© 2014
Cynthia Ayefoumi Adinortey
University of Cape Coast

UNIVERSITY OF CAPE COAST

ANTIBIOTIC RESISTANCE, PHYLOGENETIC GROUPING AND VIRULENCE POTENTIAL OF *ESCHERICHIA COLI* ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES FROM THE CAPE COAST METROPOLIS OF THE CENTRAL REGION OF GHANA

BY

CYNTHIA AYEFOUMI ADINORTEY

Thesis submitted to the Department of Molecular Biology and Biotechnology of the School of Biological Sciences, University of Cape Coast, in partial fulfilment of the requirements for the Award of Doctor of Philosophy degree in Molecular Biology and Biotechnology

SEPTEMBER, 2014

DECLARATION

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature: .... Date: ....

Name: ....

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: …………………… Date: ....

Name: ....

Co-Supervisor's Signature:……………………………… Date: ....

Name: ....

ABSTRACT

The genetic diversity of *Escherichia coli* isolated from clinical and environmental samples from Cape Coast was studied. Bacterial isolation and identification was carried out using standard biochemical tests and confirmed using Analytical Profile Index (API) 20 E kits. All test *E. coli* isolates were screened for their susceptibility to 16 antibiotics, followed by a screening for extended spectrum β-lactamase (ESBL) production. DNA was extracted from all tests *E. coli* isolates and PCR assays were performed to determine the prevalence of ESBL genes - ^{bla}TEM and ^{bla}SHV, the molecular phylogeny and the virulence potential of the *E. coli* isolates. In all 389 *E. coli* isolates were obtained comprising 261 from clinical and 128 from environmental samples. All 389 *E. coli* isolates were sensitive to imipenem whereas the least percentage sensitivity was recorded when all *E. coli* isolates were screened against ampicillin. The percentage sensitivity of the isolates recovered from environmental samples to the 16 antibiotics was higher than that of clinical isolates except for nalidixic acid, aztreonam and amikacin where isolates of environmental origin recorded lower percentages of sensitivity. Also 29.88 % of the test *E. coli* isolated from clinical samples and 12.50 % from environmental samples were phenotypically confirmed as ESBL producers. Furthermore, 42.91 % of clinical isolates and 11.72 % of the environmental isolates respectively belonged to group B2. Also 27.20 % and 26.05 % of clinical isolates belonged to group A and D respectively while the environmental isolates recorded 53.13 % and 35.16 % respectively belonging to group A and D. In addition, 3.83 % of the environmental isolates and none of the clinical isolates were found to belong to group B1. *E. coli* isolates recovered from stool samples recorded the highest percentages of enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroaggregative *E. coli* and enterohaemorrhagic *E. coli*, while *E. coli* isolates recovered from urine recorded the highest percentage of uropathogenic *E. coli*.

ACKNOWLEDGMENTS

I would like to express my profound gratitude to all persons and institutions that have contributed immensely to the successful completion and final submission of this thesis.

I wish to express my sincere gratitude to my supervisors for their guidance that enabled me stand strong amid the frustrations and challenges I encountered as I carried out my research. I am forever indebted to Dr. D. H. A. K. Amewowor from whom I have learnt a lot, not only academically, but also because of the optimistic attitude on life as a whole that he instilled in me. I would also like to express my deep appreciation to Prof. Isaac K. A. Galyuon for his relentlessness from the beginning of my programme through to the preparation of this thesis and the encouragement he offered me when things were getting tough. Many thanks also go to Dr. Daniel K. A. Asante for his guidance as I went through various stages of my programme.

I am grateful to my former Head of Department, Dr. Emmanuel Plas Otwe, for his unflinching support in making sure that facilities at the Department were working perfectly which culminated in the smooth completion of my programme.

Many thanks to all the Lecturers, Technicians, Administrators, Store keepers and Accountants of the School of Biological Sciences, University of Cape Coast, who were always ready to help anytime I fell on them for assistance. I would like to single out Dr. Denis W. Aheto and Dr. Levi

Yafeto for their valuable comments and constructive criticism that helped to reshape the write-up of this thesis.

I am grateful to Prof. Karen Krogfelt of the Serum Statens Institute, Denmark, who was very resourceful in refining some critical aspects in the course of my data collection.

I further thank Dr. Japheth A. Opintan, Messrs Nicholas Dayie, Noah Obeng Nkrumah, Michael Olu-Taiwo and Nsiah Podoo, all of the Department of Microbiology, University of Ghana Medical School, for their assistance.

I am also thankful to the Ghana Health Service for the ethical clearance and to the patients of the Central Regional Hospital from whom clinical samples were obtained.

My appreciation also goes to all colleague postgraduate and undergraduate students of the Department of Molecular Biology and Biotechnology, University of Cape Coast, as well as all my friends and for their encouragement and interest in the progress of my work.

I am thankful to the Geographic Information System Section of the Geography Department, University of Cape Coast, for the assistance in drawing, specifically for this study, a map of the Cape Coast Metropolis showing the various sites of collection.

I am ever grateful to my family especially, my parents and siblings for their spiritual and moral support, and to my lovely, patient, supportive and caring husband for being there for me at all times.

Finally, I am grateful to the Government of Ghana and the University of Cape Coast for providing the necessary financial support and academic environment for my PhD programme.

DEDICATION

To Mike, my darling husband

and Mr. and Mrs. J. A. Donkoh, my beloved parents.

TABLE OF CONTENTS

	Page
TITLE PAGE	i
DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
DEDICATION	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF PLATES	xvii
LIST OF ABBREVIATIONS	xx
CHAPTER ONE: INTRODUCTION	1
Background to the Study	1
Statement of the Problem	5
Research Questions	7
Research Objectives	8
Significance of the Study	8
CHAPTER TWO: LITERATURE REVIEW	10
Overview of the Bacterium *Escherichia coli*	10
Importance of *E. coli*	11
Habitat and Ecology of *E. coli*	13
Description, Cell Physiology and Cultural Characteristics	14
Cell Structure of the Bacterium *E. coli*	16

Clinical Categories of *E. coli* 17
Classification of *E. coli* 23
Control and Prevention of *E. coli* Related Infections 43
Methods of Isolation and Identification of *E. coli* 51
Methods Employed in Studying the Molecular Phylogeny of *E. coli* Isolates 53
Methods Employed in Antibiotic Susceptibility Testing 54
ESBL Detection Methods 57
Methods Employed in the Detection of *E. coli* Pathotypes 66
CHAPTER THREE: MATERIALS AND METHODS 68
Study Area 68
Study Population 69
Environmental Test Samples 69
Clinical Test Samples 70
Escherichia coli Isolates 71
Reference Bacterial Isolates 71
Culture Media 71
Sterile Collection Swabs 71
Antibiotics Discs 71
Analytical Profile Index (API) 20 E kits 71
Extended Spectrum Beta Lactamase Detection Disc Sets 72
Wizard Genomic DNA Purification Kits 72
Oligonucleotide Primers 72
Molecular Biology Grade Water 72

Luria Bertani Broth 72

DNA Molecular Makers 73

Accupower PCR Premixes and Accupower Multiplex PCR
Premixes 73

Other Chemicals and Reagents 73

Ethical Considerations 73

Collection of Demographic Data 74

Estimation of Sample Size 74

Methods of Sterilization 75

Preparation of Culture Media 75

Sampling Method and Experimental Design 81

Collection of Test Samples and Bacterial Isolation 82

Preparation of Serial Decimal Dilutions 86

Storage of Bacterial Isolates 87

Identification of Bacterial Isolates 87

Confirmation of Isolates Using API 20 E Commercial Kits 91

Determination of the Sensitivity of *E. coli* Isolates to Selected
Antibiotics 91

Phenotypic Confirmation of Extended Spectrum Beta Lactamases
(ESBL) Producing Strains Among *E. coli* Isolates 92

Quality Control 94

DNA Extraction 94

Determination of the Purity and Concentrations of DNA Samples 95

Confirmation of DNA Samples as Bacterial DNA 96

Detection of ESBL Genes (^{bla}SHV, and ^{bla}TEM) in Clinical and Environmental *E. coli* Isolates 97
Molecular Phylogeny of *E. coli* Isolates 98
Screening for Virulence Genes Among *E. coli* Isolates Obtained From Clinical and Environmental Samples 99
Data Analysis 104
Experimental Precautions 104
CHAPTER FOUR: RESULTS 107
Distribution of Enteric Bacteria Isolated From Clinical and Environmental Samples 107
Clinical Samples 109
Demographics of Patients From Whom Clinical Samples Were Obtained 110
Environmental Samples 111
Enteric Bacterial Species Isolated From Clinical and Environmental Samples 112
Antibiotic Susceptibility Patterns of *E. coli* Isolated From Clinical and Environmental Samples 114
Prevalence of Extended Spectrum Beta-Lactamase Producers Among *E. coli* Isolates 116
Results From DNA Extraction and Standardization of Concentration of DNA Samples 118
Prevalence of ESBL Genes (^{bla}SHV, and ^{bla}TEM) in Clinical and Environmental *E. coli* Isolates 119

Molecular Phylogeny of *E. coli* Isolates 122

Prevalence of Pathogenic *E. coli* Strains in Clinical and Environmental Samples 124

CHAPTER FIVE: DISCUSSION 136

Distribution of *E. coli* Isolates Among Clinical and Environmental Samples 136

Antibiotic Resistance of *E. coli* Isolated From Various Samples 139

Molecular Phylogeny of *E. coli* Isolated From Various Samples 146

Virulence Potential of *E. coli* Isolated From Various Samples 150

CHAPTER SIX: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 157

Summary 157

Conclusions 162

Recommendations 164

REFERENCES 166

APPENDIX I: Ethical Clearance 224

APPENDIX II: Consent Form 225

APPENDIX III: Distribution of Bacterial Isolates in Clinical and Environmental Samples 226

APPENDIX IV: Prevalence of ESBL Producers and Non-ESBL Producers Among *E. coli* Isolates Recovered From Clinical and Environmental Samples 228

APPENDIX V: Molecular Phylogeny 230

APPENDIX VI: Distribution of ESBL Genes 232

APPENDIX VII: Distribution of *E. coli* Isolates Obtained From Clinical Samples 234

APPENDIX VIII: Distribution of *E. coli* Isolates Obtained From Environmental Samples 236

LIST OF TABLES

Table		Page
1	Summary of the Virulence Determinants of Pathogenic *E. coli*	19
2	Common Pathotypes of Disease-Causing *E. coli* of Humans	27
3	*E. coli* Pathotypes and Distribution of Various Virulence Factors	67
4	Pattern of Reaction for a Typical *E. coli* Bacterium Using Standard Biochemical Tests	89
5	Characteristics of Primers Used for the Detection of ESBL Genes	100
6	Characteristics of Primers Used in Studying the Molecular Phylogeny of *E. coli* Isolates	101
7	Assays and Primers Used in the Detection of Virulent Genes Among *E. coli* Isolates	102
8	Distribution of Enteric Bacterial Isolates Recovered From Clinical and Environmental Samples	109
9	Distribution of Clinical *E. coli* Isolates With Respect to Their Sources	110
10	Age and Gender Distribution of Patients From Whom Clinical Samples Were Obtained and *E. coli* Recovered	111

11	Distribution of Environmental *E. coli* Isolates With Respect to Their Sources	112
12	Distribution of ESBL Producers and Non-ESBL Producers Among *E. coli* Isolates Recovered From Clinical and Environmental Samples	118
13	Prevalence of ^{bla}TEM and ^{bla}SHV Genes Among ESBL Producing and Non-ESBL Producing *E. coli* Isolates	120
14	Distribution of *E. coli* Isolated From Environmental and Clinical Samples Among Phylogenetic Groups	123
15	Distribution of pathotypes among *E. coli* isolates from various clinical and environmental Samples	129
16	Distribution of *E. coli* Diarrhoeagenic Pathotypes Isolated From Environmental and Clinical Samples and Genes Coding for Their Virulence	130
17	Virulence Pattern of Genes Coding for Uropathogenic Traits Among *E. coli* Strains Isolated From Environmental and Clinical Samples	131

LIST OF FIGURES

Figure		Page
1	Basic ultrastructure of *E. coli*	17
2	A positive double-disk synergy test (Jarlier *et al.,* 1988)	62
3	Geographical location of sites where samples were collected for the study	70
4	Antibiotic sensitivity pattern of *E. coli* isolates to 16 antibiotics	115
5	Phylogenetic groups of 128 *E. coli* isolated from environmental and various clinical samples as determined by the unweighted pair group method with arithmetic averages of binary character matrices using the similarity coefficient index	125
6	Phylogenetic groups of 261 *E. coli* strains isolated from clinical samples as determined by the unweighted pair group method with arithmetic averages of binary character matrix using the similarity coefficient index	126

LIST OF PLATES

Plate		Page
1	Sites of collection of environmental samples	85
2	Outer leaves of cabbage sampled	86
3	Biochemical tests using Triple Sugar Iron agar	90
4	A pair of Vernier calipers used to measure the diameters of the clear zones	92
5	Coliform bacteria growing on MacConkey after 12 hours incubation at 35 ^{o}C	108
6	Inoculated API 20E strip after 18 hours incubation period showing a pattern of reaction corresponding to that of *Escherichia coli*	113
7	Gram negative rods of a confirmed *E. coli* isolate	113
8	Inoculated Müeller Hinton agar plates each with 8 antibiotic discs after 16 hour incubation at 35 ^{o}C	116
9	Phenotypic confirmation of ESBL producers using a bacterial lawn of the test isolate showing clear zones of varying diameters around each 2nd or 3rd generation cephalosporin used	117
10	Ethidium bromide stained 1 % agarose gel showing fifteen genomic DNA samples extracted from *E. coli* isolates	118
11	Ethidium bromide stained 2.5 % agarose gel showing PCR products obtained after confirmation of DNA samples as bacterial DNA using 16S rRNA primers	119

12	Ethidium bromide-stained 2.5% agarose gel showing PCR products resulting from amplification of ^{bla}TEM gene (865 bp) and 16S rRNA gene (1500 bp) in *E. coli* isolates	121
13	Ethidium bromide-stained PCR amplification products on a 1.5% agarose gel showing the presence or absence of the ^{bla}TEM gene in *E. coli* isolates	121
14	Ethidium bromide-stained PCR amplification products on a 1.5% agarose gel showing the ^{bla}SHV gene in *E. coli* isolates	122
15	Ethidium bromide-stained 1.5% agarose gel showing multiplex PCR amplified products of *E. coli* isolates using three primer pairs for the ChuA, YjaA and TspEC2 genes for the detection of phylogenetic groups	123
16	Ethidium bromide-stained 2.5% agarose gel showing multiplex PCR amplified products of *E. coli* isolates using four primer pairs: 16 S rRNA, ChuA, YjaA and TspEC2 genes for the detection of phylogenetic groups	124
17	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting elt (322 bp), stla (170 bp) and UidA (623 bp) genes	132
18	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting eae (229 bp), bfpA (450 bp) genes and 16S rRNA (1500 bp) as internal control	132

19	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting stx1 (130 bp), stx2 (510 bp) and ial (320 bp) genes	133
20	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting stlb (190 bp) and UidA (623 bp) genes and 16S rRNA (1500 bp) as internal control	133
21	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting aggR (457 bp) and Aat (629 bp) genes, and 16S rRNA (1500 bp) as internal control	134
22	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting afa (750 bp), cnf1 (498 bp) and papC (328 bp) genes	134
23	Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting aea (602 bp), sfa (410 bp) and hlyA (534 bp) genes	135

LIST OF ABBREVIATIONS

A/E	Attaching and effacing
AAF	Aggregative adherence fimbriae
ABC	ATP Biding Cassette
ABU	Asymptomatic bacteruria
AIEC	Adherent Invasive *E. coli*
AMA	Accra Metropolitan Assembly
AMC	Amoxicillin clavulanate
APEC	Avian Pathogenic *E. coli*
API	Analytical Profile Index
AT	Annealing temperature
ATP	Adenosine Triphosphate
BDH	British Drug House
BFP	Bundle Forming Protein
bla	beta -lactamase
bp	Base pair
CDC	Center for Disease Control
CEACAM6	Carcino-embryonic Antigen-related Cell Adhesion Molecule 6
CFA	Colonization Factor Antigens
cnf	Cytotoxic necrotic factor
CLED	Cysteine Lactose Electrolyte Deficient
CLSI	Clinical Laboratory Standard Institute

CT	Cholera toxin
CV	Clavulanic acid
DAEC	Diffusely adherent *E. coli*
DAF	Decay Accelerating Factor
DDS	Double Disc Synergy
DDST	Double Disc Synergy Test
DNA	Deoxyribonucleic acid
EAEC	Enteroaggregative *E. coli*
EAF	EPEC adherence factor
EAST	Enteroaggregative *E. coli* heat stage enterotoxin
E. coli	*Escherichia coli*
ECOR	*E. coli* reference strain collection
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EMB	Eosin Methylene Blue
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum Beta Lactamase
ETEC	Enterotoxigenic *E. coli*
E-test	Epsilometer test
ExPEC	Extra-intestinal Pathogenic *E. coli*
HC	Haemorrhagic colitis
HEp-2	Human epidermoid cancer cells

HIV/AIDS	Human Immuno Deficient Virus / Acquired Immunodeficency Syndrome
HUS	Haemolytic Uremic Syndrome
LB	Luria Bertani
LCR	Ligase Chain Reaction
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharide
LT	Heat labile Toxin
MBC	Minimum bacteriocidal concentration
MIC	Minimum inhibitory concentration
MLEE	Multi Locus Enzyme Electrophoresis
MLST	Multi Locus Sequence Typing
NMEC	Neonatal Meningitis *E. coli*
NTEC	Necrotoxigenic *E. coli*
°**C**	Degree celsius
OD	Optical Density
ONT	O - Untypeable
PAI	Pathogenicity island
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
REP-PCR	Repetitive Sequence Pair-based PCR
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid

rRNA	Ribosomal RNA
ShET	Shigella enterotoxin
SHV	Sulphurhydryl Variant
SSCP	Single Strand Conformational Polymorphism
STEC	Shiga Toxin producing *E. coli*
ST	Heat stable toxin
STG	Standard Guidelines
Stx	Shiga toxin
TAE	Tris acetate EDTA
TDA	Tryptophan Deaminase
TE	Tris-EDTA
TEM	Temienora
tRNA	Transfer RNA
TSI	Triple Sugar Iron
TTP	Thrombotic ThrombocytopaenicPurpura
UCC	University of Cape Coast
UNESCO	United Nations Educational Scientific and Cultural Organization
UPEC	Uropathogenic *E. coli*
UPGMA	Unweighted pair group method with arithmetic averages
UTI	Urinary Tract Infection
UV	Ultra violet
VP	Voges - Proskauer
VTEC	Verotoxin producing *E. coli*

WHO	World Health Organization
XLD	Xylose Lysine Decarboxylase
β-lactam	Beta-lactam

CHAPTER ONE

INTRODUCTION

Background to the Study

Environmental sanitation has become an important issue of concern and budgets drawn for management of outbreaks of infectious diseases have been exerting a heavy toll on the economies of most developing countries despite the numerous interventions of Governments and the World Health Organization (WHO) (Fonkwo, 2008). As of the year 2001, countries with a per capita income of less than US$500 per year spent, on average, US$12 per person per year on health (Fonkwo, 2008). The burden of infectious diseases is a major challenge faced by most developing countries as well as many industrialized nations (WHO, 1999). Infectious diseases are illnesses caused by disease agents that are transmitted from one organism to another, either directly, by physical contact, or indirectly through an intermediate host, a vector or inanimate objects technically referred to as fomites (Ryan & Ray, 2004). The three top killer infectious diseases recorded in 2012 include in the descending order: HIV/AIDS, lower respiratory tract infections and diarrhoeal diseases (WHO, 2012).

Undoubtedly, the persistence of these infectious diseases is linked to inadequate quality drinking water, sanitation practices and/or poor hygiene usually prevailing in some areas of most developing countries (WHO, 2004; Hunter, Ramirez-Toro & Minnigh, 2010).

Generally, in Africa and for that matter Ghana, due to improper inmdustrial and domestic waste management, refuse dumps usually harbour high levels of excreted pathogens, especially in diarrhoea-endemic areas (Murray & Lopez, 1996; WHO, 2000). Improper drainage systems, during the rainy seasons, also cause floods. Floodwaters become polluted with animal and human excreta, which subsequently, contaminate farm lands, crops, water bodies as well as households (WHO, 2000). Thus, floodwaters contribute immensely to the effective spread of pathogens resulting in outbreaks of diseases and posing a serious health threat.

Every year thousands of tourists from all over the world visit the Ghana and, particularly, Cape Coast. This they do, not only because of the many prestigious historic ancient forts, castles and natural sites, such as the Kakum National Park, but also probably because of the peaceful atmosphere prevailing in the country. In 2013, Ghana was ranked the fifty-eighth most peaceful country the world (Global Peace Index, 2013). Furthermore, since the visit in July 2009 of America's first family, the number of tourists visiting Ghana has considerably increased (GMMB, 2009). Indeed, among sub-Saharan countries, Ghana can boast of many sites of attraction located in all its ten regions (Zijlma, 2013). Among the various towns and cities in Ghana, Cape Coast and its environs are certainly the most visited (Zijlma, 2013). Cape Coast, the capital of the Central Region of Ghana, is famous for its ancient forts, castles, natural resources, top Senior High Schools and various festivals. Additionally, the Central Region can boast of one of Africa's foremost natural reserve with one of the two canopy walkways in Africa

located at the Kakum National Park. Tourists during their stay usually mingle with both indigenes and other residents in their quest to experience a real feel of life in the communities. In some unfortunate situations, however, they end up contracting various infectious diseases including “traveller’s diarrhoea”, primarily caused by a pathogenic strain of *Escherichia coli* (Connor, 2013) and are either rushed to a nearby hospital or back to their country of origin for treatment.

Infections due to pathogenic bacteria, especially members of the family Enterobacteriaceae, including *Escherichia coli, Salmonella typhii, Shigella* spp., and *Klebsiella pneumonia*, are significant causes of morbidity and mortality worldwide (Howard, Scott II, Packard & Jones, 2003). Infections caused by pathogenic *E. coli* are common though usually underestimated (Siitonen, 1994). Indeed, despite it being a member of the aerobic flora of most humans, *E. coli* is the cause of a significant fraction of human bacterial disease (Siitonen, 1994; Leclerc, Mossel, Edberg & Struijk, 2001). *E. coli* is a Gram-negative bacterium which is ubiquitous in nature by virtue of its life cycle involving a transition between two distinct environments; the primary environment being the gastrointestinal tract of mammals, while soil, water and sediment represent the secondary habitat (Savageau, 1983).

E. coli is the most common cause of food-borne and water-borne human diseases worldwide causing 800,000 deaths out of 650 million cases annually, primarily in children under the age of five years (Turner *et al.,* 2006). Recently, outbreaks of infectious diseases caused by *E. coli* have been

reported in countries such as Germany, France, USA, Britain, Wales, Denmark, South Africa and Egypt (Kelland, 2011).

Ghana, a developing nation with an emerging and promising economy among other sub-Saharan countries, regrettably, is not exempted from the burden of infectious diseases caused by *E. coli*. According to the WHO (2012), the three top-killer infectious diseases recorded in 2012 in Africa include in the descending order: HIV/AIDS, lower respiratory tract infections and diarrhoeal diseases.

Furthermore, an earlier survey revealed the ten top causes of death in Ghana in the following descending order: malaria, anaemia, pneumonia, stroke, hypertension, diarrhoea, neonatal infections, typhoid fever, renal diseases and sepsis (Bosu & Acquah, 1996). More recently, a report by the Accra Metropolitan Assembly (AMA) revealed that the top five communicable diseases in the Accra Metropolis for the year 2001 were malaria, sexually transmitted infections, diarrhoea, chicken pox and enteric fever (AMA, 2006).

The pathogenic E. coli is the most important causal agent of intestinal and extra-intestinal infections. It has been *associated with a number of localized as well as systemic infections in humans, birds, cattle, pigs and many other animals* (Orskov & Orskov, 1992). Based on the site of invasion in the human body, these infections may range from mild conditions such as diarrhoea to serious life-threatening conditions like bloody diarrhoea, septicaemia, urinary tract infection, meningitis or haemolytic uremic syndrome (Croxen & Finlay, 2010).

From the genetic and clinical point of view, *E. coli* strains can be broadly categorized into three groups: commensal strains, intestinal pathogenic (i.e., enteric or diarrhoeagenic) strains, and extra-intestinal pathogenic strains (Russo & Johnson, 2003). The commensal strains are non-pathogenic and are usually harmless or beneficial while the intestinal and extra-intestinal are pathogenic causing various infections in and outside the digestive tract.

Statement of the Problem

On most local markets in Ghana it is common to see various food items including smoked fish, fresh beef and sometimes frozen chicken sold at open market places where they are most often exposed to houseflies all day through with the exemption of a few cases where these items are protected from flies and displayed in mosquito netting cages. Another observation, is the common practice of farmers to use sewage to water their crops especially vegetables. The polluted state of gutters, beaches and water bodies in Cape Coast among others the Fosu lagoon and the sea are yet a critical area of research requiring a lot of attention.

Most studies carried out in Ghana have focused on the determination of coliform counts from a variety of substrates (Obiri-Danso, Okore-Hanson, Jones, 2003; Obiri-Danso, Amoah & Jones, 2003; Agbodaze *et al*., 2005; Dodoo, Quagraine, Okai-Sam, Kambo & Headley, 2006; Pappoe *et al*., 2007; McGarvey *et al*., 2008; Addo, Mensah, Bekoe, Bonsu & Akyeh, 2009; Obiri-Danso, Adjei, Stanley & Jones, 2009). The AMA (2006) report indicates *E.*

coli could be a cause of diarrhoea and enteric fever in Accra. Furthermore, cases of urinary tract infections and gastroenteritis, especially in children below 5 years, caused by pathogenic *E. coli* are common in Ghana (Ghana Health Service, 2008).

Though a lot of research has been done in most developed and some developing countries, few studies have concentrated on the presence, distribution and antibiotics susceptibility of *E. coli* in Ghana (Agbodaze *et al*, 1988; Addy, Antepim & Frimpong, 2004; Djie-Maletz *et al*., 2008; Opintan *et al*., 2010; Namboodiri, Opintan, Lijek, Newman & Okeke, 2011; Tagoe, Nyarko, Arthur & Birikorang, 2011). Most of these studies did not look at the incidence and distribution of pathotypes of *E. coli* strains. The prevalence of antibiotic resistance in pathogenic *E. coli* strains has been increasing worldwide and a resistance pattern is now emerging (Motta *et al*., 2003). In Ghana, surveillance of antibiotic resistance has become of prime importance owing to the rising incidence of multi-drug resistance in various pathogens, such as *Staphylococcus aureus, Streptococcus pneumoniae*, *Pseudomonas aeuroginosa* and pathogenic *E. coli* (Corkish & Assoku, 1973; Sackey, Mensah, Collison & Sakyi-Dawson, 2001; Donkor & Nartey, 2008; Donkor, Nortey, Opintan, Dayie & Akyeh, 2008). A nationwide study in 2006 on bacterial resistance among Ghanaians published in 2011 (Newman, Frimpong, Donkor, Opintan & Asamoah-Adu, 2011) showed high resistance to various antibiotics. Data obtained from the Ghana Health Service (Ghana Health Service, 2008) also suggest that multi-drug resistance in *E. coli* is on the increase in the Cape Coast Metropolis. Thus, despite the fact that antibiotics

have been very helpful in controlling morbidity and mortality rates caused by *E. coli* infections, the emergence of bacterial resistance to these drugs seem to undermine their therapeutic utility. Also, the production of Extended Spectrum β-Lactamases (ESBL) as a defence mechanism against antibiotics, by members of Enterobacteriaceae, especially *Klebsiella* sp. and *E. coli*, remains an important reason for antimicrobial therapy failure (Paterson & Bonomo, 2005) and has serious consequences for infection control.

Currently, in Ghana scanty information is available on various pathotypes of *E. coli* strains, pattern of antimicrobial resistance and prevalence of ESBL in clinical as well as environmental samples. It is, therefore, necessary to investigate the various pathogenic *E. coli* strains that are prevalent in Cape Coast, their distribution pattern, their phylogenetic relatedness and their susceptibility pattern to antibiotics commonly used in Ghana to treat infections caused by *E.coli* and other related bacteria.

Research Questions

1. What is the distribution of *E. coli* in the environment and in clinical settings within the Cape Coast Metropolis?
2. What is the antibiotics susceptibility pattern of *E. coli* isolated from Cape Coast?
3. What are the prevalent molecular types of ESBL among *E. coli*?
4. Is there any genetic relatedness between environmental and clinical *E. coli* isolates?
5. What are the common pathotypes associated with *E. coli* infections?

Research Objectives

The main objective of this study was to assess the various strains of *E. coli* isolated from environmental and clinical samples within the Cape Coast Metropolis of the Central Region of Ghana.

The specific objectives were to:

1. evaluate the incidence and distribution of *E. coli* strains,
2. determine the sensitivity patterns of *E. coli* strains to antibiotics commonly used to treat *E. coli* infections,
3. determine the prevalence of extended spectrum β-lactamase (ESBL) producers among *E. coli* isolates recovered from clinical and environmental samples,
4. investigate the prevalence of ESBL genes (^{bla}SHV, and ^{bla}TEM) in *E. coli* isolates,
5. determine the molecular phylogeny of *E. coli* isolates and their genetic relatedness, and
6. investigate the prevalence and distribution of pathotypes of *E. coli* strains.

Significance of the Study

This study would provide baseline scientific data on the genetic diversity of strains of *E. coli*, knowledge on their antibiotic susceptibility profile and prevalence of ESBL among *E. coli* strains in the Cape Coast metropolis of the Central Region of Ghana. The study would also establish the pathotypes of *E. coli* strains, if any. This will provide valuable information that will help in the

prevention, treatment and prognosis of pathogenic *E. coli*-related infections in the Cape Coast Metropolis, in particular, and in Ghana as a whole.

CHAPTER TWO

LITERATURE REVIEW

This chapter reviews related literature relevant to the study area. It provides an overview of the *Escherichia coli* (*E. coli*) bacterium as well as a description of its habitat and ecology. The cell structure, physiology and classification as well as the treatment options for *E. coli* infections have also been described. The mechanism of multi-drug resistance with emphasis on the production of extended spectrum beta-lactamases is also highlighted. The methods involved in the isolation and identification as well as those used in studying the molecular phylogeny and the pathogenesis of diseases caused by *E. coli* are reviewed.

Overview of the Bacterium *Escherichia coli*

Escherichia coli (E. coli), formerly known as *Bacterium coli commune* was later renamed after its discoverer Dr. Theodor Escherich, a German paediatrician in 1885 who isolated it from faeces of newborns. For many years, *E. coli* was considered to be a commensal organism of the colon until 1935, when a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea among infants (Todar, 2011). Today, this bacterium has been studied extensively and has been known to cause various infectious diseases. Based on the site of infection, these infections may range from mild diarrhoea to life-threatening bloody diarrhoea or even the deadly haemolytic uremic syndrome. *E. coli* is a member of the family *Enterobacteriaceae* (Ewing & Edwards, 1986), which includes many genera, including known pathogens

such as *Salmonella*, *Shigella*, and *Yersinia*. *E. coli* together with other similar bacteria, including members of the genera *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella* and *Serratia*, belong to a group referred to as coliforms owing to their ability to ferment lactose with the production of acid and gas when incubated at 35 – 37 °C. *It* is widely distributed in the intestine of humans and warm-blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora that maintains the physiology of the healthy host (Neill, Tarr, Taylor & Trofa, 1994; Conway, 1995). Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that cause infections in immune-compromised hosts, especially neonates, the elderly and HIV/AIDS patients (Feng, Weagant & Grant, 2002).

Importance of *E. coli*

Owing to its abundance in animal and human excreta, *E. coli* has been used as a very good indicator of faecal pollution (Carlos *et al*., 2010). Since, *E. coli* cells are able to survive outside the body for a limited period, they serve as ideal indicator organisms for testing environmental samples for contamination (Feng *et al.*, 2002).

E. coli forms part of the gastrointestinal micro-flora of many animals where it normally exist without causing disease. It provides vitamin K, which is required for posttranslational modification of proteins involved in blood coagulation and bone metabolism and has been recognised as a protective agent against infection by pathogenic bacteria (Dam, 1935; Bentley & Meganathan, 1983; Price, 1988; Mann, 1999; Hudault, Guignot, & Servin, 2001). It is also known to prevent the establishment of opportunistic pathogenic bacteria inside the gut. Certain strains of *E. coli* have proven

probiotic effects, such as the Mutaflor isolate of *E. coli* (also known as *E. coli* Nissle 1917), which has been shown to reduce gastrointestinal colonisation by pathogenic bacteria and has efficacy for the treatment of inflammatory disorders, such as inflammatory bowel disease, through the modulation of epithelial signal transduction pathways (Lodinova-Zadnikova & Sonnenborn, 1997; Lodinova-Zadnikova, Cukrowska & Tlaskalova-Hogenova, 2003; Kamada *et al.*, 2008). This bacterium has also extensively been used as a model organism in research laboratories. Indeed, *E. coli* has been widely exploited as a cloning host in recombinant DNA technology. However, *E. coli* is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen (Kaper, Nataro & Mobley, 2004) *and has been associated with a number of localized as well as systemic infections in humans, birds, cattle, pigs and many other animals.*

Infections caused by *E. coli* are common though usually underestimated. *It is the most common cause of urinary tract infection, paediatric diarrhoea (Nataro & Kaper, 1998) and neonatal meningitis globally. It* causes an estimated 73,000 cases of infection and 61 deaths in the United States of America each year (CDC, 2011). *E. coli* and related bacteria constitute about 0.1% of gut flora (Eckburg *et al.,* 2005), and faeco-oral transmission is the major route through which the pathogenic strains of the bacterium cause disease.

In May and June 2011, an unusual shiga-toxin-producing *E. coli* strain was the subject of a bacterial outbreak that occurred in Germany and spread throughout eleven other European countries as well as certain regions in North America. The strain known as O104:H4 caused a major outbreak in Europe

(mostly Germany) with 4, 321 cases, and an unusually high number (885) of haemolytic uraemic syndrome cases and 50 deaths (European Food Safety Authority [EFSA], 2011). In the United States, 6 cases of O104:H4 infections linked to travel to Germany were identified with one death case reported (CDC, 2011).

In the developing countries, a child of less than seven years of age still has a 50% chance of dying from diarrhoeal diseases (WHO, 1980; Gillen, 1991). Cases of gastroenteritis as well as urinary tract infections caused by pathogenic *E. coli* are commonly reported in Ghana (Addy *et al*., 2004; Djie-Maletz *et al*., 2008).

Habitat and Ecology of *E. coli*

E. coli strains are common inhabitants of the terminal small intestine and large intestine of mammals and often the most abundant facultative anaerobes in this environment (Welch, 2006). They can, occasionally, be isolated in association with the intestinal tract of non-mammalian animals and insects (Welch, 2006). Although there is evidence to suggest that *E. coli* may freely replicate in tropical fresh water (Bermudez & Hazen, 1988), the presence of *E. coli* in the environment is usually considered to reflect faecal contamination and not the ability to replicate freely outside the intestine (Welch, 2006).

Usually in the gut flora, a predominant clone of *E. coli* can be found at any given time, but other less abundant clones can be found simultaneously. For example, Whittam's laboratory has demonstrated that, in a study of 3 – 6 year old healthy girls, the most abundant *E. coli* clones varied when examined on a weekly basis (Whittam, Wolfe & Wilson, 1989). It was observed that for

some rare instances a single clone could be found over a 4-week period, and in other cases an individual may have as many as 16 different clones over the same period. The average was three clones per girl over the 4-week period. Therefore, the *E. coli* strains found in the intestine are multi-clonal and fluctuate in their predominance over time. Colonization of the periurethral area or urinary tract occurs briefly, and the strains isolated at these sites are usually not the predominant clone in the stool at the same time (Schlager, Hendley, Bell & Whittam, 2002). Aside adhesins and metabolic traits that would favour one *E. coli* strain over another in their ability to colonize particular sites, the production and immunity to bacteriocins, undoubtedly, affects the dynamics of the persistence and dominance of individual strains (Riley & Gordon, 1999).

Description, Cell Physiology and Cultural Characteristics of *E. coli*

E. coli is a Gram-negative non-spore-forming, aerobic and facultative anaerobic, rod-shaped bacterium. It is motile with peritrichous flagella (though some strains are non-motile) and approximately 1.1 - 1.5 μm wide and 2 - 6 μm long and occurs either singly or in pairs (Scheutz & Strockbine, 2005). It is non-fastidious but able to grow on bile containing media, and a lactose fermenter just like members of three other genera of medical importance belonging to the family Enterobacteriaceae: *Citrobacter*, *Enterobacter* and *Klebsiella* (Cheesbrough, 2000). Their ability to ferment lactose makes them to appear as pink colonies on lactose and bile salts containing differential and selective microbiological medium, such as MacConkey Agar, while producing brilliant green and yellow colonies when

growing on Eosin Methylene Blue (EMB) Agar and Xylose Lysine Decarboxylate (XLD) Agar, respectively.

Biochemically, *E. coli* is positive for indole production and the methyl red test. Most strains are oxidase, citrate, urease and hydrogen sulphide negative. The classic differential test to primarily distinguish *E. coli* from *Shigella* and *Salmonella* is the ability to ferment lactose, which the latter two genera fail to do (Cheesbrough, 2006). Aside lactose, most *E. coli* strains can also ferment D-mannitol, D-sorbitol, and L-arabinose, maltose, D-xylose, trehalose and D-mannose (Welch, 2006).

There are limited instances where pathogenic strains differ from the commensals in their metabolic abilities. For example, commensal *E. coli* strains generally use sorbitol, but *E coli* O157:H7 does not (Feng et al., 2002). Most diarrhoeagenic strains cannot utilize D-serine as a carbon and nitrogen source, but uropathogenic and commensal faecal strains can use this enantiomer of serine (Roesch *et al*., 2003).

Most *E. coli* strains are capable of growing over a wide range of temperature (approximately 15 – 48 °C) and the growth rate is maximal in the narrow range of 37 – 42 °C (Ingraham & Marr, 1987). *Escherichia coli* can grow within a pH range of approximately 5.5–8.0 with best growth occurring at neutrality (Welch, 2006). Some diarrhoeagenic *E. coli* strains have the ability to tolerate exposure to pH 2.0; such an acid shock mimics transit through the stomach and induces expression of sets of genes involved in survival and pathogenesis (Waterman & Small, 1996).

Iron metabolism of *E. coli* is an especially well-studied topic (Braun & Braun, 2002). Ferric iron is brought into *E. coli* by chelating compounds such

as citrate, enterobactin, aerobactin, yersinabactin and haeme. These chelators each have highly specific outer membrane proteins that enable their uptake across the outer membrane where they are then brought across the cytoplasmic membrane by ATP binding cassette (ABC) transport systems. One trait that sets many of the pathogenic *E. coli* apart from the normal intestinal *E. coli* is the ability to acquire ferric iron from a wide array of chelators. The multiple gene systems enable adaptation to sites where iron might be limited by host antibacterial activities (Torres *et al*., 2001). These virulence enhancing iron acquisition systems, such as aerobactin, are often encoded by plasmids or are present on pathogenicity islands.

Cell Structure of the Bacterium *E. coli*

E. coli is a Gram-negative rod shaped bacterium with a capsule, an outer membrane, a cell wall with a thin peptidoglycan layer and a thicker lippopolyssacharide layer, a periplasmic space, a plasma membrane and a cytosol in which ribosomes and the nucleoid region can be found (Figure 1). Within the periplasmic space is a single layer of peptidoglycan. The peptidoglycan has a typical subunit structure, where the *N*-acetylmuramic acid is linked by an amide bond to a peptide consisting of L-alanine, D-glutamic acid, *meso*-diaminopimelic acid and finally D-alanine. *E. coli* are commonly motile in liquid by means of peritrichous flagella. Swarming behaviour and differentiation into hyperflagellated and elongated bacilli typical of that seen in the *Proteus* species can be observed on some solid media (Harshey, 1994).

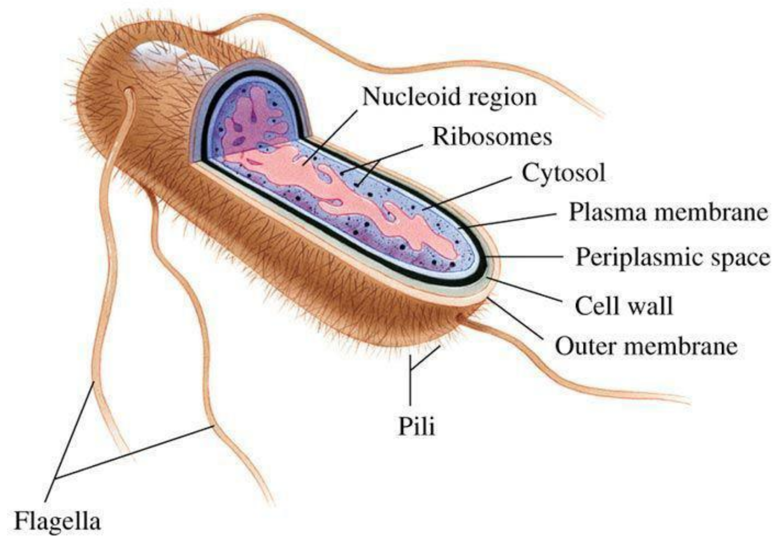


Figure 1: Basic ultrastructure of *E. coli* (Taylor, 2007)

E. coli are commonly fimbriated; the type 1 pili being the most common and are expressed in a phase switch ON or OFF manner that leads to piliated and nonpiliated states (Eisenstein, 1987). One of the traits commonly encoded on the larger genetic islands of the different pathotypes of *E. coli* are additional pili (chaperone-usher and type IV pili families and non-pili adhesins) (Bann, 2002; Schreiber & Donnenberg, 2002).

Clinical Categories of *E. coli*

Though *E. coli* has been thoroughly studied, there is a general under appreciation of the significant genetic differences between different strains. These differences determine whether the strains are able to cause disease and, if so, whether they can cause gastroenteritis or extra-intestinal infection (Nataro & Kaper, 1998). Hence, despite their usefulness, certain strains of *E. coli* are highly pathogenic due to the fact that they have acquired certain virulence factors (Table 1) and have been associated with diseases, as stated earlier (Atlas, 1995). Numerous virulence factors including adhesins, host cell

surface-modifying factors, invasins, toxins, and secretion systems are involved in *E. coli* pathogenic mechanisms (Table1). From the genetic and clinical point of view, *E. coli* strains of biological significance to humans can be grouped in into commensal strains, intestinal pathogenic (i.e. enteric or diarrhoeagenic) strains and extra-intestinal pathogenic *E. coli* also known as ExPEC (Russo & Johnson, 2000). Several pathotypes of enteric or diarrhoeagenic *E. coli* give rise to gastroenteritis but rarely cause disease outside the intestinal tract (Bien, Sokolova & Bozko, 2012). On the other hand, the ExPEC strains maintain the ability to exist in the gut without consequence; however, they have the capacity to disseminate and colonize other host niches including the blood, the central nervous system and the urinary tract, resulting in disease (Wiles, Kulesus & Mulvey, 2008).

Commensal *E. coli* strains

Commensal strains of *E. coli* are harmless or even beneficial as opposed to the pathogenic ones, because they lack virulent traits that enable the pathogenic ones to cause diseases either within or outside the gastrointestinal tract (Croxen & Finlay, 2010). However, in hosts with compromised defences, non-pathogenic *E. coli* can also be an excellent opportunistic pathogen (Nataro & Kaper, 1998). Furthermore, some strains of *E. coli* can diverge from their commensal cohorts, taking on a more pathogenic nature and the ability to cause serious diseases both within the intestinal tract and elsewhere within the host (Wiles *et al*., 2008). These strains acquire specific virulence factors (via DNA horizontal transfer of transposons, plasmids, bacteriophages, and pathogenicity islands), which

confer an increased ability to adapt to new niches and allow the bacteria to increase the ability to cause a broad spectrum of diseases (Bien *et al*., 2012).

Commensal *E. coli* can participate in extra-intestinal infections when an aggravating factor is present, such as a foreign body (e.g. urinary catheter), host compromise (e.g., local anatomical or functional abnormalities such as

Table 1: Summary of the Virulence Determinants of Pathogenic *E. coli*

Major groups	Specific examples
Adhesins	CFAI/CFAII, Type 1 fimbriae, P fimbriae, S fimbriae, Intimin (non-fimbrial adhesin)
Invasins	Haemolysins, Siderophores and siderophore uptake systems, Shigella-like "invasins" for intracellular invasion and spread
Motility/chemotaxis	Flagella
Toxins	LT toxin, ST toxin, Shiga-like toxin, Cytotoxins, endotoxin, LPS
Antiphagocytic surface properties	Capsules, K antigens, LPS
Defence against serum bactericidal reaction	LPS, K antigens
Defence against immune responses	Capsules, K antigens, LPS, Antigenic variation
Genetic attributes	Genetic exchange by transduction and conjugation, transmissible plasmids, R factors and drug resistance plasmids, Toxin and other virulence plasmids

(Todar, 2008)

urinary or biliary tract obstruction, or immune-compromise), or a high or a mixed bacterial species inoculum, such as with faecal contamination of the peritoneal cavity (Russo & Johnson, 2003).

Intestinal pathogenic strains of *E. coli*

Unlike commensal strains, intestinal pathogenic (enteric or diarrhoeagenic) strains of *E. coli* are rarely encountered in the faecal flora of healthy hosts and, instead, appear to be essentially obligate pathogens, causing gastrointestinal diseases including enteritis, enterocolitis, and colitis when ingested in sufficient quantities by a naive host (Russo & Johnson, 2000). Only in extremely rare cases are intestinal pathogenic strains responsible for infections outside the gastrointestinal tract (Tarr *et al*., 1996). There are six well-described categories of intestinal pathogenic strains of *E. coli* including enteropathogenic *E. coli* (EPEC), Shiga Toxin-producing *E. coli* (STEC) also known as Verocytotoxin-producing *E. coli* (VTEC) (comprising the sub-group enterohemorrhagic *E. coli* (EHEC)), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Scheutz & Strockbine, 2005; Croxen & Finlay, 2010). It has also been argued that, from the evolutionary point of view, the members of genus *Shigella* (*dysenteriae*, *flexneri*, *boydii*, and *sonnei*) should be classified as *E. coli* strains, a phenomenon termed taxa in disguise (Lan & Reeves, 2002). Verocytotoxin-producing *E. coli* were first described in 1977 by Konowalchuk and his co-workers (Wilshaw, Cheasty & Smith, 2000). They were recognised as significant causative agents of haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). Verocytotoxin-producing *E.*

coli illness can be fatal, especially, to children, the elderly, pregnant women and the immuno-compromised (Acheson, 2000; Wilshaw *et al*., 2000; Bell & Turnidge, 2002; Pruett *et al*., 2002).

Diseases due to intestinal pathogenic *E. coli* occur primarily in developing countries, with the exception of the enterohemorrhagic or Shiga toxin-producing strains of *E. coli* (Nataro & Kaper, 1998). The mere acquisition of these pathogens by the naïve host is usually sufficient for disease to ensue (Nataro & Kaper, 1998).

Extra-intestinal pathogenic *E. coli*

These strains are epidemiologically and phylogenetically distinct from both commensal and intestinal pathogenic strains; thus, they have been classified as extra-intestinal pathogenic strains of *E. coli* (Russo & Johnson, 2000). Extra-intestinal infections due to *E. coli* are common in all age groups and can involve almost any organ or anatomical site (Russo & Johnson, 2000). These include the urinary tract, the bloodstream, the cerebro-spinal fluid (Picard *et al,* 1999; Johnson & Stell, 2000), the respiratory tract (Johnson *et al.*, 2002), and the peritoneum (Soriano, Coll, Guarner & Al, 1995). The most common infections are urinary tract infections (which could be uncomplicated, febrile or invasive), pyelonephritis, neonatal, and post-neurosurgical meningitis and septicaemia (Scheutz & Strockbine, 2005). Other less common extra-intestinal infections due to pathogenic *E. coli* (ExPEC) have been reported, such as surgical wound infection, osteomyelitis, and myositis (Nataro & Kaper, 1998). In contrast to intestinal pathogenic strains of *E. coli,* host acquisition of an extra-intestinal pathogenic *E. coli* strain is insufficient for infection to occur; instead, entry of the organism into

an extra-intestinal site is required (Russo & Johnson, 2000). The ExPEC group comprises neonatal meningitis associated *E. coli* (NMEC), and uropathogenic *E. coli* (UPEC) eventhough septicaemia can occur with both UPEC and NMEC (Russo & Johnson, 2000). NMEC, a common inhabitant of the gastrointestinal tract, is the most frequent cause of Gram-negative-associated meningitis in newborns as it crosses the blood–brain barrier into the central nervous system (Croxen & Finlay, 2010).

E. coli has been observed to have an aetiological role in various diarrhoeal and extra-intestinal diseases of livestock (Kaper *et al.*, 2004)). An animal pathotype, known as avian pathogenic *E. coli* (APEC), causes extra-intestinal infections, primarily respiratory infections, pericarditis, and septicaemia of poultry (Kaper *et al*., 2004) and commonly associated with avian colibacilliosis (Johnson *et al.*, 2007). *E. coli* infections in animals reflect the diversity of the human *E. coli* pathotypes. ETEC infections are particularly prevalent in neonatal calves, lambs and piglets (Wray, McLaren & Carroll, 1993). ExPEC is also an important agent of mastitis in cows (Hogan & Smith, 2003). Host and tissue specificity may be related to the presentation of different fimbriae by the bacterium and differential presentation of molecules with affinity for those fimbriae by host cells (Acres, 1985). For example, *E. coli* cells isolated from diarrhoeic calves have been observed to express K99 (F5) and F41 fimbriae (ETEC-like strains) or F17 fimbriae (ExPEC-like strains) (Acres, 1985; Bertin, Girardeau, Darfeuille-Michaud & Contrepois, 1996a; Bertin, Martin, Oswald, & Girardeau, 1996b; Contrepois, Bertin, Pohl, Picard & Girardeau, 1998; Güler, Gunduz & Ok, 2008).

In addition to the eight main diarrhoeagenic pathovars of *E. coli*, other pathovars have been described but have not been well studied. Necrotoxigenic *E. coli* (NTEC) secretes two cytotoxic necrotizing factors (cfn1 and cfn2), as well as cytolethal distending toxin and infections can be isolated from human extra-intestinal infections, such as urinary tract (Croxen & Finlay, 2010). Another diarrhoeagenic *E. coli* pathotype (the cell-detaching *E. coli* or CDEC) that secretes cfn1 and a haemolysin may be associated with diarrhoea in children, yet its significance remains uncertain (Clarke, 2001; Abduch-Fabrega, Piantino-Ferreira, da Silva-Patricio, Brinkley & Affonso-Scaletsky, 2002). Perhaps the most interesting pathotype is adherent invasive *E. coli* (AIEC), which has been implicated in 36% of ileal Crohn's disease (Barnich *et al.*, 2007). AIEC adheres to carcino-embryonic antigen-related cell adhesion molecule 6 (CEACAM6) in the ileum, which is over expressed in patients predisposed to Crohn's disease (Croxen & Finlay, 2010). Adherence of AIEC also increases the expression of CEACAM6 through the stimulation of tumour necrosis factor and interferon-γ, which possibly promotes better colonization of the ileal mucosa and subsequent inflammation (Barnich *et al.*, 2007).

Classification of *E. coli*

Several schemes of classification of the bacterium *E. coli* have been described based on the serology, phylogeny as well as the pathogenesis of the bacterium.

Classification based on serology

A serotype is a group of closely related microorganisms that are distinguished by the fact that they bear a characteristic set of antigens (Todar, 2008). Bacterial serotypes are therefore defined by antibodies in the serum of the patients or animals that identify the specific type of antigen presented by the bacteria (Sabat *et al*., 2013). According to the modified Kauffman scheme, serological typing of *E. coli* is primarily based on the characterization of its three major surface antigens (Edwards & Ewing, 1972; Lior, 1996). The types of antigens are designated by letters with numbers that refer to the known subtypes of antigens, which, in turn, can be differentiated by the use of specific antibodies. The "O" antigens are somatic cell wall phospholipid-polysaccharide complexes. The "H" antigens are heat labile protein antigens found in the flagellin, which are responsible for the formation of the flagella in motile *E. coli* (Wilshaw *et al.*, 2000). The "K" antigens are the surface or capsular antigens that are acidic polysaccharides (Doyle, Zhao, Meng & Zhao, 1997). They were originally further divided into three classes: A, B and L. Only the A type of the K antigens are now considered to be important for typing antigens, because they are mainly associated with pathogenic strains of *E. coli* that cause extra-intestinal infections and not those associated with diarrhoeal disease (Wilshaw *et al*., 2000).

There are over 700 antigenic types or serotypes of *E. coli* that are known (Todar, 2008) and, among *E. coli* isolates, there is considerable variation as strains can exhibit various combinations of somatic (O and K) as well as flagellar (H) antigens. Hence, though very useful serotyping of *E. coli* is complex, with 173 O antigens, 80 K antigens, and 56 H antigens, which can

all be subdivided into partial antigens (Orskov & Orskov, 1992). The total number of *E. coli* serotypes is very high, 50 000 – 100 000 or more, but the number of frequent pathogenic serotypes is limited (Orskov & Orskov, 1992). Currently, it is only considered necessary to determine the O and H antigens to serotype strains of *E. coli* associated with diarrhoeal disease. For example, the O157: H7 strain of *E. coli* responsible for many outbreaks and sporadic cases of EHEC infection, have been linked with bovine faecal contamination of items consumed by humans, including undercooked beef, unpasteurised milk, vegetables, fruit juices or drinking water (Riley *et al.*, 1983; Borczyk, Karmali, Lior & Duncan, 1987; Morgan *et al.*, 1988; Besser *et al.*, 1993; Swinbanks, 1996; Yarze & Chase, 2000; Licence, Oates, Synge & Reid, 2001).

However, with respect to extra-intestinal pathogenic *E. coli,* strains are traditionally identified by the O:K:H serotype, such as O18:K1:H7, O6:K2:H1, and O7:K1:H- (Orskov, Orskov, Birch-Andersen, Kanamori & Svanborg-Eden, 1982). Some clonal groups are over-represented among clinical isolates as compared with faecal controls (Orskov & Orskov, 1983b; Johnson, Delavari, O'Bryan, 2001b). Some characterized pathotypes as well as the common O serogroups which affect humans are presented in Table 2.

Though *E. coli* of specific serogroup can be associated reproducibly with certain clinical syndromes, it is not in general the serological antigens themselves that confer virulence. Rather, the serotypes and serogroups serve as readily identifiable chromosomal markers that correlate with specific virulent clones (Whittman *et al.*, 1993).

Prior to the identification of specific virulence factors in diarrhoeagenic *E. coli* strains serotype analysis was the predominant means by which pathogen strains were differentiated. Though serotyping occupies a central place in the history of *E. coli*, with the advances made in molecular biology, serotyping is becoming less commonly employed (Lior, 1996). Furthermore it is limited in sensitivity and specificity, is tedious and expensive and is performed reliably only by a small number of reference laboratories (Nataro & Kaper, 1998).

Classification based on the phylogeny of *E. coli*

Phylogeny is the study of evolutionary relatedness among various groups of organisms. Studies based on DNA sequence analysis or multilocus enzyme electrophoresis (MLEE) identified clonal phylogenetic groupings of *E. coli* (Johnson, 2000). Phylogenetic studies have principally used the *E. coli* reference (ECOR) strain collection as a common reference for evolutionary comparisons (Ochman & Selander, 1984). Phylogenetic analyses have grouped *E. coli* strains into four main phylogenetic groups (A, B1, B2, and D) (Selander *et al*., 1987) and there are limited instances where host sources are associated with clonal types. Strains of these groups differ in their phenotypic characteristics, including the ability to use certain sugars, antibiotic resistance profiles and growth rate-temperature relationships (Touchon, Tenaillon, Hoede, Barbe & Baeriswly, 2009). The distribution (detection) of a range of virulence factors, thought to be involved in the ability of a strain to cause diverse diseases, also varies among strains of these phylogenetic groups (Escobar-Pàramo *et al*., 2004) indicating a role of the genetic background in the expression of *E. coli* virulence. Consequently, these

groups are differently associated with certain ecological niches, life history characteristics and propensity to cause disease (Gordon, Clermont, Tolley & Denamur, 2008).

Table 2: Common Pathotypes of Disease-Causing *E. coli* of Humans

Pathotype	Common O-serotypes	Disease characteristics	Virulence factors and mechanisms
Enteropathogenic *E. coli* (EPEC)	O126, O55, O86, O88, O103, O111, O119, O125ac, O126, O127, O128ab, O142, O145, O157, O158.	Watery diarrhoea	Initial localised adherence via bundle-forming pili (BPB) followed by intimate adherence
Enterotoxigenic *E. coli* (ETEC)	O6, O7, O8, O9, O11, O15, O17, O20, O21, O25, O27, O29, O48, O55, O56, O63, O64, O65,O71, O73, O77, O78, O85, O126, O128ac, O133, O138, O139,O86, O88, O105, O114, O115, O119, O141, O147, O148, O149, O153, O159, O166, O167.	Watery diarrhoea	Heat labile/ Heat stable enterotoxin
Enterohaemorrhagic *E. coli* (EHEC)	O26, O103, O111, O121, O145, O157*.	Water or bloody diarrhoea, haemorrhagic colitis progression to systemic diseases.	T3SS, Shiga-like toxins, enterohaemolysin, plasmid-encoded protease (EspP)
Enteroaggregative *E. coli* (EAEC)	O3, O15, O44, O86, O111, O125.	Persistent mucoid diarrhoea	Aggragative adherence, dependent on plasmid-encoded genes such as AAF-fimbriae. Also heat stable enterotoxin (EAST1) and plasmid-encoded enterotoxin (PET)
Enteroinvasive *E. coli* (EIEC)	O28ac, O29, O112ac, O115, O121, O124, O135, O136, O143, O144, O152, O159, O164, O167, O173.	Watery or bloody diarrhoea	Cellular invasion similar to Shigella spp. dependent on proteins encoded on pInv plasmid, including a T3SS.
Diffusely adherent *E. coli* (DAEC)	O126: H27	Water diarrhoea	Diffuse adherence F1845 fimbriae, AIDA-I adhesin
Extraintestinal pathogenic *E. coli* (ExPEC)	UPEC: O1, O2, O4, O6, O7, O18ac, O75, O16, O15, NMEC: O7, O18ac, O1, O6, O83	UTIs, pneumonia, wound infections, intra- abdominal infections, osteomyelitis, meningitis.	P-fimbriae, S-fimbriae, K1 capsule, α- haemolysin, CDT, CNF .

Source: Nataro & Kaper (1998) and Scheutz & Strockbine (2001).

*The most common O-serotypes of EHEC associated with human disease.

For instance, groups B2 and D strains are less frequently isolated from environment (Walk, Alm, Calhoun, Mladonicky & Whittam, 2007) than A and B1 strains (Gordon & Cowling, 2003). Furthermore, the genome size differs among these phylogeny groups, with A and B1strain having smaller genomes than B2 and D (Bergthorsson & Ochman, 1998). Phylogenetic trees of housekeeping gene sequences from the *E. coli* reference collection indicate that group D diverged first, while groups A and B1 are sister groups that separated later (Wang, Whittam & Selander, 1997). Subsequent analysis suggests that, perhaps, B2 rather than D is ancestral (Escobar-Pàramo *et al.*, 2004).

The source of *E. coli,* according to phylogeny groups, may be classified into intestinal, extra-intestinal or commensal. While the extra-intestinal pathogenic strains usually belong to groups B2 and D (Johnson and Stell, 2000); the commensal strains belong to groups A and B1 whilst the intestinal pathogenic strains belong to groups A, B1 and D (Pupo, Karadis, Lan & Reeves, 1997). A protocol for multi-locus sequence typing (MLST), which groups organisms according to nucleotide polymorphisms in selected genes, has been developed for *E. coli*, based on the sequence types of seven housekeeping genes (Wirth *et al.*, 2006). The resolution of the standard MLST method is comparable to that of MLEE. Many of the high-resolution genotyping techniques are complex, difficult to interpret or expensive, thus, there is often scope for simplification. This requires understanding of the basis of polymorphism observed for the more complex techniques. For example, sequencing of 16S rRNA loci may be simplified using PCR-RFLP (ribotyping) (Desjardins, Picard, Kaltenbock, Elion & Denamur, 1995).

Hence, *E. coli* phylogenetic groupings established by MLEE, MLST and ribosomal RNA sequencing might be predicted rapidly and reproducibly using a simple triplex PCR (Clermont *et al.*, 2000).

Classification based on the pathogenesis of pathogenic *E. coli* in humans

The loss and gain of mobile genetic elements has a pivotal role in shaping the genomes of pathogenic bacteria. Horizontal gene transfer is an important mechanism that rapidly disseminates new traits to recipient organisms (Croxen & Finlay, 2010). Acquiring these new traits is crucial in promoting the fitness and survival of a pathogen while it coevolves with its host (Erjavec & Žgur-Bertok, 2011). Large clusters of virulence genes, called pathogenicity islands (PAIs), can be found on plasmids or integrated into the chromosome in pathogenic bacteria, but they are not found in non-pathogenic bacteria (Croxen & Finlay, 2010). PAIs are usually flanked by mobile genetic elements (bacteriophages, insertion sequences or transposons) and often insert near transfer RNA (tRNA) genes (Croxen & Finlay, 2010). It is, therefore, not surprising that many of the virulence traits present in *E. coli* are carried on PAIs as well as on plasmids and prophages (Shames, Auweter & Finlay, 2009).

Each pathotype has distinguishing characteristics related to its epidemiology, pathogenesis, clinical manifestations and treatment. Among diarrhoeagenic *E. coli* pathotypes, ETEC, EPEC, EAEC, and DAEC colonise the small intestine while EIEC and EHEC preferentially colonise the large bowel prior to causing diarrhoea (Rodriguez-Ángeles, 2002). Each of the six recognized categories of diarrhoeagenic *E. coli* has unique features in their interaction with eukaryotic cells (Croxen & Finlay, 2010).

Enteropathogenic E. coli

EPEC are primarily associated with infant diarrhoea in developing countries and produce a characteristic histopathology known as attaching and effacing (A/E) on intestinal epithelial cells (Kaper *et al.*, 2004). These organisms are recognized on the basis of serotypes, such as O55:H6 and O127:H6. They are defined as those diarrhoeagenic *E. coli* strains that cause attaching and effacing (A/E) lesions on intestinal epithelium but which lack shiga toxins or verotoxins. EPEC disease, generally, results from growth of EPEC in the small intestine and is manifested by watery diarrhoea that may contain mucus but, typically, does not have blood in it. Vomiting, fever, malaise and dehydration are also associated (Nataro & Kaper, 1998). These symptoms may last for a brief period of several days, although instances of long, chronic EPEC disease have been noted (Nataro *et al.*, 1995). 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 (Welch, 2006). EPEC adheres to small bowel enterocytes, but destroy the normal microvillar architecture, inducing the characteristic attaching and effacing lesion (Nataro & Kaper, 1998). Cytoskeletal derangements are accompanied by an inflammatory response and diarrhoea (Kaper *et al.*, 2004). 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 (Welch, 2006). This is often described as an actin pedestal for the attached bacterial cell (Figure 3a). There is a specific pathogenicity island, termed the “locus of enterocyte effacement” (LEE) that encodes the genes responsible for the A/E lesion (McDaniel,

Jarvis, Donnenberg & Kaper, 1995). The LEE encodes a type III secretion system that provides the intimate adhesin, 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 signalling mechanisms including actin pedestal formation (Jerse, Yu, Tall, & Kaper, 1990; Kenny *et al*., 1997). Common to most EPEC strains are the EPEC adherence factor (EAF) plasmids, which encode an adherence factor, the bundle forming pilus (bfp) (Nataro *et al*., 1987; Donnenberg & Kaper, 1992; Sohel *et al*., 1996). Results of human volunteer studies indicate the EAF plasmid is necessary to cause disease (Levine *et al*., 1985). Although the A/E characteristic is critical for causing EPEC disease, probably through destruction of microvilli, the precise mechanism is not completely understood and may reflect the diversity of EPEC strains, since not all EPEC produce the enterotoxin, EspC (Mellies, 2001).

Attention has focused on greater understanding of atypical EPEC strains, which more commonly cause diarrhoea in industrialized nations than the typical EPEC strains (Trabulsi, Keller & Gomes, 2002). The atypical EPEC strains have animal and human reservoirs, whereas the typical isolates are almost always associated with human faecal 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) (Nataro & Kaper, 1998).

Shiga toxin producing E. coli

STEC also known as verocytotoxin producing (VTEC) *E. coli* comprises the sub-group enterohemorrhagic *E. coli* (EHEC). Infection by EHEC often leads to bloody diarrhoea, vomiting, and occasionally to kidney failure (Nataro & Kaper, 1998). Though most STEC share the ability to cause A/E lesions with EPEC, EHEC are set apart from EPEC due to their possession of shiga-like toxins and the clinical presentation of their disease (Nataro & Kaper, 1998). The elaboration of the Shiga toxin (Stx) followed by a systemic absorption of it, leads to potentially life-threatening complications (Kaper *et al*., 2004). Hence, EHEC cause disease of the large intestine that may present as simple watery diarrhoea and then progress to bloody stools with ulcerations of the bowel. In a small subset of diseased individuals there is later the onset of severe, life-threatening haemolytic-uremic syndrome (HUS) for several days (Paton & Paton, 2005). HUS involves a triad of haemolytic anaemia, thrombocytopenia and renal failure (Nataro & Kaper, 1998). The transmission of EHEC disease in humans is through ingestion of contaminated beef or foods contaminated with cattle faeces (Welch, 2006).

In cattle, the EHEC strains are transient members of the intestinal microflora, where they do not apparently cause disease (Welch, 2006). One of the remarkable features of EHEC is its low infection dose of 10–100 organisms. This microorganism has special acid-tolerance ability when compared to many other enteric bacterial pathogens (Welch, 2006). Children under the age of five are the major victims of EHEC disease, although the elderly may also exhibit bloody diarrhoea and HUS (Welch, 2006). All factors that lead to HUS are unknown except Shiga toxin, which probably plays an important role in renal injury. Purified Shiga toxin 1 (Stx-1) injected

intravenously in baboons leads to renal disease with histopathology similar to EHEC-mediated HUS (Tailor, Nouri, Lee, Kozak & Kabat, 1999). The Shiga toxin inhibits protein synthesis through cleavage of ribosomal RNA. Since EHEC do not cause bacteraemia, 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 (Welch, 2006). There are two evolutionarily related forms of Shiga toxin in *E. coli* including Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). They share approximately 55% amino acid sequence similarity. Stx1 is only different from the Shiga toxin of *Shigella dysenteriae* by a single amino acid substitution (Welch, 2006). There are many Shiga toxin-positive *E. coli* strains (STEC) that are not associated with enterohaemorrhagic colitis (Centre for Food Security and Public Health, 2009). 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 (Welch, 2006). The ubiquitous distribution of Shiga toxin genes among *E. coli* strains is due to their transmission as part of lambdoid phages (Welch, 2006). The EHEC O157:H7 strain likely originated from 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, Herbelin, Bumbaugh, Selander & Whittam, 2000; Lathem, Bergsbaken, Witowski, Perna & Welch, 2003). The precise role of pO157 in EHEC pathogenesis is unknown but may involve some putative toxin genes and a mucin-specific zinc metalloprotease, StcE

(Burland *et al*., 1998; Lathem *et al*., 2002; Grys, Siegel, Lathem & Welch, 2005).

Enterotoxigenic E. coli

Among all *E. coli* pathotypes, ETEC is the most common, particularly, in the developing world (WHO, 1999), and is increasingly recognized as an emerging enteric pathogen. Enterotoxigenic *E. coli* is the second most common cause of traveller's diarrhoea and a common cause of acute diarrheal illness in children and adults (4.5%) reporting to emergency departments and in-patient units in the United States (USA) (Cohen *et al*., 2005; Nataro *et al*., 2006). The organism is regularly imported into the developed world, because ETEC is a major cause of traveller's diarrhoea in persons who travel to developing countries in Africa and Asia (Gorbach, Kean, Evans, Evans & Bessudo, 1975; Black, 1990; Bern *et al*, 1992; Bouckenooghe, Jiang, de la Cabada; Ericsson, 2003).

ETEC diarrhoea occurs in all age groups, but mortality is most common in infants, particularly, in the most undernourished or mal-nourished infants in developing nations (Evans & Evans, 1990). The disease is characterized by watery stool, abdominal cramps, fever, malaise, and vomiting (WHO, 2007). The pathogenesis of ETEC diarrhoea involves two steps: intestinal colonization, followed by elaboration of diarrhoeagenic enterotoxin(s). Enterotoxigenic *E. coli* strains are characterized by their specialized pili, antigenically unrelated to common pili, which act as ligands to bind the bacterial cells to specific complex carbohydrate receptors on epithelial cell surfaces of the small intestine (Kaper *et al*., 2004). This

interaction results in colonization of the intestine by ETEC, with subsequent multiplication on the gut surface, therefore, these pili are termed colonization-factor antigens (CFAs) (Evans & Evans, 1990). Enterotoxigenic *E. coli* possess structures called fimbriae that are species-specific. Different types of ETEC fimbrial adhesions are used by the bacteria to colonize the gastrointestinal tract. These strains are non-invasive, but produce enterotoxins (Nataro & Kaper, 1998). The CFAs can be subdivided based on their morphological characteristics. Three major morphologic varieties exist: rigid rods, bundle-forming flexible rods, and thin flexible wiry structures. The prototype rigid rod-shaped fimbriae, CFA/I, are composed of a single protein assembled in a tight helical configuration; CFA/III is a bundle-forming pilus; and the CFA/II and CFA/IV are composed of multiple distinct fimbrial structures (Nataro & Kaper, 1998). The CFA-type pili play a major role in host specificity (Evans & Evans, 1990). ETEC carry the gene for enterotoxin production, which causes diarrhoea in humans and animals. ETEC strains cause diarrhoea through the action of two types of enterotoxins; a heat-labile toxin (LT) and a heat-stable toxin (ST) (Nataro & Kaper, 1998). These strains can express LT only, ST only, or both LT and ST. The genes coding for the production of CFAs reside on the ETEC virulence plasmids, usually on the same plasmids that carry the genes for one type or both types of *E. coli* enterotoxin. In most cases of ETEC infections, the diarrhoea is caused by CFA and both LT and ST; fewer are caused by those possessing a CFA and only one toxin (usually LT); and in very rare cases, ETEC infections may be caused by *E. coli* lacking a CFA but possessing only ST (Wilshaw *et al.*,

2000). Infection requires colonization and the release of one or more enterotoxins.

The two unrelated classes of STs (STa and STb) differ in structure and in mechanism of action (Nataro & Kaper, 1998). The genes for both classes are found predominantly on plasmids, and some ST-encoding genes have been found on transposons (Kaper *et al*., 2004). The LT and cholera toxin (CT) (involved in the pathogenesis of *Vibrio cholera*) toxins share common antigenic determinants, and their primary amino acid sequences are similar (Nataro & Kaper, 1998). The two major serogroups of LT, termed LT-I and LT-II, do not cross-react immunologically (Nataro & Kaper, 1998). LT-I is expressed by *E. coli* strains that are pathogenic for both humans and animals (Kaper *et al*., 2004). LT-II is found primarily in animal *E. coli* isolates and rarely in human isolates, but in neither animals nor humans has it been associated with disease (Nataro & Kaper, 1998).

Enteroaggregative E. coli

These organisms are defined as *E. coli* strains that do not secrete LT enterotoxin or Shiga toxin, but adhere to cultured HEp-2 cells in self-aggregates that are referred to as "stacked bricks" (Nataro *et al*., 1995). Many *E. coli* strains can mediate the "stacked brick" adhesive phenotype, but there is a subset of these that are *bona fide* human diarrhoeal pathogens (Welch, 2006). Enteroaggregative *E. coli* (EAEC) disease is watery diarrhoea that occurs in some cases with abdominal cramps, but no fever (Nataro *et al*., 1995). There is no invasion of the bloodstream. The disease seen in natural EAEC outbreaks is often reported as persistent, seemingly chronic watery

diarrhoea. 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 adhesin called "aggregative adherence fimbriae" (AAF). 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. EAEC strains often produce a heat stable enterotoxin EAST1 that is homologous to the ST1 of ETEC.

Enteroinvasive E. coli

Enteroinvasive *E. coli* (EIEC) shares virulence mechanisms with *Shigella* spp. in that it is able to invade and multiply within intestinal epithelial cells of the large intestine (Lan & Reeves, 2002). Thus, these organisms are pathogenetically so closely related to *Shigella* species that the nomenclature distinction is questionable (Lan & Reeves, 2002). 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, Toledo & Trabulsi, 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 (Welch, 2006). EIEC cause invasive inflammatory colitis and dysentery with a clinical presentation (blood and mucous stools

accompanied by fever and severe cramps) identical to the disease caused by *Shigella* species (Nataro & Kaper, 1998). EIEC/*Shigella* invades 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 neighbouring host cells by a motility process whereby actin is nucleated on one pole of the bacillus and subsequent actin polymerization propels the bacterial cell (Goldberg & Theriot, 1995). Many of genes necessary for cellular invasion and disease 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 signalling and membrane lysis are found on these plasmids. In addition, the plasmid encodes an outer membrane protein (IcsA) that is localized on one pole of the bacterium and directs the actin microfilament polymerization 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. The inability to decarboxylate lysine, a trait shared by EIEC and *Shigella*, is the result of mutations and gene rearrangements at the *cadC* gene (Welch, 2006). The decarboxylation of lysine results in cadverine, which acts as an inhibitor of inflammation and migration of neutrophils into the lamina propria. The lack of this function is hypothesized to be a pathoadaptive trait that enables EIEC/*Shigella* to cause disease (Maurelli, Fernandez, Bloch, Rode & Fasano, 1998; Fernandez *et al*., 2001; Casalino, Latella, Prosseda, & Colonna, 2003).

Diffusely adherent E. coli

The epidemiology and pathogenesis of the diffusely adherent *E. coli* (DAEC) are not well understood (Nataro & Kaper, 1998). DAEC may cause diarrhoea in very young children less than a year old (Scaletsky *et al*., 2002). They are differentiated from the other diarrhoeagenic *E. coli* by a distinct adhesion phenotype, again on HEp-2 cells (Nataro & Kaper, 1998). The adhesion is brought about by F1845 fimbriae, which belong to the Dr family of adhesins (also found in some UPEC strains) (Kaper *et al*., 2004). The Dr adhesins recognize and bind to host cell surface decay accelerating factor (DAF) (Nataro & Kaper, 1998). DAEC bound to cultured cells elicit a cytopathic phenotype and activation of signal transduction pathways (Welch, 2006). The relative significance of DAEC as a pathogen and its mechanisms for causing disease await further study (Nataro & Kaper, 1998).

The extra-intestinal infections due to *E. coli* are common in all age groups and can involve almost any organ or anatomical site (Russo & Johnson, 2000). Common examples include the urinary tract, the bloodstream, the cerebro-spinal fluid (Picard *et al.,* 1999; Johnson & Stell, 2000), the respiratory tract (Johnson, Oswald, O'Bryan, Kuskowski & Spanjaard, 2002), and the peritoneum (Soriano *et al.*, 1995). Two separate pathotypes of *E. coli* are generally recognized as causes of extra-intestinal human diseases (neonatal septicaemia/meningitis *E. coli,* (NMEC) and the urinary tract and bloodstream *E. coli,* uropathogenic *E. coli* (UPEC) (Welch, 2006). The most common infections include urinary tract infections ranging from uncomplicated to febrile to invasive, pyelonephritis, neonatal, and post-neurosurgical meningitis and septicaemia (Scheutz & Strockbine, 2005). Other less common extra-intestinal infections due to ExPEC *E. coli* such as

wound infection, osteomyelitis, and myositis have been reported (Nataro & Kaper, 1998). In contrast with intestinal pathogenic strains of *E. coli,* host acquisition of an extra-intestinal pathogenic *E. coli* strain is insufficient for infection to occur; instead, the entry of the organism into an extra-intestinal site (e.g., the urinary tract) is required (Russo & Johnson, 2000).

Septicaemia can also occur with both UPEC and NMEC. NMEC, a common inhabitant of the gastrointestinal tract, is the most frequent cause of Gram-negative-associated meningitis in new-borns as it crosses the blood–brain barrier into the central nervous system (Croxen & Finlay, 2010).

Uropathogenic E. coli

Uropathogenic *E. coli* (UPEC) are a heterogeneous group of clones (Donnenberg & Welch, 1996). Within the 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. It has been argued that approximately 60% of adult women will have a urinary tract infection (UTI) in their lifetime (Kunin, 1994). As much as 90% of all community-acquired UTIs and greater than 30% of the hospital-acquired UTIs are caused by *E. coli* (Haley *et al*., 1985). Community-wide outbreaks of UTIs by multidrug resistant UPEC clones have been reported (Manges *et al*., 2001). 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 & Orskov, 1983a; Orskov & Orskov, 1983b; Orskov & Orskov, 1985; Johnson, Roberts & Stamm, 1987; Wold, Caugant, Lidin-Janson, de Man & Svanborg, 1992). These *E. coli* strains are extraordinary in that they are especially capable of

invading the bloodstream (Johnson *et al*., 1987; Johnson, Moseley, Roberts, & Stamm, 1988; Johnson, 1991; Johnson *et al*., 1991; Johnson *et al*., 1994).

Many of the putative virulence factors for these strains are not shared with common faecal *E. coli* strains (Welch, 2006). Examples of such factors are adhesins (e.g., pap, sfa, and Dra), haemolysin (Hly), cytotoxic necrotizing factor-1 (cfn1), and the aerobactin (Aer) iron-sequestration systems (Donnenberg and Welch, 1996). A member of the autotransporter protein family, Sat, has been found as a cytotoxin of uroepithelial cells (Guyer, Henderson, Nataro & Mobley, 2000). There are additional factors that are common to all *E. coli* that are critical for pathogenesis of extraintestinal disease. The principal factors are lipopolysaccharide, capsule production and type 1 pili (Jacobsen, 2008). The type 1 pili appear to play a particularly critical role in the initial colonization of the bladder (Kisielius, Schwan, Amundsen, Duncan & Schaeffer, 1989; Connell *et al.,* 1996; Langermann *et al*., 1997; Lim *et al*., 1998; Struve & Krogfelt, 1999).

Invasion of the urinary tract basically occurs in a series of stages and is described as follows (Wiles *et al*., 2008). First, the type 1 pili-expressing UPEC secrete toxins and other virulence factors, alone or in association with outer membrane vesicles. Then, some siderophores like enterobactin and salmochelin released by UPEC scavenge iron, in competition with the host iron-chelating molecules and lipocalin 2. The type 1 pili mediate bacterial attachment to and invasion of the bladder epithelial cells, which are large terminally differentiated superficial epithelial cells that line the lumenal surface of the bladder. These epithelial cells are the primary targets of UPEC invasion. UPEC can rapidly multiply within the superficial cells, forming

large biofilm-like communities. Exfoliation of infected bladder cells facilitates bacterial clearance from the host, but leaves the smaller underlying immature cells more susceptible to infection. The efflux of UPEC from infected host cells before they complete exfoliation likely promotes bacterial dissemination and persistence within the urinary tract. During efflux, UPEC often become filamentous, probably due, in part, to mounting stress arising from increased activation of host defences.

Neonatal meningitis-associated E. coli

The pathogenesis of neonatal meningitis-associated *E. coli* (NMEC) is complex, as the bacteria must enter the bloodstream through the intestine and ultimately cross the blood-brain barrier into the central nervous system, which leads to meningeal inflammation and pleocytosis of the cerebrospinal fluid (Croxen & Finlay, 2010). Along with Group B streptococci, NMEC is the most common cause of neonatal meningitis, a severe disease with a high mortality rate and possible long-term neurological problems in survivors (Unhanand, Mustafa, Mccracken & Nelson, 1993; Stoll, Hansen, Fanaroff & Al, 2002). There is a limited number of *E. coli* serotypes associated with this disease, but greater than 80% of the strains express K1 capsule (Robbins *et al*., 1974; Sarff *et al*., 1975). It is generally thought that the newborn acquires the K1 strain from its mother during passage through the birth canal. The strain then progressively invades the bloodstream and subsequently crosses endothelial surfaces into the brain. The K1 capsule is a critical determinant in invasion across the blood-brain barrier and necessary for survival in the meninges (Hoffman, Wass, Stins, & Kim, 1999). S-fimbriae enable K1 isolates to adhere to brain microvascular endothelium (Parkkinen, Korhonen,

Hacker & Soinila, 1988). Several genes, such as ibeA, ibeB, ibeC, cnf-1 and aslA, are required for endothelial cell invasion (Prasadarao, Wass, Huang & Kim, 1999; Wass & Kim, 2000a; Badger *et al.,* 2000a; Khan *et al*., 2002). Also common to most NMEC isolates is a 100-kb plasmid that confers increased virulence (Mercer, Morelli, Heuzenroeder, Kamke & Achtman, 1984; Badger, Wass, Weissman & Kim, 2000b).

Control and Prevention of *E. coli* Related Infections

Environmental sanitation and good personal hygiene is key to successful prevention and control of the spread of infections due to pathogenic *E. coli*. Travellers visiting certain endemic localities may take specific appropriate antibiotics as a chemoprophylaxis (Nataro & Kaper, 1998). Lactating mothers may provide protection to their babies through the secretion of IgA in the breast milk (Cravioto *et al*., 1991). Currently, there are no vaccines available for the prevention of *E. coli*-related infections but trials are ongoing in a number of laboratories (Nataro & Kaper, 1998; Kelland, 2010).

Treatment of infections caused by pathogenic *E. coli* varies depending on whether the infection is caused by diarrhoeagenic or extra-intestinal isolates. Thus, most infections due to the six diarrhoeagenic or intestinal *E. coli* (ETEC, EPEC, EHEC, EAEC, EIEC and DAEC) are usually treated by providing the patient supportive care rather than administering antibiotics (Nataro & Kaper, 1998). The provision of supportive care is achieved through rehydration and adequate oxygenation while the decision to administer antibiotics is done on an individual basis (DuPont & Erickson, 1993) following the performance of a local antibiotic susceptibility pattern (Kaur, Chakraborti & Asea, 2010). Rehydration therapy is recommended for

treatment of ETEC in infants and children with ETEC diarrhoea. Treatment of EPEC infections is achieved by correcting fluid and electrolyte imbalances (Nataro & Kaper, 1998). Depending on whether the cases are mild or severe, oral or parenteral rehydration is recommended. Treatment of EHEC infections as well as that of HUS is largely limited to supportive; and the later may include dialysis, haemofiltration, transfusion of packed erythrocytes and platelet infusions (Nataro & Kaper, 1998).

Antibiotics are administered only in exceptional cases of *E. coli* infection such as in young children, patients with extra-intestinal or chronic *E. coli* infections (Nataro & Kaper, 1998). In exceptional cases, such as Traveller's diarrhoea caused by some strains of ETEC, however, the administration of antibiotics, especially, fluoroquinolones (Ciprofloxacillin, Norfloxacillin and ofloxacillin) can shorten the period of the diarrhoeal episode (Ericsson *et al*., 1990). Due to the indiscriminate use of antibiotics, the rate of emergence of antimicrobial resistance is on the increase; hence, the administration of antibiotics should not be encouraged (Nataro & Kaper, 1998). Though it has been reported that a variety of antibiotics have been proven useful in many cases, multiple antibiotic resistance is common for EPEC (Donnenberg, 1995).

Usually, the administration of antibiotic is the treatment of choice for UPEC infections (Mandell, Bennett & Mandell, 2010). In general, the frequency of resistance precludes empirical use of ampicillin, even in community acquired infections. The prevalence of resistance to first-generation cephalosporins and trimethoprim sulfamethoxazole is increasing among community-acquired strains in the United States (10 - 40%) and is

even higher outside North America (Paterson & Bonomo, 2005). Until recently, trimethoprim-sulfamethoxazole was the drug of choice for the treatment of uncomplicated cystitis in many patients (Gupta, Scholes & Stamm, 1999). Although continued empirical use of trimethoprim-sulfamethoxazole will predictably result in ever-diminishing cure rates, a wholesale switch to alternative agents (e.g., fluoroquinolones) will just as predictably accelerate the widespread emergence of resistance to these antimicrobial classes (Russo & Johnson, 2009)

In Ghana, ceftriaxone and cefotaxime are recommended cephalosporins for the treatment of infections (Stantard Treament Guidelines (STG), 2004). The infections include meningitis, typhoid fever, urinary tract infections, acute otitis media and sexually transmitted infections, such as gonorrhoea (STG, 2004).

Modes of action of antibiotics

An antibiotic is a chemical substance produced by a micro-organism, originally referred to as a natural compound produced by a fungus or another microorganism that kills pathogenic bacteria in humans or animals. Whereas the term antimicrobial is a broader one than antibiotic, it refers to any substance of natural, semi-synthetic, or synthetic origin that is used to kill or inhibit the growth of microorganisms but causes little or no host damage (Forbes, Saham, & Wesisfeld, 2007). The term antimicrobial is used synonymously with the word antibiotic by many people (Centres for Disease Control and Prevention [CDC], 2005). Antibiotics act on bacterial cells in any of five basic mechanisms (Nester, Anderson, Roberts, Pearsall & Nester, 2004).

These are:

1. inhibition of cell wall synthesis, by antibiotics such as vancomycin, bacitracin and other ß-lactam drugs;
2. disruption of the integrity of the cytoplasmic membrane (e. g. by polymixins B);
3. inhibition of protein synthesis (e.g. by aminoglycosides, tetracyclines, chloramphenicol and macrolides);
4. inhibition of nucleic acid synthesis (e.g. by fluoroquinolones and rifamycins); or
5. disruption of metabolic pathways (e. g. by trimethoprim and sulphonamides).

β-lactam antibiotics are the most common treatment for Gram-positive, Gram-negative and anaerobic bacterial infection (Ambler 1980; Kotra, Samama & Mobashery, 2002; Holten & Onusko 2000). β-lactams are a family of antibiotic agents consisting of four major groups: penicillins, cephalosporins, monobactum, and carbapenems (Kotra *et al.*, 2002). They all have a β-lactam ring, which can be hydrolyzed by β-lactamases. The groups differ from each other by additional rings e.g. Thiazolidine ring for penicillin, Cephem nucleus for cephalosporin, double ring structure for carbapenem and no ring for monobactum (Levinson, 2010). They act on bacteria by two mechanisms: (1) they incorporate in bacterial cell wall and inhibit the action of transpeptidase, which is responsible for completion of cell wall and (2) they attach to the penicillin binding proteins (PBPs) that normally suppress cell wall hydrolases, thus freeing these hydrolases, which in turn act to lyse the bacterial cell wall (Levinson, 2010).

In response to the suppressive action of antibiotics of their growth and survival, bacteria have developed certain mechanisms to subvert the adverse effects of the drugs. Several factors have been implicated in the rapid emergence of multidrug resistance, which are basically linked to the indiscriminate use of antibiotics. These include among others the acquisition of over-the-counter antibiotics, non-completion of antibiotic treatment and the addition of antibiotic to animal feed.

Antibiotic resistance in *E. coli*

Antibiotic resistance is the ability of a microorganism to withstand the effect of an antibiotic (Brooks, Butle, Morse & Jawetz, 2004). If a bacterium resists at least 2 antimicrobials then it is called multi-resistant (Refika & Marlyn, 2001) and the cross-resistance is a phenomenon of one organism acquiring resistance to one drug through direct exposure which turns out to have resistance to one or more drugs to which it has not been exposed (Biology-Online, 2005).

Multi-drug resistance has become an alarming problem faced by physicians, veterinarians and microbiologists (Todar, 2008). Multi-resistance to many clinically useful antimicrobial drugs has been found in *E. coli* (Lim *et al.,* 2007). It has been observed in a variety of sources, such as humans, wildlife, domestic animals and surface water (Sayah, Kaneene, Johnson, & Miller, 2005). Intestinal bacteria plasmids can contribute to exchanging genes encoding antibiotic resistance among them (Nirdnoy, 2005; Petridis, Bagdasarian, Waldor & Walker, 2005; Schjørring, Struve & Krogfelt, 2005). *E. coli* often carries multi-resistant plasmids (Umolu, Ohenhen, Okwu & Ogiehor, 2006), which serve as a reservoir of resistant genes that can be

transferred to other species as well as pathogens in humans and animals (Balis *et al.,* 1996; Sunde & Sorum, 2001). Boerlin *et al.* (2005) suggested a possibility of transferability of resistance and virulence genes on plasmids of pathogenic *E. coli* isolated from diarrhoea and healthy pigs, because of differences between resistance genes in pathogenic isolates and other porcine *E. coli* isolates. Other studies of resistance in *E. coli* have found such mechanisms of quinolone resistance by chromosomal mutation and plasmid-mediated resistance (Mammeri, Loo, Poirel, Martinez-Martinez & Nordmann, 2005). *E. coli* resistant to zidovidine (AZT), an effective drug in HIV treatment, has been found (Kim & Loeb, 1995).

The mechanisms by which microorganisms develop resistance are multi-varied. Antibiotic resistance in bacteria may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source (Todar, 2009). Generally, bacteria may display antibiotic resistance by one or more of the following mechanisms: they may lack receptors or targets for the antibiotic, the antibiotic target may be inaccessible, the antibiotic target may be modified to prevent the action of the drug, the antibiotic may be chemically modified or destroyed or the bacteria may elaborate alternative pathways, avoiding the drug target (Todar, 2009).

The production of β-lactamases forms an integral part of the mechanism of antimicrobial resistance in *E. coli* (Lim *et al.*, 2007). β-lactamases are enzymes produced by *E. coli* and many other bacteria that are capable of hydrolyzing cephalosporins. β-lactamases bind to the β-lactam ring

thereby preventing the drug from acting on the bacteria and ultimately rendering the drug inactive.

Several classes of β-lactamases have been identified on the basis of their amino acid sequences, substrate and inhibitor profiles; and have been classified either according to the Ambler or the Bush systems (Bush, Jacoby & Medeiros, 1995). The detection of their presence can provide useful information, especially, in monitoring the evolution of resistance and also in infection control (Forbes *et al*., 2007). The Ambler classification is the original method of β-lactamase categorisation, which orders the enzymes into 4 classes (A, B, C, and D) based on molecular structure (Ambler, 1980). Extended spectrum β-lactamases (ESBLs) are Class A β-lactamases and may be defined as plasmid-mediated enzymes that hydrolyse oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems (Bradford, 2001). They are inhibited *in vitro* by clavulanate or clavulanic acid (Paterson & Bonomo, 2005). Clavulanate is a β-lactamase inhibitor sometimes combined with penicillin group of antibiotics to overcome certain types of antibiotic resistance arising from β-lactam enzymes in some bacteria (Bradford, 2001).

Bush *et al*. (1995) also devised a classification of β-lactamases based upon their functional characteristics and substrate profile, which is widely used. Under this scheme of classification, the enzymes are divided into three major groups: group 1 - cephalosporinases, which are not inhibited by clavulanic acid; group 2 - broad spectrum enzymes which are generally inhibited by clavulanic acid (except for the 2d and 2f groups); and group 3 - metallo-β-lactamases (Dhillon & Clark, 2012). Members of group 2e are the

Extended Spectrum β-lactamases (ESBLs) and are usually associated with *E. coli* and *Klebsiella* spp infections (Bush *et al*., 1995).

Extended spectrum β-lactamases

There are various genotypes of ESBLs. Of these, the most common are the SHV, TEM, and CTX-M types (Rupp & Fey, 2003). Other clinically important types include VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC (Jacoby & Munoz-Price, 2005). When ESBLs were first recognized in the early 1980s, they were found to be point mutations of the TEM and SHV broad spectrum enzymes, which resulted in resistance to the β-lactam class of antibiotic (Knothe, Shah & Krcmery, 1983; Kliebe, Nies, Meyer, Tolxdorff-Neutzling & Wiedemann, 1985). SHV refers to sulfhydryl variant, derived from the chemical nature of the amino acids in the enzyme that are cross-linked with sulphur molecules. TEM, however, was originally found in a single strain of *E. coli* isolated from a patient named Temoniera in Greece (Medeiros, 1984). The mutations in the genes result in these enzymes having high catalytic capabilities for *β*-lactams due to low K values (i.e., high affinity) for the compounds (Knott-Hunziker, Petursson, Waley, Jaurin & Grundstrom, 1982). They have become a major cause of hospital-acquired infection, particularly in the intensivecare unit, with the majority of ESBL producers being isolated from critical care patients (Pitout *et al*., 2005; Falagas and Karageorgopoulos, 2009). TEM and SHV-types have been recognized across the world with over 100 mutations being reported as being resistant to the extended spectrum cephalosporins. This was driven by the heavy use of such antibiotics (Livermore & Hawkey, 2005).

Since the start of the 21st century, there has been increasing evidence that a shift in the genotypic make-up of ESBLs is taking place (Bush *et al*., 1995). The CTX-M genotype, originating from chromosomally encoded enzymes of the *Kluyvera* spp, has risen in prominence especially in *E. coli* and *K. pneumonia* (Livermore & Hawkey, 2005; Pitout, Nordmann, Laupland, & Poirel, 2005; Falagas & Karageorgopoulos, 2009). It is believed the genes were then conjugated onto plasmids from where they were transferred to pathogenic species, with the ability to move between different bacterial populations (Bonnet, 2004). The CTX-M enzymes appear to have a greater ability to spread and cause outbreaks (Dhillon & Clark, 2012). There are over 50 variants of CTX-M and they have been associated with numerous outbreaks of infections both in hospitals and in the community, particularly, in urinary *E. coli* isolates in nonhospital setting (Livermore & Hawkey, 2005; Falagas & Karageorgopoulos, 2009).

Methods of Isolation and Identification of *E. coli*

Assays for identification of all categories of *E. coli* are available. However, in many situations it is not necessary to implicate a specific *E. coli* pathogen in a particular patient (Nataro & Kaper, 1998).

There are many methods of isolation and identification of *E. coli*; the most commonly used include the performance of biochemical test, serotyping, phenotypic assays based on virulence and molecular detection methods using nucleic acid probes and PCR (Nataro & Kaper, 1998). Isolation of bacteria from various samples is possible provided one has an idea about the bacterium of interest and its nutritional requirements, that is a medium and other growth requirements, such as temperature, oxygen demand and/or light intensity.

Depending on the source, *E. coli* may be isolated on MacConkey, Xylose-Lysine-Decarboxylase (XLD) Agar, Cysteine Lactose Electrolyte Deficient (CLED) Agar or Eosin-Methylene-Blue (EMB) Agar (Cheesbourgh, 2006).

E. coli is a facultative anaerobe that can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions; however, growth of these organisms has also been observed at 44°C (Feng *et al*., 2012; Weagant & Feng, 2002). *E. coli* is Gram-negative, catalase-positive, oxidase-negative, non-spore forming and rod-shaped bacterium. The motility of the organism occurs due to peritrichous flagella, although some non-motile strains have also been recorded (Welch, 2006). When growing anaerobically, there is an absolute requirement for fermentable carbohydrate (Edwards & Ewing, 1972). Glucose is fermented to give acid and gas (Edwards & Ewing, 1972). These organisms are able to utilize carbon and nitrogen sources for all their metabolic and energy needs (Edwards & Ewing, 1972). *E. coli* in faecal samples are most often recovered on MacConkey or EMB agar. Merchant & Packer (1967) considered EMB agar as a suitable medium for isolation of *E. coli* from faeces and foods, because of their ability to produce distinctive colonies having greenish metallic sheen not produced by other bacteria of Enterobacteriaceae family. For epidemiological or clinical purposes, *E. coli* strains are often selected from MacConkey agar plates after presumptive visual identification of lactose-fermenting pink colonies (Nataro & Kaper, 1998). However, this method should be used only with caution, since not all of *E. coli* are lactose-fermenters, and some diarrhoeagenic *E. coli* strains are typically non lactose-fermenters (Welch, 2006). The indole test, positive in 99% of *E. coli* strains, is the single best test

for differentiation from other members of Enterobacteriaceae (Nataro and Kaper, 1998). Pathogenic *E. coli* are not distinguishable from other strains or from each other by the appearance on culture plates or by the results of the usual biochemical tests (Nataro & Kaper, 1998). To determine whether the isolated strain is one of the pathogenic strains or merely a constituent of the normal flora, additional identification techniques must be employed, which are generally limited to research laboratory settings (Welch, 2006). The O157: H7 strain is the only pathogenic strain that can be identified readily in the clinical laboratory since it forms colourless colonies when growing on Sorbitol MacConkey agar, while other strains form rose to pink colonies (March & Ratnam, 1986).

Methods Employed in Studying the Molecular Phylogeny of *E. coli* Isolates

There are various ways to determine whether bacterial strains are genetically related. Most methods use sequence variations in chromosomal genes to determine if strains share a common ancestor. Species are usually determined by sequencing of the variable regions of the 16S rRNA encoding genes but intra-species variation requires analysis of several genes or polymorphic DNA variations (Nataro & Kaper, 1998). Common methods include Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP), Repetitive Sequence Pair based PCR (REP-PCR), Pulsed Field Gel Electrophoresis (PFGE) (Romling, Grothues, Heuer & Tummler, 1992), Enterobacterial Repetitive Intergenic Consensus Sequence-Based PCR (ERIC-PCR), Multilocus Enzyme Electrophoresis (MLEE) (Selander, Caugant & Whittam, 1987), analysis by

either Southern blot or polymerase chain reaction (PCR) (Persing, Smith, Tenover & White, 1993) and Multilocus Variation Analyses (MLVA) or Multilocus Sequence Typing (MLST) (Akopyanz, Bukanov, Westblom, & Berg, 1992a; Akopyanz, Bukanov, Westblom, Kresovich & Berg, 1992b; Kawamata *et al.,* 1996; Osorio, Toranzo, Romalde & Barja, 2000; Thoreson *et al.,* 2000). A polymerase chain reaction (PCR) based method has been developed by Clermont *et al*., (2000) to characterize the phylogroups using genetic markers: chuA - a gene required for heam transport in enterohaemorrhagic O157:H7 *E. coli* (Bonacorsi *et al*., 2000); yjaA - a gene initially identified in recent complete genome sequence of *E. coli* K-12 (Blattner *et al.*, 1997); and TspE4.C2 - an anonymous DNA fragment (Bonacorsi *et al*., 2000).

Methods Employed in Antibiotic Susceptibility Testing

There are three commonly used methods for antimicrobial susceptibility testing for *in vitro* diagnosis in the laboratory; they are the diffusion susceptibility tests, dilution susceptibility tests and the diffusion and dilution susceptibility tests (Lalitha, 2004). Many factors can influence the results of antimicrobial susceptibility testing such as pH, oxygen, moisture and content of thymidine and thymine of the medium, inoculum preparation, concentration of antimicrobial drug tested in the carrier and the interpretative criteria (Lalitha, 2004). Therefore, the accuracy and reproducibility of these tests are dependent on maintaining a standard set of procedures (Clinical Laboratory Standard Institute (CLSI), 2010).

Disc diffusion methods

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria (CSLI, 2010). To standardize the inoculum density for a susceptibility test, a barium sulphate ($BaSO_4$) turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), is often used (Lalitha, 2004). The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the CLSI (CLSI, 2010). It is considered as a qualitative or semi-quantitative method. Though the test applies only to fast-growing aerobic bacteria under standard conditions, it has many advantages. The procedure, for instance, is simpler and the obtained results are comparable to the dilution technique (Lalitha, 2004). Thus, this method has been widely applied for antimicrobial susceptibility testing in clinical laboratories (Wheat, 2001). In the Kirby-Bauer method, filter paper disks containing known concentrations of an individual antimicrobial are placed on the surface of appropriate agar medium that has been inoculated with a suspension of an isolate. The culture is then incubated for 16 - 18 h at 35 ^{o}C. The antimicrobial agent diffuses into the medium creating a halogen zone of inhibition around the antimicrobial disk corresponding to the susceptibility of the isolate to the agent. The diameters of the halogen zones are measured and interpreted for susceptibility testing results into three categories: resistant, intermediate and susceptible on the basis of an appropriately interpretative criterion (CLSI, 2010). The test is designed to distinguish between sensitive and resistant members of a bacterial population, hence, bacterial isolates that fall into the

intermediate category are retested and, subsequently, categorized as sensitive or intermediate (CLSI, 2010). This test is useful because antimicrobial concentrations in paper discs are always lower than an estimation of the antibacterial effects of a drug (Lalitha, 2004).

Dilution susceptibility tests

Dilution susceptibility testing methods yield quantitative data in susceptibility of an organism to a drug (Lalitha, 2004). These methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. Antimicrobials are prepared in progressive two-fold serial dilutions in broth or agar media to give a range of concentrations (Lalitha, 2004). These methods generate the minimum inhibitory concentration (MIC), the lowest concentration of antimicrobial that can inhibit the visible growth of a microorganism after incubation and the minimum bactericidal concentration (MBC), the lowest concentration of antimicrobial that can prevent the growth of a microorganism after subculture onto antimicrobial free media (CLSI, 2010). These methods offer the potential to gain information about drug concentrations in the body tissues. Thus, dosages can be established on the basis of a pharmacokinetic description of disposition in the body (Lalitha, 2004). They are most often used to determine activity *in vitro* of new drugs and to determine MIC breakpoints (CLSI, 2010). Though very informative, this technique is expensive and labour intensive (Serrano, 2005).

Diffusion and dilution susceptibility tests

The epsilometer (E) - test is a quantitative method which is performed using a procedure with a combination of both dilution and diffusion tests

(Lalitha, 2004). This is a simple, accurate and reliable method for determining the MIC for a wide spectrum of infectious agents. E-test MICs are more precise than conventional MICs based on discontinuous two-fold serial dilutions because their antimicrobial concentration gradient is predefined (Brown & Brown, 1991).

The E-test is an 'exponential gradient' testing methodology where 'E' in E-test refers to the Greek symbol epsilon (ε). A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E-test strip is applied onto an inoculated agar plate, there is an immediate release of the drug (Brown & Brown, 1991). Following incubation, a symmetrical inhibition ellipse is produced (Lalitha, 2004). The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy (Lalitha, 2004).

ESBL Detection Methods

The detection of ESBL-production is of importance for infection control and epidemiological surveillance (Garrec, Drieux-Rouzet, Jarlier, Golmard & Robert, 2011). Aside molecular detection methods, several phenotypic methods have been developed to detect or confirm ESBL production by Enterobacteriaceae (Jarlier, Nicolas, Fournier & Philippon, 1988; Thomson & Sanders, 1992; Cormican, Marshall & Jones, 1996; M'Zali, Chanawong, Kerr, Birkenhead & Hawkey, 2000; Wiegand, Geiss, Mack, Sturenburg & Seifert, 2007; Donaldson *et al.*, 2008). Eleven phenotypic methods including two methods used for routine susceptibility testing (disc diffusion and Vitek 2 automated methods) and nine methods designed for the detection of ESBL

production are described: ESBL E-tests, combination discs, double-disc synergy (DDS), disc replacement, agar supplemented with clavulanate methods using Mueller-Hinton agar and BD Phoenix automated microbiology system, micro-scan panels as well as the three-dimensional test.

Molecular detection methods for ESBL production

A number of molecular methods are used in the molecular detection of ESBL producers. Some of these include the Polymerase Chain Reaction (PCR) with oligonucleotide primers method, the DNA probes method, the Oligotyping method, the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) method, the PCR- Single Strand Conformational polymorphism (PCR-SSCP) method, the Ligase Chain reaction (LCR) test and the Nucleotide sequencing method, which is regarded as the gold standard (Bradford, 2001). Though very useful, molecular methods, in general, are labour intensive and technically more challenging than the phenotypic detection methods (Bradford, 2001).

The disc diffusion method (DDM) for detection of ESBL production

The disc diffusion method (DDM) for detection of ESBL producers is a two-step method with the first step being a screening test for reduced susceptibility to more than one of the indicator cephalosporin (Cefpodoxime, 10 µg; Aztreonam, 30 µg; Ceftazidime, 30 µg; Cefotaxime, 30 µg; and Ceftriaxone, 30 µg). Reduced susceptibility indicates a positive result. A subsequent confirmation of ESBL production is then given by the demonstration of synergy between ceftazidime or cefotaxime and clavulanate (Drieux, Brossier, Sougakoff & Jarlier, 2008). In both steps, the Kirby-Bauer disc diffusion method is employed, as already described, for the antibiotic

susceptibility testing. This method can be used to detect the production of ESBL by *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis*. Following the incubation period, the magnitudes of the zone of inhibition around each antibiotic disc are compared with those provided by the CLSI. Measurements higher than a specific value may indicate ESBL production and this indicates that a phenotypic confirmatory test is required (CLSI, 2010). The phenotypic confirmatory testing requires that after incubation during the second step, a difference of more than or equal to 5 mm between either of the cephalosporin discs with their respective cephalosporin/clavulanate disc alone is taken to be a phenotypic confirmation of ESBL production (CLSI, 2010).

The dilution method for detection of ESBL production

Similar to the disc diffusion method, the dilution method is also in two parts: (1) the screening for potential ESBL producers followed by (2) a phenotypic confirmatory test. Screening can be done through antibiotic susceptibility testing for ESBL production by *E. coli* and *Klebsiella* spp. using Ceftazidime, Aztreonam, Cefotaxime or Ceftriaxone at a screening concentration of 1 µg ml^{-1} or Cefpodoxime at a concentration of 4 µg ml^{-1} (CLSI, 2010). The observation of growth at or above the screening concentrations may indicate ESBL production and suggest that a phenotypic confirmatory test is required (Paterson & Bomono, 2005). This phenotypic confirmatory test is performed through broth micro-dilutions assays using Ceftazidime (0.25 to 128 µg ml^{-1}), Ceftazidime plus clavulanic acid (0.25/4 to 128/4 µg ml^{-1}), Cefotaxime (0.25 to 64 µg ml^{-1}) and Cefotaxime plus clavulanic acid (0.25/4 to 64/4 µg ml^{-1}). The micro-dilution method is performed using standard methods and a phenotypic confirmation is obtained

when there is a decrease of greater than 3 two-fold in the MIC of either cephalosporin in the presence of clavulanic acid as compared to its MIC when tested alone (CLSI, 2010).

The ESBL E-tests method

ESBL E-tests have been developed in order to quantify the synergy between extended-spectrum cephalosporins and clavulanate (Drieux *et al*., 2008). In this method, E-test strips which are drug impregnated plastic strips are used with one end of each strip containing a gradient of ceftazidime (MIC test range from 0.5 to 32 µg ml^{-1} and the other with a gradient of ceftazidime plus a constant concentration of clavulanate (4 µg ml^{-1}). Similar strips for cefotaxime and cefotaxime/clavulanate are available. These strips are useful for both the screening and phenotypic confirmation of ESBL production (Drieux *et al*., 2008).

The combination disc method for detection of ESBL production

Similarly to the disk diffusion method, this method compares the sizes of the zones of inhibition of cephalosporin discs to those of the same cephalosporin plus clavulanate (M'Zali *et al*., 2000). The principle of this method is to measure the inhibition zone around a disc of cephalosporin and around a disc of the same cephalosporin plus clavulanate (Drieux *et al*., 2008). Depending on the disc type, a difference of more than or equal to 5 mm between the two diameters (i.e., corresponding to a two-fold dilution), or a zone expansion of 50 % are considered as indicating ESBL production (Carter, Oakton, Warner & Livermore, 2000; M'Zali *et al*., 2000). Besides

being easy to perform and straight forward, this method is highly sensitive and specific for all species (Drieux *et al*, 2008).

The double-disc synergy test (DDST) for detection of ESBL production

The first test specifically designed to detect ESBL production in Enterobacteriaceae was the double disc synergy test (DDST) (Jarlier *et al*., 1988). It was initially designed to differentiate between cefotaxime resistant strains, that is, those overproducing cephalosporinase and those producing ESBLs (Drieux *et al*., 2008). In this method, an agar plate is inoculated as for a routine susceptibility test. Disc containing cefotaxime (30 µg) and/or ceftazidime (30 µg) and/or cefpodoxime (10 µg) are applied 25 to 30 mm away on either side of a disc of amoxicillin-clavulanate (co-amoxiclav, 20/10 µg) (Paterson & Bonomo, 2005). ESBL production is inferred when a synergy is observed between cephalosporin and the clavulanate. Thus, the test is considered as positive when a decreased susceptibility to cefotaxime is combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as 'champagne-cork' or 'keyhole' (Drieux *et al*., 2008) (Figure 2). The major advantages of the double-disc diffusion test is that it is technically simple (Paterson & Bonomo, 2005) and cheap (Standard Unit, Evaluations and Standards Laboratory (SUESL), 2008). Additionally, the sensitivity and specificity of this method are 94.1% and 81.4%, respectively, for all species (Drieux *et al*., 2008). However, the interpretation of test result is quite subjective and optimal disc separation varies with the strain (SUESL, 2008).

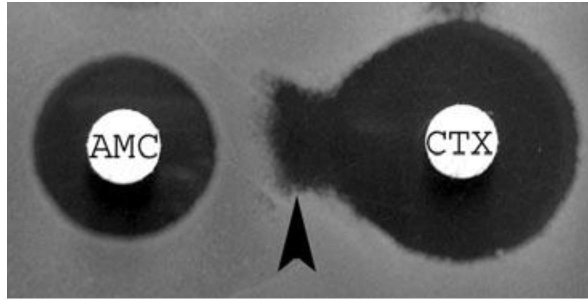


Figure 2: A positive double-disc synergy test result (Jarlier *et al.*, 1988). Cefotaxime (CTX) and amoxicillin-clavulanate (AMC) discs were placed at a distance of 30 mm from one another with the arrow indicating the characteristic shape-zone referred to as 'champagne-cork' or 'keyhole'

The disc replacement method for detection of ESBL production

In this method, three amoxicillin clavulanate discs are applied to a Mueller-Hinton agar plate inoculated with the test organism. After one hour at room temperature, these antibiotics are removed and replaced on the same spot by discs containing cefotaxime, ceftazidime and aztreonam. Control disc of these three antibiotics are simultaneously placed at least 30 mm from these locations. A positive test is indicated by a zone increase of 5 mm for the disc which have replaced the amoxicillin/ clavulanate discs compared to the control discs (Paterson & Bonomo, 2005).

The agar supplemented with clavulanate method for detection of ESBL production

This method involves the use of Mueller-Hinton agar supplemented with 4 µg/ml of clavulanate. Antibiotic discs containing ceftazidime (30 µg), cefotaxime (30 µg) and aztreonam are placed on the clavulanate-containing agar and clavulanate-free Mueller-Hinton agar plates. A difference in the

magnitude of the zone of inhibition greater than 10 mm on the two media is considered positive for ESBL production (Ho, Chow, Yuen, Ng & Chau, 1998). A major drawback of the method is the need to freshly prepare the clavulanate-containing plate since the potency of clavulanic acid begins to decrease after 72 hours (Paterson & Bonomo, 2005).

The VITEK 2 ESBL automated method for detection of ESBL production

The VITEK 2 ESBL test (Biomérieux, Marcy l'Etoile, France) is based on the simultaneous assessment of the antibacterial activity of cefepime, cefotaxime and ceftazidime, measured either alone or in the presence of clavulanate. This test relies on card wells containing 1.0 mg/L of cefepime, or 0.5 mg/L of cefotaxime or ceftazidime, either alone or associated with 10 or 4 mg/L of clavulanate, respectively (Drieux *et al*., 2008). After inoculation, cards are introduced into the VITEK 2 machine, and for each antibiotic tested, turbidity is measured at regular intervals. The proportional reduction of growth in wells containing a cephalosporin combined with clavulanate is then compared with that achieved by the cephalosporin alone and is interpreted as ESBL-positive or negative through a computerised expert system (Drieux *et al*., 2008). Though this method is highly sensitive and specific for all species (Drieux *et al.,* 2008), many laboratories that use the conventional VITEK 2 cards risk the incorrect report of ESBL producing organisms as susceptible when the minimum inhibitory concentration is 8 µg/ml (Queenan, Folena, Gownley, Wira & Bush, 2004).

The BD Phoenix automated microbiology system for detection of ESBL production

This is a short incubation system for bacterial identification and susceptibility testing (Leverstein-van Hall *et al*., 2002; Sanguinetti *et al*., 2003; Stürenburg, Sobottka, Noor, Laufs & Mack, 2004). This test uses growth response to cefpodoxime, ceftazidime, ceftriaxone and cefotaxime with or without clavulanic acid to detect the production of ESBLs. Results are usually available within 6 hours. Though this method has a high sensitivity, it has the disadvantage of low specificity (Drieux *et al*., 2008).

The micro-scan panel automated method for detection of ESBL production

This is an automated method involving the use of dehydrated panels for micro-dilution susceptibility testing. Studies with large numbers of ESBL-producing isolates show that micro-scan panels which, contained combinations of ceftazidime or cefotaxime plus β-lactamase inhibitors, are highly reliable (Thomson, Nasser, McGowan, Sebaihia & Salmond, 1999; Komatsu *et al*., 2003; Stürenburg *et al*., 2004). The disadvantage, however, of this method is that panel users reliably report ESBL producers as ceftazidime-resistant but are less likely to report them as cefotaxime-resistant or ceftriaxone-resistant (Stürenburg & Mack, 2003).

The three-dimensional test for detection of ESBL production

Two types of three-dimensional tests, direct or indirect, have been proposed (Thomson & Sanders, 1992). The direct three-dimensional test is a modification of the disc-diffusion test that generates data on both

antimicrobial susceptibility of the tested strain and substrate profile of the β-lactamase produced by this strain (Drieux *et al*., 2008). In this test, the studied organism is inoculated onto the surface of an agar plate. The antibiotic disks are placed on the agar plate 3 mm outside the strain-containing slit. Enzymatic inactivation of each antibiotic is detected by inspection of the margin of the inhibition zone in the vicinity of its intersection with the strain-containing slit. Inactivation of the antibiotic, as it diffuses through the slit, results in a distortion or discontinuity in the expected circular inhibition zone, or the production of discrete colonies in the vicinity of the inoculated slit (Drieux *et al.*, 2008).

The indirect three-dimensional test is a modification of the direct three-dimensional test, in which the surface of the agar plate is inoculated with a fully susceptible indicator strain (ATCC 25922). With the exception of this modification, the method is the same as that described for the direct three-dimensional test. This test is used for detection of β-lactams that do not give an inhibition zone by the direct test and for which information on inactivation is therefore not provided.

A major disadvantage of the three-dimensional test is that the performance of the indirect test makes the method cumbersome even though it is more sensitive than the double disc diffusion test (Vercauteren, Descheemaeker, Ieven, Sanders & Goossens, 1997). Additionally, the three-dimensional test becomes less sensitive without the performance of the indirect test (Bedenic, Randegger, Boras & Hachler, 2001).

Methods Employed in the Detection of *E. coli* Pathotypes

Pathotypes of *E. coli* represent a phenotypically diverse group of pathogens and no single method or approach can be used to detect or isolate all pathotypes of concern (Co-ordination Action Food (CAF), 2007). Consequently, methods have been developed to specifically detect or isolate certain pathotypes. These methods include biochemical tests, serotyping and examination for key virulence-associated genes (CAF, 2007). For instance using PCR, specific virulence genes can be detected and this may enable the categorization of bacterial isolates into various pathogenic groups or pathotypes (Table 3). Typing of pathogenic *E. coli* may involve the use of a variety of typing techniques including pulsed field gel electrophoresis, multiple-locus variable-number tandem repeat analysis, amplified fragment length polymorphism and ribotyping, but these are often applied to specific pathotypes during an epidemiological investigation (CAF, 2007). DNA probes may also be developed based on the sequences of genes specific to a particular pathotype (CAF, 2007).

Table 3: *E. coli* Pathotypes and Distribution of Various Virulence Factors

Virulence factors	ETEC	EPEC	STEC/ EHEC	EIEC	EAEC	UPEC	NMEC
Toxins							
Shiga toxin 1 (Stx1)			++				
Shiga toxin 2 (Stx2)			++				
Heat-labile toxin I (LTI)	++						
Heat-labile toxin II (LTII)	+[a]						
Heat-stable toxin I (STa)	++						
Heat-stable toxin II (STb)	+[a]						
Low-MW heat-stable toxin (EAST1)	+	+	++				
α-Hemolysin (Hly)			++[b]			++	
EHEC hemolysin (Ehx)			+[c]				
Cytotoxic necrotizing factor 1 (CNF1)						+	
Adhesins							
P fimbriae						++	
S fimbriae						++	+
F1C fimbriae						++	
Colonization factor antigen I (CFA/I)	+						
Colonization factor antigen II (CFA/II; CS3)	++						
Bundle-forming pilus (Bfp)		++					
Aggregative adherence fimbriae I (AAF/I)					++		
Intimin (Eae)		++	++				
Invasins							
Invasion plasmid antigen (Ipa)				++			
Iron acquisition							
Aerobactin				+		++	+
Capsule							
K1 capsule antigen						+	++
K5 capsule antigen						+	

Source: Kuhnert, Boerlin & Frey, 2000
+ occasionally detected; ++ normally detected; [a] Primarily in animal isolates; [b] In porcine isolates; [c] Predominantly strains of the EHEC sub-pathotype

CHAPTER THREE

MATERIALS AND METHODS

Study Area

The study was carried out in the Cape Coast Metropolis of the Central Region of Ghana. Cape Coast with its 5° 6′ 0″ N, 1° 15′ 0″ W geographical coordinates, is situated 165 km west of Accra on the Gulf of Guinea (Wikipedia, 2013). The city is located 30 km south of the Kakum National Park, one of the most diverse and best-preserved national parks in West Africa. The population of Cape Coast was reported in 2010 to be 169, 894 comprising 82, 810 males and 87, 084 females (Ghana Statistical Service, 2012). The metropolis covers an area of 122 km^2 (Cape Coast Metropolitan Assembly, 2006a). It is a humid area with mean monthly relative humidity varying between 85 and 99 % with the sea breeze having a moderating effect on the local climate (Cape Coast Metropolitan Assembly, 2006a). The hottest months are February and March, while the coolest period is between June and August (Cape Coast Metropolitan Assembly, 2006a). The annual rainfall ranges from 750 mm to 1000 mm and the landscape of Cape Coast is dominated by batholiths interspersed with valleys (Cape Coast Metropolitan Assembly, 2006b). Located in the valleys are several streams, the largest of which is the Kakum river while many of the streams end in wetlands and the Fosu Lagoon at Bakaano (Cape Coast Metropolitan Assembly, 2006b). In the northern parts of the Cape Coast metropolis, however, the landscape is generally low lying and is suitable for the cultivation of various crops (Cape

Coast Metropolitan Assembly, 2006b). Notable areas of development include the sectors of education, trade and tourism. The Cape Coast Castle, and other forts and castles in Ghana, are included on the United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage list. Aside the many historic and tourist sites as well as the topmost Senior High Schools in Ghana that the city has been blessed with, there is the Central Regional Hospital, which is an important referral hospital providing health care services to most residents of the region.

The sites of collection of various samples for the study were the Kakum River, near Kakumdo, the Saint Lawrence Roman Catholic Basic School at Abura, the Abura Market, the Central Regional Hospital, the Science Market of the University of Cape Coast (UCC), the Fosu Lagoon, and the Kotokuraba and Anaafo markets (Figure 3).

Study Population

The study population comprised children, adolescents and adults irrespective of their gender, selected from patients referred to the Microbiology Section of the Central Regional Hospital, Cape Coast from whom clinical samples were obtained.

Environmental Test Samples

Water samples were obtained from the sea, the Fosu lagoon and gutters/drainages around the Anaafo, Kotokuraba and Abura markets.

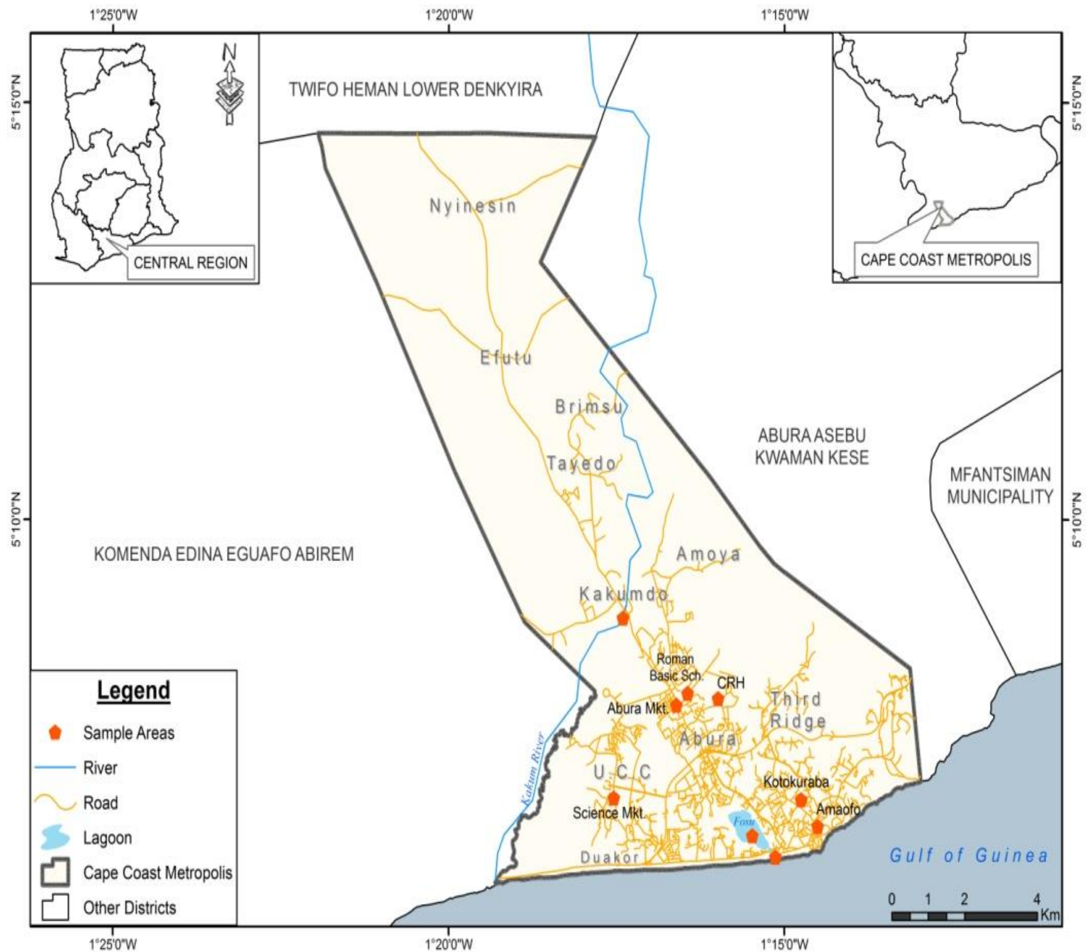


Figure 3: Geographical location of sites where samples were collected for the study

Smoked fish and fresh beef were obtained from the Kotokuraba and Abura markets.

Chicken samples were also obtained from the Kotokuraba and Abura markets.

Cabbage heads were obtained from the University of Cape Coast School of Agriculture farm, as well as the Abura and Kotokuraba markets.

Clinical Test Samples

The clinical test samples were obtained from five different types of samples including stools, urine, wounds, abscess or urogenital specimens

(high vaginal swabs) of patients reporting at the Microbiology Laboratory of the Central Regional Hospital, Cape Coast.

Escherichia coli Isolates

All *Escherichia coli* isolates screened in this study were obtained from clinical and environmental test samples.

Reference Bacterial Isolates

Reference bacterial isolates including *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia*e ATCC 700603 were obtained from the Microbiology Department of the University of Ghana Medical School.

Culture Media

Culture media including MacConkey Agar (Lab M Ltd. Topley House, UK), Müeller Hinton Agar (Lab M Ltd. Topley House, UK), Nutrient Broth (Merck, Germany) and Triple Sugar Iron Agar (Liofilchem, s.r.l. Bacteriology Products, Italy) were supplied by Cascada Enterprise Ltd. Accra, Ghana.

Sterile Collection Swabs

Sterile collection swabs (Deltalab, Rubi, Spain) were obtained from Cascada Enterprise Ltd. Accra, Ghana.

Antibiotics Discs

Antibiotics discs (Abtek, Biologicals Ltd., UK) were obtained from Cascada Enterprise Ltd. Accra, Ghana.

Analytical Profile Index (API) 20 E kits

Analytical Profile Index (API) 20 E kits (Biomérieux, France) including the API strips, Voges-Proskauer (VP) 1, VP 2, Tryptophan

deaminase (TDA) and Kovac's reagents used in the identification of *E. coli* isolates were obtained from Excite Medical Ventures, Accra, Ghana.

Extended Spectrum Beta Lactamase Detection Disc Sets

Extended Spectrum Beta Lactamase (ESBL) Detection Disc Sets (Mast Group Ltd., Merseyside, UK) were supplied by Labmeb Company Ltd., Accra, Ghana.

Wizard Genomic DNA Purification Kits

Wizard Genomic DNA Purification Kits (Promega Corporation, USA) were supplied by Anatech Instruments (Pty) Ltd., South Africa.

Oligonucleotide Primers

All oligonucleotide primers used in the screening for the presence of *Escherichia. coli* virulence genes were synthesised by Sigma Aldrich Logistik GmbH, Germany and supplied by Huge Ltd. Accra, Ghana. Additionally, all oligonucleotide primers used in the molecular phylogeny PCR assays as well as those that were used in the detection of ESBL genes, were synthesised and supplied by Bioneer Corporation, South Korea.

Molecular Biology Grade Water

Molecular biology grade water (Sigma Aldrich Logistik GmbH, Germany) was obtained from Huge Ltd. Accra, Ghana.

Luria Bertani Broth

Luria Bertani Broth (Sigma Aldrich Logistik GmbH, Germany) was obtained from Huge Ltd. Accra, Ghana.

DNA Molecular Markers

DNA molecular markers (Sigma Aldrich Logistik GmbH, Germany) were supplied by Huge Ltd. Accra, Ghana.

Accupower PCR Premixes and Accupower Multiplex PCR Premixes

Accupower PCR premixes and Accupower multiplex PCR premixes (Bioneer Corporation, South Korea) were supplied by Bioneer Corporation, South Korea.

Other Chemicals and Reagents

All other chemicals and reagents used in this study were of the AnalaR or general purpose reagent grade and were manufactured by the British Drug House (BDH) Ltd. (Poole, England). They were obtained either from the stores of the Department of Molecular Biology and Biotechnology, School of Biological Sciences or the Department of Chemistry, School of Physical Sciences, University of Cape Coast.

Ethical Considerations

The protocol of the entire study was approved by the Ghana Health Service Ethical Review Committee on Research Involving Human Subjects (GHS - ERCRIHS) prior to the commencement of collection of clinical samples (APPENDIX I). The clinical test samples were collected only after informed consent was sought and obtained from each and every patient enrolled in the study. Informed consent forms (APPENDIX II) were signed or thumb-printed by every patient or parent/ guardian.

Collection of Demographic Data

The age and gender of the patients from whom clinical samples were obtained, were recorded as samples were taken.

Estimation of Sample Size

In 2000, the total population of the Cape Coast metropolis was 118,106 (Ghana Statistical Service, 2002). Since the 2010 population and housing census came out late, the population growth was projected at 2.1 % annually (Cape Coast Metropolitan Assembly, 2006b). Given the growth rate of 2.1%, the population of the metropolis in 2010 was estimated at 145,705 and the minimum sample size for clinical samples was determined as 126 using Fisher's exact test.

Using the Fisher's exact test (Fisher, Laing, Stoeckel & Townsend, 1998) and given that the population of Cape Coast was greater than 10,000, the formula used in the determination of the sample size was:

$n = \frac{z^2pq}{d^2}$, where:

n = Required sample size

z = Standard normal deviation (standard value of 1.96 at 95% confidence level

p = Incidence of the particular characteristic under study in the target population

q = 1 – p

d = Degree of accuracy desired, usually set at 0. 05

Since there was scanty information on the prevalence of infections caused by pathogenic *E. coli*, data obtained from the Cape Coast Metropolitan Ghana Health Service was used in the calculation of the sample size for

clinical specimens. A 9% prevalence of *E. coli*-related infections (Ghana health Service, 2008) was used as the incidence of the particular characteristic under study in the target population; which, in this case was *E. coli* infections. Therefore, p = 0.09 and q = 1 – 0.09 = 0.91.

The degree of accuracy was set at 0.05. Hence,

$$n = \frac{(1.96)^2 \text{x } 0.09 \text{ x } 0.91}{(0.05)^2} = 126.$$

Methods of Sterilization

All glassware and forceps were washed with liquid soap and rinsed in several changes of tap water. They were then dried and later wrapped in sheets of greaseproof paper. These were then autoclaved at 121 °C for 15 minutes and subsequently dried in a hot air oven at 70 °C for an hour to evaporate water condensing on the various items or between the Petri dishes.

Inoculation loops and needles were heated in the spirit lamp flame until they turned red-hot and air-cooled before and after use.

All culture media and pipette tips used were sterilized by autoclaving at 121 °C for 15 minutes.

The entire surface of the working bench of the laminar flow cabinet was heavily sprayed with methylated spirit and mopped with clean tissue paper soaked with methylated spirit before and after use.

Preparation of Culture Media

MacConkey agar (without salt)

Dehydrated MacConkey Agar powder [(without salt), Lab M Ltd. Topley House, UK]: mixed peptones, 20.0 g; lactose, 10.0 g; bile, 5.0 g; neutral red, 0.05 g and agar (No.2), 13.5 g.

48.5 g of dehydrated MacConkey Agar powder (without salt) were dispersed in 1000 ml of deionised water and allowed to soak for 10 minutes with intermittent swirling to mix and get all the ingredients dissolved into solution. The mixture was then distributed into five 250-ml conical flasks and corked with a cotton wool plug. Each conical flask containing 200 ml of the mixture, was sterilized by autoclaving at 121 °C for 15 minutes after which it was allowed to cool and stored at room temperature for subsequent use.

Müeller-Hinton agar

Dehydrated Müeller-Hinton Agar powder (Lab M Ltd. Topley House, UK): beef infusion solids, 2.0 g; acid-hydrolysed casein, 17. 5 g; starch, 1.5 g and agar (No.1), 17.0 g.

38.0 g of the dehydrated Müeller-Hinton Agar powder were added to 1000 ml of deionised water and allowed to soak for 10 minutes with intermittent swirling to mix and get all the ingredients dissolved into solution. The mixture was then equally distributed into five 250 ml-conical flasks which were corked with cotton wool plugs, sterilized by autoclaving at 121 °C for 15 minutes, allowed to cool and stored at room temperature for subsequent use.

Nutrient broth

Dehydrated Nutrient Broth powder (Merck, Germany): peptone from meat, 5.0 g and meat extract, 3.0 g (pH 7.3 ± 0.2 at 25 °C).

Eight grams of Dehydrated Nutrient Broth powder were suspended in 1000 ml of demineralised water and dispensed as 1.0 ml aliquots into clean screw-cap tubes which were sterilized by autoclaving at 121°C for 15 minutes.

After autoclaving the medium was allowed to cool and then stored at room temperature for subsequent use.

Cystine lactose electrolyte deficient agar

Thirty-six grams of dehydrated Cystine Lactose Electrolyte Deficient (CLED) Agar (Laboratorios Conda S.A., Spain) [lactose, 10.0 g; casein peptone, 4.0 g; gelatin peptone, 4.0 g; beef extract, 3.0 g; L-cystine, 0.128 g; bromothymol blue, 0.02 g and bacteriological agar, 15.0 g (pH 7.3 ± 0.2 at 25 °C)] were suspended in one litre of distilled water and heated by boiling for one minute with frequent agitation until complete dissolution of all constituents. The mixture was then equally distributed into five 250 ml - conical flasks which were corked with cotton wool plugs, sterilized by autoclaving at 121 °C for 15 minutes, allowed to cool and stored at room temperature for subsequent use.

Xylose lysine deoxycholate agar

Dehydrated Xylose Lysine Deoxycholate (XLD) agar (Acumedia, Neogen Corporation, USA), 56 g [yeast extract, 3 g; lactose, 7.5 g; sucrose, 7.5 g; xylose, 3.5 g; L-Lysine, 5 g; ferric ammonium citrate, 0.8 g; phenol red, 0.08 g; sodium chloride, 5 g; sodium deoxycholate, 2.5 g; sodium thiosulphate, 6.8 g and agar, 13.5 g (pH 7.4 ± 0.2 at 25 °C)] were suspended in one litre of distilled water. The mixture was heated with frequent agitation until it reached its boiling point. No autoclaving was done. The medium was allowed to cool to 50 – 55 °C and poured into sterile Petri dishes and allowed to solidify.

Tryptone soya (or Tryptic soy) diphasic medium

Dehydrated Tryptone Soya Agar (Casein Soya Bean Digest Agar) (Acumedia Neogen Corporation, USA), 40 g (pancreatic digest of casein15.0 g, enzymatic digest of soya bean 5.0 g, sodium chloride 5.0 g and agar, 15.0 g (pH 7.3 ± 0.2 at 25°C)) were added to one litre of distilled water and the mixture was brought to the boil in order to get all components completely dissolved. Then 25 ml of the mixture were dispensed into 200 ml – medicinal flat bottles fitted with screw caps that have a central hole sealed with a rubber liner. Sterilization was done by autoclaving at 121°C for 15 minutes. After autoclaving the medium was allowed to cool with the bottles in a horizontal position thereby resulting in the preparation of an agar slope along one side of the bottles.

Dehydrated Tryptone Soya Broth also known as Tryptic Soy Broth (Biolife Italiana Srl, Italy), 30g (pancreatic digest of casein, 17.0 g; soy peptone, 3.0 g; sodium chloride, 5.0 g; dipotasium hydrogen phosphate, 2.5 g; glucose, 2.5 g (pH 7.3 ± 0.2)) were suspended in one litre of distilled water and heated to boiling. Then 0.05 g of sodium polyanethyl suphonate in addition to 0.05 g of *p*- aminobenzoic acid was added before the broth was sterilized by autoclaving at 121 °C for 15 minutes. After allowing to cool to about 50 °C, 25 ml of the sterile broth were aseptically transferred into each bottle containing an agar slope for subsequent use.

Triple sugar/ Kliger's iron agar

Triple Sugar Iron (TSI) Agar (Liofilchem s.r.l. Bacteriology Products, Italy), 64.5 g (peptospecial, 20.0 g; lactose, 10.0 g; sucrose, 10.0 g; sodium chloride, 5.0 g; beef Extract, 3.0 g; Glucose, 1.0 g; ferrous Sulphate, 0.2 g;

sodium thiosulphate, 0.3 g; phenol red, 0.025 g and agar, 12.0 g (pH 7.3 ± 0.2 at 25 °C)) were transferred in to 1000 ml of distilled water and heated until complete dissolution. 4 ml aliquots of the homogeneous mixture obtained were then transferred into clean 15 ml-screw-cap tubes which were sterilized by autoclaving at 121° C for 15 minutes. After autoclaving, the test tubes containing the sterile TSI Agar medium were placed in a slanted position and allowed to cool until the medium solidified to give a butt 25 – 30 mm deep and a slope 20 – 25 mm long. The TSI Agar slants thus prepared were stored at room temperature until the performance of the biochemical tests.

Simmon's citrate agar

Sodium Chloride, 5.0 g; Magnesium Sulphate Hydrated, 0.2 g; Ammonium Dihydrogen Phosphate, 1.0 g; Tri-Sodium Citrate Dihydrate, 3.0 g; Agar, 20.0 g; 1 % Bromthymol Blue (indicator solution), 8 ml and 990 ml of distilled water.

All ingredients except the indicator solution were mixed thoroughly with and heated to dissolve all constituents. The pH of the mixture was then adjusted to 7.0 and the bromthymol blue was added. The medium was mixed and dispensed in screw-capped tubes in aliquots of 3 ml and autoclaved at 121 °C for 15 minutes. After autoclaving, the medium was allowed to cool and solidify in a sloped position and later stored in a cool dark place until they were needed.

Christensen's urea agar

The preparation of the Christensen's Urea Agar was made in two stages: the preparation of the basal medium and that of the urea concentrate.

Basal medium: Peptone, 0.1 g; Sodium Chloride, 0.5 g; Potassium Dihydrogen Phosphate, 0.2 g; D-glucose, 0.1 g; Agar, 2.0 g and 0.2 % Phenol red, 0.6 ml were dissolved in 90 ml of distilled water with the pH of the mixture adjusted to 6.8 ± 0.2. Sterilization was done by autoclaving 121° C for 15 minutes. After autoclaving the basal medium was allowed to cool to 50 – 55 °C.

20 % Urea concentrate: Urea, 2 g was dissolved in 10 ml of sterile distilled water and kept at room temperature.

After the basal medium had cooled to 50 – 55 °C, it was aseptically mixed thoroughly with the sterile 20 % urea concentrate. The medium obtained was subsequently dispense in 3 ml amounts in sterile screw-cap tubes, allowed to solidify in a sloped position and stored in a cool dark place until they were needed (Bacteriological Analytic Manual, 1998).

Luria Bertani broth

Luria Bertani (LB) broth (Sigma Aldrich Logistik GmbH, Germany), 20 g comprising tryptone (pancreatic digest of casein), 10.0 g; Yeast Extract, 5 g and Sodium Chloride, 5.0 g were suspended in 1000 ml of distilled water. The mixture was allowed to stand for a minute and swirled for complete dissolution. Then, 1.5 ml aliquots of the homogeneous mixture obtained were then transferred into clean screw-cap tubes which were sterilized by autoclaving at 121 °C for 15 minutes. After autoclaving, the medium was allowed to cool and stored at room temperature for subsequent use.

Peptone water (1%)

Dehydrated peptone water (Oxoid Ltd., England), 15 g (peptone, 10.0 g and sodium chloride, 5.0 g (pH = 7.2 ± 0.2 at 25 °C)) were added to 1000 ml

of distilled water. The mixture was thoroughly mixed and 2 ml aliquots dispensed into 15 ml-clean screw-cap tubes and sterilized by autoclaving at 121 °C for 15 minutes.

Normal saline

Normal saline also known as physiological saline was prepared by dissolving 8.5 g of sodium chloride into 1000 ml of distilled water and allowing the solution to stand for a few seconds. After swirling the solution gently, 1 ml and 9 ml aliquots were transferred into screw-cap tubes which were sterilized by autoclaving at 121 °C for 15 minutes (Harrigan & McCance, 1966). After autoclaving, the sterile normal saline obtained were allowed to cool and subsequently stored at room temperature before use as transport medium in serial dilutions and in the performance of other tests.

Glycerol for storage of bacterial cultures

Glycerol (British Drug House Ltd, Poole, England), 0.15 ml was dispensed in clean 1.5 ml-cryovials, which were capped, sterilized by autoclaving at 121° C for 15 minutes, allowed to cool and subsequently stored at room temperature for further use.

Sampling Method and Experimental Design

The purposive sampling method was adopted, as patients enrolled in this study were those with gastroenteritis and/or urinary tract infections referred to the Microbiology Section of the Central Regional Hospital. However, random sampling was used for the collection of environmental samples.

The entire research was systematically broken down into eight consecutive experiments including (1) the collection of test samples and bacterial isolation and identification; (2) the confirmation of isolates using API 20E commercial kits; (3) the determination of the sensitivity of *E. coli* isolates to selected antibiotics; (4) the phenotypic detection of ESBL-producing strains among *E. coli* isolates; (5) DNA isolation and determination of sample purity and concentration; (6) screening for ESBL genes (^{bla}SHV, and ^{bla}TEM) in clinical and environmental *E. coli* isolates; (7) determination of the molecular phylogeny of all *E. coli* isolates obtained; and (8) screening for virulence genes among *E. coli* isolates obtained from clinical and environmental samples.

The study was conducted between June 2010 and August 2012.

Collection of Test Samples and Bacterial Isolation

Clinical samples

Stool specimens

One tablespoonful of each specimen was collected into clean dry disinfectant-free wide-necked containers from patients. In instances where faeces could not be obtained, stools were collected using sterile cotton wool swabs inserted into the rectum of the patient by an experienced nurse. A thick suspension of each stool specimen was then made in 1 ml of sterile peptone water and a loopful of the fresh emulsified faeces was inoculated on sterile Xylose Lysine Deoxycholate (XLD) agar plates which were subsequently incubated aerobically at 35°C for 12-18 hours. Presumptive *Escherichia coli* colonies were then selected and processed for storage.

Urine specimens

A 10 ml-volume of mid-stream urine was collected into sterile universal bottles from patients prior to bacterial isolation. The urine was mixed by swirling before inoculating on Cysteine Lactose Electrolyte-Deficient (CLED) agar using a sterile inoculation loop. The inoculated CLED plates were then incubated at 35°C for 12–18 hours. Presumptive *E. coli* colonies were then selected and processed for storage until required.

Blood samples

The blood samples were collected by an experienced biomedical scientist at the hospital from patients. A 10 ml-volume of blood was then transferred into a diphasic culture medium containing 25 ml of sterile Tryptic Soy broth. The blood was immediately mixed thoroughly with the broth to prevent any coagulation. The inoculated diphasic medium was then incubated at 35°C for up to 7 days with daily examinations. Sub-culturing was done anytime growth occurred and all presumptive *E. coli* colonies were then selected and processed for storage.

Urogenital specimens

Urogenital specimens collected in this study were all obtained from female patients. The collection was done by an experienced nurse using sterile vaginal swabs which were later transferred into a transport medium (Amies medium) prior to inoculation on sterile MacConkey agar plates. Incubation was done at 35 °C for 12 – 18 hours. All presumptive *E. coli* colonies were then selected and processed for storage.

Wound and aspirate specimens

Specimens were collected using sterile cotton wool swabs or syringes by a medical officer or an experienced nurse. The specimens were sent to the laboratory, examined and inoculated on MacConkey agar plates which were incubated at 35 °C for 12 – 18 hours. All presumptive *E. coli* colonies were then selected and processed for storage.

Environmental samples

Samples were obtained from water (Plate 1), fresh cabbage (Plate 2), smoked fish and fresh beef.

Each water sample (100 ml) was collected in sterile wide-mouth bottles with dust-proof ground glass stoppers from each collection site. These were immediately placed in ice in an ice-chest and transported to the laboratory for processing and microbial analysis. Samples of cabbage heads, smoked fish, fresh beef and chicken were obtained from various sampling sites, transferred into sterile translucent zip-locked polyethene bags and placed in ice-chests and covered with ice before they were sent to the laboratory for further investigations.

The pour-plate method was adopted for all water samples using MacConkey agar as the medium of choice. For each water sample, 1 ml was used as inoculum after performing a series of decimal dilutions.

A mass of 2 g of outer leaves of cabbage (Plate 2) was transferred into 2 ml sterile normal saline, vigorously shaken to dislodge all bacteria into solution and 1ml of the suspension obtained for subsequent use as inoculum following series of decimal dilutions. For each sample of smoked fish or fresh beef or chicken, 5g were aseptically transferred into another sterile translucent

x $^{1}/_{30}$

x $^{1}/_{10}$

Plate 1: Sites of collection of environmental samples.

A: Polluted part of Fosu lagoon,

B: Open gutter around the Anaafo market

x $^{1}/_{2}$

Plate 2: Outer leaves of cabbage sampled

zip-locked polyethene bag and pulverized with 45 ml of buffered 1% peptone water. A volume of 1 ml of the suspension obtained was subsequently used as inoculum following series of decimal dilutions.

A loopful of the suspension of each sample was then streaked onto sterile MacConkey agar plates, incubated at 35°C for 12-18 hr and sub-cultured to obtain pure cultures. All presumptive *E. coli* colonies were then selected and processed for storage.

Preparation of Serial Decimal Dilutions

Serial decimal dilutions were made while processing each of the environmental samples using 1 ml of water samples or 1 g of fresh cabbage leaves or fresh beef or smoked fish in 9 ml of sterile normal saline. The diluted suspensions obtained were used as inocula for microbial analysis. Dilutions ranging from 1:10 to 1:10,000 were used for inoculation depending on the type of samples.

Storage of Bacterial Isolates

All presumptive *E. coli* colonies were selected and sub-cultured onto fresh sterile MacConkey agar plates. After overnight incubation at 35°C, single colonies were transferred into a test tubes containing 1.0 ml of sterile nutrient broth and incubated at 35°C for 18 hr. After incubation, 0.85 ml of the bacterial culture was aseptically transferred into cryovials containing 0.15 ml of sterile glycerol, thereby resulting in the preparation of 15% glycerol-nutrient broth storage bacterial cultures (Lee, 2006). The 15% glycerol-nutrient broth storage bacterial cultures were subsequently vortexed and stored at -20°C for subsequent analyses.

Identification of Bacterial Isolates

The identification of all stored presumptive (pink and yellow colonies on MacConkey and CLED agar, respectively) *E. coli* recovered from all test samples screened done using various biochemical tests and Gram staining following the preparation of heat-fixed smears.

Smear preparation

Smear preparation was done according to the methods described by Brown (2007). A heat-fixed smear of each bacterial isolate was prepared before the performance of the Gram staining. For every bacterial isolate, pre-heat-fixed smear of pure bacterial culture was prepared by dropping a loopful of sterile normal saline on a clean glass slide. The inoculation loop was then flamed until red-hot, allowed to cool and, subsequently, used to pick cells from a single discrete bacterial colony. The bacteria were then transferred into a drop of sterile normal saline and mixed gently with the same inoculation

loop in a concentric manner in order to obtain a homogenous smear. The smear obtained was fixed by briefly passing the glass slide over a spirit lamp flame.

Gram staining

Gram staining was done following the protocol provided by Brown (2007). The entire heat-fixed smear was covered with crystal violet stain (0.5 g crystal violet dissolved in 100 ml of distilled water) and allowed to stand for one minute. The stain was washed off with running tap water and the stained smear covered with Gram's iodine solution (1.0g iodine, and 2.0g potassium iodide dissolved in 300 ml of distilled water). One minute thereafter, the smear was rinsed briefly with running tap water. The glass slide bearing the stained smear was tilted lengthwise over the sink and a few drops of a 1:1 alcohol-acetone solution applied. The smear was immediately rinsed with tap water, flooded with a few drops of safranin solution (0.25 g safranin dissolved in 100 ml of distilled water) and allowed to stand for 30 to 60 seconds. The glass slide was washed under running tap water again, blotted dry and the stained smear examined under an oil-immersion lens (x100) of a microscope connected to a digital camera and a monitor (Olympus Co-operation, USA) upon the addition of a drop of immersion oil.

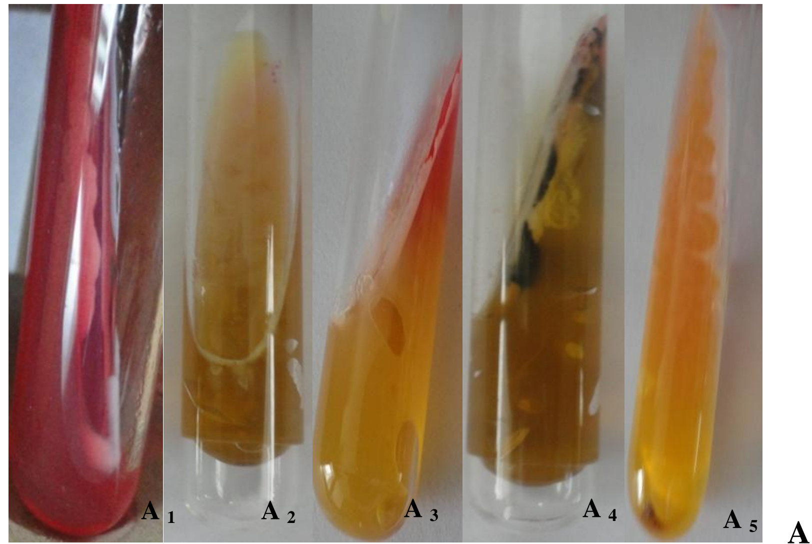
Biochemical tests

Biochemical tests were performed according to the methods described by Harrigan & McCane (1966). Three or four discrete colonies from a 24-hour-old pure culture of a presumptive *E. coli* isolate growing on MacConkey agar were transferred into 1 ml of sterile normal saline forming a suspension. The suspension was vortexed and a sterile inoculation needle dipped into it

and used to inoculate each of four identification culture media (Triple Sugar (or Kliger's) Iron Agar, Simmon's Citrate Agar, Christensen Urea Agar and Peptone water). The results obtained for the sucrose, glucose and lactose fermentations; and gas and hydrogen sulphide (H_2S) from the Triple Sugar/ Kliger's Iron Agar media as well as Citrase production on Simmon's Citrate Agar, Urease production on Christensen's Urea Agar and Indole production in Peptone water (+ Kovac's reagent) were recorded as positive are negative as the case might be. A colour change of the Simmon's citrate agar from green to blue indicated a positive reaction, while a negative reaction was recorded when the medium remained green. A colour change of the Christensen Urea agar from yellow to red indicated a positive reaction, while a negative reaction was recorded when the medium remained yellow. The appearance of red ring on the surface of the peptone water upon the addition of a few drops of the Kovac's reagent indicated a positive reaction while the appearance of a yellow indicated a negative reaction for the indole test (Table 4 and Plate 3).

Table 4: Pattern of Reaction for a Typical *E. coli* Bacterium Using Standard Biochemical Tests

Medium	Test	Reaction
Triple Sugar /Kliger's Iron agar	Sucrose fermentation	Positive
	Glucose fermentation	Positive
	Lactose fermentation	Positive
	Gas production	Positive
	H_2S production	Negative
Simmon's citrate agar	Citrase production	Negative
Christensen's urea agar	Urease production	Negative
Peptone water + Kovac's reagent	Indole production	Positive



Positive Negative Negative Positive Negative Positive

Plate 3: Biochemical tests using Triple Sugar Iron agar (A), Simmon's citrate agar (B), Christensen's urea agar (C) and Peptone water + Kovac's reagent (D). For Triple Sugar Iron agar test, A_1: red slope + red butt = No sugar fermentation, no gas or H_2S production; A_2: yellow slope + yellow butt + air bubbles = glucose, lactose and sucrose fermentation + gas production; A_3: red slope + yellow butt + air bubbles = glucose fermentation only + gas production; A_4: yellow slope + yellow butt + black staining + air bubbles = glucose, lactose and sucrose fermentation + gas and H_2S production; and A_5: yellow slope + yellow butt glucose, lactose and sucrose fermentation but gas or H_2S production

Confirmation of Isolates Using API 20 E Commercial Kits

Strips of API 20 E (Biomérieux, France) were used according to the manufacturer's instructions. Each API 20 E strip consists of 20 microtubes or wells containing dehydrated substrates. The 20 wells on each strip were filled with a suspension of the test organism and incubated at 35°C for 16 to 18 hr. The principle behind the use of this kit lies in the fact that during the incubation, metabolism of the bacteria contained in the suspension produces colour changes that are either spontaneous or revealed by the addition of reagents. Following the incubation period, the appropriate reagents including Voges-Proskauer (VP) 1, VP 2, Tryptophan Deaminase (TDA) and Kovac's reagents, were added. The reactions were then read according to a reading table and the identification of the isolates was obtained by referring to the Analytical Profile Index manual. The results obtained were recorded and used to confirm the results obtained earlier using conventional biochemical tests.

Determination of the Sensitivity of *E. coli* Isolates to Selected Antibiotics

The Modified Kirby-Bauer disk diffusion method (Cheesbrough, 2006) was used to evaluate the susceptibility or resistance of isolates to 16 selected antibiotics commonly prescribed in Ghana. These included Ampicillin (10µg), Tetracycline (30µg), Co-trimoxazole (25µg), Gentamycin (10µg), Cefuroxime (30µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) Nitrofurantoin (300 µg), Nalidixic acid (30µg), Aztreonam (30µg), Imipenem (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Amikacin (30µg) and Norfloxacin (10µg).

The bacterial suspensions of test isolates were then prepared using sterile normal saline and adjusted to the 0.5 McFarland turbidity standards

(1% Barium Chloride, 0.05 ml and 1% sulphuric acid, 9.95 ml). The suspensions were subsequently used to inoculate sterile Müeller-Hinton Agar plates to provide bacterial lawns with confluent growth.

The inoculated plates were allowed to dry on the bench top of a laminar-flow cabinet (EdgeGARD, The Baker Company, USA) for approximately 10 minutes and filter paper disks containing known concentration of a single type of antibiotics placed on their surfaces and incubated at 35°C for 16 -18 hr.

After incubation, the diameters of zones of inhibition (clear zones) that developed around each disc were measured using a pair of Vernier callipers (Rabone, Switzerland) (Plate 4) and recorded. These measurements were interpreted using guidelines provided by the Clinical Laboratory Standards Institute (CLSI, 2010) and classified as sensitive, intermediate or resistant to the various antibiotics used. All isolates that showed intermediate resistance to the various antibiotics were screened again and re-classified as sensitive or resistant.

x ½

Plate 4: A pair of Vernier calipers used to measure the diameters of the clear zones

Phenotypic Confirmation of Extended Spectrum Beta-Lactamases-Producing Strains Among *E. coli* Isolates

The prevalence of Extended Spectrum Beta-Lactamases (ESBLs) producers among the isolates was estimated before the confirmation tests were

carried out. Isolates that gave clear zones with diameters ≤ 25mm and ≤ 27mm for ceftriaxone (30µg) and cefotaxime (30µg), respectively, were considered as potential ESBL producers. The phenotypic confirmation test was carried out using special kits designed for that purpose (CLSI, 2010). Mastdiscs^{TM} ID (Mast Group Ltd., Merseyside, UK) containing three sets of second and third generation cephalosporins with and without clavulanic acid (a competitive lactamase beta inhibitor) were used according to the manufacturers' instructions. Set 1 contained Ceftazidime (30 µg) and Ceftazidime (30 µg) in combination with Clavulanic acid (10 µg); set 2 was composed of Cefotaxime (30 µg) and Cefotaxime (30 µg) in combination with Clavulanic acid (10 µg) while set 3 was made up of Cefpodoxime (30 µg) and Cefpodoxime (30 µg) in combination with Clavulanic acid (10 µg).

Bacterial lawns were prepared on Müeller-Hinton agar plates using bacterial suspensions of presumptive ESBL producers among the *E. coli* isolates recovered both from clinical and environmental sources. Using sterile forceps, each set of antibiotic discs was placed onto the surface of the inoculated medium ensuring that they were evenly spaced. Following an incubation period of 18–24 hr at 35°C, the diameters of the zones of inhibition that developed around the antibiotic discs were measured and recorded.

The sizes of the zones of inhibition for the ceftazidime, cefotaxime and cefpodoxime discs were compared with that of ceftazidime, cefotaxime and cefpodoxime plus clavulanic acid combination discs. An increase in the zone diameter of ≥ 5 mm in the presence of clavulanic acid from any or all of the three sets of antibiotics indicated the presence of ESBL in the test isolate (CLSI, 2010).

Quality Control

In order to ascertain the reliability and validity of the results, quality control tests were performed. Standard-typed strains were screened at least once a week as a quality control measure. These were *Escherichia coli* ATCC 25922 (beta-lactamase negative) and *Klebsiella pneumonia*e ATCC 700603 (beta-lactamase positive).

DNA Extraction

DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega Corporation, USA) according to the manufacturer's instructions. Each Wizard Genomic DNA Purification Kit comprised of a Nuclei Lysis Solution, a Protein Precipitation Solution, a DNA Rehydration Solution and an RNase Solution.

For each test *E. coli* isolate, 1 ml of fresh overnight culture of bacterial cells (OD_{600} = 1) growing in Luria-Bertani broth was transferred into a clean 1.5 ml microfuge tube and centrifuged at 14,000 rpm for 2 minutes to pellet the bacterial cells. The supernatant was then removed and 600 µl of Nuclei Lysis Solution gently pipetted and added to the cell pellets until the cells were re-suspended. The cells were lysed by incubating at 80°C for 5 minutes and allowed to cool to room temperature. A volume of 3µl of the RNase solution was added to the cell lysate and mixed by inverting the tube 2 to 5 times and incubated at 37 °C for 60 minutes and allowed to cool to about 25 °C. Subsequently, 200 µl of Protein Precipitation Solution was added to the RNase-treated cell lysate and the contents vortexed vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate. The mixtures were later incubated for 5 minutes on ice and centrifuged at

14,000 rpm for 3 minutes. The supernatant containing the DNA was transferred into clean 1.5 ml microfuge tubes containing 600 µl of room temperature isopropanol and gently mixed by inversion until thread-like strands of DNA formed visible mass. The contents in the tubes were centrifuged again at 14,000 rpm for 2 minutes to pellet the DNA and the supernatant was carefully poured off. Each tube was drained on clean absorbent paper and 600 µl of room temperature 70% ethanol added and the tubes gently inverted several times to wash the DNA pellet. The contents in the tube were again centrifuged at 14,000 rpm for 2 minutes, the ethanol was carefully aspirated, the tubes were then drained on clean absorbent paper and the DNA pellets allowed to air-dry for 10 to 15 minutes. Finally, DNA Rehydration solution (100 µl) was added to each tube and the DNA rehydrated by incubating the solution overnight at room temperature before storage at -20 °C. It was ensured that all DNA samples were pure before using them for subsequent analysis.

Determination of the Integrity, Purity and Concentrations of DNA Samples

The integrity of the genomic DNA samples was tested by loading 5 µl of each DNA sample on a 2% agarose gel. The loaded gel was then electrophoresed at 100 V for 60 min, visualized under an Ultraviolet Transilluminator (UVP Products, United Kingdom) and photographs taken with a digital camera.

With the aid of a Genova spectrophotometer (Jenway, UK), the purity and concentration of the DNA samples were determined and recorded. Purity was determined by taking the absorbance reading of each DNA sample at 260

nm (A_{260}) and at 280 nm (A_{280}) and the A_{260}/ A_{280} ratio calculated. A ratio between 1.8 and 2.0 denoted that the absorption in the UV range was due to nucleic acids and implied that the DNA sample was pure. A ratio lower than 1.8, however, indicated the presence of proteins and/ or other UV absorbers (contaminants). Furthermore a ratio higher than 2.0, indicated that the DNA samples were contaminated with either chloroform or phenol (Sambrook, Fritsch & Maniatis, 1989). In either case, the DNA samples were re-precipitated and the purification process repeated following the method by Hoisington, Khairallah & Gonzalez-De-Leon (1994).

The concentrations of the DNA samples were also determined using the following formula:

$$\text{DNA concentration } (\mu\text{g ml}^{-1}) = \frac{A_{260} \text{ x D x } 50\ \mu\text{g/ml}}{1000}$$

Where, A_{260} represents the measured absorbance at 260 nm and D is the Dilution factor.

The determination of the concentrations of the various DNA samples enabled their standardisation to100 ng/µl prior to the performance of various Polymerase Chain Reaction (PCR) analyses. The DNA samples that were highly concentrated were adequately diluted with sterile Tris-EDTA (TE) buffer, whereas those that had low concentrations, the DNA extraction was repeated until the required concentration was obtained.

Confirmation of DNA Samples as Bacterial DNA

The16S rRNA protocol was used to confirm DNA samples were of bacterial origin. The bacterial primers 27F(5_GAGTTTGATCCTGGCTCAG-3_) and 1492R (5_GGTTACCTTGTTACGACTT-3_) (Bioneer Cooperation, South Korea) were used in PCR amplification. The presence of a product size

of about 1500 bp was used to confirm the genus and species of the bacterial isolates (Lane, 1991).

Detection of ESBL Genes (^{bla}SHV, and ^{bla}TEM) in Clinical and Environmental *E. coli* Isolates

Two separate PCR amplification programmes were used for the detection of ^{bla}TEM and ^{bla}SHV genes, primarily, due to the fact that the primers used in their detection produce very similar amplified products (Table 5). Also the presence of 16S rRNA gene (as an internal control) was used as confirmation for bacterial DNA. In a single reaction mixture of 20 µl, 2 µl each of 1 pmol of forward and reverse primers for the 16S rRNA and ^{bla}TEM genes were added to the Accupower Multiplex PCR premix (Bioneer Corporation, South Korea) together with 10µl of sterile molecular biology grade water and 2 µl of 100 ng/µl template DNA.

The detection of the ^{bla}SHV gene was performed in a reaction mixture of 50µl containing 1µl each of 10 pmol of forward and reverse primers of the ^{bla}SHV gene, Accupower PCR premix (Bioneer Corporation, South Korea), 46 µl sterile molecular biology grade water and 2 µl of 100 ng/µl DNA.

PCR amplification was carried out using the 96-well plate of a Techne TC-512 thermal cycler (Bibby Scientific, UK). The amplification programme consisted of an initial denaturation at 95°C for 15 minutes; 30 cycles of 94°C for 30 seconds, annealing at 56°C for ^{bla}TEM and 60 °C for ^{bla}SHV primers, respectively, for 30 seconds followed by extension at 72°C for 30 seconds; and a final extension at 72°C for 7 min (Zhang, Zhang, Shen, & Wang, 2011).

For each PCR product, 10 µl were loaded in each of the wells of a 1.5 % (w/v) agarose gel containing 0.001 % (w/v) ethidium bromide (Sigma-Aldrich, Inc. USA) and a 100 bp DNA ladder (Sigma-Aldrich, Inc. USA) was used as a molecular weight marker. The PCR amplicons loaded in the agarose gel were separated by electrophoresis at 100V for one hour. Each gel was visualised under an ultraviolet transilluminator (UVP products, UK), and photographed using a digital camera.

Molecular Phylogeny of *E. coli* Isolates

A multiplex PCR was performed on all *E. coli* isolates to detect the presence or absence of any of the three genes: ChuA, a gene coding for an outer membrane protein important for haem utilization (transport in enterohaemorrhagic *E. coli*); YjaA, a hypothetical protein gene with unknown function; and TspE4C2, a DNA fragment (Clermont *et al*., 2000). The primer pair sequences used in the multiplex PCR as well as their melting temperatures and expected amplicon sizes are provided in Table 6.

Each reaction mixture of 20 µl contained 2 µl of 1 pmol of forward and 2 µl of 1 pmol of reverse primers of the three genes in addition to those of the 16S rRNA gene, as well as Accupower Multiplex PCR premix (Bioneer Corporation, South Korea), 2 µl sterile molecular biology grade water and 2 µl of 100 ng/µl DNA. A Techne TC-512 thermal cycler (Bibby Scientific, UK) was used for the amplification. The PCR amplication consisted of an initial denaturation at 95°C for 15 minutes; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; and a final extension at 72°C for 7 minutes. For each PCR product, 10 µl were loaded in each of the wells of a 1.5 % (w/v) agarose gel containing 0.001% ethidium bromide (Sigma-

Aldrich, Inc. USA) and a 100 bp DNA ladder (Sigma-Aldrich, Inc. USA) was used as a molecular weight marker. The PCR amplicons loaded in the agarose gel were separated by electrophoresis at 100V for one hour. The resolved gels were visualized under an Ultraviolet Transilluminator (UVP products) and photographed for further analyses.

The bands were scored and each *E. coli* isolate assigned to the following phylogenetic groups:($ChuA^-$, $YjaA^+$, $TspE4C2^-$) = group A; ($ChuA^-$, $TspE4C2^+$) = group B1; ($ChuA^+$, $YjaA^+$) = group B2; and ($ChuA^+$, $YjaA^-$) = group D (Abdul-Razzaq and Abdul-Lateef, 2011). Dendrograms showing the genetic relatedness of the various phylogenetic groups and the source of isolation of the *E. coli* strains were then constructed using the Power Marker V3.25 and the Mega 4 software (Nei, Tajima & Tateno, 1983).

Screening for Virulence Genes in *E. coli* Isolates Obtained From Clinical and Environmental Samples

The presence or absence of virulence genes was determined using primers specific for some virulent genes to determine the common pathotypes associated with isolates obtained in this study. Seven multiplex PCR assays comprising different sets of primer pairs were performed (Table 7). The first five assays were used to screen for the presence of diarrhoeagenic *E. coli* pathotypes (ETEC, EPEC, EHEC, EIEC and EAEC), while assays 6 and 7 were performed to detect if any uropathogenic strains were among the *E. coli* isolates recovered. For assays 1, 2, 3, 4 and 5, the PCR conditions consisted of an initial denaturation at 95°C for 15 minutes; 30 cycles of denaturation at 94°C for 1 minute followed by annealing at 55°C for 1 minute, extension at 72°C for 1 minute; and a final extension step at 72 °C for 10 min.

Table 5: Characteristics of Primers Used for the Detection of ESBL Genes

Name	Sequence (5' to 3')	AT (°C)	Product size (bp)	Reference
^{bla}TEM F	GAGTATTCAACATTTCCGTGTCGC	56	865	Zhang *et al.*, (2011)
^{bla}TEM R	TACCAATGCTTAATCAGTGAGGC			
^{bla}SHV F	ATGCGTTATATTCGCCTGTG	60	860	Zhang *et al.*, (2011)
^{bla}SHV R	TTAGCGTTGCCAGTGCTTGATC			

AT = Annealing temperature, ESBL = Extended spectrum beta-lactamase, F = forward primer, R = reverse primer.

Table 6: Characteristics of Primers Used in Studying the Molecular Phylogeny of *E. coli* Isolates

Primer name	Sequence (5' to 3')	AT (°C)	Amplicon size (bp)	References
ChuA F	GACGAACCAACGGTCAGGAT	55.9	279	Clermont *et*
ChuA R	TGCCGCCAGTACCAAAGACA	58.6		*al*. (2000)
YjaA F	TGAAGTGTCAGGAGACGCTG	53.3	211	Clermont *et*
YjaA R	ATGGAGAATGCGTTCCTCAAC	53.3		*al*. (2000)
TspE4C2 F	GAGTAATGTCGGGGCATTCA	54.5	158	Clermont *et*
TspE4C2 R	CGCGCCAACAAAGTATTACG	55.6		*al*. (2000)

AT = Annealing temperature, F = forward primer, R = reverse primer.

The PCR conditions for assays 6 and 7 also consisted of an initial denaturation at 95 °C for 12 min; 30 cycles of 30 seconds denaturation at 94°C, 30 s annealing at 60 °C and 3 minutes extension at 68 °C; and a final extension at 72 °C for 10 min. These amplifications were also carried out using the Techne TC-512 thermal cycler (Bibby Scientific, UK).

Table 7: Assays and Primers Used in the Detection of Virulent Genes Among *E. coli* Isolates

Assay	Gene	Oligonucleotide sequence (5' to 3')	Amplicon Size (bp)	Refe- rences
1	*elt* F	CTCTATGTGCACACGGAGC	322	R1
	R	CCATACTGATTGCCGCAAT		
	St1a F	TCTTTCCCCTCTTTTAGTCAGTC	170	R2
	R	CCGCACAGGCAGGATTAC		
	*uid*AF	CCAAAAGCCAGACAGAGT	623	R2
	R	GCACAGCACATCAAAGAG		
2	*eae* F	TGATAAGCTGCAGTCGAATCC	229	R2
	R	CTGAACCAGATCGTAACGGC		
	*bfpA*F	CACCGTTACCGCAGGTGTGA	450	R2
	R	GTTGCCGCTTCAGCAGGAGT		
3	*stx_1* F	GAAGAGTCCGTGGGATTACG	130	R2
	R	AGCGATGCAGCTATTAATAA		
	stx_2 F	GGGTACTGTGTGCCTGTTACTGG	510	R2
	R	GCTCTGGATGCATCTCTGGT		
	ial F	CTGGTAGGTATGGTGAGG	320	R2
	R	CCAGGCCAACAATTATTTCC		

Table 7 Continued

Assay	Gene	Oligonucleotide sequence (5' to 3')	Amplicon Size (bp)	Refe-rences
4	*st1b* F	ATTTTTCTTTCTGTATTGTCTT	190	R3
	R	CACCCGGTACAAGCAGGATT		
	*uid*A F	CCAAAAGCCAGACAGAGT	623	R2
	R	GCACAGCACATCAAAGAG		
5	*aggR* F	CTAATTGTACAATCGATGTA	457	R1
	R	AGAGTCCATCTCTTTGATAAG		
	Aat F	CTGGCGAAAGACTGTATCAT	629	R1
	R	CAATGTATAGAAATCCGCTGTT		
6	*afa1*	GCT GGG CAG CAA ACT GAT AAC TCT C	750	R4
	afa2	CAT CAA GCT GTT TGT TCG TCC GCC G		
	cfn1	AAG ATG GAG TTT CCT ATG CAG GAG	498	R5
	cfn2	CAT TCA GAG TCC TGC CCT CAT TAT T		
	pap1	GAC GGC TGT ACT GCA GGG TGT GGC G	328	R1
	pap2	ATA TCC TTT CTG CAG GGA TGC AAT A		
7	*aer1*	TAC CGG ATT GTC ATA TGC AGA CCG T	602	R5
	aer2	AAT ATC TTC CTC CAG TCC GGA GAA G		
	hlyA F	GCA TCA TCA AGC GTA CGT TCC	534	R6
	hlyA R	AAT GAG CCA AGC TGG TTA AGC T		
	sfa1	CTC CGG AGA ACT GGG TGC ATC TTA C	410	R1
	sfa2	CGG AGG AGT AAT TAC AAA CCT GGC A		

R1 = Johnson & Stell, (2000); R2 = Scaletsky *et al*. (2002); R3 = Okeke, Fayinka & Laminkanra, (2000); R4 = Le Bouguenec & Servin (2006); R5 = Yamamotto *et al*. (1995); R6 = Paton & Paton (2005).

The PCR products (10µl aliquots) were resolved in 2.5% (w/v) agarose gel (Helena Biosciences, Europe) containing 0.001% ethidium bromide (Sigma Chemical Company, USA) in 1X TAE buffer (40 mM Tris–HCl, 20 mM Glacial acetic acid, 1 mM EDTA, pH 8.5). A 20-bp DNA ladder (Sigma-Aldrich, Inc. USA) was included on each gel as a molecular size standard. Electrophoresis was performed at 100 V for an hour before the bands were visualized and photographed under an Ultraviolet Transilluminator (UVP products) for further analysis.

Data Analysis

Data were subjected to Chi-square analysis or ANOVA using SPSS software version 16 (APPENDIX III - VIII). Analyses of DNA fingerprints were also carried out and the informative band patterns of amplified DNA fragments obtained from gel electrophoresis were recorded. The presence of a given band was scored as 1 and its absence as 0 in a data matrix and analyzed using the Power Marker Software version 3.25. Dendograms of dissimilarities were then constructed using the unweighted pair group method with arithmetic averages (UPGMA) clustering technique to determine the relatedness of *E. coli* isolates with reference to their sources.

Experimental Precautions

a) The entire surface of the bench top of the laminar air flow hood was disinfected by heavily spraying with methylated spirit and mopping with clean tissue paper soaked with methylated spirit before and after use.
b) All glassware and forceps were thoroughly washed with liquid soap, rinsed in several changes of clean water and dried before use.

c) Latex gloves were worn throughout the laboratory procedures to prevent infection by pathogenic micro-organisms and contamination of cultures.

d) Nitrile gloves and protective clothing were worn during the molecular biology laboratory procedures instead of latex gloves to prevent direct contact with ethidium bromide.

e) All samples were properly labelled soon after collection.

f) Samples were thoroughly mixed before portions were taken for analyses.

g) Agar plates containing bacterial cultures were incubated in an inverted position to prevent contamination.

h) Only relatively young bacterial cultures (12 – 18 hours old) were used throughout the experiments.

i) Bacterial isolates were cryopreserved by storing in glycerol at –20 ^{o}C.

j) All used cultures were safely disposed of by autoclaving at 121 ^{o}C for 15 minutes prior to their disposal.

k) All pipettes tips were sterilized before use to prevent contamination.

l) Caps of the PCR tubes were properly fitted so as to prevent spillage while the thermocycler was in use.

m) Only pure DNA samples were used for PCR in order to obtain good quality amplification products.

n) The concentrations of all DNA samples were determined before their standardization and subsequent analyses.

o) All primers, DNA ladder and DNA samples were stored at 4 ^{o}C and -20 ^{o}C as a short and long-term storage measure respectively.

p) Great care was taken when handling chemicals and reagents, as some of them such as ethidium bromide were hazardous and mutagenic.

q) Concentrations of ethidium bromide solutions less than 0.1 % were used throughout the gel electrophoresis laboratory procedures to prevent risks associated with the chemical.

r) All 0.001 % ethidium bromide stained agarose gels were safely disposed by burying in the ground at an appropriate site.

s) Any solution that contained ethidium bromide solution was decontaminated through the addition of activated charcoal and a subsequent filtration prior to the pouring of the decontaminated solution down the drain.

CHAPTER FOUR

RESULTS

Distribution of Enteric Bacteria Isolated From Clinical and Environmental Samples

Isolates of enteric bacteria were obtained from clinical samples (stool, urine, blood, urogenital, aspirate samples and wound swabs) and environmental samples (cabbage, smoked fish, fresh beef and chicken samples and water collected from various sites including the Fosu lagoon, the Kakum river, the sea, gutters around the Abura and Anaafo markets) were collected from the Coast Cape Metropolis and identified. The results obtained from the isolation of enteric bacteria on MacConkey agar are shown on Plate 4 with red colonies representing lactose fermenters while white colonies represented non-lactose fermenters (Plate 5).

The percentage occurrence and distribution of the enteric bacterial isolates identified in this study are presented in Table 8. Out of a total of 598 enteric bacterial isolates identified, 372 isolates consisting of 252 of clinical origin and 120 of environmental origin were found to be *E. coli* while the rest (226) belonged to other genera of the family Enterobacteriaceae. Additionally, 17 isolates (9 from clinical samples and 8 from environmental samples) were found to be *Shigella* spp. The later were considered as potential enteroinvasive *E. coli* (EIEC), bringing the number of *E. coli* isolated from both clinical and environmental samples to a total of 389 (Table 8). In all the cases, the number

of enteric bacterial isolates recovered from clinical samples were significantly higher (P < 0.01) than those obtained from environmental samples except for *Klebsiella oxytoca* (P > 0.05) and *Shigella* spp. (P > 0.05) where there was no significant difference between the frequency of clinical isolates and that of environmental isolates (APPENDIX III). Due to the low frequencies and in some cases non detection of the other bacterial species such as *Pseudomonas aeruginosa*, *Pseudomonas paucimobilis*, *Proteus penneri*, *Stenotrophomonas*

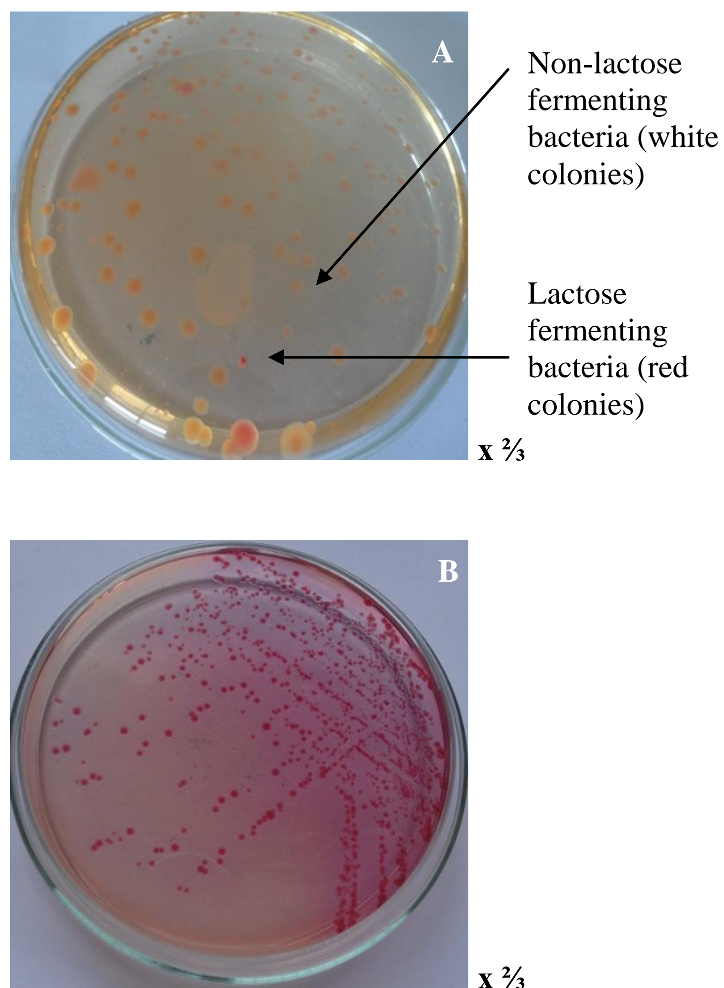


Plate 5: Coliform bacteria growing on MacConkey agar after 12 hours incubation at 35 °C. A: Mixed culture of lactose fermenting and non-lactose fermenting bacteria, B: Pure culture of *E. coli*

malthophilia, *Salmonella enterica*, *Erwinia* spp. and *Vibrio metschnikovii*, it was not possible to carry out any statistical analysis.

Clinical Samples

The distribution of *E. coli* isolates of clinical origin is shown on Table 8. Out of 382 clinical samples collected, 261 *E. coli* isolates were recovered with the stools samples recording the highest percentage occurrence (44.4 %) of isolates and blood samples recording the least (1.6 %) (Table 9).

Table 8: Distribution of Enteric Bacterial Isolates Recovered From Clinical and Environmental Samples (Percentage Occurrence (%) in Brackets)

Enteric bacterial species	Clinical	Environmental	Total
Escherichia coli	252 (65.3)	120 (56.6)	372 (62.2)
Klebsiella pneumoniae	78 (20.2)	5 (2.4)	83 (13.9)
Klebsiella oxytoca	5 (1.3)	13 (6.2)	18 (3.0)
Enterobacter spp.	5 (1.3)	55 (25.9)	60 (10.0)
Citrobacter freundii	34 (8.9)	2 (0.9)	36 (6.0)
Pseudomonas aeruginosa	0 (0.0)	3 (1.4)	3 (0.5)
Pseudomonas paucimobilis	1 (0.2)	0 (0.0)	1 (0.2)
Proteus penneri	1 (0.2)	0 (0.0)	1 (0.2)
Stenotrophomonas malthophilia	0 (0.0)	2 (0.9)	2 (0.4)
Salmonella enterica	0 (0.0)	2 (0.9)	2 (0.4)
Shigella spp.	9 (2.4)	8 (3.8)	17 (2.9)
Erwinia spp.	1 (0.2)	1 (0.5)	1 (0.2)
Vibrio metschnikovii	0 (0.0)	1 (0.5)	1 (0.2)
Total	386 (100.0)	212 (100.0)	598 (100.0)

Demographics of the Patients From Whom Clinical Samples Were Obtained

The demographics of the patients from whom clinical samples were obtained are shown in Table 10. A total of 261 clinical *E. coli* isolates comprised of 73 isolated from male patients and 188 from their female counter part. Statistical analysis revealed that there was no significant ($p > 0.05$) difference between the frequencies of the various age groups except for age group 20 – 29 years, 30 – 39 years, 40 – 49 years and above 70 years. Generally, for every age group higher numbers of *E. coli* isolates were recovered from the females than the males, except for the age group above 70 years which recorded a higher number than their female counterpart (Table 10).

Table 9: Distribution of Clinical *E. coli* Isolates With Respect to Their Sources (Percentage Occurrence (%) in Brackets)

Origin of isolates	No. of samples screened	No. of *E. coli* isolates
Urine	143	95 (36.4)
Stool	167	116 (44.4)*
Urogenital	36	21 (8.0)
Wound	17	14 (5.4)
Aspirate	11	11 (4.2)
Blood	8	4 (1.6)
Total	382	261 (100.0)

*: $P < 0.01$

Environmental Samples

In contrast to clinical samples, 128 *E. coli* isolates were recovered from 88 environmental samples screened. The highest percentage frequency of *E. coli* isolates was obtained from the Fosu lagoon (42.2 %) while the least percentage was obtained from the Kakum river as well as the Chicken samples screened (4.7 %). No isolate was recovered from seawater, cabbage and smoked fish (Table 11).

Table 10: Age and Gender Distribution of Patients From Whom Clinical Samples Were Obtained and *E. coli* Recovered (Percentage Frequency (%) in Brackets)

Age groups (years)	Male	Female	Total
0 - 9	13 (4.98)	13 (4.98)	26 (9.96)
10 - 19	3 (1.15)	3 (1.15)	6 (2.30)
20 - 29	5 (1.92)	75 (28.74)*	80 (30.66)
30 - 39	13 (4.98)	51 (19.54)	64 (24.52)
40 - 49	5 (1.92)	13 (4.98)	18 (6.90)
50 - 59	5 (1.92)	8 (3.06)	13 (4.98)
60 - 69	8 (3.06)	17 (6.51)	25 (9.57)
≥ 70	21 (8.05)	8 (3.06)**	29 (11.11)
Total	73 (27.98)	188 (72.02)	261 (100.00)

(*: $P < 0.01$; **: $P < 0.01$)

Table 11: Distribution of Environmental *E. coli* Isolates With Respect to Their Sources (Percentage Frequency (%) in Brackets)

Origin of isolates	No. of samples screened	No. of *E. coli* isolates
Anaafo market	10	22 (17.2)
Abura market	8	12 (9.4)
Kakum river	10	6 (4.7)
Fosu Lagoon	10	54 (42.2)
Seawater	10	0 (0.0)
Cabbage	16	0 (0.0)
Fresh beef	10	28 (21.8)
Smoked fish	10	0 (0.0)
Chicken	8	6 (4.7)
Total	88	128 (100.0)

Enteric Bacterial Species Isolated From Clinical and Environmental Samples

The confirmatory test performed on an *E. coli* isolate using the Analytical Profile Index (API) 20 E kit revealed a pattern of positive (+) and negative (-) reactions. The results of the various tests were recorded based on the colour change observed in the 20 substrates contained in all 20 wells of the API 20 E kit (Plate 6). Thus, *E. coli* showed a positive reaction for nine out of the 20 wells while negative reactions were recorded for the other eleven wells. The nine wells that showed positive reactions include wells containing substrates such as 2-nitrophenyl-ß-D- galactopyranoside (ONDG), L-Lysine

(LDC), L-tryptophane (IND), D-glucose (GLU), D-mannitol (MAN), D-sorbitol (SOR), L-rhamnose (RHA), D-melibiose (MEL), L-arabinose (ARA).

The results obtained from the Gram stain reaction on a confirmed *E. coli* isolate revealed Gram-negative rods (Plate 7).

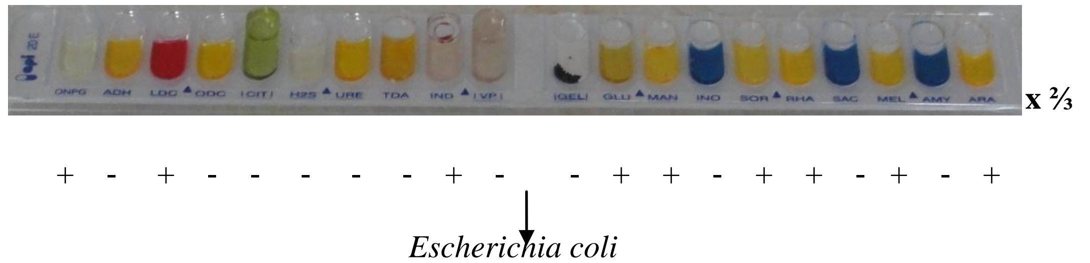


Plate 6: Inoculated API 20E strip after 18 hours incubation period showing a pattern of reaction corresponding to that of *Escherichia coli*. (+): Positive reaction; (-): Negative reaction

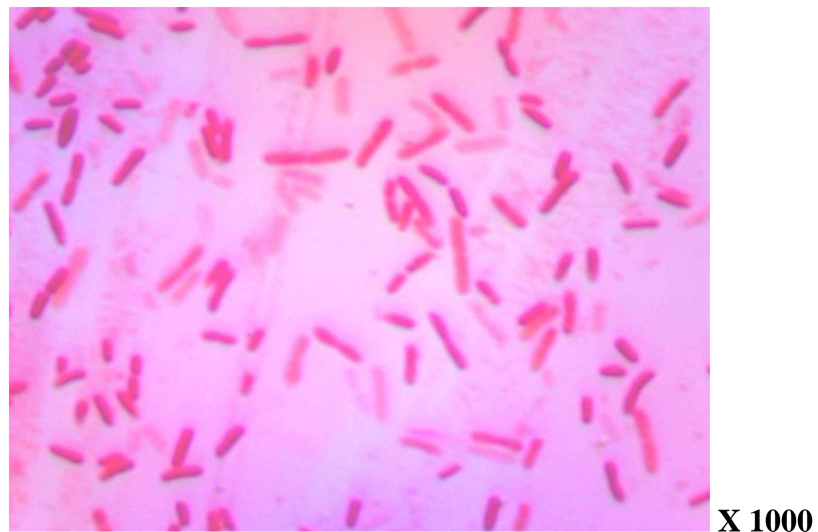


Plate 7: Gram-negative rods of a confirmed *E. coli* isolate

Antibiotic Susceptibility Patterns of *E. coli* Isolated From Clinical and Environmental Samples

The sensitivity patterns of *E. coli* isolates recovered from both clinical and environmental samples to 16 antibiotics is revealed in Figure 4.

Generally, the least percentage sensitivity was recorded when all *E. coli* isolates were screened against Ampicillin. None of the isolates recovered from the blood, wound or urogenital samples was found to be sensitive to Ampicillin.

All environmental and clinical isolates screened in this study were sensitive to Imipenem. In all categories, the percentage sensitivity of the isolates recovered from environmental samples to the 16 antibiotics was higher than those of other isolates except for Nalidixic acid, Aztreonam and Amikacin where isolates of environmental origin recorded lower percentages of sensitivity. The isolates obtained from stool samples however, recorded higher sensitivity when they were screened against those three antibiotics. The percentage sensitivity of all *E. coli* to Ampicillin (0 – 24.1 %), Tetracycline (16.0 – 28.4 %), Cotrimoxazole (16.8 – 22.0 %), Cefuroxime (27.6 – 43.2 %) and Nalidixic acid (22.1 – 47.8 %) were found to be moderately low. Culture plates obtained following the performance of the antibiotic susceptibility testing are shown on Plate 8.

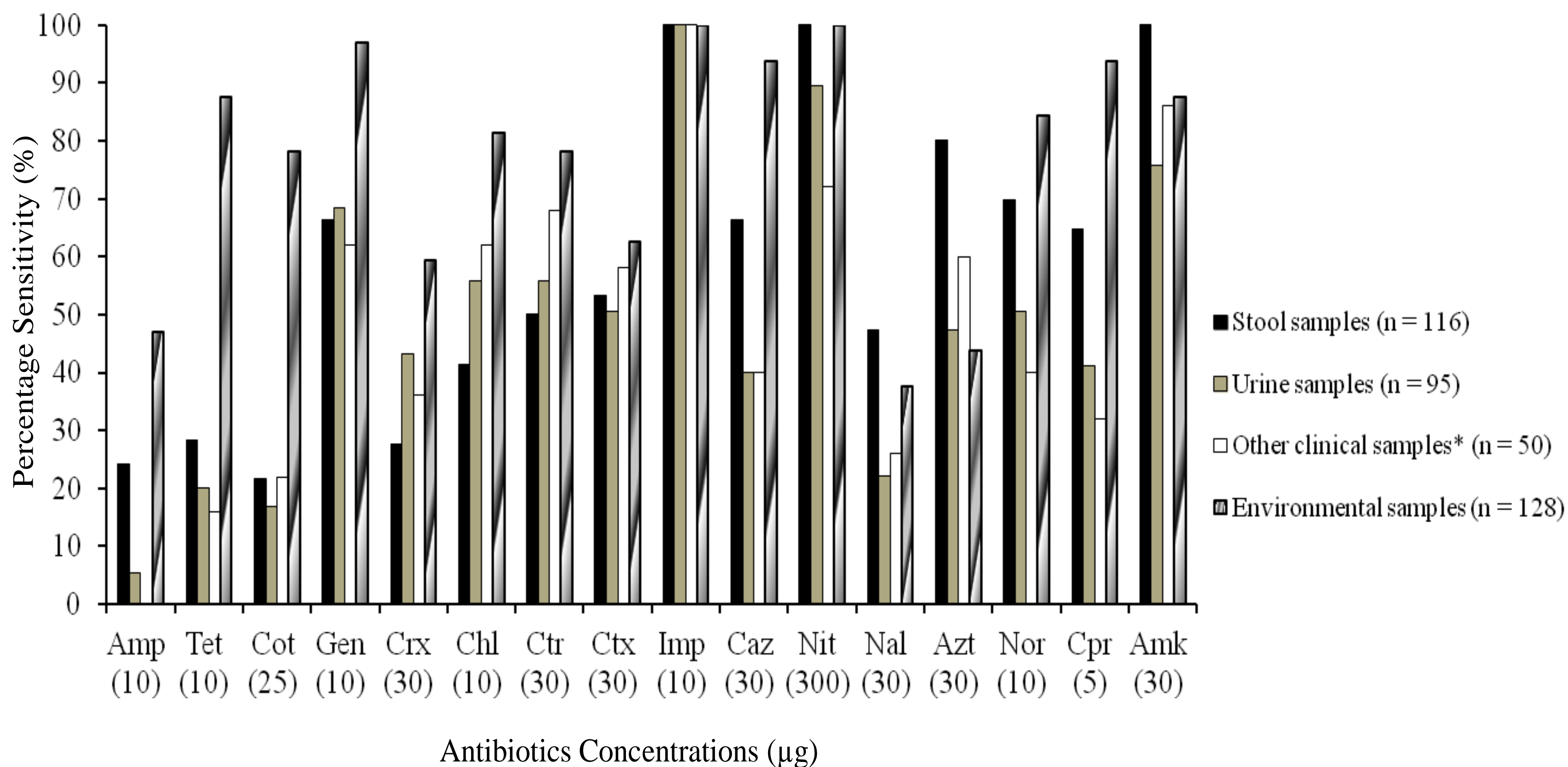
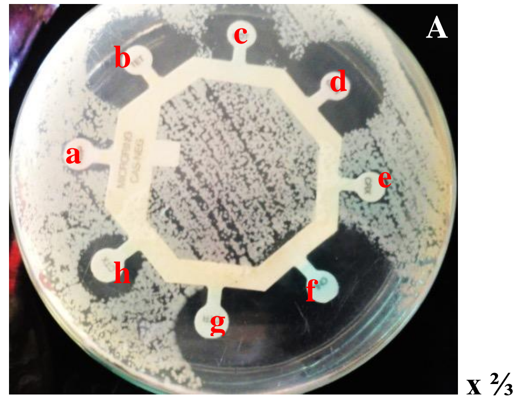


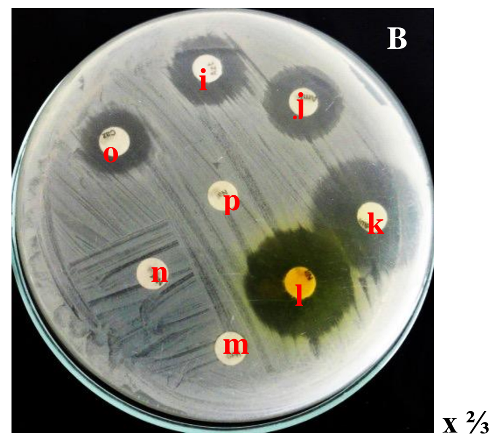
Figure 4: Antibiotic sensitivity pattern of *E. coli* isolates to 16 antibiotics

Amp = Ampicillin, Tet = Tetracycline, Cot = Co-trimoxazole, Gen = Gentamycin, Crx = Cefuroxime, Chl = Chloramphenicol, Ctr = Ceftriazone, Ctx = Cefotaxime, Imp = Imipenem, Caz = Ceftazidime, Nit = Nitrofurantoin, Nal = Nalidixic acid, Azt = Aztreonam, Nor = Norfloxacin, Cpr = Ciprofloxacin, Amk = Amikacin.

***Other clinical samples: Blood, wound, aspirate and urogenital samples**



x ⅔



x ⅔

Plate 8: Inoculated Müeller-Hinton agar plates each with 8 antibiotic discs after 16 hour incubation at 35 °C

A: (a) Ampicillin (10µg), (b) Tetracycline (30µg), (c) Co-trimoxazole (25µg), (d) Gentamycin (10µg), (e) Cefuroxime (30µg), (f) Chloramphenicol (30µg), (g) Ceftriaxone (30µg), (h) Cefotaxime (30µg)

B: (i) Nitrofurantoin (300 µg), (j) Nalidixic acid (30 µg), (k) Aztreonam (30 µg), (l) Imipenem (10 µg), (m) Ciprofloxacin (5 µg), (n) Ceftazidime (30 µg), (o) Amikacin (30 µg), (p) Norfloxacin (10µg)

Prevalence of Extended Spectrum Beta-Lactamase Producers Among *E. coli* Isolates

A phenotypic confirmation test for ESBL producers using a bacterial lawn of the isolates showed clear zones of varying diameters around each 2nd or 3rd generation cephalosporin (Plate 9).

The distribution of Extended Spectrum Beta-lactamase (ESBL) producers in all *E. coli* isolates from both clinical and environmental sources is presented in Table 12. There was a significantly ($P < 0.001$) lower percentage of ESBL producers in both clinical and environmental sources as compared to the percentage of non-ESBL producers. Additionally, the number of ESBL producers in the clinical samples was significantly ($P < 0.001$) higher than in the environmental samples. A similar trend was recorded for the non-ESBL producers ($P = 0.000$) (APPENDIX IV).

An estimated 29.88 % of *E. coli* isolates of clinical origin were found to be ESBL producers. A lower percentage of 12.50 % however of *E. coli* isolates recovered from environmental samples were found to be ESBL producers and the remaining 87.50 % were non – ESBL producers.

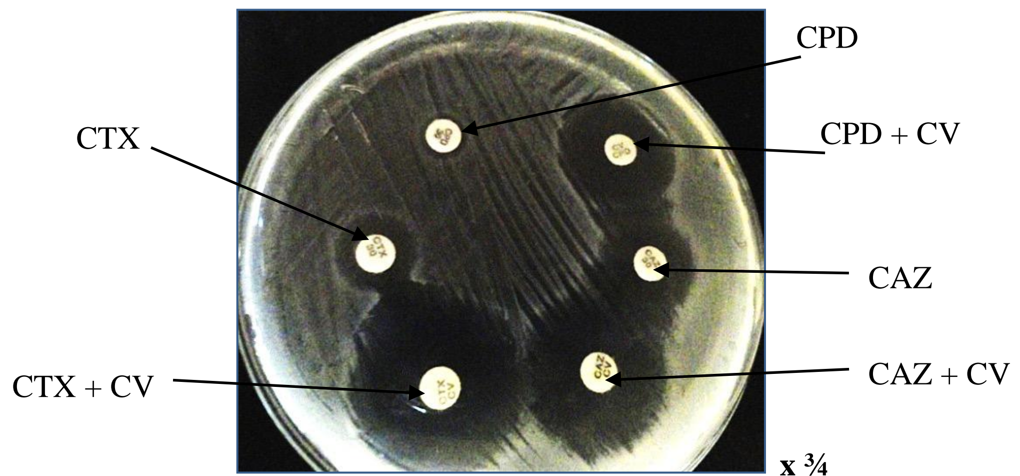


Plate 9: Phenotypic confirmation of ESBL producers using a bacterial lawn of the test isolate showing clear zones of varying diameters around each 2nd or 3rd generation cephalosporin used (CAZ = Ceftazidime (30 µg), CTX = Cefotaxime (30 µg), CPD = Cefpodoxime (30 µg), CV = Clavulanic acid (10 µg))

Table 12: Distribution of ESBL Producers and Non-ESBL Producers Among *E. coli* Isolates Recovered From Clinical and Environmental Samples (Percentages (%) in Brackets)

Sample Type	ESBL producers	Non-ESBL producers	Total
Clinical	78 (29.88)	183 (70.11)	261 (100)
Environmental	16 (12.5)	112 (87.5)	128 (100)
Total	94 (26.46)	295(73.53)	389 (100)

Results From DNA Extraction and Standardization of Concentration of DNA Samples

In all, 389 DNA samples with a concentration of 100 ng/µl were obtained excluding DNA samples used as controls. Overall, 261 DNA samples were from clinical isolates and 128 from environmental isolates. A photograph of an ethidium bromide-stained 2% agarose gel loaded with genomic DNA samples (Plate 10) indicated that the DNA samples were of high integrity. A confirmation of the DNA samples as bacterial DNA with the presence of 16S rRNA is shown in Plate 11.

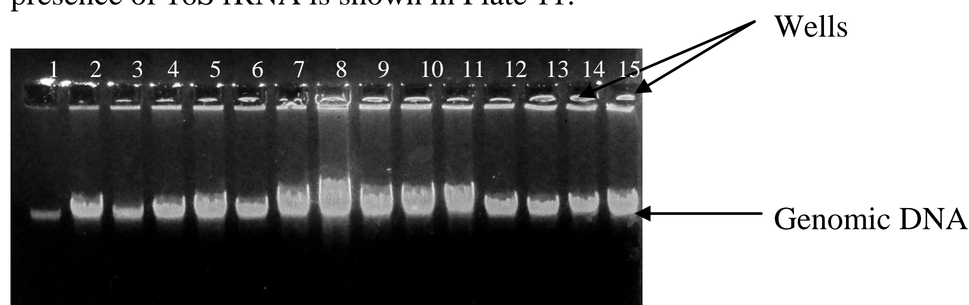


Plate 10: Ethidium bromide-stained 1 % agarose gel showing fifteen genomic DNA samples extracted from *E. coli* isolates

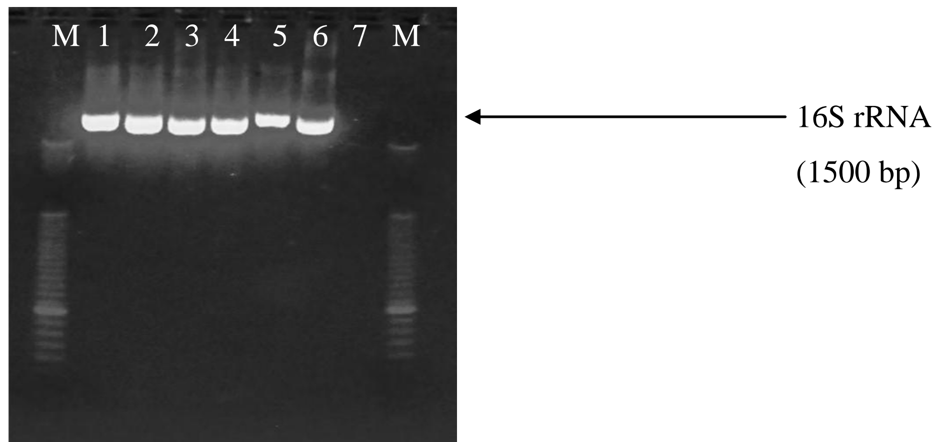


Plate 11: Ethidium bromide-stained 2.5 % agarose gel showing PCR products obtained after confirmation of DNA samples as bacterial DNA using 16S rRNA primers. Lane 1, 2, 3, 4, 5, and 6, presence of 16S rRNA gene; Lane 7, absence of 16S rRNA gene; Lane M, 20 bp molecular marker

Prevalence of ESBL Genes (^{bla}SHV and ^{bla}TEM) in Clinical and Environmental *E. coli* Isolates.

PCR amplification products separated by agarose gels for detection of ^{bla}SHV and ^{bla}TEM genes are shown on Plates 12, 13 and 14. There were highly significant ($P < 0.001$) differences between the frequencies of the various genes detected among ESBL and non-ESBL-producing *E. coli* isolates of clinical origin and the non-ESBL-producing *E. coli* isolates obtained from environmental samples (APPENDIX VI). The single detection of the ^{bla}TEM gene among phenotypically confirmed non-ESBL producers was recorded in 5.4 % and 4.9 % of *E. coli* isolates of environmental and clinical origins, respectively. However, for the ^{bla}SHV gene, 10.8 % and 18.6 % were recorded among environmental and clinical origins, respectively, for isolates that were phenotypically confirmed as non-ESBL producers. There was no detection of either gene for 12.5 % and 5.1 %, respectively, of the phenotypically

confirmed non-ESBL-producing isolates obtained from environmental and clinical samples. Similarly, there was no detection of either gene in 83.8% and 75.4 % respectively, of the phenotypically confirmed ESBL producers obtained from environmental and clinical samples (Table 13).

Among the phenotypically confirmed ESBL-producing *E. coli* isolates, 21.8% of the isolates from clinical samples possessed both ^{bla}TEM and ^{bla}SHV genes. Among phenotypically confirmed non-ESBL producers only 1.1% of isolates from clinical samples had both ESBL genes and none for environmental isolates (Table 13).

Table 13: Prevalence of ^{bla}TEM and ^{bla}SHV Genes Among ESBL Producing and Non-ESBL Producing *E. coli* Isolates (Percentages in Brackets)

Gene	Environmental isolates (n = 128)		Clinical isolate (n = 261)		Total
	ESBL +ve	ESBL -ve	ESBL +ve	ESBL -ve	
Neither ^{bla}TEM nor ^{bla}SHV	2 (12.5)	94 (83.8)	4 (5.1)	138 (75.4)	238
^{bla}TEM	4 (25.0)	6 (5.4)	20 (25.7)	9 (4.9)	39
^{bla}SHV	8 (50.0)	12 (10.8)	37 (47.4)	34 (18.6)	91
^{bla}TEM + ^{bla}SHV	2 (12.5)	0 (0.0)	17 (21.8)	2 (1.1)	21
Total	16 (100)	112 (100)	78 (100)	183(100)	389(100)

ESBL = Extended Spectrum Beta Lactamase; +ve = Positive; -ve = Negative

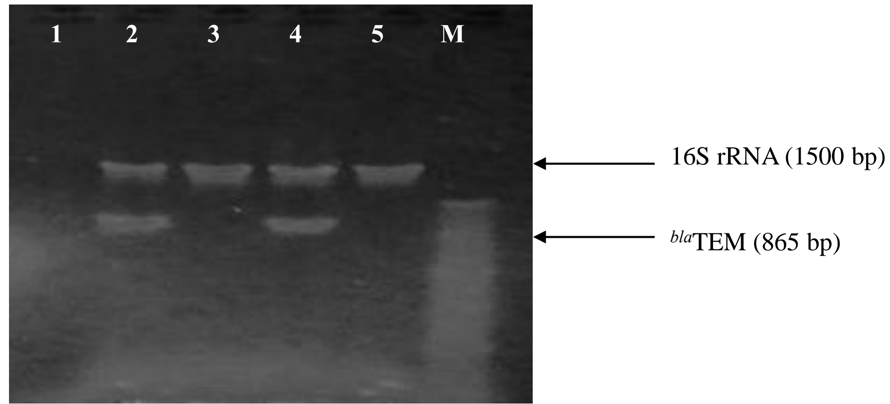


Plate 12: **Ethidium bromide-stained 2.5% agarose gel showing PCR products resulting from amplification of ^{bla}TEM gene (865 bp) and 16S rRNA gene (1500 bp) in *E. coli* isolates. Lane 1, negative control; Lane 2 and 4, presence of TEM and 16S rRNA genes; Lane 3 and 5, presence of 16S rRNA gene only; Lane M, 20-bp ladder**

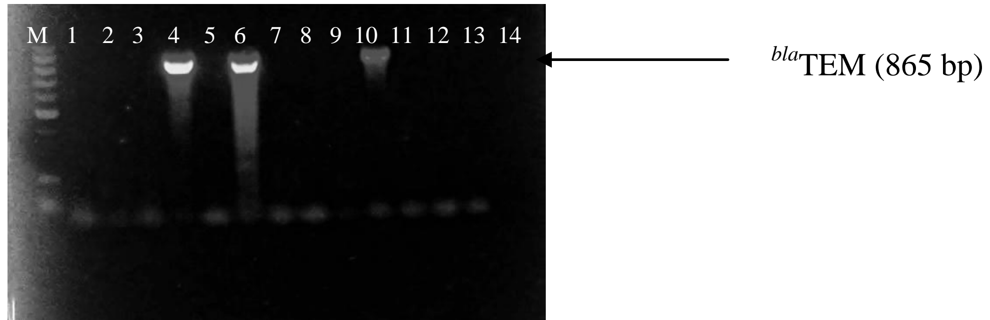


Plate 13: Ethidium bromide-stained PCR amplification products on a 1.5% agarose gel showing the presence or absence of the ^{bla}TEM gene in *E. coli* isolates. Lanes 1, 2, 3, 5, 7, 8, 9, 11, 12, 13, and 14, absence of ^{bla}TEM gene; Lanes 4, 6 and 10, presence of ^{bla}TEM gene; Lane M, 100-bp ladder

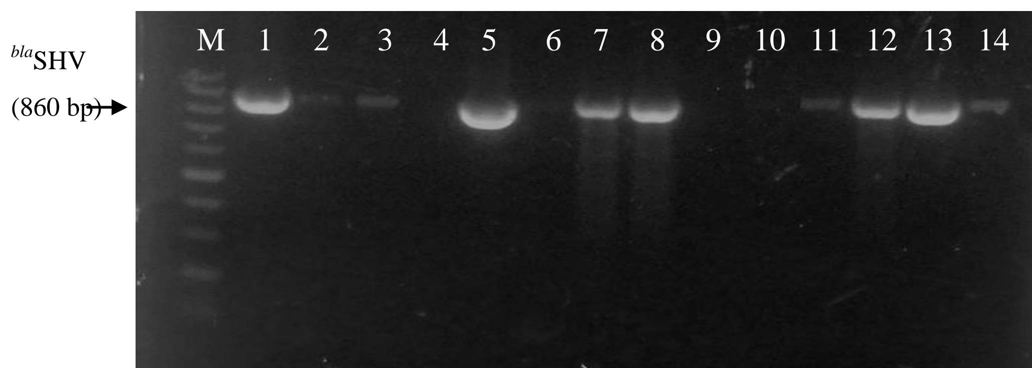


Plate 14: Ethidium bromide-stained PCR amplification products on a 1.5% agarose gel showing the ^{bla}SHV gene in *E. coli* isolates. Presence of the^{bla}SHV gene in lanes 1, 2, 3, 5, 7, 8, 11, 12, 13, and 14, and absence in lanes 4, 6, 9 and 10; Lane M, 100-bp ladder

Molecular Phylogeny of *E. coli* Isolates

The distributions of the 389 *E. coli* isolates into molecular phylogenetic groups are presented in Table 14. Majority (261) of the isolates were of clinical origin, while 128 were obtained from environmental samples. The frequencies of isolates were significantly ($P<0.01$) different among the four phylogenetic groups A, B1, B2 and D for both clinical and environmental isolates. The highest percentage of isolates obtained from clinical samples belonged to phylogenetic group B2 (37.93 %), followed by phylogenetic group A and D with 28.74 % and 28.35 %, respectively (Table 14). Members of the phylogenetic group B1 were the lowest (3.83 %).

Most of the isolates (55.47 %) from environmental samples belonged to group A, followed by group D (28.13 %), B2 (10.94 %) and the lowest recorded in group B1 (5.47 %). Ethidium bromide-stained agarose gels showing bands for PCR amplified products used for determination of genetic

relationships are presented in Plates 15 and 16, while the genetic relationships among the various *E. coli* isolates are shown in Figures 5 and 6.

Table 14: Distribution of *E. coli* Isolated From Environmental and Clinical Samples Among Phylogenetic Groups (Percentages (%) in Brackets

Phylogenetic group	Frequency of isolates obtained from various sources		Total
	Clinical	Environmental	
A	74 (28.35)*	71 (55.47)	145 (37.28)
B1	13 (4.98)	7(5.47)	20 (5.14)
B2	99 (37.93)**	14 (10.94)	113 (29.05)
D	75 (28.74)	36 (28.13)	111 (28.53)
Total	261 (100.00)	128 (100.00)	389 (100.00)

(* : P< 0.01; **: P < 0.01)

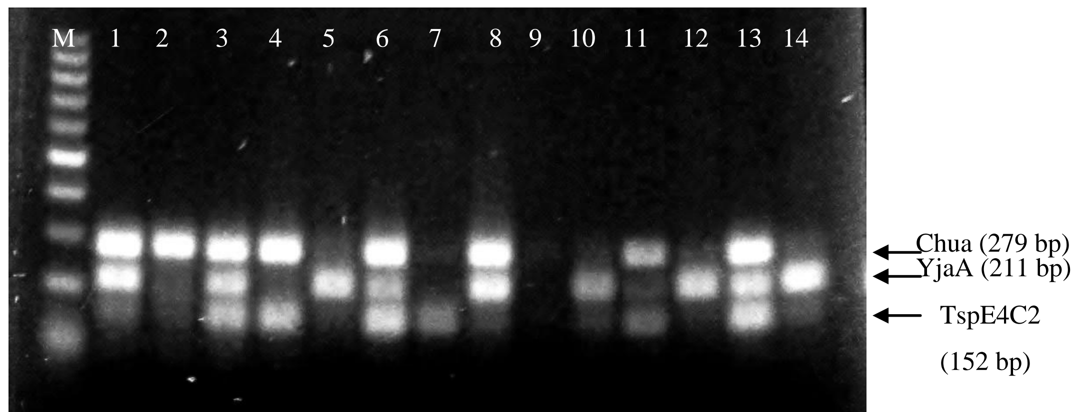


Plate 15: Ethidium bromide-stained 1.5% agarose gel showing multiplex PCR amplified products of *E. coli* isolates using three primer pairs for the ChuA, YjaA and TspEC2 genes for the detection of phylogenetic groups. Group A - lanes 5, 9, 10, 12 and, 14; Group B1 - lane 7; Group B2 - lanes 1, 3, 5, 8 and 13; and Group D - lanes 2, 4 and 11; Lane M, 100-bp molecular ladder

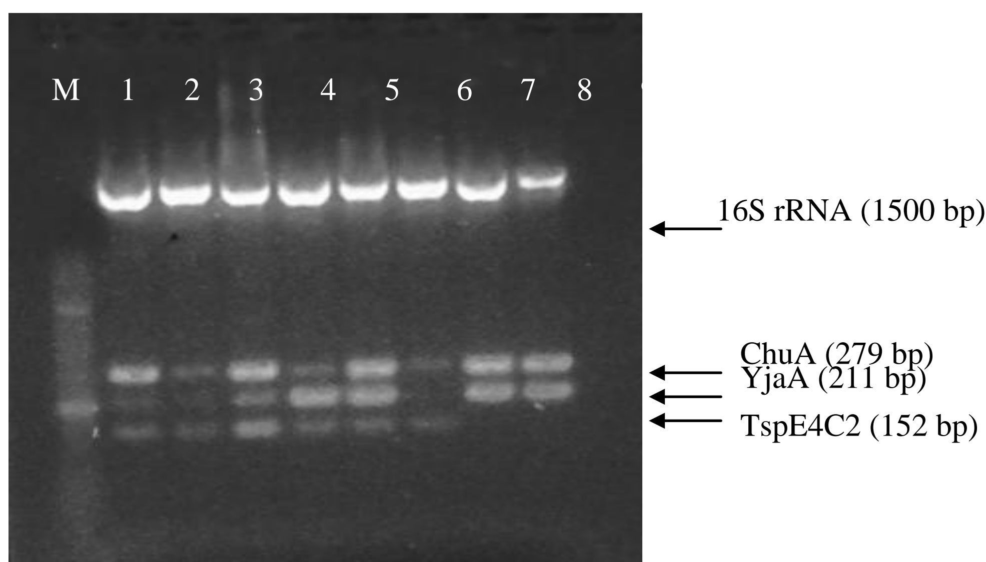


Plate 16: Ethidium bromide-stained 2.5% agarose gel showing multiplex PCR amplified products of *E. coli* isolates using four primer pairs: 16 S rRNA, ChuA, YjaA and TspEC2 genes for the detection of phylogenetic groups. Group A - lanes 2, 4 and 6; group D - lanes 1 and 3; Group B2 - lanes 5, 7 and 8; Negative control (distilled water) - Lane 9; Lane M – 100 bp molecular marker

Prevalence of Pathogenic *E. coli* Strains in Clinical and Environmental Samples

The distribution of the various *E. coli* pathotypes isolated from various clinical and environmental samples is presented in Table 15. There were highly significant ($P < 0.001$) differences among the frequencies of the various pathotypes from the various samples (Appendix VIII). Pathogenic *E. coli* isolates from environmental samples were also significantly ($P < 0.001$) fewer than isolates from clinical samples. The proportions of enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC) and enterohaemorrhagic (EHEC) *E. coli* isolates from stool samples were higher

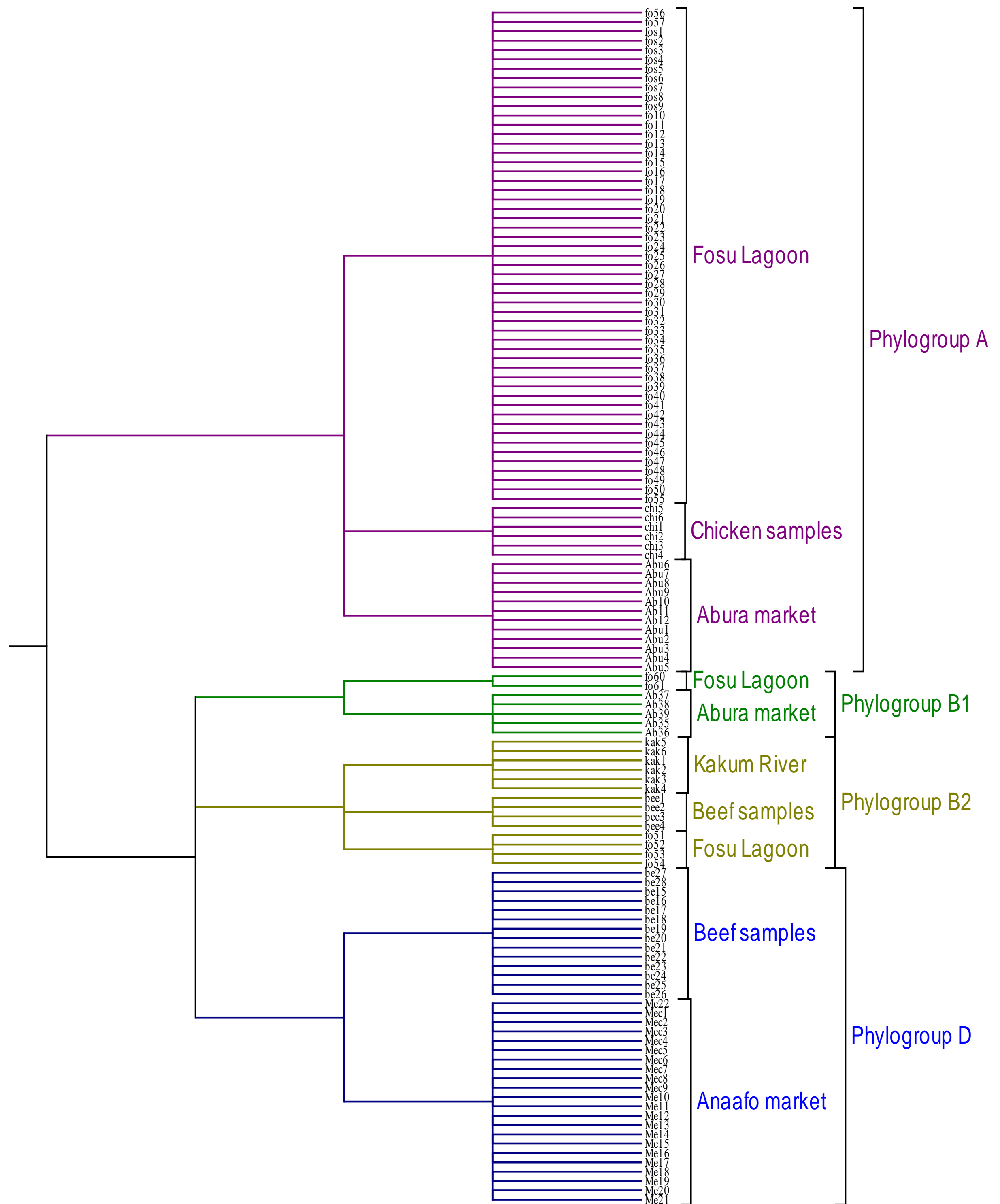


Figure 5: Phylogenetic groups of 128 *E. coli* strains isolated from environmental samples as determined by the unweighted pair group method with arithmetic averages of binary character matrix using the similarity coefficient index

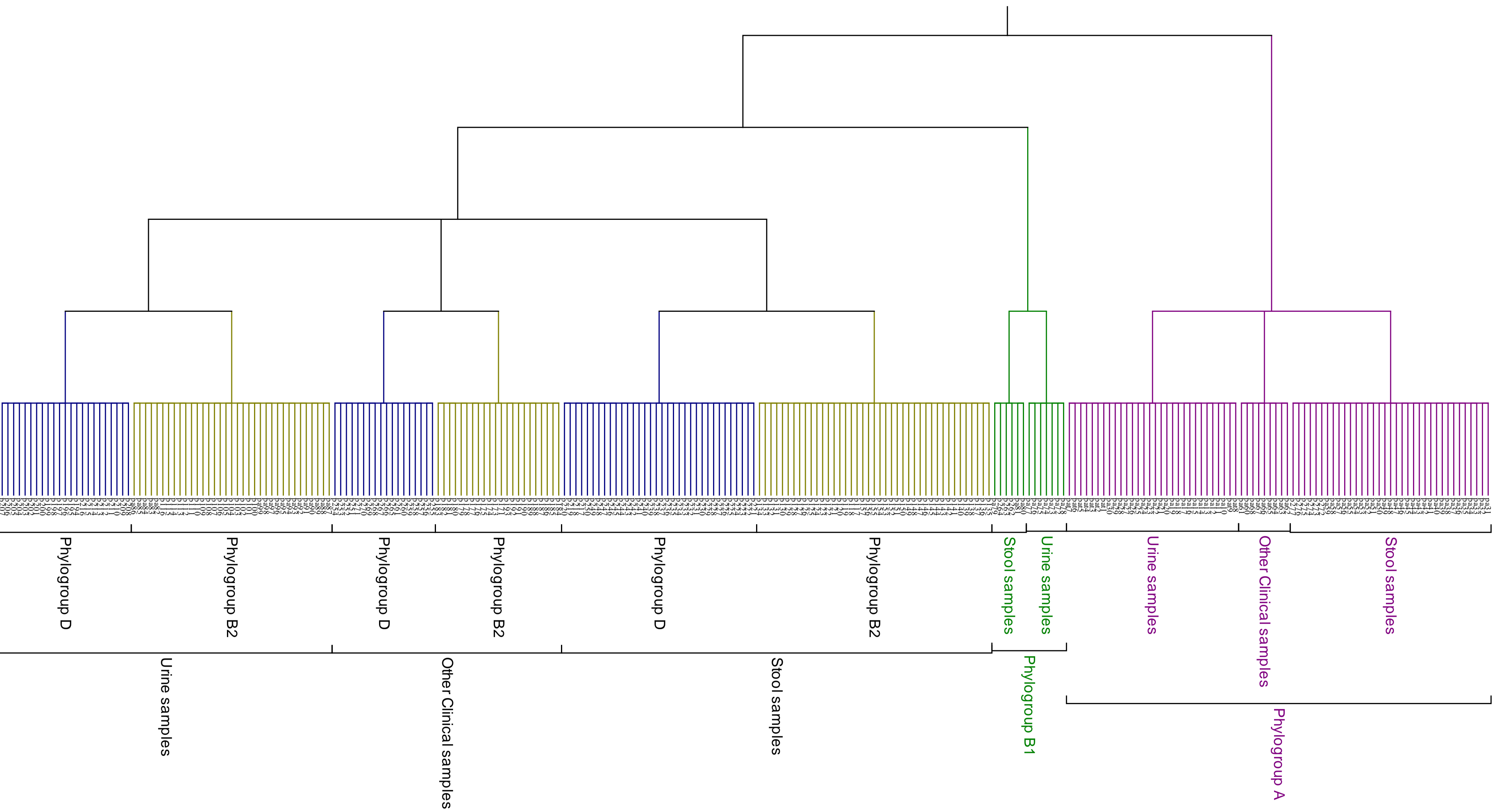


Figure 6: Phylogenetic groups of 261 *E. coli* strains isolated from clinical samples as determined by the unweighted pair group method with arithmetic averages of binary character matrix using the similarity coefficient index

than those obtained from environmental, urine and other clinical samples. Moreover, EHEC strains were found among isolates from stool samples only. EAEC and enteroinvasive *E. coli* (EIEC) strains, however, were not found in urine samples, which had the highest percentage of uropathogenic *E. coli* (UPEC) strains.

Agarose gels used for the detection of amplified products of the various *E. coli* pathotypes and the virulence genes are presented in Plates 18, 19, 20, 21, 22 and 23. The distribution of diarrhoeagenic pathotypes and virulence factors coding genes among *E. coli* strains isolated from environmental and clinical samples is presented in Table 16. The frequencies of the various diarrhoeagenic *E. coli* pathotypes differed significantly ($P < 0.01$). Generally, higher proportions of diarrhoeagenic *E. coli* pathotypes were found among *E. coli* isolates from stool samples.

Some genes were not detected in the DNA samples extracted from the various isolates. For instance, for the three genes (elt, stla and stlb) that confer enterotoxigenic traits, only stlb was detected among the isolates. Similarly, for the two genes (stx1 and stx2) that confer enterohaemorrhagic traits in *E. coli*, only stx1was detected.

Out of the 116 isolates recovered from stool samples, ETEC was the most widespread (32.76 %), followed by EPEC (26.72 %), EAEC (18.97 %) and EIEC (11.21 %) (Table 16). Among the 95 *E. coli* isolates from urine samples, ETEC and EPEC strains accounted for 2.11 % and 1.05% with no EHEC, EIEC and EAEC detected.

Isolates (50) from other clinical samples (blood, aspirate, wound and urogenital) consisted of ETEC (16.0 %), and EIEC (14.0 %), EPEC (4.0 %)

and EAEC (2.0 %) strains. However, no EHEC strain was detected among these samples.

Of the 128 *E. coli* isolates obtained from environmental samples, EIEC and ETEC strains accounted for 9.38 % and 7.03 % respectively, followed by EAEC (2.34 %) and EPEC (0.78 %) strains. Just as for the clinical samples, EHEC strains were not detected in the environmental samples.

Agarose gel profiles used in the molecular detection of uropathogenic *E. coli* strains are presented in Plates 22 and 23. The profile of genes coding for uropathogenic traits among *E. coli* isolates from environmental and clinical samples is also presented in Table 17. Of the various possible combinations of the 6 uropathogenic genes that were studied, eleven virulence patterns were recorded. All the 11 possible gene combinations were found in *E. coli* isolates from urine samples. However, the aea gene was not detected and no *E. coli* isolate with virulence trait coded for by this gene was found among the samples in this study. The most common virulence gene profiles found were for the single gene hlyA and a set of four genes: cfn1 + papC + sfa + hlyA (Table 17).

Urine samples had the highest proportion (67.37 %) of uropathogenic *E. coli* (UPEC) strains ($P < 0.01$) followed by other clinical samples (a combination of blood, wound, aspirate and urogenital samples) with 12.00 %. As low as 4.68 % and 4.31 % of isolates from environmental and stool samples, respectively, were found to be UPEC strains (Table 17).

Table 15: Distribution of Pathotypes Among *E. coli* Isolates From Various Clinical and Environmental Samples (Percentages (%) in Brackets)

Patho-types	Frequency of *E. coli* pathotypes from samples				Total
	Stool	Urine	Other[a]	Environmental[b]	
ETEC	38 (32.76)*	2 (2.11)	8 (16.00)	9 (7.03)	57 (14.65)
EPEC	31 (26.72)**	1 (1.05)	2 (4.00)	1 (0.78)	35 (9.00)
EHEC	5 (4.31)	0 (0.00)	0 (0.00)	0 (0.00)	5 (1.28)
EIEC	13 (11.21)	0 (0.00)	7 (14.00)	12 (9.38)	32 (8.23)
EAEC	22 (18.97)	0 (0.00)	4 (8.00)	3 (2.34)	29 (7.46)
UPEC	5 (4.31)	64 (67.37)***	6 (12.00)	6 (4.69)	81 (20.82)
No detection	2 (1.73)	28 (29.47)	23 (46.00)	97 (75.78)	150 (38.56)
Total	116 (100.00)	95 (100.00)	50 (100.00)	128 (100.00)	389 (100.00)

[a]: Other clinical samples; [b]: Environmental samples; UPEC = Uropathogenic *E. coli*, ETEC = Enterotoxigenic *E. coli*; EPEC = Enteropathogenic *E. coli*; EHEC = Enterohaemorrhagic *E. coli*; EIEC = Enteroinvasive *E. coli*; EAEC = Enteroaggregative *E. coli.* (*: $P < 0.01$; **: $P < 0.01$; ***: $P<0.01$)

Table 16: Distribution of *E. coli* Diarrhoeagenic Pathotypes Isolated From Environmental and Clinical Samples and Genes Coding for Their Virulence (Percentages (%) in Brackets)

Pathotypes and virulence genes	Frequency of *E. coli* pathotypes from samples			
	Stool	Urine	Other [a]	Environmental [b]
ETEC	38 (32.76)	2 (2.11)	8 (16.00)	9 (7.03)
elt	0	0	0	0
stla	38	2	8	9
stlb	0	0	0	0
elt + stla	0	0	0	0
elt + stlb	0	0	0	0
elt + stla + stlb	0	0	0	0
EPEC	31 (26.72)	1 (1.05)	2 (4.00)	1 (0.78)
eae	5	0	0	1
bfpA	2	1	1	0
eae +bfpA	24	0	1	0
EHEC	5 (4.31)	0 (0.00)	0 (0.00)	0 (0.00)
stx1	5	0	0	0
stx2	0	0	0	0
stx1 + stx2	0	0	0	0
EIEC	13 (11.21)	0 (0.00)	7 (14.00)	12 (9.38)
ial	13	0	7	12
EAEC	22 (18.97)	0 (0.00)	4 (2.00)	3 (2.34)
aggR	9	0	1	1
Aat	6	0	2	2
aggR + Aat	7	0	1	0
No detection of diarrhoeagenic genes	7 (6.03)	92 (96.84)	29 (58.00)	103 (80.47)
Total	116 (100.00)	95 (100.00)	50 (100.00)	128 (100.00)

[a] : Other clinical samples; [b] : Environmental samples; ETEC = Enterotoxigenic *E. coli*; EPEC = Enteropathogenic *E. coli*; EHEC = Enterohaemorrhagic *E. coli*; EIEC = Enteroinvasive *E. coli*; EAEC = Enteroaggregative *E. coli.*

Table 17: Virulence Pattern of Genes Coding for Uropathogenic Traits Among *E. coli* Strains Isolated From Environmental and Clinical Samples (Percentages (%) in Brackets)

No. of genes detected	Virulence pattern encountered	Frequency of *E. coli* pathotypes from samples with specific virulence profiles			
		Stool	Urine	Other[a]	Environmental[b]
1	papC	1	6	0	0
1	hlyA	2	16	1	1
2	afa + hlyA	0	3	2	0
2	cfn1 + papC	0	2	0	0
2	cfn1 + hlyA	0	3	0	0
3	cfn1 + papC + sfa	0	2	0	0
3	cfn1 + papC +hlyA	1	5	0	0
4	cfn1 + papC + sfa + hlyA	1	10	1	3
2	papC + hlyA	0	6	2	0
3	papC + sfa + hlyA	0	9	0	2
2	sfa + hlyA	0	2	0	0
No detection of uropathogenic genes		111 (95.69)	31 (32.63)	44 (88.00)	122 (95.32)
Total		116 (100.00)	95 (100.00)	50 (100.00)	128 (100.00)

[a] : Other clinical samples; [b] : Environmental samples

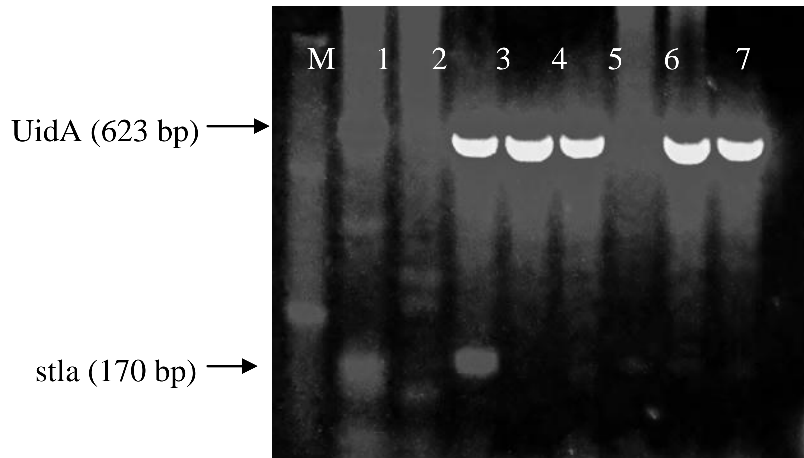


Plate 17: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting elt (322 bp), stla (170 bp) and UidA (623 bp) genes. M = 20 bp DNA ladder; Lanes 1 and 3 - UidA and stla positive; Lanes 4, 5,6, 7 and 8 - UidA positive and stla negative; Lane 2 - UidA and stla negative (negative control), No detection of elt

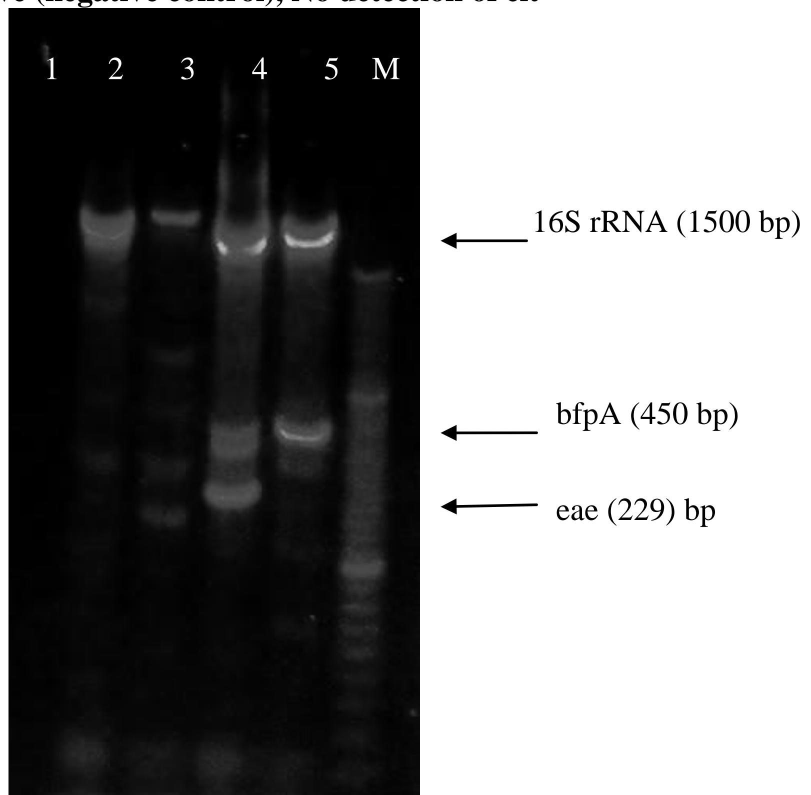


Plate 18: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting eae (229 bp), bfpA (450 bp) genes and 16S rRNA (1500 bp) as internal control. M - 20 bp ladder; Lane 1 - negative control; Lanes 2 and 3 - eae and bfpA negative; Lane 4 - eae and bfpA positive, Lane 5 - bfpA positive

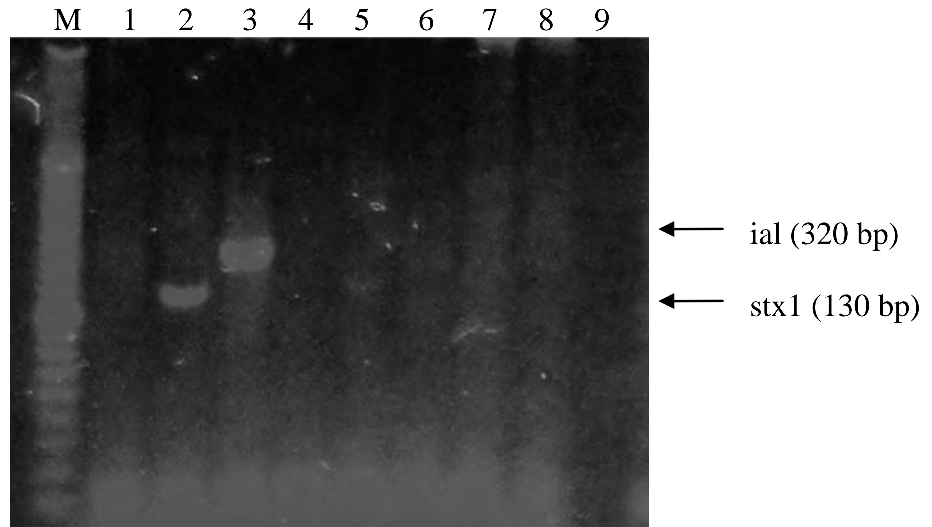


Plate 19: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting stx1 (130 bp), stx2 (510 bp) and ial (320 bp) genes. Lane M - 20 bp DNA ladder; Lanes 1, 4, 5, 6, 7, 8 and 9 - stx1 and ial negative; Lane 2 - stx1 positive; Lane 3 - ial positive; No detection of sxt2

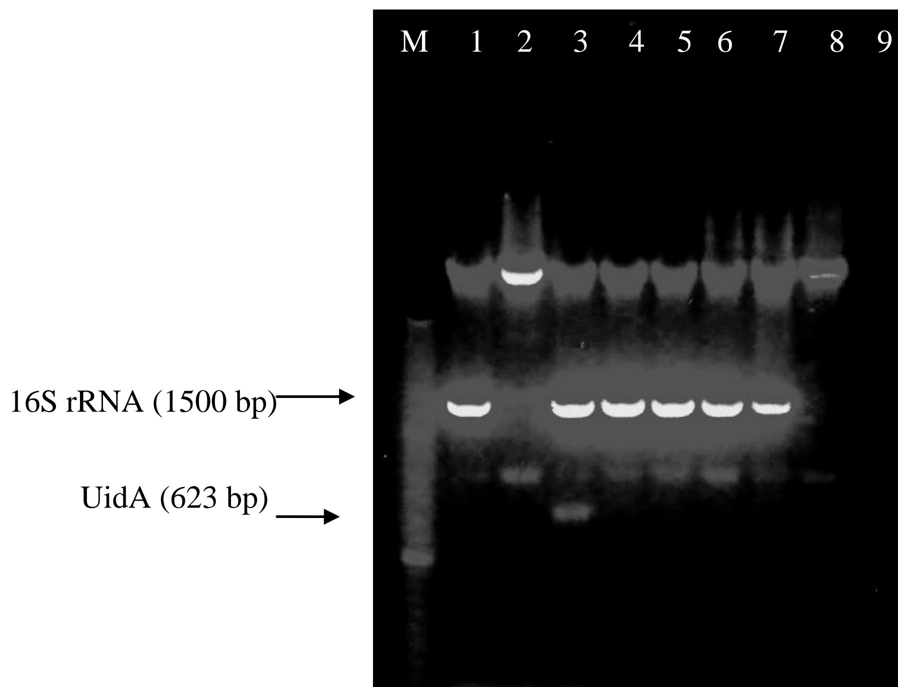


Plate 20: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting stlb (190 bp) and UidA (623 bp) genes and 16S rRNA (1500 bp) as internal control. Lane M - 20 bp DNA ladder; Lanes 1, 3, 4, 5, 6, 7 and 8 - UidA positive; Lane 2 - UidA negative; Lane 9 - negative control (molecular biology grade water); No detection of stlb

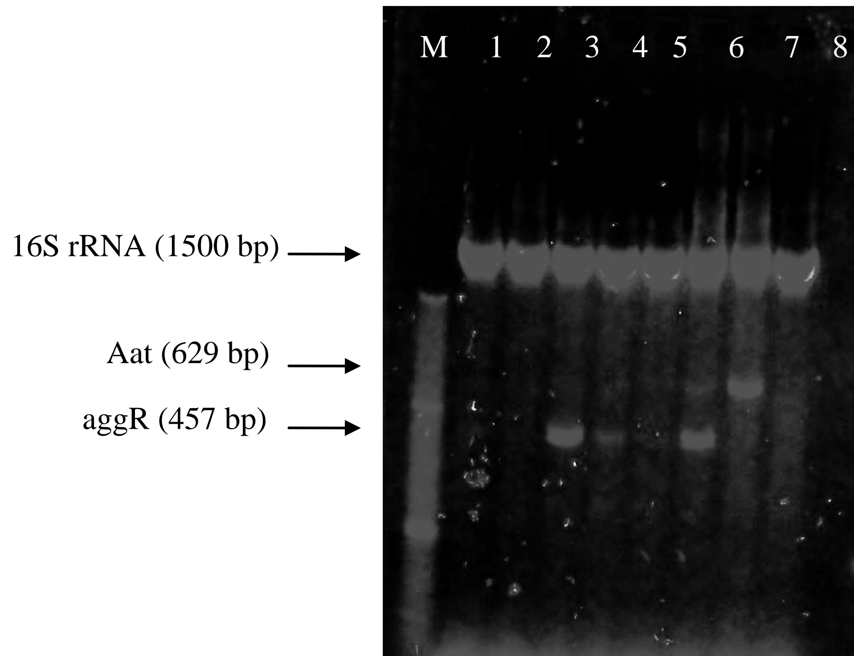


Plate 21: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting aggR (457 bp) and Aat (629 bp) genes, and 16S rRNA (1500 bp) as internal control. Lane M – 20 bp DNA ladder; Lane 9 - negative control (molecular biology grade water); Lanes 1, 2, 4, 5 and 8 - aggR and Aat negative; Lanes 3 and 6 - aggR positive, Lane 7 - Aat positive

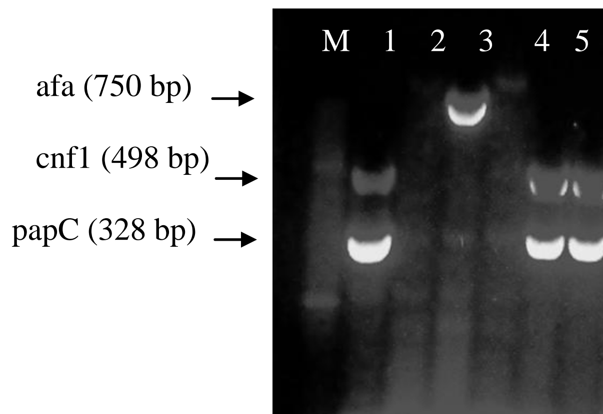


Plate 22: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting afa (750 bp), cnf1 (498 bp) and papC (328 bp) genes. M - 20 bp DNA ladder; Lanes 1, 5 and 6 - cfn1 and papC positive; Lanes 2 and 4 - cfn1, papC and afa negative; Lane 3 - afa positive

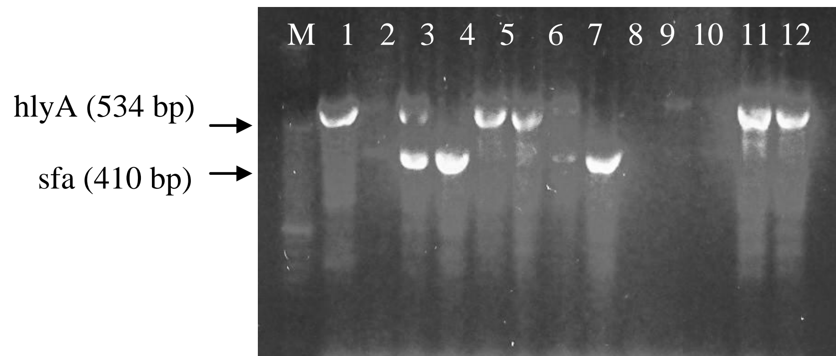


Plate 23: Ethidium bromide-stained 2.5% agarose gel revealing PCR amplified products of three primer pairs detecting aea (602 bp), sfa (410 bp) and hlyA (534 bp) genes. M - 20 bp DNA ladder; Lanes 2, 9, 10 and 11 - aea, sfa and hlyA negative; Lanes 1, 5, 6, 12 and 13 - hlyA positive; Lanes 3 and 7 - sfa and hlyA positive; Lanes 4 and 8 - sfa positive; No detection of aea

CHAPTER FIVE

DISCUSSION

The distribution, antibiotic resistance, molecular phylogeny and virulence potential of *Escherichia coli* isolates from clinical and environmental varied significantly in this study.

Distribution of *E. coli* Isolates Among Clinical and Environmental Samples

With 382 clinical and 88 environmental samples, 598 bacterial isolates were obtained, all of which belonged to the family Enterobacteriaceae. *E. coli* was widely distributed among the 382 clinical samples, with significantly ($p < 0.01$) higher frequencies obtained from urine and stool samples (Table 9). The high frequency of *E. coli* among clinical samples is not surprising since it is the most frequently isolated bacterium in most clinical settings (Clarke, Haigh, Freestone & Williams, 2003) and among the most frequently isolated in the laboratory as causative agent of infectious diseases (Orrett & Shurland, 2001).

Conversely, among environmental samples, 128 *E. coli* isolates were recovered with more than one isolate obtained from some samples while in others, no isolates were obtained. Many vegetables are grown using bovine manure and untreated irrigation waters (Solomon, Yaron & Matthews, 2002; Mukherjee, Speh, Dyck & Diez-Gonzalez, 2004). This kind of practice leads

to contamination of vegetables with enteric pathogens (Mukherjee *et al.,* 2004; Johannessen *et al.,* 2005).

In many Ghanaian and West African markets, smoked fish is mainly sold in the open exposing it to dust, houseflies and many other insects. Smoked fish in this study, was found devoid of *E. coli* probably because, essentially, the two types of smoked fish sampled (smoked Atlantic horse mackerel and smoked sardines) present an oily surface, which is not conducive for the successful growth and multiplication of most aerobic non-lipolytic bacteria. The current observations are similar to those recorded in some earlier studies in Ghana and elsewhere, where other bacterial species were isolated from various fish samples, but no *E. coli* was isolated (Novotny, Dvorska, Lorencova, Beran & Pavlik, 2004; Nyarko, Obodai, Boamponsem, Coomson, & Aniwe, 2011; Debrah, Obodai, Aheto, Ameworwor & Sarpong-Baidoo, 2011). Isolation of *E. coli* indicates faecal contamination linked to unhygienic handling practices as well as the length of the period the fish is exposed to the open air. Hence, the smoked fish sampled in this study were either fresh or handled properly or both.

Although no *E. coli* isolates were obtained from cabbage samples in this study, other closely related bacterial species, including *Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter* spp. and *Citrobacter freundii,* were isolated.

The lack of *E. coli* isolates from seawater is not surprising, since most organisms that thrive in seawater are halophiles and, thus, can tolerate the salinity and other conditions prevailing in seawater. Most enteric bacteria,

however, have a low tolerance for high salinity and when released into the sea, enteric bacteria are subjected to an immediate osmotic up-shock, which negatively affects their subsequent survival in the marine environment (Gauthier, Munro & Mohajer, 1987; Munro, Gauthier, Breittemayer & Bongio-vanni, 1989).

Among the environmental samples that tested positive for *E. coli*, the highest number of *E. coli* isolates (42.2 %) was obtained from water samples from the Fosu lagoon (Table 11). This suggests faecal contamination, which could be attributed to the high level of human activities around the lagoon. Moreover, the inadequate drainage system in the vicinity of the lagoon, as domestic wastes from households are usually collected in gutters that are connected directly or indirectly to the Fosu Lagoon, could partly contribute to this high prevalence of *E. coli*. The Kakum River, on the contrary, had the lowest number of *E. coli* isolates (4.7 %), probably, because of the nature and low level of human activity around the site of collection. Indeed, the level of human and animal activities as well as human settlements around the point of sample collection was low compared to that of the Fosu Lagoon. The gutters around the Anaafo and Abura markets had a considerable number of *E. coli* isolates (Table 11), indicating some level of faecal contamination at these sites as well.

A higher number of *E. coli* isolates (21.8 %) was obtained from fresh beef compared to the chicken samples (4.7 %). During the sample collection, it was observed that chicken was almost always sold frozen, whereas fresh beef was usually sold at butchers' shops where the sanitary conditions were

usually grossly unsatisfactory. Indeed, in most butchers' shops in Cape Coast, the meat was usually displayed on table-tops, where they stayed throughout the day exposed to unsanitary conditions which could lead to contamination. The transmission of enterohaemorrhagic *E. coli* (EHEC) in humans is primarily through the ingestion of contaminated beef or food contaminated with cattle faeces (Welch, 2006). Despite the low infection dose of 10 to 100 cells for humans, EHEC strains are usually transient members of the intestinal microflora, where they apparently do not cause any disease (Welch, 2006).

Among the clinical samples, 261 *E. coli* isolates were recovered from 73 male and 188 female patients. Among the different age groups, females of 20 – 49 years, had significantly higher ($p > 0.05$) frequencies of *E. coli* isoates than their male counterparts. However, for those aged above 70 years, a higher frequency of *E. coli* isolates was found among the males compared to females ($p < 0.01$). This could be explained by the fact that females aged 20 – 49 years were sexually active (Ghana National AIDS Control Program, 2000), while males aged above 70 years were prone to urinary tract recurrent infections (Cove-Smith & Almond, 2007). Nevertheless, the higher proportion (72 %) females among the patients screened in this study was similar to that observed in another studies conducted in Ghana (Edoh & Alomatu, 2008), which attributed the difference to the fact that women report to hospitals more frequently than men.

Antibiotic Resistance of *E. coli* Isolated From Various Samples

The sensitivity pattern of the *E. coli* isolates to 16 antibiotics varied depending on the source from which they were isolated. Consequently, the

sensitivity patterns of the *E. coli* isolates recovered from clinical samples were compared to those recovered from environmental samples in an attempt to make possible inferences as to the factors that could influence the emergence of multi-drug resistance in the environment as well as in hospital settings. The prevalence of antibiotic resistance in *E. coli* strains has been increasing progressively worldwide since the 1970s (Murray, Rensimer & Dupont, 1982; Murray *et al*., 1985; Amyes & Gemmell, 1997) and especially in the developing countries (Nys *et al*., 2004).

The percentage antibiotic sensitivity of *E. coli* isolates of environmental origin was higher than that for clinical samples (stool, urine, blood, wound and urogenital samples (Figure 4), except for the aztreonam antibiotic. The percentage sensitivity among environmental *E. coli* isolates to aztreonam was 43.8 % compared to 47.4, 60.0 and 80.2 %, respectively, for stool, urine and other clinical samples. The environmental *E. coli* isolates were also less sensitive to nalidixic acid, and amikacin antibiotics compared to *E. coli* isolates recovered from clinical samples. In many African countries with a high rate of infectious diseases, formal and informal health systems depend heavily on broad-spectrum orally administrable antibiotics (Namboodiri *et al*., 2011). Antimicrobial drug-resistant *E. coli* from human faeces and blood stream infections tend to be more similar to antimicrobial resistant and susceptible *E. coli* from retail poultry meat sources (Johnson *et al.,* 2006). These observations indicate that the selection of resistant *E. coli* is more likely to occur in animal food reservoir than in humans (Vincent *et al.,* 2010).

In most developing countries, the use of sub-therapeutic doses that are often of substandard quality (Lansang *et al*., 1990) and the partial adherence of medical professionals to an antibiotic policy, if available are among factors that contribute to the emergence and fast spread of antibiotic resistant strains (Calva, Sifuentes-Osornio, & Cerona, 1996; Okeke & Eldelman, 2001). The current study showed a moderately low percentage sensitivity of the isolates recovered from clinical samples to ampicillin (0 – 24.1 %), tetracycline (16.0 – 28.4 %), cotrimoxale (16.8 – 22.0), cefuroxime (27.6 – 43 2 %) and nalidixic acid (22.1 – 47.4 %) in this study. These findings are similar to the results of other studies (Edoh & Alomatu, 2008; Djie-Maletz *et al*., 2008; Newman *et al.*, 2011). However, Newman *et al.* (2011), found bacterial isolates, including *E. coli,* to be highly resistant to chloramphenicol instead of cefuroxime as observed in this study. Similar levels of resistance to chloramphenicol were recorded in studies conducted on children in Northern Ghana (Djie-Maletz *et al*., 2008) and the Accra Metropolis (Namboodiri *et al*., 2011). Ampicillin, tetracycline, cotrimoxazole and chloramphenicol have been reported to have been abused over a long period of time in Ghana as they are easily accessible even without prescription and are relatively inexpensive (Newman, Frimpong, Asamoah-Adu & Sampane-Donkor, 2006). The resistance of *E. coli* isolates recovered from clinical samples to cephalosporins (cefuroxime, ceftriaxone and cefotaxime) was moderately high and similar to those of *E. coli* isolates from Kumasi (KATH records, 2004 - 2008). The levels of resistance observed among the isolates obtained from clinical samples subjected to these cephalosporins were probably due to their ability to

produce extended spectrum β-lactamases (ESBL), known to hydrolyse the drugs and render them inefficient.

There was also high resistance among the isolates obtained from clinical samples to quinolones (nalidixic acid, norfloxacillin and ciprofloxacilin). For isolates obtained from stool samples 28.4, 24.1 and 21.6 % was susceptible to tetracycline, ampicillin and cotrimoxazole, respectively. These findings are similar to those reported in a study conducted on the antibiotic resistance of faecal *E. coli* isolated from healthy volunteers from eight developing countries including Ghana (Nys *et al*., 2004). Clinical isolates, generally, were resistant to ampicillin, with urinary isolates recording the highest (96.7 %) followed by faecal isolates (75.9 %). Indeed, in this study, isolates recovered from the blood, wound, aspirate and urogenital samples samples were all resistant to ampicillin, which highlights the seriousness of antibiotic resistance among patients. The isolates of environmental origin, however, were more susceptible to ampicillin (46.9 %) compared to clinical isolates. Ampicillin is a first generation cephalosporin commonly used in Ghana and worldwide. Thus, the low sensitivity of isolates to ampicillin as observed in this study connotes a high prevalence of resistance of the isolates to ampicillin, which may be due in part to the production of β-lactamases by the organisms.

Usually, resistance is conferred unto isolates through the acquisition of certain resistance genes. In Gram-negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance (Livermore, 2003). The extended spectrum β-lactamases (ESBL), particularly, have the ability to hydrolyse and cause resistance to various types of newer β-

lactam antibiotics including the extended spectrum also known as third generation cephalosporins and monobactams but not the cephamycins and carbapenems (Bradford, 2001). Therefore, it is important to detect and report ESBL-producing organisms since they remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control (Paterson & Bomono, 2005). Although *Klebsiella pneumonia* and *E. coli* remain the major ESBL producing organisms worldwide, these enzymes have also been identified in several other members of the Enterobacterioceae family and even in some non-lactose fermenters (Jacoby & Munoz-Price, 2005). ESBLs, originally reported in 1983 in a nosocomial *Klebsiella pneumonia* strain isolated in Germany, are distributed worldwide (Sarojamma & Ramakrihna, 2011). Furthermore, these enzymes used to be associated with nosocomial outbreaks of *Klebsiella pneumonia* isolates, especially in intensive care units of hospitals, but these enzymes are now found in other bacteria in the community with *E. coli* (Sarojamma & Ramakrihna, 2011).

ESBLs can be detected phenotypically using ESBL enzymes to hydrolyze different cephalosporins or genotypically using molecular techniques to detect the genes responsible for the production of these enzymes (Pitout & Laupland, 2008). The phenotypic methods are used in many developed countries for clinical diagnosis in laboratories because the tests are easy to do and cost effective, and have been incorporated in most automated susceptibility systems, making them widely accessible (Wiegand *et al.,* 2007). ESBL-producing *Klebsiella* spp. and *E. coli* are among six drug-resistant microbes to which new therapies are urgently needed (Sorlozano, Gutierrez,

Fernandez, Soto and Piedrola, 2004). However, in developing countries, such as Ghana, these tests are yet to be incorporated as part of the routine antibiotic susceptibility testing in various hospitals. Some studies though have reported the prevalence of ESBLs producers among various bacterial isolates recovered from clinical samples in Ghana (Ayisi, 2009; Feglo *et al.*, 2012). Currently, there is no documentation in Ghana on the prevalence of ESBL producers among bacterial isolates from food, water or any other environmental sample. In the current study, 29.88 % of *E. coli* isolates of clinical origin were phenotypically confirmed as ESBL producers while the rest were non-ESBL producers. The prevalence of ESBL producers among environmental samples screened was relatively lower (12.50 %). This was an indication that there was some level of multidrug resistance in the environment, suggesting that infections caused by such micro-organisms would be much more difficult to treat. This, therefore is a major contributing factor to the rapid emergence of multidrug resistance (Wellington, *et al.*, 2013) The percentage of phenotypically confirmed ESBL producers among clinical samples collected in this study was lower (29.88 %) than the 44.37 % reported in a study conducted by Ayisi (2009) in Kumasi. The presence of ESBL producers in both environmental and clinical samples suggests that there is some level of multidrug-resistant *E. coli* strains among the types of food and patients. Even though very useful, the phenotypic methods of detection of ESBL production are not able to distinguish between the specific enzymes involved (Pitout & Laupland, 2008), which could further enhance health delivery.

There are many types of ESBL, such as ^{bla}TEM, ^{bla}SHV, ^{bla}CTX, ^{bla}OXA, and ^{bla}AmpC, but the majority of them are derivatives of ^{bla}TEM and ^{bla}SHV enzymes and mostly found in *E. coli* and *K. pneumonia* (Sharma, Sharma & Ray, 2010). Several research or reference laboratories use genotypic methods for identification of specific genes responsible for the production of the ESBLs, which have the ability to detect very low levels of resistance that could be missed by phenotypic methods (Woodford & Sundsford, 2005). The molecular method commonly used is the PCR amplification of the β-lactamase TEM (^{bla}TEM) and β-lactamase SHV (^{bla}SHV) genes using oligonucleotide primers, followed by sequencing (Pitout & Laupland, 2008) to discriminate between non-ESBL parent enzymes (e.g.: ^{bla}TEM1, ^{bla}TEM2 or ^{bla}SHV1) and different variants of ^{bla}TEM or ^{bla}SHV ESBLs (e.g., ^{bla}TEM3, ^{bla}SHV2) (Bradford, 2001). In the current study, the PCR amplification of the two genes ^{bla}TEM and ^{bla}SHV was the method adopted without sequencing of the amplified products. In this study, 12.50 % and 5.10 % of phenotypically confirmed *E. coli* were negative for any of the two resistance genes possibly because they harboured some ESBL genes other than the ^{bla}TEM and ^{bla}SHV genes. It was also found that 16.2 % and 24.6 % of the phenotypically confirmed ESBL-negative *E. coli* isolates recovered from environmental and clinical samples, respectively, possessed either ^{bla}SHV or ^{bla}TEM. However, higher percentages of isolates were found to possess ^{bla}SHV only than ^{bla}TEM only among the phenotypically confirmed ESBL negative *E. coli* isolates. This suggests that ^{bla}SHV was not as good a marker as ^{bla}TEM. Similar findings were reported in a study conducted in

Pretoria, South Africa, on K. *pneumonia* isolates (Maningi *et al*., 2010). Currently, no study has been reported on the prevalence of ESBL genes in *E. coli* isolates in Ghana.

Molecular Phylogeny of *E. coli* Isolated From Clinical and Environmental Samples

E. coli strains can be divided into four main phylogenetic groups A, B1, B2 and D (Clermont, Bonacorsi & Bingen, 2000). In this study, the 389 isolates of *E. coli* obtained from environmental and clinical samples were categorized into these four phylogenetic groups (Table 12). Currently, no study has been reported on the phylogeny of *E. coli* isolated from environmental samples, such as fresh vegetables, smoked fish or water samples in Ghana or elsewhere. However, reports on phylogenetic analyses of *E. coli* isolated from clinical samples as well as animal products such as chicken, beef and pork abound (Johnson *et al*., 2003b, Vincent *et al*., 2010; Asai *et al*., 2011; Erjavec & Zgur-Bertok, 2011). A higher proportion (42.91 %) of the isolates from clinical samples belonged the phylogenetic group B2 whereas isolates from environmental samples constituted 10.94%. However, group D occurred in similar proportions for clinical (28.74 %) and environmental (28.13 %) isolates. There is a link between phylogeny and virulence (Pupo *et al*., 1997; Picard *et al*., 1999; Johnson & Stell, 2000). Consequently, extra-intestinal pathogenic *E. coli* isolates usually belong to the phylogenetic groups B2 and D, while the commensal strains belong to groups A and B1 in humans (Johnson, Delavari, Kuskowski & Stell, 2001a). Isolates belonging to group B2 are more likely to be extra-intestinal pathogenic and

hence might habour virulent genes that would enable them cause various infections outside the digestive tract, such as urinary tract infections (UTI), bacteruria and septicaemia. It was, therefore, not surprising that clinical samples recorded a higher percentage of isolates belonging to group B2 as compared to environmental isolates.

The proportion of environmental isolates recorded was relatively smaller in phylogenetic group B2 compared to clinical isolates. However, in phylogenetic groups A and B1, environmental isolates were 55.47 % and 5.47 %, respectively, whereas the respective values for clinical isolates were 28.35 % and 4.98 %. This is in agreement of reports by Johnson *et al.* (2001a) that more commensal stains are isolated from environmental samples than from clinical samples. However, in group D, similar percentages of clinical (28.74 %) and environmental (28.13 %) isolates were recorded. In a study conducted in Canada, *E. coli* isolates from retail chicken and other food sources were closely related to isolates from humans with UTI (Vincent *et al.*, 2010). Previously, extra-intestinal pathogenic *E. coli* (ExPEC) infections have been described as sporadic infections caused by bacteria that originate from the host intestinal tract (Bergeron *et al.*, 2012). However, community outbreaks have been described in the UK (Philips *et al.*, 1988), Denmark (Olesen *et al.*, 1994), the USA (Manges *et al*., 2001) and Canada (Pitout *et al*., 2005). These outbreaks therefore suggest that ExPEC can be spread from the intestinal tract of persons in the community by a common source or vehicle. In this study, majority of the *E. coli* isolates recovered from environmental samples, belonged to phylogenetic groups A and D, which is consistent with results

obtained from other studies (Jakobsen *et al*., 2010; Cortés *et al*., 2010; Asai *et al*., 2011; Bergeron *et al*., 2012).

Johnson and Stell (2000) have reported that extra-intestinal strains of *E. coli* usually belong to groups B2 and D. Pupo *et al*. (1997) have also reported that commensal strains belong to groups A and B1 while the intestinal pathogenic strains belong to groups A, B1 and D. All the 389 *E. coli* isolates grouped according to their source of isolation and their phylogeny. Clermont *et al*. (2000) have stated that the first discriminating factor is the presence or absence of the ChuA gene, which codes for an outer membrane protein important for haem utilization and transportation in enterohaemorhagic *E. coli* (EHEC). Thus, isolates that possess the ChuA gene either belong to group B2 or D, while those that lack the ChuA belong to group B1 or A (Clermont *et al*., 2000). Isolates belonging to groups B2 and D are further segregated based on the presence or absence of the YjaA gene, a hypothetical protein gene with unknown function initially identified in the complete genome sequencing of *E. coli* K12 (Clermont *et al*., 2000). Consequently, isolates that possess the ChuA gene are categorized in the group B2 while those that lack the gene belong to group D (Clermont *et al*., 2000). Similarly, among isolates belonging to groups A and B1, those that possess the TspE4C2 gene, an anonymous DNA fragment, belong to group B1 and those without belong to group A (Bonarcorsi *et al*., 2000). Though B1 remained as a single group, the other three phylogenetic groups comprised two subdivisions each. For instance B2 comprised of isolates that were Chua positive (ChuA+), YjaA positive (YjaA+) and TspE4C2 negative (TspE4C2-)

on one hand, and isolates that were ChuA+, YjaA+ and TspE4C2 positive (TspE4C2+) on the other hand. Also the phylogenetic group D comprised isolates with Chua+, YjaA negative (YjaA-) and TspE4C2- pattern on one hand and those that had a Chua+, YjaA- and TspE4C2 pattern. Finally the phylogenetic group A comprised of sub-group Ao and A1. Isolates that belonged to sub-group Ao lacked all three genes in the phylogenetic study and therefore had the following phylogenetic profile: ChuA-, YjaA- and TspE4C2-, while isolates that belonged to sub-group A1 harboured the YjaA gene but lacked the other 2 genes and hence displayed the pattern: ChuaA-, YjaA+ and TspE4C2-.

The results obtained in the current study indicated that majority of the urine isolates belonged to groups B2 and D, confirming findings by Picard (1999) and Johnson and Stell (2000). Furthermore, a sizeable number of feacal isolates also belonged to B2 and D suggesting that the patients from whom these faecal isolates were recovered were at risk of contracting UTI, particularly, in women due to their anatomical predisposition (Foxman *et al.*, 2002). A few environmental isolates were found to belong to groups B2 and D suggesting some food items could have been contaminated by faecal matter or urine, due to poor hygiene practised by the vendors. Phylogenetic group B1 was the least represented with isolates from faecal matter or urine samples. These clinical isolates, in addition to the environmental isolates in group B1 were likely to be either commensal or intestinal pathogenic *E. coli* strains according to Pupo *et al.* (1997).

The majority of the isolates in group A were predominantly (55.47 %) from environmental samples suggesting that these isolates could either be commensal or intestinal pathogenic (Pupo *et al*., 1997), since the presence of *E. coli* in food or other environmental samples, usually, implies faecal contamination (Welch, 2006). This confirms the likelihood of crops, farm lands and water bodies getting contaminated with human and animal excreta, especially during periods of heavy precipitations as a result of inadequate domestic and industrial waste management as well as poor personal hygiene. Fewer clinical isolates were also observed in phylogenetic group A compared to those in phylogenetic groups B2 and D. This suggests that the majority of the clinical isolates recovered in this study were more likely to be extra-intestinal pathogenic strains than intestinal or commensal strains of *E. coli*. It is worthy to note that currently in Ghana, no study has been published on the molecular phylogeny of *E. coli* isolated from both clinical and environmental samples.

Virulence Potential of *E. coli* Isolated From Various Samples

Intestinal and extra-intestinal *E. coli* infections are caused by strains harbouring numerous virulence factors located on plasmids, bacteriophages or the bacterial chromosome (Muhldorfer & Hacker, 1994). Several studies have shown that pathogenic *E. coli* strains may be derived from commensal strains by the acquisition of chromosomal or extra-chromosomal virulence operons (Finlay & Falkow, 1997; Ochman, Lawrence & Groisman, 2000). Intestinal *E. coli* are often referred to as diarrhoeagenic *E. coli* (DEC) and they represent any defined group of *E. coli*, which has been associated with the ability to

cause diarrhoea (Chattaway, Dallman, Okeke, & Wain, 2011). Virulence factors among *E. coli* isolates play a key role in extra-intestinal and intestinal infections (Muhldorfer & Hacker, 1994; Moyo, Maselle, Matee, Langeland, & Mylvaganam, 2007). In this study eleven virulence genes that are relevant to intestinal infections (Moyo *et al.,* 2007) and six others that play a critical role in the pathogenesis of extra-intestinal pathogenic *E. coli* (ExPEC) infections (Badri *et al*., 2009) were surveyed. The results obtained revealed that out of the three genes (stla, stlb and elt) that could confer ETEC features on the isolates, only the stla gene, which codes for heat-stable toxin was detected (Table 13). These results on faecal isolates, partly, conform to findings that all ETEC strains harbour only heat-stable enterotoxin (ST) genes, but not the heat-labile enterotoxin (LT) genes (Gunzburg, Chang, Burke, & Gracey, 1992; Okeke *et al*., 2000; Vargas *et al.,* 2004; Moyo *et al.,* 2007). No ETEC was detected among the *E. coli* isolates recovered from urine samples. However, 16 % of the *E. coli* isolates recovered from other clinical samples (blood aspirate, wound and urogenital samples) were identified as ETEC. The presence of these ETEC strains in these samples, therefore, is an indication that they might have been contracted from poor personal hygiene through faecal contamination or as a result of nosocomial infection.

ETEC are known not to cause any serious infection at body sites other than the stomach and the intestines (Nataro & Kaper, 1998). In the current study, 32.76 % of faecal and 7.03 % of environmental isolates were found to be ETEC strains. ETEC strains are a major contributor to diarrhoea among infants in developing countries and traveller's diarrhoea among visitors to

these countries (Konemon *et al*., 1997). The contamination of food with ETEC strains could pose a threat to the tourism industry in Ghana, particularly, Cape Coast, which is described as the heartbeat of tourism in Ghana.

Enteroinvasive *E. coli* (EIEC) produces dysentery-like diarrhoea, similar to that caused by *Shigella* spp, through their multiplication within epithelial cells lining of the colon (Hale, van der Woude, Braaten & Low, 1998). Detection of EIEC strains through the detection of the *ial* gene, an invasion-associated locus of the invasion plasmid found in EIEC and *Shigella* species (Moyo *et al*., 2007), showed that respectively, 11.21, 9.38 and 14.00 % of the faecal, environmental and other clinical isolates comprising isolates obtained from blood, wound, aspirate and urogenital samples, were EIEC while no EIEC was found among urine isolates. Most studies report EIEC as a minor pathogenic strain of *E. coli* (Katouli, Kühn & Möllby, 1990; Akinyemi, Oyefolu, Opere, Otunba- Payne, & Oworu, 1998; Torres *et al*., 2001). However, there are a few notable exceptions where infections caused by EIEC were health-threatening outbreaks in countries such as China, Chile, India and Senegal (Kain *et al*., 1991; Faundez *et al*., 1988; Taylor, O'Connell, Luther, & Donnenberg, 1998; Gassam-Sow *et al*., 2004). Apart from the urine isolates, the findings of this study are not in agreement with those reported in a study conducted in Tanzania, where no EIEC was detected among *E. coli* isolates from children (Moyo *et al.,* 2007).

Enteropathogennic *E. coli* (EPEC) strains were the second predominant (26.72 %) pathotypes among faecal isolates after ETEC (32.76

%). However, very low percentages were observed among the urine (1.05 %) and other clinical isolates (4.00 %). They are Shiga toxin (*stx*) negative *E. coli* strains able to produce attachment and effacement (A/E) lesions on intestinal cells, which are detectable *in vitro* by a positive *eae* or FAS test (Badri *et al*., 2009). In this study, the various isolates assigned to the EPEC pathotype was based on the detection of at least one of the two genes *eae* and *bfpA* in the genome of the test *E. coli* isolates. Typical EPEC strains are well noted for causing gastroenteritis in infants (Nataro & Kaper, 1998). The prevalence of EPEC observed in this study, therefore, indicates that they may be a source of ill-health in adults as well. Indeed, even though EPEC is primarily an infection among infants, several outbreaks of diarrhoea due to EPEC have been reported in healthy adults (Costin, Voiculescu & Gorcea, 1964; Viljamen *et al*., 1990). A very low percentage (0.78 %) of EPEC was recorded among environmental *E. coli* isolates, indicating that it may not be a public health threat. However, if foodstuffs, such as vegetables, are watered with water contaminated by faecal matter and proper hygiene is not observed it might pose some health threat considering the high prevalence among stool isolates. Reports on studies conducted in South Africa and Morocco on pathogenic *E. coli* isolates from river sources and food found diarrhoeagenic *E. coli* (DEC) such as EPEC in samples collected from both sources (Badri *et al*., 2009).

Shiga toxin *E. coli* (STEC) are shiga toxin (*stx*) positive *E. coli* strains capable of producing *stx* (Badri *et al*., 2009). Those which cause haemorrhagic colitis and haemolytic uremic syndrome (HUS) in children are referred to as enterohaemorrhagic *E. coli* (EHEC) according to Tarr (1995).

EHEC strains were the least (4.31 %) represented pathotype among the *E. coli* isolates recovered in this study and found among faecal isolates alone (Table 15). *Stx*-producing *E. coli* can be found in the faecal flora of a wide range of animals including cattle, sheep, goats, pigs, cats, dogs, chickens and gulls, but the most important among them is cattle in terms of human infection (Nataro & Kaper, 1998). Of the many EHEC strains that exist, strains of the serotype O157:H7 have been implicated in a number of life-threatening infections in many industrialized nations as well as developing countries (Bell *et al*., 1994; Nataro & Kaper, 1998). However, EHEC is much less frequently isolated in developing countries than other DEC, such as ETEC and EPEC (Nataro & Kaper, 1998), as observed in the current study.

Enteroaggregative *E. coli* (EAEC) strains were more prevalent among faecal isolates (18.97 %), followed by environmental (2.34 %) and other clinical (2.00 %) isolates based on the detection of the two plasmid borne genes - *Aat* and *AggR* (Table 16). Just like ETEC, EAEC stains have been reported to cause traveller's diarrhoea (Nataro & Kaper, 1998; Okeke, 2009) and persistent diarrhoea in infants and young children living in countries with poor sanitation. The prevalent rates found in this study were lower compared to findings by Adachi, Mathewson, Jiang, Ericsson & DuPont, (2002), where 44 % of Mexican table sauces contained viable EAEC. In Texas, a much cleaner city, EAEC the strains were not found in any sauces in the same study (Adachi *et al.*, 2002). Furthermore, two consecutive outbreaks which affected 24 individuals in Italy were linked to unpasteurized cheese (Scavia, Staffolani, & Fisichella, 2008). In Japan also, it was reported that children who ate

infected school lunches had severe diarrhoea with EAEC detected as the causal agent in 10 % of the cases (Itoh, Nagano, Kunishima & Ezaki, 1997). Indeed, food has been recognized as an effective vehicle for the transmission of EAEC as compared to ETEC (Kaur, Kumar, Ray & Kaur, Chakraborti, 2009). Thus, the detection of EAEC strain among environmental samples (water, fresh cabbage, smoked fish and beef) suggests that the health of people could be endangered if proper hygiene is not observed. It has been argued that there are inconsistencies using the identification of the aggregative adherence (AA) gene for detection of EAEC (Okeke *et al.*, 2010). Thus, the use of the gold standard for EAEC identification, a HEp-2 adherence test among cultured human cell lines, should be used in future studies for definitive results, since logistics available did not permit the use of this test.

The prevalence of uropathogenic *E. coli* (UPEC) in this study was 67.37, 4.68, 4.31 and 12.00 % respectively for urine, environmental, faecal and other clinical isolates comprising isolates obtained from blood, wound, aspirate and urogenital samples. Virulence factors associated with UPEC pathotypes include several combinations of certain somatic, capsular and flagellar antigen, the ability to adhere to the uroepithelial cells by frimbrial or afrimbrial adehesins, the production of toxins, siderophores or iron acquisition systems, serum resistance mechanisms and invasions (Yamamoto, 2007). The fact that a relatively high percentage (67.37 %) of urine isolates was recorded as UPEC strains, was not surprising since *E. coli* has been reported as a major causative agent of urinary tract infections (UTI) (Yamamoto *et al.,* 1995a). The distribution of genes for various virulence factors such as pilus associated

with pyelonephritis (pap), S fimbriae (sfa), afrimbrial adhesin 1 (afa1), haemolysin (Hly), aerobactin (aer) and cytotoxic necrotic factor 1 (cnf1) were adopted as useful markers for the detection of UPEC as proposed by Yamamoto *et al.* (1995b) for the diagnosis of UTI. The genes for all these virulence factors were detected, except the *aer* gene for aerobactin.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The distribution, antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia coli* isolated from clinical and environmental samples collected from the Cape Coast metropolis of the Central Region of Ghana were investigated. The study was approved by the Ethical Review Committee of the Ghana Health Service. Clinical samples comprising stool, urine and aspirate samples as well as wound and urogenital swabs were obtained from patients that reported to the Central Regional Hospital, Cape Coast, Ghana. Signed informed consent forms were obtained from every patient before he/ she was enrolled into the study. Environmental samples including water samples collected from the sea, Fosu lagoon, Kakum river, gutters around the Anaafo and Abura markets as well as cabbage sampled from the School of Agriculture farm and Science market of the University of Cape Coast and the Abura and Kotokuraba markets. Smoked fish and fresh beef were also obtained from those markets.

Bacterial isolation was done for all samples using MacConkey, Xylose Lysine Deoxycholate and Cystine Lactose Electrolyte Deficient Agar media. Bacterial identification was carried out using standard biochemical tests and Gram staining followed by confirmation using analytical profile index (API) 20 E kits. All *Shigella* isolates recovered in this study were noted and treated

as potential enteroinvasive *E. coli* (EIEC) and thus were added to the total number of test *E. coli* isolates.

All test *E. coli* isolates were screened against 16 antibiotics and the pattern of sensitivity observed for isolates recovered from clinical samples was recorded and compared to that of isolates obtained from environmental samples. The 16 antibiotics were: Ampicillin (10µg), Tetracycline (30µg), Co-trimoxazole (25µg), Gentamycin (10µg), Cefuroxime (30µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) Nitrofurantoin (300 µg), Nalidixic acid (30µg), Aztreonam (30µg), Imipenem (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Amikacin (30µg) and Norfloxacin (10µg).

The prevalence of extended spectrum beta lactamase (ESBL) producers among test *E. coli* isolates of environmental origin as well as that of isolates of clinical origin were determined followed by a phenotypic confirmatory test using ESBL detection disc sets.

DNA extraction from all test *E. coli* isolates was performed and the DNA samples obtained were loaded and electrophorezed in a 1 % agarose gel to check the integrity of the samples. The purity and concentration of each DNA was determined and all samples were standardized to a concentration of 100 ng/ µg before the performance of various polymerase chain reaction (PCR) assays. The detection of the ESBL genes: ^{bla}TEM and ^{bla}SHV was achieved by performing two separate PCR amplifications using the 16 S rRNA gene as an internal control. The amplification products obtained were separated electrophoretically along side with a 100 bp DNA ladder at 100 V

for an hour on a 1.5 % agarose gel containing 0.001 % ethidium bromide.The molecular phylogeny of all test *E. coli* isolates was studies multiplex PCR which enabled the detection of the presence or absence of any of three genes: ChuA, YjaA and TSPE4C2. The analysis of the pattern of bands obtained resulted in the assignment of the isolates to either of the A, B1, B2 or D phylogenetic groups. The molecular phylogeny of the isolates and that of isolates obtained from environmental samples were compared and inferences were made. A dendogram of dissimilarity was constructed to assess the inter relatedness of the test *E. coli* isolates. A virulence gene profiling was carried out on all DNA samples using specific primers to get the common pathotypes associated with *E. coli* isolates obtained from Cape Coast. This was achieved by running 7 different PCR assays targeted at the categorization of the isolates into ETEC, EPEC, EHEC, EIEC, EAEC and UPEC pathotypes.

In all 598 bacterial isolates were recovered and identified from which 372 were confirmed as *E. coli* while 17 as *Shigella* spp. bringing the number of test *E. coli* isolates to a total of 389. The 389 test *E. coli* isolates comprised 261 isolates of clinical origin and 120 of environmental origin. Among the clinical samples, stool samples recorded significantly the highest number of test *E. coli* isolates followed by urine samples while blood samples recorded the least number of *E. coli* test isolates. Among the environmental samples, the Fosu lagoon significantly recorded the highest number of test *E. coli* isolates followed by fresh beef while the Kakum river and chicken samples recorded the least ($p = 0.000$). No *E. coli* or *Shigella* was isolated from the seawater, cabbage and smoked fish samples. The analysis of the demographic

data revealed that 261 clinical isolates comprised of 73 isolated from male patients and 188 from their female counterpart. Statistical analysis revealed that there was no significant ($p>0.005$) difference between the frequencies of the various age groups except for age group 20 – 29 years, 30 – 39 years, 40 – 49 years and above 70 years.

For every age group higher numbers of *E. coli* isolates were recovered from the females than the males, except for the age group above 70 years which recorded a higher number than their female counter part. Generally, the least percentage sensitivity was recorded when all *E. coli* isolates were screened against ampicillin and none of the isolates recovered from the blood, wound or urogenital samples was found to be sensitive to ampicillin. All environmental and clinical isolates screened in this study were sensitive to Imipenem. In all categories, the percentage sensitivity of the isolates recovered from environmental samples to the 16 antibiotics was higher than that of other isolates except for nalidixic acid, aztreonam and amikacin where isolates of environmental origin recorded lower percentages of sensitivity. The isolates obtained from stool samples however, recorded higher sensitivity when they were screened against those three antibiotics. The percentage sensitivity of all *E. coli* to ampicillin (0 – 24 %), tetracycline (16.0 – 28.4 %), cotrimoxazole (16.8 – 22.0 %), cefuroxime (27.6 – 43.2 %) and nalidixic acid (22.1 – 47. 8%) were found to be moderately low.

An estimated 29.88 % of the test *E. coli* isolated from clinical samples and 12.50 % of those obtained from environmental samples were phenotypically confirmed as ESBL producers. Out of the isolates that were

phenotypically confirmed as ESBL producers, 12. 5 % and 5. 1 % of environmental and of clinical origin respectively were found not harbouring either of ^{bla}TEM and ^{bla}SHV genes. An estimated 25 % each isolates recovered from environmental and clinical samples respectively that were phenotypically confirmed ESBL producers harboured the ^{bla}TEM gene while the single detection of ^{bla}SHV was recorded in 50.0 % and 47.4 % respectively of environmental and clinical isolates that were phenotypically confirmed as ESBL producers. Lower percentages however were obtained for the double detection of both genes among phenotypically confirmed ESBL producers as isolates of environmental origin recorded 12.5 % while those of clinical origin recorded 21.8 %.

The results obtained from the molecular phylogeny revealed that 42.91 % of clinical isolates belonged to B2 as opposed to the 11.72 % of environmental isolates. Also 26. 05 % and 27. 20 % of clinical isolates belonged to group D and A respectively as compared to environmental isolates which recorded 35.16 % and 53. 13 % respectively. The group B1 was the least represented as no environmental isolate and 3. 83 % of the clinical isolates were found belonging to that group.

The isolates recovered from stool samples recorded higher percentages of enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and enterohaemorragic *E. coli* (EHEC) as compared to isolates obtained from environmental, urine and other clinical samples. Additionally, EHEC strains were obtained only from isolates recovered from stool samples. No EAEC and EIEC strains however were

obtained from isolates recovered from urine samples. The highest percentage of uropathogenic *E. coli* (UPEC) strains was obtained from isolates recovered from urine samples.

Conclusions

This study underlines the importance of understanding the distribution, antimicrobial resistance, phylogeny as well as the composition of virulent strains and diversity of *Escherichia coli* strains isolated from clinical and environmental sources. The data obtained represent baseline information for enhanced clinical and epidemiological monitoring and healthcare delivery within the Cape Coast Metropolis in the Central Region of Ghana. This study highlights the role of various environmental sources including retail fresh vegetables, beef, chicken as well as polluted water bodies and gutters as reservoirs for the transmission and spread of pathogenic *E. coli* in the community.

Out of 382 clinical samples including: urine, stool, urogenital, wound, aspirates and blood samples that were screened, 261 *E. coli* were isolated. Stool samples recorded the highest percentage of *E. coli* (44.4 %), followed by urine samples (36.4 %); while blood recorded the least percentage of (1.6 %). Out of 88 environmental samples including: the Anaafo and Abura markets, the Kakum river, the Fosu lagoon, seawater, cabbage, fresh beef, smoked fish and chicken samples, that were screened, 128 *E. coli* were isolated. The Fosu lagoon recorded the highest percentage of *E. coli* (44.2 %), followed by fresh beef samples (21.8 %); while no *E. coli* was isolated from sea water, cabbage and smoked fish (0 %).

Generally, the least percentage sensitivity of the *E. coli* isolates was recorded for Ampicillin. However, none of the *E. coli* isolates obtained from blood, wound or urogenital samples was found to be sensitive to Ampicillin. The percentage sensitivity of environmental *E. coli* isolates was higher than clinical *E. coli* isolates except for Nalidixic acid, Aztreonam and Amikacin eventhough *E. coli* isolates obtained from stool samples recorded higher sensitivity to those three antibiotics. All environmental and clinical *E. coli* isolates were sensitive to Imipenem.

Furthermore, 29.88 % and 12.50 % of *E. coli* isolates of clinical and environmental origin respectively were found to be ESBL producers; the rest in both cases, were non-ESBL producers.

Additionally, 5.4 % and 4.9 % of environmental and clinical isolates respectively that were phenotypically confirmed non-ESBL producers were fond to habour only ^{bla}TEM; while, 10.8 % and 18.6 % of environmental and clinical isolates respectively that were phenotypically confirmed non-ESBL producers, were found to habour only ^{bla}SHV. There was no detection of either genes in 83.8 % and 75.4 % of environmental and clinical isolates respectively phenotypically confirmed ESBL producers.

The molecular phylogeny revealed that among *E. coli* isolates, group B_2 was highly represented (37.93 %), followed by group A (28.74 %) and group D (28.35 %), while group B_1 was the least represented (3.83 %). Among environmental E. coli isolates however, group A was highly represented (55.47 %) followed by group D (28.13 %)and group B_2 (10.94 %)while group B_1 was the least represented (5.47 %).

The proportions of enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC) from stool, were significantly higher ($P < 0.01$) than those obtained from environmental, urine and other clinical samples. EHEC was found only in isolates from stool, while EAEC and interoinvasive *E. coli* (EIEC) were not found in urine samples. *E. coli* isolates obtained from urine samples recorded the highest proportion of uropathogenic *E. coli* (UPEC).

This study has established a background database for further studies on pathogenic as well as commensal strains of *E. coli* in Ghana.

Recommendations

This study presents a number of limitations, which could be addressed through the implementation of the following recommendations:

1. The detection of β-lactamase genes ^{bla}TEM and ^{bla}SHV was made using genomic DNA of *E. coli* isolates. A better understanding, however, of extended spectrum β-lactamases (ESBL) production can be achieved by detecting ^{bla}TEM and ^{bla}SHV genes on bacterial chromosome and plasmids. Further molecular studies are needed to characterize the types of ESBL prevailing in the country including those harbouring the ^{bla}CTM gene, an emerging type of ESBL.
2. There is also the need to match the genotypes of *E. coli* isolated from various sources using more informative molecular techniques such as multi-locus sequence typing (MLST) and enzymatic digestion followed by pulse field gel electrophoresis.

3. In this study the environmental isolates were under sampled as compared to clinical isolates, thereby preventing a strong basis of comparison between these two groups. Therefore, further studies are needed which will address this limitation.
4. It was not possible to include standard strains of pathogenic *E. coli* in the PCR assays for use as positive control due to the difficulty in obtaining these strains. Further studies are needed which would tackle this issue.
5. A more comprehensive approach toward the control of *E. coli* related infections should be put in place to limit the spread of *E. coli* into the community.
6. Also an integrated approach should be put in place to properly regulate both the sale and consumption of antibiotics so as to limit their abuse in the country and ultimately minimize the emergence of antibiotic resistance.
7. For lack of data availability at the hospital, the detailed clinical history of patients could not be recorded. This could have enabled a better interpretation of results by relating clinical symptoms to pathogenicity, which could have culminated in obtaining valuable information on the molecular epidemiology of the pathogen. Therefore, further studies are needed which will address this limitation.
8. The inabilitiy to perform HEp-2 assays for the identification of enteroaggregative *E. coli* (EAEC) also represents a limitation of this study. Further studies are required to address this limitation.

REFERENCES

Abduch-Fabrega, V. L., Piantino-Ferreira, A. J., da Silva-Patricio F. R., Brinkley, C. & Affonso-Scaletsky, I. C., (2002). Cell-detaching *Escherichia coli* (CDEC) strains from children with diarrhoea: identification of a protein with toxigenic activity. *Federation of European Microbiological Societies Microbiology Letters*, *217*, 191 - 197.

Abdul-Razzaq, M. S. & Abdul-Lateef, L. A. (2011). Molecular phylogeny *of Escherichia coli* isolated from clinical samples in Hilla, Iraq. *African Journal of Biotechnology*, *10* (70), 15783 - 15787.

Accra Metropolitan Assembly [AMA] (2006). Know more about the AMA: Heath Sector. Retrieved June 2, 2011, from www.ama.com

Acheson, D. W. K., (2000). How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? *Journal of Food Protection. 63* (6), 819 - 821.

Acres, S. D. (1985). Enterotoxigenic *Escherichia coli* infections in newborn calves: A Review. *Journal of Dairy Science*, *68*, 229 - 256.

Adachi, J. A., Mathewson, J. J., Jiang, Z. D., Ericsson, C. D. & DuPont, H. L. (2002). Enteric pathogens in Mexican sauces of popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Annals of Internal Medicine, 136*, 884 - 7.

Addo, K. K., Mensah, G. I., Bekoe, M., Bonsu, C. &. Akyeh, M. L. (2009). Bacteriological quality of sachet water produced and sold in Teshie-

Nungua suburbs of Accra, Ghana. *African Journal of Food, Nutrition, Agriculture and Development*, *9*, 1019 - 1030.

Addy, P. A. K., Antepim, G. & Frimpong, E. H. (2004). Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. *East African Medical Journal, 81* (7), 353-357

Agbodaze, D., Abrahams, C.A. & Arai, S. (1988). Enteropathogenic and enterotoxigenic *Escherichia coli* as aetiological factors of infantile diarrhoea in rural and urban Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 82* (3), 489 - 491.

Agbodaze, D., Nmai, P. N. A., Robertson, F. C., Yeboah-Manu, D., Owusu-Darko, K., & Addo, K. K. (2005). Microbiological quality of "Khebab" consumed in the Accra metropolis. *Ghana Medical Journal, 39* (2), 46 - 49.

Akinyemi, K. O., Oyefolu, A. O., Opere, B., Otunba- Payne, V. A. & Oworu, A. O. (1998). *Escherichia coli* in patients with acute gastroenteritis in Lagos, Nigeria. *East African Medical Journal, 75*, 512 - 515.

Akopyanz, N., Bukanov, N. O., Westblom, T. U. & Berg, D. E. (1992). PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. *Nucleic Acids Research, 20*, 6221 - 6225.

Ambler, R. P. (1980). The structure of beta-lactamases. *Philosophical Transactions of the Royal Society of London, Series B*, *289* (1036), 321 – 331.

Amyes, S. G. B & Gemmell, C. G. (1997). Antibiotic resistance. *Journal of Medical Microbiology*, *46*, 436 – 446.

Asai, T., Masani, K., Sato, C., Hiki, M., Usui, M., Baba, K., Ozawa, M., Harada, K., Aoki, H. & Sawada, T. (2011). Phylogenetic groups and cephalosporin resistance genes of *Escherichia coli* from diseased food-producing animals in Japan. *Acta Veterinaria Scandinavica*, *53* (52), 1 - 5.

Atlas, R. M. (1995). *Principles of Microbiology* (1st ed.) London, UK: Mosby-Year BooK, Inc.

Ayisi, L. A. (2009). *Extended spectrum beta-lactamase production among Escherichia coli and Klebsiella species at the Komfo Anokye Teaching Hospital in Kumasi, Ghana.* MPhil Thesis. Retrieved October 2010, from www.dspace.knust.edu.gh:8080/jspui/bitstream/.../113/.../ESBL@KATH.pd.

Bacteriological Analytical Manual (1998). BAM Media M40: Christensen's Urea Agar. Retrieved July 5, 2011, from www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM.

Badger, J. L., Wass, C. A. & Kim, K. S. (2000a). Identification of *Escherichia coli* K1 genes contributing to human brain microvascular endothelial cell invasion by differential fluorescence induction. *Molecular Microbiology, 36*, 174 – 182.

Badger, J. L., Wass, C. A., Weissman, S. J. & Kim, K. S. (2000b). Application of signature-tagged mutagenesis for identification of *Escherichia coli* K1 genes that contribute to invasion of human brain microvascular endothelial cells. *Infection and Immunity, 68*, 5056 – 5061.

Badri, S., Filliol, I., Carle, I., Hassar, M., Fassouane, A. & Cohen, N. (2009). Prevalence of virulence genes in *Escherichia coli* isolated from food in Casablanca. *Food Control, 20*, 560 - 564.

Balis, E., Vatopoulos, A. C., Opoulou, M. K., Mainas, E., Hatzoudis, G., Kontogianni, V., Malamou-Lada, H., Kitsou-Kiriakopoulou, S. & Kalapothaki, V. (1996). Indications of *in vitro* transfer of an epidemic R plasmid from *Salmonella enteritidis* to *Escherichia coli* of the normal human gut flora. *Journal of Clinical Microbiology, 34* (4), 977 - 979.

Bann, J. G. (2002). Adhesive pili of the chaperone-usher family. In M. S. Donnenberg (Ed.), *Escherichia coli Virulence Mechanisms of a Versatile Pathogen,* (pp. 289 – 306). New York: Academic Press.

Barnich N., Carvalho F. A., Glasser A. L., Darcha C., Jantscheff P., Allez, M., Peeters, H., Bommelaer, G., Desreumaux, P., Colombel, J. F. & Darfeuille-Michaud, A. (2007). CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. *Journal of Clinical Investigation, 117*, 1566 - 1574.

Bedenic, B., Randegger, C. C., Boras, A. & Hachler, H. (2001). Comparison of five different methods for detection of SHV extended-spectrum-lactamases. *Journal of Chemotherapy*, *13*(1), 24 - 33.

Bell, B. P., Goldoft, M., Griffin, P. M., Davis, M. A., Gordon, D. C., Tarr, P. I., Bartleson, C. A., Lewis, J. H., Barrett, T. J. & Wells, J. G. (1994). A multistate outbreak of *Escherichia coli* O157: H7-associated bloody diarrhoea and haemolytic uremic syndrome from hamburgers: The Washington Experience. *Journal of the American Medical Association, 272*, 1349 - 1353.

Bell, J. M. & Turnidge, J. D. (2002). High rates of multi-resistance in bacteraemia *Escherichia coli*: Results from the SENTRY Asia-Pacific (APAC) Surveillance Program, 1998-2001. *Gateway to the USA National Library of Medicine.* Retrived August 16, 2010, from http://www.gateway.nlm.nih.gov/MeetingAbstracts/102267313.html.

Bentley, R. & Meganathan, R. (1983). Vitamin K biosynthesis in bacteria-precursors, intermediates, enzymes and genes. *Journal of Natural Products, 46*, 44 - 59.

Bergeron, C. R., Prussing, C., Boerlin, P., Daignault, D., Dutil, L., Reid-Smith, R. J., Zhanel, G. G. & Manges, A. R. (2012). Chicken as Reservoir for Extraintestinal Pathogenic *Escherichia coli* in Humans, Canada. *Emerging Infectious Diseases*, *18*(3), 415 – 421.

Bergthorsson, U. & Ochman, H., (1998). Distribution of chromosome length variation in natural isolates of *Escherichia coli*. *Molecular Biology and Evolution*, *15*, 6 - 16.

Bermudez, M., & Hazen, T. C. (1988). Phenotypic and genotypic Comparison of *Escherichia coli* from pristine tropical waters. *Applied Environmental Microbiology*, *54*, 979 - 983.

Bern, C., Martines, J., de Zoysa, I. & Glass, R. I. (1992). The magnitude of the global problem of diarrheal disease: A ten-year update. *Bulletin of the World Health Organization*, *70*, 705 - 14.

Bertin, Y., Girardeau, J. P., Darfeuille-Michaud, A. & Contrepois, M. (1996a). Characterization of 20K fimbria, a new adhesin of septicaemic and diarrhoea-associated *Escherichia coli* strains, that belongs to a family of adhesins with N-acetyl-D-glucosamine recognition. *Infection and Immunity, 64*, 332 - 342.

Bertin, Y., Martin, C., Oswald, E. & Girardeau, J. P. (1996b). Rapid and specific detection of F17-related pilin and adhesin genes in diarrheic and septicaemic *Escherichia coli* strains by multiplex PCR. *Journal of Clinical Microbiology*, *34*, 2921 - 2928.

Besser, R. E., Lett, S. M., Weber, J. T., Doyle, M. P., Barrett, T. J., Wells, J. G. & Griffin, P. M. (1993). An outbreak of diarrhoea and haemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *Journal of the American Medical Association*, *269*, 2217 - 2220.

Bien, J., Sokolova, O. & Bozko, P. (2012). Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *International Journal of Nephrology, 681473*, 1 - 15.

Biology Online (2005). Definition of cross-resistance. Retrieved August 19, 2010, from http://www.biologyonline.org/dictionary/Cross-resistance.

Black, R. E. (1990). Epidemiology of travellers' diarrhoea and relative importance of various pathogens. *Reviews of Infectious Dis*eases, *12*, 73-9.

Blattner, F. R., Plunkett, G., Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado, V. 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. & Shao, Y. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science*, *277*, 1453 - 1474.

Boerlin, P., Travis, R., Gyles, C. L., Reid-Smith, R., Lim, N. J. H., Nicholson, V., McEwen, S. A., Friendship, R. & Archambault, M. (2005). Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Applied Environmental Microbiology, 71* (11), 6753 - 6761.

Bonacorsi, S. P., Clermont, O., Tinsley, C., Le Gall, I., Beaudoin, J. C., Elion, J., Nassif, X. & Bingen, E., (2000). Identification of regions of the *Escherichia coli* chromosome specific for neonatal meningitis-associated strains. *Infection and Immunity, 68*, 2096 – 2101.

Bonnet, R. (2004). Growing group of extended-spectrum *ß*-lactamases: the CTX-M enzymes. *Antimicrobial Agents and Chemotherapy*, *48* (1), 1 – 14.

Borczyk, A. A., Karmali, M. A., Lior, H. & Duncan, L. M. (1987). Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. *Lancet, 1*(8524), 98.

Bosu, W. K. & Acquah, S. (1996). Susceptibility of urinary tract bacteria to antibiotics in Cape Coast, Ghana. *East African Medical Journal*, *73*(7), 468 - 470.

Bouckenooghe, A. R., Jiang, Z. D., de la Cabada, F. J., Ericsson, C. D. & DuPont, H. L. (2002). Enterotoxigenic *Escherichia coli* as cause of diarrhoea among Mexican adults and US travellers' in Mexico. *Journal of Travel Medicine*, *9*, 137 - 140.

Bradford, P. A. (2001). Extended-spectrum *β*-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*, *14*(4), 933 – 951.

Braun, V. and Braun, M. (2002). Active transport of iron and siderophore antibiotics. *Current Opinion in Microbiology, 5*(2), 194 - 201.

Brooks, G. F., Butle, J. S., Morse, S. A. & Jawetz, M. (2004). *Adelberg's Medical Microbiology* (23rd International Edition). San Franscisco: McGraw-Hill Companies.

Brown, A. E. (2007). *Benson's Microbiological Applications: Laboratory Manual in General Microbiology* (10th ed) New York, NY: McGraw-Hill Companies.

Brown, D. F. J., & L. Brown. (1991). Evaluation of the E test, a novel method of quantifying antimicrobial activity. *Journal of Antimicrobial Chemotherapy*, *27*, 185 - 190.

Burland, V., Shao, Y., Perna, N. T., Plunkett, G., Sofia, H. J. & Blattner, F. R. (1998). The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic Acids Research*, *26*, 4196 - 4204.

Bush, K., Jacoby, G. A. & Medeiros, A. A. (1995). A functional classification scheme for *β*-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, *39* (6), 1211 – 1233.

Calva, J. J., Sifuentes-Osornio, J. &. Cerona, C. (1996). Antimicrobial Resistance in Fecal Flora: Longitudinal Community-Based Surveillance of Children from Urban Mexico. *Antimicrobial Agents and Chemotherapy*, *40*(7), 1699 – 1702.

Cape Coast Metropolitan Assembly (2006a). Retrieved November 28, 2012, from http://www.centralregion.gov.gh/index.php?option=com_k2&view=item&id=43:capecoast&Itemid=135.

Cape Coast Metropolitan Assembly (2006b). Retrieved May 28, 2009, from http://www.ghanadistricts.com/districts.

Carlos, C., Pires, M. M., Stoppe, N. C, Hachich, E. M., Sato, M. I. Z., Gomes, T. A. T., Amaral, L. A. & Ottoboni, L. M. M. (2010). *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BioMed Central Microbiology*, *10*, 161.

Carter, M.W., Oakton, K. J., Warner, M. & Livermore, D.M. (2000). Detection of extended-spectrum beta-lactamases in *Klebsiellae* with

the Oxoid combination disk method. *Journal of Clinical Microbiology*, *38*, 4228 – 4232.

Casalino, M., Latella, M. C., Prosseda, G. & Colonna, B. (2003). CadC is the preferential target of a convergent evolution driving enteroinvasive *Escherichia coli* toward a lysine decarboxylase-defective phenotype. *Infection and Immunity, 71*, 5472 – 5479.

CDC (2005). What is an antibiotic? In National Antimicrobial Resistance Monitoring System (NARMS), Department of Health and Human Services. Frequently asked questions about antibiotic resistance. Retrieved August 16, 2008, from: http://www.cdc.gov/narms/faq_pages.

CDC (2011). Germany Outbreak. Retrived June 27, 2011, from http://wwwnc.cdc.gov/travel/notices/outbreak-notice/2011-germany-europe-e-coli.htm.

Centre for Food Security and Public Health (2009). *Enterohemorrhagic Escherichia coli infections.* USA.

Chattaway, M. A., Dallman, T., Okeke, I. N. & Wain, J. (2011). Enteroaggregative *E. coli* O104 from an outbreak of HUS in Germany 2011, could it happen again? *Journal of Infection in Developing Countries*, *5* (6), 425 – 436.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries.* Part 2 (1st ed.), Cambridge: Cambridge University Press.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. Part 2 (2nd ed.), Cambridge: Cambridge University Press.

Clarke, S. C. (2001). Diarrhoeagenic *Escherichia coli* - an emerging problem? *Diagnostic Microbiology and Diseases, 41*, 93 – 98.

Clarke, S. C., Haigh, R. D., Freestone, P. P. E. & Williams, P. H. (2003). Virulence of Enteropathogenic *Escherichia coli*, a Global Pathogen. *Clinical Microbiology Reviews*, *16* (3), 365 – 378.

Clermont, O., Bonacorsi, S. & Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology, 66*, 4555 - 4558.

Clinical and Laboratory Standards Institute (CLSI) (2010). Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Information Supplement. *CLSI document M100-S20*. CLSI, Wayne, Pennsylvania, USA.

Co-ordination Action Food (CAF) (2007). Methods for Detection and Molecular Characterisation of Pathogenic *Escherichia coli*. O'Sullivan, J.; Bolton, D. J., Duffy, G., Baylis, C., Tozzoli, R., Wasteson, Y. and Lofdahl, S (Eds.).

Cohen, M. B., Nataro, J. P., Bernstein D. I., Hawkins, J., Roberts, N. & Staat, M. A. (2005). Prevalence of diarrhoeagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. *Journal of Pediatrics*, *146*(1), 54 - 61.

Connell, I., Agace, W., Klemm, P., Schembri, M., Marild, S. & Svanborg, C. (1996). Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. *Proceedings of the National Academy of Science of the United State of America, 93*, 9827 – 9832.

Connor, B. (2013). Travelers' health, Center for Disease Control and Prevention. Retrieved March 10, 2015, from: www.nc.cdc.gov.

Contrepois, M., Bertin, Y., Pohl, P., Picard, B. & Girardeau, J. P. (1998). A study of relationships among F17 a producing enterotoxigenic and non -enterotoxigenic *Escherichia coli* strains isolated from diarrhoeic calves. *Veterinary Microbiology, 64*, 75 - 81.

Conway, P. L. (1995). Microbial ecology of the human large intestine. In G. R. Gibson and G. T. Macfarlane, (Eds.), *Human colonic bacteria: role in nutrition, physiology, and pathology* (pp. 1 – 24). Boca Raton, USA: CRC Press.

Corkish J. D. & Assoku, R. K. G. (1973). Public health hazard of antibiotic resistance in enterobacterial (*E. coli* and *Salmonella)* isolates from poultry in Ghana. *Ghana Medical Journal, 12*, 430 – 432.

Cormican, M. G., S. A. Marshall, & R. N. Jones (1996). Detection of extendedspectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. *Journal of Clinical Microbiology*, *34*, 1880 - 1884.

Cortés, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J. E., Blanco, M., López, C., Andreu, A., Navarro, F., Alonso, M. P., Bou, G., Blanco, J. & Llagostera, M. (2010). Isolation and characterization of potentially pathogenic antimicrobial resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Applied Environmental Microbiology*, *76*, 2799 – 805. Doi: 10.1128/AEM.02421-09.

Costin, I., Voiculescu, D. & Gorcea, V. (1964). An outbreak of food poisoning in adults associated with *Escherichia coli* serotype 86:B7:H34. *Pathologia et Microbiologia (Basel), 27*, 68 – 78.

Cove-Smith, A. & Almond, M. (2007). Management of urinary tract infections in the elderly. *Trends in Urology Gynaecology & Sexual Health*, July/August, 31 – 34.

Cravioto A., Tello A., Villafán, H., Ruiz J., Del Vedovo, S. & Neeser, J. R. (1991). Inhibition of localized adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk. *Journal of Infectious Diseases*, *163*, 1247 – 1255.

Croxen, M. A. & Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews: Microbiology*, *8*, 26 - 38.

Dam, H. (1935). The antihaemorrhagic vitamin of the chick. *Biochemical Journal, 29*, 1273-1285.

Debrah, J. S., Obodai, E. A., Aheto, D. W., Ameworwor, D. H. A. K., & Sarpong-Baidoo, M. (2011). Coliform and *Escherechia coli* levels in the Yellow Fin Tuna (*Thunnus albacares*) landed at the dixcove beach in Ghana. *Journal of Microbiology and Biotechnology Research*, *1*(4), 198 – 205.

Desjardins, P., Picard, B., Kaltenbock, B., Elion, J. & Denamur, E. (1995). Sex in *Escherichia coli* does not disrupt the clonal structure of the population: evidence from random amplified polymorphic DNA and

restriction fragment-length polymorphism. *Journal of Molecular Evolution, 41*, 440 - 448.

Dhillon, R. H. P. & Clark, J. (2012). ESBLs: A clear and Present Danger? Diarrhoeagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. *Critical Care Research and Practice*, *625170*, 1 – 11.

Djie-Maletz, A., Reither, K., Danour, S., Anyidoho, L. Danikuu, F., Ziniel, P. Weitzel, T., Wagner, J., Saad, E., Bienzle, U., Stark, K., Seidu-Korkor, A., Mockenhaupt, F. P. & Ralf, I. (2008). High rate of resistance to locally used antibiotics among enteric bacteria from children in Northern Ghana *Journal of Antimicrobial Chemotherapy, 61*, 1315 – 1318.

Dodoo, D. K., Quagraine, E. K., Okai-Sam, F., Kambo D. J. & Headley, J. V. (2006). Quality of sachet water in the Cape Coast Municipality. *Journal of Environmental Science and Health*, *3*, 329 - 342.

Donaldson, H., McCalmont, M., Livermore, D. M., Rooney, P. J., Ong, G., McHenry, E., Campbell, R. & McMullan, R. (2008). Evaluation of the VITEK 2 AST 481 N-054 test card for the detection of extended-spectrum beta-lactamase production in 482 *Escherichia coli* with CTX-M phenotypes. *Journal of Antimicrobial Chemotherapy, 62*, 1015 - 7.

Donkor, E. S. & Nartey, E. (2008). Nasal colonisation of drug resistant bacteria in Ghanaian children less than five years. *The Internet Journal of Microbiology, 5* (2). DOI: 10.5580/16d5.

Donkor, E. S., Nortey, T., Opintan, J. A., Dayie, N. & Akyeh, M. L. (2008). Antimicrobial susceptibility of *Salmonella typhi* and *Staphylococcus aureus* isolates and the effect of some media on susceptibility testing results. *The Internet Journal of Microbiology, 4*(2). Doi: 10.5580/33a.

Donnenberg, M. S. (1995). Enteropathogenic *Escherichia coli*. In: M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg & R. L. Guerrant (eds.) *Infections of the gastrointestinal tract*. New York: Raven Press.

Donnenberg, M. S. & Kaper J. B. (1992). Enteropathogenic *Escherichia coli*. *Infection and Immunity*, *60*, 3953 - 61.

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

Doyle, M. P., Zhao, T., Meng, J. & Zhao, S. (1997). *Escherichia coli* O157:H7. In M.P. Doyle, L. R. Beuchat, & T. D. Montville (Eds.), *Food Microbiology: Fundamentals and frontiers* (pp. 171 - 191). Washington, D.C., USA: ASM Press.

Drieux, L., Brossier, F., Sougakoff, W. & Jarlier, V. (2008). Phenotypic detection of extended-spectrum *ß*-lactamase production in Enterobacteriaceae: review and bench guide. *Clinical Microbiology and Infection*, *14* (Supplement 1), 90 – 103.

DuPont, H. L. & Ericsson, C. D. (1993). Prevention and treatment of traveler's diarrhea. *The New England Journal of Medicine*, *328*, 1821 – 1827.

Eckburg P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*, 1635 - 1638.

Edoh, D. & Alomatu, B. (2008). Comparison of antibiotic resistance pattern between laboratories in Accra East, Ghana. *Nigerian Annals of Natural Sciences, 8*(1), 10 – 18.

Edwards, R. & Ewing, W. N. (1972). *Identification of Enterobacteriaceae.* (3rd ed.). Minnesota, USA: Burgess Publishing Co.

Eisenstein, B. I. (1987). Fimbriae. In C. Roy, C. Frederick, Neidhardt 3rd, & J. L. Ingraham (Eds.) *Escherichia coli* and *Salmonella Typhimurium*. (1st ed.). (pp. 84 – 90) Washington, DC: ASM Press.

Ericsson, C. D., DuPont, H. L., Mathewson, J. J., West, M. S., Johnson, P. C. & Bitsura, J. A. M. (1990). Treatment of traveller's diarrhoea with sulfamethoxazole and trimethoprim and loperamide. *Journal of the American Medical Association,* 263, 257 – 261.

Ericsson, C.D. (2003). Travelers' diarrhea. *International Journal of Antimicrobial Agents*, *21*, 116 - 124.

Erjavec, M. S. & Žgur-Bertok, D. (2011). Extended Characterization of Human Uropathogenic *Escherichia coli* Isolates from Slovenia. Chapter 3. In A. Nikibakhsh (Ed.), *Clinical Management of Complicated Urinary Tract Infection*, Doi: 10.5772/24699.

Escobar-Paramo, P., Clermont, O., Blanc-Potard, A. B., Bui, H., Le Bouguenec, C. & Denamur, E. (2004). A specific genetic background

is required for acquisition and expression of virulence factors in *Escherichia coli*. *Molecular Biology and Evolution, 21*, 1085 – 1094.

European Food Safety Authority (EFSA) (2011). Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. European Food Safety Authority *Journal, 9*, 2274.

Evans, D. J. Jr. & Evans, D. G. (1990). Colonization factor antigens of human pathogens. Current Topics, *Escherichia coli* in diarrhoeal disease. *Microbiology and Immunology*, *151*, 129.

Ewing, W. & Edward, P. R. (1986). *Edwards and Ewing's Identification of Enterobacteriaceae,* 4th ed. New York, Elsevier.

Falagas, M. E. & Karageorgopoulos, D. E. (2009). Extended - spectrum *ß*-lactamase-producing organisms. *Journal of Hospital Infection*, *73*(4), 345–354.

Faundez, G., Figueroa, G., Troncoso, M., & Cabello, F. (1988). Characterization of enteroinvasive *Escherichia coli* strains isolated from children with diarrhoea in Chile. *Journal of Clinical Microbiology*, *26*(5), 928 – 932.

Feglo, P., Adu-Sarkodie, Y., Ayisi, L. A., Jain, R., Spurbeck, R. R., Springman, A. C., Engleberg, N. C., Newton, D. W., Xi, C. & Walk, S. T. (2012). Emergence of a 1 novel ESBL-producing, fluoroquinolone-resistant clone of extraintestinal pathogenic *Escherichia coli* in Kumasi, Ghana. *Journal of Clinical Microbiology, 51*(2), 728 - 30.

Feng, P., Weagant, S. & Grant, M. (2002). Enumeration of *Escherichia coli* and the Coliform Bacteria. Bacteriological Analytical Manual (8th ed.) Retrieved May 3, 2012, from www.fda.gov/Food/foodScienceResearch/Laboratory Method.

Fernandez, I. M., Silva, M., Schuch, R., Walker, W. A., Siber, A. M., Maurelli, A. T. & McCormick, B. A. (2001). Cadaverine prevents the escape of *Shigella flexneri* from the phagolysosome: A connection between bacterial dissemination and neutrophil transepithelial signaling. *Journal of Infectious Diseases, 184*, 743 – 753.

Finlay, B. B. & Falkow, S., (1997). Common themes in microbial pathogenicity revisited. *Molecular Microbiology Reviews, 61*, 136 – 169.

Fisher, A. A., Laing, E. J., Stoeckel, E. J. & Townsend, W. J. (1998). *Handbook for Family Planning Operations Research Design*. (2nd ed.) New York: Population Council.

Fonkwo, P. N. (2008). Pricing infectious disease: The economic and health implications of infectious diseases. *European Molecular Biology Organization Reports, Science and Society*, *9*, 513 – 517.

Forbes, B. A., Saham, D. F. & Wesisfeld, A. S. (2007). *Bailley and Scotte's. Diagnostic Microbilogy.* Chicago, USA: Mosby.

Foxman, B. (2003). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Disease-a-Month*, *49*(2), 53 – 70.

Foxman, B., Manning, S. D., Tallman, P., Bauer, R., Zhang, L., Koopman, J. S., Gillespie, B., Sobel, J. D. & Marrs, C. F. (2002). Uropathogenic

Escherichia coli are more likely than commensal *E. coli* to be shared between heterosexual sex partners. *American Journal of Epidemiology, 156*(12), 1133 – 1140.

Garrec, H., Drieux-Rouzet, L. Jarlier, V., Golmard, J. L. & Robert, J. (2011). Comparison of Nine Phenotypic Methods for Detection of Extended-Spectrum Lactamase Production by *Enterobacteriaceae. Journal of Clinical Microbiology*, *49*(3), 1048 – 1057.

Gassama-Sow, A., Sow, P. S., Gueye, M., Gueye-N'diaye, A., Perret, J. L., M'boup, S. & Aidara-Kane, A. (2004). Characterization of pathogenic *Escherichia coli* in human immunodeficiency virus-related diarrhoea in Senegal. *Journal of Infectious Diseases, 189*, 75 – 78.

Gauthier, M. J., Munro, P. M. & Mohajer, S. (1987). Influence of salts and sodium chloride on the recovery of *Escherichia coli* from seawater. *Current Microbiology, 15*, 5 – 10.

Ghana Health Service (2008). Cape Coast Metropolitan Health Records. Cape Coast.

Ghana National AIDS Control Program (2000). Ghana Behavioral Surveillance Survey, 2000. Unpublished report, Research International, Family Health International. Accra, Ghana.

Ghana Statistical Service (2002). The 2000 Population and Housing Census. *Special Report on Urban Localities*. Accra, Ghana Statistical Service.

Ghana Statistical Service (2012), *Population and Housing Census Final Results*. Retrieved September 12, 2012, from

www.statsghana.gov.gh.

Gillen, C. M. (1991). Gastro-intestinal disorders. *Applied Physiology*, *67*, 101 - 104.

Global Peace Index (2013). Institute for Economics and Peace Report. Retrieved March 4, 2015, from www.visionofhumanity.org/pdf/.../2013_Global_Peace_Index_Report

GMMB (2009). Ghana Museums and Monuments Board Statistics of visitation to the Cape Coast Castle.

Goldberg, M. B., & Theriot, J. A. (1995). *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proceedings of the National Academy of Science in the United States of America*, *92*, 6572 – 6576.

Gorbach, S. L., Kean, B. H., Evans, D. G., Evans, D. J. Jr. & Bessudo, D. (1975). Travelers' diarrhoea and investigation and control. *The New England Journal of Medicine, 292* (18), 933 – 936.

Gordon, D. M. & Cowling, A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology, 149*, 3575 – 3586.

Gordon, D. M., Clermont, O., Tolley, H. & Denamur, E. (2008). Assigning Escherichia coli strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. *Environ Microbiol, 10*, 2484–2496.

Grys, T. E., Siegel, M. B., Lathem, W. W. & Welch, R. A. (2005). The StcE protease contributes to intimate adherence of enterohaemorrhagic

Escherichia coli O157:H7 to host cells. *Infection and Immunity*, *73*, 1295 - 1303.

Güler, L., Gunduz, K. & Ok, U. (2008). Virulence Factors and Antimicrobial Susceptibility of *Escherichia coli* Isolated from Calves in Turkey. *Zoonoses and Public Health, 55*, 249 - 257.

Gunzburg, S. T., Chang, B. J., Burke, V. & Gracey, M. (1992). Virulence factors of enteric *Escherichia coli* in young Aboriginal children in north-west Australia. *Epidemiology and Infection, 109*, 283 – 289.

Gupta, K., Scholes, D. & Stamm, W. E. (1999). Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *Journal of the American Medical Association, 281*(8): 736-738.

Guyer, D. M., Henderson, I. R., Nataro, J. P. & Mobley, H. L. (2000). Identification of Sat, an autotransporter toxin produced by uropathogenic *Escherichia coli.* *Molecular Microbiology*, *38*, 53 – 66.

Hale, W. B., van der Woude, M. W., Braaten, B. A. & Low, D. A. (1998). Regulation of uropathogenic *Escherichia coli* adhesin expression by DNA methylation *Molecular Genetics Metabolism*, *65*(3), 191 - 6.

Haley, R. W., Culver, D. H., White, J. W., Morgan, W. M., Emori, T. G., Munn, V. P. & Hooton, T. M. (1985). The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. *American Journal of Epidemiology, 121*, 182 – 205.

Harrigan, W. F. & McCane, M. E. (1966). *Laboratory Methods in Microbiology* (1st ed.). London: New York Academic Press.

Harshey, R. M. (1994). Bees aren't the only ones: Swarming in Gram-negative bacteria. *Molecular Microbiology*, *13*, 389 – 394.

Ho, P. L., Chow, K. H., Yuen, K. Y., Ng, W. S. & Chau, P. Y. (1998). Comparison of a novel, inhibitor-potentiated disc diffusion test with other methods for the detection of extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae. Journal of Antimimicrobial Chemotherapy*, *42*, 49 -54.

Hoffman, J. A., Wass, C., Stins, M. F. & Kim, K. S. (1999). The capsule supports survival but not traversal of *Escherichia coli* K1 across the blood-brain barrier. *Infection and Immunity*, *67*, 3566 – 3570.

Hogan, J. & Smith, K. L. (2003). Coliform mastitis. *Veterinary Research, 34*, 507 - 519.

Hoisington, D., Khairallah, M. & Gonzalez-De-Leon, D. (1994). *Laboratory Protocols: CIMMYT Applied Biotechnology Centre* (2nd ed.), Mexico, D. F.: CIMMYT.

Holten, K. B., & Onusko, E. M. (2000). Appropriate Prescribing of Oral Beta-Lactam Antibiotics. *American Family Physician*, *62*, 611 - 20.

Howard, D. H., Scott II, D., Packard, R. & Jones, D. (2003). The Global Impact of Drug Resistance. *Clinical Infectious Diseases*, *36* (Suppl 1): S4–10.

Hudault, S., Guignot, J. & Servin, A. L. (2001). *Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection. *Gut, 49*, 47 - 55.

Hunter, P. R., Ramirez-Toro, G. I. & Minnigh, H. A. (2010). Impact of diarrhoeal illness of a community educational intervention to improve drinking water quality in rural communities in Puerto Rico. *BioMed Central Public Health*, *10* (219), 1- 11.

Ingraham, J. L., & Marr, A. G. (1987). In F. C. Neidhardt (Ed.) *Escherichia coli* and *Salmonella typhimurium* (pp: 1570 –1572). Washington, DC: ASM Press.

Itoh, Y., Nagano, I., Kunishima, M. & Ezaki, T. (1997). Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable: H10 associated with a massive outbreak of gastrointestinal illness. *Journal of Clinical Microbiology, 35*, 2546 – 2550.

Jacoby, G. A. & Munoz-Price, L. S. (2005). The new beta-lactamases. *The New England Journal of Medicine*, *352* (4), 380 – 391.

Jakobsen, L., Kurbasic, A., Skjøt-Rasmussen, L., Ejrnaes, K., Porsbo, L. J., Pedersen, K. Jensen, L. B., Emborg, H. D., Agersø, Y., Olsen, K. E., Aarestrup, F. M., Frimodt-Møller, N. & Hammerum, A. M. (2010). *Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. *Foodborne Pathogens and Diseases*, *7*(5), 537 – 47. Doi: 10.1089/fpd.2009.0409

Jarlier, V., Nicolas, M. H., Fournier, G. & Philippon, A. (1988). Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Reviews of Infectious Diseases*, *10*, 867 – 878.

Jerse, A. E., Yu, J., Tall, B. D. & Kaper, J. B. (1990). A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proceedings of the National Academy of Science of the United States of America, 87*, 7839 - 7843.

Johannessen, G. S., Bengtsson, G. B., Heier, B. T., Bredholt, S., Wasteson, Y. & Rørvik, L. M. (2005). Uptake of *E. coli* O157:H7 from organic manure into crisphead lettuce. *Applied and Environmental Microbiology, 71*, 2221 - 2225.

Johnson, J. R, & Stell, A. L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *Journal of Infectious Diseases*, *181*, 261 – 72.

Johnson, J. R. (1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clinical Microbiology Reviews*, *4*, 80 – 128.

Johnson, J. R., Clermont, O., Menard, M., Kuskowski, M. A., Picard, B. & Denamur, E. (2006). Experimental mouse lethality of *Escherichia coli* isolates in relation to accessory traits, phylogenetic group, and clinical source. *Journal of Infectious Diseases*, *194*,1141 – 1150.

Johnson, J. R., Delavari, P. & O'Bryan, T. (2001b). *Escherichia coli* O18:K1:H7 isolates from acute cystitis and neonatal meningitis exhibit common phylogenetic origins and virulence factor profiles. *Journal of Infectious Diseases, 183*, 425 – 434.

Johnson, J. R., Delavari, P., Kuskowski, M. & Stell, A. L. (2001a). Phylogenetic distribution of extra-intestinal virulence-associated traits in *Escherichia coli. Journal of Infectious Diseases*, *183*, 78 – 88.

Johnson, J. R., Gajewski, A., Lesse, A. J. & Russo, T. A. (2003a). Extraintestinal pathogenic *Escherichia coli* as a cause of invasive non- urinary infections. *Journal of Clinical Microbiology*, *41*, 5798 – 5802.

Johnson, J. R., Goullet, P., Picard, B., Moseley, S. L. Roberts, P. L. & Stamm, W. E. (1991). Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. *Infection and Immunity*, *59*, 2311 – 2315.

Johnson, J. R., Murray, A. C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M. A. & Smith, K. E. (2003b). Isolation and molecular characterization of nalidixic acid-resistant extra-intestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrobial Agents Chemotherapy*, *47*, 2161 – 2168.

Johnson, J. R., O'Bryan, T. T., Kuskowski, M. A. & Maslow, J. N., (2001c). Ongoing horizontal and vertical transmission of virulence genes and

papA alleles among *Escherichia coli* blood isolates from patients with diverse-source bacteremia. *Infection and Immunity, 69*, 5363 – 5374.

Johnson, J. R., Orskov, I., Orskov, F., Goullet, P., Picard, B., Moseley, S. L., Roberts, P. L. & 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.

Johnson, J. R., Oswald, E., O'Bryan, T. T., Kuskowski, M. A. & Spanjaard, L. (2002). Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal bacterial meningitis in the Netherlands. *Journal of Infectious Disease, 185*, 774 - 784.

Johnson, J. R., Roberts, P. L., & Stamm, W. E. (1987). P fimbriae and other virulence factors in *Escherichia coli* urosepsis: Association with patients' characteristics. *Journal of Infectious Diseases*, *156*, 225 – 229.

Johnson, J.R., Moseley, S., Roberts, P. & Stamm, W. E. (1988). Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. *Infection and Immunity*, *56*, 405 – 412.

Johnson, T. J., Kariyawasam, S., Wannemuehler, Y., Mangiamele, P., Johnson, S. J., Doetkott, C., Skyberg, J. A., Lynne, A. M., Johnson, J. R. & Nolan, L. K. (2007). The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with

human extraintestinal pathogenic *E. coli* genomes. *Journal of Bacteriology*, *189*, 3228 - 3236.

Kain, K. C., Barteluk, R. L., Kelly, M. T., Xin, H., Hua, G. D., Yuan, H., Proctor, E. M., Byrne, S. & Stiver, H. G. (1991). Etiology of childhood diarrhoea in Beijing, China. *Journal of Clinical Microbiology*, *29*, 90 – 95.

Kamada, N., Maeda, K., Inoue, N., Hisamatsu, T., Okamoto, S., Hong, K. S., Yamada, T., Watanabe, N., Tsuchimoto, K., Ogata, H. & Hibi, T. (2008). Nonpathogenic *Escherichia coli* strain Nissle 1917 inhibits signal transduction in intestinal epithelial cells. *Infection and Immunity, 76*, 214 - 220.

Kaper, J. B. (1996). Defining EPEC, In Proceedings of the International Symposium on Enteropathogenic *Escherichia coli* (EPEC). *Revista de Microbiologia*, *27*, 130 - 3.

Kaper, J. B., Morris, J. G. Jr, & Levine, M. M. (1995). Cholera. *Clinical Microbiology Review, 8*(1), 48 - 86.

Kaper, J. B., Nataro, J. P. & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology, 2*, 123 - 140.

KATH records, 2004 – 2008. Komfo-Anokye Teaching Hospital Health Records, Kumasi, Ghana.

Katouli, M., Kühn, I. & Möllby, R. (1990). Evaluation of the stability of biochemical phenotypes of *Escherichia coli* upon sub-culturing and storage. *Journal General Microbiology, 136*, 1681 – 1688.

Kaur P, Chakraborti A. & Asea, A. (2010). Enteroaggregative *Escherichia coli*: An Emerging Enteric Food Borne Pathogen. *Interdisciplinary Perspective on Infectious Diseases*, *2010*, 254159.

Kaur, H., Kumar, P., Ray, P., Kaur, J. & Chakraborti, A. (2009). Biofilm formation in clinical isolates of group streptococci from north India. *Microbial Pathogenesis*, *46*, 321 – 327.

Kawamata, O., Yoshida, H., Hirota, K., Yoshida, A, Kawaguchi, R., Shiratori, Y. & Omata, M. (1996). Nested-polymerase chain reaction for the detection of Helicobacter pylori infection with novel primers designed by sequence analysis of urease A gene in clinically isolated bacterial strains. *Biochemical and Biophysical Research Communications*, *219*, 266 - 272.

Kelland, K. (2011). Killer *E. coli* strain described as deadliest yet. *Reuters.* Retrieved June 2, 2011, from www.reuters.com.

Kenny, B., DeVinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. & Finlay, B. B. (1997). Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell, 91*, 511 - 520.

Khan, A., Yamasaki, S., Sato, T., Ramamurthy, T., Pal, A., Datta, S., Chowdhury, N. R., Das, S. C., Sikdar, A., Tsukamoto, T., Bhattacharya, S. K., Takeda, Y. & Nair, G. B. (2002). Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef and humans, Calcutta, India. *Emerging Infectious Diseases, 8*, 54 - 62.

Kim, B. & Loeb, L. A. (1995). A screen in *Escherichia coli* for nucleoside analogs that target human immunodeficiency virus (HIV) reverse transcriptase: co expression of HIV reverse transcriptase and herpes simplex virus thymidine kinase. *Journal of Virology, 69*(10), 6563 - 6566.

Kisielius, P. V., Schwan, W. R., Amundsen, S. K., Duncan, J. L. & Schaeffer, A. J. (1989). In vivo expression and variation of *Escherichia coli* type 1 And P Pili in the urine of adults with acute urinary tract infections. *Infection and Immunity, 57*, 1656 – 1662.

Kliebe, C., Nies, B. A. Meyer, J. F., Tolxdorff-Neutzling, R. M. & Wiedemann, B. (1985) Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. *Antimicrobial Agents and Chemotherapy*, *28*(2), 302 – 307.

Knothe, H., Shah, P. & Krcmery, V. (1983). Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection*, *11*(6), 315 – 317.

Knott-Hunziker, V. Petursson, S., Waley, S. G., Jaurin, B. & Grundstrom, T. (1982). The acyl-enzyme mechanism of beta-lactamase action. The evidence for class C Beta-lactamases. *Biochemical Journal*, *207*(2), 315 – 322.

Komatsu, M., Aihara, M., Shimakawa, K., Iwasaki, M., Nagasaka, Y., Fukuda, S., Matsuo, S. & Iwatani, Y. (2003). Evaluation of Microscan ESBL confirmation panel for Enterobacteriaceae

producing, extended spectrum beta-lactamases isolated in Japan. *Diagnostic Microbiology and infectious Disease*, *46*, 125 - 130.

Kotra, L. P., Samama, J. & Mobashery, S., (2002). Beta-lactamases and resistance to beta-lactam antibiotics. In K. Lewis, A. A. Salyers, H. W. Taber & R. G. Wax (Eds.), *Bacterial resistance to antimicrobials*, 123 - 60.

Kuhnert, P., Boerlin, P. & Frey, J. (2000). Target genes for virulence assessment of *Escherichia coli* isolates from water, food and the environment. *Federation of European Microbiological Societies Microbiology Reviews*, 24 (1), 107 – 117.

Kunin, C. M. (1987). *Detection, Prevention and Management of Urinary Tract Infections* (4th ed.). Philadelphia: Lea and Febiger.

Kunin, C. M. (1994). Urinary tract infections in females. *Clinical Infectious Diseases*, 18, 1 – 10.

Lalitha, M. K. (2004). *Manual on antimicrobial susceptibility testing*. Indian Association of Medical Microbiologists: Tamil Nadu, India pp. 1 – 53. Retrieved August 19, 2009, from www.ijmm.org/documents/Antimicrobial.doc

Lan, R. & Reeves, P. R. (2002). *Escherichia coli* in disguise: molecular origins of Shigella. *Microbes and Infection, 4*(11), 1125 – 1132.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematics.* E. Stackebrandt, and M. Goodfellow (Eds.), (pp. 115-175). New York, NY: John Wiley and Sons.

Langermann, S., Palaszynski, S., Barnhart, M., Auguste, G., Pinkner, J., Burlein, J. & Al, E. (1997). Prevention of mucosal *Escherichia coli* infection by Fim-H-adhesin-based systemic vaccination. *Science*, *276*, 607 – 611.

Lansang, M. A., Lucas-Aquino, R., Tupasi, T. E., Mina, V. S., Salazar, L. S., Juban, N., Limjoco, T. T., Nisperos, L. E. & Kunin, C. M. (1990). Purchase of antibiotics without prescription in Manila, the Philippines: Inappropriate choices and doses. *Journal of Clinical Epidemiology*, *43*, 61 - 67.

Lathem, W. W., Bergsbaken, T., Witowski, S. E., Perna, N. T. & Welch, R. A. (2003). Acquisition of StcE, a C1 esterase inhibitor-specific metalloprotease, during the evolution of *Escherichia coli* O157:H7. *Journal of Infectious Diseases, 187*, 1907 - 1914.

Lathem, W. W., Grys, T. E., Witowski, S. E., Torres, A. G., Kaper, J. B., Tarr, P. I. & Welch, R. A. (2002). StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Molecular Microbiology, 45*, 277 - 288.

Le Bouguenec, C. & Servin, A. L. (2006). Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. *Federation of European Microbiological Societies Microbiology Letters*, *256*, 185 – 194.

Leclerc, H., Mossel, D. A. A., Edberg, S. C. & Struijk, C. B. (2001). Advances in the bacteriology of the coliform group: their suitability

as markers of microbial safety. *Annual Reviews of Microbiology, 55*, 201 - 234.

Lee, Y. K. (2006). *Microbial Biotechnology: Principles and Applications*. Retrieved June 12, 2011, from www.books.google.com.gh.

Leverstein-van Hall, M. A., Fluit, A. C., Paauw, A., Box, A. T., Brisse, S. & Verhoef, J. (2002). Evaluation of the Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 automated instruments for detection of extended-spectrum beta-lactamases in multi-resistant *Escherichia coli* and *Klebsiella* spp. *Journal of Clinical Microbiology*, *40*, 3703 – 3711.

Levine, M. M., Nataro, J. P., Karch, H., Baldini, M. M., Kaper, J. B., Black, R. E., Clements, M. L. & O'Brien A. D. (1985). The diarrheal response of humans to some classic serotypes of enteropathogenic Escherichia coli is dependent on a plasmid encoding an enteroadhesiveness factor. *Journal of Infectious Diseases*, *152*, 550 – 559.

Levinson, W. (2010). *Review of medical microbiology and immunology*. 11th ed. (pp. 85 - 93), New York: Lange.

Licence, K., Oates, K. R., Synge, B. A. & Reid, T. M. (2001). An outbreak of *E. coli* O157 infection with evidence of spread from animals to man through contamination of a private water supply. *Epidemiology and Infection, 126*, 135 - 138.

Lim, J. K., Gunther, N. W. T., Zhao, H., Johnson, D. E., Keay, S. K. & Mobley, H. L. (1998). *In vivo* phase variation of *Escherichia coli* type

1 fimbrial genes in women with urinary tract infection. *Infection and Immunity*, *66*, 3303 – 3310.

Lim, J. Y., Li, J., Sheng, H., Besser, T. E., Potter, K. & Hovde, C. J. (2007). *Escherichia coli* O157:H7 colonization at the rectoanal junction of long-duration culture-positive cattle. *Applied and Environmental Microbiology, 73*, 1380 - 1382.

Lior, H. (1996). Classification of *Escherichia coli*. In C. L. Gyles (Ed.) *Escherichia coli in domestic animals and humans* (pp. 31 – 72), Wallingford, United Kingdom: CAB International.

Livermore, D. M. (2003). Bacterial resistance: origins, epidemiology, and impact. *Clinical Infectious Diseases*, *36*, S11 - S23.

Livermore, D. M. & Hawkey, P. M. (2005). CTX-M: changing the face of ESBLs in the UK. *Journal of Antimicrobial Chemotherapy*, *56*(3), 451 – 454.

Lodinova-Zadnikova, R. & Sonnenborn, U. (1997). Effect of preventive administration of a nonpathogenic *Escherichia coli* strain on the colonization of the intestine with microbial pathogens in newborn infants. *Biology of the Neonate, 71*, 224 - 232.

Lodinova-Zadnikova, R., Cukrowska, B. & Tlaskalova-Hogenova, H. (2003). Oral administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). *International Archives of Allergy Immunology, 131*, 209 - 211.

M'Zali, F. H., Chanawong, A., Kerr, K. G., Birkenhead, D. & Hawkey, P. M. (2000). Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *Journal of Antimicrobial Chemotherapy*, *45*, 881 - 885.

Mammeri, H., Loo, M. V. D., Poirel, L., Martinez-Martinez, L. & Nordmann, L. (2005). Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrobial Agents and Chemotherapy, 49*(1), 71 – 76.

Mandell, G. L., Bennett, J. E. & Mandell, D. R. (2010). *Douglas and Bennett's principles and practice of infectious diseases* (7th ed.). Philadelphia, PA: Churchill Livingstone/Elsevier.

Manges, A. R., Johnson, J. R., Foxman, B., O'Bryan, T. T., Fullerton, K. E. & Riley, L. W. (2001). Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group, *New England Journal of Medicine, 345*(14), 1007 – 1013.

Maningi, N. E., Ehlers, M. M., Osman, A. G., Ismail, N. A., Kock, M. M. & Hoosen, A. A. (2010). Prevalence of ESBL and MBL genes among *Klebsiella pneumoniae* isolates in Pretoria. Retrieved April 12, 2012, from www.docstoc.com.

Mann, K. G. (1999). Biochemistry and physiology of blood coagulation. *Thrombosis and Haemostasis, 82*, 165 - 174.

March, S. B. & Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with haemorrhagic colitis. *Journal of Clinical Microbiology*, *23*, 869.

Maurelli, A. T., Fernandez, R. E., Bloch, C. A., Rode, C. K. & Fasano, A. (1998). Black holes and bacterial pathogenicity: A large genomic deletion that enhances the virulence of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Proceedings of the National Academy of Science of the United States of America*, *95*, 3943 – 3948.

McDaniel, T. K., Jarvis, K. G., Donnenberg, M. S. & Kaper, J. B. (1995). A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proceedings of the National Academy of Science of the United States of America, 92*, 1664 - 1668.

McGarvey, S. T., Buszin, J., Reed, H., Rahman, Z., Andrzejewski, C., White, M. J., Smith, D. C. & Awusabo-Asare, K. A (2008). Community and household determinants of water quality in coastal Ghana. *Journal of Water and Health, 6(3), 339 – 349.*

Medeiros, A. A. (1984). β-lactamases. *British Medical Bulletin, 40*, 18 – 27.

Mellies, J. L., Navarro, F., Okeke, I., Friedrickson, J., Nataro, J. P. & Kaper, J. B. (2001). espC pathogenicity island of enteropathogenic *Escherichia coli* encodes an enterotoxin. *Infection and Immunity*. *69*, 315 - 324.

Mercer, A. A., Morelli, G., Heuzenroeder, M., Kamke, M. & Achtman, M. (1984). Conservation of plasmids among *Escherichia coli* K1 isolates of diverse origins. *Infection and Immunity*, *46*, 649 – 657.

Merchant, I. A. & Packer, R. A. (1967). *Veterinary Bacteriology and Virology* (6th ed.). Iowa State University Press: USA.

Morgan, G. M., Newman, C., Palmer, S. R., Allen, J. B., Shepherd, W., Rampling, A. M., Warren, R. E., Gross, R. J., Scotland, S. M. & Smith, H. R. (1988). First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157.H7 in the UK. *Epidemiology and Infection 101*, 83 - 91.

Motta, R. N., Oliveira, M. M., Magallaes, P. S., Dias, A. M., Aragao, L. P., Forti, A. C. & Carvallo, C. B. M. (2003). Plasmid mediated extended spectrum β-lactamase producing strains of *Enterobacteriaceae* isolated from Diabetes foot infection. *Brazilian Journal of Infectious Diseases*, *2*, 129 - 134.

Moyo, S., Maselle, S. Y., Matee, M. I., Langeland, N. & Mylvaganam, H. (2007). Identification of diarrhoeagenic *Escherichia coli* isolated from children in Dar es Salaam, Tanzania. *BioMed Central Infectious Diseases, 7*, 92. Doi: 10.1186/1471-2334-7-92.

Mühldorfer, I. & Hacker, J. (1994). Genetic aspects of *Escherichia coli* virulence. *Microbial Pathogenesis, 16*, 171 - 181.

Mukherjee, A., Speh, D., Dyck, E. & Diez-Gonzalez, F. (2004). Preharvest Evaluation of Coliforms, *Escherichia coli, Salmonella,* and *Escherichia coli* O157:H7 in Organic and Conventional Produce Grown by Minnesota Farmers. *Journal of Food Protection, 67*(5), 894 – 900.

Munro P. M., Gauthier, J. M., Breittemayer, A. & Bongio - vanni, J. (1989).

Influence of osmoregulation process on starvation survival of *Escherichia coli* in seawater. *Applied Environmental Microbiology*, *55*, 2017 – 2024.

Murray B. E., Rensimer, E. R. & Dupont, H. L. (1982). Emergence of high-level trimethoprim resistance in faecal *Escherichia coli* during oral administration of trimethoprim or trimethoprim - sulphamathoxazole. *New England Journal of Medicine, 306*, 130 - 135.

Murray, B. E., Alvarado, T., Kim, K. H., Vorachit, M., Jayanetra, P., Levine, M. M., Prenzel, I., Fling, M., Elwell, L. & McCracken, G. H. (1985). Increasing resistance to trimethoprim - sulphamethoxazole among isolates of *Escherichia coli* in developing countries. *Journal of Infectious Diseases, 152*, 1107 - 1113.

Murray, C. J. L. & Lopez, A. D. (eds) (1996). *The Global Burden of Disease*, Vol. II, Global Health Statistics: A compendium of incidence, prevalence and mortality estimates for over 200 conditions, Harvard School of Public Health on behalf of the World Health Organization and The World Bank, Cambridge, MA.

Namboodiri, S. S., Opintan, J. A., Lijek, R. S., Newman, M. J. & Okeke, I. N. (2011). Quinolone resistance in *Escherichia coli* from Accra, Ghana. *BioMed Central Microbiology*, *11*(44), 1 - 9.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiolgy Reviews*, *11*, 142 – 201.

Nataro, J. P., Kaper, J. B., Robins-Browne, R., Prado, V., Vial, P. & Levine, M. M. (1987). Patterns of adherence of diarrhoeagenic *Escherichia*

coli to HEp-2 cells. *Pediatric Infectious Disease Journal*, *6*, 829 – 831.

Nataro, J. P., Mai, V., Johnson, J., Blackwelder, W. C., Heimer, R. Tirrell, S., Edberg, S. C., Braden, C. R., Glenn, M. J. Jr. & Hirshon, J. M. (2006). Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. *Clinical Infectious Diseases*, *43*, 402 – 407.

Nataro, J. P., Y. Deng, S. Cookson, A. Cravioto, S. J. Savarino, L. D. Guers, Levine, M. M. & Tacket, C. O. (1995). Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. *Journal of Infectious Diseases*, *171*, 465 – 468.

Nei, M., Tajima, F. & Tateno, Y (1983). Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. *Journal of Molecular Evolution, 19*, 153 - 170.

Neill, M. A., Tarr, P. I., Taylor, D. N. & Trofa, A. F. (1994). *Escherichia coli* In Y. H. Hui, J. R. Gorham, K. D. Murrell & D. O. Cliver (Eds.), *Foodborne Disease Handbook: Diseases Caused by Bacteria*, (vol. 1) (pp. 169 – 213). New York: Marcel Dekker.

Nester, E. W. Anderson, D. G. Roberts, C. E. Pearsall, N. N. & Nester, M. T. (2004), *Microbiology: a human's perceptive.* 4th ed. Washington: Mc-Graw Hill Publishers.

Newman, M. J., Frimpong, E., Asamoah-Adu, A. & Sampane-Donkor, E. (2006). Resistance to Antimicrobial Drugs in Ghana. *Technical Report Series,* No. 5.

Newman, M. J., Frimpong, E., Donkor, E. S., Opintan, J. A. & Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana. *Infection and Drug Resistance, 4*, 215 – 220.

Nirdnoy, W. (2005). The contribution of plasmids to antibiotic resistance in *Campylobacter jejuni*: Mahidol University, Faculty of graduate studies, Dissertation. Bangkok, Thailand.

Novotny, L., Dvorska, L., Lorencova, A., Beran, B. & Pavlik, I. (2004). Fish: A potential source of bacterial pathogens for human beings. *Vetenary Medicine*, *49*(9), 343 - 358.

Nyarko, H. D., Obodai, E. A., Boamponsem, L. K., Coomson, S. S & Aniwe, Y. (2011). Microbial profile of smoked sardine (*Sardilella aurita)* at smoking sites and market centres of Tema, Ghana. *Archives of Applied Science Research, 3*(3), 443 – 453.

Nys, S., Okeke I. N., Kariuki, S., Dinant, G. J., Driessen, C. & Stobberingh, E. E. (2004). Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries. *Journal of Antimicrobial Chemotherapy*, *54*(5), 952 - 955.

Obiri-Danso, K, Amoah, P. & Jones, K. (2003b). Impact of wastewater use on fresh vegetables sold in Kumasi (Ghana). *152nd Meeting of the Society of General Microbiology*, FdBev 09, 44, Edinburgh.

Obiri-Danso, K., Adjei, B., Stanley, K. N. & Jones, K. (2009). Microbiological quality and metal levels in wells and boreholes water in some peri-urban communities in Kumasi, Ghana. *African Journal of Environmental Science and Technology, 3*(1), 059 - 066.

Obiri-Danso, K., Okore-Hanson, A. & Jones, K. (2003a). The microbiological quality of drinking water sold on the streets in Kumasi, Ghana. *Letters in Applied Microbiology*, *37*, 334 - 339.

Ochman, H., & Selander, R. K. (1984). Standard reference strains of *Escherichia coli* from natural populations. *Journal of Bacteriology, 157*, 690 – 693.

Ochman, H., Lawrence, J. G. & Groisman, E. A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature*, *405*, 299 - 304.

Okeke, I. N. (2009). Diarrheagenic *Escherichia coli* in sub-Saharan Africa: Status, Uncertainties and Necessities. *Journal of Infection in Developing Countries*, *3*(11), 817 - 842.

Okeke, I. N., & Edelman, R. (2001). Dissemination of antibiotic-resistant bacteria across geographic borders. *Clinical Infectious Diseases, 33*(3), 364 - 369.

Okeke, I. N., Fayinka, S. T. & Laminkanra, A. (2000). Antibiotic resistance in *Escherichia coli* from Nigerian students. *Emerging Infectious Diseases*, *6*, 393 – 396.

Okeke, I. N., Wallace-Gadsden, F., Simons, H. R., Matthews, N., Labar, A. S., Hwang, J. & Wain, J. (2010). Multi-locus sequence typing of enteroaggregative *Escherichia coli* isolates from Nigerian children uncovers multiple lineages. *PLOS ONE, 5*(11), e14093.

Opintan, J. A., Newman, M. J., Ayeh-Kumi, P. F., Affrim, R., Gepi-Attee, R., Sevilleja, J. E. A. D., Roche, J. K., Nataro J. P., Warren, C. A. & Guerrant, R. L. (2010). Pediatric diarrhoea in southern Ghana:

etiology and association with intestinal inflammation and malnutrition. *The American Journal of Tropical Medicine and Hygiene*, *83*(4), 936 - 43.

Orrett, F. A. & Shurland, S. M. (2001). Prevalence of Resistance to Antimicrobials of *Escherichia coli* Isolates from Clinical Sources at a Private Hospital in Trinidad. *Japan Journal of Infectious Diseases*, *54*, 64 – 68.

Orskov, F. & Orskov, I. (1983a). 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.

Orskov, F. & Orskov, I. (1992). *Escherichia coli* serotyping and disease in man and animals. *Canadian Journal of Microbiology*, *38*, 699 - 704.

Orskov, I., & Orskov, F. (1983b). Serology of *Escherichia coli* fimbriae. *Progress in Allergy 33*, 80 – 105.

Orskov, I., & Orskov, F. (1985). *Escherichia coli* in extra-intestinal infections. *Journal of Hygiene (London), 95*, 551 – 575.

Orskov, I., Orskov, F., Birch-Andersen, A., Kanamori, M. & Svanborg-Eden, C. (1982). O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. *Scandinavian Journal of Infectious Diseases*, *33*, 18 – 25.

Osorio, C. R., Toranzo, A. E. Romalde, J. L. & Barja, J. L. (2000). Multiplex PCR assay for ureC and 16S rRNA genes clearly discriminates

between both subspecies of *Photobacterium damselae*. *Diseases of Aquatic Organisms, 40*, 177 - 183.

Pappoe, A. N. M., Asmah, A. E., Owusu Ansah, E., Opoku-Boahen, Y., Akotoye, H. K., Ameyaw, Y., & Lamptey, S. O. (2007). Short-term investigations into underground water quality in Cape Coast and its environs in Ghana. *Journal of Natural Sciences, 3*(1), 62 – 70.

Parkkinen, J., Korhonen, T. K., Hacker, P. A. & Soinila, S. (1988). Binding sites of the rat brain for *Escherichia coli* S - fimbriae associated with neonatal meningitis. *Journal of Clinical Investigation*, *81*, 860 – 865.

Paterson, D. I. & Bonomo, R. A. (2005). Extended spectrum ß-lactamase: A Clinical update. *Clinical Microbiology Reviews 18*, 657 - 686.

Paton, A. W. & Paton, J. C. (2005). Multiplex PCR for Direct Detection of Shiga Toxigenic *Escherichia coli* Strains Producing the Novel Subtilase Cytotoxin, Journal of Clinical Microbiology, *43*(6), 2944 – 2947.

Persing, D. H., Smith, T. F., Tenover, F. C. & White, T. J. (1993). *Diagnostic molecular Microbiology, Principles and Applications*. Washington, DC, USA: ASM Press.

Petridis, M., Bagdasarian, M., Waldor, M. K. & Walker, E. (2005). Horizontal transfer of shiga toxin and antibiotic resistance genes among *Escherichia coli* strains in house fly (*Diptera Muscidae*) gut. *Journal Medical Entomology, 43*(2), 288 – 295.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J. & Denamur, E. (1999). The link between phylogeny and

virulence in *Escherichia coli* extraintestinal infection. *Infection and Immunity*, *67*, 546 – 553.

Pitout, J. D. D., Nordmann, P. Laupland, K. B. & Poirel, L. (2005). Emergence of Enterobacteriaceae producing extended spectrum *ß*-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*, *56* (1), 52 – 59.

Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet Infectious Diseases*, *8*, 159 - 166.

Prasadarao, N. V., Wass, C. A., Huang, S. H. & Kim, K. S. (1999). Identification and characterization of a novel Ibe10 binding protein that contributes to Escherichia coli invasion of brain microvascular endothelial cells. *Infection and Immunity*, *67*, 1131 – 1138.

Price, P. A. (1988). Role of vitamin-K-dependent proteins in bone metabolism. *Annual Reviews in Nutrition*, *8*, 565 - 583.

Pruett, J. R., Biela, W. P., Lattuada, T., Mrozinski, C. P., Babour, P. M., Flowers, W. M., Osborne, R. S., Reagan, W., Theno, J. O., Cook, D., McNamara, A. M. & Rose, B. (2002). Incidence of *Escherichia coli* O157:H7 in frozen beef patties produced over an 8-hour shift. *Journal of Food Protection, 65*(9), 1363 - 1370.

Pupo, G. M., Karadis, D. K. R., Lan, R. & Reeves, P. R. (1997). Evolutionary relationships among pathogenic and non-pathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. *Infection and Immunity*, *64*, 2685 – 2692.

Queenan, A. M., Folena, B., Gownley, C., Wira, E. & Bush, K. (2004). Effects of inoculum and beta-lactamase activity in AmpC and extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. *Journal of Clinical Microbiology*, *42*, 269 - 275.

Refika, H. & Marlyn, S.W. (2001). Advanced cystic fibrosis lung disease in children, Current opinion in pulmonary medicine. *Pulmonary Medicine, 7*(6), 448 - 453.

Reid, S. D., Herbelin, C. J., Bumbaugh, A. C., Selander, R. K., & Whittam, T. S. (2000). Parallel evolution of virulence in pathogenic *Escherichia coli*. *Nature*, *406*, 64 – 67.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A. & Cohen, M. L. (1983). Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*. *308*, 681 - 685.

Riley, M. A., & Gordon, D. M. (1999). The ecological role of bacteriocins in bacterial competition. *Trends in Microbiology*, *7*, 129 – 133.

Robbins, J. B., McCracken, G. H. Jr., Gotschlich, E. C., Orskov, F., Orskov, I. & Hanson L. A. (1974). *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *New England Journal of Medicine*, *290*, 1216 – 1220.

Rodriguez-Ángeles, G. (2002). Principal characteristics and diagnosis of the pathogenic groups of *Escherichia coli*. *Salud Pública de México, 44* (5), 464 – 475.

Roesch, P. L., Redford, P., Batchelet, S., Moritz, R. L., Pellett, S., Haugen, B. J., Blattner, F. R. & Welch, R. A. (2003). Uropathogenic *Escherichia coli* use d-serine deaminase to modulate infection of the murine urinary tract. *Molecular Microbiology*, *49*, 55 – 67.

Romling, U., Grothues, D., Heuer, T. & Tummler, B. (1992). Physical genome analysis of bacteria. *Electrophoresis*, *13*, 626 - 631.

Rubino, S., Cappuccinelli, P. & Kelvin, D. J. (2011). *Escherichia coli* (STEC) serotype O104 outbreak causing haemolytic syndrome (HUS) in Germany and France. *Journal of infections in Developping countries, 5*(6), 437-440.

Rupp, M. E. & Fey, P. D. (2003). Extended spectrum *ß*-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. *Drugs*, *63*(4), 353 – 365.

Russo, T. & Johnson, J. (2003). Medical and economic impact of extra-intestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and Infection, 5*, 449 – 456.

Russo, T. A. & Johnson, J. R. (2000). Proposal for a new inclusive designation for extra-intestinal pathogenic isolates of *Escherichia coli*: ExPEC. *Journal of Infectious Diseases, 181*, 1753 - 1754.

Russo, T. A. & Johnson, J. R. (2009). Extra-intestinal Pathogenic *Escherichia coli*. In A. D.T. Barrett and L. R. Stanberry (Eds.). *Vaccines for*

Biodefense and Emerging and Neglected Diseases (pp. 939 – 961), Elsevier Inc.

Ryan, K. J. & Ray, C.G., (2004). *Sherris Medical Microbiology* (4th ed.). New york: McGraw Hill.

Sabat, A. J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J. M., Laurent, F., Grundmann, H. & Friedrich, A. W. (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance, *Eurosurveillance*, *18*(4), 1 – 15.

Sackey, B. A., Mensah, P., Collison, E. & Sakyi-Dawson, E. (2001). Campylobacter, *Salmonella*, *Shigella* and *Escherichia coli* in live and dressed poultry from metropolitan Accra. *International Journal of Food Microbiology, 71*, 21 – 28.

Sambrook, J., Fritsch, E. F. & Maniatis, T., (1989). *Molecular Cloning, A laboratory manual* (2nd ed.). USA: Cold Spring Harbor Laboratory Press.

Sanguinetti, M., Posteraro, B., Spanu, T., Ciccaglione, D., Romano, L., Fiori, B., Nicoletti, G., Zanetti, S. & Fadda, G. (2003). Characterization of clinical isolates of Enterobacteriaceae from Italy by the BD Phoenix extended-spectrum beta-lactamase detection method. *Journal of Clinical Microbiology, 41*, 1463–1468.

Sarff, L. D., McCracken, G. H., Schiffer, M. S., Glode, M. P., Robbins, J. B., Orskov, I. & Orskov, F. (1975). Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. *Lancet I*, 1099 – 1104.

Sarojamma, V. & Ramakrishna, V. (2011). Prevalence of ESBL producing Klebsiella pneumoniae isolates in Tertiary Care Hospital. *ISRN Microbiology*, *318348*, 1 – 5.

Savageau, M. A. (1983). *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. *American Naturalist, 122*, 732 - 744.

Sayah, R. S., Kaneene, J. B., Johnson, Y. & Miller, R. A. (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Applied Environmental Microbiology, 71*(3), 1394 – 1404.

Scaletsky, I. C. (2002). Cell-detaching *Escherichia coli* (CDEC) strains from children with diarrhoea: identification of a protein with toxigenic activity. *Federation of European Microbiological Societies Microbiology Letters*, *217*, 191 – 197.

Scavia, G., Staffolani, M. & Fisichella, S. (2008). Enteroaggregative *Escherichia coli* associated with a foodborne outbreak of gastroenteritis, *Journal of Medical Microbiology*, *57*(9), 1141 – 1146.

Scheutz, F. & Strockbine, N. A. (2001). Genus I: *Escherichia*. In G. Garrity, D. J. Brenner, N. R. Krieg & J. R. Staley (Eds.), *Bergey's Manual of Systematic Bacteriology*, Volume 2: Part B: The Gammaproteobacteria (pp. 607-624), Baltimore: Williams & Wilkins.

Scheutz, F. & Strockbine, N. A. (2005). *Escherichia.* In G. M. Garrity, D. J. Brenner, N. R. Krieg & J. T. Staley (Eds.), *Bergey's manual of systematic bacteriology* (pp. 607 – 624), New York, NY: Springer

Schjørring, S., Struve, C. & Krogfelt, K. A. (2005). Plasmid transfer from *Klebsiella pneumoniae* to *Escherichia coli*. Paper presented at the *15th European Congress of Clinical Microbiology and Infectious Diseases*, Denmark, 2 - 5 April, 2005. Copenhagen: Blackwell Synery. Retrieved August 17, 2009, from http://www.blackwellpublishing.com/eccmid15/abstract.asp?id=37125.

Schlager, T. A., Hendley, J. O., Bell, A. L. & Whittam, T. S. (2002). Clonal diversity of *Escherichia coli* colonizing stools and urinary tracts of young girls. *Infection and Immunity*, *70*, 1225 – 1229.

Schreiber, W., & M. S. Donnenberg. (2002). Type IV pili. *In* M. S. Donnenberg (Ed.) *Escherichia coli Virulence Mechanisms of a Versatile Pathogen* (p. 417). Academic Press: New York.

Selander, R. K., Caugant, D. A. & Whittam, T. S. (1987). Genetic structure and variation in natural populations of *Escherichia coli*. In F. C. Neidhardt (Ed.) *Escherichia coli and Salmonella typhimurium*. ASM Press. Washington, DC. *2*, 1625 – 1648.

Selander, R. K., Caugant, D. A., Ochman, H., Musser, J. M., Gilmour, M. N. & Whittam T. S. (1986). Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Applied Environmental Microbiology*, *51*, 873 - 884.

Serrano, P. H. (2005). Responsible use of antibiotics in aquaculture. *FAO Fisheries technical paper,* No. 469, Rome: FAO, 97 pp.

Shames, S. R., Auweter, S. D. & Finlay, B. B. (2009). Co-evolution and exploitation of host cell signalling pathways by bacterial pathogens. *International Journal of Biochemistry and Cell Biology, 41*, 380 – 389.

Sharma, J., Sharma, M. & Ray, P. (2010). Detection of TEM & SHV genes in *Escherichia coli* & *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian Journal of Medical Research 132*, 332-336.

Siitonen, A. (1994). What makes *Escherichia coli* pathogenic? *Annals of Medicine, 4*, 229 - 231.

Silva, R. M., Toledo, M. R. & Trabulsi, L. R. (1980). Biochemical and cultural characteristics of invasive *Escherichia coli*. *Journal of Clinical of Microbiology*, *11*, 441 – 444.

Sohel, I., Puente, J. L., Ramer, S., Bieber, D. Wu, C. Y. & Schoolnik, G. K. (1996). Enteropathogenic *Escherichia coli*: Identification of a gene cluster coding for bundleforming pilus morphogenesis. *Journal of Bacteriology*, *178*, 2613 – 2628.

Solomon, E. B., Yaron, S. & Matthews, K. R. (2002) Transmission of *Escherichia coli* O157:H7 from Contaminated Manure and Irrigation Water to Lettuce Plant Tissue and Its Subsequent Internalization. *Applied and Environmental Microbiology*, *68*(1), 397 – 400.

Soriano, G., Coll, P., Guarner, C. & Al., E. (1995). *Escherichia coli* capsular polysaccharide and spontaneous bacterial peritionitis. *Hepatology*, *21*, 668 – 673.

Sorlozano, A., Gutierrez, J., Fernandez, F., Soto, M. J. & Piedrola, G., (2004). A preliminary study on the presence of extended-spectrum beta-lactamases (ESBL) in clinical isolates of *Escherichia coli* in Granada (Spain). *Annals of Microbiology*, *54*, 227 – 232.

Standard Unit, Evaluations and Standards Laboratory (SUESL) (2008). *National Standard Method. Laboratory Detection and reporting of bacteria with extended spectrum beta-lactamases* (Ref. No. QSOP 51i2.2), (pp. 6 – 15).

Stantard Treatment Guidelines (STG) (2004). *Ministry of Health, Ghana National Drug Programme* (5th ed.). (pp. 298 – 450), Accra: Yamens Press Ltd.

Stoll, B., Hansen, N., Fanaroff, A. & Al, E. (2002). Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *New England Journal of Medicine*, *347*, 240 – 247.

Struve, C., & Krogfelt, K. A. (1999). *In vivo* detection of *Escherichia coli* type 1 fimbrial expression and phase variation during experimental urinary tract infection. *Microbiology*, *145* (10), 2683 – 2690.

Stürenburg, E. & Mack, D. (2003). Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *Journal of Infection, 47* (4), 273 - 295.

Stürenburg, E., Sobottka, I., Noor, D., Laufs, R. & Mack, D. (2004). Evaluation of a new cefepimeclavulanate ESBL E-test to detect extended-spectrum beta - lactamases in an Enterobacteriaceae strain collection. *Journal of Antimicrobial Chemotherapy*, *54*, 134 – 138.

Sunde, M. & Sorum, H. (2001). Self-transmissible multi-drug resistance plasmids in *Escherichia coli* of the normal intestinal flora of healthy swine. *Microbial Drug Resistance, 7* (2), 191 - 196.

Swinbanks, D. (1996). Japan shuns radishes after 'possible link' to *E. coli*. *Nature, 382*, 567.

Tagoe, D. N. A, Nyarko, H., Arthur, S. A. &. Birikorang, E. (2011). A Study of Antibiotic Susceptibility Pattern of Bacterial Isolates in Sachet Drinking Water Sold in the Cape Coast Metropolis of Ghana. *Research Journal of Microbiology, 6, 153 - 158.*

Tailor, C. S., Nouri, A., Lee, C. G. Kozak, C. & Kabat, D. (1999). Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukaemia viruses. *Proceedings of the National Academy of Science of the United State of America*, *96*, 927 – 932.

Tarr, P. I. (1995). *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. *Clinical Infectious Dieases, 20*, 1-8.

Tarr, P. I., Fouser, L. S., Stapleton, A. E., Wilson, R. A., Kim, H. H., Vary, J. C., & Clausen, C. R. (1996). Haemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga toxin-producing *Escherichia coli* O103:H2. *New England Journal of Medicine*, *335*, 635 – 638.

Taylor, D. (2007). *E. coli* Infection: Symptoms and treatment. Retrieved April 2, 2014, from www.steadyhealth.com.

Taylor, K. A., O'Connell, C. B., Luther, P. W. & Donnenberg, M. S. (1998). The EspB protein of enteropathogenic *Escherichia coli* is targeted to the cytoplasm of infected HeLa cells. *Infection and Immunity*, *66*, 5501 - 5507.

Thomson, K. S., & Sanders, C. C. (1992). Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. *Antimicrobial Agents and Chemotherapy, 36*, 1877 - 1882.

Thomson, N. R., Nasser, W., McGowan, S., Sebaihia, M. & Salmond, G. P. (1999). *Erwinia carotovora* has two KdgR-like proteins belonging to the IclR family of transcriptional regulators: Identification and characterization of the RexZ activator and the KdgR repressor of pathogenesis. *Microbiology, 145*, 1531 – 1545.

Thoreson, M. A., Anastasiadis, P. Z., Daniel, J. M., Ireton, R. C., Wheelock, M. J., Johnson, K. R., Hummingbird, D. K. & Reynolds, A. B. (2000). Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. *Journal of Cell Biology, 148*, 189 - 202.

Todar, K (2008). Todar's Online Textbook of Bacteriology. Retrieved January 7, 2009, from http://www.textbookofbacteriology.net/e.coli.htm

Todar, K. (2009). Todar's Online Textbook of Bacteriology. Retrieved December 15, 2010, from http://www.textbookofbacteriology.net/e.coli.htm

Todar, K. (2011). Todar's Online Textbook of Bacteriology. Retrieved June 23, 2011, from http://www.textbookofbacteriology.net/e.coli.htm.

Torres, M. E., Pirez, M. C., Schelotto, F., Varela, G., Parodi, V., Allende, F., Falconi, E., Dell'Acqua, L., Gaione, P., Mendez, M. V., Ferrari, A. M., Montano, A., Zanetta, E., Acuna, A. M., Chiparelli, H. & Ingold, E. (2001). Etiology of children's diarrhoea in Montevideo, Uruguay: associated pathogens and unusual isolates. *Journal of Clinical Microbiology*, *39*, 2134 - 2139.

Touchon, M., Tenaillon, O., Hoede, C., Barbe, V. & Baeriswly, S. (2009). Organized Genome Dynamic in the *Escherichia coli* species Results in Highly Diverse Adaptive Paths. *PLOS Genetics*, *5* (1), e1000344.

Trabulsi LR, Keller R & Gomes, T. A. (2002). Typical and atypical enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases*, *8*, 508 - 513.

Turner, S. M., Chaudhuri, R. R., Jiang, Z. D., DuPont, H., Gyles, C., Penn, C. W., Pallen, M. J. & Henderson, I. R. (2006). Phylogenetic comparisons reveal multiple acquisitions of the toxin genes by enterotoxigenic *Escherichia coli* strains of different evolutionary lineages. *Journal of Clinical Microbiology*, *44*, 4528 - 4536.

Umolu, P. I., Ohenhen, E. R., Okwu, I. G. & Ogiehor, I. S. (2006). Multiple Antibiotic resistant index and plasmid of *Escherichia coli* in beef in Ekpoma. *American Journal of Science, 2* (3), 1 - 4.

Unhanand, M., Mustafa, M., Mccracken, G. & Nelson, J. (1993). Gram negative enteric bacillary meningitis: a twenty-year experience. *Journal of Pediatrics*, *122*, 15 – 21.

Vargas, M., Gascon, J., Casals, C., Schellenberg, D., Urassa, H., Kahigwa, E., Ruiz, J., & Vila, J. (2004). Etiology of diarrhoea in children less than five years of age in Ifakara, Tanzania. *American Journal of Tropical Medicine and Hygiene*, *70*, 5536 - 5539.

Vercauteren, E., Descheemaeker, P., Ieven, M., Sanders, C. C. & Goossens, H. (1997). Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian teaching hospital. *Journal of Clinical Microbiology*, *35*, 2191 – 2197.

Viljamen, M., Peltola, T., Junnila, S., Olkkonen, L., Järvinen, H., Kuistila, M. & Huovinen, P. (1990). Outbreak of diarrhoea due *Escherichia coli* O111:B4 in schoolchildren and adults: association of Vi antigen-like reactivity. *Lancet*, *336*, 831 - 834.

Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., Reid-Smith, R. J., Tellier, P., Tellis, P. A., Ziebell, K. & Manges, A. R. (2010). Food Reservoir for *Escherichia coli* Causing Urinary Tract Infections. *Emerging Infectious Diseases, 16* (1), 88 – 95.

Walk, S. T., Alm, E. W., Calhoun, L. M., Mladonicky, J. M. & Whittam, T. S. (2007). Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. *Environmental Microbiology*, *9*, 2274 - 2288.

Wang, F. C., Whittam, T. S. & Selander, R. K. (1997). Evolutionary genetics of the isocitrate dehydrogenase gene (icd) in *Escherichia coli* and *Salmonella enterica. Journal of Bacteriology, 179*, 6551 - 6559.

Waterman, S. R. & Small, P. L. (1996). Characterization of the acid resistance phenotype and rpoS alleles of shigalike toxin-producing *Escherichia coli*. *Infection and Immunity*, *64*, 2808 – 2811.

Weagant, S. D. & Feng, P. (2002). Comparative Analysis of a Modified Rapid Presence-Absence Test and the standard MPN Method for Detecting *Escherichia coli* in Orange Juice. *Food Microbiology, 19*, 111 - 115.

Welch, R. A. (2006). The genus *Escherichia. Prokaryotes 6*, 60 – 71.

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M. & Williams A. P. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *The Lancet Infectious Diseases, 13*(2), 155 - 65. doi: 10.1016/S1473-3099(12)70317-1.

Wheat, P. F. (2001). History and development of antimicrobial susceptibility testing methodology. *Journal of Antimicrobial Chemotherapy, 48*, 1 - 4.

Whittam, T. S., Wolfe, M. L. & Wilson, R. A. (1989**).** Genetic relationships among *Escherichia coli* isolates causing urinary tract infections in humans and animals. *Epidemiology and Infection*, *102*, 37 – 46.

WHO (1980). *Diarrhoeal Disease Control Program, A Manual for the Treatment of Acute Diarrhoea*, Geneva: (WHO/CDD/SER/802).

WHO (1999). New frontiers in the development of vaccines against enterotoxigenic (ETEC) and enterohaemorrhagic (EHEC) *E. coli* infections. *Weekly Epidemiological Record, 13*, 98 – 100.

WHO (2000). *Global Water Supply and Sanitation Assessment*. World Health Organization, Geneva.

WHO (2004). The World Health Report 2004 - Changing History. Retrieved September 8, 2009, from http://www.who.int/whr/2004/en/.

WHO (2006). Ghana Mortality Country Fact Sheets. Retrieved May 25, 2012, from www.who.int/whosis/mort/profiles/mort_afro_gha_ghana.pdf.

WHO (2007). *Foodborne disease outbreaks: Guidelines for investigation and control* Geneva, Switzerland, WHO 2007, 21.

WHO (2012). The top 10 causes of death, *2012 WHO Report,* Fact sheet No. 310. Retrieved March 5th, 2015, from www.who.int.

Wiegand, I., Geiss, H. K., Mack, D., Sturenburg, E. & Seifert, H. (2007). Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. *Journal of Clinical Microbiology*, 45, 1167 - 1174.

Wikipedia (2013) Cape Coast. Retrieved June 21, 2013, from http://en.wikipedia.org/wiki/Cape_Coast.

Wiles, T. J., Kulesus, R. R. & Mulvey, A. M. (2008). Origins and Virulence Mechanisms of Uropathogenic *Escherichia coli. Experimental and*

Molecular Pathology, *85*(1), 11 – 19. doi: 10.1016/j.yexmp.2008.03.007.

Wilshaw, G. A., Cheasty, T. & Smith, H. R. (2000). *Escherichia coli*. In B. M. Lund, T. C. Baird-Parker & G. W. Gould, (Eds.), *The microbiological safety and quality of food II* (pp. 1136 -77). Maryland, USA: Aspen Press.

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., Karch, H., Reeves, P. R., Maiden, M. C., Ochman, H. & Achtman, M. (2006). Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Molecular Microbiology, 60*, 1136 - 1151.

Wold, A. E., Caugant, D. A., Lidin-Janson, G., de Man, P. & Svanborg, C. (1992). Resident colonic *Escherichia coli* strains frequently display uropathogenic characteristics. *Journal of Infectious Diseases*, *165*, 46 – 52.

Woodford, N. & Sundsfjord, A. (2005). Molecular detection of antibiotic resistance: when and where? *Journal of Antimicrobial Chemotherapy*. *56*, 259-261.

Wray, C., McLaren, I. M. & Carroll, P. J. (1993). *Escherichia coli* isolated from farm-animals in England and Wales between 1986 and 1991. *Veterinary Record, 133*, 439 – 442.

Yamamoto, S. (2007) Molecular epidemiology of uropathogenic *Escherichia coli*. *Journal of Infection and Chemotherapy*, *13*, 68 – 73.

Yamamoto, S., Terai, A., Yuri, K., Kurazono, H., Takeda, Y., & Yoshida, O. (1995a). Detection of Urovirulence factors in *Escherichia coli* by

multiple polymerase chain reactions. *Federation of European Microbiological Societies, 12*, 85 - 90.

Yamamoto, S., Tsukamoto, T., Terai, A., Kurazono, H., Takeda, Y. & Yoshida, O. (1995b). Distribution of virulence factors in *Escherichia coli* isolated from urine of cystitis patients. *Microbiology and Immunology, 39*, 401 – 404.

Yarze, J. C. & Chase, M. P. (2000). *E. coli* O157:H7 - another waterborne outbreak! *American Journal of Gastroenterology, 95*, 1096.

Zhang, C., Zhang, X., Shen, Y. & Wang, J. (2011) Study on the Resistance of Extended Spectrum β-Lactamases. *Journal of Animal and Veterinary Advances, 10* (8), 1032 – 1036.

Ziljma, A. (2013). 10 Top places to visit in Ghana. Retrieved April 4, 2012, from www.goafrica.about.com.

APPENDICES

Ethical Clearance

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

Research and Development Divison
Ghana Health Service
P. O. Box MB 190
Accra

My Ref. :GHS-ERC: 3
Your Ref. No

28th July 2010

Tel: −233- 0302 681109
Fax + 233-0302 685424
Email: Hannah.Frimpong@ghsmail.org

CYNTHIA A. DONKOH- Principal Investigator

ETHICAL CLEARANCE - ID NO: GHS-ERC: 09/5/10

The Ghana Health Service Ethical Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Genetic Diversity of Escherichia Coli Strains Isolated from various sources in the Cape Coast Metropolis of the Central Region of Ghana"

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol

SIGNED...

PROFESSOR ALBERT GEORGE BAIDOE AMOAH
(GHS-ERC CHAIRMAN)

Cc: The Director, Research and Development Division, GHS, Accra

APPENDIX II

Consent Form

Title of study: "Antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia coli* isolated from various sources in the Cape Coast Metropolis of the Central Region of Ghana"

Information: (To be read or translated to patients/guardians in their own mother tongue)

Dear Sir/Madam,

We kindly ask you to enrol into this study, which we will proceed to describe.

The study in a few words:

The purpose of this study is to access the genetic differences between *E. coli* isolates from different sources including urine, stool and water samples. *E. coli* is a micro-organism usually found harmless in the large intestine of human beings and other warm-blooded animals. There are however some pathogenic strains that colonize other body sites such as the urinary tract, the digestive tract and sometimes the meninges of the brain thereby causing serious infections. Although a lot is known about this micro-organism, little research has been done about its genetic diversity especially in Ghana where environmental conditions are different from those prevailing in developed countries.

You will not receive any direct benefits when you take part in this study. Your participation will however help to provide information that could help in proper diagnosis, treatment and prognosis of *E. coli* infections. This study is a requirement of my PhD program.

Methods:

We would like to start by stressing that this study is strictly voluntary. If you decide not to participate, it will have no consequences for the treatment you will receive or are currently receiving. Your decision will be respected without any further discussion.

If you agree to take part in the study, you will donate 8 to 10 mL of urine or blood (approximately 2 teaspoons) or wound specimens and/or about a spoonful portion of stools as the case may be. A bacterial culture will be obtained from each sample. Further analyses will be made on the *E. coli* isolates including genomic analyses, serology and antibiotics susceptibility testing. The collection of urine/stool usually causes no discomfort. All subjects will receive appropriate treatment as necessary. All information gathered would be treated in strict confidentiality. If you have any question(s), please feel free to ask the physician in charge.

Yours sincerely,
Cynthia A. Donkoh,
Department of Molecular Biology and Biotechnology, UCC.

I,……………………………………………….........., having understood the study, after having the consent form thoroughly explained to me in English/Twi/Fanti/Ga/Ewe/ Hausa language do hereby agree to enrol and participate in this study.

...
Signature/thumbprint of volunteer* and date
* A parent's signature is required if applicant is under the age of 18.

...
Signature of Physician in charge and date

If you have any question(s), please feel free to ask the physician in charge or any of the people listed below:

Dr. Isaac Galyuon,
Dean, School of Biological Sciences, University of Cape Coast
Dr. Damian Amewowor, Department of Molecular Biology and Biotechnology, University of Cape Coast.

APPENDIX III

Distribution of Bacterial Isolates in Clinical and Environmental Samples

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
E. coli	372	1.32	.468	1	2
K. pneum	83	3.0602	.23938	3.00	4.00
K. oxytoca	18	5.7222	.46089	5.00	6.00
Citrobacter	36	9.0556	.23231	9.00	10.00
Shigella sp	17	21.4706	.51450	21.00	22.00
Enterobacter	60	7.9167	.27872	7.00	8.00

Chi-Square Test

Frequencies

E. coli

	Observed N	Expected N	Residual
E. coli clinical	252	186.0	66.0
E. coli environmental	120	186.0	-66.0
Total	372		

K. pneum

	Observed N	Expected N	Residual
K. pneum clinical	78	41.5	36.5
K. pneum environmental	5	41.5	-36.5
Total	83		

Citrobacter

	Observed N	Expected N	Residual
Citrobacter clinical	34	18.0	16.0
Citrobacter Environmental	2	18.0	-16.0
Total	36		

APPENDIX III CONTINUED

***Shigella s*p**

	Observed N	Expected N	Residual
Shigella clinical	9	8.5	.5
Shigella Environmental	8	8.5	-.5
Total	17		

K. oxytoca

	Observed N	Expected N	Residual
K. oxytoca clinical	5	9.0	-4.0
K. oxytoca environmental	13	9.0	4.0
Total	18		

Enterobacter

	Observed N	Expected N	Residual
Enterobacter clinical	5	30.0	-25.0
Enterobacter Environmental	55	30.0	25.0
Total	60		

Test Statistics

	E. coli	K. pneum	K. oxytoca	Citrobacter	Shigella sp	Enterobacter
Chi-Square	46.839[a]	64.205[b]	3.556[c]	28.444[d]	.059[e]	41.667[f]
df	1	1	1	1	1	1
Asymp. Sig.	.000	.000	.059	.000	.808	.000

APPENDIX IV

Prevalence of ESBL Producers and Non-Esbl Producers Among *E. Coli* Isolates Recovered From Clinical and Environmental Samples.

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Clinical samples	261	1.70	.459	1	2
Environmental samples	128	1.88	.332	1	2
ESBL clinical environmental	94	1.17	.378	1	2
Non-ESBL clinical/environmental	295	1.38	.486	1	2

Chi-Square Test

Frequencies

Clinical samples

	Observed N	Expected N	Residual
ESBL	78	130.5	-52.5
NON -ESBL	183	130.5	52.5
Total	261		

Environmental samples

	Observed N	Expected N	Residual
ESBL	16	64.0	-48.0
NON-ESBL	112	64.0	48.0
Total	128		

ESBL clinical/environmental

	Observed N	Expected N	Residual
Clinical	78	47.0	31.0
Environmental	16	47.0	-31.0
Total	94		

APPENDIX IV CONTINUED

Non-ESBL clinical/environmental

	Observed N	Expected N	Residual
Clinical	183	147.5	35.5
Environmental	112	147.5	-35.5
Total	295		

Test Statistics

	Clinical samples	Environmental samples	ESBL clinical/environmental	Non-ESBL clinical/environmental
Chi-Square	42.241[a]	72.000[b]	40.894[c]	17.088[d]
df	1	1	1	1
Asymp. Sig.	.000	.000	.000	.000

APPENDIX V

Molecular Phylogeny

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Clinical	261	2.67	1.170	1	4
Environmental	92	2.55	1.345	1	4
A row	145	1.4897	.50163	1.00	2.00
B1 row	20	1.3500	.48936	1.00	2.00
B2 row	113	1.1239	.33093	1.00	2.00
D row	111	1.3243	.47024	1.00	2.00

Chi-Square Test

Frequencies

Clinical

	Observed N	Expected N	Residual
A	74	65.2	8.8
B1	13	65.2	-52.2
B2	99	65.2	33.8
D	75	65.2	9.8
Total	261		

Environmental

	Observed N	Expected N	Residual
A	35	23.0	12.0
B1	7	23.0	-16.0
B2	14	23.0	-9.0
D	36	23.0	13.0
Total	92		

APPENDIX V CONTINUED

A row

	Observed N	Expected N	Residual
Clinical	74	72.5	1.5
environmental	71	72.5	-1.5
Total	145		

B1 row

	Observed N	Expected N	Residual
Clinical	13	10.0	3.0
Environmental	7	10.0	-3.0
Total	20		

B2 row

	Observed N	Expected N	Residual
Clinical	99	56.5	42.5
Environmental	14	56.5	-42.5
Total	113		

D row

	Observed N	Expected N	Residual
Clinical	75	55.5	19.5
Environmental	36	55.5	-19.5
Total	111		

Test Statistics

	Clinical	Environmental	A row	B1 row	B2 row	D row
Chi-Square	61.927[a]	28.261[b]	.062[c]	1.800[d]	63.938[e]	13.703[f]
df	3	3	1	1	1	1
Asymp. Sig.	.000	.000	.803	.180	.000	.000

APPENDIX VI

Distribution of ESBL Genes

Descriptive Statistics

	N
Clinical ESBL +ve	78
Clinical ESBL -ve	183
Environmental ESBL +ve	16
Environmental ESBL -ve	112

Chi-Square Test

Frequencies

Clinical ESBL +ve

	Observed N	Expected N	Residual
No gene	4	19.5	-15.5
TEM bla	20	19.5	.5
SHV bla	37	19.5	17.5
SHVbla + TEM bla	17	19.5	-2.5
Total	78		

Clinical ESBL -ve

	Observed N	Expected N	Residual
No gene	138	45.8	92.2
TEM bla	9	45.8	-36.8
SHV bla	34	45.8	-11.8
SHV bla +TEM bla	2	45.8	-43.8
Total	183		

APPENDIX VI CONTINUED

Environmental ESBL +ve

	Observed N	Expected N	Residual
No gene	2	4.0	-2.0
TEM bla	4	4.0	.0
SHV bla	8	4.0	4.0
SHV bla + TEM bla	2	4.0	-2.0
Total	16		

Environmental ESBL -ve

	Observed N	Expected N	Residual
No gene	94	37.3	56.7
TEM bla	6	37.3	-31.3
SHV bla	12	37.3	-25.3
Total	112		

Test Statistics

	Clinical ESBL +ve	Clinical ESBL -ve	Environmental ESBL +ve	Environmental ESBL -ve
Chi-Square	28.359[a]	260.388[b]	6.000[c]	129.500[d]
df	3	3	3	2
Asymp. Sig.	.000	.000	.112	.000

APPENDIX VII

Distribution of *E. coli* Isolates Obtained From Clinical Samples

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
wound	31	1.45	.506	1	2
Urogenital	57	1.37	.487	1	2
Aspirate	22	1.50	.512	1	2
Blood	16	1.31	.479	1	2
Urine	238	1.40	.491	1	2
Stool	283	1.41	.493	1	2

Chi-Square Test

Frequencies

Wound: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Screened wound	17	15.5	1.5
isolated wound	14	15.5	-1.5
Total	31		

Urogenital: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Samples screened	36	28.5	7.5
E. coli isolates	21	28.5	-7.5
Total	57		

APPENDIX VII CONTINUED

Aspirate: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Samples Screened	11	11.0	.0
E. coli isolates	11	11.0	.0
Total	22		

Blood: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Samples screened	11	8.0	3.0
E. coli isolates	5	8.0	-3.0
Total	16		

Urine: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Samples screened	143	119.0	24.0
E. coli isolates	95	119.0	-24.0
Total	238		

Stools: samples screened/*E. coli* isolates

	Observed N	Expected N	Residual
Samples screened	167	141.5	25.5
E. coli isolates	116	141.5	-25.5
Total	283		

Test Statistics

	Wound samples screened/*E. coli* isolates	Urogenital samples screened/ *E. coli* isolates	Aspirate samples screened /E. coli isolates	Blood samples screened/ *E. coli* isolates	Urine samples screened/ *E. coli* isolates	Stool samples screened/*E. coli* isolates
Chi-Square	.290[a]	3.947[b]	.000[c]	2.250[d]	9.681[e]	9.191[f]
df	1	1	1	1	1	1
Asymp. Sig.	.590	.047	1.000	.134	.002	.002

APPENDIX VIII

Distribution of *E. Coli* Isolates Obtained From Environmental Samples

Descriptive Statistics

	N
Anaafo	32
Abura	20
Kakum	16
Fosu	64
Sea	10
Cabbage	16
Fresh beef	38
Smoked fish	10
Chicken	14

Chi-Square Test

Frequencies

Anaafo

	Observed N	Expected N	Residual
Samples screened	10	16.0	-6.0
E. coli isolates	22	16.0	6.0
Total	32		

Abura

	Observed N	Expected N	Residual
Samples screened	8	10.0	-2.0
E. coli isolates	12	10.0	2.0
Total	20		

APPENDIX VIII CONTINUED

Kakum

	Observed N	Expected N	Residual
Samples screened	10	8.0	2.0
E. coli isolates	6	8.0	-2.0
Total	16		

Fosu

	Observed N	Expected N	Residual
Samples screened	10	32.0	-22.0
E. coli isolates	54	32.0	22.0
Total	64		

Sea

	Observed N	Expected N	Residual
Samples screened	10	10.0	.0
Total	10[a]		

Cabbage

	Observed N	Expected N	Residual
Samples screened	16	16.0	.0
Total	16[a]		

Fresh beef

	Observed N	Expected N	Residual
Samples screened	10	19.0	-9.0
E. coli isolates	28	19.0	9.0
Total	38		

APPENDIX VIII CONTINUED

Smoked fish

	Observed N	Expected N	Residual
Samples screened	10	10.0	.0
Total	10[a]		

Chicken

	Observed N	Expected N	Residual
Samples screened	6	7.0	-1.0
E. coli isolates	8	7.0	1.0
Total	14		

Test Statistics

	Anaafo	Abura	Kakum	Fosu	Fresh beef	Chicken
Chi-Square	4.500[a]	.800[b]	1.000[c]	30.250[d]	8.526[e]	.286[f]
df	1	1	1	1	1	1
Asymp. Sig.	.034	.371	.317	.000	.004	.593