivate to consequently inhibit cell wall synthesis. This change is actually rendered by the expression of a certain *mecA* gene in some strains of these bacteria, which is hypothesised to have been induced by the excessive use of penicillin. Expression of *mecA* gene results in an alternative PBP (PBP2a) that has a low affinity for most  $\beta$ -lactam antibiotics, thereby allowing these strains to replicate in the presence of methicillin and related antibiotics (Forbes *et al.*, 1998). Some antimicrobial resistance is brought about by multiple changes in the bacterial genome. For example, isoniazid resistance of *Mycobacterium tuberculosis* results from changes in the following genes: *katG* gene which encodes a catalase; *inhA* gene which is the target for isoniazid; the *oxyR* gene and neighboring *aphC* gene and their intergenic region (Forbes *et al.*, 1998).

### **2.5.5 Biological versus Clinical Resistance**

Biological resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent than has been previously observed. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is then said to have achieved clinical resistance. It is important to note



that often, biological resistance and clinical resistance do not necessarily coincide. From a clinical laboratory and public health perspective it is important to realise that biological development of antimicrobial resistance is an ongoing process, while clinical resistance is dependent on current laboratory methods and established cut-offs (Forbes *et al.*, 1998). Our inability to reliably detect all these processes with current laboratory procedures and criteria should not be perceived as evidence that they are not occurring. (Forbes *et al.*, 1998).

Intrinsic resistance is the innate ability of a bacterial species to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics, which allows tolerance of a particular drug or antimicrobial class. This can also be called “insensitivity” since it occurs in organisms that have never been susceptible to that particular drug. Such natural insensitivity can be due to: lack of affinity of the drug for the bacterial target inaccessibility of the drug into the bacterial cell extrusion of the drug by chromosomally encoded active exporters innate production of enzymes that inactivate the drug (Forbes *et al.*, 1998).

Intrinsic resistance of a pathogen of concern is important in practice to avoid inappropriate and ineffective therapies. For bacterial pathogens which are naturally insensitive to a large number of classes of antimicrobials, such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*, this consideration can pose a limitation in the range of options for treatment and thus consequently further increase the risk for emergence of acquired resistance (Forbes *et al.*, 1998, Giguere, 2006).



### **2.5.6 Acquired Resistance**

Acquired resistance is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible (Microbiology model, 2011). This can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes or from a combination of these two mechanisms. Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of each particular bacterial species. Laboratory methods are therefore needed to detect acquired resistance in bacterial species that are not intrinsically resistant (Microbiology model, 2011). These same methods are used for monitoring rates of acquired resistance as a means of combating the emergence and spread of acquired resistance traits in pathogenic and non-pathogenic bacterial species. Acquired resistance results from successful gene change and/or exchange that may involve: mutation or horizontal gene transfer via transformation, transduction or conjugation (Microbiology model, 2011).

### **2.5.7 Mutation**

A mutation is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait which it codes for. Any change in a single base pair may lead to a corresponding change in one or more of the amino acids for which it codes, which can then change the enzyme or cell structure that consequently changes the affinity or effective activity of the targeted antimicrobials. In prokaryotic genomes, mutations frequently occur due to base changes caused by exogenous agents, DNA polymerase errors, deletions, insertions and duplications (Gillespie, 2002). For prokaryotes, there is a constant rate of

spontaneous mutation of about 0.0033 mutations per DNA replication that is relatively uniform for a diverse spectrum of organisms (Gillespie, 2002). The mutation rate for individual genes varies significantly among and within genes (Gillespie, 2002).

### **2.5.8 Horizontal Gene Transfer**

Horizontal gene transfer, or the process of swapping genetic material between neighboring “contemporary” bacteria, is another means by which resistance can be acquired. Many of the antibiotic resistance genes are carried on plasmids, transposons or integrons that can act as vectors that transfer these genes to other members of the same bacterial species, as well as to bacteria in another genus or species (Torrence and Isaacson, 2003). Horizontal gene transfer may occur via three main mechanisms: transformation, transduction or conjugation (Torrence and Isaacson, 2003).

Transformation involves uptake of short fragments of naked DNA by naturally transformable bacteria. Transduction involves transfer of DNA from one bacterium into another via bacteriophages. Conjugation involves transfer of DNA via sexual pilus and requires cell –to–cell contact. DNA fragments that contain resistance genes from resistant donors can then make previously susceptible bacteria express resistance as coded by these newly acquired resistance genes.

Conjugation and Plasmids Conjugation was first described in 1946 by Lederberg and Tatum, based on studies showing that the intestinal bacteria *E. coli* uses a process resembling sex to exchange circular, extra chromosomal elements, now known as plasmids (Torrence and Isaacson, 2003).

### **2.5.9 Detecting antimicrobial resistance**

Historically, veterinary practitioners prescribed antibiotics based on expected mode of action, spectrum of activity and clinical experience. With the emergence and spread of antimicrobial resistance, treatment of bacterial infections has become increasingly difficult and is no longer as straightforward as it was many years prior. Practitioners now need to consider that the particular pathogen they wish to treat may be resistant to some or all of the available antibiotics, thus making antimicrobial susceptibility testing a standard procedure. Antimicrobial susceptibility testing methods are in vitro procedures used to detect antimicrobial resistance in individual bacterial isolates (Watt and Lindeman, 2006). Because these laboratory detection methods can determine resistance or susceptibility of an isolate against an array of possible therapeutic candidates, antimicrobial susceptibility testing results can be a useful clinical guideline in selecting the best antibiotic treatment option for each particular patient. These same methods can also be used for monitoring the emergence and spread of resistant microorganisms in the population (Watt and Lindeman, 2006). Clinical Breakpoints are threshold values established for each pathogen-antibiotic (i.e., bug-drug) combination indicating at what level of antibiotic the isolate should be considered to be sensitive, intermediate or resistant. The interpretative criteria for these are based on extensive studies that correlate laboratory resistance data with serum achievable levels for each antimicrobial agent and a history of successful and unsuccessful therapeutic outcomes (Watt and Lindeman, 2006). Although veterinary laboratories originally based interpretations on standards established using human pathogens, it became apparent by the early 1980s that such an approach did not reliably predict clinical outcomes when applied to veterinary practice. Subsequently, groups within organisations that set standards were

created for the purpose of developing veterinary-specific standards (Watt and Lindeman, 2006).

Standard conditions for these assays have been established based on extensive batteries of laboratory testing. Guidelines and recommendations for these are continuously updated by certain organizations worldwide, such as CLSI, EUCAST, OIE, BSAC, SFM, SRGA and CDS. Of these, those which specify antimicrobial testing methods and interpretative criteria for veterinary pathogens are: the CLSI in the USA, OIE in EU and CDS-AST in Australia (Watt and Lindeman, 2006; Turnidge and Peterson, 2007).

#### **2.5.10 Laboratory approaches and strategies**

Some points to consider when deciding whether or not to conduct antimicrobial susceptibility testing should include: clinical relevance of the isolate, purity of the isolate, logical panel of antimicrobial agents to be tested (i.e., do not include antibiotics to which the isolate is known to have intrinsic resistance) availability of test methodology, resources and trained personnel standardization of testing valid interpretation of results cost efficiency effective means to communicate results and interpretation to end-users.

Most often, interpretation is reduced to whether the isolate is classified as susceptible, intermediately susceptible, or resistant to a particular antibiotic. It should, however, be remembered that these invitro procedures are only approximations of invivo conditions which can be very different depending on the nature of the drug, the nature of the host and the conditions surrounding the interaction between the antibiotic and the target pathogen. One critical aspect is following standardised procedures that can generate reproducible

results, i.e. quality control. Aspects of quality control include: standardised bacterial inoculum size culture conditions (growth medium, pH, cation concentration blood and serum supplements and thymidine content) incubation conditions (atmosphere, temperature, duration) concentration of antimicrobials for testing (Microbiology model, 2011).

### **2.5.11 Test Methods in Detecting Antimicrobial Resistance**

There are currently several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotic treatment. This data may be utilised as guidelines for chemotherapy, or at the population level as indicators of emergence and spread of resistance based on passive or active surveillance. Some examples of antibiotic sensitivity testing methods are:

- (a) Dilution method (broth and agar dilution method) (Microbiology model, 2011)
- (b) Disk-diffusion method E-test (Microbiology model, 2011)
- (c) Automated methods (Microbiology model, 2011)

Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test genotypic methods such as PCR and DNA hybridisation methods (Microbiology model, 2011)

Selection of the appropriate method will depend on the intended degree of accuracy, convenience, urgency, availability of resources, availability of technical expertise and cost.

Interpretation should be based on veterinary standards whenever possible, rather than on human medical standards, which may not always be applicable. Among these available tests, the two most commonly used methods in veterinary laboratories are the Disk-diffusion method (Microbiology model, 2011) and the broth microdilution method (Microbiology model, 2011).





## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Sampling Areas

The research was conducted in the Accra metropolis. Accra Metropolitan Area (AMA) is one of the districts in the Greater Accra Region. It covers an area of 173 km<sup>2</sup>. It shares boundaries with the Ledzokuku-Krowor Assembly on the East and on the Northern. The Western frontiers are the Ga East, Ga West, and the Ga South district. The Southern boundary of AMA is the Gulf of Guinea.



**Figure 1: Map of Accra Metropolitan Assembly**

Whole dressed chicken were purchased from open markets, commercial cold stores and farms where chicken processing is done. The selected markets included Kaneshie, Makola, Agbogboloshie, Tuesday (Mamprobi), Dansoman, Adabraka and Mallam Atta, Madina and Mallam. Also, samples were purchased from super markets at Osu and Accra Mall. Samples were also purchased from local farms from where chicken processing (dressing) is carried out at Laterbiokorshie, Sukura, Abokobi, Teiman, Nungua, Dansoman and Sakaman.

### **3.2 Sampling Methods**

A total of fifty (50) fresh chicken carcasses were sampled. Twenty-five (25) fresh chicken carcasses were sampled from open markets and meat shops that retailed imported chicken and twenty-five (25) from the local farms engaged in chicken processing. Samples collected were kept in sterilised zip bags and stored in ice chest containing ice cubes to maintain the storage temperature at 0°C (ICMSF, 1986). They were taken to the Microbiology Laboratory of the Animal Research Institute. The samples were worked on immediately they were taken to the laboratory. Sampling and laboratory work was done from February, 2015 to April, 2015, a period of 3 months.

### **3.3 Laboratory Techniques**

#### **3.3.1 Preparation of Samples**

The carcasses were allowed to thaw to room temperature of 30°C. Using a sterile microbiological swab dipped in 0.1% peptone water [Liofilchem s.r.l. BACTERIPLOGY PRODUCTS, 610038, Italy], swabs were taken from each carcass from three sampling sites the vent area, under the wing and from the breast muscle. The tip of the swab stick containing the suspected organisms with the sample was carefully broken into sterile MacCartney bottle containing 5ml of 0.1% sterile peptone water (Merck, Darmstadt-Germany) to form the neat. The neat was then incubated at 37°C in a bacteriological incubator (Wagtech) for 15 minutes just before use to enable the suspected microorganisms to adjust to incubation temperature.

#### **3.3.2 Culture Techniques**

From each sample, a sterile loop full of the neat was aseptically streaked onto sheep Blood agar (Merck, Darmstadt-Germany) and MacConkey agar (Merck, Darmstadt-Germany) using the plate out technique (Heritage *et al*, 1996).

Cultures were incubated aerobically and anaerobically in a bacteriological incubator (Wagtech-England) at 37°C for 18- 24hours. The microorganisms on the media were examined for their colonial morphology. Mixed or impure colonies on primary media (Blood agar and MacConkey agar) were purified by subculturing onto selected secondary media based on organisms of interest or study.

### 3.3.3 Isolation and Identification

After overnight incubation, colonial morphology of organisms based on their physiological characteristics were studied for size, shape, outline, colour and change in medium on various media. Standard microbiological techniques including Gram staining, examinations of cellular morphology of organisms using light microscopy magnified at 100x with oil immersion were carried out. Biochemical tests such as, Motility Indole Urea (MIU) [Liofilchem s.r.l. BACTERIOLOGY PRODUCTS, 610236, Italy], Catalyst, Triple Sugar Iron (TSI) [Oxoid, CM0277, Hampshire-England], Indole Methyl Red Voges-Proskauer and Citrate [IMViC Test] were conducted. Carbohydrates oxidation/fermentation (O/F Test) to detect gas and or acid production among others were also used to isolate and identify meat spoilage and poisoning organisms.

### 3.3.4 Culture, Isolation and Identification of *Salmonella* sp. and *Shigella* sp.

One (1) ml of the neat sample was added to 20ml of double strength Selenite F broth [Oxoid, CM 395, L. 121, Hampshire- England]. This was mixed thoroughly and incubated at 37°C in a bacteriological incubator (Wagtech -England) for 18-24 hours.

Using plate-out technique, subcultures were made from the broth aseptically on to *Salmonella* sp. *Shigella* (SS) agar [Oxoid, CM 533, Hampshire- England] and Xylose Lysine Deoxycholate (XLD) [Liofilchem s.r.l. BACTERIOLOGY PRODUCTS, 610060, Italy]. Cultures were incubated in a bacteriological incubator (Wagtech-England) at 37°C for 24-48 hours and examined for the physiological characteristics of colonies on the media (colonial morphology). Colonies showing slight to heavy blackening due to hydrogen

sulphide production were isolated and identified as *Salmonella* sp. while creamy sparkling colonies showing no hydrogen sulphide production were isolated as suspected *Shigella* sp. Organisms were differentiated and confirmed using biochemical test such as Motility Indole Urea (MIU) [Liofilchem s.r.l. BACTERIOLOGY PRODUCTS, 610236, Italy] and Triple Sugar Iron (TSI) [Oxoid, CM0277, Hampshire-England].

### **3.3.5 Culture, isolation and identification of *Escherichia coli***

All lactose fermenting colonies on MacConkey agar were selected and aseptically subcultured on to Eosin Methylene Blue agar (EMBA) [Scharlau Chemie S.A., 01-068, Barcelona-Spain] to isolate and identify *E. coli*. Cultures were incubated at 45°C in a bacteriological incubator for 24-48 hours.

After incubation, colonies showing metallic green sheen, observed at an angle of 45° to a light source, were isolated and identified to be *E. coli*. The organisms were confirmed using cell morphology and Indole Methyl Red Vorges-Proskeur Citrate [IMViC Test].

Cell morphology was examined based on physiological characteristics of the media used. The cells were then stained using Gram staining technique and cell morphology was identified under a light microscope of 100x magnification.



### 3.4 Antibiotic Susceptibility Test (Stokes Diffusion Diagnostic Technique)

#### 3.4.1 Antibiotic Susceptibility Testing

Stoke's disc diffusion assay was performed to assess the antibiotic susceptibility pattern of all *Salmonella* sp. and *E. coli* isolates.

The antibiotic susceptibility testing of test organisms were carried out on Muller-Hinton agar (BIOTEC Laboratory LTD, Ipswich-United Kingdom, 3/154) with antibiotic disc (Abtek Biologicals Ltd, Liverpool- United Kingdom ) using the multiple disc diffusion technique against the following antibiotics and their concentrations: Ampicillin (10 µg), Chloramphenicol (30 µg), Cefotaxime (30 µg) Ceftriaxone (30 µg), Gentamicin (10 µg), Cefuroxime (30 µg), Cotrimoxazole (25 µg), and Tetracycline (10 µg/ml). This Technique was based on the standard operating procedure (SOP) adapted from Clinical and Laboratory Standards Institute (CLSI) of United States of America. After overnight incubation, zones of inhibition were measured with the aid of a long meter rule at three different angles and the average zone of inhibition in millimeters (mm) based on the value obtained were reported as Sensitive, Intermediate and Resistant. To standardise the inoculum density for a susceptibility test, a BaCl<sub>4</sub> turbidity standard, equivalent to 1 McFarland standard was used by strictly following the SOP for the preparation and standardisation.

### **3.4.2 Media Preparation**

Mueller-Hinton (MH) agar (BIOTEC Laboratory Ltd, Ipswich-United Kingdom, 3/154), the recommended media for Antibiotic Susceptibility Testing (AST) was reconstituted according to the manufacturer's instructions. To ensure equal depth of media as a requirement for AST, aliquoted 15ml of MH were dispensed into each Mac Cartney bottles and sterilised by autoclaving. Content of each bottle was aseptically poured into 9cm sterile petri dishes, allowed to cool, set and solidify.

### **3.4.3 Purification of selected bacteria**

Selected test organisms were purified by subculturing onto Nutrient agar [Oxoid, CM 3, Hampshire- England] to obtain discrete colonies. The cultures were incubated in a bacteriological incubator at 37°C for 18-24 hours.

### **3.4.4 Preparation of inoculum**

After incubation, 3-5 purified and discrete colonies of the test organisms were selected aseptically with a sterile inoculating loop and placed into 5ml of 0.1% sterile peptone water. This was emulsified to obtain homogenous solution comparable to 1 MacFarland standard solution. Just before AST the homogenous solution was incubated at 37°C for 10-15min in a bacteriological incubator.

### **3.4.5 Test Procedure**

For the AST, the Stokes Diffusion Techniques was used. The Mueller Hinton medium plates were dried for 10-15min in a bacteriological incubator. After drying, the plates were flooded with between 0.2-0.5ml of the prepared inoculum and allowed to stand for 2-3minutes to enable the test organism to seed into the media. A sterile forceps was used to aseptically pick the orthodox AST multi disc paper and firmly pressed onto the media to enhance contact between the disc and the test organism. Each plate containing one isolate was incubated at 37°C for 18-24 hours in a bacteriological incubator. Zone of inhibition of the various antibiotics discs were measured to the nearest millimeter (mm) using a long meter rule at three different angles. The average zone of inhibition in millimeters (mm) was calculated and recorded.

### **3.4.6 Interpretation of Results**

Antibiotic disc with the average zone of inhibition less than 2mm indicated that the test organism was resistant to the selected antibiotic. Disc with average zone of inhibition greater than but less than 3mm indicated that the reaction of the test organism was intermediate to the selected antibiotic. Zone of inhibition greater than 3mm indicated that the test organism was sensitive to the antibiotics selected and can be used to treat any infection caused by the test organism (Cheesbrough, 1984).

## CHAPTER FOUR

### RESULTS

#### 4.1 Organisms Isolated

Isolates from swabs taken from three sampling sites (the wing, the breast muscle and the vent area) of each of the chicken carcass are as shown in Table 1 below

**Table 1: Frequency (%) Distribution of isolates**

<b>Bacteria</b>	<b>Total</b>	<b>(%)</b>	<b><math>\lambda^2</math></b>	<b>P value</b>
<i>E. coli</i>	70	46.98	0.54	0.46
<i>Salmonella</i> sp.	79	53.02	0.54	0.46
<i>Shigella</i> sp.	0	0		

N= 149.

A total of 149 isolates from the 150 swabs prepared from the 50 samples were investigated. Analysis using  $\lambda^2$  statistic suggested that there were equal chances of finding isolates of *Salmonella* sp., and *E. coli*. No sample however proved positive for *Shigella* sp.

#### 4.2 Isolates by Chicken Anatomical Sites

Result for the frequency (%) distribution of isolates from respective anatomical sites is shown in Table 2. Percent distribution of isolates per chicken anatomical site ranged from 16.78-17.45% for *Salmonella* sp. and 12.75-17.45% for *E. coli*

**Table 2: Frequency (%) distribution of isolates by Chicken anatomical sites**

Anatomical sites	Total	Pathogen	%	$\lambda^2$	P. value
Breast	29.53	<i>Salmonella</i> sp.	16.78	0.37	0.83
		<i>E. coli</i>	12.75	0.37	0.83
Vent	36.24	<i>Salmonella</i> sp.	18.79	0.37	0.83
		<i>E. coli</i>	17.45	0.37	0.83
Wing	34.23	<i>Salmonella</i> sp.	17.45	0.37	0.83
		<i>E. coli</i>	16.78	0.37	0.83
N=149	100.00				

Values obtained showed a tendency for higher incidence of detection of *Salmonella* sp. compared to *E. coli* in all the three anatomical sites sampled (Table 2). However, based on the  $\lambda^2$  statistics, the likelihood of isolating *E. coli* or *Salmonella* sp. was not dependent ( $p > 0.05$ ) on anatomical site.



### 4.3 Organisms Isolated From Different Sampling Sources

Retail of fresh chicken is done in open markets, meat shops, cold stores and in the local farms in the Accra metropolis. Retail of fresh chicken is done for both local and imported chicken. Chicken is stored in refrigerators before retailing.

Table 3 below shows the distribution of isolates from different sources of the fresh whole chicken.

**Table 3: Frequency (%) distribution of isolates from different sampling sources**

Source	%	$\lambda^2$	P value	Bacteria	%	$\lambda^2$	P-value
Imported	28.9	26.6	0.0001	<i>Salmonella</i> sp.	13.42	1.02	0.31
				<i>E. coli</i>	15.44	1.02	0.31
Local	71.1	26.6	0.0001	<i>Salmonella</i> sp.	39.60	1.02	0.31
				<i>E. coli</i>	31.54	1.02	0.31

N=149

There was a significant difference ( $p < 0.05$ ) between the number of isolates from the locally dressed chicken (71.1%) compared to that from the imported chicken (28.9%) (Table 3). The likelihood of finding *E. coli* and /or *Salmonella* sp. in locally processed chicken was more than twice for imported chicken.

Both *E. coli* (15.44%) and *Salmonella* sp. (13.42%) were isolated from imported chicken. Similarly, *E. coli* (31.54%) and *Salmonella* sp. (39.60%) were isolated from locally dressed

chicken. The incidence of *E. coli* was greater by 16.10% in locally dressed chicken compared to imported chicken. Also, a greater number of *Salmonella* sp. (39.60%) was observed in locally dressed chicken compared to 13.42% recorded in imported chicken.

#### 4.4 Organisms Isolated From Markets and Farms

Table 4 below shows the type of organisms isolated from different outlets. The outlets were the open markets and the farms.

**Table 4: Frequency (%) distribution of isolates from different market outlets**

Outlet	Total (%)	Type of Bacteria	(%)	$\lambda^2$	P. value
Open market	49.00	<i>Salmonella</i> sp.	27.52	0.568	0.451
		<i>E. coli</i>	21.48	0.568	0.451
Farm gate	51.00	<i>Salmonella</i> sp.	25.50	0.568	0.451
		<i>E. coli</i>	25.50	0.568	0.451
N=149	100.00				

The number of *Salmonella* sp. isolates from the markets (27.52%) was similar to that from the local farms (25.50%). Similarly the number of *E. coli* isolates from the farms (25.50%) was not different from those isolated from the markets (21.48%). Thus there was equal likelihood ( $p > 0.05$ ) of finding *E. coli* or *Salmonella* sp. in retail chicken regardless of whether the chicken was purchased in open markets or farms.

#### 4.5 Distribution of antibiotic sensitivity of all isolates

Table 5 below shows the distribution of the antibiotic sensitivity of the isolates to different antibiotics.

**Table 5: Frequency (%) distribution of antibiotic sensitivity of all isolates**

Antibiotic results	(%)	$\chi^2$	P. value
Intermediate	1.3	206.4	0.0001
Resistant	10.1	206.4	0.0001
Sensitive	88.6	206.4	0.0001

N=149

Majority of the isolates (*Salmonella* sp. and *E. coli*) (88.60%) were sensitive to the test antibiotics whereas 10.1% were resistant to at least one tested antibiotic. Therefore, there is a higher likelihood of finding *E. coli* and /or *Salmonella* sp. sensitive to the tested antibiotics and 10% likelihood of finding a resistant isolate ( $p < 0.05$ ).



#### 4.6 Antibiotic sensitivity of isolates

Table 6 below shows how the isolates reacted to the various antibiotics tested in the study.

**Table 6: Frequency (%) distribution of antibiotic sensitivity of isolates**

Antibiotic results	Total (%)	Isolate	(%)	$\lambda^2$	P-value
Intermediate	1.3	<i>Salmonella</i>	0.0	0.54	0.46
		<i>E. coli</i>	1.3	0.54	0.46
Resistant	10.0	<i>Salmonella</i>	6.0	0.54	0.46
		<i>E. coli</i>	4.0	0.54	0.46
Sensitive	88.6	<i>Salmonella</i>	47.0	0.54	0.46
		<i>E. coli</i>	41.6	0.54	0.46
N=149	100.0				

*Salmonella* sp. (47.0%; n=79) were sensitive to at least one antibiotic. Similarly for *E. coli*, (41.6%; n=70) were sensitive to at least one antibiotic. However, the difference in the number of *E. coli* and *Salmonella* sp. isolates sensitive or resistant to the tested antibiotics were not significantly different ( $p>0.05$ ).

#### 4.7 Distribution of sensitivity of isolates to different antibiotics

Sensitivity of the various isolates to antibiotics is as presented in table 7 below.

**7: Frequency (%) distribution of sensitivity of isolates to different antibiotics**

Antibiotics	Bacteria	Sensitive			Intermediate			Resistant		
		(%)	$\lambda^2$	P. value	(%)	$\lambda^2$	P. value	(%)	$\lambda^2$	P. value
CTR	<i>E. coli</i>	1.34	2.55	0.27	4.03	2.55	0.27	41.61	2.55	0.2794
	<i>Salmonella</i> sp	0	2.55	0.27	6.04	2.55	0.27	46.98	2.55	0.2794
CTX	<i>E. coli</i>	0.67	1.13	0.56	5.37	1.13	0.56	40.94	1.13	0.5662
	<i>Salmonella</i> sp	0	1.13	0.56	6.04	1.13	0.56	46.98	1.13	0.5662
CHL	<i>E. coli</i>	0	20.28	0.0001	8.86	20.28	0.0001	36.91	20.28	0.0001
	<i>Salmonella</i> sp	4.7	20.28	0.0001	24.83	20.28	0.0001	23.49	20.28	0.0001
CRX	<i>E. coli</i>	0	42.88	0.0001	9.4	42.88	0.0001	37.58	42.88	0.0001
	<i>Salmonella</i> sp	2.01	42.88	0.0001	36.91	42.88	0.0001	14.09	42.88	0.0001
GEN	<i>E. coli</i>	2.01	2.38	0.3	4.7	2.38	0.3	40.27	2.38	0.3
	<i>Salmonella</i> sp	4.7	2.38	0.3	2.68	2.38	0.3	45.64	2.38	0.3
COT	<i>E. coli</i>	1.34	4.45	0.1	31.54	4.45	0.1	14.09	4.45	0.1
	<i>Salmonella</i> sp	3.36	4.45	0.1	40.94	4.45	0.1	8.72	4.45	0.1
TET	<i>E. coli</i>	2.01	11.63	0.003	31.54	11.64	0.003	13.42	11.63	0.003
	<i>Salmonella</i> sp	0.67	11.63	0.003	47.65	11.64	0.003	4.7	11.63	0.003
AMP	<i>E. coli</i>	3.36	11.57	0.003	32.89	11.57	0.003	10.74	11.57	0.003
	<i>Salmonella</i> sp	2.01	11.57	0.003	48.32	11.57	0.003	2.68	11.57	0.003

N=149

CTR; Ceftriaxone (30 µg), CTX; Cefotaxime (30ug), CHL; Chloramphenicol (30 µg),

CRX; Cefuroxime (30ug), GEN; Gentamycin (10 µg), COT; Cotrimoxazole (25µg), TET;

Tetracycline (10 µg), AMP; Ampicillin (10 µg).



A greater percentage of *Salmonella* sp. isolates (24-47%) were resistant to TET, Ampicillin (AMP), Chloramphenicol (CHL) and Cefuroxim (CRX) (Table 7). The number of *E. coli* isolates resistant to the source antibiotics (9.4 – 31.5%) was significantly ( $p < 0.05$ ) less compared to *Salmonella* sp. isolates. There was a greater likelihood of finding *Salmonella* sp. which were resistant to Tetracycline (TET), Ampicillin (AMP), Chloramphenicol (CHL) and Cefuroxime (CRX) than *E. coli*.



#### 4.8 Distribution of antibiotic sensitivity Isolates (*Salmonella* sp., *E. coli*) of imported and locally processed chicken

Table 8 shows the antibiotic sensitivity of the isolates from imported fresh retail chicken against isolates from locally processed fresh chicken

**Table 8: Frequency (%) distribution of antibiotic sensitivity of imported and locally processed chicken**

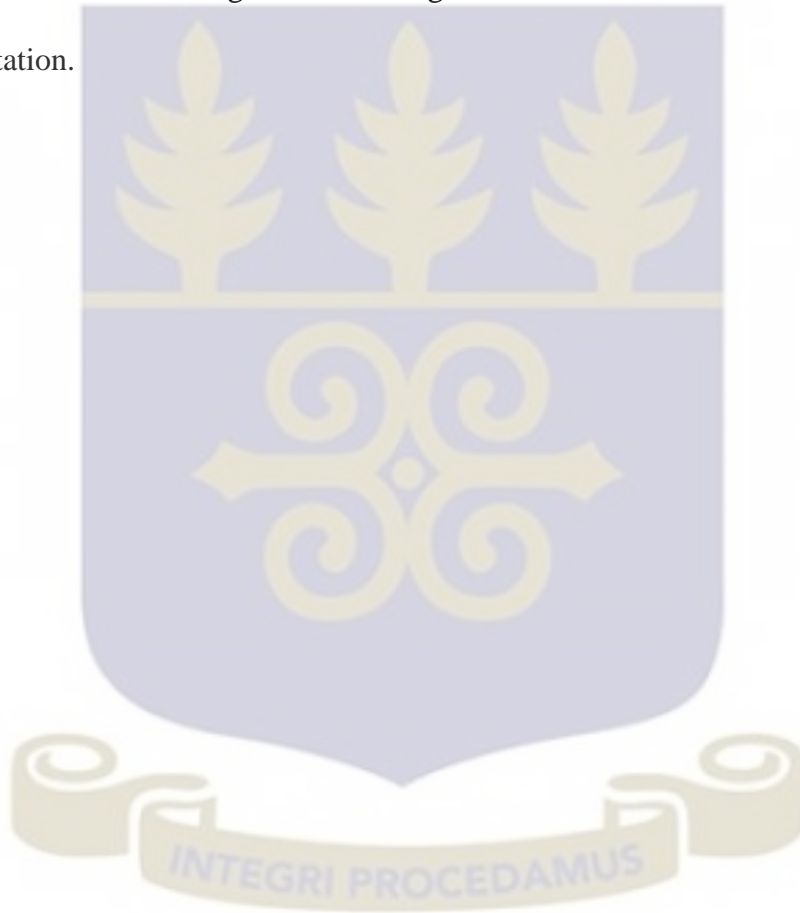
Antibiotics	Resistance	% of Imported	% of Local	$\chi^2$	P. value
CHL	Intermediate	1.23	3.7	8.04	0.017
	Resistant	6.17	34.57	8.04	0.017
	Sensitive	24.69	29.69	8.04	0.017
CRX	Intermediate	-	-	-	-
	Resistant	2.47	33.33	13.16	0.0003
	Sensitive	29.63	31.57	13.16	0.0003
CTR	Intermediate	1.23	1.23	17.6	0.0002
	Resistant	16.05	61.73	17.6	0.0002
	Sensitive	14.81	4.97	17.6	0.0002
TET	Intermediate	2.47	1.23	19.77	0.0001
	Resistant	14.81	61.73	19.77	0.0001
	Sensitive	14.81	25	19.77	0.0001
AMP	Intermediate	6.17	2.47	6.47	0.039
	Resistant	24.69	56.79	6.47	0.039
	Sensitive	1.23	8.64	6.47	0.039

N=149

CHL; Chloramphenicol (30 µg), CRX; Cefuroxime (30ug), CTR; Ceftriaxone (30 µg), TET; Tetracycline (10 µg) AMP; Ampicillin (10 µg), S; Sensitive, I; Intermediate, R; Resistant

Majority of the isolates (*Salmonella* sp. and *E. coli*) that are resistant to CHL, CRX, CTR, TET and AMP were from locally produced dressed chicken.

This could be attributed to the long use and abuse of antibiotics on the farms for the treatments of infection caused by bacteria in both ruminants and poultry. The long use and abuse of these antibiotics against these organisms makes them build resistant through genetic mutation.



## CHAPTER FIVE

### DISSCUSSION

#### 5.1 Bacteria Isolated

Out of the total number of isolates (n=149), 79 representing 53.02% were *Salmonella* sp. Although the value obtained was higher than 13(6.8%) of values obtained by Sackey *et al.*, (2001), our finding is lower than 60% reported by Antunes *et al.*, (2003) in Portugal. Also, the figure obtained is close to 40% obtained by Tavechio *et al.*, (2002) in Sao Paulo, Brazil, 52.00% by Hassanein *et al.*, (2011) in Egypt and 33% by Adeyanju *et al.*, (2014) in Nigeria. A similar study conducted by Uyttendaele *et al.*, (1999), Zhao *et al.*, (2001), Dominguez *et al.*, (2002) and Jorgensen *et al.*, (2002) showed a prevalence of 36%, 36%, 40% and 25% for *Salmonella* sp., respectively.

The high incidence of *Salmonella* sp. isolates from the fresh chicken in Accra metropolis could be attributed to the husbandry practices and dressing and processing methods of chickens from the local farms and storage conditions of the imported frozen chicken in Accra. The figure (53.02%) is likely to reduce if husbandry practices and slaughtering processes are improved in the local poultry farms.

*E. coli* isolates from this study was 46.98%. This figure is comparable to 48.4% incidence of *E. coli* isolates in Morocco (Cohen *et al.*, 2007). However, our finding is lower than figures obtained in similar experiments that recorded 98% (Saikia and Joshi, 2010) and 78% (Odwar *et al.*, (2014)) in India and Kenya respectively. Also, higher prevalence rates of 100% of *E. coli* contamination compared to 46.98% recorded in this study were recorded in Vietnam (Thangh *et al.*, 2009) and in Cameroon, Siakia and Josh, (2010).

The presence of *E. coli* in the chicken samples was an indicator of faecal contamination (Bitton, 2005). This may have resulted from contamination from the environment, personnel or by virtue of processing including contaminated water.

There was no significant difference between the number of *Salmonella* sp. and *E. coli* isolated ( $p>0.05$ ). Although the types of organisms isolated depended upon where the samples were taken and upon the stage of processing (Frazier and Westhoff, 1988), results from this study suggested that, regardless of anatomical sites, the likelihood of isolating *E. coli* or *Salmonella* sp. was not different.

No *Shigella* sp was isolated from the samples collected. This is contrary to earlier report by Sackey *et al.*, (2001) who found 6.9% *Shigella* sp. in samples collected from 97 carcasses of chicken and 87 chicken parts of chicken from markets in Accra. However, our finding confirms the low levels of *Shigella* sp. in Ghana as reported by Sackey *et al.*, (2001). This is an indication that the prevalence of *Shigella* sp. in poultry products in the Accra metropolis is low and hence foodborne diseases caused by *Shigella* sp. from chicken is likely to be low. This could mean among other factors that farmers are supplying poultry with clean water free from *Shigella* contamination (DuPont, 2000).

## 5.2 Distribution of Isolates from Different Sources

About 80 percent of imported frozen chicken to Ghana is processed into choice cuts, mainly chicken leg quarters and wings, with the remaining 20 percent being frozen whole chickens and gizzards (Ghana Poultry Report Annual, 2013). Major retail outlets for fresh chicken in Accra metropolis are traders in open markets, cold stores as well as from local farms. Both locally produced and imported chicken are stored in freezer units during retail.



The bulk of imported poultry products come in the form of choice cut parts shipped in either brown boxed-packages (US origin) or white boxes with branded names (Brazil origin) with most weighing approximately 10-15 kg. Locally produced birds are sold to retailers and individuals as live or processed whole birds during the festive occasions of Christmas and Easter (Ghana Poultry Report Annual, 2013).

A significant difference ( $p \leq 0.05$ ) between the number of isolates from the locally dressed chicken (71.17%) compared to that from the imported chicken (28.86%) (Table 3) was observed. This suggested that, the likelihood of finding *E. coli* and /or *Salmonella* sp. in locally processed chicken was more than twice for imported chicken. This observation underscores the need for improved carcass handling practices on farm and at slaughter points in order to eliminate/minimize contamination and its subsequent food safety challenges and risks to human health. The public health implications of this observation cannot be overemphasized. The lack of standards and a functional food safety policy in Ghana is a major obstacle to ensuring consumer safety.

With most chicken local slaughtering and processing is done in the open and processing equipment (knives, pans and tables) are not properly cleaned, increasing the risk of contamination dramatically. This is a health concern to both processors and consumers. It is also a concern to the local poultry industry since this may lead to a shift to higher consumption of the imported frozen chicken.

This situation can be controlled or averted by implementing effective Critical Control Points (CCPs) at the various processing stages of the local processing plants to reduce pathogenic bacteria contamination of carcasses (Rose *et al.*, 2001). Minimum pathogenic bacteria counts must be put in place and strictly implemented.

### 5.3 Distribution of Isolates from Different Outlets

Data from this study indicated that the number of *Salmonella* sp. isolated from the markets (27.52%) was similar to that from the local farms (25.50%). Similarly the number of *E. coli* isolates (25%) from the farms was not different from those isolated (21.48%) from the open markets. Thus, there was equal likelihood ( $p < 0.05$ ) of finding *E. coli* or *Salmonella* sp. in retail chicken regardless of the market outlet. More *Salmonella* sp and *E. coli* (71.1%) were isolated from fresh local chicken than from the imported fresh chicken (28.9%) (Table 3). There was therefore a high likelihood of finding the target organisms (*Salmonella* sp and *E. coli*) in locally processed fresh chicken than imported fresh chicken ( $p < 0.05$ ). However, there was equal likelihood of finding similar levels of *Salmonella* sp contamination and *E. coli* in imported chicken and locally processed chicken ( $p > 0.05$ ). Although the isolates may be gut derived, the act of processing, washing, handling and packaging is suggested to have evenly distributed bacteria to all anatomical sites.

### 5.4 Antibiotic Sensitivity of Isolates

Results of the antibiotic sensitivity tests for isolates (Table 5) indicated that 10 % of the isolates tested ( $n=149$ ) had at least one or multiple resistance to the antibiotics tested. However, it is not known whether multiple antibiotic resistances is controlled by several or a specific gene (Davies and Davies, 2010). Although a greater percentage of the isolates were susceptible to the antibiotics tested, there is a 10% chance that the antibiotics tested could not cure *Salmonella* sp and or *E. coli* infections as found in this study. This finding is of great importance to public health. The use of antibiotics in the local farms must be managed to reduce the incidence of antibiotic resistance.

### 5.5 Antibiotic Sensitivity for *E. coli* and *Salmonella sp.* isolates

No significant difference was observed between the sensitivity of *E. coli* and *Salmonella sp.* isolates to the tested antibiotics. A decreased resistance to CTR, CTX and GEN (Table 7) compared to CHL, CRX, COT, TET and AMP for both isolates was observed. No significant ( $P>0.05$ ) difference was observed between *E. coli* and *Salmonella sp.* isolates resistance to COT (Table 7). However, a greater percentage of *Salmonella sp.* isolates was resistant to CHL, CRX and TET and AMP respectively. This observation raises the hypothesis that the rate of acquisition of antibiotic resistance genes is higher among *Salmonella sp.* isolates compared to *E. coli*.

The sustained efficacy of antibiotics is a major concern in public health due of the evolution and acquisition of antibiotic resistance genes which are often acquired through horizontal gene transfer among bacteria. The occurrence of such antibiotic resistance in retail poultry constitutes a direct exposure of humans to antibiotic resistant pathogenic bacteria and this should be of great public health concern. Although the application of heat may kill such organisms, cross contamination is always a risk in meat handling and retail. It was interesting to note that majority of the isolates (*Salmonella sp.* and *E. coli*) that were resistant to CHL, CRX, CTR, TET and AMP were isolated from locally produced and dressed chicken (Table 8).

The long use and indiscretionary abuse of antibiotics in our environments for the treatments of infections among both animals and humans, could sustain the build-up resistant genes and evolution of new ones.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

*E. coli* and *Salmonella* sp. have been isolated from both imported and locally dressed chicken sold in the Accra metropolis.

More *Salmonella* sp. contamination of fresh chicken were recorded than *E. coli* contamination.

The prevalence of *E. coli* and *Salmonella* sp. is high in locally dressed chicken (71.14%) compared to the imported chicken (28.86%).

*Salmonella* sp. and *E. coli* isolates showed some varying degrees of resistance to all antibiotics tested. However resistance to COT, TET, and AMP were high (over 70%).

*Salmonella* sp. isolated showed more resistance to COT, TET and AMP (over 40%) compared to *E. coli* isolates.

Resistance among isolates from locally dressed chicken is significantly higher from that of imported chicken.

#### 6.2 Recommendations

Processing procedures must be evaluated with the objective of identifying critical control points in the production process in order to reduce the rate of contamination of poultry products. Additionally, the use of antibiotics in the local farms must be managed to reduce the incidence of antibiotic resistance. Furthermore, studies should be conducted in other

regions to ascertain the prevalence of these pathogens and their antibiotic resistance in poultry products. Lastly, a genetic characterisation of antibiotic genes in the *E. coli* and *Salmonella* sp isolates will be imperative in understanding their diversity and transmission mechanisms.





## REFERENCES

- Abraham, E. P. and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Nature*. 146: 837-837.
- 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(1): 8-12.
- Adeyanju, G. T., and Olayinka, I. (2014). *Salmonella* sp. and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springer Plus*. 3: 139.
- Adu-Gyamfi, A., Torgby-Tetteh, W., Appiah, V. (2012). Microbiological quality of chicken sold in Accra and determination of D10-Value of *E. coli*. *Food and Nutrition Sciences*. 3: 693-698.
- Ahmad, M. U. D., Sarwar, A., Najee, M. I., Nawaz, M., Anjum, A. A. and Alian, M. A. (2013). Assessment of Microbial Load of Raw Meat at Abattoirs and Retail Outlets. *Journal of Animal and Plant Sciences*. 23(3): 745-748.
- Alekshun, M. N. and Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128: 1037-1050.
- American Public Health Association (APHA), "Shigellosis," in Control Of Communicable Diseases Manual: 556-60, edited by Heymann, David L., (19th Ed. 2008).
- Antunes, P., Reu, C., Sousa, J. C., Peixe, L. and Pestana, N. (2003). Incidence of *Salmonella* sp. from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology*. 82: 97-103.
- Austin, C. C. and Wilkins M. J. (1998). Reptile associated *Salmonellosis*. *Journal of the American Veterinary Medical Association*. 212-6: 866-867.

- Baumler, A. J., Hargis, B. M. and Tsois R M. (2000). Tracing the origins of *Salmonella* sp. outbreaks. *Science*. 287: 50-52.
- Bailey, J .S., Cox, N. A., Craven, S. E. and Cosby, D. E. (2002). Serotype tracking of *Salmonella* sp. through integrated broiler chicken operations. *Journal of Food Protection*. 65-5: 742-745.
- Bean, N. H. and Potter, M. E. (1992). *Salmonella* sp. serotypes from human sources. U.S. *Animal Health Association*. 488-491.
- Bern, C., Martines, J., de zoysa, I., (1992). The magnitude of the global problem of diarrhoeal diseases: a ten-year update. *Bulletin WHO*, v. 70, p.705-714.
- Berger-Bächi, B. (2002). Resistance Mechanisms of Gram Positive Bacteria. *International Journal of Medical Microbiology*. 292: 27-35.
- Bermudez, M. and Hazen, T. C. (1988). Phenotypic and genotypic comparison of *Escherichia coli* from pristine tropical waters. *Applied and Environmental Microbiology*. 54: 979-983.
- Blattner, F. R., Plunkett, G 3<sup>rd</sup>. Bloch, C A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science*. 277(5331): 1453-62.
- Bush, (1988). Beta-lactamase inhibitors from laboratory to clinic. *Clinical Microbiology Reviews*. 1(1): 10-9-103.
- Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F. and Coque, T. M. (2008). Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. *Clinical Microbiology and Infection*. 14: 144-153.

- Capita, R., Alvarez-Astorga, M., Alonso-Calleja, C., Moreno, B., and Garcia-Fernandez, M. C. (2003). Occurrence of *Salmonellae* in retail chicken carcasses and their products in Spain. *International Journal of Food Microbiology*, 81, 169–173.
- Casalino, M., Latella, M.C., Prosseda, G. and Colonna, B. (2003). Preferential target of a convergent evolution driving enteroinvasive *Escherichia coli* toward a lysine decarboxylase-defective phenotype. *Infection and Immunity*. 71 (10): 5472-5479.
- Centers for Disease Control and Prevention (2009). National Center for Zoonotic, Vector Borne, and Enteric Diseases, “*Shigellosis*, General Information. <http://www.cdc.gov/nczved/divisions/dfbmd/Diseaseseases/shigellosis/>. Retrieved: February 6, 2015.
- Cheesbrough, M. (1984). *Medical Laboratory Manual for Tropical Countries*. Volume II: Microbiology. 350-352.
- Cohen, N., Ennaji, H., Bouchri, B., Hassar, M. and Kari, H. (2007). Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). *Journal of Applied Poultry Research*. 16: 502-508.
- Collins, C. H. and Lyne, P. M. (2004). *Microbiological Methods*. The Ashes, Hadlow, Kent, UK (Eighth Edition). 205-213.
- Conway, P. and Macfarlane, G. (1995). Microbial Ecology of the Human Large Intestine. In: *Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology*, Gibson, G. and G. MacFarlane (Eds.). CRC Press, London. 1- 24.
- Corrier, D. E., Hinton Jr, A., Hargis, B. and De Loach, J. R. (1992). Effect of used litter from floor pens of adult broilers on *Salmonella* sp. colonization of broiler chicks. *Avian Diseases*. 36:897-902.
- Cox, N., Bailey, J., Berrang, M., Buhr, R. and Mauldin J. (1994). Automated spray sanitizing of broiler hatching eggs. Total bacteria and coliform recovery after using an egg spraying machine. *Journal of Applied Poultry Research*. 3: 234-237.

Cramer, E.H, Blanton C.J, and Otto C., “Shipshape: Sanitation Inspections on Cruise Ships, 1990-2005,” CDC Vessel Sanitation Program, Journal of Environmental Health, Vol. 70, No. 7, 15-21 (March 2008). <http://www.cdc.gov/nceh/vsp/pub/biblio/cramer08.pdf>

Cristina Paiva de Sousa (2006). *Escherichia coli* as a specialized bacterial pathogen. REVISTA DE BIOLOGIA E CIÊNCIAS DA TERRA. Volume 6- Número 2 - 2°. ISSN 1519-5228

Dahal, N. (2007) Prevalence and antimicrobial resistance of *Salmonella* in imported chicken carcasses in Bhutan. 1, 9, 14, 15. <http://www.moa.gov.bt/ncah/download/files/pub7mc3308ib>. Retrieved: February 20, 2015.

Davies, P. R., Morrow, W. E.M., Jones, F. T., Deen, J., Fedorka-Cray, P. J. and Harris, I. T. (1997). Prevalence of *Salmonella* sp. in finishing swine raised in different production systems in North Carolina, USA. *Epidemiology and Infection*. 119: 237-244.

Deibel, C., Kramer, S., Chakraborty, T. and Ebel, F. (1998). EspE, a novel secreted protein of attaching and effacing bacterial, is directly translocated into effected host cells, where it appears as a tyrosine-phosphorylated 90kDa protein. *Molecular Microbiology*. 28: 463-474.

Dominguez, C., Gomez, I. and Zumalacarregui, J. (2002). Prevalence of *Salmonella* sp. and *Campylobacter* in retail chicken meat in Spain. *International Journal of Food Microbiology*. 72: 165–168.

Donnenberg, M. S. and Welch, R. A. (1996). Virulence determinants of uropathogenic *E. coli*. In: H. L. a. J. W. W. Mobley (Eds.) *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management*. ASM Press Washington, DC. 135–174.

- Downes, F. P. and Ito, K. (Eds.). (2001). *Compendium of Methods for the Microbiological Examination of Foods* (4th ed.). American Public Health Association. Washington, DC, USA. 24-31.
- DuPont, H. L.(2000)“*Shigella* species (bacillary dysentery),” in Mandell, Douglas, and Bennett’s *Principles And Practice of Infectious Diseases*. Fifth Edition,. p. 2363-9, Mandell, Bennett, and Dolan, Editors)
- Ebel, E. D., David, M. J, and Mason, J. (1992). Occurrence of *Salmonella* sp. enteritis diseases in the U.S. Commercial egg industry. Report on a national spent hen survey. *Avian Diseases*. 36: 646-654.
- Edel, W. (1994) *Salmonella* sp. enteritis diseases eradication program in poultry breeder flocks in the Netherlands. *International Journal of Food Microbiology*. 21: 171-178.
- European Food Safety Authority (2007). The community summary report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance and foodborne outbreaks in the European Union. *The EFSA Journal*.130: 3-352.
- Eisenstein, B. I. and Zaleznik, D. F. (2000). *Enterobacteriaceae*: in (Mandell, Bennet, and Dolin,)ed. *Principle and Practices of Infectious Diseases*, 5th Edition, Churchill Livingstone. 7. London: Academic Press. 43–54.
- Eriksson De, R., Mallinson, C. L., Tablante, E. T., Morales, N. L., Park, R. A., Carr, L. E. and Joseph, S. W. (2001). Effect of dry litter and airflow in reducing *Salmonella* sp. and *Escherichia coli* populations in the broiler production environment. *Journal of Applied Poultry Research*. 10: 245-251.
- Fabrega, A. and Vila, J. (2013). "*Salmonella* sp. enterica Serovar typhimurium. Skills to succeed in the Host: Virulence and Regulation". *Clinical Microbiology Reviews* 26 (2): 308– 341.



- Fagundes-Neto, U., Scaletsky, I. C. (2000). The gut at war: the consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. *Sao Paulo Medical Journal*. 118: 21-29.
- Ferris, K. E. and Miller, D. A. (1993). *Salmonella* sp. serotypes from animals and related sources. U.S. Animal Health Association, Richmond, VA.440-454.
- Food and Agriculture Organization (2010). Smallholder poultry production – livelihoods, food security and sociocultural significance, by K. N. Kryger, K. A. Thomsen, M. A. Whyte and M. Diseases. FAO Smallholder Poultry Production Paper No. 4. Rome. 30-51.
- Food Standards Agency. (2003). UK wide survey of *Salmonella* sp. and *Campylobacter* of Fresh Frozen Chicken on Retail Sale. [www.foodstandards.gov.uk](http://www.foodstandards.gov.uk). Retrieved: February 10, 2015.
- Forbes, B. A., Sahm, D. F. and Weissfeld, A. S. (1998). Bailey and Scott's Diagnostic Microbiology, 10th edn. Mosby Inc., St. Louis Missouri, USA. 52-57.
- Foskett, P., Ceserani, V. and Kinton, R. (2003). The Theory of Catering. 10th ed. Hodder and Stoughton. London. 531.
- Fotadar, U., Zaveloff, P. and Terracio, L. (2005). "Growth of *Escherichia coli* at elevated temperatures". *Journal of Basic Microbiology*. 45 (5): 403–4.
- Frazier, W. C. and D.C. Westhoff(1988). *Food Microbiol*. 4: McGraw-Hill Book Company. NewYork, NY.
- Gaastra, W. and Svennerholm, A. M. (1996). Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). *Trends in Microbiology*. 4: 444–452.
- Gabriel Bitton (2005). Microbial indicators of fecal contamination: application to microbial source tracking. Florida Storm water Association 719 East Park Avenue, Tallahassee, 32301. P 5-39.



- Gast, R. K. (1997). Paratyphoid infection. Diseases of poultry. Iowa State University Press. 97-121.
- Gast, R. K. and Beard, C. W. (1990). Isolation of *Salmonella* sp. enteritis diseases infection in laying hens. *Avian Diseases*. 34: 991-993.
- Gates, J. C. (1987). BASIC FOODS. 3rd ed. Library of Congress Cataloging –in Publication Data. Harcourt Brace Javanovich College Publishers. U.S.A.31.
- Ghana Poultry Report Annual (2013). Assessments of Commodity and Trade Issues. Ghana Agricultural Network Information. 1-8.
- Giguère, S. (2006). Antimicrobial Drug Action and Interaction: Introduction of Antimicrobial therapy in Veterinary Medicine 4th edn , Giguère, S., Prescott, J. F., Baggot, J. D., Walker, R. D. and Dowling, P.M, eds. Blackwell Publishing, Ames Iowa, USA.
- Gillespie, S. H. (2002). Antibiotic Resistance in the Absence of selective Pressure. *International Journal of Antimicrobial Agents*. 17: 171-176.
- Goldberg, M. B. and Theriot, J. A. (1995). *Shigella flexneri* surface protein, A is sufficient to direct actin-based motility. *Proceedings of the National Academy of Science of the USA*.92: 6572–6576.
- Gracey, J. F. and Collins, D. S. (1994). Meat hygiene. Bailliere Tindall. 9th edn. Bath Press, London, UK. 41-45.
- Grys, T. E., Siegel, M. B., Lathem, W. W. and Welch, R. A. (2005). The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. *Infection and Immunity*. 73:1295–1303.
- Hassanein, R., Sohaila, F. H. A., Ashraf, M. A. M., Moemen, A. M., and Khalid, I. E. (2011). Detection and identification of *Salmonella* species in minced beef and chicken meats by using Multiplex PCR in Assiut city. *Veterinary World*.4(1): 5-11.

Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K., Han, C. G., Ohtsubo, E., Nakayama, K., Murata, T., Tanaka, M., Tobe, T., Iida, T., Takami, H., Honda, T., Sasakawa, C., Ogasawara, N., Yasunaga, T., Kuhara, S., Shiba, T., Hattori, M. and Shinagawa, H. (2001). Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Research*. 8(1): 11-22.

Hooper, L. V. and Gordon, J. I. (2001). Commensal Host-Bacterial relationship in the gut. *Science*.292: 1115-1118.

International Commission on Microbiological Specifications for Foods (1986).

Microorganisms in Foods. Sampling for Microbiological Analysis. Principles and Specific Applications, 2<sup>nd</sup> edn. Toronto: Toronto University Press. (ISBN 0-8020-5692-8).

Ingledeu, W. and Poole, R. (1984). "The respiratory chains of *Escherichia coli*". *Microbiological Reviews*. 48 (3): 222–71.

Irwin, R. J., Poppe, C., Messier, S., Jinley, G. G, and Oggel, J. (1994). A national survey to estimate the prevalence of *Salmonella* sp. among Canadian registered commercial turkey flocks. *Canadian Journal of Veterinary Research*. 58: 263-267.

Iyer, A., Barbour, E., Azhar, E., Qadri, I., Chaudhary, A., Jaouni, S., Qari, M, Bahijri, S., Abuzenadah, A., Jadidi, A., Hosseni, S. D., Homayounimehr, A., Hamidi, A., Ghani, S. and Rafiee, B. (2012). Simple and rapid detection of *Salmonella* sp. from cattle feces using polymerase chain reaction (PCR) in Iran. *African Journal of Microbiology Research*. 6: 5210-5214.

James P. Nataro and James B. Kaper (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbial Rev*; 11(1): 142–201.

Jay, J. M. (2000): Modern Food Microbiology. Gaithersburg, Maryland: Aspen Publishers, Inc. 21-35.

- Johnson, J. R., Orskov, I., Orskov, F., Goulet, P., Picard, B., Moseley, S. L., Roberts, P. L. and Stamm, W. E. (1994). O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing urosepsis. *Journal of Infectious Diseases*.169: 119–126.
- Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R. A., Bolton, F. J., Frost, J. A., Odwar, A. J., Kikuvi, G., Ngumo, K. J. and Kariuki, S. (2014). A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes*. 7: 627.
- Joyce Arua Odwar, Gideon Kikuvi, James Ngumo Kariuki and Samuel Kariuki (2014). A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes* 20147:627 DOI: 10.1186/1756-0500-7-627.
- Julian Davies and Dorothy Davies (2010). Origins and Evolution of Antibiotic Resistance *Microbiol Mol Biol Rev*. 74(3): 417–433.
- Kitchell, A. G., Ingram, G. C. and Hudson, W. R. (1973). Microbiological sampling in abattoirs. In: *Sampling Microbiological Monitoring of the Environment* (eds Board R. G. and Lovelock, D W), Academic Press, London, pp. 43-61.
- Knutton, S., Rosenshine, I., Pallen, M.J., Nisan, I., Neves, B.C., Bain, C., Wolff, C., Dougan, G., and Frankel, G.(1998). A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO Journal*. 17: 2166–2176.
- Kotloff, K. L, Winickoff, J.P., Ivanoff, B., Clemens, J. D., Swerdlow, D. L. Sansonetti P. J., Adak G.K., and Levine, M. M. (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization*.77 (8).

- Kubitschek H.E (1990). "Cell volume increase in *Escherichia coli* after shifts to richer media". *Journal of Bacteriology*. **172** (1): 94–101. PMC 208405 . PMID 2403552.
- Kusters, J. G., Mulders-Kremers, G. A. W. M., van Doornik, C. E. M. and Van Der Zeijst, B. M. (1993). Effects of multiplicity of infections, bacterial protein synthesis, and growth phase on adhesion to and invasion of human cell lines by *Salmonella* sp. Typhimurium. *Infection and Immunity*. 61: 5013-5020.
- Kweon, M. N. (2008). *Shigellosis: the current status of vaccine development*. *Infectious Diseases* . 21(3): 313-318.
- Levine, M. M., Nataro, J. P., Karch, H., Baldini, M. M. and Kape, J. B. (1985). The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an entero-adhesiveness factor. *Journal of Infectious Diseases*. 152: 550–559.
- Lillard, H. S. (1990). The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. *Journal of Food Protection*. 53: 202- 204.
- Macleod, S. and Douglas, F. (1999). *Food and Drink Service in the Restaurant* Bath Press. Avon . London. 20.
- Mallinson, E. T., De Rezende, C. E., Tablane, N. T., Carr, L. E., and Joseph, S. W. (2000). A management technique to identify prime locations of *Salmonella* sp. contamination on broiler and layer farms. *Journal of Applied Poultry Research*. 9: 364-370.
- Manges, A. R., Johnson, J. R., Foxman, B. O., Bryan, T. T., Fullerton, K. E. and Riley, L. W. (2001). Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *England Journal of Medicine*. 345: 1007– 1013.
- Mather, E. (2001). *The Science of Pre-Harvest Food Safety*. National Food Safety and Toxicology Center. [www.agriculture.gov](http://www.agriculture.gov). Retrieved in April 2015.

- Maung San Myint (2004). Epidemiology of *Salmonella* Contamination of poultry meat Products: Knowledge Gaps in the farm to store products
- McIlroy, S. G., McCracken, R .M., Neilland, S. D. and O'brien, J. J. (1989). Control, prevention and eradication of *Salmonella* sp. enteritis diseases infection in broiler and broiler breeder flocks. *Veterinary Record*.125: 5445-548.
- Meyerson, L. A. and Reaser, J. K. (2002). Biosecurity moving toward a comprehensive approach. *Bio Science*. 52: 593-600.
- Microbiology model (2011). Michigan State University Board of Trustees. East Lansing, MI 48824. 39-41
- Miranda, J. M., Vázquez, B. I., Fente, C. A., Barros-Velázquez, J., Cepeda, A. and Franco, C.M. (2008). Evolution of resistance in poultry intestinal *Escherichia coli* during three commonly used antimicrobial therapeutic treatments in poultry. *Journal of Poultry Science*. 87:1643-1648.
- Morton, N. (1993). Despite intensive efforts, egg-related *Salmonellosis* outbreaks continue. *South Dakota Journal of Medicine*. 46(6). 189–191.
- Mulvey, M. A., Schilling, J. D. and Hultgren, S. J. (2001). Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infection and Immunity*. 69(7): 4572-9.
- Murase. T., Senjyu, K., Maeda, T., Tanaka, M., Sakae, H., Matsumoto, Y., Kaneda, Y., Ito, T. and Otsuki, K. (2001). Monitoring of chicken houses and an attached egg-processing facility in a laying farm for *Salmonella* contamination. *Journal of Food Protection*. 64: 1912-1916.
- Naravaneni, R. and Jamil, K. (2005). Rapid detection of food-borne pathogens by using molecular techniques. *Journal of Medical Microbiology*. 54: 51–54.



- Nataro, J. P. and Kaper, J. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*. 11: 142-201.
- Nataro, J. P., Maher, K. O., Mackie, P. and Kaper, J. B. (1987). Characterization of plasmids encoding the adherence factor of enteropathogenic *Escherichia coli*. *Infection and Immunity*. 55: 2370–2377.
- Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threlfall, J., Scheutz, F., van der, J. and Kruse, H. (2010). Food-borne Diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*. 1 (139): 3-15.
- Noël, J. M. and Boedeker, E. C. (1997). Enterohemorrhagic *Escherichia coli*: a family of emerging pathogens. *Dig Diseases*. 15: 67.
- Norrung, B., Andersen, J. B. K. and Buncic, S. (2009). Main concerns of pathogenic microorganisms in meat safety of meat and processed meat. F. Toldrá. (Springer New York). 3-29.
- Notermans, S. and Hoogenboom-Verdegaal, A. (1992). Existing and emerging food borne Diseases International Journal of Food Microbiology. Organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO Journal*. 17: 2166- 2176.
- Orskov, F. and Orskov, I. (1983). From the National Institutes of Health: Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the Enterobacteriaceae and other bacteria. *Journal of Infectious Diseases*. 148: 346–357.
- Panisello, P. J., Rooney, R., Quantick, P. C. and Stanwell-Smith, R. (2000). Application of foodborne Diseases outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*. 59: 221-234.
- Perna, N. T., Plunkett, G 3<sup>rd</sup>., Burland, V., Mau, B., Glasner, J. D., Rose, D. J., Mayhew, G. F., Evans, P. S., Gregor, J, Kirkpatrick, H. A., Pósfai, G., Hackett, J., Klink, S. Boutin,



- A., Shao, Y., Miller, L., Grotbeck, E.J., Davis, N. W., Lim, A., Dimalanta, E. T., Potamouisis, K. D., Apodaca, J., Anantharaman, T. S., Lin, J., Yen, G., Schwartz, D. C., Welch, R. A. and Blattner, F. R. (2001). Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature*. 409(6819): 529-33.
- Pickering, L. K., Obirg, T. G. and Stapleton, F. B. (1994). Hemolytic-uremic syndrome and enterohemorrhagic *Escherichia coli*. *Pediatric Infectious Disease Journal*. 13: 459-476.
- Plummer, R. A. S., Blissett, S. J. and Dodd, C. E. R. (1995). *Salmonella* contamination of retail chicken products sold in the UK. *Journal of Food Protection*. 58: 843-846.
- Pointon, A. M., Sexton, P., Dowsett, T., Saputra, A., Kiermeier, M., Lorimer, G., Holds, G. A., Pomeroy, B. S., Nagaraja, K. V., Ausherman, L. T., Peterson, I. L. and Friendshuh, K. A. (1989). Studies on feasibility of producing *Salmonella* sp.-free turkeys. *Avian Diseases*. 33:1-7.
- Poppe, C., Irwin, R. J., Forsberg, C. M., Clarke, R. C. and Oggel, J. (1991). The prevalence of *Salmonella* sp. enteritis Diseases and other *Salmonella* sp. among Canadian registered commercial layer flocks. *Epidemiology and Infection* 106:259-270.
- Rodriguez, D. C., Tauxe, R. V. and Rowe, B. (1990). International increase in *Salmonella* sp. enteritis Diseases: A new pandemic. *Epidemiology and Infection*. 105: 21-27.
- Rose, B. E., Hill, W. E., Umholtz, R., Ransom, G. M. and James, W. O. (2001). Testing for *Salmonella* sp. in raw meat and poultry products collected at federally inspected Establishments in the United States. *Journal of Food Protection*. 65: 937-947.
- Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp.362-8.
- Sackey, B. A., Mensah, P., Collison, E. and Sakyi-Dawson, E. (2001). *Campylobacter*, *Salmonella* sp., *Shigella* and *Escherichia coli* in live and dressed poultry from metropolitan Accra. *International Journal of Food Microbiology*. 71(1): 21-8.

- Saikia, P. and Joshi, S. R. (2010). Retail market poultry meats of North-East India – a microbiological survey for pathogenic contaminants. *Research in Microbiology*. 5(1): 36-43
- Sato, Y., Fukui, S., Kurusu, H., Kitazawa, I., Kuwamoto, R. and Aoyagi, T. (1999). *Salmonella* typhimurium infection in domesticated fowl in a children's zoo. *Avian Diseases*. 43(3): 611-615
- Scaletsky, I. C., Fabbriotti, S. H., Carvalho, R. L., Nunes, C. R., Maranhão, H. S., Morais, M. B., Fagundes-Neto, U. (2002). Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: A case control study. *Journal of Clinical Microbiology*. 40(2):645–658.
- Schroeder, G. N. and Hilbi, H (2008). Molecular pathogenesis of *Shigella* sp.: controlling host cell signaling, invasion, and death by type III secretion. *Clinical Microbiology Reviews*. 21(1): 134-156.
- Scotland, S. M., Smith, H. R. and Rowe, B. (1991). *Escherichia coli* O128 strains from infants with diarrhea commonly show localized adhesion and positivity in the fluorescent actin staining test but do not hybridize with an enteropathogenic *Escherichia coli* adherence factor probe. *Infection and Immunity*. 59: 1569-1571.
- Seligmann, R. and Lapinsky, Z. (1970). *Salmonella* sp. findings in poultry as related to conditions prevailing during transportation from the farm to the processing plant. *Refuahveterinarith*. 27:7-14.
- Servin, A. L. (2005). Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clinical Microbiology Reviews*. 18: 264-292.
- Silva, R. M., Toledo, M. R. and Trabulsi, L. R. (1980). Biochemical and cultural characteristics of invasive *Escherichia coli*. *Journal of Clinical Microbiology*. 11: 441–444.

- Snoeyenbos, G. H., Carlson, V. L., Smyser, C. F. and Olesiuk, O. M. (1969). Dynamics of *Salmonella* sp. infection in chicks reared on litter. *Avian Diseases*. 13: 72-83.
- Soultos, N., Koi, P. and Madden, R. H. (2003). Presence of *Listeria* and *Salmonella* sp. in retail chicken in Northern Ireland. *Letters in Applied Microbiology* 37,421–423
- Sousa, C.P. and Dubreuil, J. D. (2001). Distribution and expression of the astA gene (EAST1 toxin) in *Escherichia coli* and *Salmonella* sp. *International Journal of Medical Microbiology*. 291: 15-20.
- Spangler, B. D. (1992). Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxin. *Microbiology Reviews*. 56: 622–647.
- Straver, J. M., Janssen, A. F. W., Linnemann, A. R., Van Boekel, M. A. J. S., Beumer, R. R. and Zwietering, M. H. (2007). Number of *Salmonella* on chicken breast fillet at retail level and its implications for public health risk. *Journal of Food Protection*. 70 (9): 2045-2055.
- Struve, C. and Krogfelt, K. A. (1999). In vivo detection of *Escherichia coli* type 1 fimbrial expression and phase variation during experimental urinary tract infection. *Journal of Microbiology*. 145 (10): 2683-90.
- Taylor, C. S., Nouri, A., Lee, C. G., Kozak, C., and Kabat, D. (1999). Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukemia viruses. *Proceedings of the National Academy of Sciences, U S A*. 96 (3); PMC15327
- Tara, S. (2003). A focus on *Salmonella* sp. State/Country of Publication: Maryland. 8-10.
- Tauxe, R.V. (2002). Emerging food-borne pathogens. *International Journal of Medical Microbiology*. 78: 31-41.

- Tavechio, A. T., Ghilardi, A.C.R., Peresi, J. T. M., Fuzihara, T.O., Yonamine, E. K., Jakabi., M and Fernandes, S.A. (2002). Serotypes of *Salmonella* sp isolated from nonhuman sources in Sao Paulo, Brazil. *Journal of Food Protection*.65: 1041–1044.
- Thangh Huong CT, Hai Duong NT, Thu Hien NT (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. *J Sci Dev*. 7(2):181–186.
- Todd, C. D. (1999). Foodborne and waterborne Diseases in developing countries- Africa and the Middle East. Dairy, *Food Environment Sanitation*. 21: 110–122.
- Torrence, M. E. and Isaacson, R. E. (2003). Microbial Food Safety in Animal Agriculture: Current Topics. Oxford: Blackwell. 97-104
- Trabulsi, L. R., Keller, R. and Gomes. T. A. T. (2002). Typical and atypical enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases*. 8: 508–513.
- Trampel, D. W., Hasiak, R. J., Hoffman, L. J. and De Bey, M. C. (2000). Recovery of *Salmonella* sp. from water, equipment, and carcasses in turkey processing plants. *Journal of Applied Poultry Research*. 9: 9-34.
- Turnidge, J. and Peterson, D. L. (2007). Setting and Revising Antibacterial Susceptibility Breakpoints. *Clinical Microbiology Reviews*. 20(3): 391-408.
- Ukut, I. O., Okonko, I. O., Ikpoh, I. S., Nkang, A. O., Udeze, A. O., Babalola, T. A., Mejeha, O K., Fajobi, E. A. (2010). Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 9(1):89-100.
- United States Department of Agriculture (USDA) (1995). Pathogen reduction; hazard analysis and Critical control point (HACCP) systems. *Fed Reg*. 60: 6774-6889.

- Uyttendaele, M. R., Debevere, J. M., Lips, R. M. and Neyts, K. D. (1998). Prevalence of *Salmonella* sp. in poultry carcasses and their products in Belgium. *International Journal of Food Microbiology*. 40: 1-8.
- Uyttendaele, M., De Troy, P. and Debevere, J. (1999). Incidence of *Salmonella* sp., *Campylobacter jejuni*, *Campylobacter coli*, and *Listeria monocytogenes* in poultry carcass and different types of poultry products for sale on the Belgian retail market. *Journal of Food Protection*. 62: 735–740.
- Van de Giessen., A. W., Dufrenne, A. G., Ritmeester, W. S., Berkers, P. A. T. A, Van Leeuwen, W. J. and Notemans, H. W. (1991) *Salmonella* sp. contamination of poultry flocks in the Netherlands. *Vet Q*. 13: 41-46.
- Venter P, Lues, J. F. R. and Theron. H. (2000). Quantification of bioaerosols in automated chicken egg production plants. *Poultry Science*. 83:1226-1231.
- Walsh, C. (2000). Molecular Mechanisms that Confer Antibacterial Drug Resistance. *Nature*. 406: 775-781.
- Waltman, W. D., Horne A. M., Pirkle, C. and Johnson, .D. C. (1992). Prevalence of *Salmonella* sp. enteritis diseases in spent hens. *Avian Diseases*. 36:251-255.
- Watts, J. L. and Lindeman, C. J. (2006). Antimicrobial susceptibility testing of bacteria of veterinary origin. Antimicrobial resistance in bacteria of animal origin, FM Aarestrup, ed. ASM Press, Washington DC, USA.
- White, D. and Cox, E. (2013). Fighting the Impact of Antibiotic-Resistant Bacteria. Consumer Health Information. [www.fda.gov/consumer](http://www.fda.gov/consumer). Retrieved: January 20, 2015.
- Whitfield, C. and Roberts, I. S. (1999). Structure, assembly and regulation of expression of N capsules in *Escherichia coli*. *Molecular Microbiology*. 31: 1307–1319.
- World Health Organization (2012). Diseases: Shigellosis". Initiative for Vaccine Research (IVR).



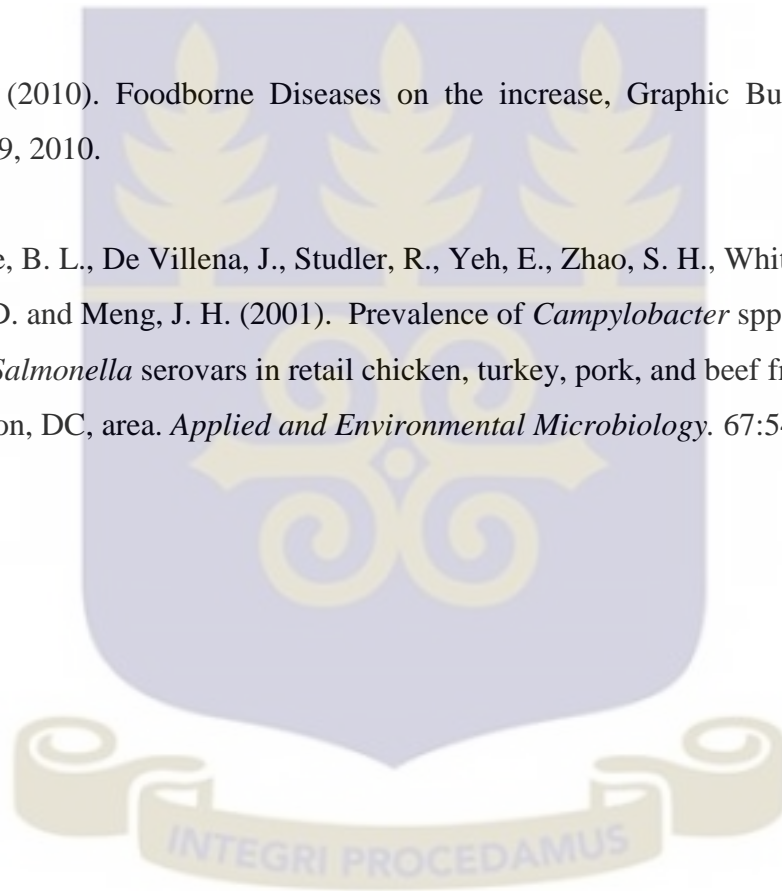
Williams, J. E. and Benson, S. T. (1978). Survival of *Salmonella* sp. typhimurium in poultry feed and litter of three temperatures. *Avian Diseases*. 22:742-747.

Williams, J. E. (1981). *Salmonella* sp. in poultry feeds: a worldwide review. *World's Poultry Science Journal*. 37: 97-106.

Wilson, I. G. (2002). *Salmonella* sp. and *Campylobacter* contamination of raw retail chickens from different producers: a six year survey. *Epidemiology and Infection*. 129: 634–645.

Yeboah, A. L. (2010). Foodborne Diseases on the increase, Graphic Business. Tuesday, October 19, 2010.

Zhao, C. W., Ge, B. L., De Villena, J., Studler, R., Yeh, E., Zhao, S. H., White, D. G., Wagner, D. and Meng, J. H. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, DC, area. *Applied and Environmental Microbiology*. 67:5431–5436.





## APPENDIX

## Appendix 1: Analyzed data using Statistical Analysis System (SAS)

## SAS Syntax

```

options ls = 100 ps = 100 nocenter formdlim = '-';
data bact;
input Isol $ Subb $ OUTL $ Shop    Source $ Anat $ CTRS CTR $    CTXS CTX $
      CHLS CHL $ CRXS CRX $ GENTS    GENT $ COTS    COT $ TETS
      TET $ AMP AMP $ @@;

;;
proc print data = bact;
run;
PROC sort data = bact;
by isol;
run;
proc freq data = bact;
tables anat/chisq;
tables source/chisq;
tables CTR/chisq;
tables CTX/chisq;
tables CHL/chisq;
tables CRX/chisq;
tables GENT/chisq;
tables COT/chisq;
tables TET/chisq;
tables AMP/chisq;
by isol;
run;
Proc freq data = bact;
tables isol anat isol*anat /chisq;
tables isol source isol*source/chisq;
tables isol CTR isol*CTR/chisq;
tables isol CTX isol*CTX/chisq;
tables isol CHL isol*CHL/chisq;
tables isol CRX isol*CRX/chisq;
tables isol GENT isol*GENT/chisq;
tables isol COT isol*COT/chisq;
tables isol TET isol*TET/chisq;
tables isol AMP isol*AMP/chisq;
run;
Proc freq data = bact;
tables CHL*source/chisq;
tables CRX*source/chisq;
tables COT*source/chisq;
tables TET*source/chisq;

```

tables AMP\*source/chisq;  
run;

