Phylogenetic analysis of drug resistant environmental *Escherichia coli*

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF DHAKA FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF DHAKA
DHAKA-1000

SUBMITTED BY
EXAMINATION ROLL NO. 1
REGISTRATION NO. 053
SESSION: 2015-2016

Dhaka University Institutional Repository

Certification

It is thereby certified that student bearing Roll no: 1, Registration no: 053 has carried out the research work entitled "Phylogenetic analysis of drug resistant environmental *Escherichia coli*" for the partial fulfillment of her Master of Philosophy Degree in Microbiology from the University of Dhaka, Bangladesh under our academic supervision in the Environmental Microbiology Laboratory, Department of Microbiology, University of Dhaka.

Supervisor
Professor Dr. Sunjukta Ahsan
Department of Microbiology,
University of Dhaka Dhaka-1000
Bangladesh

Co-supervisor
Professor Humaira Akhter
Department of Microbiology,
University of Dhaka Dhaka-1000
Bangladesh

Acknowledgement

At first and foremost, I express my gratitude to omnipotent God, the beneficial, the merciful, for blessings, benediction guidance, protection, help, mental power and wisdom in all aspects of my life.

I am inundated to express my respect, sincere gratitude and heartfelt thanks to my supervisor Dr. Sunjukta Ahsan, Professor, Department of Microbiology, University of Dhaka, for her endless inspiration, extensive support, scholastic guidance, inertness patience and excellent counsel in writing my thesis paper as accurately as possible and to complete the research.

I wish to thank my co-supervisor Professor Humaira Akhter, Department of Microbiology, University of Dhaka, for her encouragement over my days in the department.

I am also grateful to the chairman Dr. Sabita Rezwana Rahman and all of my respected teachers of the Department of Microbiology, University of Dhaka.

I am extremely grateful to all the members of the Environmental Microbiology Lab, the laboratory staffs and employees of the Department of Microbiology, University of Dhaka for their constant help and encouragement during the present study.

Finally, to my parents and brother, I am indebted for their invaluable affection, inspiration, encouragement and continuous support for completion of this study.

Author

December, 2020

Dhaka University Institutional Repository

DEDICATED TO

MY BELOVED PARENTS AND

BROTHER

Abstract

Escherichia coli can be both harmful causing disease in human or harmless in the environment. Considering the diverse nature of *E. coli*, the main aim of this study was to understand the current dynamics of *E. coli* in the environment. One hundred and eighty four isolates from different environmental sources including human (n=60), animal (n=54), prawn (n=25) and the abiotic environment (n=45) were investigated. All of the test isolates harbored *uspA* and *uidA* genes confirming their identity. Fifty randomly selected isolates represented the same ARDRA (Amplified Ribosomal DNA Restriction Analysis) pattern indicating that the 16s rDNA sequences were conserved. Quadruplex PCR was used for phylogrouping, in which, environmental B1 (46.74%) was found to be the predominant group, followed by commensal group A (28.26%), B2 (1.63%), C (8.15%), D (10.67%), E (3.26%) and F (2.17%). Phylogroups A and B1 were found in all environments, whereas pathogenic B2, D and sister group F were present only in human UTI samples. Both Shannon diversity index for human (1.32) and nonhuman (1.17) and Simpsons diversity index (0.64) for *E. coli* phylogroup diversity were significantly different ($p>0.05$) indicating that the two environments are different in terms of phylogroup diversity. According to Pianka's Pairwise index of similarity the value between human and non-human sources was 0.48, indicating low similarity. The most prevalent virotype was EPEC (1.33%, n=150) followed by ETEC (0.67%, n=150). The *eae* gene was absent indicating no recent fecal contamination occurred. Class-1 Integron was present only in 30% (n=150) isolates whereas plasmid was detected in 58.67% (n=150) isolates, of which 80.68% isolates were resistant to all of the antibiotics tested. It was observed that there was no specific correlation between the occurrence of Class I Integron or plasmids and multidrug resistance. *E. coli* of human origin were predominantly resistant to Azithromycin (60%) whereas non-human host isolates were mostly resistant to Cefixime (32.73%). Overall, *E. coli* isolated from human were more resistant to most of the antibiotics tested compared to their non-human relatives. In terms of diversity in antibiotic resistance, there was no significant difference between the resistance patterns of human and non-human *E. coli*. This study indicates that environmental *E. coli* has adapted to live in different environmental types including the human gut, which is of particular concern since these isolates are able to harbour hitherto unknown and potentially harmful genes from the environment. On the other hand, commensal *E. coli* are predominant residents of the animal gut which contradicts our general understanding about commensals.

Dhaka University Institutional Repository

Table of Contents

Chapter 1: Introduction and Literature Review

1.1	Introduction	1-2
1.2	Review of literature	3-29
1.2.1	*Escherichia coli*	3
1.2.2	Indicator bacteria	4
1.2.3	Total Coliforms and Fecal Coliforms	4-5
1.2.4	Common sources of *E. coli*	5-6
1.2.5	*E. coli* as indicator	7
1.2.6	Microbial source tracking	7-9
1.2.7	*E. coli* source tracking	9
1.2.8	*E. coli* typing	10-11
1.2.8.1	Rapid detection and identification of *Escherichia coli* with PCR using universal primers and restriction endonuclease digestions	10-11
1.2.9	Identification of *Escherichia coli* by targeting *uidA* and *uspA* genes	12
1.2.10	Phylo-group determination of *Escherichia coli*	12-15
1.2.11	Patho-types of *Escherichia coli*	15-16
1.2.11.1	Enterotoxigenic *Escherichia coli* (ETEC)	16-17
1.2.11.2	Enterohemorrhagic *Escherichia coli* (EHEC)	17-18
1.2.11.3	Enteropathogenic *Escherichia coli* (EPEC)	18-19
1.2.11.4	Enteroaggregative *Escherichia coli* (EAEC)	20
1.2.11.5	Enteroinvasive *Escherichia coli* (EIEC)	21
1.2.12	Antibiotic resistance	22-25
1.2.13	Multidrug resistance	25-26
1.2.14	Plasmid mediated antibiotic resistance	27-28
1.2.15	Integron mediated antibiotic resistance	28-29
1.3	Aims and Objectives	30
1.3.1	General Objectives	30
1.3.2	Specific objectives	30

Chapter 2: Materials and Methods

2.1	Sample Collection	31-37
2.2	General Procedure and Equipment	37-39
2.2	Sterilization	37
2.2.2	Maintenance of media, reagents and solution	37-38
2.2.3	Sample collection and processing	38
2.2.4	Most Probable Number method (MPN)	38
2.2.5	Sample preparation for Most Probable Number (MPN)	39
2.2.6	Resuscitation of bacteria from stock	39
2.3	Preliminary Identification of *Escherichia coli*	39
2.3.1	Isolation using selective media	39
2.3.2	Observation of colony morphology	39
2.3.3	Microscopic Examination	40
2.4	Biochemical Tests	40-41
2.4.1	Kligler's Iron Agar (KIA) Test	40-41
2.4.2	Citrate Utilization Test	41
2.4.3	Motility, Indole, Urease (MIU) Test	41
2.4.4	Methyl Red (MR) Test	41-42
2.4.5	Voges–Proskauer (VP) test	42
2.5	DNA Extraction	42
2.5.1	Preparation of Template DNA	42-43
2.5.2	Determination of DNA concentration and purity	43
2.6	Molecular characterization	44
2.6.1	PCR amplification procedure	44-49
2.7	PCR Cycle	49-50
2.8	Restriction Endonuclease Digestion	50
2.9	Electrophoretic Analysis of Amplified DNA Product	51
2.10	Determination of the Antibiotic Resistance Pattern of the isolates	51-53
2.11	Plasmid Profiling	53-54

Chapter-3: Results

3.1	Presumptive identification by Most Probable Number (MPN) method	55-70
3.2	Identification based on cultural characteristics of *E. coli*	70
3.2.1	Phenotypic confirmation of test isolates by culture based properties	70-71
3.2.2	Biochemical Identification of *E. coli*	71-79
3.2.2.1	Kligler's Iron Agar (KIA) Test	71
3.2.2.2	Citrate Utilization Test	72
3.2.2.3	Methyl Red (MR) Test	72
3.2.2.4	Indole Production Test	73
3.3	Molecular characterization of *E. coli*	79-80
3.3.1	Confirmation of identity of test isolates by using PCR with universal primers	79-80
3.4	Amplified ribosomal DNA restriction analysis (ARDRA)	80-81
3.5	Detection of *uidA* and *uspA* gene for identification of *E. coli*	81-82
3.6	Phylogroup diversity of *E. coli* isolates	82-84
3.7	Analysis of Phylogroup diversity	87-94
3.8	Determination of Patho-types of *E. coli* from Human and Non Human Host	94-97
3.9	Molecular detection of *eae* gene among environmental isolates	97
3.10	Detection of *intl*-1 gene among environmental *E. coli*	97-98
3.11	Co-existance of class 1 Integron and antibiotic resistance	98-101
3.12	Plasmid profiling in *E coli* samples	101-102
3.12.1	Plasmid number variation of size among isolates	102-107
3.12.2	Co-existence of plasmid and Class-1 Integron among *E. coli* isolates	107-109
3.13	Antibiotic Resistance Pattern of *E. coli*	110-120
3.13.1	Overall sensitivity and resistance profile of isolated *E. coli* to different antibiotics	112-113

3.13.2	**Comparison of antibiotic resistance pattern of *E. coli* isolated from Human and Non Human sources**	**114-115**
3.13.3	**Plasmid mediated antibiotic resistance pattern of environmental *E. coli* isolates**	**115-120**
3.14	**Correlation of antibiotic resistance pattern between Human and Non Human *E. coli* isolates**	**120-122**
3.15	**Molecular profiling of tested *E. coli***	**122-131**

Chapter- 4: Discussion

4.1	**Discussion**	**132-142**
4.2	**Future endeavors**	**142**
4.3	**Concluding Remarks**	**142-143**

Chapter- 5: Reference

5	**Reference**	**144-169**

Appendix

Appendix-I	**Media Composition**	**1-5**
Appendix-II	**Chemical Composition**	**6-7**
Appendix-III	**Instruments and Apparatus**	**8**
Appendix-IV	**Tables related to *E. coli* phylogrouping and antibiotic sensitivity**	**9-24**
Appendix-V	**Calculation formula of Similarity and Diversity indices**	**24-32**

Dhaka University Institutional Repository

List of Tables

Table Number	Title of Table	Page
1.1	**Quadruplex Genotype**	**14-15**
2.1	**Environmental Samples collected from different locations**	**31-34**
2.2	**Details of Human samples from a local hospital of Dhaka, Bangladesh**	**34-37**
2.3	**Composition of Master Mix for PCR**	**44**
2.4	**Sequence of primers used in universal PCR**	**45**
2.5	**Sequence of *uidA* and *uspA* gene primers**	**45-46**
2.6	**Sequence of *eae* and *intl* gene primers**	**46**
2.7	**Primer sequences for the identification of *E. coli* Phylo-groups**	**47-48**
2.8	**Primer sequences for the identification of *E. coli* Patho-types**	**48-49**
2.9	**Annealing Temperature for separate PCR**	**50**
2.10	**Antibiotics used in the susceptibility testing of *Escherichia coli***	**52**
3.1	**MPN of Water Samples collected from different places**	**55-59**
3.2	**MPN of Soil Samples collected from different places**	**59-62**
3.3	**MPN of Prawn Samples collected from different markets**	**62-65**
3.4	**MPN of Animal Samples collected from different places**	**65-68**
3.5	**Colony morphology of *Escherichia coli* on MacConkey and Eosin Methylene Blue plates**	**70**
3.6	**Biochemical characteristics of re-confirmed 150 *E. coli* isolates**	**73-79**
3.7	**Pairwise Pianka's index of similarity among the hosts analyzed**	**87**
3.8	**Comparison of Human and Non Human *E. coli* Phylogroup diversity by Shannon diversity index**	**88**
3.9	**Comparison of Human and Animal *E. coli* Phylogroup's diversity by Shannon diversity index**	**90**
3.10	**Comparison of Phylogroup diversity of *E. coli* isolated from Cow and Chicken by Shannon diversity index**	**91**
3.11	**Comparison of Phylogroup diversity among *E. coli* isolated from Soil and Water by Shannon diversity index**	**92**
3.12	**Comparison of Phylogroup diversity of *E. coli* isolated from Water and Prawn *E. coli* by Shannon diversity index**	**93**
3.13	**Shannon's and Simpson's diversity index of each host analyzed**	**94**
3.14	**Number and size of plasmids extracted from *E. coli* isolated from different sources**	**102-107**
3.15	**Plasmid and Class-1 Integron profiling of *E. coli* isolates**	**107-109**
3.16	**Plasmid profiling and percentage of antibiotic resistance among environmental *E. coli* isolates**	**115-119**
3.17	**Comparison of Resistance diversity of human and non human *E. coli* isolates using Shannon Diversity Index**	**120-121**
3.18	**Comparison of antibiotic resistance diversity of human and animal *E. coli* using Shannon Diversity Index**	**121-122**

3.19	Molecular profiling of *E. coli* bacteria isolated from different sources of environment	123-131

List of Figures

Figure	Title	Page
1.1	**Relationships between total coliform and fecal coliform bacteria**	**5**
1.2	**Flow chart of the universal PCR and RFLP for detection and identification of *E.coli***	**11**
1.3	**Pathogenesis of ETEC infection**	**17**
1.4	**Steps of STEC infection**	**18**
1.5	**Steps of EPEC infection**	**19**
1.6	**Steps of EAEC infection**	**20**
1.7	**Steps of EIEC infection**	**21**
1.8	**Diarrheagenic *Escherichia coli* infection with six pathotypes**	**22**
1.9	**Bacterial mechanisms of drug resistance**	**25**
1.10	**Mechanism of Multi-drug resistance of bacteria**	**26**
1.11	**Bacterial plasmid**	**28**
1.12	**Class 1 Integron**	**29**
3.1	**Different types of samples collected for analysis by MPN method**	**69**
3.2	**Samples analyzed by MPN method**	**70**
3.3	**Representative *Escherichia coli* colonies on MacConkey (left) and EMB (right) agar plates**	**71**
3.4	**Kligler's Iron Agar Test of representative test isolates**	**71**
3.5	**Simmons Citrate test of representative test isolates**	**72**
3.6	**A representative Methyl Red Test Result**	**72**
3.7	**A representative Indole Production Test Result**	**73**
3.8	**Gel image of 16s rDNA amplicon for *Escherichia coli***	**80**
3.9	**HaeIII, EcoRI and HindIII digestion patterns of universal PCR products**	**81**
3.10	**Amplification of *uidA* and *uspA* genes from both human and non-human host isolated *E. coli* samples**	**82**
3.11	**Distribution of different phylo-groups among isolates**	**83**
3.12	**Gel showing confirmation of A/C phylogroup with C-specific PCR**	**83**
3.13	**Gel showing confirmation of D/E and E/Clade 1 isolates with E- specific PCR**	**84**
3.14	**Graphical presentation of the distribution of Phylogenetic groups among the test isolates**	**84**
3.15	**Graphical presentation of the distribution of Phylogenetic groups among Human Host isolates**	**85**
3.16	**Graphical presentation of the distribution of Phylogenetic groups of *E. coli* from Non-Human Host**	**85**
3.17	**Graphical presentation of the distribution of Phylogenetic markers among *E. coli* isolates**	**86**
3.18	**Comparison of *E. coli* phylogroups's diversity between Human and Non Human hosts**	**89**
3.19	**Comparison of *E. coli* phylogroups's diversity between Human and Animal hosts**	**90**

3.20	**Comparison of phylogroup diversity of *E. coli* isolated from Cow and Chicken**	**91**
3.21	**Comparison of phylogroup diversity of *E. coli* isolated from Soil and Water**	**92**
3.22	**Comparison of phylogroup diversity of *E. coli* isolated from prawn and water**	**93**
3.23	**Resolution of *bfpA* and *eaeA* genes by agarose gel electrophoresis**	**95**
3.24	**Detection of *elt* gene by agarose gel electrophoresis**	**96**
3.25	**Graphical representation of the presence of ETEC, EPEC, EHEC, EAEC and EIEC among the test isolates**	**96**
3.26	**Resolution of *eae* gene by agarose gel electrophoresis**	**97**
3.27	**Agarose gel resolution of *intl*-1 PCR amplicon by agarose gel electrophoresis**	**98**
3.28	**Graphical representation of Antibiotic Resistance pattern of Class 1 Integron carrying *E. coli* isolated from Human**	**99**
3.29	**Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Animal Host isolates**	**99**
3.30	**Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Soil isolates**	**100**
3.31	**Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Water isolates**	**100**
3.32	**Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Prawn isolates**	**101**
3.33	**Agarose gel electrophoresis of isolates showing respective band patterns for plasmids**	**102**
3.34	**Graphical representation of the susceptibility profile of *E. coli* isolated from human**	**110**
3.35	**Graphical representation of the susceptibility profile of *E. coli* isolated from non human isolates to different antibiotics**	**110**
3.36	**Clear zones in antibiogram following Kirby and Bauer method**	**111**
3.37	**Graphical representation of antibiotic susceptibility pattern of prawn *E.coli* isolates**	**112**
3.38	**Graphical representation of antibiotic susceptibility pattern of soil *E.coli* Isolates**	**112**
3.39	**Graphical representation of antibiotic susceptibility pattern of water *E. coli* isolates**	**113**
3.4O	**Graphical representation of antibiotic susceptibility pattern of animal Host *E.coli* isolates**	**113**
3.41	**Graphical representation of antibiotic resistance pattern of *E. coli* isolated from Human Host**	**114**
3.42	**Graphical representation of antibiotic resistance pattern of *E. coli* isolates from Non Humanl sources**	**114**
3.43	**Graphical representation of antibiotic resistance pattern of Human *E. coli* isolates carrying multiple plasmids**	**119**
3.44	**Comparison of Antibiotic Resistance pattern between Human and Non Human *E. coli***	**121**

3.45	**Shannon Diversity indices of antibiotic resistance profile of human and animal *E. coli***	**122**

List of Abbreviations

ATCC	American Type Culture Collection
bp	Base pair
kbp	Kilobase pair
cm	Centimeter
cfu	Colony Forming Unit
EDTA	Ethylene diamine tetra-acetic acid
TAE	Tris-acetate EDTA
et al.	And others
L	Liter
M	Molar
mL	Milliliter
mM	Millimole
min	Minute
PCR	Polymerase Chain Reaction
µl	Microliter
%	Percentage
°C	Degree Celsius
pH	Negative logarithm of hydrogen ion concentration
spp.	Species (plural)
sp.	Species (singular)
e.g.	Examplia gratia
g	gram
h	hours
µg	Microgram
PVC	Poly Vinyl Chloride
TE	Tris-EDTA
ETEC	Entero-toxigenic *Escherichia coli*
EHEC	Entero-hemorrhagic *Escherichia coli*
EIEC	Entero-invasive *Escherichia coli*
EAEC	Entero-aggregative *Escherichia coli*
EPEC	Entero-pathogenic *Escherichia coli*
mm	Millimeter
EMB	Eosine Methylene Blue
NB	Nutrient Broth
NA	Nutrient Agar
PBS	Phosphate Buffer Saline
LB	Luria Bertani
MHB	Mueller-Hinton Broth
MHA	Mueller-Hinton Agar

BGLB	**Brilliant Green Lactose Broth**
MPN	**Most Probable Number**
PBS	**Phosphate Buffer Saline**
TSB	**Tryptic Soy Broth**
UV	**Ultraviolet**
sec	**Second**
AMC	**Amoxicillin-Clavulanic Acid**
AZM	**Azithromycin**
CRO	**Ceftriaxone**
C	**Chloramphenicol**
CIP	**Ciprofloxacin**
CFM	**Cefixime**
Gen	**Gentamicin**
COT	**Co-trimoxazole**
TE	**Tetracycline**
F-300	**Nitrofurantoin**
psi	**pounds per square inch**

Introduction and Literature Review

1.1 **Introduction**

E. coli is a common bacteria found in the human intestine. Under certain conditions, *E. coli* can become pathogenic i.e. it gains the ability to cause disease. *E. coli* is often used to study bacterial adaption (Sleight *et al*., 2008), experimental evolution (Cooper *et al*., 2008; Ostrowski *et al.,* 2008) and speciation (Dykhuizen and Green, 1991; Ochman and Groisman, 1994; Konstantinidis, Ramette and Tiedje, 2006; Retchless and Lawrence, 2007). As the bacterium is adapted to conditions in the intestine, its occurrence in the environment indicates recent fecal shedding from the body. Moreover, as the environmental conditions are significantly different from what exists in the human intestine, *E. coli* fails to survive long outside of the human host. For these reasons, *E. coli* has been used as an indicator of recent fecal contamination and represents a threat to human and environmental health (Hartstra *et al.,* 2015). *E. coli* has been long considered one of the best indicators for the presence of potentially pathogenic bacteria (Wang *et al*., 2011). For a long time *E. coli*, however, has become a common resident of the environment. Many of these are multi-drug resistant, having acquired the resistance determinant while residing in the human body or in the environment. Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Antimicrobial exercise and/or especially abused is considered to be the most vital selecting force to antimicrobial resistance of bacteria (Moreno *et al*., 2000; Okeke *et al*., 1999). Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into people via food or direct contact with infected animals. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens (Van den Bogaard *et al*., 2000; Schroeder *et al*., 2002). However, once a pathogenic/ commensal strain enters a particular environment, it may change its property and survivability owing to gene transfer between pathogenic (disease-causing) and non-pathogenic forms. Under such conditions, it may be difficult to identify the origin of the isolate. It can also render the use of the bacterium as an indicator of water pollution difficult or questionable. It is therefore, important to understand the phylogeny of this bacterium so a clearer picture of the source of different isolates can be stated with confidence. This is essential to identify the current distribution of pathogenic and commensal *E. coli* and assess its suitability as an indicator. A few rapid methods are reported for identification

of *E. coli*. Phylogeny is the study of evolutionary relatedness among various groups of organism. Phylogenetic analysis of a bacteria helps to analyze its origin. The distribution (presence/absence) of a variety of genes thought to enable a strain to cause extra-intestinal disease also varies among strains of the four phylo-groups like A, B1, B2 and D (Johnson *et al*., 2001). Eight recognized phylogroups of *E. coli* with seven belonging to *E. coli* sensu stricto (A, B1, B2, C, D, E, F) and one corresponding to *Escherichia* clade I have been recognized (Clermont *et al*., 2013). The virulent extra-intestinal strains belong mainly to group B2 and to a lesser extent to group D (Bingen *et al*., 1998; Picard *et al*., 1999; Johnson *et al*., 2001) whereas most commensal strains belong to group A and phylogroup B1 represents the environmental strains (Picard *et al*., 1999; Walk *et al*., 2007). Special attention has been given to the development of tools allowing the rapid and universal identification of the clones or clonal complexes/ phylogroups is crucial, as a strain's ecological niche, lifestyle and propensity to cause disease vary with its phylogenetic origins (Picard *et al.,* 1999; Gordon and Cowling., 2003; Walk *et al*., 2009; Ratajczak *et al*., 2010). Phylogroups B2 and D include pathogenic strains of *E. coli* (Carlos *et al*., 2010). The presence of phylo-group E, formerly considered as a small set of unassigned strains, is now well recognized. Another phylo group named F is also now known and consists of strains that form a sister group of phylogroup B2; more recently, a phylo-group C has been suggested for a group of strains closely related but distinct from phylogroup B1 (Clermont *et al*., 2013). Recently Clermont and colleagues declared that some strains belong to a group intermediate between the F and B2 phylo-groups, designated as phylo-group G (Clermont *et al*., 2019). *E. coli* are often used in microbial source tracking (MST) as an indicator for existence of other bacterial contamination in water. *E. coli* containing combinations of one, two, all or none of these genes were categorized into seven subgroups. *E. coli* isolates are characterized into different pathotypes according to the presence of specific virulence factors. The main pathotypes in swine diseases are enterotoxigenic *E. coli* (ETEC), characterized by the presence of the toxins STa, STb and LT and F4, F5, F6, F18 or F41 fimbria; enteropathogenic *E. coli* (EPEC), which carries the *eae* gene; Shiga toxigenic or verotoxigenic *E. coli* (STEC or VTEC), characterized by the presence of factors such as F18a/b fimbria and Stx2 toxin and finally Uropathogenic *E. coli* (UPEC) which carries at least one of the following genes: *cnf, hly, bfp, eae, sfa, pap, iha* and *usp* (de Brito *et al.,*1999; Afset *et al*., 2008; Campos *et al*., 2008). In the present study, we want to analyze the origin of multi drug resistant *E. coli* isolated from the environment.

1.2 Review of literature

1.2.1 *Escherichia coli*

E. coli was first discovered in 1885 by Theodor Escherich, a German bacteriologist *E. coli* has since been commonly used for biological laboratory experiment and research. *E. coli* is a facultative (aerobic and anaerobic growth) gram-negative, rod shaped bacteria that can be commonly found in animal feces, lower intestines of mammals and even on the edge of hot springs. They grow best at 37°C. *E. coli* is a Gram-negative organism that cannot sporulate. Therefore, it is easy to eradicate by simple boiling or basic sterilization. *E. coli* and its related species are named as 'enteric bacteria' because they mostly live in the intestinal tracts of human and other animal species (Minnock *et al.,* 2000). *E. coli* can also be classified into hundreds of strains on the basis of different serotypes. *E. coli* is cited as one of the most widespread causative agents of foodborne illness. In Bangladesh, which is a low-lying deltaic region and also in other riverine areas in Asia, floods have become more frequent and also more devastating. In these regions, waterborne diseases are common and flow of some diseases have a characteristic pattern, increasingly exponentially at certain predicted periods of the year but remaining endemic all year round (Kotloff, 1999; Kosek *et al*., 2003). Most *E. coli* strains are harmless but some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K_2 and preventing colonization of the intestine with pathogenic bacteria. *Escherichia coli* (*E. coli*) bacteria normally live in the intestines of people and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food or through contact with animals or persons. *Escherichia coli* strains are commonly present in the gastrointestinal tracts of warm blooded animals including humans (Kaper, 2004). Currently *E. coli* is used as an indicator of fecal contamination in fresh water system (United States Environmental Protection Agency, 1986). There may be several sources of *E. coli* that contribute to high counts of this bacterium in water ways, on beaches, including humans, farm, wild animals, waterfowl, pets and environmental reservoirs (Ishii *et al.,* 2006 and 2007)

1.2.2 Indicator bacteria

The use of an organism that can serve as a surrogate for another is called an indicator organism. An indicator of microbial water quality is generally something (not necessarily bacteria), which has entered the water at the same time as feces, but is easier to measure than the full range of microorganisms which pose the health risk. To be an ideal assessor of fecal contamination, an indicator organism should meet as many of the following criteria as possible:

1. The organism should be present whenever enteric (intestinal) pathogens are present.
2. The organism should be useful for all types of water.
3. The organism should have a longer survival time than the hardiest enteric pathogen.
4. The organism should not grow in water.
5. The organism should be found in warm blooded animal's intestines.
6. The testing method should be easy to perform.

1.2.3 Total Coliforms and Fecal Coliforms: 'Coliform' was the term first used in the 1880s to describe rod-shaped bacteria isolated from human faeces. The coliform group of bacteria, is a functionally-related group which all belong to a single taxonomic family (*Enterobacteriaceae*) and comprises many genera and species. Total coliforms are a group of closely related bacterial genera that all share a useful diagnostic feature: the ability to metabolize the sugar lactose, producing both acid and gas as byproducts. There are many selective growth media available to take advantage of these metabolic characteristics in traditional testing protocols. Total coliforms are not very useful for testing recreational or shellfishing waters. Total coliforms are useful when it is necessary for testing treated drinking water where contamination by soil or plant material would be a concern. A more fecal-specific indicator is the fecal coliform group, which is a subgroup of the total coliform bacteria. Fecal coliforms are widely used to test recreational waters and are approved as an indicator by the U.S. Food and Drug Administration's National Shellfish Sanitation Program (NSSP) for classifying shellfishing waters. About 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes and cause a variety of lesions in immune-compromised hosts including poultry (Daini *et al*., 2008; Mailk *et al*., 2013). *E. coli* bacteria are good indicator organisms of fecal contamination because they generally live longer than pathogens are found in greater number and are less risky to collect or culture in a laboratory than pathogens. In short, there is no direct correlation between numbers of any indicator and enteric pathogens (Grabow, 1996).

To eliminate the ambiguity in the term 'microbial indicator', the following three groups are now recognized:

- **General (process) microbial indicators**: A group of organisms that demonstrates the efficacy of a process such as total heterotrophic bacteria or total coliforms for chlorine disinfection.
- **Fecal indicators (such as *E. coli*)**: A group of organisms that indicates the presence of fecal contamination such as the bacterial groups thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present.
- **Index organisms and model organisms**: A group/or species indicative of pathogen presence and behavior respectively such as *E. coli* as an index for *Salmonella* and F-RNA coli phages as models of human enteric viruses.

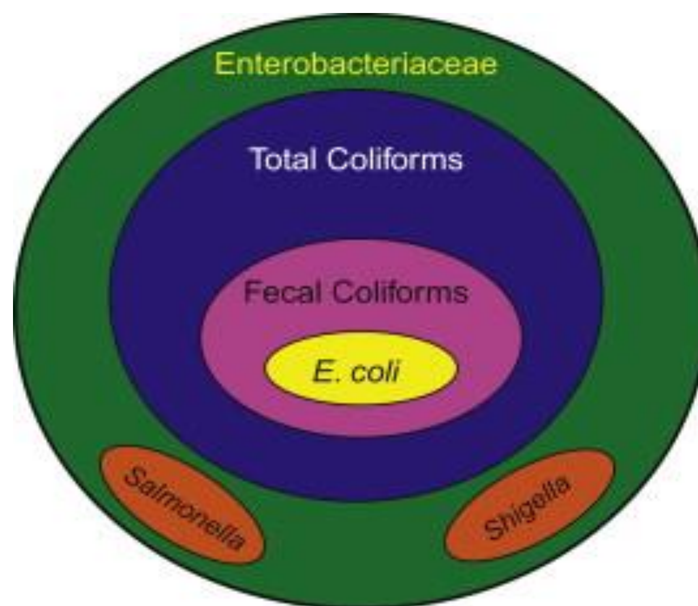


Figure 1.1: Relationships between total coliform and fecal coliform bacteria (Gerba, 2009).

1.2.4 Common sources of *E. coli*

E. coli is normally live in the intestines of cattle. Certain serotypes of *E. coli*, such as *E. coli* O157:H7, have also been found in the intestines of chickens, deer, sheep, and pigs. These bacteria cause human illness when they are ingested and can lead to *E. coli* infection through various modes of transmission, including through food and water sources, animal to animal contact and person to person contact in daycares and other settings. Hemolytic Uremic Syndrome (HUS) secondary to

E. coli 0157:H7 infection was known as 'Hamburger Disease'. Fast food items like pizza and cookie dough may also be contaminated with *E. coli*. Ground beef and other meat products contaminated with *E. coli* lead to plague at both meat industry and within public. Improper sanitation, cross-contamination and a failure to cook meat to a high temperature to kill *E. coli* have all been found to have contribution to *E. coli* outbreaks associated with restaurant food. Ingestion of raw milk and unpasteurized cheeses act as risk factors for *E. coli* infection. *E. coli* and other pathogens are shed in the feces of livestock such as cows and goats and contaminate milk during the milking process. Before or after harvest fresh fruits and vegetables can become contaminated with *E. coli* also. Several *E.coli* O157:H7 outbreaks have been traced in unpasteurized fruit juices and cider prepared from apples which are usually picked up from the ground. Water has been recognized as the source of several *E. coli* outbreaks. Several outbreaks with *E. coli* has been identified due to animal to person transmission. Person to person transmission of *E. coli* has also been known to occur between diseased persons and their caregivers. Although most *E. coli* strains are harmless, certain strains are pathogenic and cause diseases like watery diarrhea, bloody diarrhea, urinary tract infection, meningitis and sepsis, which may lead to death (Nataro, 1998; Gyles, 2007). Usually zoonotic bacterial pathogen is responsible for waterborne outbreaks in humans through contaminated drinking and recreational water both at unindustrialized and developed countries (Rosenberg *et al.*, 1977; Probert, 2017). Contamination from possible human sources include discharge of waste water, sewage leaks and failing septic tanks as well as municipal, residential, medical and industrial waste facilities. *E. coli* contamination from animal sources include runoff from animal farms, land application of animal manure, pet wastes from parks and wildlife like raccoons and deer (Cho *et al*., 2018). In metropolitan watersheds, fecal indicator bacteria are considerably interrelated with human density (Frenzel and Couvillion, 2002). The scientists assumed that the levels of *E. coli* at the pristine site possibly came from wildlife, such as deer and elk, living the area (Niemi and Niemi, 1991). Likewise, recent environmental investigations constantly have recovered considerable *E. coli* populations from soils and fresh water environments (Ishii *et al*., 2006; Walk *et al*., 2009) indicating that naturalized (innocuous) strains (Walk *et al*., 2009) may be prevalent in nature.

1.2.5 *E. coli* as indicator

Escherichia coli plays a dominating role the predominant members of the facultative anaerobic portion of the human colonic normal flora (Krieg, 1984). The bacterium's only natural resident is the large intestine of warm-blooded animals and with some exceptions, *E. coli* generally does not survive well outside of the intestinal tract, its presence in environmental samples, food or water usually indicates recent fecal contamination or poor sanitation practices in food-processing facilities. The extent of fecal pollution, lack of hygienic practices and storage conditions are responsible for the presence of large number of *E. coli* in these samples (Krieg, 1984). *E. coli* is commonly used to assess the quality of water in the field of water purification. The *E. coli*-index can indicate the amount of human feces in the water. The reason why *E. coli* is used as an indicator is due to a significant larger amount of *E. coli* in human feces than other bacterial organisms. Most strains of "*E. coli*" are helpful to their hosts; however, more and more newly discovered strains are contributing into existing population through mutation and evolution. Some can cause severe disease, such as *E. coli* O157: H7. *E. coli* would track bacterial pathogens and most viruses, but would not be useful for parasitic cysts. *E.coli* has decreased oxidant susceptibility, so at source drinking water it is present in very few numbers. Virtually all sources of drinking water are from well-protected environments which should have a low fecal challenge. *E.coli* act as an important biological indicator in case of distribution water. The public health threat comes from sewage intrusion, which will have a very high concentrations of *E. coli* (10^8-10^9 per ml).

1.2.6 Microbial source tracking

Microbial source tracking (MST) describes a set of methods and strategy for investigation of fecal pollution sources in environmental waters which is based on the association of certain fecal microorganisms with a particular host (Harwood *et al*., 2014). Fecal contamination of drinking water sources, harvestable shellfish and recreational waters are responsible for human exposure to pathogenic microorganisms (Napier *et al*., 2017). Therefore continuous monitoring and proper protection of these waters are required. Traditionally, fecal indicator bacteria (FIB) have been used to detect the quality of environmental waters and to assess the associated public health risks (Griffith *et al*., 2009). Microbial water quality monitoring with FIB have several disadvantages as these bacteria can survive and multiply outside of the host (Byappanahalli *et al*., 2003) and a poor correlation have been found between FIB and pathogen presence (Ahmed *et al*., 2013). In case of

identifying the origin or source of fecal contamination, detection of FIB is unable to indicate the source (Field and Samadpour, 2007). Therefore, microbial source tracking (MST) techniques have been developed over the last decade to unequivocally identify the sources and origins of fecal pollution. The number and range of potential host sources included in MST studies must be deliberately chosen to suit the water body and particular questions associated with it (Hagedorn *et al.*, 2011). Microbial Source Tracking (MST) methods are designed to distinguish between human and non-human sources of fecal contamination and some methods are used to differentiate between fecal contaminations originating from individual animal species (Griffith *et al.,* 2003). MST, the active area of research is very much effective to provide important information to effectively manage water resources (Stoeckel *et al.*, 2004). MST methods are typically divided into two categories. The first category is called library-dependent, relying on isolate-by-isolate identification of bacteria cultured from various fecal sources and water samples and comparing them to a "library" of bacterial strains from known fecal sources. These fingerprints are then compared to developed libraries for classification. The use of fecal bacteria to determine the host animal source of fecal contamination is based on the concept strains from different host animals can be differentiated based on phenotypic or genotypic markers (Layton *et al.*, 2006). Different sources (e.g., human, pets, livestock and wildlife) of indicator bacteria (e.g., *E. coli* or *Enterococcus*) can be identified depending on library-dependent methods. However, these methods are very costly and require more time and experienced personnel completing the analysis due to the time it takes to develop a library. Another method known as library-independent which work based on the detection of a specific host associated genetic marker or gene target identified in the molecular material isolated from a water sample. One of the advantage of these methods is that they can help identify sources based on a known host-specific characteristic (genetic marker) of the bacteria without the requirement of a "library". Polymerase chain reaction (PCR) is used in case of library independent method to amplify a gene target that is specifically found in a host population (Shanks *et al.,* 2010). However, it is possible to identify and characterize the origin of fecal contamination by using appropriate method and appropriate indicator (Simpson *et al.,* 2002). MST based on identification of specific molecular markers can provide a more complete picture of the land uses and environmental health risks associated with fecal pollution loading in a watershed than is currently possible with traditional indicators and methods (Jenkins *et al.*, 2009). In theory, genetic marker sequences are used in case of host-specific PCR (library-independent

MST) that are specific both to fecal bacteria and to the host species that produced the feces, allowing discrimination among different potential sources (Field *et al*., 2003). Host-specific PCR act as an efficient method for characterizing a microbial population without first culturing the organisms (Scott *et al*., 2002). Furthermore, these methods are cost effective, rapid and potentially more specific than library-dependent methods. It is expected that these host-specific molecular methods can measure the amount of microbial DNA present in the water sample rather than simply detecting a presence or absence of microbial DNA by using the quantitative polymerase chain reaction (qPCR) technique that (Santo Domingo *et al*., 2007).

1.2.7 *E. coli* source tracking

Waterborne pathogens remain a leading cause of morbidity and mortality globally. Fecal-oral route act as the mode of transmission of pathogens which are typically found in water supplies (Simpson, 2004). *Escherichia coli* has been used to assess the quality of water since the early 1900s to identify contaminated water sources, as they are a normal inhabitant of the GI tract and found in most mammals including humans, livestock and wildlife (Klein and Houston, 1897). The possible sources of water contamination include manure used for agricultural purposes, sewage overflows, ineffective aseptic water decontamination systems and false analysis of water quality (Lee and Wong, 2009). In order to determine the source of *E. coli* isolates, a number of methods like ribotype analysis, pulse- field gel electrophoresis, antibiotic resistance profiling, rep-PCR DNA fingerprinting and ERIC-PCR have been studied for their effectiveness, efficiency and reliability to correctly identify infected hosts (Dombek *et al.*, 2000). Often DNA fingerprinting and other library based methods correlate to misclassification and continuous library enlargement due to the diversity of *E. coli* strains from fecal sources (Lyautey *et al*., 2010). For MST applications limited success has been achieved using *E. coli*, although this bacterium is widely used to determine water quality (Harwood *et al*., 2014). One study revealed the evidence of a potentially human-specific strain of *E. coli* that belongs to the B2 clonal subgroup VIII with an 081 serotype (Clermont *et al.,* 2008). However, genetic markers like *chuA*, *yjaA, arpA* and TspE4.C2 fragment are recently used for phylogenetic grouping of *E. coli* which is applied in MST.

1. 2. 8 *E. coli* typing

1.2.8.1 Rapid detection and identification of *Escherichia coli* with PCR using universal primers and restriction endonuclease digestions: In many developing countries isolation of organisms in culture is often hindered by the use of antibiotics by a large portion of patients prior to their arrival at a center where culture facilities are available. However the currently available methods like latex agglutination (LA) and counter immune electrophoresis require the presence of ≥ CFU of organisms per ml for optimal sensitivity (Davis and Fuller, 1991; Feigin, 2009). Commercial DNA probes have become available in recent years for the diagnosis of a number of infectious diseases but this test also requires more than 10^3 organisms to find out a positive result (Pozzi *et al.*, 1989). A rapid and accurate identification of bacterial strains is necessary when it involves outbreak cases in hospitals (Kong *et al.*, 2011). In this situation a rapid and sensitive method named ARDRA (Amplified Ribosomal DNA Restriction Analysis) was developed to detect and identify *E.coli* isolated from different environmental samples. Among these, amplified ribosomal DNA restriction analysis (ARDRA) was considered as one of the useful method to give accurate subtyping results (Dijkshoorn *et al.*, 1998; Jawad *et al.*, 1998; Shin *et al.*, 2004). Because of this reason, a PCR program and one set of PCR primers was designed based on the conserved sequence of the 16S rRNA genes. Different restriction patterns were found for universal PCR products from different bacteria. Besides, PCR products from different isolates from different sources of the same bacteria were found to have the same restriction length pattern. These results formed the basis for identification of bacteria in a more significant way. The procedure for the use of PCR-RFLP or ARDRA for detection and identification of bacterial pathogens requires only 1 day to complete whereas the conventional methods require at least 2 days and most of the phenotypic methods require specific media. So, researchers can get result 1 day earlier by this method of universal PCR-RFLP than the conventional methods. This PCR-RFLP procedure can reduce the unnecessary use of broad spectrum antibiotic therapies but it is not much cost effective comparing to the conventional methods. One pair of primers, designated as U1 and U2, with sequences conserved among all of these bacteria was selected for this purpose. The sequence of primer is 5′-CCAGCAGCCGCGGTAATACG-3′, corresponding to nucleotides 518 to 537 of the *E. coli* 16S rRNA gene and that of U2 is 5′-ATCGG (C/T) TACCTTGTTACGACTTC-3′, corresponding to nucleotides 1513 to 1491 of the same gene. PCR performed with these two primers is referred to as the universal PCR. The expected size of a PCR product generated from

all of these DNA samples was 996 bp. The PCR products were then digested with three different restriction enzymes named *HaeIII, EcoRI, HindIII* in order to determine whether there is a restriction fragment length polymorphism that would be helpful to identify different types of bacteria. PCR products from different isolates of one species of bacteria would have the same restriction fragment length polymorphism pattern, which could assist to be confirmed about the identification protocols. Figure 3 here summarizes different stages of the PCR-RFLP protocols in brief.

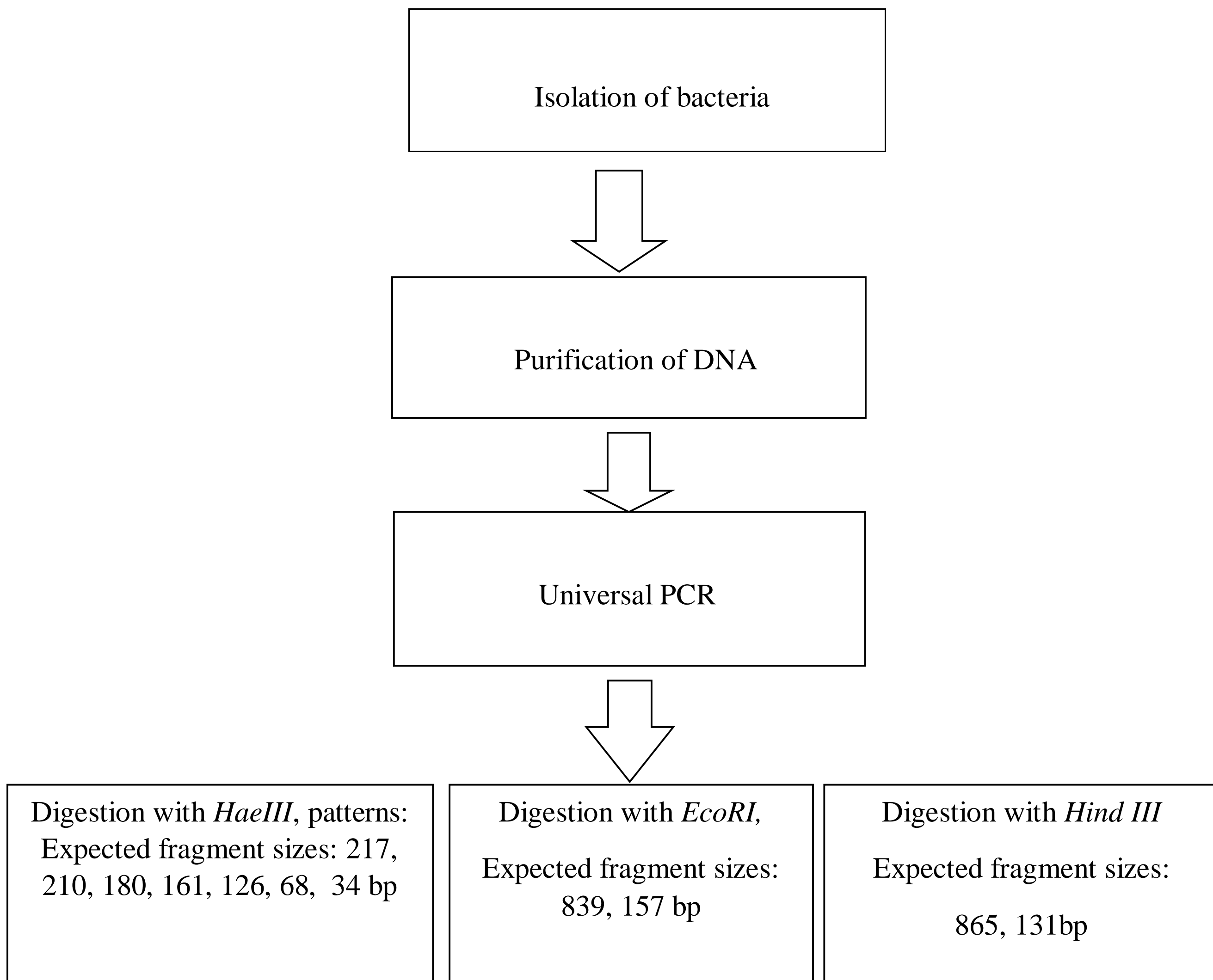


Figure 1.2: Flow chart of the universal PCR and RFLP for detection and identification of *E. coli.*

1.2.9 Identification of *Escherichia coli* by targeting *uid*A and *usp*A genes: The β glucoronidase or GUD was first isolated from *E. coli* and it is an inducible enzyme that is encoded by the *uid*A gene in *E. coli* (Jefferson *et al*., 1986). *Escherichia coli* β- glucuronidase has a monomeric molecular weight of 68, 200 which appears to function as a tetramer and hydrolyzes β-glucuronides as substrate. Detection of *uid*A gene from environmental samples appears to be an effective tool to differentiate unique *E. coli* populations and should be useful for the characterization of *E. coli* dynamics in secondary environment. B - glucuronidase activity have been detected among phototrophic and auxotropic variants of *E. coli* and other members of *Enterobacteriaceae* responsible for urinary tract infection. It has been found that the *uid*A gene considered to be very specific to *E.coli* and present in single copy per genome; however, primers specific to this region also able to amplify some species of *Shigella* (Bej *et al*., 1991). Bej *et al*. (1991) also noticed that the carboxyl end of the *uid*A gene is also unique and conserved in *E. coli* and *Shigella* spp. Molina *et al*. (2015) used two primer pairs like *uidA* and *lacZ* to identify *E. coli*. Because of the limitations of *uid*A primers, one of the *E. coli* specific primer set for flanking region of *usp*A (Chen *et al*., 1998) was incorporated (Godambe *et al*., 2017). The universal stress protein (*usp*A) is a 13.5 kDa cytoplasmic protein. Heat shock and osmotic stress are responsible to increase the synthesis of the protein. Conditions such as nutritional starvation and the presence of toxic agents also stimulated its production. The over production of *usp*A genes allows the organisms to better cope up with stresses by largely unknown mechanisms. *usp*A is widely present within bacterial genomes. It is hypothesized that *uspA* is especially more important to the recovery of *E.coli* following starvation of any nutrients. The use of two molecular markers (*uid*A and flanking region of *usp*A) which is specific for the *E. coli* would be used effectively for the confirmation of the presence of *E. coli*. The pair of DNA marker (*uspA* and *uidA*) is more perfect for the confirmation of *E. coli* than any single DNA marker (Godambe *et al*., 2017). In this study detection of *uid*A and *usp*A gene was done by using specific primers in a multiplex PCR.

1.2.10 Phylo-group determination of *Escherichia coli*

Phylogenetic grouping of *E. coli* strains based on genetic markers, *chuA*, *yjaA* and TspE4.C2 DNA fragment, was recently studied in application for MST. Based on the study by Clermont *et al.,* (2000) and recently Carlos *et al.* (2010), *E. coli* can be classified into four phylogenetic groups: A, B1, B2, or D and then into subgroups: A0, A1, B1, B2, B3, D1 and D2. The presence of phylo

group E, formerly a small set of unassigned strains of which 0157: H7 is the best known member, is now well recognized (Tenaillon *et al*., 2010). A phylo group F is also now recognized and consists of strains that form a sister group to phylo group B2 (Jaureguy *et al*., 2008; Clermont *et al*., 2011). More recently, a phylo group C has been suggested for a group of strains closely related to but distinct from, phylo group B1 which includes strains from environmental sources (Moissenet *et al*., 2010; Clermont *et al*., 2011). Walk and colleages (2009) reported on several novel lineages of *E. coli* that are genetically distinct but phylogenetically indistinguishable from *E. coli. Escherichia* clade I, one of these cryptic lineages should also be considered a phylo group of *E. coli* based on the amount of recombination detected between strains belonging to clade I and *E. coli* (Luo *et al*., 2011). Thus at present there are eight recognized phylogroups of *E. coli* with seven belonging to *E. coli sensu stricto* (A, B1, B2, C, D, E, F) and one corresponding to *Escherichia* clade I. Clermont and colleagues developed a new PCR-based method that enables an *E. coli* isolate to be dispensed to one of the eight phylogroups and which allows isolates that are members of the cryptic clades (II to V) of *Escherichia* to be identified. The groups were determined based on the presence or absence of *chuA*, *yjaA* and DNA fragment TspE4.C2. The new available genomic data were used to modify the *chu*A, *yja*A and TspE4.C2 primer sequences in order to avoid polymorphisms in the nucleotide sequence used for primer annealing and exclude amplification of TspE4.C2 and *chu*A in strains belonging to cryptic clade I and clades III, IV and V respectively (Clermont *et al*., 2011) and further an additional gene target *arp*A was added, thus making the new method a quadruplex PCR. The inclusion of *arp*A serves two purposes. First, it acts as an internal control for DNA quality, as with its addition all *E.coli* and clade 1 strains are expected to yield at least one PCR product using the quadruplex PCR. Second, the inclusion of *arp*A enables strains belonging strains (*chu*A+, *yja*A-, TspE4.C2), to be distinguished because *arp*A is present in all *E. coli* with the exceptions of strains belonging to phylogroups B2 and F (Clermont *et al*., 2013). *arp*A is also absent in cryptic clades II, III, IV, V as well as *Escherichia albertii* and *Escherichia fergusonii*. In order to identify strains belonging to phylogroups C and E, two additional allele specific PCR primer pairs were designed (Lescat *et al*., 2013). The main purpose of Clermont phylotyping method on the basis of this quadruplex method is to determine the phylogenetic background of *E. coli* strains.

Table 1.1 Quadruplex Genotype

arpA (400bp)	*chuA* (288bp)	*yjaA* (211bp)	TspE4.C2 (152bp)	Phylogroup	Next step
+	-	-	-	A	
+	-	-	+	B1	
-	+	-	-	F	
-	+	+	-	B2	
-	+	+	+	B2	
-	+	-	+	B2	Could be confirmed by testing *ibeA* gene
+	-	+	-	A or C	Screen using C-specific primers. If C+ then C, else A
+	+	-	-	D or E	Screen using E-specific primers. If E+ then E, else D

+	+	-	+	D or E	Screen using E-specific primers. If E+ then E, else D

1.2.11 Pathotypes of *Escherichia coli*

E. coli consists of a diverse group of bacteria. Some *E. coli* strains cause urinary tract infections, bacteremia and bacterium related diarrhea and are also the main cause of neonatal meningitis in human and animals. In 1999, the Centers for Disease Control and Prevention assessed that there were 269,060 circumstances of gastroenteritis caused by *E. coli* in the United States alone. Pathogenic *E. coli* strains can be distinguished from their nonpathogenic complements by the presence of virulence genes, which code for adherence and colonization, invasion, cell surface molecules, secretion, transport and siderophore formation (Finlay and Falkow, 1997). Pathogenic *E. coli* strains are characterized into pathotypes. Six pathotypes are related with diarrhea and collectively are stated as diarrheagenic *E. coli*. Shiga toxin-producing *E. coli* (STEC). STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC). This pathotype is one of the most commonly known reason for foodborne outbreaks in the world. Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) (Zinnah *et al*., 2007). STEC is responsible for bloody diarrhea in addition to possibly fatal diseases in humans, including hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (Nataro, 1998; Paton, 1998; Kaper, 2004; Mainil, 2005). Some pathogenic STEC strains also bring a chromosomally localized pathogenicity island stated as the locus enterocyte effacement (LEE) and these strains are often called enterohemorrhagic *E. coli* (EHEC) (Kaper, 2004). It has been found that contaminated fruits, vegetables and water have been related to *E. coli* 0157:H7 outbreaks (Ferens and Hovde, 2011). Diarrhea is considered the most common type of symptom of such type of infection which can cause death in immune-compromised individuals such as the very young and the elderly, due to dehydration from prolonged illness (Kinge *et al*., 2010). *E. coli* is the reason of 80-85% of

urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5-10% (Nicolle, 2002).With its variety of pathologies, *E. coli* is a key of human morbidity and mortality around the world. Each year *E. coli* causes in excess of two million deaths due to infant diarrhoea (Kotloff *et al*., 1999 ; Kosek *et al.,* 2003) and extraintestinal infections (mainly septicaemia derived from urinary tract infection) (Russo and Johnson, 2003) and is also responsible for nearly 150 million cases of uncomplicated cystitis (Russo and Johnsons, 2003). Since humans and animals carry so many *E. coli* cells that may create commensal or antagonistic interactions with their hosts it is compulsory to express the genetic and population determinants that develop commensal strains to adopt a pathogenic behavior. Kinge *et al* (2010) report that *E. coli* that has been linked to well-known antibiotic resistance gene pools and these genes are shifted into the normal flora of humans and animals, where they exert a strong selective pressure for the development and extent of resistance *E. coli* strains.

1.2.11.1 Enterotoxigenic *Escherichia coli* (ETEC): ETEC are bacteria that inhabit the small intestine and cause severe diarrhea, dysentery, abdominal cramps and fever. Infection caused by ETEC can be life threatening due to significant fluid loss and severe dehydration. ETEC is responsible to cause 280-400 million diarrheal episodes per year in children under 5 years of age, resulting in 300,000 to 500,000 deaths (WHO, 2006). Often ETEC is the first enteric infection experienced by infants in low resource countries and in endemic areas almost all children have had 1, ETEC diarrhea episode in their first year of life. Because of natural immunity which develop following several incidents of the disease, ETEC is less predominant in children 5 years and older, as well as in adults (Walker and Black, 2010). Malnutrition, growth stunting and cognitive deficits in children are responsible for ETEC infection. One study revealed that malnutrition and dehydration lead productivity loss of 15 to 20% in adult life (Qadri *et al*., 2005). The progress of ETEC as a gastrointestinal pathogen is not new (Clarke, 2001). Because of the gaining of pathogenicity island, different types of virulence genes are transferred from one organism to another (Clarke, 2001). Non-pathogenic *E. coli* retains many of the genes requisite for host cell interaction but lack certain genes to make them fully pathogenic (Groisman and Ochman, 1994). Similarity has found between the heat labile (LT) enterotoxin of ETEC, which is encoded in communicable plasmid with cholera toxin (CT) (Qadri *et al*., 2005).

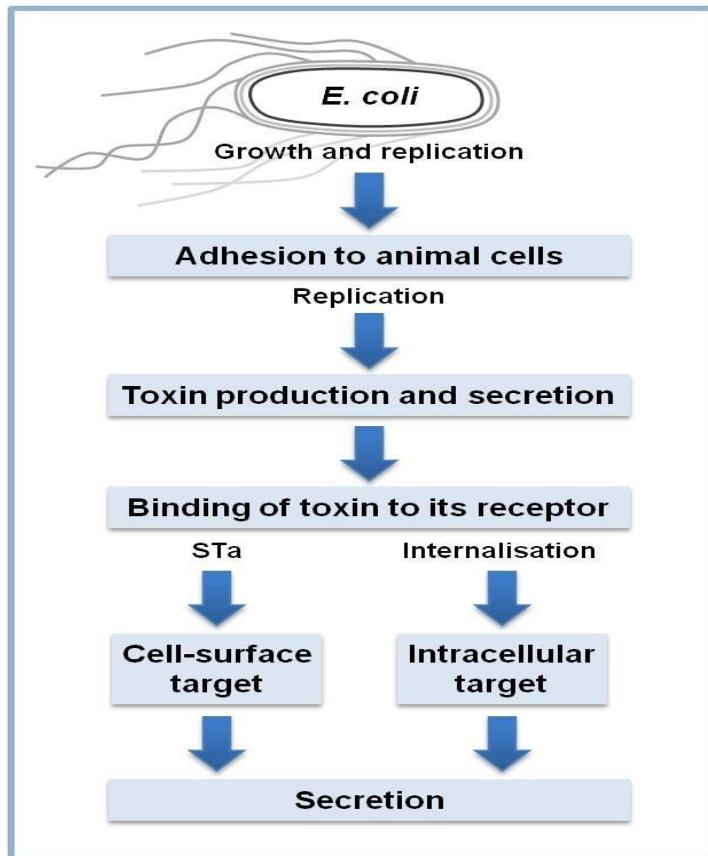


Figure 1.3: Pathogenesis of ETEC infection (Dubreuil, 2013).

1.2.11.2 Enterohemorrhagic *Escherichia coli* (EHEC): Enterohemorrhagic *Escherichia coli* (EHEC) serotype 0157:H7 is a human pathogen responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) worldwide. Cattle is the main reservoir of this pathogen. Infection has also been seen in other ruminants like sheep, goats, deer while other mammals such as pigs, horses, rabbits, dogs and cats, birds like chickens and turkeys have been found infected too (WHO, 2018). Primary sources of STEC outbreaks are raw or undercooked meat products, raw milk and faecal contamination of vegetables. Features of *E. coli* serotype 0157:H7 infection includes abdominal cramps and bloody diarrhea as well as the fatal complication hemolytic uremic syndrome (HUS) (Karmali *et al.,* 1983; Karmali, 1989; Griffin and Tauxe, 1991). In humans, EHEC settles the large intestine (Phillips *et al.,* 2000). EHEC releases shiga toxin that binds to endothelial cells with the expression of Gb3, permitting absorption into the bloodstream and distribution of the toxin to other organs (Sandvig, 2001). The Gb3 expressing tissues and cell types varies among hosts and the dissemination of Gb3 marks the pathology of toxin mediated disease

to cells expressing Gb3 (Pruimboom-Brees *et al*., 2000). STEC is heat-sensitive. In preparing food at home, be sure to follow basic food hygiene practices such as cook thoroughly. According to WHO (2018) five keys to safer food is a key measure to prevent infections with foodborne pathogens such as STEC.

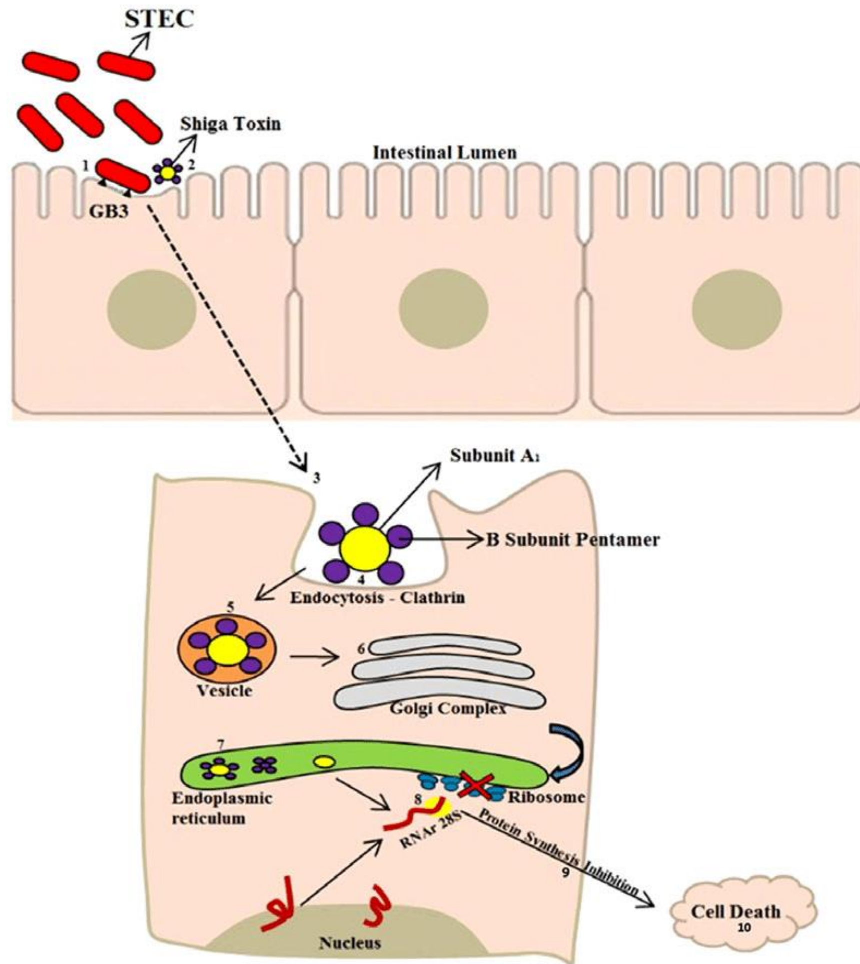


Figure 1.4: Steps of STEC infection (Castro *et al.*, 2017).

1.2.11.3 Enteropathogenic *E. coli* (EPEC): EPEC is responsible for infantile diarrhea. Enteropathogenic *E. coli* (EPEC) are important diarrheal pathogens of young children. Globally diarrhea considered as the second leading cause of death in children younger than 5 years accounting for 1.3 million deaths annually (Black, 2010). EPEC, one of the diarrheagenic *E. coli* pathotypes are among the most important pathogens infecting children worldwide because of their high prevalence in both the community and hospital setting (Mercado *et al*., 2011) and because they are one of the main causes of persistent diarrhea (Abba *et al*., 2009). EPEC were originally

serogroup defined *E. coli* associated with infantile diarrhea. EPEC most commonly causes acute diarrhea and may also cause persistent diarrhea. After Rota virus and ETEC infections in the community setting we found that EPEC had the second highest severity score and ORS usage (Ochoa *et al*., 2009). A trademark phenotype of EPEC is the capability to produce attaching and effacing (A /E) lesions (Nataro *et al*., 1998). Intestinal cell attachment is mediated by an outer membrane protein called intimin, encoded by *eae*, which is currently used for the molecular diagnosis of EPEC. Moreover, EPEC are categorized into typical and atypical strains based on the presence of plasmid *E.coli* adherence factor (EAF).There are two important operons on this plasmid, *bfp* and *per*, the first encoding the type 4 bundle forming pilus (BFP) and the second encoding a transcriptional activator called plasmid encoded regulator (Per). All EPEC strains lack the genes to produce shiga toxin (*stx*). *E.coli* strains that are ea*e+bfpA+stx-* are classified as typical EPEC (tEPEC), most of these strains belong to classic O:H serotypes and produce the localized adherence (LA) phenotype linked with the production of BFP (Trabulsi *et al.,* 2002). On the other hand, *E. coli* strains that are ea*e+ bfpA- stx-* are classified as atypical EPEC (aEPEC). These strains display localized-like (LAL), diffuse (DA) or aggregative adherence (AA) patterns. The LAL pattern in aEPEC is responsible for the formation of common pilus and other known adhesins (Scaletsky *et al.,* 2010). In a current study of hospitalized diarrheal patients in India, EPEC was found to cause 3.2% diarrhoea in children younger than 5 years of age (Nair *et al.,* 2010). Prolonged and persistent periods of infection in children establish a major percentage of the global burden of diarrheal disease (Moore, 2011).

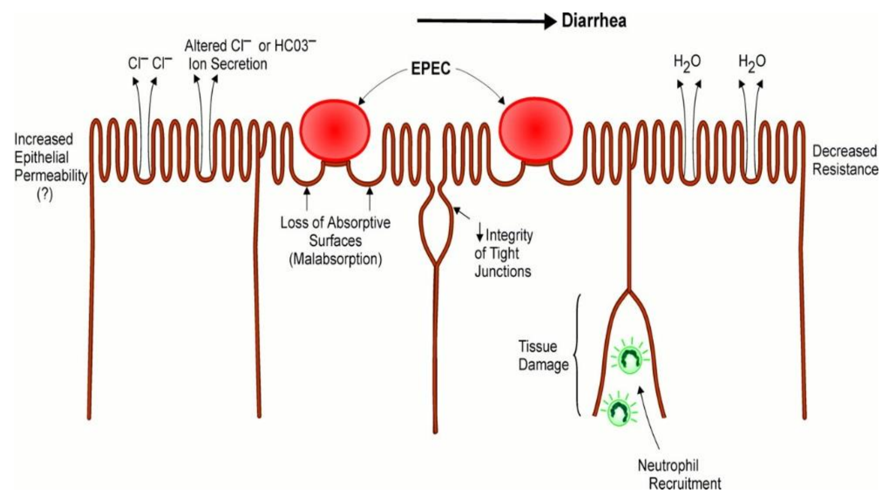


Figure 1.5: Steps of EPEC infection (Vallance and Finlay, 2000).

1.2.11.4 Enteroaggregative *E. coli* (EAEC): EAEC is the most recently identified diarrheagenic *E. coli* and is increasingly recognized as an emerging enteric pathogen and cause of persistent diarrhea and malnutrition in children and HIV infected persons living in developed countries. In USA, it is recommended as the second common cause of traveler's diarrhea and is a common cause of acute diarrheal illness in children and adults presenting to emergency departments and inpatient units (Nataro *et al*., 2006). The USA National Institutes of Health has characterized EAEC as a group B potential bioterrorism agent (Huang *et al*., 2004). EAEC strains has the ability to produce a 'stacked-brick' appearance when they are incubated with HEp-2 epithelial cells in culture (Nataro and Kaper, 1998).The aggregative phenotype elements of EAEC are enclosed in a large plasmid that carries a number of virulence genes that are under the control of the master AggR regulator. EAEC diarrheal infection has more often been connected with the presence of fecal leukocytes and lactoferrin (Greenberg *et al*., 2002; Mercado *et al*., 2011).

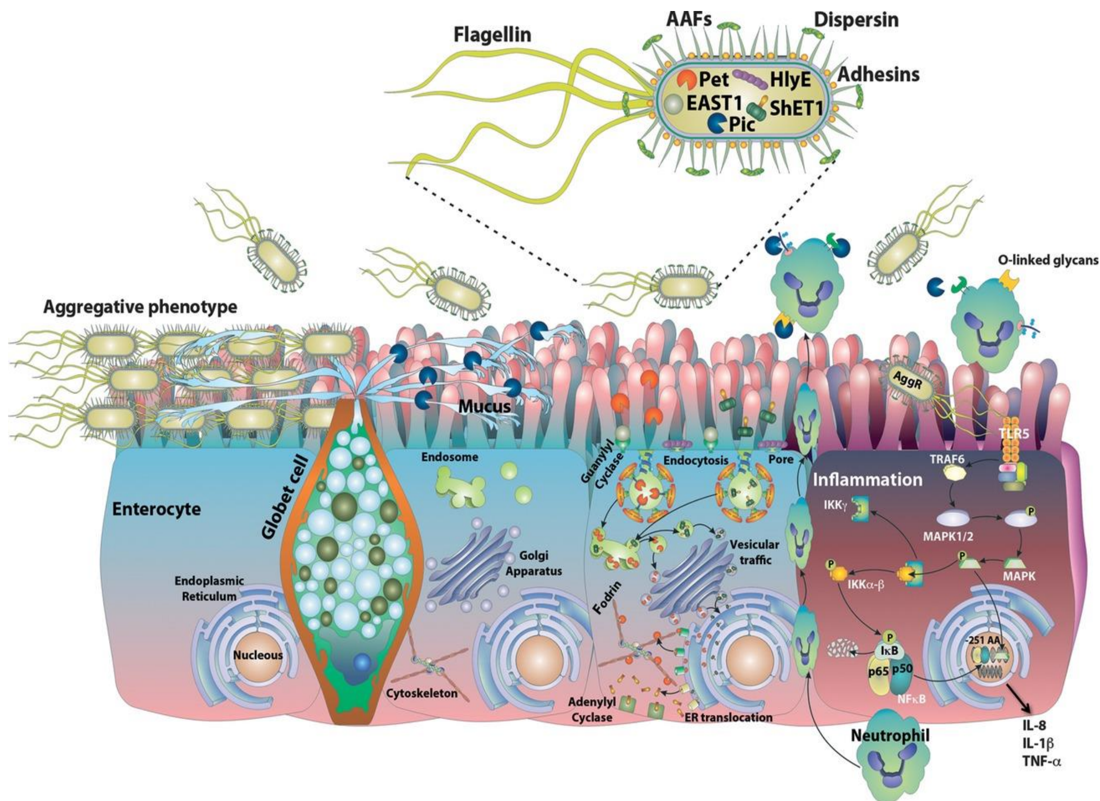


Figure 1.6: Steps of EAEC infection (Garcia and Garcia, 2012).

1.2.11.5 Enteroinvasive *Escherichia coli* (EIEC): EIEC are a group of intracellular pathogens able to enter epithelial cells of colon, multiply within them and move between adjacent cells with a mechanism similar to *Shigella*. Molecular analysis confirms that EIEC are extensively spread among *E. coli* phylogenetic groups and resemble to bio serotypes (Pasqua *et al*., 2017). EIEC cause sporadic cases of infection but have been associated in outbreaks also and sometimes involving large number of circumstances (Pasqua *et al*., 2017). An EIEC 096:H9 strain, a serotype never defined before for EIEC was isolated from cooked vegetables (Escher *et al*., 2014). EIEC was isolated in a case of traveler's diarrhea in Spain in 2013 (Michelacci *et al*., 2016). The role played by EIEC in endemic diarrheal disease has not been investigated extensively. Enteroinvasive *Escherichia coli* (EIEC) is a pathogenic form of *E.coli* that causes dysentery similar to *Shigella*, but the symptoms produced by it is less severe compared to *Shigella* (DuPont *et al*., 1971; Lan *et al*., 2004; Van den Beld and Reubsaet, 2012). EIEC are highly invasive and they utilize adhesion proteins to bind to and enter intestinal cells. They produce no toxins, but severely damage the intestinal wall through mechanical cell destruction.

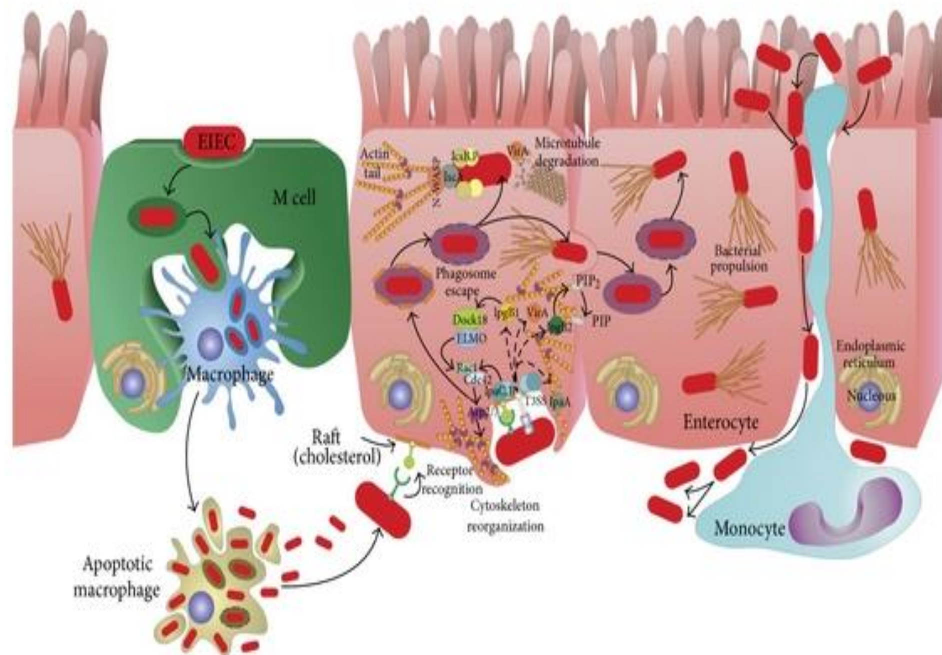


Figure 1.7: Steps of EIEC infection (Garcia *et al*., 2013).

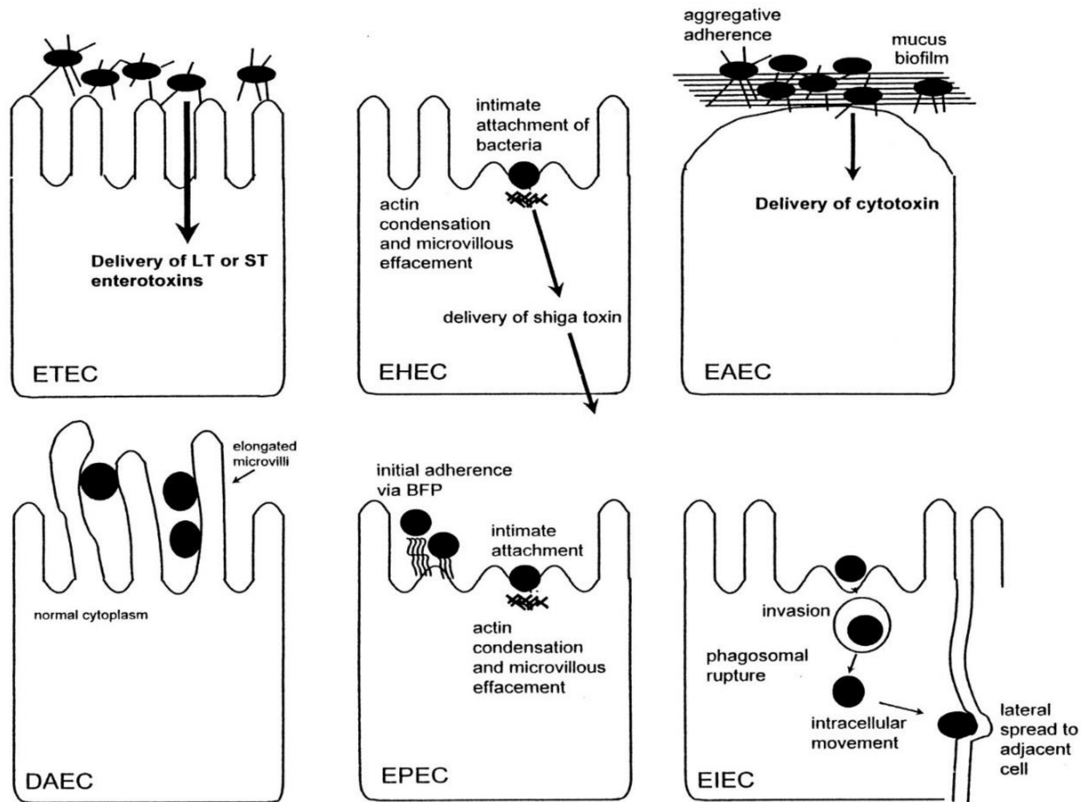


Figure 1.8: Diarrheagenic *Escherichia coli* infection with six pathotypes (Nataro and Kaper, 1998).

1.2.12 Antibiotic resistance

Antimicrobial resistance also known as antibiotic resistance or drug resistance occurs when microbes survive exposure to agents because of genetic changes in the microbes. Viruses, fungi and parasites can become resistant but the greatest problems have occurred with bacteria. Antimicrobial resistance is a progressively international problem and emerging antimicrobial resistance has become a public health concern worldwide (Kaye *et al*., 2000). Bangladesh is a main contributor to this because of its poor healthcare standards, beside with the misuse and overuse of antibiotics (Ahmed *et al*., 2019). One recent study revealed that antibiotic consumption had been increased about 39% in 76 countries of this planet from 15 years's time (2000-2015) (Haque, 2019). Another one recent study revealed that not much time is remaining to observe *Escherichia coli* and *Klebsiella pneumoniae* will be resistant throughout the planet aganist third generation cephalosporins and carbapenems (Alvarez-Uria *et al*., 2018). A variety of foods and environmental

sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (Bager and Helmuth, 2001; Schroeder *et al*., 2002; Anderson *et al*., 2003). Antibiotic resistance can be the result of mutations and acquisition of resistance encoding genes. The World Health Organization states that antibiotic resistance is "a growing public health threat of broad concern that threatens the achievements of modern medicine." Several important organizations, like the centers for Disease Control and Prevention (CDC), infectious diseases Society of America, World Economic Forum and the World Health organization (WHO) have declared antibiotic resistance as a 'global public health concern' (Michael *et al*., 2014; Spellberg *et al*., 2016). The World Health Association demanded WHO to propose a global action plan to contest the antibiotic resistance problem (Hoffman *et al*., 2015). In America, by 2015, President Barack Obama under the references of the US President's Council of Advisors on Science and Technology ordered the National Security Council to draft an inclusive national action plan to tackle antibiotic resistance (Ventola, 2015; Landers and Kavanagh, 2016). Bacteria that are resistant to multiple antibiotics are considered multi drug resistant (MDR) or superbugs. Common types of drug-resistant bacteria include MRSA (methicillin-resistant *Staphylococcus aureus*), VRSA (vancomycin-resistant *S. aureus*), ESBL (extended spectrum beta-lactamase), VRE (vancomycin-resistant *Enterococcus*) and MRAB (multidrug-resistant *A. baumannii*). While most are hospital acquired and some are community-acquired infections. In the simplest cases, drug-resistant organisms may have developed resistance to first-line antibiotics thereby demanding the use of second-line agents. Development and spread of antibiotic resistance has become a worldwide health threat and is often interconnected with overuse and misuse of clinical and veterinary chemotherapeutic agents. The use of antibiotics as growth promoters and food enhancers is linked with economic gains (Durso and Cook, 2014). Modern industrial-scale animal feeding operations rely extensively on veterinary pharmaceuticals, including antibiotics to augment animal growth. A percentage of antimicrobial resistance has risen from application of antimicrobial drugs in food animals with subsequent spread of resistant bacteria and resistance characters between animals and their products as well as the environment (McEwen and Fedorka-Cray, 2002). These antibiotic-resistant bacteria and antimicrobial resistance genes of animal source can simply be transferred to humans by a number of ways (Van *et al*., 2020). Bacterial resistance to antibiotics increasingly hinders treatment of life-threatening illnesses. Misuse and overuse of antibiotics plays a critical role in development of resistance and

there is evidence that agricultural use of antibiotics is a contributor to the aggregation of resistance in the environment (Levy and Marshall, 2004; Gilchrist *et al.*, 2007). Nearly 10 million kilograms of antibiotics per year (likely an under estimation because of the lack of reporting requirements) are used in animal agriculture in the United States alone (Sarmah *et al.*, 2006). Antibiotics are administered to beef cattle to treat and prevent disease and to promote growth (Phillips *et al.*, 2004; Shuford and Patel, 2005; Khan *et al.*, 2008). It is estimated that antibiotic utilization will increase by 67% by the year 2030, with almost twice this increase in countries such as China, Brazil, India, South Africa and Russia (Boeckel *et al.*, 2015). Antibiotics used for growth promotion are added to livestock feed and after ingestion are incompletely metabolized and poorly absorbed in the gastrointestinal tract, resulting in excretion of parent compounds and metabolites (Wegener, 2003; Shuford and Patel, 2005; Boxall *et al.*, 2006; Khan *et al.*, 2008; Chee-Sanford *et al.*, 2009). Upon excretion, these compounds may be transported into the environment beyond feed yard boundaries via application of manure waste onto agricultural fields, runoff and as reported here, airborne particulate matter (PM) (Wegener, 2003; Chee-Sanford *et al.*, 2009). Once in the environment, antibiotics can facilitate *de novo* development of bacterial antibiotic resistance and provide a selective advantage for bacteria that acquire resistance either in treated animals or in the environment (Gilchrist *et al.*, 2007; Chee-Sanford *et al.*, 2009). The most vital matters to monitor during antibiotic treatment are duration of treatment, toxicity of the medication and cost. Antibiotic resistance is a public health threat and characteristic of pathogens causing different diseases. It is normally not a problem of disease pathology but one of limited therapy choices (Samie *et al.*, 2009), thus containment approaches must be improved to the needs of specific disease control and treatment programs.

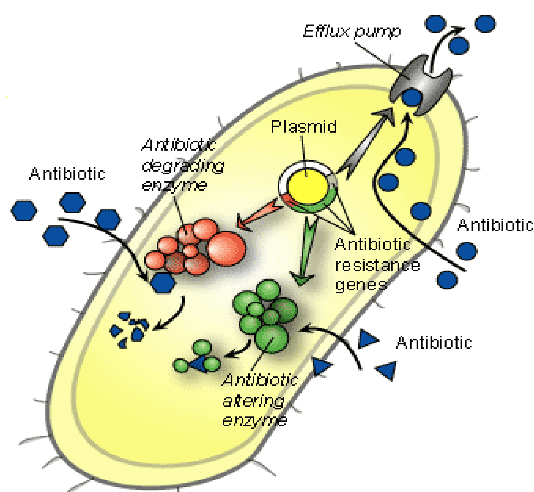


Figure 1.9: Bacterial mechanisms of drug resistance (Todar, 2004).

1.2.13 Multidrug resistance

Antibiotic resistance and in particular multidrug resistance (MDR) are public health threats. Multidrug resistant infections are related with poorer clinical outcomes and higher cost of treatment than other infections (Giske *et al*., 2008; Chang *et al*., 2015) and there is concern that the emergence of pan-resistant strains will render some infections untreatable (Falagas and Bliziotis, 2007). Multiple antibiotic resistance in human pathogens has increased over the past decades and increased over the past decades and challenged our ability to treat bacterial pathogens (Alekshun and Levy, 2007). Multidrug resistant (MDR) was well-defined as developed non-susceptibility to at least one agent in three or more antimicrobial groups (Basak *et al*., 2016). More and more bacteria are becoming resistant to our common antibiotics and to make matters worse, more and more are becoming resistant to all known antibiotics. The problem is known as multi-resistance and is commonly defined as one of the most significant future threats to public health. In 2011, WHO declared 'combat drug resistance: no action today, no cure tomorrow' (Sharma, 2011). Antibiotic resistance can arise in bacteria in our environment and in our bodies. Antibiotic resistance can then be transferred to the bacteria that cause human diseases, even if the bacteria are not related to each other. Various microorganisms have persisted for thousands of years by their ability to adjust to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. This process enables some bacteria to face the action of certain antibiotics, rendering the antibiotics ineffective. In recent years, strains of multidrug resistant organisms have become

expanded worldwide (Cohen, 2000). Abuse and overuse of antibiotics in the clinic has resulted in the appearance of several antibiotic resistant bacterial strains (Goldman, 2004). The discovery of penicillin in 1928 was followed by the discovery and commercial production of many other antibiotics. We now take for approved that any contagious disease is curable by antibiotic therapy. Antibiotics are manufactured at an estimated scale of about 100,000 tons annually worldwide and their use had a profound impact on the life of bacteria on earth. More strains of pathogens have become antibiotic resistant and some have become resistant to many antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance. Since poultry has been suggested as a reservoir for multidrug-resistant *E. coli* strains causing extraintestinal infections (Extraintestinal pathogenic *E. coli*, ExPEC) in humans, the phylogenetic analysis of strains circulating in both hosts can add useful data for the evaluation of the potential zoonotic risk (Johnson *et al*., 2005; Collignon and Angulo, 2006; Nordstrom *et al*., 2013).

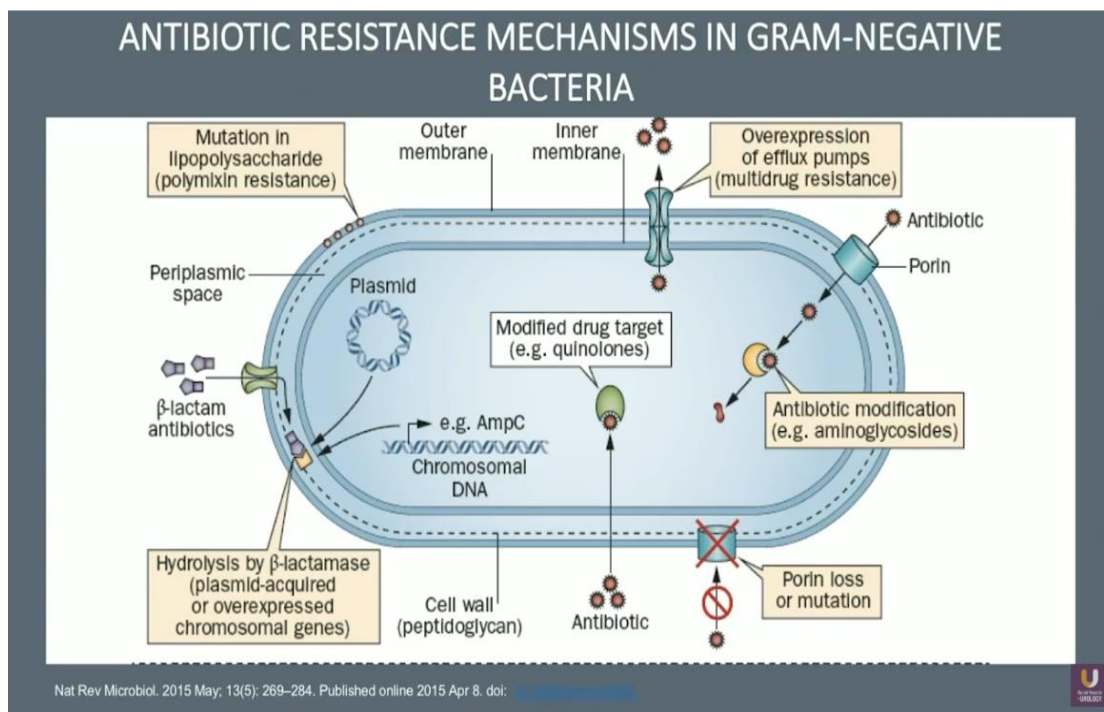


Figure 1.10: Mechanism of Multi-drug resistance of bacteria (Zowawi *et al*., 2015).

1.2.14 Plasmid mediated antibiotic resistance

In 1952, Joshua Lederberg coined the term plasmid in reference to any extra-chromosomal transmissible element. Antibiotic resistance plasmids are bacterial extra-chromosomal elements that carry genes conferring resistance to one or more antibiotics. They are notorious for their ability to transfer by conjugation between bacterial species and are significantly involved in the emergence and dissemination of multiple drug resistance associated with bacterial infections in humans. Multiple antibiotic resistances present a serious and growing clinical problem with regard to bacterial infections in humans. Resistance genes are commonly found on plasmids, which are small extra-chromosomal elements commonly found in bacteria. Plasmids can vary widely with regard to their size and copy number in the cell. Mostly plasmids can be found in bacteria but they are also present in multicellular organisms and archaea. Plasmids are important vehicles for the communication of genetic information between bacteria (Shintani *et al.*, 2015). Plasmids are commonly able to move from one bacterial cell to another by a mechanism known as conjugation which involves cell-to-cell contact followed by transfer of a copy of plasmid DNA from a donor to a recipient. Bacterial plasmids serve as the frame on which antibiotic resistance genes are gathered by transposition (transposable elements and ISCR mediated transposition) and site-specific recombination mechanisms (integron gene cassettes) (Bennett, 2008). "IncP-1 plasmids are very effective 'vehicles' for carrying antibiotic resistance genes between bacterial species. Plasmids act as significant genetic tools for manipulation and analysis of microorganisms through the introduction, alteration or exclusion of target genes (Frost *et al.*, 2005; Skovgaard, 2008). New plasmids have been informed with the current revolution in nucleotide sequencing (Shintani *et al.*, 2015). Therefore, it does not matter much in what environment, in what part of the world or in what bacterial species antibiotic resistance arises.

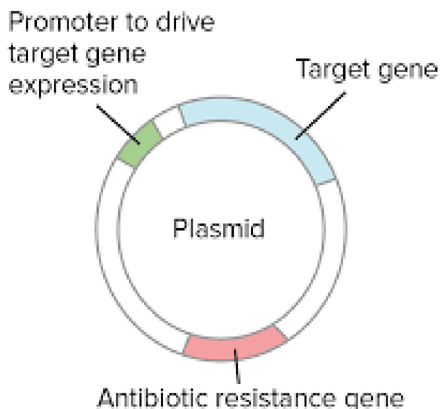


Figure 1.11: Bacterial plasmid (Alton *et al*., 2015).

1.2.15 Integron mediated antibiotic resistance

Integrons are mobile genetic elements able to acquire and rearrange open reading frames (ORFs) inserted in gene cassette units and alter them to functional genes by confirming their correct appearance. They were initially identified as a mechanism used by Gram-negative bacteria to gather antibiotic resistance genes and express multiple resistance phenotypes in cooperation with transposons. The class 1 integrons that are widely distributed in pathogens from clinical settings are part of a more diverse group of class 1 integrons found on the chromosomes of environmental bacteria (Gillings *et al*., 2008). An integron is defined as a genetic element that retains a site, *attI*, at which additional DNA in the form of gene cassettes can be incorporated by site-specific recombination and which encodes an enzyme, integrase that facilitates these site-specific recombination events. Gene cassettes are distinct genetic components that may exist as free, circular, non-replicating DNA molecules when moving from one genetic site to another (Collis and Hall, 1992) but which are normally found as linear sequences that constitute part of a larger DNA molecule such as a plasmid or bacterial chromosome. These integrons are potentially mobile elements (namely, transposons or defective transposon derivatives) that constitute a site-specific recombination system capable of integrating and expressing the genes in cassette structures. Integrons comprise three essential components located within the 5 conserved segment (CS): an integrase gene, *IntI1* which encodes a site-specific recombinase; an adjacent attI1 site which is recognized by the integrase and acts as a receptor for gene cassettes and a promoter region, P. There are four distinct classes of multi resistant integrons each encoding a distinct integrase gene (*IntI 1, IntI 2, IntI 3* and I*ntI 9*). Class 1 integrons, located on plasmids and transposons make up

the majority of the integrons found in clinical isolates and are associated with the MDR seen in the hospital environment (Freijo *et al.,* 1998). Integrons were constituents of the first resistance plasmids reported, conferring resistance to aminoglycosides, chloramphenicol and sulphonamides. One of the most recent additions to the list of drug-resistance gene cassettes is one with bla_{IMP} (Arakawa *et al*., 1995) a gene which encodes a metallo-β-lactamase conferring resistance to carbapenems such as imipenem. Multidrug resistant *Pseudomonas aeruginosa* harbours integrons and other mobile genetic elements such as plasmids and transposons, which easily disseminate antibiotic resistance genes among clinical strains of *Pseudomonas aeruginosa* (Odumosu *et al.,* 2013).The presence of integrons are associated with antimicrobial resistance and are being increasingly reported worldwide (Fluit and Schmitz, 1999; Lee *et al*., 2001; Salem *et al*., 2010). Several studies have revealed the relation of antimicrobial resistance of *P. aeruginosa* to the presence of one or more of these genetic elements (da Fonseca and Vicente, 2011). Genes carried by integrons usually encodes multiple resistance mechanisms such as resistance to beta-lactams, aminoglycosides and other antimicrobial agents (Elbourne and Hall, 2006; Jeong *et al*., 2009).

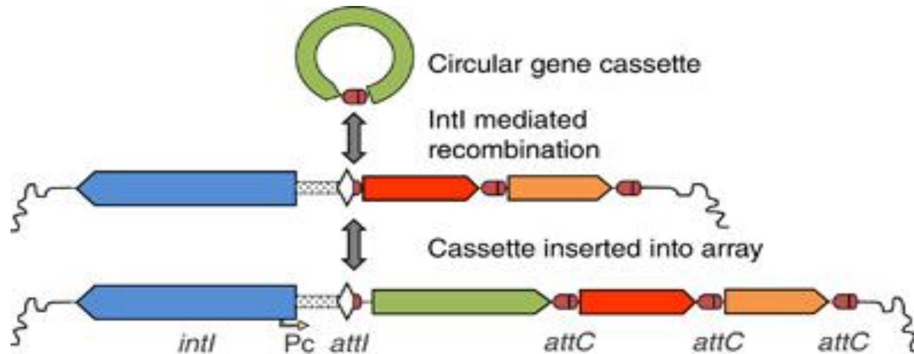


Figure 1.12: Class 1 Integron (Gillings e*t al*., 2015).

1.3 Aims and Objectives

1.3.1 General Objectives

Escherichia coli may be present in the environment both as harmful and harmless forms. The main aim of this study is to know the current status of this bacterium in the environment along with different characteristics like to know their source, their origin by phylotyping, antibiotic resistance profile, detecting the presence of antibiotic resistance gene transfer mediators including to detect the presence of virulence genes.

1.3.2 Specific objectives

This study addresses the following objectives:

1. Isolation and identification of *Escherichia coli* from different types of environment.
2. Investigation of phylogeny by molecular methods
3. To explore the distribution of selected virulence genes among isolated *E. coli* samples
4. To investigate the antibiotic resistance pattern of *E. coli* isolates
5. To observe the prevalence of integron among test isolates
6. To extract plasmids present in isolated *E. coli*
7. To identify the antibiotic resistance mediators
8. Analysis of the results

Materials and Methods

2.1. Sample Collection

An assemblage of 400 samples were collected randomly from different environmental sources such as soil, water, prawn, animal, street foods and human. Each sample was processed on the same day. Later the samples were grown on Nutrient agar plate and then maintained in glycerol broth as stock.

Among these samples 184 (Table 2.1) were confirmed to contain *E. coli* by different biochemical tests and PCR amplification procedure targeting *E. coli* specific genes.

Table 2.1. Environmental Samples collected from different locations

Source of environmental *E. coli* samples		
Designated number in this thesis paper		**Sample collection site**
Prawn Samples	P0, P7, P10, P11, P12, P20, P21, P22, P24, P26, P27, P29, P34, P35, P37, P41, P43, P45, P50, P52, P54, P56, P58, P59, P60	Two different markets named Anando bazar and Polashi bazar of Dhaka city which are located near to Dhaka university.
	W13	Tap water from Siddhesori, Dhaka
	W14	Tap water from Mirpur 11, Dhaka
	W27	Shahidulla Hall pond of Dhaka University
	W31	Jagannath Hall pond of Dhaka University

Water Samples	W42	Tap water from Mirpur Taltola, Dhaka
	W46	Tap water from Dhaka Cantonment
	W49	Tap water from Mohammadpur, Dhaka
	W50	A pond of Chandpur at Puran Bazar
	W55, W56	Two different ponds of Chandpur near the railway station
	W81	A pond of Chandpur near the sweeper colony
	W84	A river of Chandpur named Dakatia
	W85	Buriganga river, Dhaka
	W86	A pond from Mymensingh
	W88	A pond from Tangail
	W89, W90	Two different ponds from Barishal
	W91, W92, W93	Three different pondss from Kishoregonj
	S4, S5, S6	Mokarram Bhabaen area, University of Dhaka

Soil Samples	S9, S11, S12	Curzon Hall area, University of Dhaka
	S20, S23	Uttara sector 12, Dhaka
	S31, S33	Uttara sector 11, Dhaka
	S48	Area close to swimming pool of Dhaka University
	S49	Area close to Mathematics Department of Dhaka University
	S51	Basabo, Dhaka
	S56	Shahidullah Hall area of Dhaka University
	S65	Ekushey Hall area of Dhaka University
	S74	Norsingdi
	S76	Chandpur
	S78	Mymensingh
	S79, S80	Ramna park lake, Dhaka
	S82, S84	Kishoregonj
	S85	Dhanmondi, Dhaka
	A1, A2, A6, A7, A8, A32, A34, A35, A36, A37, A39, A40, A42	Farm cow faeces
	A9, A10	Sheep faeces

Animal Samples	A12, A13, A14, A16, A18, A19, A20, A21, A22, A23, A24, A31, A62, A63 A64, A68, A69, A70, A71, A76	Faeces from chicken
	A26	Faeces from turkey
	A27, A28	Faeces from duck
	A30, A54, A56, A57, A58, A59	Goat faeces
	A43, A44, A46, A47, A48, A49, A50, A51, A52, A53	From domestic cow faeces
Street Food Samples	SF-V(5)	Velpuri from a roadside food court
	SF-6	Salad from a roadside food court

Details of the clinical *E. coli* samples are shown in Table 2.2.

Table 2.2. Details of Human samples from a local hospital of Dhaka, Bangladesh

Designated number in this thesis paper	Sample source	Patient's Age	Patient's Sex
47697	Urine	65 years	Female
30987	Stool	5 months	Male
30955	Stool	5 months	Female

47509	Urine	74 years	Female
47508	Urine	48 years	Female
47770	Urine	3 years	Female
47990	Urine	40 years	Female
8996	Stool	5 months	Male
25733	Urine	54 years	Female
C/O Saidul	Stool	3 years and 3 months	Male
26170	Stool	42 years	Male
407	Urine	67 years	Male
464	Urine	50 years	Female
774	Stool	1 year	Male
755	Stool	60 years	Male
397	Stool	12 years	Male
394	Urine	50 years	Male
779	Pus	60 years	Male
496	Urine	72 years	Male
032	Stool	45 years	Male
075	Stool	64 years	Male
9312	Urine	50 years	Female
914	Urine	37 years	Male
064	Urine	80 years	Female
343	Urine	27 years	Female

425	Urine	45 years	Female
521	Stool	7 years	Male
265	Stool	-	Male
340	Stool	-	Male
588	Stool	13 years	Female
647	Urine	48 years	Female
168	Urine	37 years	Male
685	Urine	48 years	Male
564	Urine	12 years	Female
689	Urine	45 years	Female
595	Urine	27 years	Female
3491	Urine	50 years	Female
534	Urine	23 years	Female
585	Tracheal aspirate	19 years	Female
9	Pus	-	-
13	Pus	-	-
14	Pus	-	-
68	Pus	-	-
180`	Stool	7 years	Male
176	Stool	9 months	Female
940	Stool	6 years	Female
657	Stool	16 years	Female

544	Stool	35 years	Female
938	Stool	7 years	Male
823	Urine	59 years	Female
038	Urine	1 year	Male
380	Urine	6 years	Male
ME 3	Urine	-	-
ME 4	Urine	-	-
ME 5	Urine	-	-
ME 6	Urine	-	-
ME 7	Urine	-	-
ME 8	Urine	-	-
ME 9	Urine	-	-
ME 10	Urine	-	

2.2. General Procedure and Equipment

2.2.1 Sterilization

All the necessary equipment, relevant glassware, culture media and appropriate reagents were sterilized by means of an autoclave machine which was run at 15 p.s.i for 20 minutes .

2.2.2 Maintenance of media, reagents and solution

Maintenance of necessary media, reagents and solution is really important to get any potent result. Dehydrated media were always kept in a dry place in tightly-sealed containers at 2-25°C, whereas prepared media were stored below 8°C and definitely protected from direct light. Any freshly

prepared Tryptic Soy Broth (TSB), Nutrient Broth (NB), Nutrient Agar (NA), Tryptic Soy Agar (TSA). Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), Brilliant Green Lactose Broth (BGLB) were under routine contamination check before use. Other reagents and chemicals were always maintained in a refrigerator at 4°C. Stock culture of isolates and every PCR reagents such as primers, master-mix, DNA ladder, PCR products, restriction enzymes etc. were stored at -20°C and always handled aseptically.

2.2.3 Sample collection and processing

Water samples were collected in UV treated plastic bottles and soil, animal, prawn and food samples were collected in UV treated plastic bags by wearing protective gloves, face masks. Samples were transferred to the laboratory and processed as soon as possible. In case of prawn and street food samples, 10g of samples were mixed with 90 ml of Phosphate Buffer Saline (PBS) by using a blender for proper grinding and mixing. The vessel and the related equipment used in the grinding were washed every time after processing of each sample with 70% ethanol solution and then with autoclaved distilled water to avoid any cross contamination. After that the samples were tested by using MPN method to find out the desired bacteria.

2.2.4 Most Probable Number method (MPN)

MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It is based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculum placed into a set number of culture media tubes. Positive growth response after incubation may be indicated by such observations as gas production in fermentation tubes or visible turbidity in broth tubes, depending upon the type of media used. The sample should be diluted in such a manner that higher dilutions of the sample will result in fewer positive culture tubes in the series. The number of sample dilutions to be prepared is generally based on the expected population contained within the sample. If particularly high microbial populations are expected, the sample must be diluted to a range where the MPN can be obtained. Most reliable results occur when all tubes at the lower dilution are positive and all tubes at the higher dilution are negative. Generally tenfold serial dilutions are used in either a 3, 5 or 10 tube MPN series. In the present study, 3 tube MPN series was used.

2.2.5 Sample preparation for Most Probable Number (MPN)

A total of 9 empty tubes were used, of which the first set of 3 tubes contained 9 ml, the second set of 3 tubes contained 9.9 ml and the last set contained 9.99 ml of BGLB media. Then 1ml of homogenate was added to the first 3 tube series, 0.1 ml to the second 3 tube series and finally 0.01 ml of homogenate was added to the last 3 tube series. This series of tubes represented 1.0, 0.1, and 0.01 g of sample. The tubes were then incubated at 37°C for 24 hours to observe visible growth.

2.2.6 Resuscitation of bacteria from stock

Samples from glycerol broth stock were revived in Tryptic Soy Broth (TSB) and Nutrient broth (NB). For resuscitation, one loop-full culture was inoculated into TSB or NB and after incubation at 37°C for 18-24 hours growth could be visualized in broth. After getting turbid growth one loop-full culture from TSB or NB was streaked onto NA plate and then subcultured onto Eosin Methylene Blue (EMB) agar and MacConkey agar plates for an incubation period of 18-24 hours at 37°C.

2.3 Preliminary Identification of *Escherichia coli*

2.3.1 Isolation using selective media

All the isolates were identified by conventional biochemical tests in the laboratory. We also used plate based assays to further confirm them. Selective media were used to identify them. EMB (Eosin Methylene Blue) plates were used to identify *Escherichia coli* where they produced characteristic greenish metallic sheen. MacConkey agar plates were used too and after 24 hours of incubation time period at 37°C pink colonies were observed which were further streaked in nutrient agar plate. After another 24 hours incubation single colonies were subjected to biochemical tests for confirmation.

2.3.2. Observation of colony morphology

Colony morphology on different culture media was observed cautiously and various information about the morphological characteristics such as shape, size, elevation, margin, opacity, texture etc. were recorded after a period of 24 hours of incubation time.

2.3.3. Microscopic Examination

One drop of normal saline was taken on a clean glass slide and loop-full of bacterial culture transferred on to the drop of saline. A thin smear was prepared and the slide was air dried. Heat fixation was done after by passing the slide over a flame. The smear was flooded with crystal violet and was allowed to stand for 45 seconds. The crystal violet was washed away with a gentle steam of water. Gram's iodine (mordant) was applied to the slide and after 1 minute, was washed away. The smear was decolorized by adding 95% alcohol to the slide and was washed away after 10 seconds. Finally the slide was flooded with the counter stain safranin allowed to stand for 1 minute. The slide was washed and dried and was ready to be visualized under bright field microscope.

2.4. Biochemical Tests

Further confirmation of test isolates were done by conventional biochemical tests. Biochemical tests were performed for the identification of the isolates according to the methods described in Microbiology Laboratory Manual (Cappucino *et al.,* 1996). The tests included Kligler's Iron Agar (KIA) test, Citrate test, Motility, Indole, Urease (MIU) test, lndole test, Methyl Red test, Voges–Proskauer test.

2.4.1. Kligler's Iron Agar (KIA) Test

The test was performed to assess dextrose utilization in oxidative/fermentative mode by stabbing the butt and streaking the slant through a needle containing fresh culture. KIA media was allowed to incubate at 37°C for 18 to 24 hours. After incubation, results were recorded for changes in color of the butt, slant and H_2S or other gas production. In a test-tube containing KIA media, a yellow slant indicates fermentation of lactose by organism after incubation period. A yellow butt shows that the organism ferments glucose. Black precipitation in the butt suggests hydrogen sulfide production. Production of any other gases except hydrogen sulfide gas is ensured by cracking or any bubbles in the media. If any isolate ferments glucose only, the entire tube turns yellow due to the effect of the acid produced on phenol red. Because the organism quickly exhausts a minimum amount of glucose present in the tube and begins oxidizing amino acid for energy. Ammonia is thus produced and pH rises. Within 24 hours the phenol red indicator reverts it to the original red color in the slant. Since the butt having limited oxygen, bacteria are unable to oxidize amino acid

there. The butt thus remains yellow. A lactose (+)ve bacteria can turn the butt and slant yellow and these will remain the same for 48 hours because of high level of acid production from the abundant sugar. If the gas being produced is hydrogen sulfide, it reacts with the ferrous sulfate and precipitates out as a black precipitate (ferric sulfide) in the butt.

2.4.2. Citrate Utilization Test

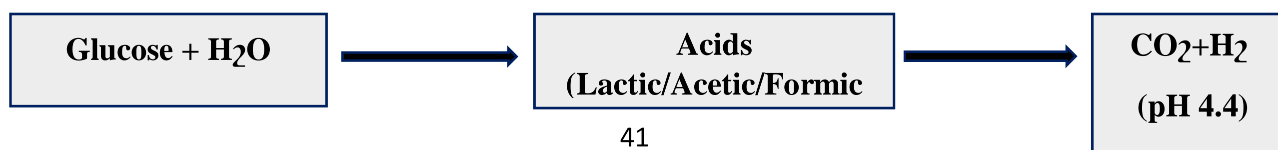
The Simmons citrate test was performed to assess citrate fermentation/utilization ability of isolates. This media contains sodium citrate as the sole source of carbon, ammonium di-hydrogen phosphate as a sole source of nitrogen and bromothymol blue as pH indicator. The slant was streaked by a needle containing fresh culture and the media was allowed to incubate at 37°C for 18 to 24 hours. After incubation results were recorded for changes in color. If the media turned into blue, it indicates that the isolate is citrate (+) ve. Tubes with no color change means citrate (-) ve isolate.

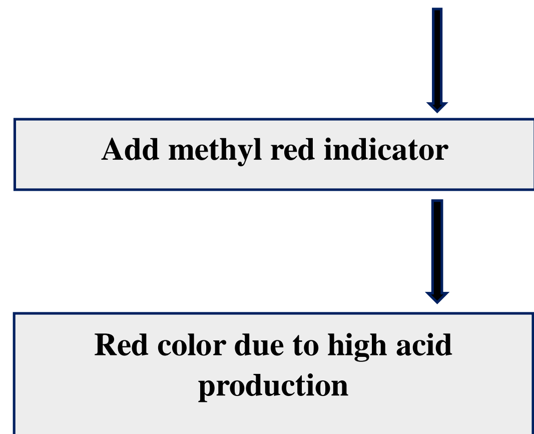
2.4.3. Motility, Indole, Urease (MIU) Test

Tubes containing MIU medium were inoculated with straight wire. Stabbing the medium was done carefully to a depth not touching the bottom. Motile organism dispersed through the medium leaving the stabbed line and made the tube turbid. Pink coloration of the MIU medium indicates a positive test for urease and no change in color was recorded as negative. Indole test was performed separately. Sterile peptone broth was inoculated by a fresh culture with a sterile needle. The tube was incubated at 37°C for 18 to 24 hours. After incubation, 8-10 drops of Kovac's reagent was added to the tube and the tube was shaken gently. The formation of cherry red ring indicated positive indole test, whereas no ring indicated negative results.

2.4.4. Methyl Red (MR) Test

The freshly grown isolates were inoculated into 5 ml of MR-VP broth media. The test-tubes were incubated for 18-24 hours at 37°C. After incubation 3-4 drops of methyl red reagent were added and shaken well. A distinct red color throughout the broth indicated positive result and any yellowish color was recorded as negative result.





2.4.5. Voges–Proskauer (VP) test

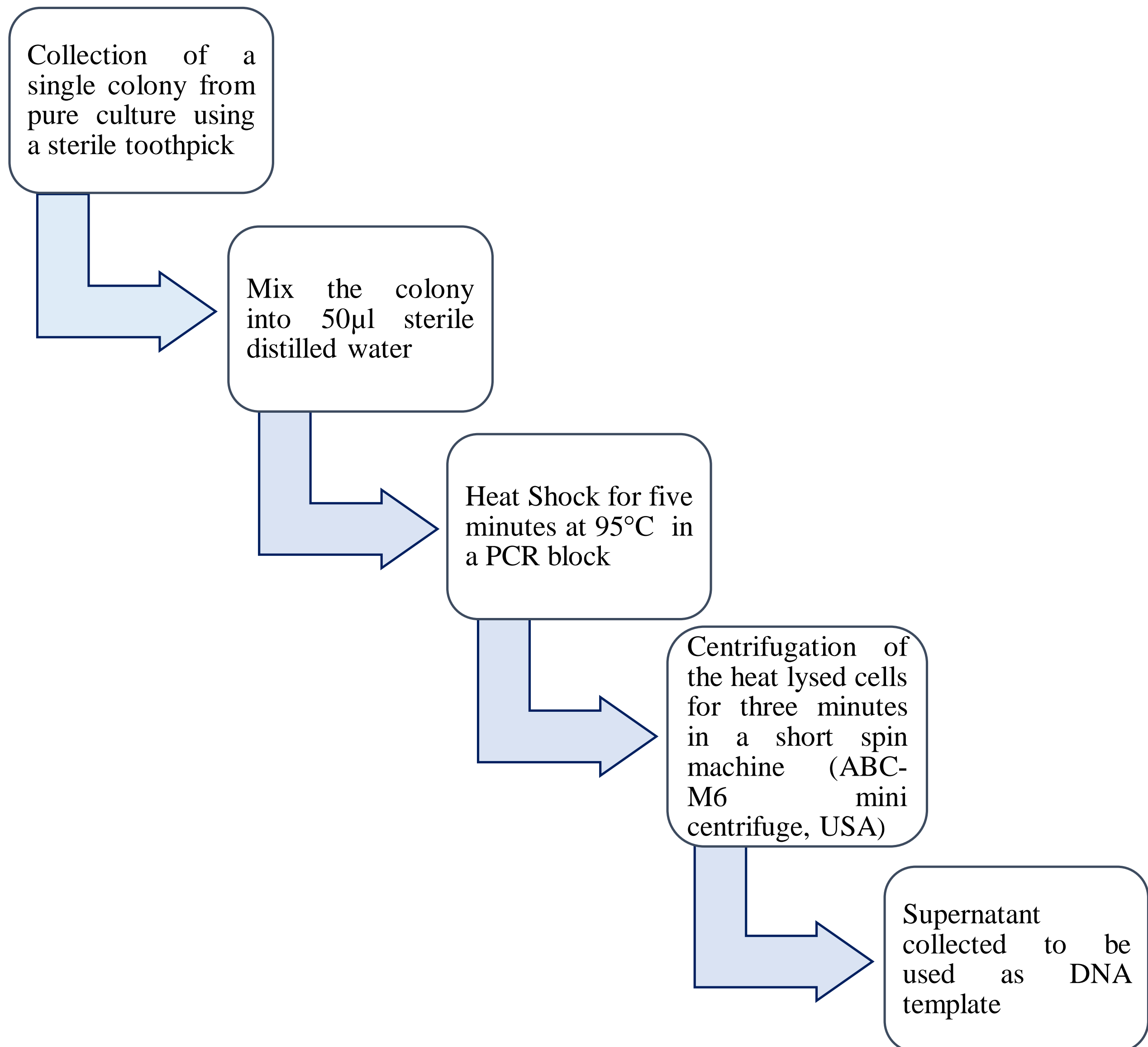
For this test, VP medium was inoculated with fresh culture for 24-48 hours at 37°C. After this time 3ml of 5% alcoholic α-naphthol solution was added into medium followed by 1 ml of potassium hydroxide creatine solution. The tubes were then shaken vigorously for1-2 minutes. Appearance of crimson ruby color in the medium indicated the production of acetyl methyl carbinol (acetoin).

2.5. DNA Extraction

2.5.1. Preparation of Template DNA

For any subsequent PCR template preparation was the most crucial part and fresh culture of bacterial samples were an emergence for this procedure. So every time fresh overnight culture

along with autoclaved distilled water and sterile toothpick were used.



2.5.2. Determination of DNA concentration and purity

The DNA concentrations were measured after purifying it using Colibri Microvolume Spectrometer (Titertek-Berthold, Berthold Detection System GmbH, Bleichstrasse, Pforzhem,Germany) at absorbance 260 nm. Elution buffer from the PCR product purification kit was used as blank. The ratio of absorbance at 260 nm and 280 nm provides the purity. Generally a ratio of 1.8 indicates pure DNA and any variation from 1.8 indicates contamination.

2.6. Molecular characterization

2.6.1. PCR amplification procedure

Amplification procedure was performed in a 25µl reaction volume except in the case of quadruplex PCR and 16s rDNA PCR which were performed in a 50 ul reaction volume contained in a PCR tube. For each specimen 2-3µl of template DNA was used. The reaction volume was prepared by mixing the following reagents (Table 2.3) with the template DNA. The primers used in different PCR reaction are mentioned in Tables 2.4 - 2.8. The master mix was prepared in a sterile eppendorf. A volume of 23µl was transferred but in case of 16s rDNA and quadruplex PCR, 47 µl transferred into each 0.2 ml PCR tube and then the corresponding DNA templates were added to each of that tube. All these work were done inside a PCR work station. The PCR tubes were then transferred to a DNA thermal cycler for amplification of DNA. For the amplification of different target gene different annealing temperature were used.

The annealing temperature for every PCR reaction are mentioned here in table 2.9

Table 2.3. Composition of Master Mix for PCR

Reagents	Volume in microliter		
	Quadruplex PCR	Duplex PCR	Singleplex PCR
Autoclaved distilled water	19	7.5	7.5
Magnesium chloride	0.5	0.25	0.25
Dimethyl sulfoxide (DMSO)	0.5	0.25	0.25
Forward Primer 1	0.25	0.75	1.25
Reverse Primer 1	0.25	0.75	1.25
Forward Primer 2	0.25	0.75	
Reverse Primer 2	0.25	0.75	

Forward primer 3	0.25		
Reverse Primer 3	0.25		
Forward Primer 4	0.25		
Reverse Primer 4	0.25		
Taq 2X Master mix	25	12.5	12.5
Template DNA	3	2	2
Total Volume	50	25	25

Table 2.4. Sequence of primers used in universal PCR

Primer Name	Sequence (5′ to 3′)	Tm °C	Amplicon Size (bp)	Reference
Universal (U1) Forward	5′-CCAGCAGCCGCGGTAATACG-3′	54.3	996 bp	Lu *et al.*, 2000
Universal (U2) Reverse	5′-ATCGG(C/T)TACCTTGTTACGACTTC-3′	65.1	996 bp	Lu *et al.*, 2000

Table 2.5. Sequence of *uidA* and *uspA* gene primers

Primer Name	Sequence (5′ to 3′)	Target gene	Tm °C	Amplicon Size (bp)	Reference
uidA Forward	5′-TATGGAATTTCGCCGATTTT -3′	*uidA*	52.3	166 bp	Bej *et al.*, (1991

uidA Reverse	5′-TGTTTGCCTCCCTGCTGCGG -3′		64.6	166 bp	Bej *et al*., (1991
uspA Forward	5′-CCGATACGCTGCCAATCAGT-3′	*uspA*	60.5	884 bp	Bej *et al*., (1991)
uspA Reverse	5′-ACGCAGACCGTAGGCCAGAT-3′		62.5	884 bp	Bej *et al*., (1991)

Table 2.6. Sequence of *eae* and *intl* gene primers

Primer Name	Sequence (5′ to 3′)	Target gene	T_m °C	Amplicon size (bp)	Reference
Eae Forward	5′-CCCGAATTCGGCACAAGCATAAGC-3′	*eae*	78.4	863bp	Zhang *et al*., (2002)
Eae Reverse	5′-CCCGGATCCGTCTCGCCAGTATTCG-3′		74.2	863 bp	Zhang *et al*., (2002)
intl-1Forward	5′-ACATGTGAGGCGACGCACGA-3′	*intl1*	61.9	539 bp	Goldstein *et al*., (2001)
intl-1Reverse	5′-ATTTCTGTCCTG GCTGGCGA-3′		59.2	539 bp	Goldstein *et al*., (2001)

Table 2.7. Primer sequences for the identification of *E. coli* Phylo-groups

Primer Name	Target Gene	Sequence	T_m °C	Amplicon size (bp)	Reference
chuA.1b	*chuA*	5′-ATGGTACCGGACGAACCAAC-3	60.5	288 bp	Clermont *et al*., (2013)
chuA.2		5′-TGCCGCCAGTACCAAAGACA-3′	60.5	288 bp	Clermont *et al*., (2013)
yjaA.1b	*yjaA*	5′-CAAACGTGAAGTGTCAGGAG-3	60.5	211 bp	Clermont *et al*., (2013)
yjaA.2b		5′-AATGCGTTCCTCAACCTGTG-3′	59.4	211 bp	Clermont *et al*., (2013)
TspE4C2.1b	TspE4.C2	5′-CACTATTCGTAAGGTCATCC-3′	59.4	152 bp	Clermont *et al*., (2013)
TspE4C2.2b		5′-AGTTTATCGCTGCGGGTCGC-3′	58.4	152 bp	Clermont *et al*., (2013)
AceK.f	*arpA*	5′-AACGCTATTCGCCAGCTTGC-3′	60.5	400 bp	Clermont *et al*., (2013)
ArpA1.r		5′-TCTCCCCATACCGTACGCTA-3′	60.5	400 bp	Clermont *et al*., (2013)
ArpAgpE.f	*arpA*	5′-GATTCCATCTTGTCAAAATATGCC-3	60.1	301bp	Lescat *et al*., (2012)

ArpAgpE.r		5′-GAAAAGAAAAAGAATTCCCAAGAG-3	58.4	301bp	Lescat *et al.*, (2012)
trpAgpC.1	*trpA*	5′-AGTTTTATGCCCAGTGCGAG-3′	58.4	219 bp	Lescat *et al.*,(2012)
trpAgpC.2		5′-TCTGCGCCGGTCACGCCC-3′	65.3	219 bp	Lescat *et al.*, (2012)
trpBA.f	*trpA*	5′-CGGCGATAAAGACATCTTCAC-3	59.4	489bp	Lescat *et al.*, (2012)
trpBA.r		5′-GCAACGCGGCCTGGCGGAAG-3′	68.7	489 bp	Lescat *et al.*, (2012)

Table 2.8. Primer sequences for the identification of *E. coli* Patho-types

Reference Strain	**Primer Sequence**	**Target Gene**	**Tm °C**	**Amplicon Size (bp)**	**Reference**
EPEC	5′TGATAAGCTGCAGTCGAATCC-3′	*eaeA*	54.8	229	Hegde *et al.*, (2012)
	5′CTGAACCAGATCGTAACGGC-3′		55.7		
	5′CACCGTTACCGCAGGTGTGA-3′	*bfpA*	59.9	450	Hegde *et al.*, (2012)
	5′GTTGCCGCTTCAGCAGGAGT-3′		60.6		

ETEC	5′CTCTATGTGCACACGG AGC-3′	*Elt*	53.3	322	Hegde *et al.*, (2012)
	5′CCATACTGATTGCCGC AAT-3′		55.8		
EIEC	5′CTGGTAGGTATGGTG AGG-3′	*Ial*	51.2	320	Hegde *et al.*, (2012)
	5′CCAGGCCAACAATTA TTTCC-3′		51.9		
EAEC	5′CTGGCGAAAGACTGT ATCAT-3′	CVD432	52.2	630	Hegde *et al.*, (2012)
	5′CAATGTATAGAAATC CGCTGTT-3′		50.8		
EHEC	5′GCATCATCAAGCGTA CGTTCC-3′	*hlyA*	56.5	534	Hegde *et al.*, (2012)
	5′AATGAGCCAAGCTGG TTAAAGCT-3′		57.5		

2.7. PCR Cycle:

The PCR was performed using the following conditions:

Initial denaturation--- 95°C…10 minutes

Denaturation---95°C…1 minute

Annealing --X°C…1 minute

Extension---72°C…1 minute

} 35 or 40 cycles

Final Extension---72°C…10 minutes

Table 2.9. Annealing Temperature for separate PCR

PCR name	Annealing Temperature °C
Universal PCR	**55°C**
***uidA* and *uspA* gene detection**	**55.2°C**
Phylo-genetic grouping (Quadruplex PCR)	**59°C**
Patho-types Detection	**55°C**
***eae* gene detection**	**56°C**
***intl* -1 gene Detection**	**59°C**

2.8. Restriction Endonuclease Digestion:

The restriction enzyme digestion patterns of the universal primer PCR products from different species of bacteria are different. It was done for rapid detection and identification of bacterial isolates. The restriction enzymes used were HaeIII, HindIII and EcoRI from New England Biolabs^{TM}.

1. Initially a master-mix was produced comprising of 7.25µL autoclaved, filter sterilized distilled water, 2µL Buffer and 0.75µL restriction enzyme solution.

2. 10µL of the master-mix was dispensed into PCR tubes and 10µL purified DNA was added to each of the tubes making a total volume of 20µL.

3. This was incubated at 37°C for 30 minutes and horizontal gel electrophoresis in 2% agarose gel in TAE buffer at room temperature was performed at 80V.

2.9. Electrophoretic Analysis of Amplified DNA Product:

TAE or Loading Buffer (1 L of 50X buffer): It contains 242 g Tris base, 57.19 ml glacial acetic acid, 100 ml of 0.5M Na-EDTA (pH 8.0) and water upto 1 L. Agarose was purchased from Invitrogen, USA.

Staining Solution: Ethidium bromide was dissolved in (10 μg/μ1) dH_20 and stored at 4° C and protected from light.

DNA molecules were resolved electrophoretically in an agarose gel (1.5% w/v analytical grade agarose). Agarose 1.5 g was dissolved in IX TAE (100 ml) at the appropriate concentration by heating in the microwave and then the gel were poured into the tray. After solidification, 10 µL of PCR product was mixed with 1µL of gel loading dye and loaded into the slots of the gel with the aid of a micropipette. 1X TAE buffer was used for electrophoresis. Then the gel was stained with staining solution containing ethidium-bromide for 30 minutes and de-stained with distilled water for 15 minutes. The Et-Br stained DNA bands were observed on a UV trans-illuminator (Gel Doc, Bio-Rad, USA). Photographs were taken using Gel Doc machine attached to a computer and bands were analyzed with "Quantity One" software. The PCR product sizes were estimated using the 1kB or 100bp marker.

2.10. Determination of the Antibiotic Resistance Pattern of the isolates:

The isolates were subjected to antimicrobial susceptibility testing by disk diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI *et.al*. 2015) using commercial antimicrobial disks. The antibiotic disks used in this study were: Tetracycline (30 µg), Ciprofloxacin (5 µg), Amoxicillin-clavulanic Acid (20 µg), Nitrofurantoin (300 µg), Azithromycin (15µg), Ceftriaxone (30 µg), Cefixime (5 µg), Chloramphenicol (30 µg), Gentamycin (10 µg) and Co-trimoxazole.(25 µg).

The method described by Bauer and Kirby (1969) was followed. An inoculating needle was touched to a freshly grown, well isolated colony on NA plate and then inoculated into 1 ml of Muller-Hinton Broth (MHB). The culture were then incubated in a shaker at 37°C for 4 hours to obtain the actively growing culture, equivalent to 0.5 McFarland standard (1.5x 108 CFU/mL). A sterile cotton swab was dipped into the standard suspension, excess broth was purged by pressing and rotating the swab firmly against the inside wall of the tube above the fluid. The swab was then streaked evenly in three directions over the entire surface of the agar plate to obtain a uniform inoculum. A final sweep was made of the agar rim with the cotton swab. This plate was then allowed to dry for three to five minutes before the disks were applied. Antibiotic impregnated disks were then applied to the surface of the inoculated plates with sterile syringe needle. All disks were gently pressed down onto the agar with sterile forceps to ensure complete contact with the agar surface. Within 15 minutes after the disks were applied, the plates were inverted and placed in an incubator at 37°C. After overnight incubation, the plates were examined for zone of inhibition and the diameter of the zone of inhibition was measured to the nearest whole millimeter by a ruler. The zone diameters for individual antimicrobial agents was then translated into susceptible, intermediate or resistant categories according to the CLSI guidelines (2015).

Table 2.10. Antibiotics used in the susceptibility testing of *Escherichia coli*

Name of the Antibiotic disks	Abbreviation	Concentration µg	Sensitivity (mm)	Intermediate (mm)	Resistance (mm)
Nitrofurantoin	F-300	300	≥17	15-16	≤14
Amoxicillin-Clavulanic Acid	AMC	20	≥18	14-17	≤13
Azithromycin	AZM	15	≥18	14-17	≤13
Ceftriaxone	CRO	30	≥23	20-22	≤19
Chloramphenicol	C	30	≥18	13-17	≤12
Cefixime	CFM	5	≥19	16-18	≤15
Ciprofloxacin	CIP	5	≥31	21-30	≤20

Gentamicin	Gen	10	≥15	13-14	≤12
Co-trimoxazole	COT	25	≥16	11-15	≤10
Tetracycline	TE	30	≥15	12-14	≤11

2.11. Plasmid Profiling:

Isolation of Plasmid DNA

Plasmid DNA was isolated from the samples using Alkaline Lysis Method.

Reagents:

1. **Solution 1**: This solution included glucose (0.9 gm), 1M Tris HCL (2.5ml), 0.5M EDTA (2ml) and distilled water (up to 100ml). It was autoclaved at 115°C for about 10 minutes.
2. **Solution 2**: Freshly prepared solution 2 and an autoclaved McCartney bottle were important for this method. It was prepared with distilled water (9ml), 10% SDS (1 ml) and sodium hydroxide (0.08gm).
3. **Solution 3**: This solution was prepared with potassium acetate (14.721gm) and glacial acetic acid (5.75ml) and distilled water (up to 50ml). It was also autoclaved like solution 1 at 115°C for 10 minutes.
4. **Others**: 99% ice cold ethanol, 70% ethanol.

Procedure of Plasmid Extraction

1. A single colony from a fresh overnight culture plate of the test bacteria was inoculated in 5 ml Luria Bertani Broth (LB broth) and incubated at 37°C overnight.
2. 1mL of this fresh *E. coli* culture was transferred to a 1.5 mL micro centrifuge receiver tube.
3. This was centrifuged at 12000 rpm for 5 minutes and the supernatant was removed completely.
4. The pellet was re-suspended in 100 µL Solution 1.

5. 200 µL solution 2 was then added to the micro centrifuge tube and mixed gently but thoroughly and kept that in ice for 5 minutes.

6. 150 µL solution 3 was added next and mixed by inverting the tube and it was kept again in the ice for 5-7 minutes.

7. This was centrifuged for 15 minutes at 12000 rpm.

8. The clarified supernatant was transferred to a fresh, sterile eppendorf and it was mixed with double volume of ice cold ethanol (99%). It was mixed gently but thoroughly and incubated for 30 minutes at 4°C.

9. It was then centrifuged at 12000 rpm for 10 minutes and the supernatant was discarded.

10. Then 500µL Wash Solution (70% ethanol) was added and tapped with fingers to mix the wash solution with the pellet. It was then centrifuged at 12000 rpm for 5-7 minutes.

11. Supernatant was discarded and the procedure of step 10 was repeated for the 2nd time.

12. Then the eppendorf was dried.

13. After that 50µL TE buffer was added with the solution and this resultant solution contained plasmid.

Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gels in a TAE buffer at room temperature at 85 volts for 1.5 hours. The gel was stained with Et-Br for 25 minutes and destained with distilled water for 5 minutes. DNA bands were visualized and photographed using Gel Documentation with UV trans-illuminator. The size of the unknown plasmid DNA was determined on the basis of its mobility through agarose gel and was compared with the mobility of the known size marker. 1kb DNA ladder (GeneON, UK) was used as marker.

Results

3.1 Presumptive identification by Most Probable Number (MPN) method

In Most Probable Number method a series of tubes containing selective Brilliant Green Lactose Bile (BGLB) media were inoculated with test portions of different types of samples and incubated at 37°C. Each tube containing gas was regarded as presumptively positive for coliform. Subsequent confirmatory test with selective EMB and MacConkey agar media were performed. Variable number of coliforms were obtained from different sites and types of samples. Most Probable Number are outlined according to sample types in tables 3.1-3.4. Water samples contained *E. coli* in the range from W1 to W93. In soil samples, the count ranged from S1 to S85. Prawn Samples contained *E. coli* in the range from P0 to P64 and animal samples contained *E. coli* in the range from A1 to A76.

Table 3.1 MPN of Water Samples collected from different places

Number of tubes giving a positive reaction				MPN Index	95% confidence limit	
Sample ID	3 of 1 ml	3 of 0.1 ml	3 of 0.01ml	Per ml (g)	Lower	Upper
W1	0	0	0	<0.3	---	0.95
W2	1	0	0	0.36	0.017	1.8
W3	0	0	0	<0.3	---	0.95
W4	1	0	0	0.36	0.017	1.8
W5	0	0	0	<0.3	---	0.95
W6	0	0	0	<0.3	---	0.95
W7	2	0	0	0.92	0.14	3.8
W8	0	0	0	<0.3	---	0.95
W9	3	3	0	24.3	4.2	100.4

W10	3	3	0	24.3	4,2	100.4
W11	0	0	0	<0.3	---	0.95
W12	3	2	1	15.3	3.7	42.4
W13	3	1	0	4.3	0.90	18.4
W14	3	3	1	46.3	9.0	200.4
W15	3	0	0	2.3	0.46	9.4
W16	0	0	0	<0.3	---	0.95
W17	3	1	0	4.3	0.90	18.4
W18	0	0	0	<0.3	---	0.95
W19	0	0	0	<0.3	0.90	0.95
W20	0	0	0	<0.3	---	0.95
W21	0	0	0	<0.3	---	0.95
W22	1	0	0	0.36	0.017	1.8
W23	0	0	0	<0.3	---	0.95
W24	0	0	0	<0.3	---	0.95
W25	0	0	0	<0.3	---	0.95
W26	0	0	0	<0.3	---	0.95
W27	3	3	3	>110.3	42.0	---
W28	0	0	0	<0.3	---	0.95
W29	0	0	0	<0.3	---	0.95
W30	0	0	0	<0.3	---	0.95
W31	3	1	0	4.3	0.90	18.4
W32	2	1	0	1.5	0.37	4.2
W33	0	0	0	<0.3	---	0.95
W34	0	0	0	<0.3	---	0.95
W35	0	0	0	<0.3	---	0.95
W36	0	0	0	<0.3	---	0.95

W37	1	0	0	0.36	0.017	1.8
W38	0	0	0	<0.3	---	0.95
W39	0	0	0	<0.3	---	0.95
W40	1	0	0	0.36	0.017	1.8
W41	0	0	0	<0.3	---	0.95
W42	3	0	0	2.3	0.46	9.4
W43	0	0	0	<0.3	---	0.95
W44	0	0	0	<0.3	---	0.95
W45	0	0	0	<0.3	---	0.95
W46	3	1	0	4.3	0.90	18.4
W47	0	0	0	<0.3	---	0.95
W48	3	0	0	2.3	0.46	9.4
W49	3	1	0	4.3	0.90	18.4
W50	0	0	0	<0.3	---	0.95
W51	1	0	0	0.36	0.017	1.8
W52	1	0	0	0.36	0.017	1.8
W53	3	3	3	>110.3	42.0	---
W54	3	3	3	>110.3	42.0	---
W55	3	3	3	>110.3	42.0	---
W56	3	3	2	110.3	18.0	410.4
W57	3	3	3	>110.3	42.0	---
W58	3	3	3	>110.3	42.0	---
W59	3	2	1	15.3	3.7	42.4
W60	3	3	3	>110.3	42.0	---
W61	1	0	0	0.36	0.017	1.8
W62	3	0	0	2.3	0.46	9.4
W63	3	3	1	46.3	9.0	200.4

W64	3	1	0	4.3	0.90	18.4
W65	3	3	0	24.3	4.2	100.4
W66	3	3	3	>110.3	42.0	---
W67	3	3	3	>110.3	42.0	---
W68	3	3	3	>110.3	42.0	---
W69	3	3	3	>110.3	42.0	---
W70	3	3	3	>110.3	42.0	---
W71	3	3	3	>110.3	42.0	---
W72	3	3	3	>110.3	42.0	---
W73	3	3	3	>110.3	42.0	---
W74	3	3	3	>110.3	42.0	---
W75	3	3	3	>110.3	42.0	---
W76	3	3	3	>110.3	42.0	---
W77	3	3	3	>110.3	42.0	---
W78	3	3	3	>110.3	42.0	---
W79	3	3	3	>110.3	42.0	---
W80	1	1	1	1.1	0.36	3.8
W81	3	3	3	>110.3	42.0	---
W82	1	1	1	1.1	0.36	3.8
W83	0	0	0	< 0.3	---	0.95
W84	3	3	3	>110.3	42.0	---
W85	3	3	3	>110.3	42.0	---
W86	3	3	3	>110.3	42.0	---
W87	1	0	0	0.36	0.017	1.8
W88	3	3	3	>110.3	42.0	---
W89	0	0	0	< 0.3	---	0.95
W90	3	3	3	>110.3	42.0	---

W91	3	3	3	>110.3	42.0	---
W92	3	3	3	>110.3	42.0	---
W93	3	3	3	>110.3	42.0	---

Table 3.2 MPN of Soil Samples collected from different places

Number of tubes giving a positive reaction				MPN Index	95% confidence limit	
Sample ID	3 of 1 ml	3 of 0.1 ml	3 of 0.01ml	Per g (ml)	Lower	Upper
S1	3	3	3	>110.3	42.0	---
S2	3	3	2	110.3	18.0	410.4
S3	1	3	0	1.6	0.45	4.2
S4	3	3	3	>110.3	42.0	---
S5	3	3	3	>110.3	42.0	---
S6	3	3	3	>110.3	42.0	---
S7	3	3	3	>110.3	42.0	---
S8	3	2	0	9.3	1.8	42.4
S9	3	3	2	110.3	18.0	410.4
S10	3	2	0	9.3	1.8	42.4
S11	3	3	2	110.3	18.0	410.4
S12	3	3	3	>110.3	42.0	---
S13	3	3	3	>110.3	42.0	---

S14	3	3	3	>110.3	42.0	---
S15	3	3	3	>110.3	42.0	---
S16	3	3	3	>110.3	42.0	---
S17	3	3	0	24.3	4.2	100.4
S18	3	3	0	24.3	4.2	100.4
S19	3	3	1	46.3	9.0	200.4
S20	3	1	0	4.3	0.90	18.4
S21	3	0	0	2.3	0.46	9.4
S22	3	3	0	24.3	4.2	100.4
S23	3	3	2	110.3	18.0	410.4
S24	3	2	0	9.3	1.8	42.4
S25	3	3	2	110.3	18.0	410.4
S26	3	3	3	>110.3	42.0	---
S27	3	3	1	46.3	9.0	200.4
S28	3	3	2	110.3	18.0	410.4
S29	3	3	3	>110.3	42.0	---
S30	3	3	3	>110.3	42.0	---
S31	3	3	3	>110.3	42.0	---
S32	3	3	1	46.3	9.0	200.4
S33	3	3	3	>110.3	42.0	---
S34	3	3	3	>110.3	42.0	---
S35	3	1	0	4.3	0.90	18.4
S36	3	3	3	>110.3	42.0	---
S37	3	3	3	>110.3	42.0	---
S38	3	3	0	24.3	4.2	100.4
S39	3	3	0	24.3	4.2	100.4
S40	3	3	2	110.3	18.0	410.4

S41	3	3	3	>110.3	42.0	---
S42	3	3	2	110.3	18.0	410.4
S43	3	3	0	24.3	4.2	100.4
S44	3	3	3	>110.3	42.0	---
S45	3	3	3	>110.3	42.0	---
S46	3	3	2	110.3	18.0	410.4
S47	3	3	3	>110.3	42.0	---
S48	3	3	3	>110.3	42.0	---
S49	3	3	3	>110.3	42.0	---
S50	3	3	3	>110.3	42.0	---
S51	3	3	3	>110.3	42.0	---
S52	3	3	3	>110.3	42.0	---
S53	3	3	3	>110.3	42.0	---
S54	3	3	0	24.3	4.2	100.4
S55	3	2	2	21.3	4.0	43.4
S56	3	3	0	24.3	4.2	100.4
S57	3	3	3	>110.3	42.0	---
S58	3	3	2	110.3	18.0	410.4
S59	3	3	3	>110.3	42.0	---
S60	3	2	0	9.3	1.8	42.4
S61	3	3	3	>110.3	42.0	---
S62	3	3	3	>110.3	42.0	---
S63	3	3	3	>110.3	42.0	---
S64	3	3	3	>110.3	42.0	---
S65	3	3	3	>110.3	42.0	---
S66	3	3	3	>110.3	42.0	---
S67	3	3	3	>110.3	42.0	---

S68	3	2	3	29.3	9.0	100.4
S69	3	2	3	29.3	9.0	100.4
S70	0	2	0	0.62	0.12	1.8
S71	3	2	3	29.3	9.0	100.4
S72	3	2	0	9.3	1.8	42.4
S73	3	2	1	15.3	3.7	42.4
S74	3	3	3	>110.3	42.0	---
S75	3	3	0	24.3	4.2	100.4
S76	3	3	3	>110.3	42.0	---
S77	1	0	2	1.1	0.36	3.8
S78	3	3	3	>110.3	42.0	---
S79	3	3	3	>110.3	42.0	---
S80	3	3	3	>110.3	42.0	---
S81	1	1	1	1.1	0.36	3.8
S82	3	3	3	>110.3	42.0	---
S83	1	0	1	0.72	0.13	1.8
S84	3	3	3	>110.3	42.0	---
S85	3	3	3	>110.3	42.0	---

Table 3.3 MPN of Prawn Samples collected from different markets

Number of tubes giving a positive reaction				MPN Index	95% confidence limit	
Sample	3 of 1 ml	3 of 0.1 ml	3 of 0.01 ml	Per g (ml)	lower	Upper
P0	3	3	3	>110.3	42.0	---

P1	3	3	3	>110.3	42.0	---
P2	3	3	3	>110.3	42.0	---
P3	3	1	0	6.4	1.7	18.4
P4	3	3	3	>110.3	42.0	---
P5	3	3	3	>110.3	42.0	---
P6	3	3	3	>110.3	42.0	---
P7	3	3	3	>110.3	42.0	---
P8	3	3	3	>110.3	42.0	---
P9	3	3	3	>110.3	42.0	---
P10	3	3	0	24.3	4.2	100.4
P11	3	0	0	2.3	0.46	9.4
P12	3	3	3	>110.3	42.0	---
P13	3	3	3	>110.3	42.0	---
P14	3	3	3	>110.3	42.0	---
P15	3	3	3	>110.3	42.0	---
P16	3	3	3	>110.3	42.0	---
P17	3	3	3	>110.3	42.0	---
P18	3	3	3	>110.3	42.0	---
P19	3	3	3	>110.3	42.0	---
P20	3	3	3	>110.3	42.0	---
P21	3	3	3	>110.3	42.0	---
P22	3	3	3	>110.3	42.0	---
P23	3	3	3	>110.3	42.0	---
P24	3	3	3	>110.3	42.0	---
P25	3	3	3	>110.3	42.0	---
P26	3	3	3	>110.3	42.0	---
P27	3	3	3	>110.3	42.0	---
P28	3	3	3	>110.3	42.0	---

P29	3	3	3	>110.3	42.0	---
P30	3	3	3	>110.3	42.0	---
P32	3	3	3	>110.3	42.0	---
P33	3	3	3	>110.3	42.0	---
P34	3	3	3	>110.3	42.0	---
P35	3	3	3	>110.3	42.0	---
P36	3	3	3	>110.3	42.0	---
P37	3	3	3	>110.3	42.0	---
P38	3	3	3	>110.3	42.0	---
P39	3	3	3	>110.3	42.0	---
P40	3	3	3	>110.3	42.0	---
P41	3	3	3	>110.3	42.0	---
P42	3	3	3	>110.3	42.0	---
P43	3	3	3	>110.3	42.0	---
P44	3	3	3	>110.3	42.0	---
P45	3	3	0	24.3	4.2	100.4
P46	3	3	2	110.3	18.0	410.4
P47	3	3	3	>110.3	42.0	---
P48	3	3	1	46.3	9.0	200.4
P49	3	3	3	>110.3	42.0	---
P50	2	1	1	2.0	0.45	4.2
P51	3	3	3	>110.3	42.0	---
P52	0	1	0	0.30	0.015	1.1
P53	3	2	0	9.3	1.8	42.4

P54	3	3	1	46.3	9.0	200.4
P55	3	3	3	>110.3	42.0	---
P56	3	3	1	46.3	9.0	200.4
P57	3	2	2	21.3	4.0	43.4
P58	2	0	0	0.92	0.14	3.8
P59	1	1	1	1.1	0.36	3.8
P60	2	2	1	2.8	0.87	9.4
P61	0	0	0	<0.3	---	0.95
P62	3	1	0	4.3	0.90	18.4
P63	1	1	1	1.1	0.36	3.8
P64	3	3	1	46.3	9.0	200.4

Table 3.4 MPN of Animal Samples collected from different places

Number of tubes giving a positive reaction				MPN Index	95% confidence limit	
Sample ID	3 of 1 ml	3 of 0.1 ml	3 of 0.01ml	Per g (ml)	Lower	Upper
A1	3	3	3	>110.3	42.0	---
A2	3	3	3	>110.3	42.0	---
A3	3	3	3	>110.3	42.0	---
A4	3	3	3	>110.3	42.0	---
A5	3	3	3	>110.3	42.0	---
A6	3	3	3	>110.3	42.0	---
A7	3	3	3	>110.3	42.0	---

A8	3	3	3	>110.3	42.0	---
A9	3	3	3	>110.3	42.0	---
A10	3	3	3	>110.3	42.0	---
A11	3	3	3	>110.3	42.0	---
A12	3	3	3	>110.3	42.0	---
A13	3	3	3	>110.3	42.0	---
A14	3	3	3	>110.3	42.0	---
A15	3	3	3	>110.3	42.0	---
A16	3	3	3	>110.3	42.0	---
A17	3	3	3	>110.3	42.0	---
A18	3	3	3	>110.3	42.0	---
A19	3	3	3	>110.3	42.0	---
A20	3	3	3	>110.3	42.0	---
A21	3	3	3	>110.3	42.0	---
A22	3	3	3	>110.3	42.0	---
A23	3	3	3	>110.3	42.0	---
A24	3	3	3	>110.3	42.0	---
A25	3	3	3	>110.3	42.0	---
A26	3	3	3	>110.3	42.0	---
A27	3	3	3	>110.3	42.0	---
A28	3	3	3	>110.3	42.0	---
A29	3	3	3	>110.3	42.0	---
A30	3	3	3	>110.3	42.0	---
A31	3	3	3	>110.3	42.0	---
A32	3	3	3	>110.3	42.0	---
A33	3	3	3	>110.3	42.0	---
A34	3	3	3	>110.3	42.0	---

A35	3	3	3	>110.3	42.0	---
A36	3	3	3	>110.3	42.0	---
A37	3	3	3	>110.3	42.0	---
A38	3	3	3	>110.3	42.0	---
A39	3	3	3	>110.3	42.0	---
A40	3	3	3	>110.3	42.0	---
A41	3	3	3	>110.3	42.0	---
A42	3	3	3	>110.3	42.0	---
A43	3	3	3	>110.3	42.0	---
A44	3	3	3	>110.3	42.0	---
A45	3	3	3	>110.3	42.0	---
A46	3	3	3	>110.3	42.0	---
A47	3	3	3	>110.3	42.0	---
A48	3	3	3	>110.3	42.0	---
A49	3	3	3	>110.3	42.0	---
A50	3	3	3	>110.3	42.0	---
A51	3	3	3	>110.3	42.0	---
A52	3	3	3	>110.3	42.0	---
A53	3	3	3	>110.3	42.0	---
A54	3	3	3	>110.3	42.0	---
A55	3	3	3	>110.3	42.0	---
A56	3	3	3	>110.3	42.0	---
A57	3	3	3	>110.3	42.0	---
A58	3	3	3	>110.3	42.0	---
A59	3	3	3	>110.3	42.0	---
A60	3	3	3	>110.3	42.0	---
A61	3	3	3	>110.3	42.0	---

A62	3	3	3	>110.3	42.0	---
A63	3	3	3	>110.3	42.0	---
A64	3	3	3	>110.3	42.0	---
A65	3	3	3	>110.3	42.0	---
A66	3	3	3	>110.3	42.0	---
A67	3	3	3	>110.3	42.0	---
A68	3	3	3	>110.3	42.0	---
A69	3	3	3	>110.3	42.0	---
A70	3	3	3	>110.3	42.0	---
A71	3	3	3	>110.3	42.0	---
A72	3	3	3	>110.3	42.0	---
A73	3	3	3	>110.3	42.0	---
A74	3	3	3	>110.3	42.0	---
A75	3	3	3	>110.3	42.0	---
A76	3	3	3	>110.3	42.0	---

Figure 3.1: Different types of samples collected for analysis by MPN method.

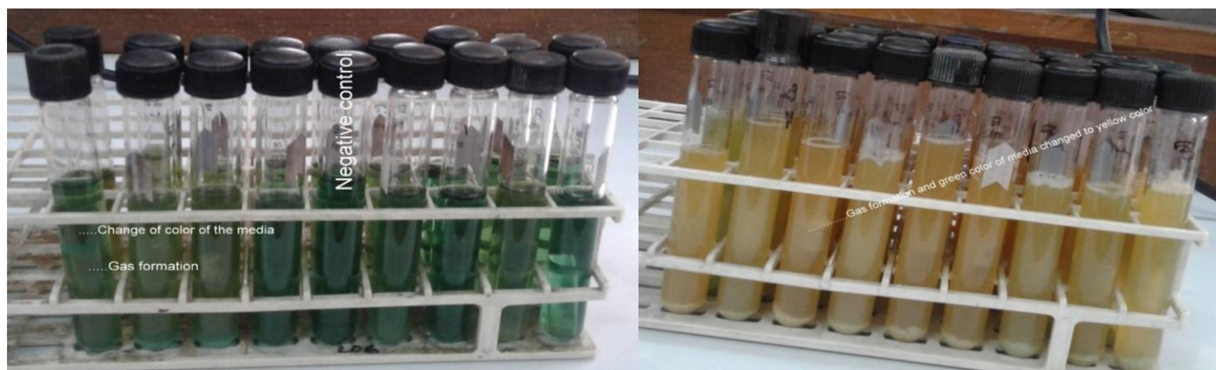


Figure 3.2: Samples analyzed by MPN method where gas formation and color change of green media towards yellowish indicate positive result.

3.2 Identification based on cultural characteristics of *E. coli*

3.2.1 Phenotypic confirmation of test isolates by culture based properties

After MPN study isolated samples were then identified as *E. coli* through some of the distinctive cultural characteristics. A total of 184 isolates were confirmed to be *E. coli* after observing lactose fermenting pink colonies on MacConkey Agar plates and greenish metallic sheen on Eosin Methylene Blue agar (Fig 3.3) following 18-24 hours incubation at 37°C. The colony morphologies are summarized in the table 3.5.

Table 3.5 Colony morphology of *Escherichia coli* on MacConkey and Eosin Methylene Blue plates

	MacConkey	**Eosin Methylene Blue**
Size	Medium	Medium
Shape	Circular	Circular
Margin	Entire	Entire
Elevation	Flat	Flat
Pigmentation	Pink	Green sheen

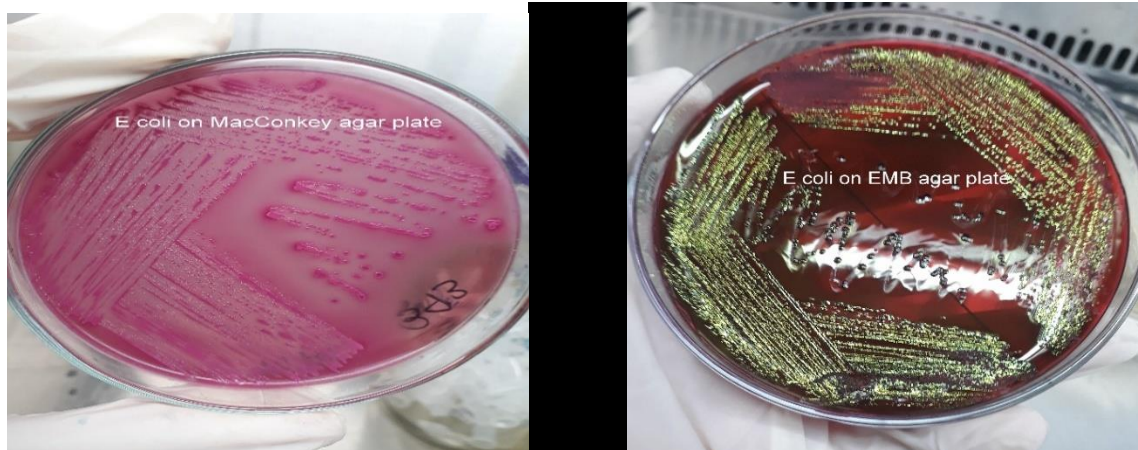


Figure 3.3: Representative *Escherichia coli* colonies on MacConkey (left) and EMB (right) agar plates.

3.2.2 Biochemical Identification of *E. coli*

Isolates were confirmed through common biochemical reactions such as Kligler's Iron Agar (KIA), Methyl red test, Indole production test, Citrate Utilization Test etc.

3.2.2.1 Kligler's Iron Agar (KIA) Test

Kligler's Iron agar is used to determine if bacteria can ferment glucose and /or lactose and if they can produce hydrogen sulfide or other gases. Among 150 isolates, 98.67% produced yellow slant and yellow butt whereas 97.33% produced gas. Hence lactose and glucose were fermented.

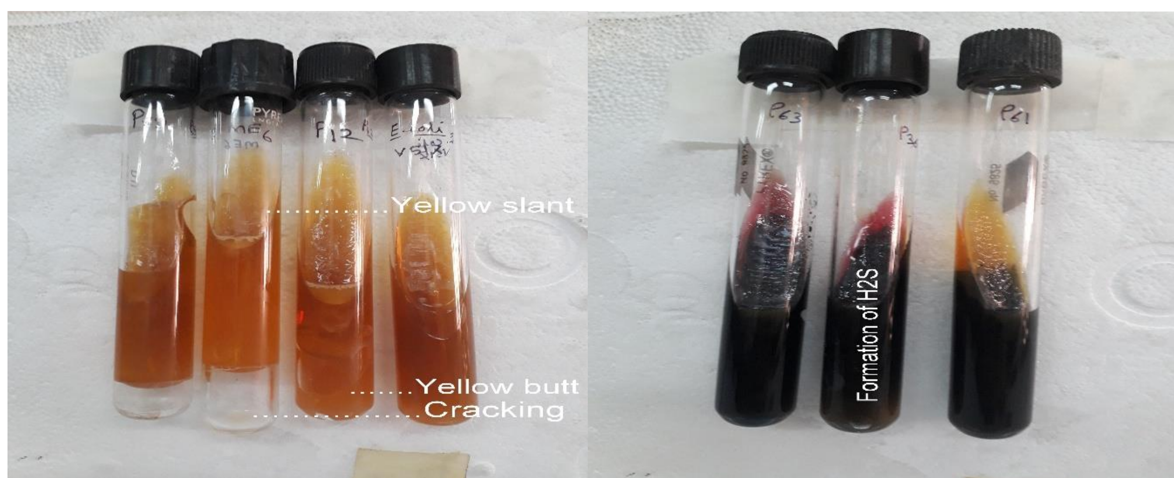


Figure 3.4: Kligler's Iron Agar Test of representative test isolates where lactose and glucose were fermented and gas was produced by *E. coli* (left) without forming Hydrogen

Sulphide whereas non *E. coli* isolates produced Hydrogen Sulphide without completely fermenting lactose and glucose (right).

3.2.2.2 Citrate Utilization Test

Many microorganisms can utilize citrate as their sole carbon source for energy. During citrate utilization, the medium becomes alkaline, the carbon dioxide that is generated combines with sodium and water to form sodium carbonate, an alkaline product. The bromothymol indicator in the medium changes color from green to deep Prussian blue. One hundred and fifty tested isolates (100%) were citrate negative i.e. they were unable to use citrate as a carbon source and hence the color of the medium was green.

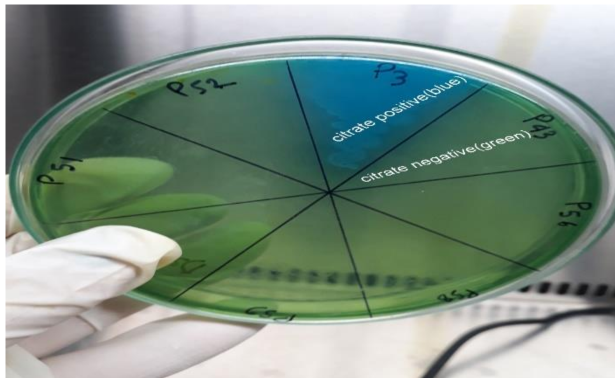


Figure 3.5: Simmons Citrate test of representative test isolates where *E. coli* were citrate negative (Green) and non *E. coli* isolates were citrate positive (Blue).

3.2.2.3 Methyl Red (MR) Test

All isolates metabolized glucose to produce acids such as lactic, acetic, formic acids and as a result pH dropped to 4.2-4.4. At this pH the methyl red indicator turned red in color.

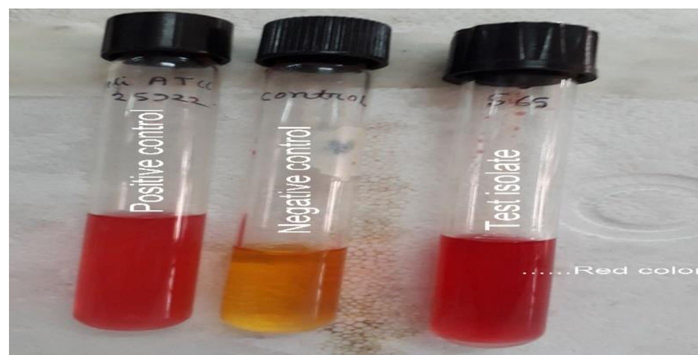


Figure 3.6: A representative Methyl Red Test Result.

3.2.2.4 Indole Production Test

Tryptophanase is an enzyme that hydrolyzes tryptophan and produces indole. Tryptophan is present in peptone water and the presence of indole is detected by adding Kovac's reagent which produces a cherry red color. All of the isolates that were *E. coli* showed indole positive result i.e. were able to produce tryptophanase.

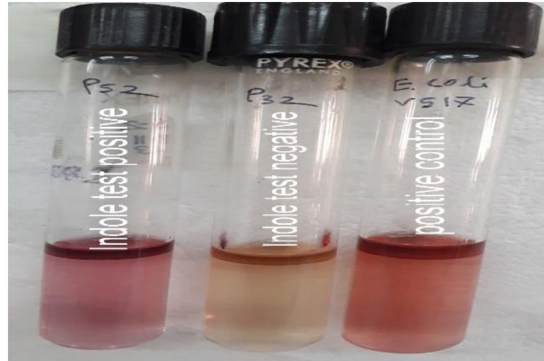


Figure 3.7: A representative Indole Production Test Result.

Table 3.6 Biochemical characteristics of re-confirmed 150 *E. coli* isolates from different sources including both Human and Non-Human hosts

	Kligler's Iron Agar test				MIU test					
Sample No	Slant (Lactose)	Butt (Dextrose +Sucrose)	Gas	H_2S	Motility	Indole	Urea	Citrate	Methyl Red	VP
P0	A	A	+	-	+	+	-	-	+	-
P7	A	A	+	-	+	+	-	-	+	-
P10	A	A	+	-	+	+	-	-	+	-
P11	A	A	+	-	+	+	-	-	+	-
P12	A	A	+	-	+	+	-	-	+	-
P20	A	A	+	-	+	+	-	-	+	-
P21	A	A	+	-	+	+	-	-	+	-

P22	A	A	+	-	+	+	-	-	+	-
P24	A	A	+	-	+	+	-	-	+	-
P26	A	A	+	-	+	+	-	-	+	-
P27	A	A	+	-	+	+	-	-	+	-
P29	A	A	+	-	+	+	-	-	+	-
P34	A	A	+	-	+	+	-	-	+	-
P35	A	A	+	-	+	+	-	-	+	-
P37	A	A	+	-	+	+	-	-	+	-
P41	A	A	+	-	+	+	-	-	+	-
P43	A	A	+	-	+	+	-	-	+	-
P45	A	A	+	-	+	+	-	-	+	-
P50	A	A	+	-	+	+	-	-	+	-
P52	A	A	+	-	+	+	-	-	+	-
P54	A	A	+	-	+	+	-	-	+	-
P56	A	A	+	-	+	+	-	-	+	-
P58	A	A	+	-	+	+	-	-	+	-
P59	A	A	+	-	+	+	-	-	+	-
P60	A	A	-	-	+	+	-	-	+	-
W13	A	A	+	-	+	+	-	-	+	-
W14	A	A	+	-	+	+	-	-	+	-
W27	A	A	+	-	+	+	-	-	+	-
W31	A	A	+	-	+	+	-	-	+	-
W42	A	A	+	-	+	+	-	-	+	-
W46	A	A	+	-	+	+	-	-	+	-
W49	A	A	+	-	+	+	-	-	+	-
W50	A	A	+	-	+	+	-	-	+	-
W55	A	A	+	-	+	+	-	-	+	-

W81	A	A	+	-	+	+	-	-	+	-
W84	A	A	+	-	+	+	-	-	+	-
W85	A	A	+	-	+	+	-	-	+	-
W86	A	A	+	-	+	+	-	-	+	-
W88	A	A	+	-	+	+	-	-	+	-
W89	A	A	+	-	+	+	-	-	+	-
W90	A	A	+	-	+	+	-	-	+	-
W91	A	A	+	-	+	+	-	-	+	-
W92	A	A	+	-	+	+	-	-	+	-
W93	A	A	+	-	+	+	-	-	+	-
S4	A	A	+	-	+	+	-	-	+	-
S5	A	A	+	-	+	+	-	-	+	
S6	A	A	+	-	+	+	-	-	+	-
S9	A	A	+	-	+	+	-	-	+	-
S11	A	A	+	-	+	+	-	-	+	-
S12	A	A	+	- +		+	-	-	+	-
S20	A	A	+	- +		+	-	-	+	-
S23	A	A	+	-	+	+	-	-	+	-
S31	A	A	+	-	+	+	-	-	+	-
S33	A	A	+	-	+	+	-	-	+	-
S48	A	A	+	-	+	+	-	-	+	-
S49	A	A	+	-	+	+	-	-	+	-
S51	A	A	+	-	+	+	-	-	+	-
S56	A	A	+	-	+	+	-	-	+	-

S65	A	A	+	-	+	+	-	-	+	-
S74	A	A	+	-	+	+	-	-	+	-
S76	A	A	+	-	+	+	-	-	+	-
S78	A	A	+	-	+	+	-	-	+	-
S79	A	A	+	-	+	+	-	-	+	-
S80	A	A	+	-	+	+	-	-	+	-
S82	A	A	+	-	+	+	-	-	+	-
S84	A	A	+	-	+	+	-	-	+	-
S85	A	A	+	-	+	+	-	-	+	-
A1	A	A	+	-	+	+	-	-	+	-
A2	A	A	+	-	+	+	-	-	+	-
A6	A	A	+	-	+	+	-	-	+	-
A7	A	A	+	-	+	+	-	-	+	-
A8	A	A	+	-	+	+	-	-	+	-
A9	A	A	+	-	+	+	-	-	+	-
A10	A	A	+	-	+	+	-	-	+	-
A12	A	A	+	-	+	+	-	-	+	-
A13	A	A	+	-	+	+	-	-	+	-
A14	K	A	+	-	+	+	-	-	+	-
A18	A	A	+	-	+	+	-	-	+	-
A19	A	A	+	-	+	+	-	-	+	-
A20	A	A	+	-	+	+	-	-	+	-
A21	A	A	+	-	+	+	-	-	+	-
A23	A	A	+	-	+	+	-	-	+	-
A26	A	A	+	-	+	+	-	-	+	-
A27	A	A	+	-	+	+	-	-	+	-

A28	K	K	+	-	+	+	-	-	+	-
A30	A	A	+	-	+	+	-	-	+	-
A31	A	A	+	-	+	+	-	-	+	-
26170	A	A	+	-	+	+	-	-	+	-
47508	A	A	+	-	+	+	-	-	+	-
823	A	A	+	-	+	+	-	-	+	-
380	A	A	+	-	+	+	-	-	+	-
180	K	A	+	-	+	+	-	-	+	-
47509	A	A	+	-	+	+	-	-	+	-
47990	A	A	+	-	+	+	-	-	+	-
25733	A	A	+	-	+	+	-	-	+	-
C/O Saidul	A	A	+	-	+	+	-	-	+	-
544	A	A	+	-	+	+	-	-	+	-
657	A	A	+	-	+	+	-	-	+	-
938	A	A	+	-	+	+	-	-	+	-
940	A	A	+	-	+	+	-	-	+	-
038	A	A	+	-	+	+	-	-	+	-
176	A	A	+	-	+	+	-	-	+	-
8996	A	A	+	-	+	+	-	-	+	-
30955	A	A	+	-	+	+	-	-	+	-
47770	A	A	+	-	+	+	-	-	+	-
47697	A	A	+	-	+	+	-	-	+	-
30987	A	A	+	-	+	+	-	-	+	-
407	A	A	+	-	+	+	-	-	+	-
394	A	A	+	-	+	+	-	-	+	-
464	A	A	-	-	+	+	-	-	+	-
496	A	A	+	-	+	+	-	-	+	-

779	A	A	+	-	+	+	-	-	+	-
755	A	A	-	-	+	+	-	-	+	-
774	A	A	+	-	+	+	-	-	+	-
397	A	A	+	-	+	+	-	-	+	-
075	A	A	-	-	+	+	-	-	+	-
064	A	A	+	-	+	+	-	-	+	-
032	A	A	+	-	+	+	-	-	+	-
914	A	A	+	-	+	+	-	-	+	-
9312	A	A	+	-	+	+	-	-	+	-
425	A	A	+	-	+	+	-	-	+	-
343	A	A	+	-	+	+	-	-	+	-
585	A	A	+	-	+	+	-	-	+	-
534	A	A	+	-	+	+	-	-	+	-
647	A	A	+	-	+	+	-	-	+	-
689	A	A	+	-	+	+	-	-	+	-
595	A	A	+	-	+	+	-	-	+	-
564	A	A	+	-	+	+	-	-	+	-
168	A	A	+	-	+	+	-	-	+	-
685	A	A	+	-	+	+	-	-	+	-
588	A	A	-	-	+	+	-	-	+	-
265	A	A	+	-	+	+	-	-	+	-
521	A	A	+	-	+	+	-	-	+	-
340	A	A	+	-	+	+	-	-	+	-
3491	K	A	+	-	+	+	-	-	+	-
9	A	A	+	-	+	+	-	-	+	-
13	A	A	+	-	+	+	-	-	+	-

14	A	A	+	-	+	+	-	-	+	-
68	A	A	+	-	+	+	-	-	+	-
ME3	A	A	+	-	+	+	-	-	+	-
ME4	A	A	+	-	+	+	-	-	+	-
ME5	A	A	+	-	+	+	-	-	+	-
ME6	A	A	+	-	+	+	-	-	+	-
ME7	A	A	+	-	+	+	-	-	+	-
ME8	A	A	+	-	+	+	-	-	+	-
ME9	A	A	+	-	+	+	-	-	+	-
ME10	A	A	+	-	+	+	-	-	+	-
SF-V(5)	A	A	+	-	+	+	-	-	+	-
SF-6	A	A	+	-	+	+	-	-	+	-
ATCC 25922	A	A	+	-	+	+	-	-	+	-

3.3 Molecular characterization of *E. coli*

3.3.1 Confirmation of identity of test isolates by using PCR with universal primers

Molecular genotyping technique involves the amplification of 16s rRNA gene for genera identification. So amplification of 16s rDNA and after that some other molecular typing help to confirm the isolates. Figure 3.8 represents the amplicon corresponding to 16s rRNA gene.

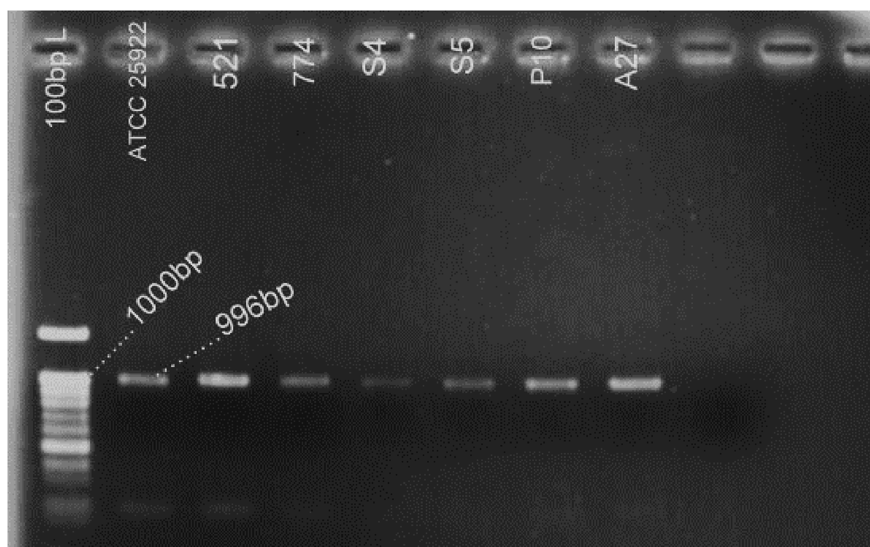


Figure 3.8. Gel image of 16s rDNA amplicon for *Escherichia coli*. *Escherichia coli* 25922 was used as positive control and here 521, 774 were Human, S4, S5 were Soil, P10 was Prawn and A27 was Animal *Escherichia coli* isolates. The ladder was a 100bp Ladder from GeneON (UK).

Randomly selected 50 isolates that successfully amplified the 16s rRNA fragment after the PCR reaction produced an amplicon of 996 bp in size. After purification of these PCR products were digested with HaeIII, EcoRI and HindIII to determine whether there was a genetic diversity among the isolates. Samples from the same species were supposed to provide the same length pattern and different patterns would indicate samples from different sources.

3.4 Amplified ribosomal DNA restriction analysis (ARDRA)

The purpose of this typing was to study the microbial density and diversity among the isolates collected from diverse human and non-human Host isolates like water, soil, prawn and animal. The isolates could be grouped according to the similarity of their restriction patterns into different ARDRA types. This rapid protocol needed the purified products of 16s rDNA amplicon from different isolates and it gave rise to the same restriction fragment length pattern. The corresponding fragment size after digestion with the three different restriction endonuclease enzymes like EcoRI, HaeIII, HindIII remained same for every single isolate. This rapid and sensitive method thus ensured the confirmation of the test isolates to be *E. coli* and also indicated that there was no

difference among the isolates in terms of their sequence change based on the specific restriction enzyme selection used.

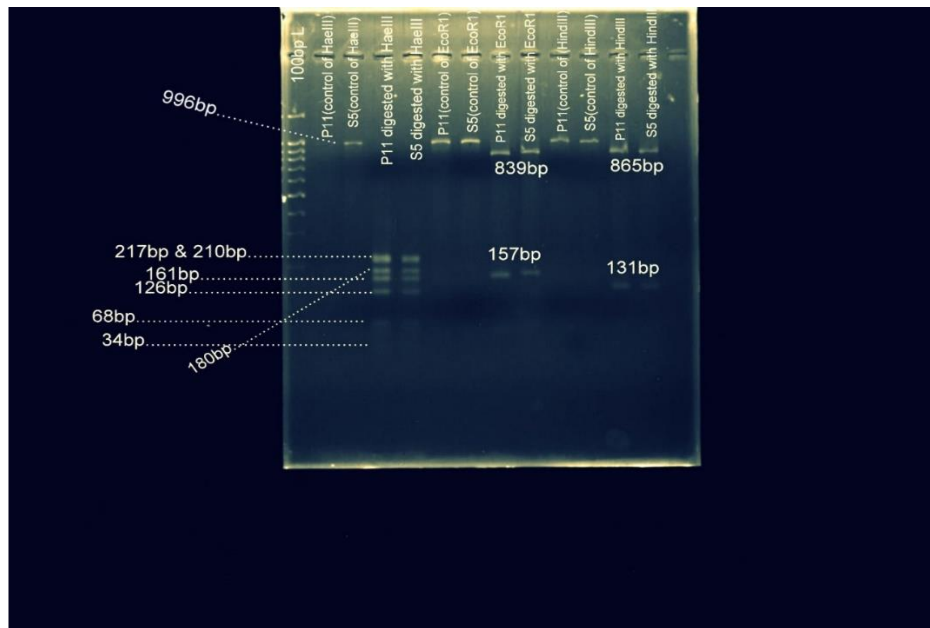


Figure 3.9: HaeIII, EcoRI and HindIII digestion patterns of universal PCR products. Samples in lanes 3 and 4 were HaeIII digested PCR products from the control bacteria of lane 1 and 2. Lane 7 and 8 containing fragments of control DNA (Lane 5 and 6) after digestion with EcoRI enzyme. Fragments in lane 11 and 12 were the HindIII digested product of control DNA in lane 9 and 10. The ladder used was a 100bp Ladder from GeneON(UK).

3.5 Detection of *uidA* and *uspA* gene for identification of *E. coli*

A total of 184 presumptive *E. coli* samples isolated from different sources were confirmed by means of some biochemical characteristics and followed by a rapid method consisting of a PCR assay along with RFLP patterns. After that these isolates were re-confirmed by two *E. coli* specific primers for the marker gene (*uspA* and *uidA*). Every isolates were positive for both sets of primers. The *uidA* gene has been shown to be very specific to *E. coli*; but primers specific to this region also amplifies few species of *Shigella* (Bej *et al*., 1991). To address this short coming of *uidA* primers, we included *E. coli* specific primer set for flanking region *uspA* (Chen *et al*., 1998).

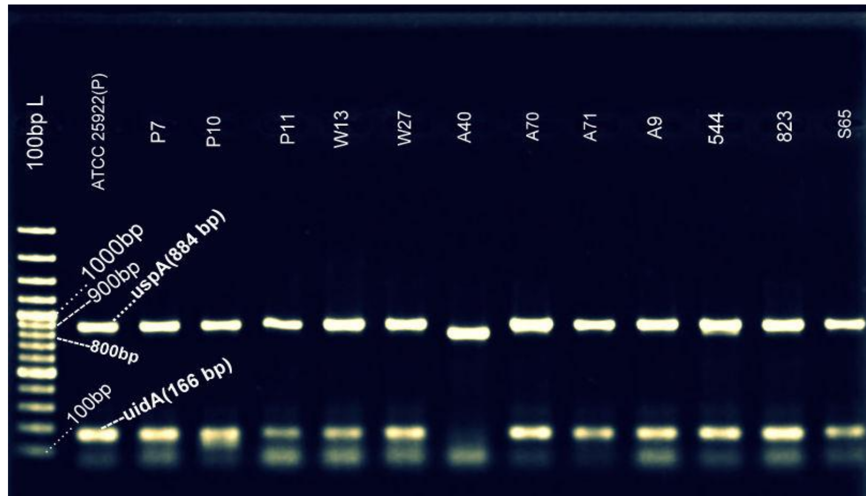


Figure 3.10: Amplification of *uidA* and *uspA* genes from both human and non-human host isolated *E. coli* samples. *Escherichia coli* 25922 was used as a positive control. The ladder used was a 100bp ladder from GeneRuler (UK). All of these isolates contained the specific amplicon for both *uidA* and *uspA* gene amplification.

3.6 Phylogroup diversity of *E. coli* isolates

Phylogenetic analysis revealed the origin of the test isolates based on the presence of different genetic markers including *chuA, yjaA*, TspE4.C2 and *arpA*. The isolates belonged to one of 7 phylogroups: A, B1, B2, C, D, E and F. The most prevalent phylogroup was group B1. Among the 184 (Human=60, animal=54, prawn=25, water=20, soil=23, street food=2) isolates analyzed, 46.74% (86 isolates) were included in phylogroup B1 (*arpA* and TspE4.C2 +), representing environmental isolates. Of the remaining, 28.26% isolates belonged to phylogroup A, (*arpA* +), indicating commensal origin. Only 1.63% isolates belonged to phylogroup B2 (*chuA* and *yjaA* +), representing pathogenic isolates, 8.15% isolates were included in phylogroup C which were both *arpA* and *yjaA* genes positive, reconfirmed with C-specific PCR and were closely related to environmental isolates in terms of phylogeny. Isolates which showed positive results at C-specific PCR were included in phylogroup C otherwise they were classified as A (Clermont *et al*., 2013). Of these, 10.67% belonged to phylogroup D and 3.26% isolates belonged to phylogroup E (*arpA*+ and *chuA*+). These isolates were reconfirmed with E-specific PCR. Isolates which contained E-specific PCR amplicons were grouped as phylogroup E, otherwise they were categorized as phylogroup D (Clermont *et al*., 2013). Phylogroup F included 2.17% of isolates which contained only *chuA* gene.

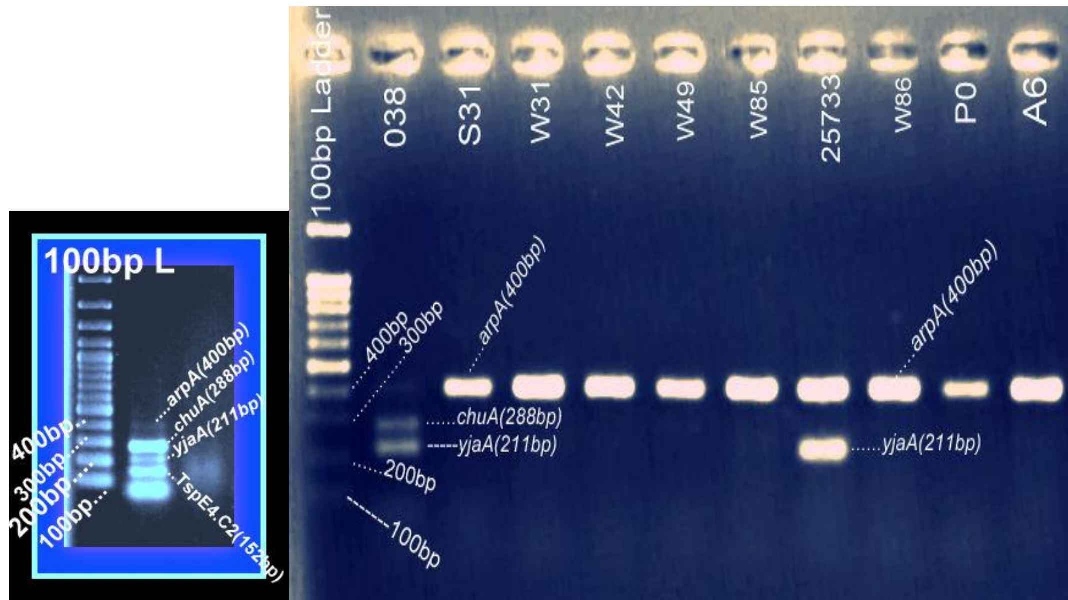


Figure 3.11: Distribution of different phylogroups among isolates. The gel on the left shows resolution of bands generated by PCR on known *E. coli*. The second gel shows different phylogenetic markers within the test isolates. The ladder used was a 100bp ladder from GeneRuler (UK).

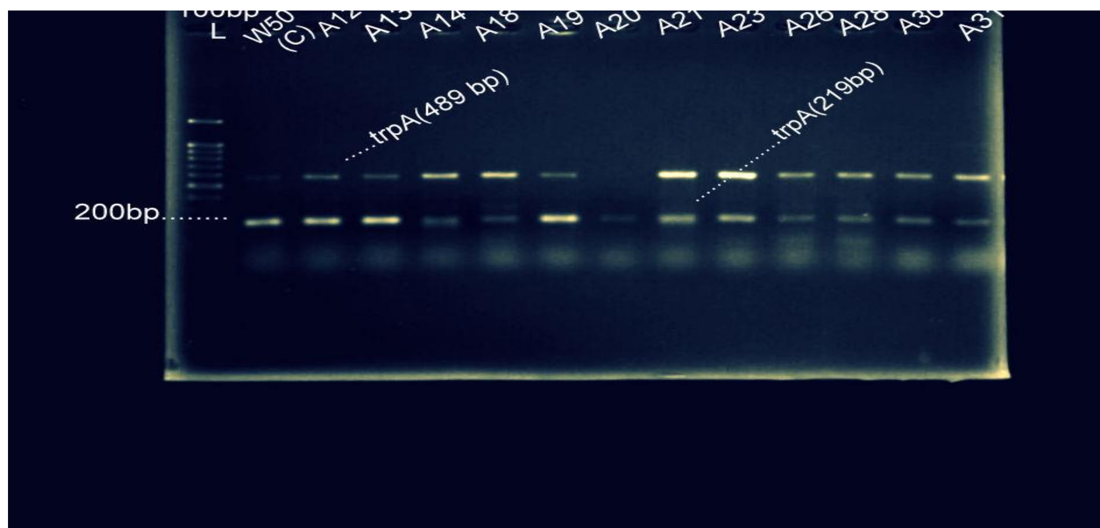


Figure 3.12: Gel showing confirmation of A/C phylogroup with C-specific PCR. Isolates which contain *trpA* gene are categorized in phylogroup C.

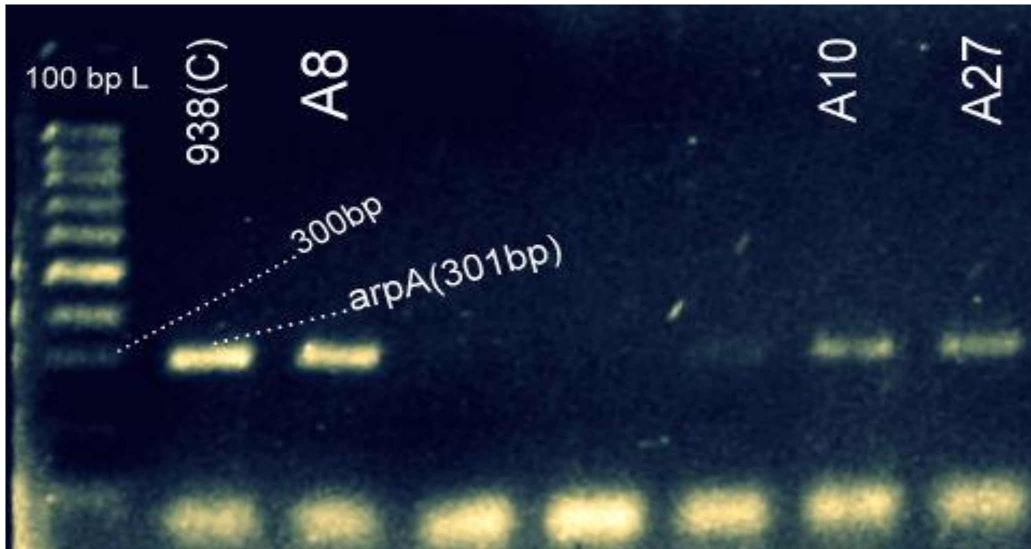


Figure 3.13: Gel showing confirmation of D/E and E/Clade 1 isolates with E- specific PCR. (Human host isolate 938 was used as a positive control). Animal isolates denoted A8, A10 and A27 contained *arpA* amplicon, specific for Phylogroup E (Clermont *et al*., 2013).

The summarized distribution of seven different phylogenetic groups of 182 isolates among 184 isolates are given in Figure 3.14.

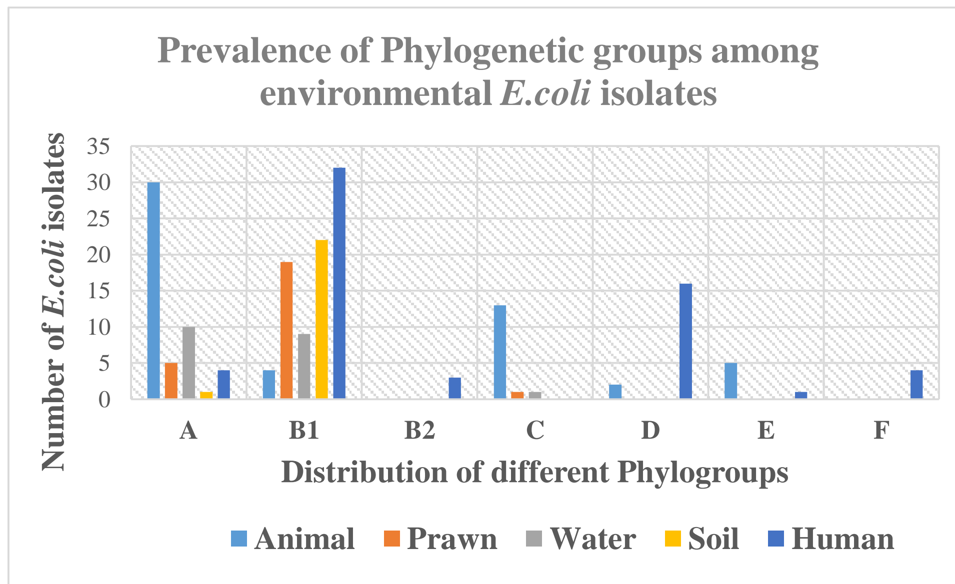


Figure 3.14: Graphical presentation of the overall distribution of Phylogenetic groups among the test isolates.

The overall distribution of different phylogroups among the test isolates is shown in Figure 3.15.

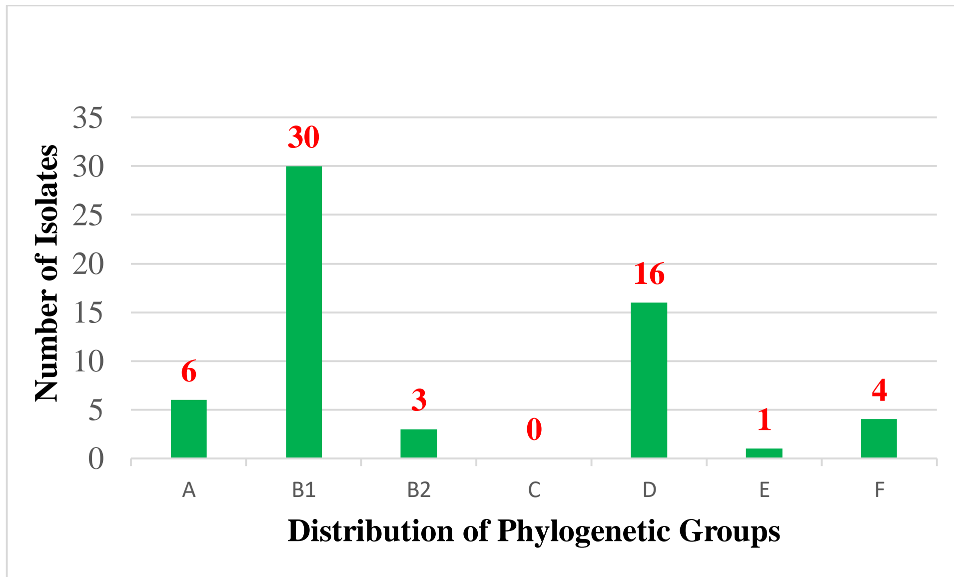


Figure 3.15: Graphical presentation of the distribution of Phylogenetic groups among Human Host isolates.

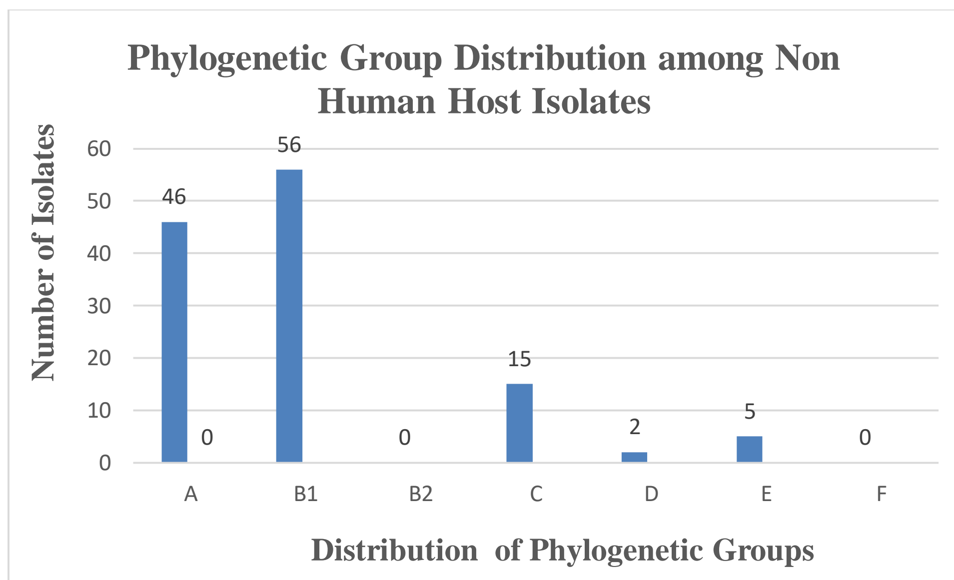


Figure 3.16: Graphical presentation of the distribution of Phylogenetic groups of *E. coli* from Non-Human Host.

According to the revised Clermont phylotyping method (Clermont *et al.,* 2013) described earlier, the test isolates were assigned to different phylogroups. This graphical presentation (Figure 3.14) reveals the fact that phylogroup A and B1 existed within all of these different environments viz. human, water, soil, street food, prawn and animal. The abundance of phylogroup B1 among these

isolates indicated that environmental *E. coli* are prevalent among these human and non-human hosts. So, group B1 (environmental *E. coli*) was the most common and then group A (commensal organism) existed within human, animal, prawn, soil and water isolates. Phylogroup B2 (virulent strains) was isolated from only within human whereas phylogroup C (closely related but distinct from group B1) was present only in Non-Human host *E. coli* isolates. However, *E. coli* isolates belonging to phylogroup D (extraintestinal virulent strain) and E (new group, unassigned group) were found in human and animal hosts whereas phylogroup F (related and sister group of pathogenic B2) existed only among *E. coli* isolated from human.

Figure 3.15 shows that *E. coli* isolated from human predominantly belonged to phylogroup B1 (50%), which represented environmental *E. coli* followed by group D (26.67%) pathogenic *E. coli*. Surprisingly, commensal phylogroup A accounted for only 10% of the isolates. Of the isolates, 5% were B2, 1.67% was E and 6.67% were F representing phylogenetic origins other than commensal *E. coli*. In non-human host (water, soil, animal, street food and prawn), phylogroup B1 was predominant (45.16%), followed by A (37%), C (12%), E (4%) and D (1.61%) in decreasing order (Table 3.10 and figure 3.16). As in human host, *E. coli* phylogroup B1 was also the dominating group in non-human host *E. coli* isolates.

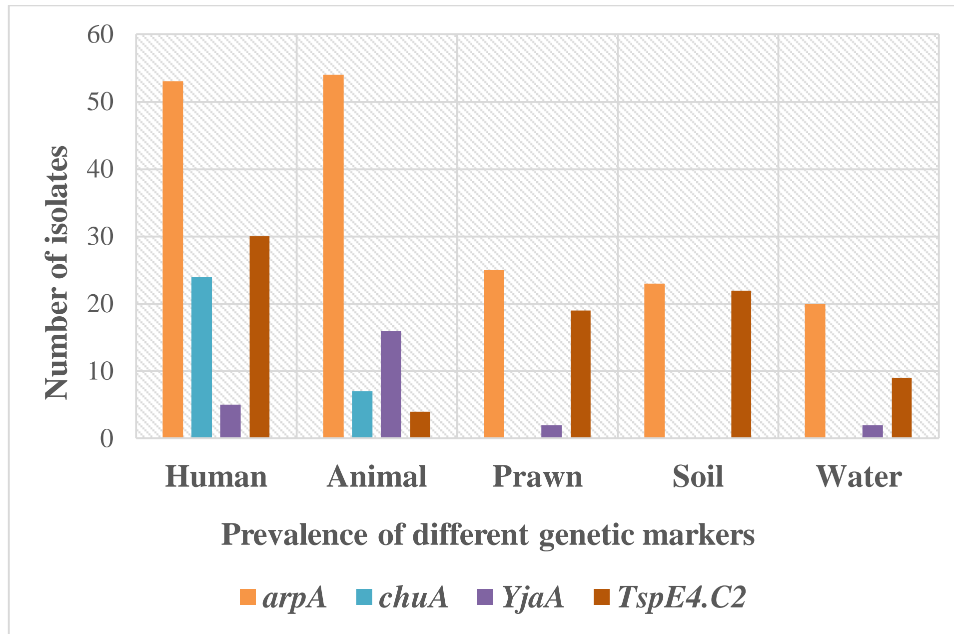


Figure 3.17: Graphical presentation of the distribution of Phylogenetic markers among *E. coli* isolates in different environments.

Figure 3.17 reveal that the phylogenetic marker *arpA* was present in 95% of *E. coli* isolates, the DNA fragment TspE4C2 was present in 48.65% isolates, *chuA* remained in 16.85% isolates whereas *yjaA* existed only within 13.59% isolates.

Similarity in the distribution of the different phylogroups in various environments was determined by calculating Pianka's index (Table 3.7).

3.7 Analysis of Phylogroup diversity with different indices

Table 3.7 Pairwise Pianka's index of similarity among the hosts analyzed

Pair	Pianka's index
Human vs Non-Human	0.48
Human vs Animal	0.77
Water vs Soil	0.70
Prawn vs Water	0.84

Cow vs Chicken	0.58

Table 3.7 shows that 48% similarity existed between the phylogroups of Human and Non-human Host *E. coli* isolates which include water, soil, prawn and animal isolates whereas 77% similarity existed between the phylogroups of Human and Animal Host isolates, 58% similarity existed between the phylogroups of Cow and Chicken *E. coli* isolates whereas 70% similarity was present between soil and water *E. coli* phylogroups and 84% similarity was present between prawn and water *E. coli* phylogroups which was comparatively high than the percentage similarity of other source isolates.

Different environments analyzed showed variations in diversity of the phylogroups. Comparisons were made between different types of environment to investigate whether there was any difference in terms of diversity. Shannon's index of diversity was used for this analysis.

Table 3.8 Comparison of Human and Non-Human *E. coli* Phylogroup diversity by Shannon diversity index

Site	Human	Non Human
Total	60	124
Richness	6	5
H	1.32786	1.17842
Variance	0.012196	0.004095
Confidence Interval	0.220871003	0.127984374
t	1.170827696	
df	101.5182978	
Critical value	1.983731003	
p	0.244423049	

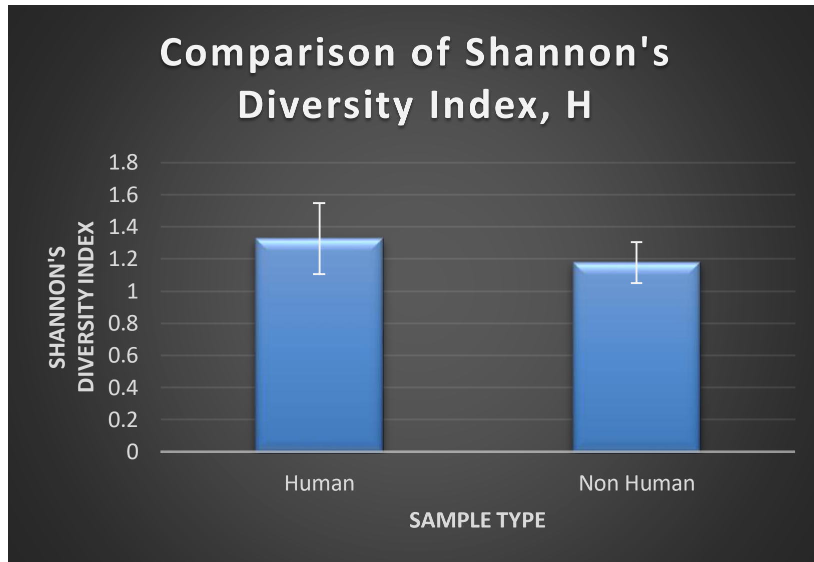


Figure 3.18: Comparison of *E. coli* phylogroup diversity between Human and Non-Human hosts.

Table 3.8 and figure 3.18 show the values of different variables such as degree of freedom (df), t test, p value and critical values for comparison. The bars at figure 3.18 indicated the values of different Shannon Diversity indexes (H) for both Human and Non-human host *E. coli* phylogroups.

The Shannon Diversity indices of *E. coli* from human was 1.33 and for Non-human host was 1.17. These differences in diversity were found to be statistically significant ($p>0.05$) and the value of t was within the critical value which indicated that similarity existed between the phylogroup diversity pattern of *E. coli* isolated from human and Non-human host.

Table 3.9 Comparison of Human and Animal *E. coli* Phylogroup diversity by Shannon Diversity index

Site	Human	Animal
Total	60	54
Richness	6	5
H	1.32786	1.20456
Variance	0.012196	0.01286
Confidence Interval	0.220871	0.22683
t	0.778899112	
df	113.2865427	
Critical value	1.981180359	
p	0.437667238	

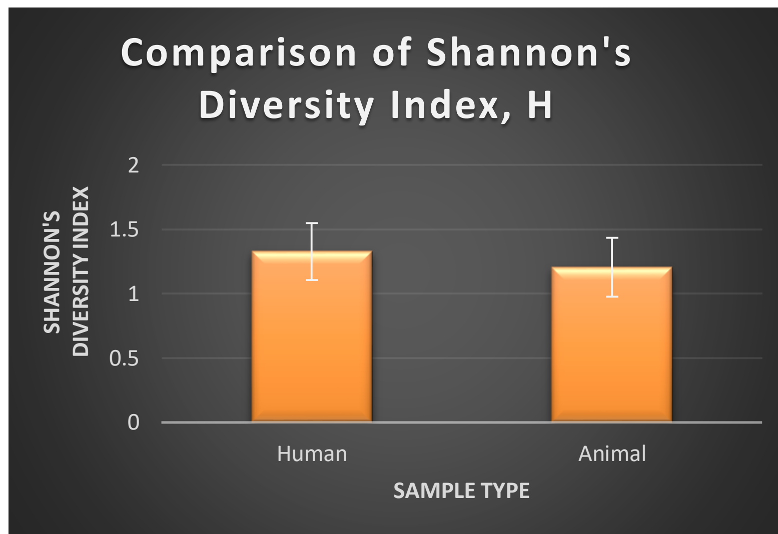


Figure 3.19: Comparison of *E. coli* phylogroup diversity between Human and Animal hosts.

Table 3.9 and figure 3.19 show that the Shannon Diversity indices for *E. coli* from human host was 1.33 and for animal host was 1.20. These differences in diversity were found to be statistically significant because here ($p > 0.05$) and the value of t was within the critical value which indicated that similarity existed between the phylogroup diversity pattern of human and animal host *E. coli* isolates.

Table 3.10 Comparison of Phylogroup diversity of *E. coli* isolated from Cow and Chicken by Shannon diversity index

Site	Cow	Chicken
Total	23	21
Richness	4	3
H	0.73017	0.9303
Variance	0.042398	0.00303
Confidence Interv	0.4118155	0.11002
t	0.939008466	
df	26.25376866	
Critical value	2.055529439	
p	0.356368590	

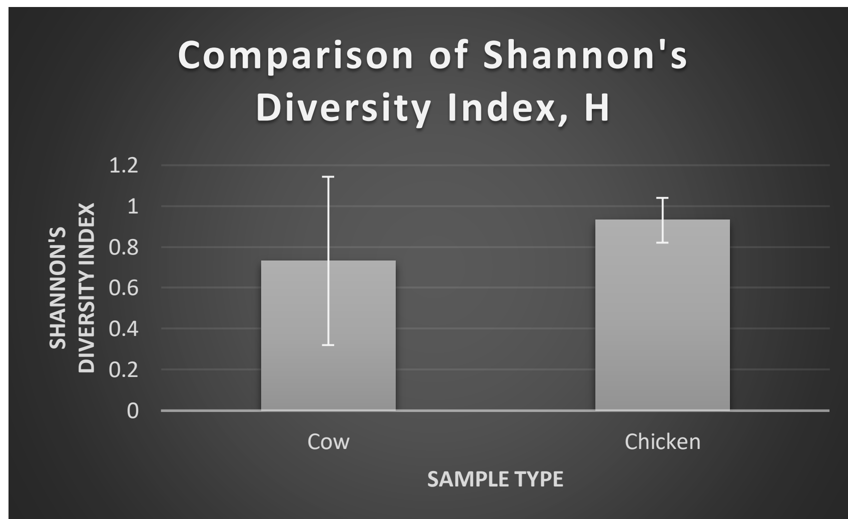


Figure 3.20: Comparison of phylogroup diversity of *E. coli* isolated from Cow and Chicken.

Table 3.10 and figure 3.20 show that the Shannon Diversity indices for cow and chicken were 0.73 and 0.93, respectively. These differences in diversity were found to be statistically significant ($p>0.05$).

Table 3.11 Comparison of Phylogroup diversity among *E. coli* isolated from Soil and Water by Shannon diversity index

Site	Soil	Water
Total	23	20
Richness	2	3
H	0.1788449	0.85569
Variance	0.0182214	0.01468
Confidence Interval	0.2699734	0.24235
t	3.731280159	
df	42.93850744	
Critical value	2.018081703	
p	0.000565705	

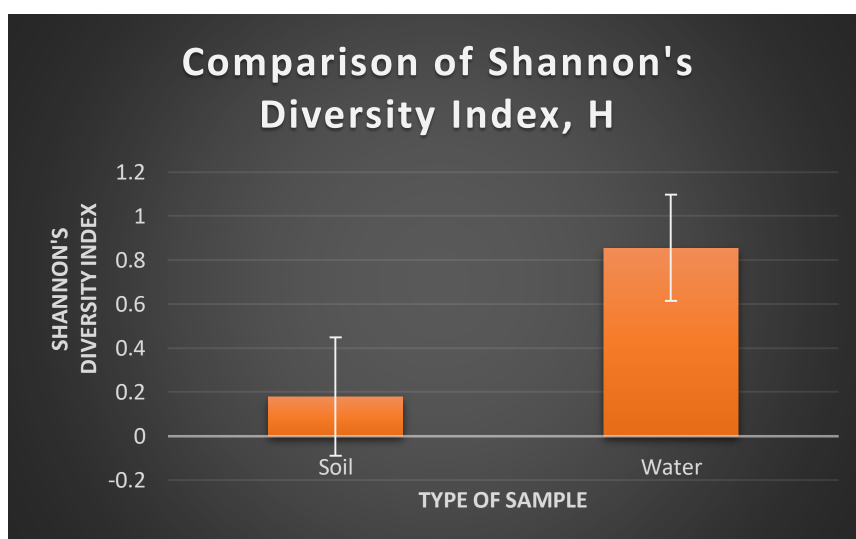


Figure 3.21: Comparison of phylogroup diversity of *E. coli* isolated from Soil and Water.

Shannon Diversity Indices for *E. coli* isolated from water and soil were 0.86 and 0.17, respectively (Table 3.11 and figure 3.21). These differences in diversity were not statistically significant ($p<0.05$).

Table 3.12 Comparison of phylogroup diversity of *E. coli* isolated from Water and Prawn *E. coli* by Shannon diversity index

Site	Prawn	Water
Total	25	20
Richness	3	3
H	0.66	0.85569
Variance	0.0238072	0.01468
Confidence Interval	0.3085917	0.24235
t	0.99744179	
df	44.28893062	
Critical value	2.015367574	
p	0.324004132	

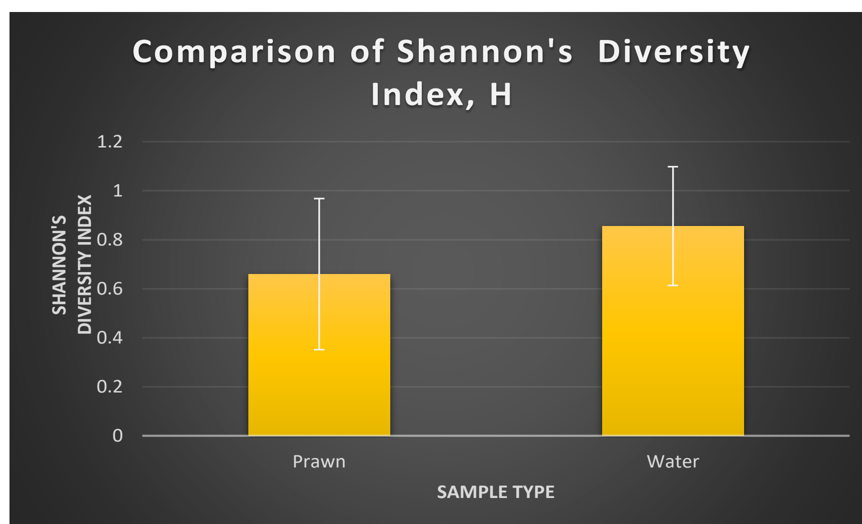


Figure 3.22: Comparison of phylogroup diversity of *E. coli* isolated from Prawn and Water.

Shannon diversity indices of *E. coli* phylogroups isolated from water and prawn were 0.86 and 0.66, respectively (Table 3.12 and Figure 3.22). These differences in diversity were statistically significant (p>0.05).

Table 3.13 Shannon's and Simpson's diversity index of each host analyzed

<table>
<tr><th rowspan="4">Shannon Diversity Index (H)</th><th>Human</th><th colspan="2">Animal</th><th>Prawn</th><th>Water</th><th>Soil</th></tr>
<tr><td rowspan="3">1.33</td><td colspan="2">1.2</td><td rowspan="3">0.66</td><td rowspan="3">0.86</td><td rowspan="3">0.17</td></tr>
<tr><td>Cow</td><td>Chicken</td></tr>
<tr><td>0.73</td><td>0.93</td></tr>
<tr><th>Simpson Diversity Index (D)</th><td>0.64</td><td colspan="2">0.63</td><td>0.39</td><td>0.57</td><td>0.09</td></tr>
</table>

3.8 Determination of Pathotypes of *E. coli* from Human and Non-Human Host

In order to determine the different pathotypes of *E. coli*, an array of seven different primer pairs were used in five different PCR assays. All of these PCR assays showed 100% specificity in identifying the reference strains. Nonspecific bands were not visualized. Figure 3.23 and figure 3.24 show the PCR products of the two different PCR assays derived from pure cultures of reference strains of EPEC, ETEC and the test isolates from different sources.

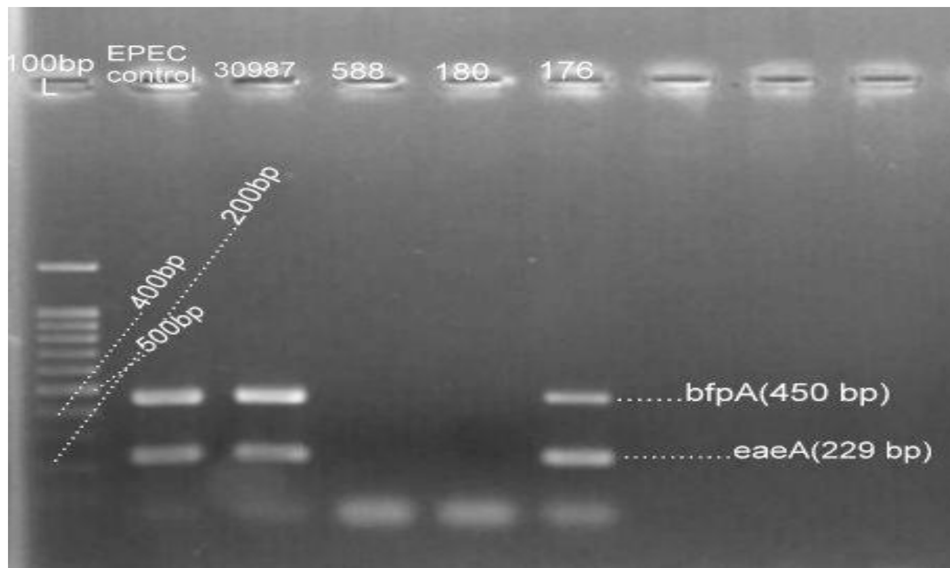


Figure 3.23: Resolution of *bfpA* and *eaeA* genes by agarose gel electrophoresis. The ladder used was a 100 bp ladder from GeneON(UK). EPEC reference strain was used as the positive control here and two Human host isolates named 30987 and 176 harboring *bfpA* and *eaeA* genes.

Out of the 150 isolates analyzed for the detection of EPEC path-type only two were positive for *bfpA* and *eaeA* genes. *eaeA* is a chromosomal gene responsible for the production of A/E lesions (attaching and effacing) lesions at human intestinal epithelium cells. Due to the presence of *bfpA* (bundle-forming pili) EPEC is capable of forming micro-colonies, resulting in a localized adherence pattern. The presence of these two genes accounted for only 1.33% of the isolates being EPEC (30987 and 176) (Figure 3.23).

A total 150 isolates were studied further for the detection of ETEC path- type. This multiplex PCR assay was designed to detect the presence of *elt* gene, encoding the heat-labile toxin LT-1 and *stla* encoding heat labile toxin carried by entero-toxigenic *E. coli* (ETEC) (Lasaro *et al.*, 2008). The *elt* gene specific amplicon (322 bp) was present in 1 isolate (0.67%, n=150)) (isolate no. 534, Figure 3.24) indicating that the isolate might be Enter-toxigenic *E. coli*.

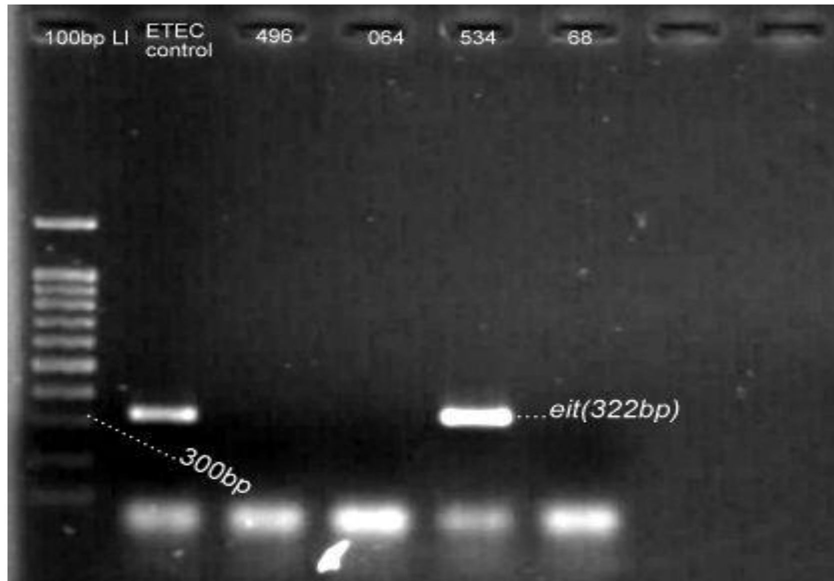


Figure 3.24: Detection of *elt* gene by agarose gel electrophoresis. The ladder was 100 bp ladder from GeneON (UK). A previously identified laboratory ETEC strain was used as a reference strain. The Human host isolate 534 was found to harbor the *elt* gene, characteristic of ETEC.

The distribution of five different patho-types among the 150 isolates are given in the following graph (Figure 3.25).

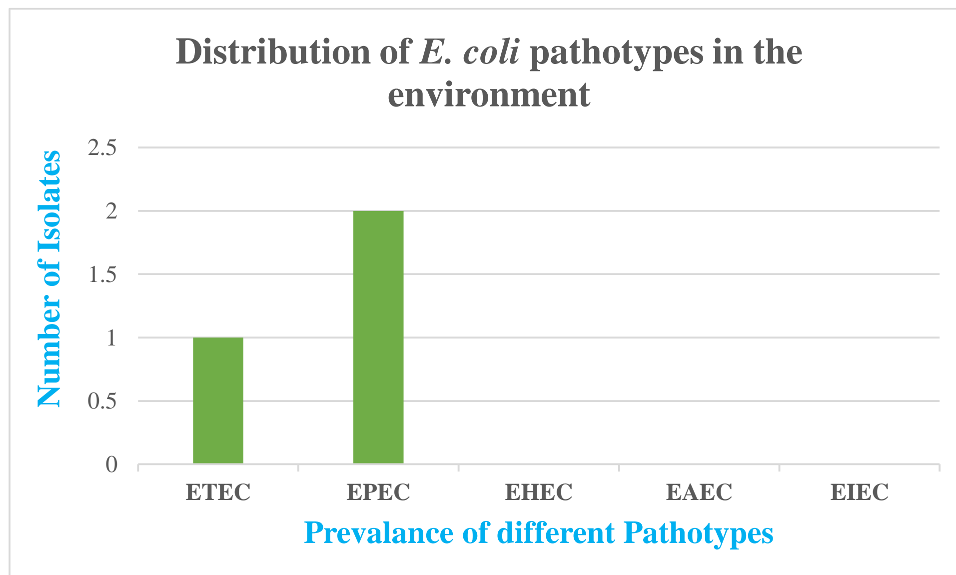


Figure 3.25: Graphical representation of the presence of ETEC, EPEC, EHEC, EAEC and EIEC among the test isolates.

Five different PCR assays were followed along with seven different pairs of primers (Table 2.8) in order to study a number of environmental isolates (Hedge *et al.*, 2012). This study did not detect any EHEC , EAEC or EIEC strains.

3.9 Molecular detection of *eae* gene among environmental isolates

The *eae* gene is generally correlated to virulent *E. coli* that has caused recent fecal contamination. The PCR product of virulence gene *eae*, which is 863bp, was not detected in any of the test isolates. A singleplex PCR with the specified primers (Table 2.6) was used to detect this gene (Zhang *et al.*, 2002). This indicated that none of our isolates were present due to recent fecal contamination.

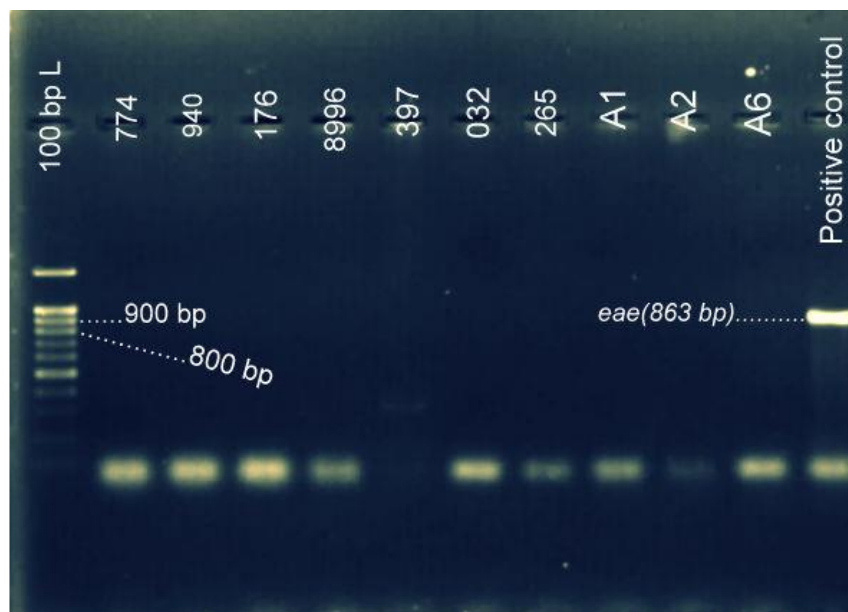


Figure 3.26: Resolution of *eae* gene by agarose gel electrophoresis. The ladder used here was a 100 bp ladder from GeneON (UK) and the reference strain *Escherichia coli* 0157:H7 was used as a positive control for this PCR assay.

3.10 Detection of *intl*-1 gene among environmental *E. coli*

Integrons are mobile genetic elements which can carry different genes such as antibiotic resistance gene. Integron profiling was performed to understand any role of Integrons in antibiotic resistance pattern. Among 150 isolates, 30% (45 isolates) contained the PCR amplicon for Class 1 Integron. Figure 3.27 shows the result of Integron analysis.

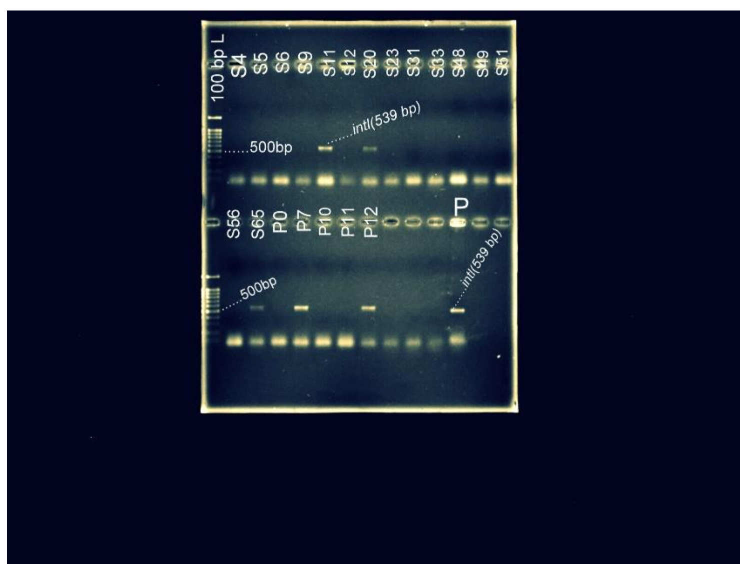


Figure 3.27: Agarose gel resolution of *intl*-1 PCR amplicon by agarose gel electrophoresis Here the ladder used was a 100 bp ladder from GeneON (UK). A laboratory reference *E. coli* isolated from street food and known to contain *intl-1* gene was used as the positive control in the PCR and denoted as P (Positive Control). Isolates numbered S11, S20, S65, P7 and P12 showed desired amplicon of the same size (539 bp).

3.11 Co-existence of class 1 Integron and antibiotic resistance

Integrons are mobile genetic elements that may carry antibiotic resistance genes. In this study we found that 30% (among 150 isolates) of our isolates from different sources carried class 1 Integrons and among them 28% were drug resistant while 25.33% were Multi Drug Resistant (MDR).

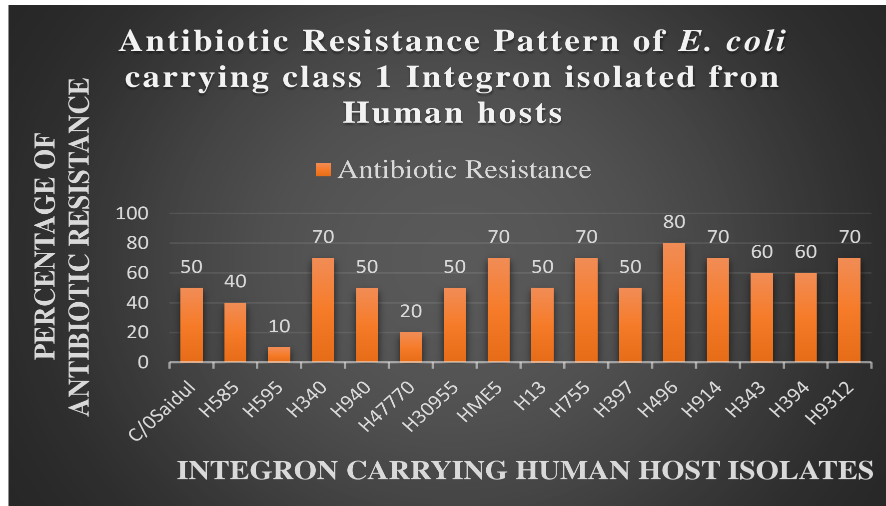


Figure 3.28: Graphical representation of Antibiotic Resistance pattern of Class 1 Integron carrying *E. coli* isolated from Human.

Figure 3.28 shows that among 60 isolates from human, 25% were multidrug resistant and carried Class 1 Integron, of which 33.33% isolates were resistant to 7, 13.33% were resistant to 6 , 33.33% were resistant to 5 , 6.67% were resistant to 8 and 6.67% were resistant to 4 different types of antibiotics. Only 1 isolate named H 595 was resistant to only one antibiotic.

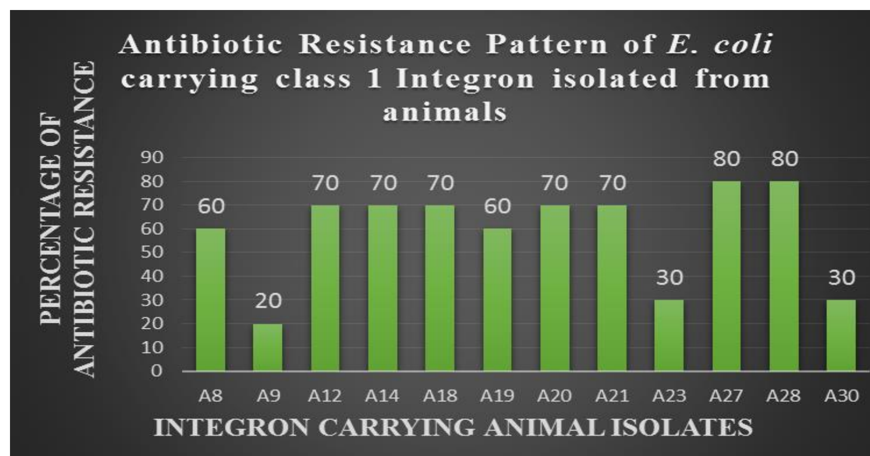


Figure 3.29: Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Animal Host isolates.

Figure 3.29 shows that among 20 *E. coli* isolated from animal, 60% were multidrug resistant and carried class 1 Integrons. Among these, 16.67% were resistant to 8, 41.67% were resistant to 7, 16.67% were resistant to 6 and 3, whereas 8.33% were resistant to 2 different types of antibiotics.

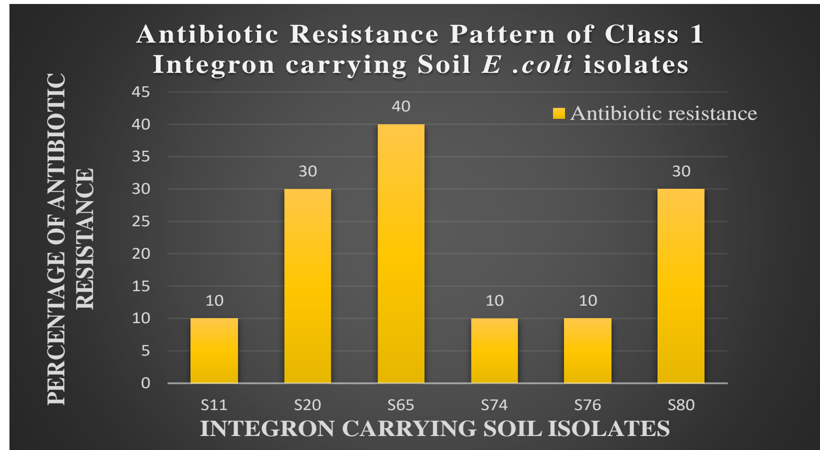


Figure 3.30: Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Soil isolates.

Among 23 *E. coli* isolated from soil, 34.78% carried Class 1 Integron. Of these, 37.5% isolates were multidrug resistant where 12.5% isolates showed resistance to 4 , 25% were resistant to 3 and 37.5% isolates were resistant to only one type of antibiotic (Figure 3.30).

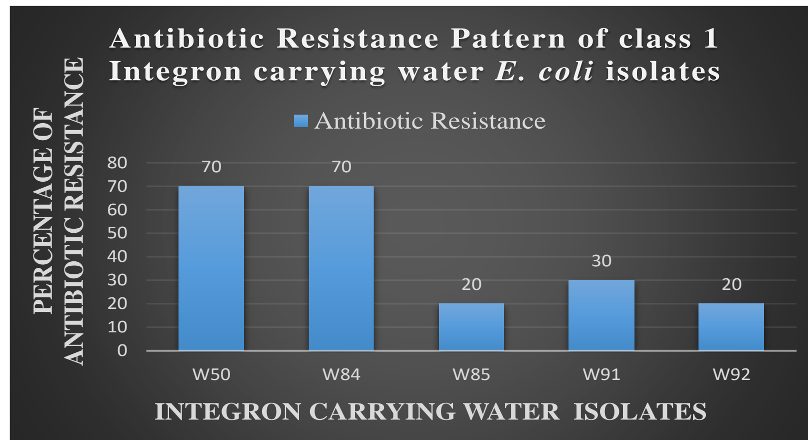


Figure 3.31: Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Water isolates.

Five isolates carrying class 1 Integrons among 20 water isolates and all of these Integron carrying isolates (25%) were multidrug resistant (Figure 3.31). Among these Integron carrying isolates 40% were resistant against 7 different types of antibiotics, 20% were resistant against 3 different types of antibiotics while 40% showed resistance against 2 different types of antibiotics.

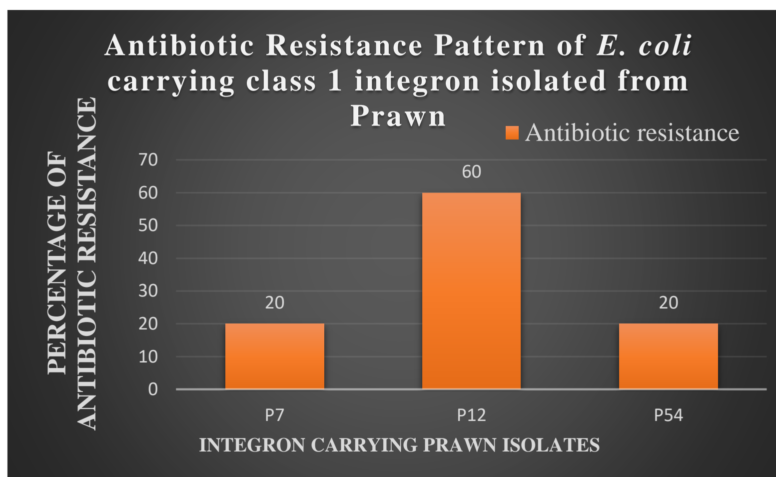


Figure 3.32: Graphical representation of Antibiotic Resistance pattern with Class 1 Integron carrying Prawn isolates.

Among 25 prawn *E. coli* isolates only 3 isolates (12%) carrying class 1 Integrons and all of these isolates were multidrug resistant. Among these multidrug resistant isolates 33.33% isolates showed resistance against six different types of antibiotics and 66.67% were resistant to two different types of antibiotics (Figure 3.32).

3.12 Plasmid profiling in *E coli* samples

Plasmid profile analysis of the *E.coli* isolates was analyzed in 1% gel to understand the possible relation in between number of plasmids and percentage of antibiotic resistance properties among the isolates. Moreover, co-existence of plasmids and class-1 Integron were also examined. In our study, 88 (58.67%) of the isolates showed the presence of plasmids of various sizes. A representative gel showing plasmids extracted from the test isolates is shown in figure 3.33.

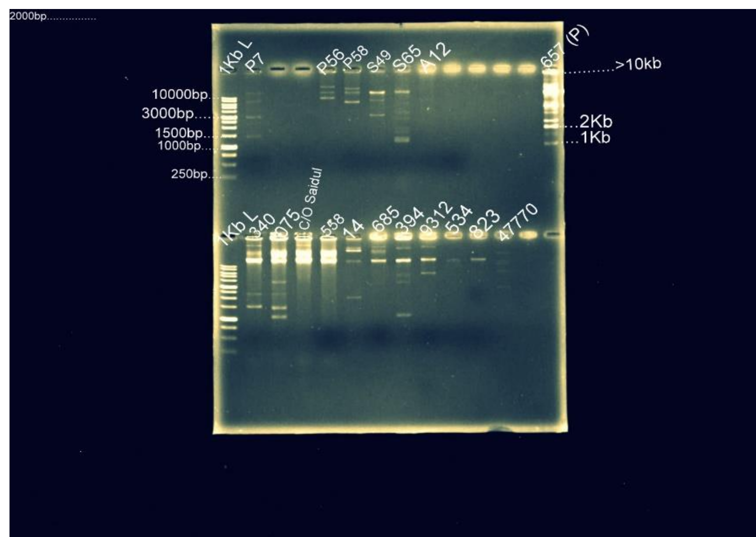


Figure 3.33: Agarose gel electrophoresis of isolates showing respective band patterns for plasmids. The ladder used here was a 1 kb ladder of GeneON (UK). Sample no.657 used here as a positive control which was previously analyzed and it is a Human Host isolate. At this gel, the plasmids of upper lanes like P7, P56 and P58 were from prawn isolates, S49, S65 were from soil isolates, A12 from animal host isolates whereas all of the plasmids at lower lanes were from Human host isolates.

3.12.1 Plasmid number variation of size among isolates

A total of 150 isolates were studied for the presence of the plasmid DNA. Among these isolates 88 (58.67%) isolates were found to carry plasmid. Fifty one (57.95%) of these samples were isolated from Human host, eighteen (20.45%) were from prawn, twelve (13.63%) from soil and only seven (7.95%) were isolated from water. The number and size variation of plasmids found in *E.coli* samples are mentioned in table 3.19.

Table 3.14 Number and size of plasmids extracted from *E. coli* isolated from different sources

Sample types	Sample ID	Number of plasmid	Size of plasmid
Human	9	3	>10kb, 3kb, 2kb
Human	689	1	>10kb

Human	564	1	>10kb
Human	779	1	>10kb
Human	380	1	>10kb
Human	168	1	>10kb
Human	343	1	>10kb
Human	407	1	>10kb
Human	585	1	>10kb
Human	3491	1	>10kb
Human	ME9	1	>10kb
Human	O38	1	>10kb
Human	30955	1	>10kb
Human	68	3	>10kb, 2.5kb, 2kb
Human	774	3	All >10kb
Human	47697	2	Both >10kb
Human	823	2	Both >10kb
Human	521	2	Both >10kb
Human	176	2	Both >10kb
Human	26170	2	Both >10kb
Human	180	2	Both >10kb
Human	O32	2	Both >10kb
Human	940	2	Both >10kb
Human	588	2	Both >10kb

Human	265	3	All >10kb
Human	C/O Saidul	3	All >10kb
Human	685	4	All >10kb
Human	464	4	All >10kb
Human	755	6	Three >10kb, 4kb, 2.5kb, 2kb
Human	938	6	Three >10kb, 5.5kb, 2.5kb, 2kb
Human	9312	3	Two >10kb, 6kb
Human	394	4	Two>10kb, 3kb, 2.5kb
Human	8996	2	>10kb, 1.5kb
Human	47990	2	>10kb, 3kb
Human	O75	4	>10kb, 3kb, 1.5kb, 1kb
Human	647	4	>10kb, 4.8kb, 2kb
Human	340	3	All >10kb
Human	14	6	Three >10kb, 2kb,1.5kb,1kb
Human	397	7	Four >10kb, 8kb, 4kb, 3kb
Human	544	7	Three >10kb, 4kb, 3.5kb, 3kb, 1.5kb
Human	938	7	Three>10kb, 5.5kb, 2.5kb, 1.5kb,1kb

Human	657	8	Three >10kb, 5kb, 2.5kb, Two 2kb, 1kb
Human	496	3	Two >10kb, 1.5kb
Human	47770	4	>10kb, 3kb, 2.5kb, 2kb
Human	595	2	2.5kb, 2kb
Human	914	1	2kb
Human	25733	2	4kb, 2kb
Human	425	1	5kb
Human	O64	3	5kb, 2.5kb, 2kb
Human	13	3	6kb, 2.5kb, 2kb
Human	534	1	8kb
Prawn	P0	1	>10kb
Prawn	P10	1	>10kb
Prawn	P11	1	>10kb
Prawn	P12	1	>10kb
Prawn	P26	1	>10kb
Prawn	P27	1	>10kb
Prawn	P29	1	>10kb
Prawn	P37	1	>10kb
Prawn	P45	1	>10kb
Prawn	P50	1	>10kb
Prawn	P52	1	>10kb

Prawn	P54	1	>10kb
Prawn	P60	1	>10kb
Prawn	P34	5	Two >10kb, 8kb, 5kb, 2.5kb
Prawn	P58	2	>10kb, 6kb
Prawn	P56	2	>10kb, 8kb
Prawn	P41	1	3.5kb
Prawn	P7	3	6kb, 3kb,1.5kb
Soil	S31	1	>10kb
Soil	S33	1	>10kb
Soil	S48	1	>10kb
Soil	S51	1	>10kb
Soil	S78	1	>10kb
Soil	S80	1	>10kb
Soil	S84	1	>10kb
Soil	S56	2	>10kb, 1.5kb
Soil	S79	2	>10kb, 3kb
Soil	S76	3	>10kb, 6kb, 2.5 kb
Soil	S65	1	1.5kb
Soil	S49	1	3kb
Water	W81	1	>10kb
Water	W85	1	>10kb

Water	W93	1	>10kb
Water	W88	3	>10kb, 3kb, 2kb
Water	W86	2	>10kb, 6kb
Water	W92	3	>10kb, 6kb, 2.5 kb
Water	W84	5	Three >10kb, 2.5kb, 2kb

3.12.2 Co-existence of plasmid and Class-1 Integron among *E. coli* isolates

Out of the total 150 isolates, 58.67% isolates were found to harbor plasmids of different sizes and on the contrary Integron profiling showed that 29.33% of the isolates revealed the desired amplicon (539 bp) of Class-1 Integron specific PCR. It was one of the aims of this study to investigate the co-existence of plasmids and Class-1 Integron among the isolates. But it was seen that isolates that contained plasmids did not contain any Integron and *vice versa* except 23 isolates that were from Human and Prawn.

Table 3.15 Plasmid and Class-1 Integron profiling of *E. coli* isolates

Sample ID	Presence of *intl*-1	Presence of Plasmid	Sample ID	Presence of *intl*-1	Presence of Plasmid
ME 5	(+)ve	(-)ve	47697	(-)ve.	(+)ve
940	(+)ve	(+)ve	8996	(-)ve	(-)ve
755	(+)ve	(+)ve	774	(-)ve	(+)ve
340	(+)ve	(+)ve	O32	(-)ve	(+)ve
C/O Saidul	(+)ve	(+)ve	588	(-)ve	(+)ve
397	(+)ve	(+)ve	938	(-)ve	(+)ve
30955	(+)ve	(+)ve	176	(-)ve	(+)ve
585	(+)ve	(+)ve	657	(-)ve	(+)ve
47770	(+)ve	(+)ve	30987	(-)ve	(-)ve
394	(+)ve	(+)ve	O75	(-)ve	(+)ve
914	(+)ve	(+)ve	265	(-)ve	(+)ve

343	(+)ve	(+)ve	544	(-)ve	(+)ve
595	(+)ve	(+)ve	521	(-)ve	(+)ve
496	(+)ve	(+)ve	26170	(-)ve	(+)ve
13	(+)ve	(+)ve	14	(-)ve	(+)ve
ME 3	(-)ve	(-)ve	180	(-)ve	(+)ve
ME 4	(-)ve	(-)ve	531	(-)ve	(-)ve
ME 6	(-)ve	(-)ve	689	(-)ve	(-)ve
ME 7	(-)ve	(-)ve	647	(-)ve	(+)ve
ME 8	(-)ve	(-)ve	3491	(-)ve	(+)ve
ME 9	(-)ve	(+)ve	685	(-)ve	(+)ve
ME 10	(-)ve	(-)ve	25733	(-ve)	(+)ve
479990	(-)ve	(+)ve	O38	(-ve)	(+ve)
47509	(-ve)	(-ve)	380	(-)ve	(+)ve
823	(-)ve	(+)ve	47508	(-)ve	(-)ve
425	(-)ve	(+)ve	9312	(+)ve	(+)ve
O64	(-)ve	(+)ve	168	(-)ve	(+)ve
564	(-)ve	(+)ve	779	(-)ve	(+)ve
407	(-)ve	(+)ve	464	(-)ve	(+)ve
9	(-)ve	(+)ve	68	(-)ve	(+)ve
W13	(-)ve	(-)ve	S4	(-)ve	(-)ve
W14	(-)ve	(-)ve	S5	(-)ve	(-)ve
W27	(-)ve	(-)ve	S6	(-)ve	(-)ve
W31	(-)ve	(-)ve	S9	(-)ve	(-)ve
W42	(-)ve	(-)ve	S11	(+)ve	(-)ve
W46	(-)ve	(-)ve	S12	(+)ve	(-)ve
W49	(-)ve	(-)ve	S20	(+)ve	(-)ve
W50	(+)ve	(-)ve	S23	(-)ve	(-)ve
W55	(-)ve	(-)ve	S31	(-)ve	(+)ve
W56	(-)ve	(-)ve	S33	(-)ve	(+)ve
S48	(-)ve	(+)ve	S49	(-)ve	(+)ve
S51	(-)ve	(+)ve	S56	(-)ve	(+)ve
S65	(+)ve	(-)ve	P0	(-)ve	(+)ve
P7	(+)ve	(+)ve	P10	(-)ve	(+)ve
P11	(-)ve	(+)ve	P12	(+)ve	(+)ve
P20	(-)ve	(-)ve	P21	(-)ve	(-)ve
P22	(-)ve	(-)ve	P24	(-)ve	(-)ve
P26	(-)ve	(+)ve	P27	(-)ve	(+)ve
P29	(-)ve	(+)ve	P34	(-)ve	(+)ve
P35	(-)ve	(-)ve	P37	(-)ve	(+)ve

P41	(-)ve	(+)ve	P43	(-)ve	(-)ve
P45	(-)ve	(+)ve	P50	(-)ve	(+)ve
P52	(-)ve	(+)ve	P54	(+)ve	(+)ve
P56	(-)ve	(+)ve	P58	(-)ve	(+)ve
P59	(-)ve	(-)ve	P60	(-)ve	(+)ve
A1	(-)ve	(-)ve	A2	(-)ve	(-)ve
A6	(-)ve	(-)ve	A7	(-)ve	(-)ve
A8	(+)ve	(-)ve	A9	(+)ve	(-)ve
A10	(-)ve	(-)ve	A12	(+)ve	(-)ve
A13	(-)ve	(-)ve	A14	(+)ve	(-)ve
A18	(+)ve	(-)ve	A19	(+)ve	(-)ve
A20	(+)ve	(-)ve	A21	(+)ve	(-)ve
A23	(+)ve	(-)ve	A26	(-)ve	(-)ve
A27	(+)ve	(-)ve	A28	(+)ve	(-)ve
A30	(+)ve	(-)ve	A31	(-)ve	(-)ve
SF-V(5)	(+)ve	(-)ve	SF-6	(-)ve	(-)ve
S74	(+)ve	(-)ve	S76	(+)ve	(+)ve
S78	(+)ve	(+)ve	S79	(-)ve	(+)ve
S80	(+)ve	(+)ve	S82	(-)ve	(-)ve
S84	(-)ve	(+)ve	S85	(-)ve	(-)ve
W81	(-)ve	(+)ve	W84	(+)ve	(+)ve
W85	(+)ve	(+)ve	W86	(-)ve	(+)ve
W88	(-)ve	(+)ve	W89	(-)ve	(-)ve
W90	(-)ve	(-)ve	W91	(-)ve	(+)ve
W92	(-)ve	(+)ve	W93	(-)ve	(-)ve

3.13 Antibiotic Resistance Pattern of *E. coli*

Antibiotic susceptibility test of 150 isolates was performed against 10 antibiotics.

Figure 3.34: Clear zones in antibiogram following Kirby and Bauer method (Bauer *et al.*, 1966).

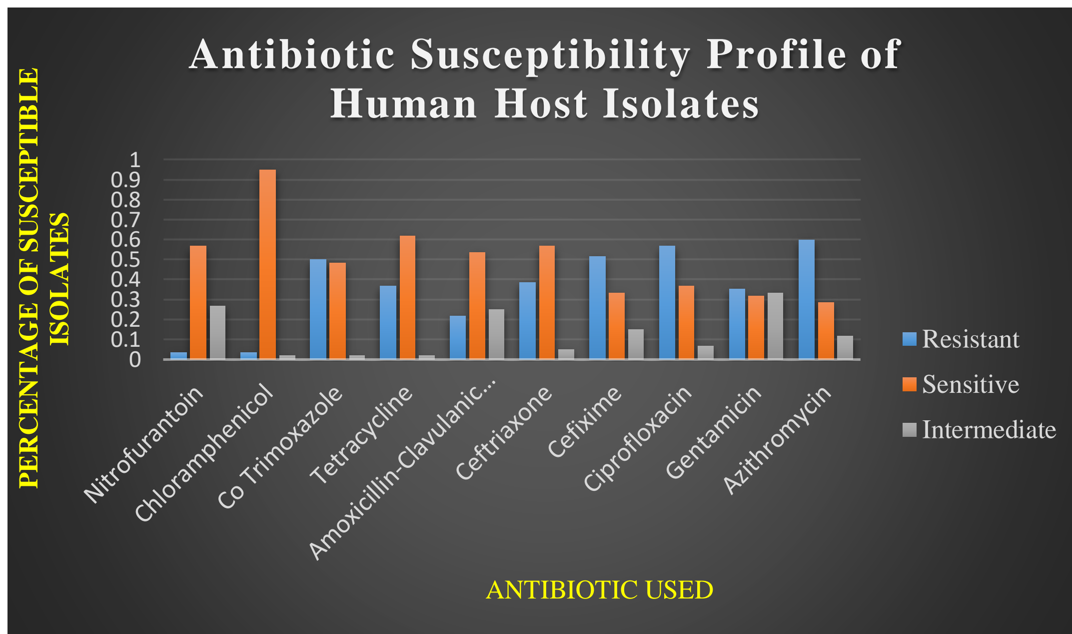


Figure 3.35: Graphical representation of the susceptibility profile of *E. coli* isolated from human.

All of the Human host *E. coli* isolates (n=60) were tested for their antibiotic susceptibility to 10 different antibiotics. The order of resistance obtained in this study includes Nitrofurantoin (3.33%), Chloramphenicol (3.33%), Co-Trimoxazole (50%), Tetracycline (37%), Amoxicillin-Clavulanic acid (21.67%), Ceftriaxone (38.33%), Cefixime (51.67%), Ciprofloxacin (56.66%), Gentamicin

(35%), Azithromycin (60%). *E. coli* ATCC 25922 was used as a positive control of sensitivity for antibiogram.

Resistance percentage of these isolates for the 10 different antibiotics and comparison of resistant, intermediate and sensitive strains are given in figure 3.35.

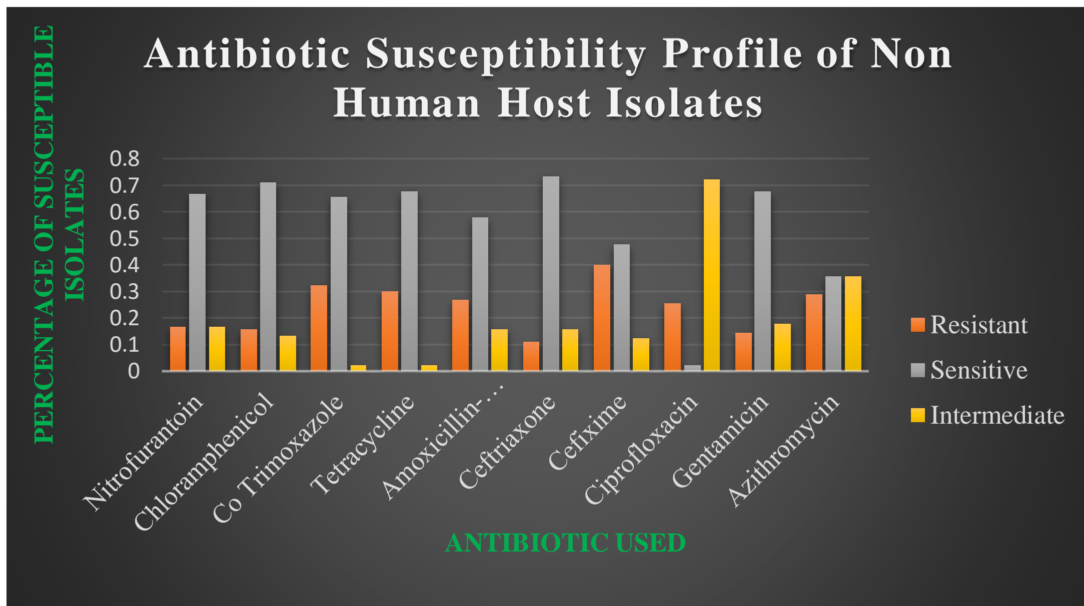


Figure 3.36: Graphical representation of the susceptibility profile of *E. coli* isolated from non-human isolates to different antibiotics.

All of the *E.coli* isolated from non-human sources (n=90) were tested for their antibiotic susceptibility to 10 different antibiotics. The resistance percentages obtained includes Nitrofurantoin (16.67%), Chloramphenicol (15.56%), Co-Trimoxazole (32.22%), Tetracycline (30%), Amoxicillin-Clavulanic acid (26.67%), Ceftriaxone (11.11%), Cefixime (40%), Ciprofloxacin (25.56%), Gentamicin (14.45%), Azithromycin (28.89%) (Figure 3.36).

3.13.1 Overall sensitivity and resistance profile of isolated *E. coli* to different antibiotics

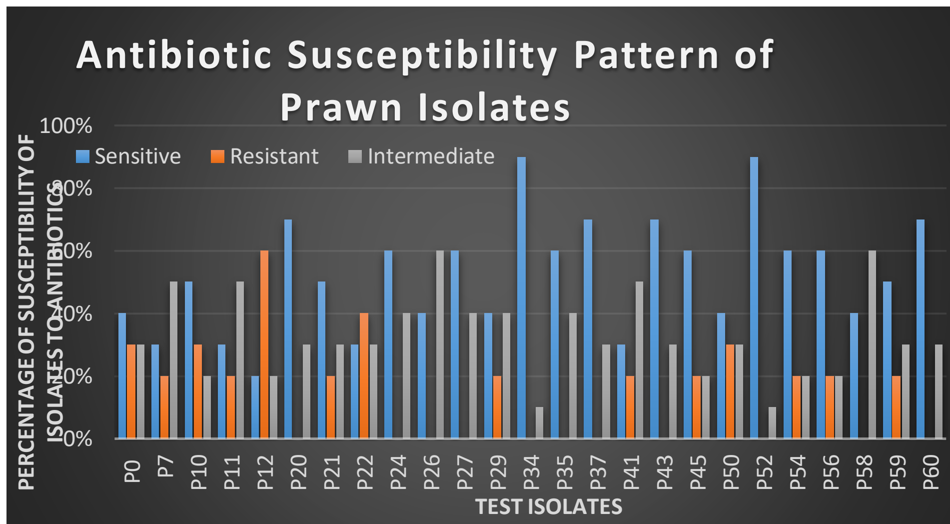


Figure 3.37: Graphical representation of antibiotic susceptibility pattern of prawn *E. coli* isolates.

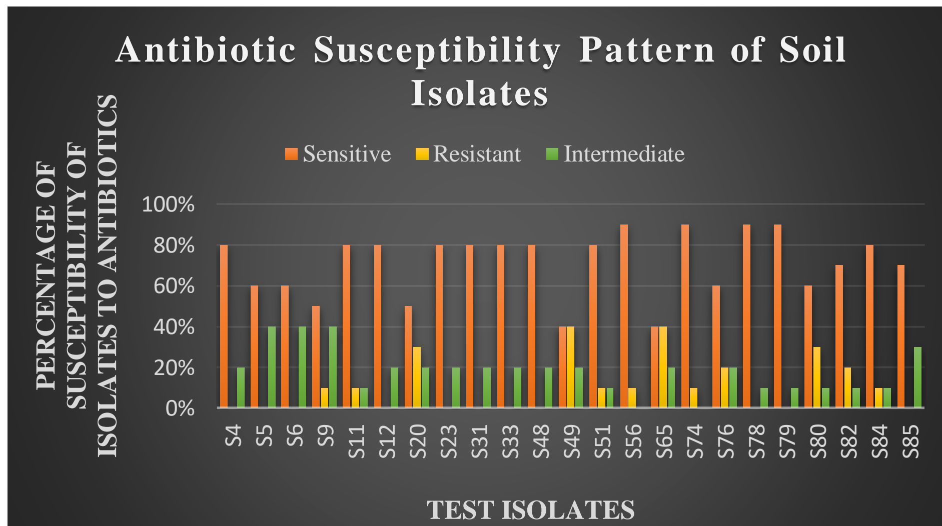


Figure 3.38: Graphical representation of antibiotic susceptibility pattern of soil *E. coli* Isolates.

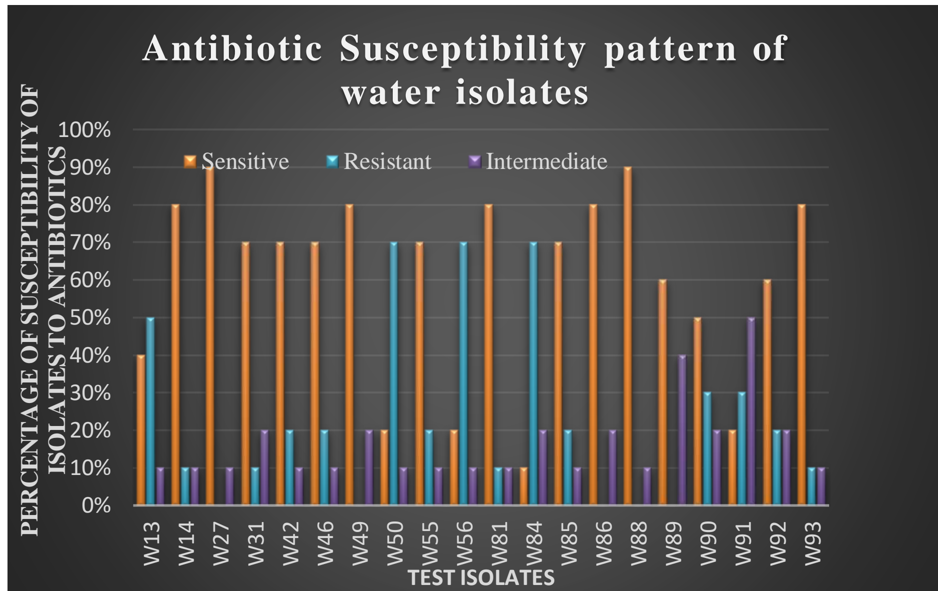


Figure 3.39: Graphical representation of antibiotic susceptibility pattern of water *E. coli* isolates.

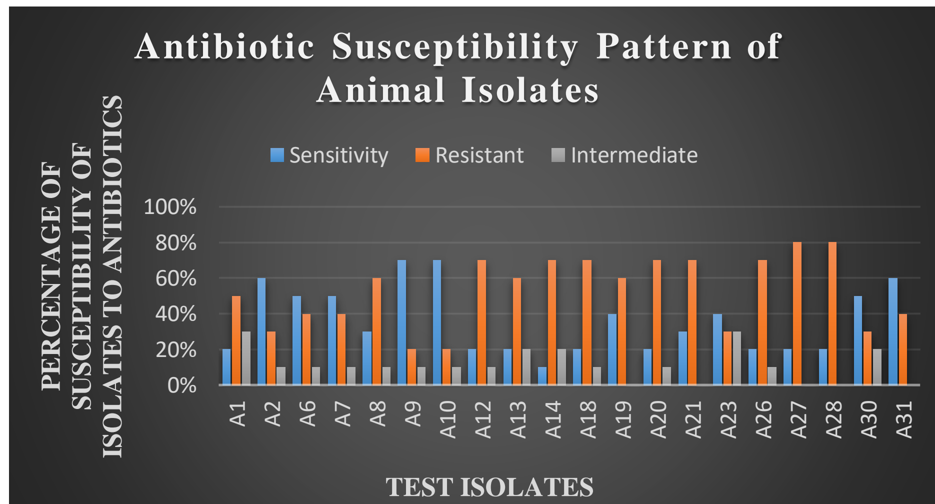


Figure 3.40: Graphical representation of antibiotic susceptibility pattern of animal *E. coli* isolates.

Figure 3.37, figure 3.38, figure 3.39 and figure 3.40 show the antibiotic susceptibility pattern of different types of Non-Human host *E.coli* isolates such as prawn, soil, water and animal host respectively.

3.13.2 Comparison of antibiotic resistance pattern of *E. coli* isolated from Human and Non-Human sources

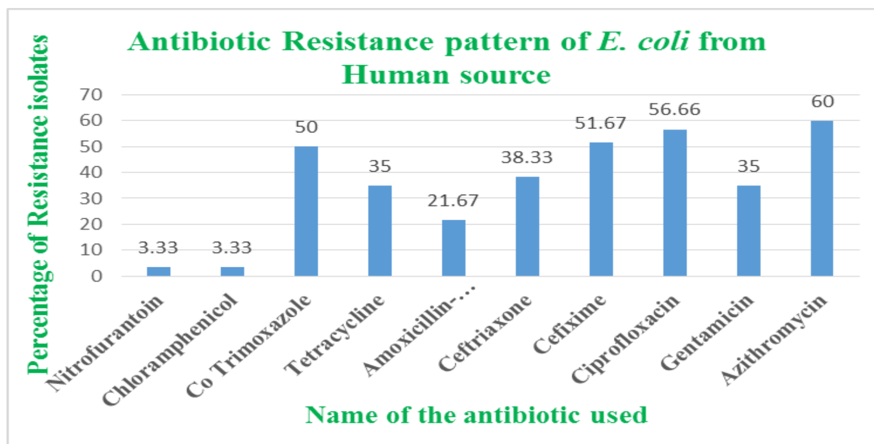


Figure 3.41: Graphical representation of antibiotic resistance pattern of *E. coli* isolated from Human Host.

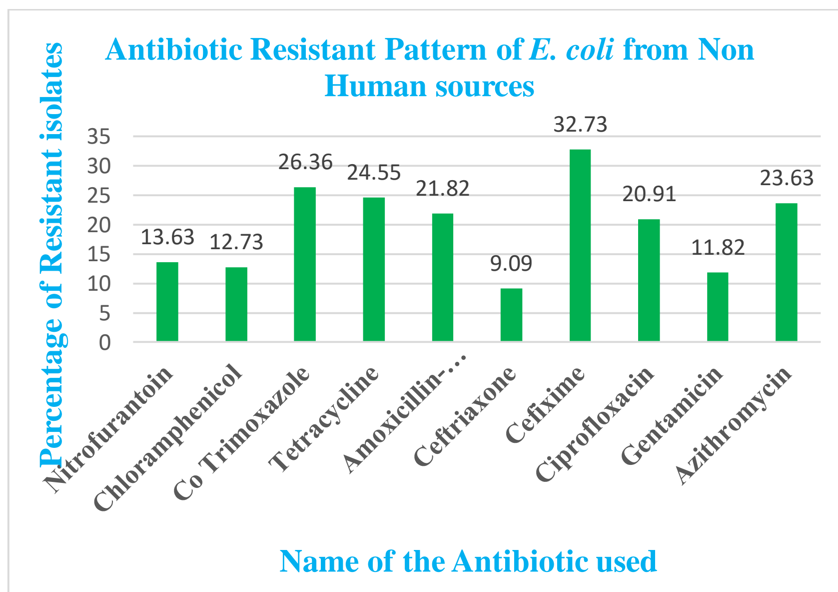


Figure 3.42: Graphical representation of antibiotic resistance pattern of *E. coli* isolates from Non-Human sources.

Figure 3.41 shows that *E. coli* isolated from human showed highest percentage of resistance to Azithromycin (60%) and lowest percentage of resistance to two groups of antibiotics viz.

Nitrofurantoin and Chloramphenicol (3.33%) whereas figure 3.42 shows that non-human *E. coli* isolates were predominantly resistant to Cefixime (32.73%) and showed lowest percentage of antibiotic resistance to Ceftriaxone (9.09%). On the contrary, human isolates showed 51.67% and 38.33% resistance to Cefixime and Ceftriaxone, respectively whereas non-human *E. coli* isolates showed 23.63% resistance to Azithromycin and 12.63% resistance to Nitrofurantoin and 12.73% resistance to Chloramphenicol. Therefore, the percentages of resistance of human *E. coli* were greater for most of the tested antibiotics when compared to non-human *E. coli*.

3.13.3 Plasmid mediated antibiotic resistance pattern of environmental *E. coli* isolates

It was postulated that plasmids are harboring some of the antibiotic resistance genes in their bacterial hosts. It was seen that 88 (58.67%, n=150) *E. coli* isolates carried plasmids and of these 71 (80.68%, n=88) were resistant to 10 different types of antibiotics (Table 3.16).

Table 3.16 Plasmid profiling and percentage of antibiotic resistance among environmental *E. coli* isolates

Sample ID	Number of plasmids present	Percentage of antibiotic resistance
25733	2	20
496	3	80
s585	1	40
464	4	40
47990	2	50
47697	2	0
9	3	60
14	6	60
689	1	40
685	4	0

647	4	20
13	3	50
68	3	80
595	2	10
914	1	70
564	1	50
779	1	80
380	1	20
394	4	40
9312	3	70
168	1	0
343	1	60
407	1	10
534	1	10
823	2	20
47770	4	20
O64	3	10
397	7	50
544	7	10
3491	1	50
S31	1	0
S33	1	0

S48	1	0
S49	1	40
S51	1	10
S56	1	10
S65	1	40
P0	1	30
P7	3	20
P10	1	30
P11	1	20
P12	1	60
P26	1	0
P27	1	0
P29	1	20
P34	5	0
ME9	1	20
O38	1	30
425	1	10
P37	1	0
P41	1	20
P45	1	20
P50	1	30
P52	1	0

P54	1	20
P56	1	20
P58	2	0
P60	1	0
938	6	20
8996	2	40
774	3	60
521	2	10
170	2	70
30955	1	60
265	3	60
755	6	70
657	7	10
340	3	70
O75	4	90
26170	2	50
180	2	30
O32	2	50
C/O Saidul	3	50
940	2	50
588	2	70
938	7	20

W81	1	10
W84	5	70
W85	1	20
W86	2	0
W88	3	0
W92	3	20
W93	1	10
S76	3	20
S78	1	0
S79	2	0
S80	1	30
S84	1	10

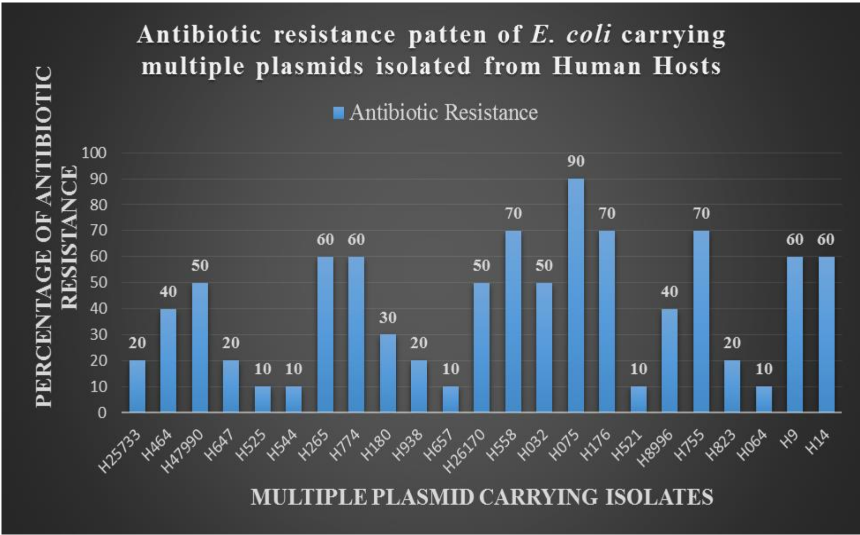


Figure 3.43: Graphical representation of antibiotic resistance pattern of Human *E. coli* isolates carrying multiple plasmids.

Figure 3.43 shows that among 23 multiple plasmid carrying isolates, one isolate named 075 was resistant to nine different antibiotics, three isolates were resistant to seven different antibiotics, four were resistant to six antibiotics, three were resistant to five antibiotics, two were resistant to four antibiotics, one was resistant to three antibiotics, four were resistant to two different antibiotics and five isolates were resistant to only one antibiotic, 78.26% (n=18) of the multiple plasmid carrying isolates were Multidrug resistant (MDR), that is, resistant to more than one type of antibiotics.

3.14 Correlation of antibiotic resistance pattern between Human and Non-Human *E. coli* isolates

Statistical analysis was performed on the antibiotic resistance patterns of human and non-human *E. coli* isolates to determine if differences were statistically significant.

Table 3.17 Comparison of Resistance diversity of human and non-human *E. coli* isolates using Shannon Diversity Index

Site	Resistant human host isolates	Resistant non-human host isolates
Total	213	217
Richness	10	10
H	2.11	2.23
Variance	0.001039	0.019278
Confidence Intervel=	0.045585085	0.196356818
t value=	0.86	
df=	241.28	

Critical value=	1.969856213	
p=	0.390643728	

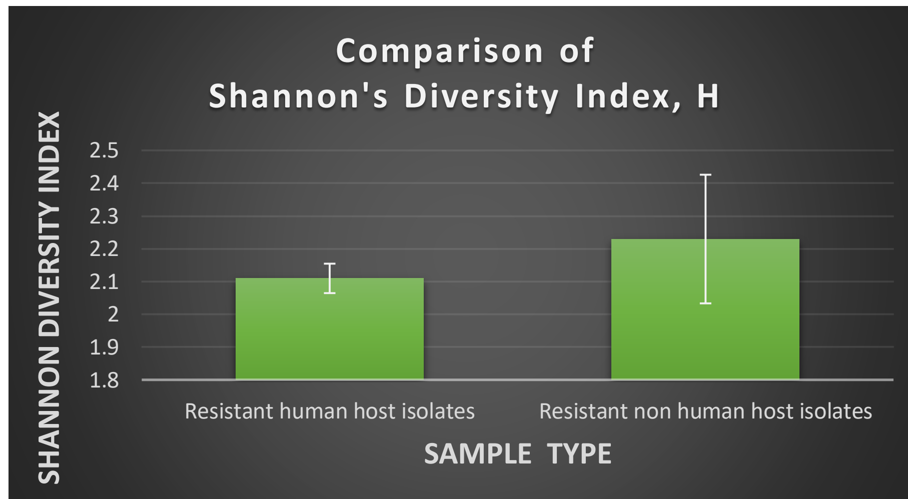


Figure 3.44: Comparison of Antibiotic Resistance pattern between Human and Non-Human *E. coli.*

According to Table 3.17 and figure 3.44, the indices for *E. coli* isolated from human was 2.11 and for non-human host was 2.23. These differences in diversity were not statistically significant ($p>0.05$). The value of t is within the critical value which indicated that similarity existed between the antibiotic resistance pattern of human and non-human *E. coli* isolates.

Table 3.18 Comparison of antibiotic resistance diversity of human and animal *E. coli* using Shannon Diversity Index

Site	Resistant Human isolates	Resistant animal isolates
Total	213	105
Richness	10	10
H	2.11	1.93

Variance	0.001039	0.004569
Confidence intervel	0.045585085	0.095592887
t value	0.58	
df	233.43	

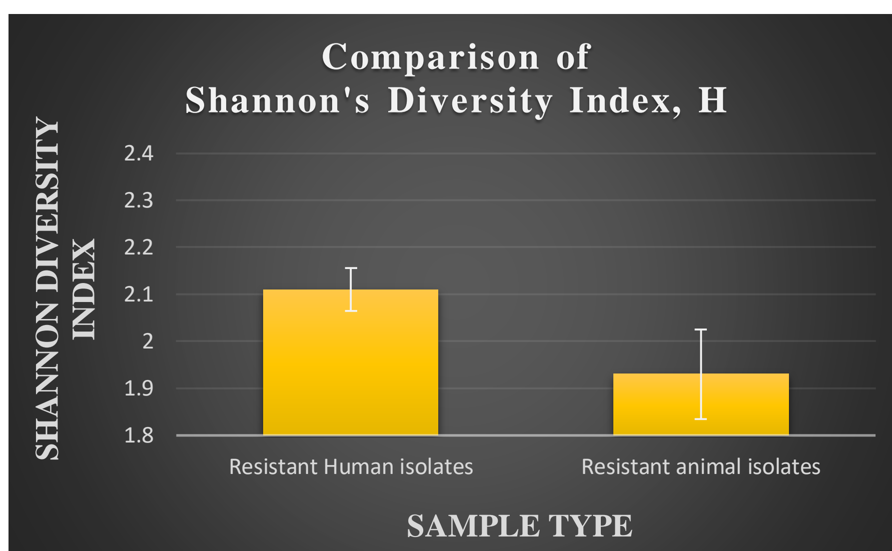


Figure 3.45: Shannon Diversity indices of antibiotic resistance profile of human and animal *E. coli.*

According to Table 3.18 and figure 3.45 the indices for human isolates was 2.11 and for animal isolates was 1.93. These differences in diversity were not statistically significant ($p>0.05$). The value of t, is within the critical value which indicated that similarity existed between the antibiotic resistance patterns of human and animal isolates. The bars in figure 3.45 indicate the values of Shannon Diversity indices (H) for resistant *E. coli* isolated from human and those isolated from animal, respectively.

3.15 Molecular profiling of tested *E. coli*

The overall molecular profiling of *E. coli* isolated from different environmental sources is summarized in Table 3.19.

Table 3.19 Molecular profiling of *E. coli* bacteria isolated from different sources of environment

Sample No.	PhyloGroup	Presence of different virulence genes	Presence of *eae* gene	Presence of *intl-1*	Presence of Plasmid
25733	D	(-)ve	(-)ve	(-)ve	(+)ve
47990	B1	(-)ve	(-)ve	(-)ve	(+)ve
C/O Saidul	B1	(-)ve	(-)ve	(+)ve	(+)ve
O38	B2	(-)ve	(-)ve	(-)ve	(+)ve
588	B1	(-)ve	(-)ve	(-)ve	(+)ve
774	A	(-)ve	(-)ve	(-)ve	(+)ve
O32	B1	(-)ve	(-)ve	(-)ve	(+)ve
3491	D	(-)ve	(-)ve	(-)ve	(+)ve
585	D	(-)ve	(-)ve	(+)ve	(+)ve
647	D	(-)ve	(-)ve	(-)ve	(+)ve
689	B1	(-)ve	(-)ve	(-)ve	(-)ve
534	B1	*elt*(+)ve	(-)ve	(-)ve	(+)ve
595	A	(-)ve	(-)ve	(+)ve	(+)ve
265	B1	(-)ve	(-)ve	(-)ve	(+)ve
340	B1	(-)ve	(-)ve	(+)ve	(+)ve
521	B1	(-)ve	(-)ve	(-)ve	(+)ve
564	B1	(-)ve	(-)ve	(-)ve	(+)ve

168	B2	(-)ve	(-)ve	(-)ve	(+)ve
30987	B1	*eae and bfpA(+)ve*	(-)ve	(-)ve	(-)ve
180	D	(-)ve	(-)ve	(-)ve	(+)ve
8996	B1	(-)ve	(-)ve	(-)ve	(-)ve
47509	B1	(-)ve	(-)ve	(-)ve	(-)ve
940	B1	(-)ve	(-)ve	(+)ve	(+))ve
176	B1	*eae and bfpA(+)ve*	(-)ve	(-)ve	(+)ve
47508	B1	(-)ve	(-)ve	(-)ve	(-)ve
47770	D	(-)ve	(-)ve	(+)ve	(+) ve
26170	D	(-)ve	(-)ve	(-)ve	(+)ve
30955	D	(-)ve	(-)ve	(+)ve	(+)ve
47697	B2	(-)ve	(-)ve	(-)ve	(+)ve
ME3	B1	(-)ve	(-)ve	(-)ve	(-)ve
ME4	B1	(-)ve	(-)ve	(-)ve	(-)ve
ME5	B1	(-)ve	(-)ve	(+)ve	(-)ve
ME6	B1	(-)ve	(-)ve	(-)ve	(-)ve
ME7	B1	(-)ve	(-)ve	(-)ve	(-)ve
ME8	B1	(-)ve	(-)ve	(-)ve	(-)ve
ME9	A	(-)ve	(-)ve	(-)ve	(+)ve
ME10	B1	(-)ve	(-)ve	(-)ve	(-)ve
9	B1	(-)ve	(-)ve	(-)ve	(+)ve
13	B1	(-)ve	(-)ve	(+)ve	(+)ve

14	B1	(-)ve	(-)ve	(-)ve	(+)ve
68	F	(-)ve	(-)ve	(-)ve	(+)ve
755	F	(-)ve	(-)ve	(+)ve	(+)ve
397	B1	(-)ve	(-)ve	(+)ve	(+)ve
779	B1	(-)ve	(-)ve	(-)ve	(+)ve
407	F	(-)ve	(-)ve	(-)ve	(+)ve
496	B1	(-)ve	(-)ve	(+)ve	(+)ve
914	D	(-)ve	(-)ve	(+)ve	(+)ve
O75	D	(-)ve	(-)ve	(-)ve	(+)ve
343	F	(-)ve	(-)ve	(+)ve	(+)ve
425	A	(-)ve	(-)ve	(-)ve	(+)ve
380	A	(-)ve	(-)ve	(-)ve	(+)ve
938	E	(-)ve	(-)ve	(-)ve	(+)ve
394	D	(-)ve	(-)ve	(+)ve	(+)ve
O64	D	(-)ve	(-)ve	(-)ve	(+)ve
464	D	(-)ve	(-)ve	(-)ve	(+)ve
9312	D	(-)ve	(-)ve	(+)ve	(+)ve
544	B1	(-)ve	(-)ve	(-)ve	(+)ve
657	D	(-)ve	(-)ve	(-)ve	(+)ve
823	B1	(-)ve	(-)ve	(-)ve	(+)ve
685	B1	(-)ve	(-)ve	(-)ve	(+)ve
S4	A	(-)ve	(-)ve	(-)ve	(-)ve

S5	B1	(-)ve	(-)ve	(-)ve	(-)ve
S6	B1	(-)ve	(-)ve	(-)ve	(-)ve
S9	B1	(-)ve	(-)ve	(-)ve	(-)ve
S11	B1	(-)ve	(-)ve	(+)ve	(-)ve
S12	B1	(-)ve	(-)ve	(+)ve	(-)ve
S20	B1	(-)ve	(-)ve	(+)ve	(-)ve
S23	B1	(-)ve	(-)ve	(-)ve	(-)ve
S31	A	(-)ve	(-)ve	(-)ve	(+)ve
S33	B1	(-)ve	(-)ve	(-)ve	(+)ve
S48	B1	(-)ve	(-)ve	(-)ve	(+)ve
S49	B1	(-)ve	(-)ve	(-)ve	(+)ve
S51	B1	(-)ve	(-)ve	(-)ve	(+)ve
S56	B1	(-)ve	(-)ve	(-)ve	(+)ve
S65	B1	(-)ve	(-)ve	(+)ve	(-)ve
P0	A	(-)ve	(-)ve	(-)ve	(+)ve
P7	B1	(-)ve	(-)ve	(+)ve	(-)ve
P10	B1	(-)ve	(-)ve	(-)ve	(+)ve
P11	B1	(-)ve	(-)ve	(-)ve	(+)ve
P12	B1	(-)ve	(-)ve	(+)ve	(+)ve
P20	A	(-)ve	(-)ve	(-)ve	(-)ve
P21	B1	(-)ve	(-)ve	(-)ve	(-)ve
P22	B1	(-)ve	(-)ve	(-)ve	(-)ve

P24	B1	(-)ve	(-)ve	(-)ve	(-)ve
P26	B1	(-)ve	(-)ve	(-)ve	(+)ve
P27	B1	(-)ve	(-)ve	(-)ve	(+)ve
P29	B1	(-)ve	(-)ve	(-)ve	(+)ve
P34	C	(-)ve	(-)ve	(-)ve	(+)ve
P35	B1	(-)ve	(-)ve	(-)ve	(-)ve
P37	B1	(-)ve	(-)ve	(-)ve	(+)ve
P41	B1	(-)ve	(-)ve	(-)ve	(+)ve
P43	B1	(-)ve	(-)ve	(-)ve	(-)ve
P45	B1	(-)ve	(-)ve	(-)ve	(+)ve
P50	A	(-)ve	(-)ve	(-)ve	(+)ve
P52	B1	(-)ve	(-)ve	(-)ve	(+)ve
P54	B1	(-)ve	(-)ve	(+)ve	(+)ve
P56	B1	(-)ve	(-)ve	(-)ve	(+)ve
P58	B1	(-)ve	(-)ve	(-)ve	(+)ve
P59	B1	(-)ve	(-)ve	(-)ve	(-)ve
P60	B1	(-)ve	(-)ve	(-)ve	(+)ve
W13	B1	(-)ve	(-)ve	(-)ve	(-)ve
W14	A	(-)ve	(-)ve	(-)ve	(-)ve
W27	B1	(-)ve	(-)ve	(-)ve	(-)ve
W31	A	(-)ve	(-)ve	(-)ve	(-)ve
W42	A	(-)ve	(-)ve	(-)ve	(-)ve

W46	B1	(-)ve	(-)ve	(-)ve	(-)ve
W49	A	(-)ve	(-)ve	(-)ve	(-)ve
W50	B1	(-)ve	(-)ve	(+)ve	(-)ve
W55	B1	(-)ve	(-)ve	(-)ve	(-)ve
W56	B1	(-)ve	(-)ve	(-)ve	(-)ve
A1	B1	(-)ve	(-)ve	(-)ve	(-)ve
A2	B1	(-)ve	(-)ve	(-)ve	(-)ve
A6	A	(-)ve	(-)ve	(-)ve	(-)ve
A7	B1	(-)ve	(-)ve	(-)ve	(-)ve
A8	E	(-)ve	(-)ve	(+)ve	(-)ve
A9	B1	(-)ve	(-)ve	(+)ve	(-)ve
A10	E	(-)ve	(-)ve	(-)ve	(-)ve
A12	C	(-)ve	(-)ve	(+)ve	(-)ve
A13	C	(-)ve	(-)ve	(-)ve	(-)ve
A14	C	(-)ve	(-)ve	(+)ve	(-)ve
A18	C	(-)ve	(-)ve	(+)ve	(-)ve
A19	C	(-)ve	(-)ve	(+)ve	(-)ve
A20	C	(-)ve	(-)ve	(+)ve	(-)ve
A21	C	(-)ve	(-)ve	(+)ve	(-)ve
A23	C	(-)ve	(-)ve	(+)ve	(-)ve
A26	C	(-)ve	(-)ve	(-)ve	(-)ve
A27	E	(-)ve	(-)ve	(+)ve	(-)ve

A28	C	(-)ve	(-)ve	(+)ve	(-)ve
A30	C	(-)ve	(-)ve	(+)ve	(-)ve
A31	C	(-)ve	(-)ve	(-)ve	(-)ve
SF-V(5)	A	(-)ve	(-)ve	(+)ve	(-)ve
SF-6	B1	(-)ve	(-)ve	(-)ve	(-)ve
S74	B1	(-)ve	(-)ve	(+)ve	(-)ve
S76	B1	(-)ve	(-)ve	(+)ve	(+)ve
S78	B1	(-)ve	(-)ve	(+)ve	(+)ve
S79	B1	(-)ve	(-)ve	(-)ve	(+)ve
S80	B1	(-)ve	(-)ve	(+)ve	(+)ve
S82	B1	(-)ve	(-)ve	(-)ve	(-)ve
S84	B1	(-)ve	(-)ve	(-)ve	(+)ve
S85	B1	(-)ve	(-)ve	(-)ve	(-)ve
W81	B1	(-)ve	(-)ve	(-)ve	(+)ve
W84	B1	(-)ve	(-)ve	(+)ve	(+)ve
W85	A	(-)ve	(-)ve	(+)ve	(+)ve
W86	A	(-)ve	(-)ve	(-)ve	(+)ve
W88	B1	(-)ve	(-)ve	(-)ve	(+)ve
W89	A	(-)ve	(-)ve	(-)ve	(-)ve
W90	A	(-)ve	(-)ve	(-)ve	(-)ve
W91	C	(-)ve	(-)ve	(+)ve	(-)ve
W92	A	(-)ve	(-)ve	(+)ve	(+)ve

W93	B1	(-)ve	(-)ve	(-)ve	(+)ve
A16	A	---	---	---	---
A22	A	---	---	---	---
A24	A	---	---	---	---
A32	A	---	---	---	---
A34	A	---	---	---	---
A35	A	---	---	---	---
A36	A	---	---	---	---
A37	A	---	---	---	---
A39	A	---	---	---	---
A40	A	---	---	---	---
A42	A	---	---	---	---
A43	A	---	---	---	---
A44	A	---	---	---	---
A46	D	---	---	---	---
A47	A	---	---	---	---
A48	A	---	---	---	---
A49	A	---	---	---	---
A50	A	---	---	---	---
A51	A	---	---	---	---
A52	A	---	---	---	---
A53	A	---	---	---	---

A54	A	---	---	---	---
A56	D	---	---	---	---
A57	A	---	---	---	---
A58	A	---	---	---	---
A59	A	---	---	---	---
A62	A	---	---	---	---
A63	A	---	---	---	---
A64	A	---	---	---	---
A68	A	---	---	---	---
A69	E	---	---	---	---
A70	E	---	---	---	---
A71	C	---	---	---	---
A76	A	---	---	---	---

Discussion

4.1 Discussion

Escherichia coli, a bacterium widely distributed in the environment has long been used as an indicator of fecal pollution. According to Bekal *et al*. (2003) the bacterium *E. coli* is a normal inhabitant of the intestinal tract of human and warm-blooded animals. Despite being usually harmless, various *E. coli* strains have acquired genetic determinants (virulence genes) rendering them pathogenic for both humans and animals. *E. coli* may be present in different environments both as harmful and harmless forms. In this study, the aim was to determine whether the definitions of 'commensal', 'environmental' and 'pathogenic' still mean different categories of *E. coli* and to what extent the different types have moved into different environments and adapted there. As a result, the present study was designed to determine the distribution of the different phylogroups in the environment in order to identify the source of *E. coli* in different environment. It was also designed to investigate the pathotypes, antibiotic resistance pattern, and mediators of resistance among *E. coli* isolated from different environments.

The isolates were obtained from different environments such as water, soil, prawn, animal, street foods and from human. Samples were initially enriched in specific medium (BGLB). Standard biochemical tests (e.g. KIA, Indole, MR_VP test ability to utilize citrate etc.) were used in the initial screen to identify *E. coli.* Most of the isolates passed as *E.coli* in the initial screen as they also produced colonies with green metallic sheen on EMB agar and typical pink colonies on MacConkey agar media. Molecular identification was done by using two *E. coli* specific primer pairs considered as marker genes (*usp*A and *uid*A) where *usp*A amplifies a universal stress protein specific region and *uid*A gene encodes β - glucuronidase enzyme. The phenotypic expression of the latter gene gives a positive result in the 4-Methylumbelliferyl-β-D-Glucuronidase test (MUG test). The detection of *uid*A is important as its presence ensures that the test isolates were fecal in origin. All of the 184 isolates were positive for *uid*A and *usp*A genes. The co-existence of these two genes confirmed the isolates to be *Escherichia coli* without the need of any sequencing which would be more time consuming and costly. In another study (Godambe *et al*., 2017) it was estimated that 149 isolates (79%) from the food samples tested were positive for both of these marker genes. They found that 43 (22%) of the *E. coli* isolates were positive for any of the two primer sets [*uid*A (9%) and flanking region of *usp*A (13%)]. The *uid*A gene has been shown to be very specific to *E. coli*; however primers specific to this region also amplify few species of *Shigella*

(Bej *et al*., 1991). However the combination of these two genes were found to be very specific to *E. coli* identification and in this study all of the tested isolates were found to contain both of these genes which was in contrast to the findings of Godambe *et al*. (2017).

ARDRA (Amplified Ribosomal DNA Restriction Analysis) was used to type 50 randomly selected isolates. One set of previously described PCR primers was used (Lu *et al*., 2000). PCR products from different isolates of the same bacteria were found to have the same restriction pattern indicating that the 16s rDNA sequence was conserved. These results formed one of the basis for identification of bacteria in this study. This method requires only one day to complete. Conventional methods for detection and identification of bacterial pathogens require at least 2 days. The universal PCR method will provide physicians with results at least 1 day earlier than conventional methods. Although the cost of using the universal primer PCR for diagnosis is higher than the conventional methods, the universal primer PCR coupled with restriction enzyme analysis can rapidly detect and identify pathogens so that the unnecessary use of broad-spectrum antibiotic therapies can be minimized. This will be useful in patient care for diagnosis. Rådström *et al*., (1994) described the use of a semi-nested PCR method with genes or species specific primers to detect and identify *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae* and 24 different species of bacteria. All of these studies used multiple sets of PCR primers to detect or identify bacteria. In this study, we detected bacteria with only one set of PCR primers and used restriction enzyme analysis rather than species-specific probes or sequencing for identifying bacteria. In a similar study (Ventura *et al*., 2000) at which PCR-ARDRA was performed using a set of four restriction enzymes, able to differentiate fourteen species of *Lactobacillus*. They described the PCR-ARDRA procedure as a reliable and rapid method for identifying *Lactobacillus* species from intestinal and vaginal microflora at species and subspecies level. According to (Kong *et al*., 2011) ARDRA provides an accurate, rapid and definitive approach towards the identification of the species level in the genus *Acinetobacter*. They applied ARDRA to confirm the identity of *A. baumannii* strains as well as to differentiate between the subspecies (Vaneechoutte *et al*., 2001). In our study this PCR-RFLP work showed that there was no polymorphism detected among the test isolates. Clermont and colleagues (Clermont *et al.,* 2013) estimated that over 95% of *E. coli* isolates can be correctly assigned to a phylogroups (A, B1, B2, C, D, E, F). This study demonstrated that this extended quadruplex PCR assay of Clermont phylotyping method offered some advantages over the previous triplex PCR method (Clermont *et*

al., 2000). With the accumulation of whole genome sequence data Clermont and colleagues declared that some strains belong to a group intermediate between the F and B2 phylogroups, designated as phylogroup G (Clermont *et al*., 2019). The presence of these genetic markers helped to detect the origin of the test isolates without considering the matter of their collection sources. In our study we observed predominance (46.74%) of phylogroup B1 [*arp*A (+)ve and TspE4C2 (+)ve] among the test isolates. This was followed by phylogroup A (28.26%). Among non-human (animal, prawn, street food, soil and water) *E.coli* isolates, phylogroup B1 covered 45.16% whereas in human host isolates this value remained at 50%. Among non-human isolates, 37% of the isolates belonged to phylogroup A whereas 10% of human *E. coli* were phylogroup A. This finding was unexpected considering the fact that Group A generally contains human commensal strains and group B1 represents environmental *E. coli* strains (Escobar-Páramo *et al.,* 2006; Walk *et al*., 2007). However in tropical areas both groups A and B1 have been found to be prevalent among human strains (Escobar-Páramo *et al.,* 2004). Strains isolated from animals have been reported to fall mostly into group B1 (Ishii *et al*., 2007; Carlos *et al*., 2010) suggesting an association between phylogenetic groups and host species. In another study by Coura and colleages (2015) where B1 was found as the main phylogroup of *E. coli* isolated from domestic animals followed by phylogroup A. Müştak and colleagues (2015) declared that group B1 and C were the predominant groups with 45% and 37% occurrence, respectively, in mastitis isolates. They defined phylogroup C as that which included strains closely related but distinct from group B1 (Müştak *et al*., 2015). These studies were, however, in contrast with our study where we found phylogroup A (55.56%) as the dominating group among animal host isolates. In case of phylogroup C, the finding was similar to that of Müştak *et al.* (2015). We isolated 7.41% B1 strains from animal hosts all of which were isolated from herbivorous mammals such as cow and sheep. This phylogroup was totally absent among the isolates collected from birds where the dominating group was phylogroup C (52.38%), which is related but distinct from B1. This finding was in contrast to the findings of Carlos *et al*. (2010) where phylogroup A (76.92%) was the dominating group among the chicken *E. coli* isolates.

Carlos *et al.* (2010) also observed that phylogroup B1 was prevalent among the herbivorous hosts. Within the same host species, geography, climate, diet, body mass, sex, age, hygiene level, inter alia may be associated with the distribution of phylogroups (Gordon and Cowling, 2003; Escobar-Páramo *et al*., 2006). In our study 50% of 60 human isolates were grouped into phylogroup B1

whereas a similar proportion was found in a study by Stoppe *et al*. (2017), who found 48.3% of 116 human host isolates to belong to group A. In the same study, 44% of the isolates from waste water was grouped under phylogroup A. In our study 50% of the isolates from both tap and surface water was grouped under phylogroup A. Our findings were, therefore, very similar to the findings of Stoppe *et al.* (2017).

A shift from group A to B2 as the most frequent *E. coli* phylogroup in human was observed by Tenaillon *et al*. (2010) in France in 1980 and 2000. Group A was found to be the most common phylogroup in Africa (Mali and Benin), Asia (Pakistan), Europe (Croatia) and South America (French, Guiana, Colombia and Bolvia) (Tenaillon *et al*., 2010). In our study, *E. coli* isolated from animal and water mostly belonged to group A, isolates from human, prawn and soil were predominantly B1. Walk *et al*. (2007) demonstrated that the majority of the *E. coli* strains that are able to persist in the environment belong to the B1 phylogenetic group. Some authors have suggested group A to be the best adapted to different environments (Skurnik *et al*., 2008; Anastasi *et al*., 2012). In some countries in Asia, however, phylogroup prevalence has shifted from A (Kyoto, Japan and Seoul, Korea) (Kanamaru *et al*., 2006; Lee *et al.,* 2010) to B2 (Beijing, China and Tokyo, Japan) or D (Jeonnam province, Korea) (Unno *et al*., 2009). However, this is not the case in Bangladesh as observed in our study. In addition to phylogroup B1 and A, we found group D, C, E, F and B2 among the 184 isolates at the rate of 9.78%, 8.15%, 3.26%, 2.17% and 1.63%, respectively. In case of 60 human host isolates, phylogroups B2 and D comprised 5% (n=3) and 26.67% (n=16) respectively of the total human isolates, where in the findings of Stoppe *et al.* (2017) these two groups were as 16.4% (n=19) and 30.2% (n=35) among 116 human host isolates. We also isolated phylogroup E and F at the rates of 1.67% (n=1) and 6.67%, (n=4) respectively, from human but Stoppe *et al.* (2017) did not find any C, E or F phylogroup either from human or from waste water samples, a finding which was in contrast to ours. In this study among *E. coli* isolated from water 5% of the isolates belonged to phylogroup C.

In a recent study (Son *et al*., 2009), the genetic diversity of *E. coli* strains isolated from feces and farm environment were evaluated and results of that study revealed that group B1 (64%) and A (22%) were found to be the most prevalent groups, followed by group D (11%) and group B2 (4%). In our study, the phylogroup distribution pattern of *E. coli* among animal host (cow, goat, sheep, chicken, duck) isolates were in the order of A (55.55%), C (24.07%), E (9.26%), B1 (7.41%)

and D (3.7%), respectively, with group A being the prevalent phylogroup. Mateus *et al.* (2013) investigated canine isolates from pyometra and cystitis for virulence genotypes and prevalence of phylogroups. The prevalence of group B2 was found to be significantly high at 94% in pyometra and 39% in cystitis isolates. Henriques *et al*. (2014) also compared *E. coli* strains isolated from cows with clinical metritis and bitches with pyometra and found that predominant group was B2 (93%) after group A (7%). However, this could be attributed to the fact that the host was diseased in both cases. Müştak and colleagues isolated phylogroups C, E and F for the first time from acute bovine mastitis cases. Osugui *et al*. (2014) revealed that the most prevalent phylogroup was B2 (62%), D (18%) and B1 (16%) in UTIs of dogs and cats. In all of these cases, the hosts were afflicted and this could explain the predominance of pathogenic phylogroups. Bovine fecal isolates displayed a higher percentage of E (40%) than Soil Before Grazing (SBG) (7%) or pasture sample suggesting that phylogroup E was primarily bovine associated and less able to maintain populations in pasture soil (NandaKafle *et al*., 2017). Among soil *E. coli* isolates (n=23) only phylogroup A and B1 were present at the rate of 5% (n=1) and 95% (n=22) respectively.

Phylogroup B1 has been previously reported to be the predominant phylogroup isolated from feces of domesticated and wild animals as well as soil and surface water samples (Tenaillon *et al*., 2010; Bergholz *et al*., 2011; Berthe *et al*., 2013; Tymensen, *et al*., 2015; NandaKafle *et al*., 2017). The predominance of phylogroup B1 may be, in part, due to its dominance in hosts, enhanced survival of phylogroup B1 isolates in extra host environments due to a unique set of stress tolerance traits (Berthe *et al*., 2013; Méric *et al*., 2013; NandaKafle, 2017) or explained by the existence of some clades in B1 that appear more readily grow in sediment and/or soil habitats (Walk *et al*., 2007). Likewise, in our study phylogroup B1 was found as the dominating group isolated from soil samples. The diversity indices (Shannon Diversity Index) showed that greater diversity was found in *E. coli* strains isolated from human than in non-human hosts including animal, prawn, soil and water. However among *E. coli* of animal origin higher diversity was observed in chicken compared to cow. Surprisingly prawn and soil *E. coli* diversity index values were similar in this study. In a study by Morcatti *et al*. (2015) *E. coli* isolated from poultry showed greater diversity than water buffalo and cattle which was similar to our finding. In our study the highest Shannon diversity index was found in human (Carlos *et al*., 2010). The Shannon index obtained for chicken and cow were almost similar to that of Carlos *et al*. (2010). According to Morcatti *et al*. (2015) some

characteristics such as diet and gut morphology may account for the differences in the diversity indices in case of animal isolates of *E. coli*.

Pianka's index was used to evaluate the phylogroup overlap by using the formula: $O=\sum p_j p_k \surd^{\surd} \sum p_j^2 \sum p_k^2$, where p_j and p_k are phylogroup portions in the hosts j and k, respectively. The results were expressed as percentages (Pianka, 1973). Pianka's index of similarity was highest between prawn and water and least between human and non-human sources. Non-human sources included a variety of environments with their inherent features. Consequently, pairwise comparison between different environments were variable depending on what type of environments were compared. For example, non-human sources included animals that were either omnivorous or herbivorous and a comparison between cow (herbivore) and chicken (omnivore) showed moderate similarity index (0.58). Abiotic environments included water and soil in which case water receives run-off and disposals whereas soil may not be affected to the same extent. Hence similarity between soil and water was relatively higher (0.7) than that of cow and chicken (0.58). Prawns are filter feeders and may accumulate different pathotypes just as water can harbor different pathotypes from various sources; possibly because of this similarity index between prawn and water was high (0.84). On the other hand, human diet includes both animal and plant food whereas animals selected for this study were either omnivorous or herbivorous. Consequently, the similarity index for human and animal was moderately high (0.77). Overall, the similarity index for human and non-human was relatively low (0.48) owing to variations in the natures of the different environments selected.

Diarrheagenic *E. coli* strains are classified into five main categories according to the presence of different virulence genes. In order to determine the pathotype distribution in the test isolates, several PCR assays were performed. In the present study we used multiplex PCR by combining seven primer pairs specific for EAEC, EIEC, EHEC, EPEC and ETEC in a single reaction. Out of 150 *E. coli* 1.33% (n=2) belonged to EPEC and 0.67% (n=1) to ETEC. None of the isolate was EAEC, EIEC or EHEC pathotype. Salem *et al*. (2011) worked on *Escherichia coli* pathotypes also obtained similar results among 30 *E. coli* isolates, ETEC (76.6%, n=23) acted as the predominant pathotype. ETEC has also been isolated from surface water samples in Bangladesh suggesting that water is an important reservoir and mode of spread of ETEC infection (Begum *et al*., 2007). Most

E. coli diarrhea cases are caused by ETEC (233 million; 95% UI 154-380 million) worldwide; however, other pathovars also cause significant disease such as EPEC and increasingly, EAEC (Pires *et al*., 2015). According to our phylogenetic study, isolates carrying virulence genes in human represented environmental phylogroups. Two of the *bfpA* and *eaeA* gene (representative for EPEC) carrying isolates were obtained from two infants suffering from diarrhea. Among children with persistent diarrhea from developed countries, atypical EPEC are the most common pathogens isolated (Afset *et al*., 2004; Nguyen *et al*., 2006). An *elt* gene (representative for ETEC) carrying isolate was surprisingly obtained from urine of a patient. This might represent an opportunistic infection as ETEC is uncommon in UTI cases.

In our study it was important to determine if the isolates reached a particular environment by recent fecal contamination because a bacteria of fecal origin might be either commensal or pathogenic as opposed to environmental. Discrimination between a commensal or a pathogenic *E. coli* and an environmental *E. coli* becomes difficult as all belong to the same species and contain the same identification genes (*uspA* and *uidA*). Detection of the *eae* gene which is usually present in *E. coli* can help determine if an isolate reached the environment because of recent fecal contamination since this gene tends to be lost easily after *E. coli* enters the abiotic environment. In our study, the *eae* gene was absent indicating that all environmental *E. coli* have been in that environment for a reasonably long time. Similar result was found in a study by (Lee, 2011) where none of the isolates contained any *eae* gene.

Escherichia coli may cause a variety of diarrheal and infectious diseases in hosts when it exists as a pathogen. A recent study done from 2000 to 2015 revealed that antibiotic consumption has increased by around 39% in 76 countries of this planet in 15 years' time (Haque, 2019). The antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Bartoloni *et al*., 2006). In this study, *E. coli* isolates of human fecal origin were more resistant than non-human (animal, prawn, soil and water) isolates of *E. coli* to a total of 8 antibiotics among 10 antibiotics tested. This would be expected as humans often undergo antibiotic treatment. In case of nitrofurantoin and chloramphenicol, however, non-human *E. coli* isolates were more resistant than human *E. coli* isolates. Whether these antibiotics are used in animal feed resulting in Bangladesh remains to be investigated. These drugs were known to be highly effective against *E. coli* found in a study (Mazumdar *et al*., 2006). In our study, 51.67% of the isolates from human

exhibited resistance to Trimethoprim-Sulfamethoxazole, 5% were resistant to chloramphenicol and 38.34% were sensitive to Tetracycline; whereas in case of isolates from non-human sources 34.44% exhibited resistance to Trimethoprim-Sulfamethoxazole, 28.89% to Chloramphenicol and 32.22% were resistant to Tetracycline. Thus, over the years, *E. coli* has acquired resistance to all three antibiotics compared (Trimethoprim-Sulfamethoxazole, Chloramphenicol and Tetracycline) in both human and non- human sources, with the exception of Chloramphenicol to which resistance has decreased in human isolates. This could be attributed to the low level of Chloramphenicol use in recent years. In earlier studies *E. coli* was found to be highly sensitive to Nitrofurantoin (76% sensitive, n=58) in case of clinical isolates (Christopher *et al.*, 2013) but in our study among 60 human isolates, only 3.33% were found sensitive to Nitrofurantoin. Non- human isolates showed low resistance against Gentamicin (9.09%) and higher resistance to Cefixime (32.73%). Consistent step-up in *E.coli* resistance to Ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Oteo *et al.* (2005). Ciprofloxacin resistance of 25.8% *E. coli* were resistant to Ciprofloxacin in a study conducted in Portugal and 24.3% in Italy, while in Germany and the Netherlands the proportions were 15.2% and 6.8%, respectively (Bonten *et al.*, 1990). A relatively high percentage (89.4%) of Ciprofloxacin resistance was observed in Bareilly-India (Christopher *et al.*, 2013). The findings of Christopher *et al.* (2013) is similar to our study (56.66% for human host isolates). This may be supported by the fact that their study location is close to ours and living conditions are similar to what would be found in Bangladesh, hence similar percentages in antibiotic resistance was observed. Tadesse and colleagues (2017) observed high rate of resistance of *E. coli* to Ampicillin, Amoxyclav, Amoxicillin and Ciprofloxacin. However, resistance was lower (20.91%) in isolates of non-human origin. Overall, isolates from non-human sources showed comparatively less resistance than isolates from human. The reason for the high percentage of antibiotic resistance may be due to increase in an irrational consumption rate, transmission of resistant isolates between people, self-medication and noncompliance with medication and sales of substandard drug. *E. coli* from human and non-human (animal, soil, water, prawn and street food) origin were found to be more resistant to the family of third generation Cephalosporins such as Cefixime. This could be attributed to the high indiscriminate usage of third generation antibiotics by the public favoring development of antibiotic resistance. It is known that the emergence of extended spectrum beta-lactamases has threatened the empirical use of Cephalosporins and Ciprofloxacins (Kiffer *et al.*, 2007; Pondei *et al.*, 2012). Globally antibiotics are used as a growth promoter in live-stock. In a

study, a global map of 228 countries was drawn which depicted the consumption of antibiotics in livestock; it was estimated that the total antibiotic consumption was 63,151 tons in 2010 (Van Boeckel *et al*., 2015). In our study, all isolates from animal hosts (chicken, duck, cow, goat and sheep) were Multi Drug Resistant (MDR) and among them two duck isolates showed resistance to eight of the ten antibiotics tested. We compared the diversity of antibiotic resistance among isolates from human and non-human sources. The diversity index values for resistance pattern were not significantly different. The diversity index values for antibiotic sensitivity pattern of isolates from human and animal origin were also not significantly different. Microorganisms use various mechanisms to develop drug resistance such as recombination of foreign DNA in bacterial chromosome, horizontal gene transfer and alternation in genetic material (Klemn *et al*., 2006). Several other mechanisms such as antibiotic efflux or poor drug penetration resulting in the reduction of the intracellular concentration of antibiotic, modification of the antibiotic target site due to posttranslational target modification or genetic mutation of the target and inactivation of the antibiotic by modification or hydrolysis (Floyd *et al*., 2010; Ogawa *et al*., 2012). The resistance pattern of microorganisms vary from country to country, state to state, large hospital to small hospital and hospital to community. In Pakistan, the problem of drug resistance is high because of overuse and misuse of antibiotics (Iqbal *et al*., 2010; Tanvir *et al*., 2012). One of the most contributing factor to the spread of antimicrobial resistance in bacteria has been attributed to the fact that in most developing countries, diarrheal diseases are treated with an inadequate regimen of antimicrobials and often without first identifying the pathogen (Ram *et al*., 2008). Unfortunately, in Bangladesh there is no systematic national surveillance of antibiotic resistance and insufficient data is available to quantify the problem (Abdul *et al*., 2008). Currently globally antimicrobial resistance is considered as one of the top public health issues for treating infectious diseases (Alvarez-Uria, *et al*., 2018). Another recent study stated that *Escherichia coli* and *Klebsiella pneumoniae* will be resistant through the planet against third generation Cephalosporins and Carbapenems before long (Alvarez-Uria, *et al*., 2018). Therefore antimicrobial resistance has been found in all areas of flora and fauna and evolved as a grave issue and global public health threat for mankind (Ferri *et al*., 2017).

Bacterial antibiotic resistance is often associated with the presence of plasmids. Determining the presence of plasmids in antibiotic resistant bacteria and establishing co-relation between the presence of plasmid and antibiotic resistant is important because spread of resistance via plasmids

can lead to outbreaks or endemic occurrence (Meyer *et al*., 1993). Plasmid profiles of 150 isolates were studied to demonstrate this co-relation. In this study, 88 (58.67%) of the isolates showed the presence of plasmids of various sizes. Fifty one (57.95%) of these samples were isolated from Human host, eighteen (20.45%) were from prawn, twelve (13.63%) from soil and only seven (7.95%) were isolated from water. The smallest plasmid was a 1 kb plasmid in human host isolates and in non-human host isolates it was 1.5kb. The largest plasmid was more than 10 kb and was common for *E. coli* isolated from both human and non-human. This plasmid was found in all isolates except isolate 595, 914, 25733, 425, 064, 13, 534 (isolates from human origin) and, P7, P41 S49, S65 (isolates from non-human sources). The presence of plasmids found in the present study was different from previous findings (Lina *et al*., 2014). 80.68% *E. coli* carrying multiple plasmids showed greater than 50% antibiotic resistance to all of the 10 tested antibiotics. In the present study plasmids were found to be present in all the different sources including human, animal, prawn, soil and water and may have distributed via horizontal gene transfer. Smillie and colleagues (2010) estimated that >50% of all plasmids can be transferred by conjugation. Different factors may contribute to the long-term stability of plasmids in the environment (Lopatkin *et al*., 2017). Another recent work has shown that other factors like positive selection coupled with compensatory adaption can help to explain the long term persistence of plasmid in the environment (San Millan *et al*., 2014; Yano *et al*., 2016). In the present study the mobile genetic element integron was present in 30% (45/150) of isolates. Integrons are strongly associated with strains isolated from the clinical environment (Martinez-Freijo *et al*., 1998; Chang *et al*., 2000b; Yu *et al*., 2004). Integrons have also been found in bacteria from healthy hosts and from environmental samples (Rosser and Young, 1999; Petersen *et al*., 2000). Similarly 37% (44/120) class 1 Integrons was detected in another study (Cocchi, *et al*., 2007). A much higher prevalence of Class 1 Integrons was found by Lina *et al*. (2014) who reported 50% (20/40) Class 1 Integron containing ESBL producers. Previous reports have shown higher prevalence of Class 1 Integron in *E. coli* : 64.4% in isolates from swine with diarrhea (Kang *et al*., 2005), 62% in intensive-care and surgical-unit isolates from hospitals in nine European countries (Martinez-Freijo *et al*., 1998), 59% in isolates from calf diarrhea cases (Du *et al*., 2005), 52% in various clinical isolates (Chang *et al*., 2000) and 45% in urinary-tract isolates (White *et al*., 2001). Likewise, this study also revealed the presence of Class 1 Integrons in *E. coli* from different sources such as human (35.55%, n=16), animal (27.27%, n=12), prawn (6.81%, n=3), soil (18.18%, n=8), water (11.36%, n=5) and street food

(50%, n=1). A total of 60% (12/20) of *E. coli* isolated from animal contained Class 1 integron. Of these, 58.33% was detected in chicken, 16.67% in duck and 8.33% in each of cow, goat, sheep and street food. These findings were found to be similar with the findings of (Cocchi, *et al*., 2007). According to Cocchi *et al*. (2007) this is probably a result of the fact that farm animals that are raised for economic purposes are more exposed to an antibiotic pressure than the other animals. Most of these Class 1 Integron containing isolates belong to phylogroup B1 (environmental strains) and C (sister group of B1). But Cocchi *et al*. (2007) showed that nonpathogenic commensal strains (phylogroup A) represent an important reservoir of Integrons which was in contrast to our findings. Cocchi *et al.* (2007) found lower percentages of B2 and D strains (virulent strains) among Class 1 Integron carrying isolates which result was in similar to our findings. In this study we tried to analyse the relationship between the presence of Class 1 Integron and Multidrug resistance. However, it was observed that there was no specific correlation.

4.2 Future endeavors

The current study revealed some important facts about *Escherichia coli* isolated from diverse environments. However, including a larger number of isolates can increase the statistical validity of the study. Sequencing the phylogroup specific amplicons may help to sub-group the isolates and identify more specific origin related or novel phylogenetic markers. Hence this study should be continued with more focal points.

4.3 Concluding Remarks

In summary the results described in this thesis may contribute to a better understanding of the distribution pattern and phylodynamics of *E. coli* in the environment. From this study it can be concluded that Phylogroup B1 (environmental origin) was the predominant phylogroup in our study. The predominance of group B1 in human, instead of commensal group A, indicates a shift in the gut of the *E. coli* population from commensal to environmental. Neither of the isolates contained *eae* gene, an indicator of recent fecal contamination, which confirms the presence of the isolates in the environment over a reasonably long period of time. *E. coli* isolated from human showed an alarming rate of antibiotic resistance to the antibiotics tested as well as the presence of multiple plasmids. They may act as a reservoir of antibiotic resistance genes which can be transferred to other bacteria. Class I Integron was also detected in many isolates, which gives them the potential of antibiotic resistance gene transfer. From the dynamic movement and adaptability

of environmental *E. coli*, phylogroup B1 in different environment including the human gut, it may be concluded that the demarcation line between environmental and commensal *E. coli* will become questionable in the near future.

References

- ✓ Abba, K., Sinfield, R., Hart, C. A., & Garner, P. (2009). Pathogens associated with persistent diarrhoea in children in low and middle income countries: systematic review. *BMC Infectious Diseases*, *9*(1), 88.
- ✓ Abdul, J. K. P., Abdul Rahim, K., Abdul, H. Y. S., & Sanaullah, K. (2008). Current antibiotic susceptibility in Khyber Teaching Hospital Peshawar (NWFP) Pakistan. *Gomal University Research Journals*, *13*, 224-229.
- ✓ Afset, J. E., Bevanger, L., Romundstad, P., & Bergh, K. (2004). Association of atypical enteropathogenic *Escherichia coli* (EPEC) with prolonged diarrhoea. *Journal of Medical Microbiology*, *53*(11), 1137-1144.
- ✓ Afset, J. E., Anderssen, E., Bruant, G., Harel, J., Wieler, L., & Bergh, K. (2008). Phylogenetic backgrounds and virulence profiles of atypical enteropathogenic *Escherichia coli* strains from a case-control study using multilocus sequence typing and DNA microarray analysis. *Journal of Clinical Microbiology*, *46*(7), 2280-2290.
- ✓ Ahmed, W., Sritharan, T., Palmer, A., Sidhu, J. P. S., & Toze, S. (2013). Evaluation of bovine feces-associated microbial source tracking markers and their correlations with fecal indicators and zoonotic pathogens in a Brisbane, Australia, reservoir. *Applied and Environmental Microbiology*, *79*(8), 2682-2691.
- ✓ Ahmed, I., Rabbi, M. B., & Sultana, S. (2019). Antibiotic resistance in Bangladesh: A systematic review. *International Journal of Infectious Diseases*, *80*, 54-61.
- ✓ Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, *128*(6), 1037-1050.
- ✓ Alton, E. W., Armstrong, D. K., Ashby, D., Bayfield, K. J., Bilton, D., Bloomfield, E. V., ... & Carvelli, P. (2015). Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. *The Lancet Respiratory Medicine*, *3*(9), 684-691.
- ✓ Alvarez-Uria, G., Gandra, S., Mandal, S., & Laxminarayan, R. (2018). Global forecast of antimicrobial resistance in invasive isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *International Journal of Infectious Diseases*, *68*, 50-53.
- ✓ Anastasi, E. M., Matthews, B., Stratton, H. M., & Katouli, M. (2012). Pathogenic *Escherichia coli* found in sewage treatment plants and environmental waters. *Applied and Environmental Microbiology*, *78*(16), 5536-5541.

- ✓ Anderson, A. D., Nelson, J. M., Rossiter, S., & Angulo, F. J. (2003). Public health consequences of use of antimicrobial agents in food animals in the United States. *Microbial Drug Resistance*, *9*(4), 373-379.
- ✓ Arakawa, Y., Murakami, M., Suzuki, K., Ito, H., Wacharotayankun, R., Ohsuka, S., ... & Ohta, M. (1995). A novel integron-like element carrying the metallo-beta-lactamase gene blaIMP. *Antimicrobial Agents and Chemotherapy*, *39*(7), 1612-1615.
- ✓ Bager, F., & Helmuth, R. (2001). Epidemiology of resistance to quinolones in *Salmonella*. *Veterinary Research*, *32*(3-4), 285-290.
- ✓ Bartoloni, A., Pallecchi, L., Benedetti, M., Fernandez, C., Vallejos, Y., Guzman, E., ... & Strohmeyer, M. (2006). Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerging Infectious Diseases*, *12*(6), 907.
- ✓ Basak, S., Singh, P., & Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: A study. *Journal of Pathogens*, *2016*.
- ✓ Begum, Y. A., Talukder, K. A., Nair, G. B., Khan, S. I., Svennerholm, A. M., Sack, R. B., & Qadri, F. (2007). Comparison of enterotoxigenic *Escherichia coli* isolated from surface water and diarrhoeal stool samples in Bangladesh. *Canadian Journal of Microbiology*, *53*(1), 19-26.
- ✓ Bej, A. K., Dicesare, J. L., Haff, L., & Atlas, R. M. (1991). Detection of *Escherichia coli* and *Shigella spp*. in water by using the polymerase chain reaction and gene probes for uid. *Applied and Environmental Microbiology*, *57*(4), 1013-1017.
- ✓ Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J., & Harel, J. (2003). Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. *Journal of Clinical Microbiology*, *41*(5), 2113-2125.
- ✓ Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, *153*(S1), S347-S357.
- ✓ Bergholz, P. W., Noar, J. D., & Buckley, D. H. (2011). Environmental patterns are imposed on the population structure of *Escherichia coli* after fecal deposition. *Applied and Environmental Microbiology*, *77*(1), 211-219.
- ✓ Berthe, T., Ratajczak, M., Clermont, O., Denamur, E., & Petit, F. (2013). Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and

their survival in estuary water. *Applied and Environmental Microbiology*, *79*(15), 4684-4693.

- ✓ Bingen, E., Picard, B., Brahimi, N., Mathy, S., Desjardins, P., Elion, J., & Denamur, E. (1998). Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. *The Journal of Infectious Diseases*, *177*(3), 642-650.
- ✓ Black, R. E., Cousens, S., Johnson, H. L., Lawn, J. E., Rudan, I., Bassani, D. G., ... & Eisele, T. (2010). Global, regional, and national causes of child mortality in 2008: a systematic analysis. *The Lancet*, *375*(9730), 1969-1987.
- ✓ Blyton, M. D., & Gordon, D. M. (2017). Genetic attributes of *E. coli* isolates from chlorinated drinking water. *PLoS ONE*, *12*(1), e0169445.
- ✓ Bonten, M., Stobberingh, E., Philips, J., & Houben, A. (1990). High prevalence of antibiotic resistant *Escherichia coli* in faecal samples of students in the south-east of The Netherlands. *Journal of Antimicrobial Chemotherapy*, *26*(4), 585-592.
- ✓ Boxall, A. B., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E., & Levy, L. S. (2006). Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry*, *54*(6), 2288-2297.
- ✓ Byappanahalli, M. N., Shively, D. A., Nevers, M. B., Sadowsky, M. J., & Whitman, R. L. (2003). Growth and survival of *Escherichia coli* and *Enterococci* populations in the macro-alga Cladophora (Chlorophyta). *FEMS Microbiology Ecology*, *46*(2), 203-211.
- ✓ Campos, T. A. D., Lago, J. C., Nakazato, G., Stehling, E. G., Brocchi, M., Castro, A. F. P. D., & Silveira, W. D. D. (2008). Occurrence of virulence-related sequences and phylogenetic analysis of commensal and pathogenic avian *Escherichia coli* strains (APEC). *Pesquisa Veterinaria Brasileira*, *28*(10), 533-540.
- ✓ Carlos, C., Pires, M. M., Stoppe, N. C., Hachich, E. M., Sato, M. I., Gomes, T. A., ... & Ottoboni, L. M. (2010). *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC Microbiology*, *10*(1), 1-10.
- ✓ Castro, V. S., Carvalho, R. C. T., Conte-Junior, C. A., & Figuiredo, E. E. S. (2017). Shiga-toxin producing *Escherichia coli*: pathogenicity, supershedding, diagnostic methods,

occurrence, and foodborne outbreaks. *Comprehensive Reviews in Food Science and Food Safety*, *16*(6), 1269-1280.

- ✓ Chang, C. Y., Chang, L. L., Chang, Y. H., Lee, T. M., Li, Y. H., & Chang, S. F. (2000). Two new gene cassettes, dfr17 (for trimethoprim resistance) and aadA4 (for spectinomycin/streptomycin resistance), inserted in an *Escherichia coli* class 1 integron. *Journal of Antimicrobial Chemotherapy*, *46*(1), 87-89.
- ✓ Chang, C. Y., Chang, L. L., Chang, Y. H., Lee, T. M., & Chang, S. F. (2000). Characterisation of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of *Escherichia coli* from Taiwan, ROC. *Journal of Medical Microbiology*, *49*(12), 1097-1102.
- ✓ Chang, H. H., Cohen, T., Grad, Y. H., Hanage, W. P., O'Brien, T. F., & Lipsitch, M. (2015). Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiology and Molecular Biology Reviews*, *79*(1), 101-116.
- ✓ Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., ... & Aminov, R. I. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of Environmental Quality*, *38*(3), 1086-1108.
- ✓ Chen, J., & Griffiths, M. W. (1998). PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. *Letters in Applied Microbiology*, *27*(6), 369-371.
- ✓ Cho, S., Hiott, L. M., Barrett, J. B., McMillan, E. A., House, S. L., Humayoun, S. B., ... & Frye, J. G. (2018). Prevalence and characterization of *Escherichia coli* isolated from the Upper Oconee Watershed in Northeast Georgia. *PloS ONE 13*(5), e0197005.
- ✓ Christopher, A. F., Hora, S., & Ali, Z. (2013). Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Annals of Tropical Medicine and Public Health*, *6*(3), 285.
- ✓ Clarke, S. C. (2001). Diarrhoeagenic *Escherichia coli* an emerging problem? *Diagnostic Microbiology and Infectious Disease*, *41*(3), 93-98.

- ✓ Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology*, *66*(10), 4555-4558.
- ✓ Clermont, O., Lescat, M., O'Brien, C. L., Gordon, D. M., Tenaillon, O., & Denamur, E. (2008). Evidence for a human-specific *Escherichia coli* clone. *Environmental Microbiology*, *10*(4), 1000-1006.
- ✓ Clermont, O., Olier, M., Hoede, C., Diancourt, L., Brisse, S., Keroudean, M., ... & Denamur, E. (2011). Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. *Infection, Genetics and Evolution*, *11*(3), 654-662.
- ✓ Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environmental Microbiology Reports*, *5*(1), 58-65.
- ✓ Clermont, O., Dixit, O. V., Vangchhia, B., Condamine, B., Dion, S., Bridier-Nahmias, A., ... & Gordon, D. (2019). Characterization and rapid identification of phylogroup G in *Escherichia coli*, a lineage with high virulence and antibiotic resistance potential. *Environmental Microbiology*, *21*(8), 3107-3117.
- ✓ Cocchi, S., Grasselli, E., Gutacker, M., Benagli, C., Convert, M., & Piffaretti, J. C. (2007). Distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. *FEMS Immunology & Medical Microbiology*, *50*(1), 126-132.
- ✓ Cohen, M. L. (2000). Changing patterns of infectious disease. *Nature*, *406*(6797), 762-767.
- ✓ Collignon, P., & Angulo, F. J. (2006). Fluoroquinolone-resistant *Escherichia coli*: food for thought. *The Journal of Infectious Diseases*, *194*(1), 8-10.
- ✓ Collis, C. M., & Hall, R. M. (1992). Gene cassettes from the insert region of integrons are excised as covalently closed circles. *Molecular Microbiology*, *6*(19), 2875-2885.
- ✓ Cooper, T. F., Remold, S. K., Lenski, R. E., & Schneider, D. (2008). Expression profiles reveal parallel evolution of epistatic interactions involving the CRP regulon in *Escherichia coli*. *PLoS Genet*, *4*(2), e35.
- ✓ da Fonseca, É. L., & Vicente, A. C. P. (2011). Spread of the qnrVC quinolone resistance determinant in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy*, *55*(1), 457-457.

- ✓ Daini, O. A., & Adesemowo, A. (2008). Antimicrobial susceptibility Patterns and R-Plasmids of Clinical strains of *Escherichia coli*. *Journal of Basic Applied Sciences*, *2*, 397-400.
- ✓ Davis, T. E., & Fuller, D. D. (1991). Direct identification of bacterial isolates in blood cultures by using a DNA probe. *Journal of Clinical Microbiology*, *29*(10), 2193-2196.
- ✓ de Brito, B. G., da Silva Leite, D., Linhares, R. E. C., & Vidotto, M. C. (1999). Virulence-associated factors of uropathogenic *Escherichia coli* strains isolated from pigs. *Veterinary Microbiology*, *65*(2), 123-132.
- ✓ Dombek, P. E., Johnson, L. K., Zimmerley, S. T., & Sadowsky, M. J. (2000). Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. *Applied and Environmental Microbiology*, *66*(6), 2572-2577.
- ✓ Du, X., Shen, Z., Wu, B., Xia, S., & Shen, J. (2005). Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic *Escherichia coli*. *FEMS Microbiology Letters*, *245*(2), 295-298.
- ✓ Dubreuil, J. D. (2013). Antibacterial and antidiarrheal activities of plant products against enterotoxinogenic *Escherichia coli*. *Toxins*, *5*(11), 2009-2041.
- ✓ DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., ... & Kalas, J. P. (1971). Pathogenesis of *Escherichia coli* diarrhea. *New England Journal of Medicine*, *285*(1), 1-9.
- ✓ Durso, L. M., & Cook, K. L. (2014). Impacts of antibiotic use in agriculture: what are the benefits and risks? *Current Opinion in Microbiology*, *19*, 37-44.
- ✓ Dusek, N., Hewitt, A. J., Schmidt, K. N., & Bergholz, P. W. (2018). Landscape-scale factors affecting the prevalence of *Escherichia coli* in surface soil include land cover type, edge interactions, and soil pH. *Applied and Environmental Microbiology*, *84*(10).
- ✓ Dijkshoorn, L., van Harsselaar, B., Tjernberg, I., Bouvet, P. J., & Vaneechoutte, M. (1998). Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. *Systematic and Applied Microbiology*, *21*(1), 33-39.
- ✓ Dykhuizen, D. E., & Green, L. (1991). Recombination in *Escherichia coli* and the definition of biological species. *Journal of Bacteriology*, *173*(22), 7257-7268.

- ✓ Elbourne, L. D., & Hall, R. M. (2006). Gene cassette encoding a 3-N-aminoglycoside acetyltransferase in a chromosomal integron. *Antimicrobial Agents and Chemotherapy*, *50*(6), 2270-2271.
- ✓ Escher, M., Scavia, G., Morabito, S., Tozzoli, R., Maugliani, A., Cantoni, S., ... & Torresani, E. (2014). A severe foodborne outbreak of diarrhoea linked to a canteen in Italy caused by enteroinvasive *Escherichia coli*, an uncommon agent. *Epidemiology & Infection*, *142*(12), 2559-2566.
- ✓ Escobar-Páramo, P., Grenet, K., Le Menac'h, A., Rode, L., Salgado, E., Amorin, C., ... & Denamur, E. (2004). Large-scale population structure of human commensal *Escherichia coli* isolates. *Applied and Environmental Microbiology*, *70*(9), 5698-5700.
- ✓ Escobar-Páramo, P., Le Menac'h, A., Le Gall, T., Amorin, C., Gouriou, S., Picard, B., & Denamur, E. (2006). Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. *Environmental Microbiology*, *8*(11), 1975-1984.
- ✓ Estrada-Garcia, T., & Navarro-Garcia, F. (2012). Enteroaggregative *Escherichia coli* pathotype: a genetically heterogeneous emerging foodborne enteropathogen. *FEMS Immunology & Medical Microbiology*, *66*(3), 281-298.
- ✓ Falagas, M. E., & Bliziotis, I. A. (2007). Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? *International Journal of Antimicrobial Agents*, *29*(6), 630-636.
- ✓ Feigin, R. D. (2009). Feigin & Cherry's textbook of pediatric infectious diseases. Philadelphia, PA: Saunders/Elsevier.
- ✓ Ferens, W. A., & Hovde, C. J. (2011). *Escherichia coli* O157: H7: animal reservoir and sources of human infection. *Foodborne Pathogens and Disease*, *8*(4), 465-487.
- ✓ Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: a global emerging threat to public health systems. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2857-2876.
- ✓ Field, K. G., Bernhard, A. E., & Brodeur, T. J. (2003). Molecular approaches to microbiological monitoring: fecal source detection. In *Coastal Monitoring through Partnerships* (pp. 313-326). Springer, Dordrecht.

- ✓ Field, K. G., & Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research*, *41*(16), 3517-3538.
- ✓ Finlay, B. B., & Falkow, S. (1997). Common themes in microbial pathogenicity revisited. *Microbiology and Molecular Biology Reviews*, *61*(2), 136-169.
- ✓ Fleisher, J. M. (1990). The effects of measurement error on previously reported mathematical relationships between indicator organism density and swimming-associated illness: a quantitative estimate of the resulting bias. *International Journal of Epidemiology*, *19*(4), 1100-1106.
- ✓ Fleisher, J. M. (1991). A reanalysis of data supporting US federal bacteriological water quality criteria governing marine recreational waters. *Research Journal of the Water Pollution Control Federation*, 259-265.
- ✓ Floyd, J. L., Smith, K. P., Kumar, S. H., Floyd, J. T., & Varela, M. F. (2010). LmrS is a multidrug efflux pump of the major facilitator superfamily from *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *54*(12), 5406-5412.
- ✓ Fluit, A., & Schmitz, F. J. (1999). Class 1 integrons, gene cassettes, mobility, and epidemiology. *European Journal of Clinical Microbiology and Infectious Diseases*, *18*(11), 761-770.
- ✓ Frenzel, S. A., & Couvillion, C. S. (2002). Fecal-indicator bacteria in streams along a gradient of residential development 1. *JAWRA Journal of the American Water Resources Association*, *38*(1), 265-273.
- ✓ Friedman, M. S., Roels, T., Koehler, J. E., Feldman, L., Bibb, W. F., & Blake, P. (1999). *Escherichia coli* O157: H7 outbreak associated with an improperly chlorinated swimming pool. *Clinical Infectious Diseases*, *29*(2), 298-303.
- ✓ Frost, F. J., Craun, G. F., & Calderon, R. L. (1996). Waterborne disease surveillance. *Journal-American Water Works Association*, *88*(9), 66-75.
- ✓ Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: the agents of open source evolution. *Nature Reviews Microbiology*, *3*(9), 722-732.
- ✓ Gerba, C. P. (2009). Indicator Microorganisms. In Environmental Microbiology (pp. 485-499). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-370519-8.00023-7

- ✓ Gilchrist, M. J., Greko, C., Wallinga, D. B., Beran, G. W., Riley, D. G., & Thorne, P. S. (2007). The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives*, *115*(2), 313-316.
- ✓ Gillings, M. R., Krishnan, S., Worden, P. J., & Hardwick, S. A. (2008). Recovery of diverse genes for class 1 integron-integrases from environmental DNA samples. *FEMS Microbiology Letters*, *287*(1), 56-62.
- ✓ Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M., & Zhu, Y. G. (2015). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *The ISME Journal*, *9*(6), 1269-1279.
- ✓ Giske, C. G., Monnet, D. L., Cars, O., & Carmeli, Y. (2008). Clinical and economic impact of common multidrug-resistant gram-negative bacilli. *Antimicrobial Agents and Chemotherapy*, *52*(3), 813-821.
- ✓ Gizachew, Y., Solomon, A., Daniel, A., Sisay, Y., & Afework, K. (2010). Bacteriological profile and resistant pattern of clinical isolates from pediatric patients, Gondar University Teaching Hospital, Gondar, northwest Ethiopia. *Ethiopian Medical Journal*, *48*(4), 293-300.
- ✓ Godambe, L. P., Bandekar, J., & Shashidhar, R. (2017). Species specific PCR based detection of *Escherichia coli* from Indian foods. *3 Biotech*, *7*(2), 1-5.
- ✓ Goldman, E. (2004). Antibiotic abuse in animal agriculture: Exacerbating drug resistance in human pathogens. *Human and Ecological Risk Assessment*, *10*(1), 121-134.
- ✓ Goldstein, C., Lee, M. D., Sanchez, S., Hudson, C., Phillips, B., Register, B., ... & Maurer, J. J. (2001). Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrobial Agents and Chemotherapy*, *45*(3), 723-726.
- ✓ Gordon, D. M., & Cowling, A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology*, *149*(12), 3575-3586.
- ✓ Grabow, W. O. K. (1996). Waterborne diseases: Update on water quality assessment and control. *SA Waterbulletin*, *22*(2), 193-202. (12), 3575-3586.
- ✓ Greenberg, D. E., Jiang, Z. D., Steffen, R., Verenker, M. P., & DuPont, H. L. (2002). Markers of inflammation in bacterial diarrhea among travelers, with a focus on

enteroaggregative *Escherichia coli* pathogenicity. *The Journal of Infectious Diseases*, *185*(7), 944-949.

- ✓ Griffin, P. M., & Tauxe, R. V. (1991). The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiologic Reviews*, *13*(1), 60-98.
- ✓ Griffith, J. F., Weisberg, S. B., & McGee, C. D. (2003). Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples. *Journal of Water and Health*, *1*(4), 141-151.
- ✓ Griffith, J. F., Cao, Y., McGee, C. D., & Weisberg, S. B. (2009). Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality. *Water Research*, *43*(19), 4900-4907.
- ✓ Groisman, E. A., & Ochman, H. (1994). How to become a pathogen. *Trends in Microbiology*, *2*(8), 289-294.
- ✓ Gyles, C. L. (2007). Shiga toxin-producing *Escherichia coli*: an overview. *Journal of Animal Science*, *85*(suppl_13), E45-E62.
- ✓ Hagedorn, C., Blanch, A. R., & Harwood, V. J. (Eds.). (2011). *Microbial source tracking: methods, applications, and case studies*. Springer Science & Business Media.
- ✓ Hartstra, A. V., Bouter, K. E., Bäckhed, F., & Nieuwdorp, M. (2015). Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care*, *38*(1), 159-165.
- ✓ Harwood, V. J., Staley, C., Badgley, B. D., Borges, K., & Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews*, *38*(1), 1-40.
- ✓ Haque, M. (2019). Antibiotic Use, Antibiotic Resistance, and Antibiotic Stewardship–A Global Public Consequences. *Bangladesh Journal of Medical Science*, *18*(2), 169-170.
- ✓ Hegde, A., Ballal, M., & Shenoy, S. (2012). Detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Indian Journal of Medical Microbiology*, *30*(3), 279.
- ✓ Hendricks, L. D., Wood, D. E., & Hajduk, M. E. (1978). Haemoflagellates: commercially available liquid media for rapid cultivation. *Parasitology*, *76*(3), 309-316.
- ✓ Henriques, S., Silva, E., Lemsaddek, A., Lopes-da-Costa, L., & Mateus, L. (2014). Genotypic and phenotypic comparison of *Escherichia coli* from uterine infections with

different outcomes: clinical metritis in the cow and pyometra in the bitch. *Veterinary Microbiology*, *170*(1-2), 109-116.

- Hoffman, S. J., Caleo, G. M., Daulaire, N., Elbe, S., Matsoso, P., Mossialos, E., ... & Røttingen, J. A. (2015). Strategies for achieving global collective action on antimicrobial resistance. *Bulletin of the World Health Organization*, *93*, 867-876.
- Huang, D. B., Koo, H., & DuPont, H. L. (2004). Enteroaggregative *Escherichia coli*: an emerging pathogen. *Current Infectious Disease Reports*, *6*(2), 83.
- Iqbal, T., Naqvi, R., & Akhter, S. F. (2010). Frequency of urinary tract infection in renal transplant recipients and effect on graft function. *JPMA-Journal of the Pakistan Medical Association*, *60*(10), 826.
- Ishii, S., Ksoll, W. B., Hicks, R. E., & Sadowsky, M. J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Applied and Environmental Microbiology*, *72*(1), 612-621.
- Ishii, S., Yan, T., Shively, D. A., Byappanahalli, M. N., Whitman, R. L., & Sadowsky, M. J. (2006). *Cladophora (Chlorophyta*) *spp.* harbor human bacterial pathogens in nearshore water of Lake Michigan. *Applied and Environmental Microbiology*, *72*(7), 4545-4553.
- Ishii, S., Hansen, D. L., Hicks, R. E., & Sadowsky, M. J. (2007). Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. *Environmental Science & Technology*, *41*(7), 2203-2209.
- Jaureguy, F., Landraud, L., Passet, V., Diancourt, L., Frapy, E., Guigon, G., ... & Picard, B. (2008). Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. *BMC Genomics*, *9*(1), 560.
- Jawad, A., Snelling, A. M., Heritage, J., & Hawkey, P. M. (1998). Comparison of ARDRA and rec A-RFLP analysis for genomic species identification of *Acinetobacter spp*. *FEMS Microbiology Letters*, *165*(2), 357-362.
- Jefferson, R. A., Burgess, S. M., & Hirsh, D. (1986). beta-Glucuronidase from *Escherichia coli* as a gene-fusion marker. *Proceedings of the National Academy of Sciences*, *83*(22), 8447-8451.
- Jenkins, M. W., Tiwari, S., Lorente, M., Gichaba, C. M., & Wuertz, S. (2009). Identifying human and livestock sources of fecal contamination in Kenya with host-specific Bacteroidales assays. *Water Research*, *43*(19), 4956-4966.

- ✓ Jeong, J. H., Shin, K. S., Lee, J. W., Park, E. J., & Son, S. Y. (2009). Analysis of a novel class 1 integron containing metallo-β-lactamase gene VIM-2 in *Pseudomonas aeruginosa. The Journal of Microbiology*, *47*(6), 753-759.
- ✓ Johnson, J. R., Delavari, P., Kuskowski, M., & Stell, A. L. (2001). Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. *The Journal of Infectious Diseases*, *183*(1), 78-88.
- ✓ Johnson, J. R., Delavari, P., O'Bryan, T. T., Smith, K. E., & Tatini, S. (2005). Contamination of retail foods, particularly turkey, from community markets (Minnesota, 1999–2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli*. *Foodborne Pathogens & Disease*, *2*(1), 38-49.
- ✓ Kanamaru, S., Kurazono, H., Nakano, M., Terai, A., Ogawa, O., & Yamamoto, S. (2006). Subtyping of uropathogenic *Escherichia coli* according to the pathogenicity island encoding uropathogenic-specific protein: Comparison with phylogenetic groups. *International Journal of Urology*, *13*(6), 754-760.
- ✓ Kang, S., Lee, D., Shin, S., Ahn, J., & Yoo, H. (2005). Changes in patterns of antimicrobial susceptibility and class 1 integron carriage among *Escherichia coli* isolates. *Journal of Veterinary Science*, *6*(3), 201.
- ✓ Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, *2*(2), 123-140.
- ✓ Karmali, M., Petric, M., Steele, B., & Lim, C. (1983). Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *The Lancet*, *321*(8325), 619-620.
- ✓ Karmali, M. A. (1989). Infection by verocytotoxin-producing *Escherichia coli*. *Clinical Microbiology Reviews*, *2*(1), 15-38.
- ✓ Kaye, K. S., Fraimow, H. S., & Abrutyn, E. (2000). Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management. *Infectious Disease Clinics of North America*, *14*(2), 293-319.
- ✓ Khan, S. J., Roser, D. J., Davies, C. M., Peters, G. M., Stuetz, R. M., Tucker, R., & Ashbolt, N. J. (2008). Chemical contaminants in feedlot wastes: concentrations, effects and attenuation. *Environment International*, *34*(6), 839-859.

- ✓ Kiffer, C. R., Mendes, C., Oplustil, C. P., & Sampaio, J. L. (2007). Antibiotic resistance and trend of urinary pathogens in general outpatients from a major urban city. *International Brazilian Journal of Urology*, *33*(1), 42-49.
- ✓ Kinge, C. N. W., Ateba, C. N., & Kawadza, D. T. (2010). Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, North-West Province, South Africa. *South African Journal of Science*, *106*(1-2), 44-49.
- ✓ Klein, E., & Houston, A. C. (1897). Report on Bacteriological Evidence of Presumably Recent and therefore Dangerous Sewage Pollution of elsewise Potable Waters. *Supplement to XXVII Annual. Report of the Local Government Board, containing Report of the Medical Officer for*, *98*, 318.
- ✓ Klemm, P., Roos, V., Ulett, G. C., Svanborg, C., & Schembri, M. A. (2006). Molecular characterization of the *Escherichia coli* asymptomatic bacteriuria strain 83972: the taming of a pathogen. *Infection and Immunity*, *74*(1), 781-785.
- ✓ Kong, B. H., Hanifah, Y. A., Yusof, M. Y., & Thong, K. L. (2011). Amplification of amplified ribosomal DNA restriction analysis in identification of *Acinetobacter baumannii* from a Tertiary Teaching Hospital, Malaysia. *Tropical Biomedicine*, *28*(3), 563-568.
- ✓ Konstantinidis, K. T., Ramette, A., & Tiedje, J. M. (2006). The bacterial species definition in the genomic era. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1475), 1929-1940.
- ✓ Kosek, M., Bern, C., & Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization*, *81*, 197-204.
- ✓ Kotloff, K. L., Winickoff, J. P., Ivanoff, B., Clemens, J. D., Swerdlow, D. L., Sansonetti, P. J., ... & Levine, M. M. (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization*, *77*(8), 651.
- ✓ Krieg, N. R., & Holt, J. G. (1984). *Bergey's Manual of Systematic Bacteriology*, *1*, 837-942.
- ✓ Lan, R., Alles, M. C., Donohoe, K., Martinez, M. B., & Reeves, P. R. (2004). Molecular evolutionary relationships of enteroinvasive *Escherichia coli* and *Shigella spp. Infection and Immunity*, *72*(9), 5080-5088.

- ✓ Landers, T., & Kavanagh, K. T. (2016). Is the Presidential Advisory Council on Combating Antibiotic Resistance missing opportunities? *American Journal of Infection Control*, *44*(11), 1356-1359.
- ✓ Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., & Sayler, G. (2006). Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and Environmental Microbiology*, *72*(6), 4214-4224.
- ✓ Lee, J. C., Oh, J. Y., Cho, J. W., Park, J. C., Kim, J. M., Seol, S. Y., & Cho, D. T. (2001). The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of *Escherichia coli* in Korea. *Journal of Antimicrobial Chemotherapy*, *47*(5), 599-604.
- ✓ Lee, A., & Wong, E. (2009). Optimization and the robustness of BOX A1R PCR for DNA fingerprinting using trout lake *E. coli* isolates. *Jounal of Experimental Microbiology and Immunology*, *13*, 104-113.
- ✓ Lee, S., Yu, J. K., Park, K., Oh, E. J., Kim, S. Y., & Park, Y. J. (2010). Phylogenetic groups and virulence factors in pathogenic and commensal strains of *Escherichia coli* and their association with blaCTX-M. *Annals of Clinical & Laboratory Science*, *40*(4), 361-367.
- ✓ Lee, C. C. Y. (2011). Genotyping *Escherichia coli* isolates from duck, goose, and gull fecal samples with phylogenetic markers using multiplex polymerase chain reaction for application in microbial source tracking. *Journal of Experimental Microbiology and Immunology*, *15*, 130-135.
- ✓ Lescat, M., Clermont, O., Woerther, P. L., Glodt, J., Dion, S., Skurnik, D., ... & Catzeflis, F. (2013). Commensal *Escherichia coli* strains in Guiana reveal a high genetic diversity with host-dependant population structure. *Environmental Microbiology Reports*, *5*(1), 49-57.
- ✓ Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*, *10*(12), S122-S129.
- ✓ Lina, T. T., Khajanchi, B. K., Azmi, I. J., Islam, M. A., Mahmood, B., Akter, M., ... & Cravioto, A. (2014). Phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* in Bangladesh. *PloS ONE, 9*(10), e108735.

- ✓ Lopatkin, A. J., Meredith, H. R., Srimani, J. K., Pfeiffer, C., Durrett, R., & You, L. (2017). Persistence and reversal of plasmid-mediated antibiotic resistance. *Nature Communications*, *8*(1), 1-10.
- ✓ Lu, J. J., Perng, C. L., Lee, S. Y., & Wan, C. C. (2000). Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. *Journal of Clinical Microbiology*, *38*(6), 2076-2080.
- ✓ Luo, C., Walk, S. T., Gordon, D. M., Feldgarden, M., Tiedje, J. M., & Konstantinidis, K. T. (2011). Genome sequencing of environmental *Escherichia coli* expands understanding of the ecology and speciation of the model bacterial species. *Proceedings of the National Academy of Sciences*, *108*(17), 7200-7205.
- ✓ Lyautey, E., Lu, Z., Lapen, D. R., Wilkes, G., Scott, A., Berkers, T., ... & Topp, E. (2010). Distribution and diversity of *Escherichia coli* populations in the South Nation River drainage basin, eastern Ontario, Canada. *Applied and Environmental Microbiology*, *76*(5), 1486-1496.
- ✓ Mailk, S., Kumar, A., Verma, A. K., Gupta, M. K., Sharma, S. D., Sharma, A. K., & Rahal, A. (2013). Incidence and drug resistance pattern of collibacillosis in cattle and buffalo calves in Western Uttar Pradesh in India. *Journal of Animal Health and Production 1*(1), 15-19.
- ✓ Mainil, J. G., & Daube, G. (2005). Verotoxigenic *Escherichia coli* from animals, humans and foods: who's who? *Journal of Applied Microbiology*, *98*(6), 1332-1344.
- ✓ Mandal, P., Kapil, A., Goswami, K., Das, B., & Dwivedi, S. N. (2001). Uropathogenic *Escherichia coli* causing urinary tract infections. *Indian Journal of Medical Research*, *114*, 207.
- ✓ Martinez-Freijo, P., Fluit, A. C., Schmitz, F. J., Grek, V. S., Verhoef, J., & Jones, M. E. (1998). Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *The Journal of Antimicrobial Chemotherapy*, *42*(6), 689-696.
- ✓ Mateus, L., Henriques, S., Merino, C., Pomba, C., da Costa, L. L., & Silva, E. (2013). Virulence genotypes of *Escherichia coli* canine isolates from pyometra, cystitis and fecal origin. *Veterinary Microbiology*, *166*(3-4), 590-594.

- ✓ Mazumdar, K., Dutta, N. K., Dastidar, S. G., Motohashi, N., & Shirataki, Y. (2006). Diclofenac in the management of *E. coli* urinary tract infections. *in vivo*, *20*(5), 613-619.
- ✓ McEachran, A. D., Blackwell, B. R., Hanson, J. D., Wooten, K. J., Mayer, G. D., Cox, S. B., & Smith, P. N. (2015). Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. *Environmental Health Perspectives*, *123*(4), 337-343.
- ✓ McEwen, S. A., & Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. *Clinical Infectious Diseases*, *34*(Supplement_3), S93-S106.
- ✓ Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, *5*(5), 607.
- ✓ Mercado, E. H., Ochoa, T. J., Ecker, L., Cabello, M., Durand, D., Barletta, F., ... & Cleary, T. G. (2011). Fecal leukocytes in children infected with diarrheagenic *Escherichia coli*. *Journal of Clinical Microbiology*, *49*(4), 1376-1381.
- ✓ Méric, G., Kemsley, E. K., Falush, D., Saggers, E. J., & Lucchini, S. (2013). Phylogenetic distribution of traits associated with plant colonization in *Escherichia coli*. *Environmental Microbiology*, *15*(2), 487-501.
- ✓ Meyer, K. S., Urban, C., Eagan, J. A., Berger, B. J., & Rahal, J. J. (1993). Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Annals of Internal Medicine*, *119*(5), 353-358.
- ✓ Michael, C. A., Dominey-Howes, D., & Labbate, M. (2014). The antimicrobial resistance crisis: causes, consequences, and management. *Frontiers in Public Health*, *2*, 145.
- ✓ Michelacci, V., Prosseda, G., Maugliani, A., Tozzoli, R., Sanchez, S., Herrera-León, S., ... & Morabito, S. (2016). Characterization of an emergent clone of enteroinvasive *Escherichia coli* circulating in Europe. *Clinical Microbiology and Infection*, *22*(3), 287-e11.
- ✓ Minnock, A., Vernon, D. I., Schofield, J., Griffiths, J., Parish, J. H., & Brown, S. B. (2000). Mechanism of Uptake of a Cationic Water-Soluble Pyridinium Zinc Phthalocyanine across the Outer Membrane of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, *44*(3), 522-527.

- ✓ Moissenet, D., Salauze, B., Clermont, O., Bingen, E., Arlet, G., Denamur, E., ... & Vu-Thien, H. (2010). Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum β-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. *Journal of Clinical Microbiology*, *48*(7), 2459-2463.
- ✓ Molina, F., López-Acedo, E., Tabla, R., Roa, I., Gómez, A., & Rebollo, J. E. (2015). Improved detection of *Escherichia coli* and coliform bacteria by multiplex PCR. *BMC Biotechnology*, *15*(1), 48.
- ✓ Moore, S. R. (2011). Update on prolonged and persistent diarrhea in children. *Current Opinion in Gastroenterology*, *27*(1), 19-23.
- ✓ Morcatti Coura, F., Diniz, S. D. A., Silva, M. X., Mussi, J. M. S., Barbosa, S. M., Lage, A. P., & Heinemann, M. B. (2015). Phylogenetic group determination of *Escherichia coli* isolated from animals samples. *The Scientific World Journal*, *2015*.
- ✓ Moreno, M. A., Domınguez, L., Teshager, T., Herrero, I. A., Porrero, M. C., de Ávila, L. D. S. A., ... & de Valladolid, L. D. S. A. (2000). Antibiotic resistance monitoring: the Spanish programme. *International Journal of Antimicrobial Agents*, *14*(4), 285-290.
- ✓ Müştak, H. K., Günaydin, E., Kaya, İ. B., Salar, M. Ö., Babacan, O., Önat, K., ... & Diker, K. S. (2015). Phylo-typing of clinical *Escherichia coli* isolates originating from bovine mastitis and canine pyometra and urinary tract infection by means of quadruplex PCR. *Veterinary Quarterly*, *35*(4), 194-199.
- ✓ Nair, G. B., Ramamurthy, T., Bhattacharya, M. K., Krishnan, T., Ganguly, S., Saha, D. R., ... & Takeda, Y. (2010). Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. *Gut Pathogens*, *2*(1), 4.
- ✓ NandaKafle, G., Seale, T., Flint, T., Nepal, M., Venter, S. N., & Brözel, V. S. (2017). Distribution of diverse *Escherichia coli* between cattle and pasture. *Microbes and Environments*, ME17030.
- ✓ Napier, M. D., Haugland, R., Poole, C., Dufour, A. P., Stewart, J. R., Weber, D. J., ... & Wade, T. J. (2017). Exposure to human-associated fecal indicators and self-reported illness among swimmers at recreational beaches: a cohort study. *Environmental Health*, *16*(1), 103.

- Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, *11*(1), 142-201.
- Nataro, J. P., Mai, V., Johnson, J., Blackwelder, W. C., Heimer, R., Tirrell, S., ... & Hirshon, J. M. (2006). Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New haven, Connecticut. *Clinical Infectious Diseases*, *43*(4), 402-407.
- Navarro-Garcia, F., Serapio-Palacios, A., Ugalde-Silva, P., Tapia-Pastrana, G., & Chavez-Dueñas, L. (2013). Actin cytoskeleton manipulation by effector proteins secreted by diarrheagenic *Escherichia coli* pathotypes. *BioMed Research International*, *2013*.
- Neu, H. C. (1992). The crisis in antibiotic resistance. *Science*, *257*(5073), 1064-1073.
- Nguyen, R. N., Taylor, L. S., Tauschek, M., & Robins-Browne, R. M. (2006). Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerging Infectious Diseases*, *12*(4), 597.
- Nicolle, L. E. (2002). Urinary tract infection: traditional pharmacologic therapies. *The American Journal of Medicine*, *113*(1), 35-44.
- Niemi, R. M., & Niemi, J. S. (1991). Bacterial pollution of waters in pristine and agricultural lands. *Journal of Environmental Quality*, *20*(3), 620-627.
- Nordstrom, L., Liu, C. M., & Price, L. B. (2013). Foodborne urinary tract infections: a new paradigm for antimicrobial-resistant foodborne illness. *Frontiers in Microbiology*, *4*, 29.
- Ochman, H., & Groisman, E. A. (1994). The origin and evolution of species differences in *Escherichia coli* and *Salmonella typhimurium*. In *Molecular Ecology and Evolution: Approaches and Applications* (pp. 479-493). Birkhäuser, Basel.
- Ochoa, T. J., Ecker, L., Barletta, F., Mispireta, M. L., Gil, A. I., Contreras, C., ... & Cleary, T. G. (2009). Age-related susceptibility to infection with diarrheagenic *Escherichia coli* among infants from Periurban areas in Lima, Peru. *Clinical Infectious Diseases*, *49*(11), 1694-1702.
- Odumosu, B. T., Adeniyi, B. A., & Chandra, R. (2013). Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant *Pseudomonas aeruginosa* from Southwest Nigeria. *Annals of Clinical Microbiology and Antimicrobials*, *12*(1), 29.
- Ogawa, W., Onishi, M., Ni, R., Tsuchiya, T., & Kuroda, T. (2012). Functional study of the novel multidrug efflux pump KexD from *Klebsiella pneumoniae*. *Gene*, *498*(2), 177-182.

- ✓ Okeke, I. N., Lamikanra, A., & Edelman, R. (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases*, *5*(1), 18.
- ✓ Ostrowski, E. A., Woods, R. J., & Lenski, R. E. (2008). The genetic basis of parallel and divergent phenotypic responses in evolving populations of *Escherichia coli. Proceedings of the Royal Society B: Biological Sciences*, *275*(1632), 277-284.
- ✓ Osugui, L., de Castro, A. P., Iovine, R., Irino, K., & Carvalho, V. M. (2014). Virulence genotypes, antibiotic resistance and the phylogenetic background of extraintestinal pathogenic *Escherichia coli* isolated from urinary tract infections of dogs and cats in Brazil. *Veterinary Microbiology*, *171*(1-2), 242-247.
- ✓ Oteo, J., Lázaro, E., de Abajo, F. J., Baquero, F., Campos, J., & members of EARSS, S. (2005). Antimicrobial-resistant invasive *Escherichia coli*, Spain. *Emerging Infectious Diseases*, *11*(4), 546.
- ✓ Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., ... & Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. *Frontiers in Microbiology*, *8*, 2390.
- ✓ Paton, J. C., & Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical Microbiology Reviews*, *11*(3), 450-479.
- ✓ Perkins, M. D., Mirrett, S., & Reller, L. B. (1995). Rapid bacterial antigen detection is not clinically useful. *Journal of Clinical Microbiology*, *33*(6), 1486-1491.
- ✓ Perna, N. T., Plunkett, G., Burland, V., Mau, B., Glasner, J. D., Rose, D. J., ... & Pósfai, G. (2001). Genome sequence of enterohaemorrhagic *Escherichia coli* O157: H7. *Nature*, *409*(6819), 529-533.
- ✓ Petersen, A., Guardabassi, L., Dalsgaard, A., & Olsen, J. E. (2000). Class I integrons containing a dhfrI trimethoprim resistance gene cassette in aquatic *Acinetobacter spp*. *FEMS Microbiology Letters*, *182*(1), 73-76.
- ✓ Phillips, A. D., Navabpour, S., Hicks, S., Dougan, G., Wallis, T., & Frankel, G. (2000). Enterohaemorrhagic *Escherichia coli* O157: H7 target Peyer's patches in humans and cause attaching/effacing lesions in both human and bovine intestine. *Gut*, *47*(3), 377-381.

- ✓ Phillips, I., Casewell, M., Cox, T., de Groot, B., Friis, C., Jones, R., ... & Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A reply to critics. *Journal of Antimicrobial Chemotherapy*, *54*(1), 276-278.
- ✓ Pianka. (1973). ER. The structure of lizard communities. *Annual Review of Ecology and Systematics*, *4*(1), 53.
- ✓ Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., ... & Denamur, E. (1999). The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infection and Immunity*, *67*(2), 546-553.
- ✓ Pires, S. M., Fischer-Walker, C. L., Lanata, C. F., Devleesschauwer, B., Hall, A. J., Kirk, M. D., ... & Angulo, F. J. (2015). Aetiology-specific estimates of the global and regional incidence and mortality of diarrhoeal diseases commonly transmitted through food. *PloS ONE*, *10*(12), e0142927.
- ✓ Pondei, K., Oladapo, O., & Kunle-Olowu, O. E. (2012). Anti-microbial susceptibility pattern of micro-organisms associated with urinary tract infections in a tertiary health institution in the Niger Delta Region of Nigeria. *African Journal of Microbiology Research*, *6*(23), 4976-4982.
- ✓ Pontius, F. W. (1990). Water quality and treatment: a handbook of community water supplies. In *Water quality and treatment: a handbook of community water supplies* (pp. 1194-1194).
- ✓ Pozzi, G., Oggioni, M. R., & Tomasz, A. (1989). DNA probe for identification of *Streptococcus pneumoniae*. *Journal of Clinical Microbiology*, *27*(2), 370-372.
- ✓ Probert, W. S., Miller, G. M., & Ledin, K. E. (2017). Contaminated stream water as source for *Escherichia coli* O157 illness in children. *Emerging Infectious Diseases*, *23*(7), 1216.
- ✓ Pruimboom-Brees, I. M., Morgan, T. W., Ackermann, M. R., Nystrom, E. D., Samuel, J. E., Cornick, N. A., & Moon, H. W. (2000). Cattle lack vascular receptors for *Escherichia coli* O157: H7 Shiga toxins. *Proceedings of the National Academy of Sciences*, *97*(19), 10325-10329.
- ✓ Qadri, F., Svennerholm, A. M., Faruque, A. S. G., & Sack, R. B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical Microbiology Reviews*, *18*(3), 465-483.

- ✓ Rådström, P., Bäckman, A., Qian, N. Y., Kragsbjerg, P., Påhlson, C., & Olcén, P. (1994). Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae,* and *Streptococci* using a seminested PCR strategy. *Journal of Clinical Microbiology*, *32*(11), 2738-2744.
- ✓ Ram, S., Vajpayee, P., & Shanker, R. (2008). Contamination of potable water distribution systems by multi antimicrobial-resistant enterohemorrhagic *Escherichia coli*. *Environmental Health Perspectives*, *116*(4), 448-452.
- ✓ Ratajczak, M., Laroche, E., Berthe, T., Clermont, O., Pawlak, B., Denamur, E., & Petit, F. (2010). Influence of hydrological conditions on the *Escherichia coli* population structure in the water of a creek on a rural watershed. *BMC Microbiology*, *10*(1), 222.
- ✓ Retchless, A. C., & Lawrence, J. G. (2007). Temporal fragmentation of speciation in bacteria. *Science*, *317*(5841), 1093-1096.
- ✓ Rosenberg, M. L., Koplan, J. P., Wachsmuth, I. K., Wells, J. G., Gangarosa, E. J., Guerrant, R. L., & Sack, D. A. (1977). Epidemic diarrhea at Crater Lake from enterotoxigenic *Escherichia coli*: a large waterborne outbreak. *Annals of Internal Medicine*, *86*(6), 714-718.
- ✓ Rosser, S. J., & Young, H. K. (1999). Identification and characterization of class 1 integrons in bacteria from an aquatic environment. *Journal of Antimicrobial Chemotherapy*, *44*(1), 11-18.
- ✓ Russo, T. A., & Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and infection*, *5*(5), 449-456.
- ✓ Salem, M. M., Muharram, M., & Alhosiny, I. M. (2010). Distribution of classes 1 and 2 integrons among multi drug resistant *E. coli* isolated from hospitalized patients with urinary tract infection in Cairo, Egypt. *Australian Journal of Basic and Applied Sciences 4*(3), 398-407.
- ✓ Salem, I. B., Ouardani, I., Hassine, M., & Aouni, M. (2011). Bacteriological and physico-chemical assessment of wastewater in different region of Tunisia: impact on human health. *BMC Research Notes*, *4*(1), 144.
- ✓ Samie, A., Guerrant, R. L., Barrett, L., Bessong, P. O., Igumbor, E. O., & Obi, C. L. (2009). Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroeal

human stools from Vhembe district, South Africa. *Journal of Health, Population, and Nutrition*, *27*(6), 739.

- Sandvig, K. (2001). Shiga toxins. *Toxicon*, *39*(11), 1629-1635.
- San Millan, A., Peña-Miller, R., Toll-Riera, M., Halbert, Z. V., McLean, A. R., Cooper, B. S., & MacLean, R. C. (2014). Positive selection and compensatory adaptation interact to stabilize non-transmissible plasmids. *Nature Communications*, *5*(1), 1-11.
- Santo Domingo, J. W., Bambic, D. G., Edge, T. A., & Wuertz, S. (2007). Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. *Water Research*, *41*(16), 3539-3552.
- Sarmah, A. K., Meyer, M. T., & Boxall, A. B. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, *65*(5), 725-759.
- Scaletsky, I. C., Aranda, K. R., Souza, T. B., & Silva, N. P. (2010). Adherence factors in atypical enteropathogenic *Escherichia coli* strains expressing the localized adherence-like pattern in HEp-2 cells. *Journal of Clinical Microbiology*, *48*(1), 302-306.
- Schroeder, C. M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D. G., ... & Meng, J. (2002). Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Applied and Environmental Microbiology*, *68*(2), 576-581.
- Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R., & Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology*, *68*(12), 5796-5803.
- Shanks, O. C., White, K., Kelty, C. A., Hayes, S., Sivaganesan, M., Jenkins, M., ... & Haugland, R. A. (2010). Performance assessment PCR-based assays targeting Bacteroidales genetic markers of bovine fecal pollution. *Applied and Environmental Microbiology*, *76*(5), 1359-1366.
- Sharma, A. (2011). Antimicrobial resistance: no action today, no cure tomorrow. *Indian Journal of Medical Microbiology*, *29*(2), 91.
- Shin, M. G., Kim, S. H., Lee, J. C., Cho, D., Kee, S. J., Shin, J. H., ... & Ryang, D. W. (2004). A Comparison of ID 32 GN System with Amplified Ribosomal DNA Restriction Analysis for Identification of *Acinetobacter baumannii*. *Annals of Laboratory Medicine*, *24*(2), 107-112.

- ✓ Shintani, M., Sanchez, Z. K., & Kimbara, K. (2015). Genomics of microbial plasmids: classification and identification based on replication and transfer systems and host taxonomy. *Frontiers in Microbiology*, *6*, 242.
- ✓ Shuford, J. A., & Patel, R. (2005). Antimicrobial growth promoter use in livestock-implications for human health. *Reviews in Medical Microbiology*, *16*(1), 17-24.
- ✓ Sleight, S. C., Orlic, C., Schneider, D., & Lenski, R. E. (2008). Genetic basis of evolutionary adaptation by *Escherichia coli* to stressful cycles of freezing, thawing and growth. *Genetics*, *180*(1), 431-443.
- ✓ Simpson, J. M., Santo Domingo, J. W., & Reasoner, D. J. (2002). Microbial source tracking: state of the science. *Environmental Science & Technology*, *36*(24), 5279-5288.
- ✓ Simpson, H. (2004). Promoting the management and protection of private water wells. *Journal of Toxicology and Environmental Health, Part A*, *67*(20-22), 1679-1704.
- ✓ Skovgaard, N. (2008). Plasmids. Current Research and Future Trends, Georg Lipps (Ed.), Caister Academic Press (2008), viii+ 264 pp, hardback,£ 150 www. horizonpress. com/pla, ISBN: 978-1-904455-35-6.
- ✓ Skurnik, D., Bonnet, D., Bernède-Bauduin, C., Michel, R., Guette, C., Becker, J. M., ... & Boutin, J. P. (2008). Characteristics of human intestinal *Escherichia coli* with changing environments. *Environmental Microbiology*, *10*(8), 2132-2137.
- ✓ Smillie, C., Garcillán-Barcia, M. P., Francia, M. V., Rocha, E. P., & de la Cruz, F. (2010). Mobility of plasmids. *Microbiology and Molecular Biology Reviews*, *74*(3), 434-452.
- ✓ Son, I., Van Kessel, J. A. S., & Karns, J. S. (2009). Genotypic diversity of *Escherichia coli* in a dairy farm. *Foodborne Pathogens and Disease*, *6*(7), 837-847.
- ✓ Spellberg, B., Srinivasan, A., & Chambers, H. F. (2016). New societal approaches to empowering antibiotic stewardship. *Jama*, *315*(12), 1229-1230.
- ✓ Stoeckel, D. M., Mathes, M. V., Hyer, K. E., Hagedorn, C., Kator, H., Lukasik, J., ... & Wiggins, B. A. (2004). Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. *Environmental Science & Technology, 38*(22), 6109-6117.
- ✓ Stoppe, N. D. C., Silva, J. S., Carlos, C., Sato, M. I., Saraiva, A. M., Ottoboni, L. M., & Torres, T. T. (2017). Worldwide phylogenetic group patterns of *Escherichia coli* from commensal human and wastewater treatment plant isolates. *Frontiers in Microbiology*, *8*, 2512.

- Strausbaugh, L. J. (1997). Emerging infectious diseases: a challenge to all. *American Family Physician*, *55*(1), 111-117.
- Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. (2017). Antimicrobial resistance in Africa: a systematic review. *BMC Infectious Diseases*, *17*(1), 616.
- Tanvir, R., Hafeez, R., & Hasnain, S. (2012). Prevalence of multiple drug resistant *Escherichia coli* in patients of urinary tract infection registering at a diagnostic laboratory in Lahore Pakistan. *Pakistan Journal of Zool*ogy, *44*(3), 707-12.
- Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal *Escherichia coli*. *Nature Reviews Microbiology*, *8*(3), 207-217.
- Todar, K. (2004). Todar's Online Textbook of Bacteriology.
- Trabulsi, L. R., Keller, R., & Gomes, T. A. T. (2002). 10.321/eid0805. Typical and Atypical Enteropathogenic *Escherichia coli*. *Emerging infectious diseases*, *8*(5), 508.
- Tymensen, L. D., Pyrdok, F., Coles, D., Koning, W., McAllister, T. A., Jokinen, C. C., ... & Neumann, N. F. (2015). Comparative accessory gene fingerprinting of surface water *E scherichia coli* reveals genetically diverse naturalized population. *Journal of Applied Microbiology*, *119*(1), 263-277.
- United States. Environmental Protection Agency. Environmental Criteria, & Assessment Office. (1986). *Air quality criteria for lead* (Vol. 4). US Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.
- Unno, T., Han, D., Jang, J., Lee, S. N., Ko, G., Choi, H. Y., ... & Hur, H. G. (2009). Absence of *Escherichia coli* phylogenetic group B2 strains in humans and domesticated animals from Jeonnam Province, Republic of Korea. *Applied and Environmental Microbiology*, *75*(17), 5659-5666.
- US Environmental Protection Agency. Office of Water Regulations and Standards. (1986). *Ambient water quality criteria for bacteria-1986*.
- Vallance, B. A., & Finlay, B. B. (2000). Exploitation of host cells by enteropathogenic *Escherichia coli*. *Proceedings of the National Academy of Sciences*, *97*(16), 8799-8806.

- ✓ Van, T. T. H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of Global Antimicrobial Resistance*, *20*, 170-177.
- ✓ Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., ... & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654.
- ✓ Van den Beld, M. J. C., & Reubsaet, F. A. G. (2012). Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *European Journal of Clinical Microbiology & Infectious Diseases*, *31*(6), 899-904.
- ✓ Van den Bogaard, A. E., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. *International Journal of Antimicrobial Agents*, *14*(4), 327-335.
- ✓ Van Lerberghe, W. (2008). *The world health report 2008: primary health care: now more than ever*. World Health Organization.
- ✓ Vaneechoutte, M., & Heyndrickx, M. (2001). Application and analysis of ARDRA patterns in bacterial identification, taxonomy and phylogeny. In *New approaches for analysis of microbial typing data. Editors: L. Dijkshoorn, K. Towner, and M. Struelens. ISBN Hardbound 0444 507 40X., 2001. Elsevier. 357 p. Chapter 9* (pp. 211-247).
- ✓ Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, *40*(4), 277.
- ✓ Ventura, M., Casas, I. A., Morelli, L., & Callegari, M. L. (2000). Rapid amplified ribosomal DNA restriction analysis (ARDRA) identification of *Lactobacillus spp*. isolated from fecal and vaginal samples. *Systematic and Applied Microbiology*, *23*(4), 504-509.
- ✓ 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*(9), 2274-2288.
- ✓ Walk, S. T., Alm, E. W., Gordon, D. M., Ram, J. L., Toranzos, G. A., Tiedje, J. M., & Whittam, T. S. (2009). Cryptic lineages of the genus *Escherichia*. *Applied and Environmental Microbiology*, *75*(20), 6534-6544.
- ✓ Walker, C. F., & Black, R. E. (2010). Diarrhoea morbidity and mortality in older children, adolescents, and adults. *Epidemiology & Infection*, *138*(9), 1215-1226.

- ✓ Wang, J., Wang, W., Xu, W., Wang, X., & Zhao, S. (2011). Mercury removals by existing pollutants control devices of four coal-fired power plants in China. *Journal of Environmental Sciences*, *23*(11), 1839-1844.
- ✓ Wegener, H. C. (2003). Antibiotics in animal feed and their role in resistance development. *Current Opinion in Microbiology*, *6*(5), 439-445.
- ✓ White, P. A., McIver, C. J., & Rawlinson, W. D. (2001). Integrons and gene cassettes in the enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, *45*(9), 2658-2661.
- ✓ Witte, W. (1998). Medical consequences of antibiotic use in agriculture. *Science*, *279*(5353), 996-997.
- ✓ World Health Organization. (2006). Future directions for research on enterotoxigenic *Escherichia coli* vaccines for developing countries. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, *81*(11), 97-104.
- ✓ World Health Organization. (2018). WHO *expert consultation on rabies: third report* (Vol. 1012). World Health Organization.
- ✓ Yano, H., Wegrzyn, K., Loftie-Eaton, W., Johnson, J., Deckert, G. E., Rogers, L. M., ... & Top, E. M. (2016). Evolved plasmid-host interactions reduce plasmid interference cost. *Molecular Microbiology*, *101*(5), 743-756.
- ✓ Yu, H. S., Lee, J. C., Kang, H. Y., Jeong, Y. S., Lee, E. Y., Choi, C. H., ... & Cho, D. T. (2004). Prevalence of dfr genes associated with integrons and dissemination of dfrA17 among urinary isolates of *Escherichia coli* in Korea. *Journal of Antimicrobial Chemotherapy*, *53*(3), 445-450.
- ✓ Zinnah, M. A., Bari, M. R., Islam, M. T., Hossain, M. T., Rahman, M. T., Haque, M. H., ... & Islam, M. A. (2007). Characterization of *Escherichia coli* isolated from samples of different biological and environmental sources. *Bangladesh Journal of Veterinary Medicine*, *5*(1 & 2), 25-32.
- ✓ Zowawi, H. M., Harris, P. N., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., ... & Paterson, D. L. (2015). The emerging threat of multidrug-resistant Gram-negative bacteria in urology. *Nature Reviews Urology*, *12*(10), 570.

Appendix

Appendix-I

Media Composition

Mueller- Hinton Agar

Beef Infusion	2.0g
Bacto casamino acid	17.5g
Starch	1.5g
Agar	15g
Distilled Water	1000 ml
pH	7.3
Sterilized at 121°C under 15lbs pressure for 15 minutes.	

Mac-Conkey Agar:

Peptone	17g
Protease peptone	3g
Lactose	10g
Bile Salt	1.5g
NaCl	5g
Neutral Red	0.03g
Bacteriological Agar	13.5g
Distilled Water	1000 ml
pH	7.1
Sterilized at 121°C under 15lbs pressure for 15 minutes.	

Nutrient Agar:

Peptone	0.5g
Beef Extract	0.03g
NaCl	0.05g
Agar	1.5g
Distilled water	1000 ml
pH	7.2
Sterilized at 121°C under 15 p.s.i pressure for 15 minutes.	

Motility, Indole, Urease Agar:

Peptone	3%
NaCl	0.5%
Urea	2%
Mono Potassium Phosphate	0.2%
Phenol Red	0.0005%
Agar	0.4%
Distilled Water	100 ml
pH	7

Tryptic Soy Agar:

Casein Peptone	17g
Soya Peptone	3g
Sodium Chloride	5g
Dipotassium Phosphate	2.5g
Agar	15g
Dextrose	2.5g
pH	7.3
Sterilized at 121°C under 15 p.s.i pressure for 15 minutes.	

Simmons Citrate Agar:

Magnesium Sulfate	0.02%
NaCl	0.5%
Sodium Citrate	0.2%
Di potassium Phosphate	0.1%
Mono potassium phosphate	0.1%
Bromothymol Blue	0.008%
Agar	2%
Distilled Water	100 ml
pH	7
Sterilized at 121°C under 15 p.s.i pressure for 15 minutes.	

Eosine Methylene Blue Agar:

Peptone	10g
Lactose	5g
Sucrose	5g
Dipotassium phosphate	2g
Agar	13.5g
Eosine Y	0.4g
Methylene Blue	0.065g
Distilled Water	Upto 1L
pH	7.2
Boiled to completely dissolve and then autoclaved at 121°C for 15 minutes.	

Brilliant Green Lactose Broth:

Peptone	10.0
Lactose	10.0
Ox-bile	20.0
Brilliant green	0.0133
Distilled water	Upto 1L
pH	7.2±0.2
Sterilized at 121°C under 15 p.s.i pressure for 15 minutes.	

Kligler's Iron Agar:

Beef extract	3.0
Yeast extract	3.0
Peptone	15.0
Protease peptone	5.0
lactose	10.0
Dextrose	1.0
Ferrus sulphate	0.2
Sodium chloride	5.0
Sodium thiosulfate	0.3
Phenyl Red	0.024
BactoAgar	15.0
Distilled water	1000 ml
pH	7.4 ± 0.2
Sterilized at 121°C under 15 p.s.i pressure for 15 minutes	

Appendix-II

Chemical Composition

Hydrogen Peroxide:

3% aqueous solution of hydrogen peroxide was prepared from the absolute solution.

Kovac's reagent

p-Ddimethylaminobezaldehyde: 5.0 g

amyl-alcohol: 75.0 g

HCI: 25.0 g

Methyl red solution

Methyl red: 0.04 g

Ethanol: 40 ml

Distilled water: 100 ml

McFarland 0.5 standard:

$BaCl_2$(0.048 M): 1.75 % (w/v)

H_2SO_4 (0.36N): 1.0%.(w/v)

α- napthol reagent

α- napthol: 50.0 g

Ethanol (45%): 95 ml

Oxidase reagent

Tetramethyl paraphenylance- diamine dihydrachloride: 1.0 g

Distilled water: 100 ml

Safranin 0 (Certified): 2.5 g

Ethanol (95%): 10 ml

Distilled water: 100 ml

Safranin O was dissolved in the ethanol and water was then added.

Buffers and Reagents

Tris-EDTA buffer (TE buffer):

Tris-HCI: 10Mm

EDTA: 5Mm

One ml of 1 M Tris-HCI (pH 8) and 0.2 ml of 0.5M EDTA (pH 8.0) were added to 98.8 ml distilled water to make 1000m1TE buffer solution.

0.5 M EDTA

18.612 of EDTA was dissolved in 70 ml distilled water and then pH was adjusted at 8.0 with 10N NaOH. Further distilled water was added to make 100 ml. the solution was then autoclaved and stored at room temperature.

1 M Tris- HCI:

12.1 g of Tris base was dissolved in 80 distilled water and was adjusted to 8.0 with concentrated HCI. Distilled water was then added to make 100 ml solution. The solution was then autoclaved and stored at 4° C temperature.

10X Tris Borate EDTA (TBE) stock Electrophoresis buffer:

Tris base (108 g), 40 ml 0.5 EDTA (pH 8.0) and 55 g boric acid were dissolved in 700 ml distilled water. Distilled water was further added to make 1 liter solution. The pH was adjusted to 8.3.

Loading buffer:

Bromophenol blue: 0.15% (w/v)

SDS: 0.5(w/v)

EDTA: 0.15 M

Glycerol: 50% (v/v)

Appendix-III

Apparatus used in the study

Autoclave (Model HL-42E)	Tokyo, Japan
Centrifuge machine	Sigma, USA
Class-II A1 biological safety cabinet	Thermo Forma, USA
Duran bottle	Scott, Germany
Electric balance model no. 210S	Sartorius, Germany
Eppendorf tubes (1.5mL)	Eppendorf, Germany
Freezer (-20°C)	Thermo Forma, USA
Fridge (4°C)	West frost
Fridge 8°C model no. MIR-253	Japan
Gel Documentation	Bio-rad, USA
Incubator	Memmert, Germany
Incubator, WTB binder, model no. D-78502	Germany
Glassware	Pyrex brand, USA
Magnetic stirrer	Corning, UK
Microcertrifuge, Eppendorf centrifuge	Germany
Micropipettes	Eppendorf, Germany
Micropipette tips	Lab systems, Finland
Microscope	Olympus, Japan
Microwave oven, model no. CE2933N	Samsung, Korya
PCR machine	MJ Research, USA
Power supply	BIO_RAD, USA
pH meter, model no. MP 220	Toledo, Germany

Appendix-IV

Tables related to *E. coli* phylogrouping and antibiotic sensitivity test are mentioned in this section.

Quadruplex genotypes of isolated *E. coli* Phylo-groups

		Quadruplex Genotype						
Sample Type	**Sample ID**	***arpA* (400 bp)**	***chuA* (288 bp)**	***yjaA* (211 bp)**	**TspE4.C2 (152 bp)**	**Phylo-group**	**Next Step (C or E PCR)**	**Quadruplex Phylogroup**
Human	25733	+	+	-	-	D/E	E-	D
Human	47990	+	-	-	+	B1		B1
Human	C\O Saidul	+	-	-	+	B1		B1
Human	O38	-	+	+	-	B2		B2
Human	588	+	-	-	+	B1		B1
Human	774	+	-	-	-	A		A
Human	O32	+	-	-	+	B1		B1
Human	3491	+	+	-	-	D/E	E-	D
Human	585	+	+	-	-	D/E	E-	D
Human	647	+	+	-	-	D/E	E-	D
Human	689	+	-	-	+	B1		B1
Human	534	+	-	-	+	B1		B1
Human	595	+	-	-	-	A		A
Human	265	+	-	-	+	B1		B1
Human	340	+	-	-	+	B1		B1
Human	521	+	-	-	+	B1		B1
Human	564	+	-	-	+	B1		B1
Human	168	-	+	+	-	B2		B2
Human	30987	+	-	-	+	B1		B1
Human	180	+	+	-	-	D/E	E-	D
Human	8996	+	-	-	+	B1		B1
Human	47509	+	-	-	+	B1		B1
Human	940	+	-	-	+	B1		B1
Human	176	+	-	-	+	B1		A
Human	47508	+	-	-	+	B1		B1
Human	47770	+	+	-	-	D/E	E-	D
Human	26170	+	+	-	-	D/E	E-	D

Human	30955	+	+	-	-	D/E	E-	D
Human	47697	-	+	+	-	B2		B2
Human	ME3	+	-	-	+	B1		B1
Human	ME4	+	-	-	+	B1		B1
Human	ME5	+	-	-	+	B1		B1
Human	ME6	+	-	-	+	B1		B1
Human	ME7	+	-	-	+	B1		B1
Human	ME8	+	-	-	+	B1		B1
Human	ME9	+	-	-	-	A		A
Human	ME10	+	-	-	+	B1		B1
Human	9	+	-	-	+	B1		B1
Human	13	+	+	-	-	D/E	E-	D
Human	14	+	-	-	+	B1		B1
Human	68	-	+	-	-	F		F
Human	755	-	+	-	-	F		F
Human	397	+	-	-	+	B1		B1
Human	779	+	-	-	+	B1		B1
Human	407	-	+	-	-	F		F
Human	496	+	-	-	+	B1		B1
Human	914	+	+	-	-	D/E	E-	D
Human	075	+	+	-	-	D/E	E-	D
Human	343	-	+	-	-	F		F
Human	425	+	-	+	-	A/C	C-	A
Human	380	+	-	-	-	A		A
Human	938	+	+	+	-	E/Clade 1	E+	E
Human	394	+	+	-	-	D/E	E-	D
Human	064	+	+	-	-	D/E	E-	D
Human	464	+	+	-	-	D/E	E-	D
Human	9312	+	+	-	-	D/E	E-	D
Human	544	+	-	-	+	B1		B1
Human	657	+	+	-	-	D/E	E-	D
Human	823	+	-	-	+	B1		B1
Human	685	+	-	-	+	B1		B1
Soil	S4	+	-	-	+	A		A
Soil	S5	+	-	-	+	B1		B1
Soil	S6	+	-	-	+	B1		B1
Soil	S9	+	-	-	+	B1		B1
Soil	S11	+	-	-	+	B1		B1
Soil	S12	+	-	-	+	B1		B1
Soil	S20	+	-	-	+	B1		B1
Soil	S23	+	-	-	+	B1		B1
Soil	S31	+	-	-	-	A		A
Soil	S33	+	-	-	+	B1		B1
Soil	S48	+	-	-	+	B1		B1

Soil	S49	+	-	-	+	B1		B1
Soil	S51	+	-	-	+	B1		B1
Soil	S56	+	-	-	+	B1		B1
Soil	S65	+	-	-	+	B1		B1
Soil	S74	+	-	-	+	B1		B1
Soil	S76	+	-	-	+	B1		B1
Soil	S78	+	-	-	+	B1		B1
Soil	S79	+	-	-	+	B1		B1`
Soil	S80	+	-	-	+	B1		B1
Soil	S82	+	-	-	+	B1		B1
Soil	S84	+	-	-	+	B1		B1
Soil	S85	+	-	-	+	B1		B1
Prawn	P0	+	-	-	-	A		A
Prawn	P7	+	-	-	+	B1		B1
Prawn	P10	+	-	-	+	B1		B1
Prawn	P11	+	-	-	+	B1		B1
Prawn	P12	+	-	-	+	B1		B1
Prawn	P20	+	-	+	-	A/C	C-	A
Prawn	P21	+	-	-	+	B1		B1
Prawn	P22	+	-	-	+	B1		B1
Prawn	P24	+	-	-	+	B1		B1
Prawn	P26	+	-	-	+	B1		B1
Prawn	P27	+	-	-	+	B1		B1
Prawn	P29	+	-	-	+	B1		B1
Prawn	P34	+	-	+	-	A/C	C+	C
Prawn	P35	+	-	-	+	B1		B1
Prawn	P37	+	-	-	+	B1		B1
Prawn	P41	+	-	-	+	B1		B1
Prawn	P43	+	-	-	+	B1		B1
Prawn	P45	+	-	-	+	B1		B1
Prawn	P50	+	-	-	-	A		A
Prawn	P52	+	-	-	+	B1		B1
Prawn	P54	+	-	-	+	B1		B1
Prawn	P56	+	-	-	+	B1		B1
Prawn	P58	+	-	-	+	B1		B1
Prawn	P59	+	-	-	+	B1		B1
Prawn	P60	+	-	-	+	B1		B1
Water	W13	+	-	-	+	B1		B1
Water	W14	+	-	-	-	A		A
Water	W27	+	-	-	+	B1		A
Water	W31	+	-	-	-	A		A
Water	W42	+	-	-	-	A		A
Water	W46	+	-	-	+	B1		B1

Water	W49	+	-	-	-	A		A
Water	W50	+	-	-	+	B1		B1
Water	W55	+	-	-	+	B1		B1
Water	W56	+	-	-	+	B1		B1
Water	W81	+	-	-	+	B1		B1
Water	W84	+	-	-	+	B1		B1
Water	W85	+	-	-	-	A		A
Water	W86	+	-	-	-	A		A
Water	W88	+	-	-	+	B1		B1
Water	W89	+	-	+	-	A/C	C-	A
Water	W90	+	-	-	-	A		A
Water	W91	+	-	+	-	A/C	C+	C
Water	W92	+	-	-	-	A		A
Water	W93	+	-	-	+	B1		B1
Animal	A1	+	-	-	+	B1		B1
Animal	A2	+	-	-	+	B1		B1
Animal	A6	+	-	-	-	A		A
Animal	A7	+	-	-	-	B1		B1
Animal	A8	+	+	-	-	D/E	E+	E
Animal	A9	+	-	-	+	B1		B1
Animal	A10	+	+	+	-	E/clade 1	E+	E
Animal	A12	+	-	+	-	A/C	C+	C
Animal	A13	+	-	+	-	A/C	C+	C
Animal	A14	+	-	+	-	A/C	C+	C
Animal	A16	+	-	-	-	A		A
Animal	A18	+	-	+	-	A/C	C+	C
Animal	A19	+	-	+	-	A/C	C+	C
Animal	A20	+	-	+	-	A/C	C+	C
Animal	A21	+	-	+	-	A/C	C+	C
Animal	A22	+	-	-	-	A		A
Animal	A23	+	-	+	-	A/C	C+	C
Animal	A24	+	-	-	-	A		A
Animal	A26	+	-	+	-	A/C	C+	C
Animal	A27	+	+	+	-	E or Clade 1	E+	E
Animal	A28	+	-	+	-	A/C	C+	C
Animal	A30	+	-	+	-	A/C	C+	C
Animal	A31	+	-	+	-	A/C	C+	C
Animal	A32	+	-	-	-	A		A
Animal	A34	+	-	-	-	A		A
Animal	A35	+	-	-	-	A		A

Animal	A36	+	-	-	-	A		A
Animal	A37	+	-	-	-	A		A
Animal	A39	+	-	-	-	A		A
Animal	A40	+	-	-	-	A		A
Animal	A42	+	-	-	-	A		A
Animal	A43	+	-	-	-	A		A
Animal	A44	+	-	-	-	A		A
Animal	A46	+	+	-	-	D/E	E-	D
Animal	A47	+	-	-	-	A		A
Animal	A48	+	-	-	-	A		A
Animal	A49	+	-	-	-	A		A
Animal	A50	+	-	-	-	A		A
Animal	A51	+	-	-	-	A		A
Animal	A52	+	-	-	-	A		A
Animal	A53	+	-	-	-	A		A
Animal	A54	+	-	-	-	A		A
Animal	A56	+	+	-	-	D/E	E-	D
Animal	A57	+	-	-	-	A		A
Animal	A58	+	-	-	-	A		A
Animal	A59	+	-	-	-	A		A
Animal	A62	+	-	+	-	A/C	C-	A
Animal	A63	+	-	-	-	A		A
Animal	A64	+	-	-	-	A		A
Animal	A68	+	-	-	-	A		A
Animal	A69	+	+	-	-	D/E	E+	E
Animal	A70	+	+	-	-	D/E	E+	E
Animal	A71	+	-	+	-	A/C	C+	C
Animal	A76	+	-	-	-	A		A
Street Food	SF-6	+	-	-	+	B1		B1
Street Food	SF-V(5)	+	-	-	-	A		A
Positive Control	ATCC 25922	-	+	+	-	B2		B2

Diversity of phylogenetic groups in different environment

Phylogenetic groups	Animal	Prawn	Water	Soil	Human
A	30	5	10	1	4
B1	4	19	9	22	32
B2	0	0	0	0	3
C	13	1	1	0	0
D	2	0	0	0	16
E	5	0	0	0	1
F	0	0	0	0	4

Distribution of phylogenetic groups among *E. coli* isolated from Human

Distribution of Phylogenetic Groups	Number of Isolates
A	6
B1	30
B2	3
C	0
D	16
E	1
F	4

Distribution of phylogenetic groups among *E. coli* isolated from non-human hosts

Distribution of Phylogenetic Groups	Number of Isolates
A	46
B1	56
B2	0
C	15
D	2
E	5
F	0

Antibiotic Sensitivity pattern of *E. coli* isolates

Sample type	Sample ID	Nitrofurantoin (F-300)	Chloramphenicol (C 30)	Co-Trimoxazole (COT 25)	Tetracycline (TE 30)	Amoxicillin-Clavulanic acid (AMC 30)	Ceftriaxone (CRO 30)	Cefixime (CFM 5)	Ciprofloxacin (CIP 5)	Gentamicin (Gen 10)	Azithromycin (AZM 30)
Human	25733	I	S	S	S	S	S	R	I	R	S
Human	496	S	S	R	R	R	R	R	R	R	R
Human	585	S	S	S	S	I	R	R	R	I	R
Human	464	I	S	S	S	S	R	R	R	S	R
Human	47990	S	S	S	S	R	R	R	R	S	R
Human	47697	S	S	S	S	S	S	S	I	S	I
Human	47509	S	S	S	S	S	S	S	S	S	S
Human	038	S	S	S	S	I	R	R	R	S	I
Human	3491	S	S	R	S	I	R	R	R	I	R
Human	689	S	S	R	R	S	S	S	R	S	R
Human	647	S	S	R	S	S	S	S	I	S	R
Human	595	S	S	S	R	I	S	S	S	S	S
Human	168	S	S	S	S	S	S	S	I	S	S
Human	564	S	S	R	I	S	R	R	R	S	R
Human	47770	S	S	R	S	S	S	S	I	S	R
Human	394	S	S	R	R	S	S	S	R	I	R
Human	407	I	S	R	R	R	R	R	R	R	R
Human	779	S	S	R	R	R	R	R	R	R	R
Human	534	S	S	S	S	I	S	S	S	I	R
Human	685	S	S	S	S	S	S	S	I	I	S
Human	544	S	S	R	S	S	S	S	I	I	I
Human	265	I	S	R	S	R	R	R	R	S	R
Human	774	I	S	R	S	I	R	R	R	R	R
Human	180	I	S	R	S	I	I	R	I	I	R
Human	397	I	S	R	R	I	I	R	R	I	R
Human	940	S	S	R	R	S	S	S	R	R	R
Human	938	S	S	R	R	S	S	I	I	I	I
Human	30955	S	S	R	S	I	R	R	R	I	R
Human	657	S	S	S	S	S	S	S	I	I	R
Human	30987	S	S	S	S	S	S	S	S	I	S
Human	26170	I	R	R	R	I	S	S	I	R	R
Human	588	I	S	R	R	R	R	R	R	I	R

Human	075	I	R	R	R	R	R	R	R	R	R
Human	032	I	S	S	S	I	S	R	I	I	I
Human	176	S	S	R	R	R	S	R	R	R	R
Human	521	I	S	S	S	I	S	S	R	I	S
Human	340	S	S	R	R	I	R	R	R	R	R
Human	8996	I	S	R	R	I	S	I	R	S	R
Human	755	I	I	I	R	R	R	R	R	R	R
Human	C/O Saidul	I	S	R	R	I	I	S	R	R	R
Human	9312	R	S	R	S	S	R	R	R	R	R
Human	343	S	S	R	R	S	R	R	R	S	R
Human	425	S	S	S	S	S	S	S	I	R	S
Human	823	S	S	S	S	S	R	R	I	I	I
Human	914	I	S	R	R	S	R	R	R	R	R
Human	380	S	S	S	S	S	S	I	R	I	R
Human	064	S	S	S	S	S	S	S	I	R	S
Human	47508	S	S	S	S	S	S	S	I	S	S
Human	9	R	S	S	S	R	S	R	R	R	R
Human	13	S	S	R	S	S	R	R	R	R	I
Human	14	S	S	S	R	R	R	R	R	S	R
Human	68	S	S	R	R	R	R	R	R	R	R
Animal	A1	R	R	I	R	R	I	R	I	S	S
Animal	A2	S	S	S	R	R	S	R	I	S	S
Animal	A6	S	S	S	R	R	S	R	R	S	I
Animal	A7	S	S	S	R	R	S	R	R	S	I
Animal	A8	R	R	R	R	R	S	R	I	S	S
Animal	A9	S	S	S	S	R	S	R	I	S	S
Animal	A10	S	S	S	S	R	S	R	I	S	S
Animal	A12	I	R	R	R	R	S	R	R	S	R
Animal	A13	R	S	R	R	R	I	R	I	S	R
Animal	A14	R	R	R	R	R	S	R	I	I	R
Animal	A18	R	R	R	R	R	I	R	R	S	S
Animal	A19	S	R	R	R	R	S	R	R	S	S
Animal	A20	S	R	R	R	R	S	R	R	S	I
Animal	A21	S	R	R	R	R	S	R	R	S	R
Animal	A23	S	I	R	S	R	S	R	I	S	I
Animal	A26	R	R	R	R	R	S	R	R	S	I
Animal	A27	R	R	R	R	R	S	R	R	S	R
Animal	A28	R	R	R	R	R	S	R	R	S	R
Animal	A30	S	S	I	R	R	S	R	I	S	S
Animal	A31	S	R	S	S	R	S	R	R	S	S
Prawn	P0	S	I	S	S	I	S	R	I	R	R
Prawn	P7	S	I	R	R	S	S	I	I	I	I
Prawn	P10	S	S	S	S	I	S	R	I	R	R
Prawn	P11	R	S	S	S	I	I	R	I	I	I

Prawn	P12	R	S	S	R	I	S	R	I	R	R
Prawn	P20	S	I	S	S	S	S	S	I	S	I
Prawn	P21	S	I	S	S	S	S	R	I	I	R
Prawn	P22	S	I	S	R	I	S	I	R	R	R
Prawn	P24	I	S	S	S	S	S	S	I	I	I
Prawn	P26	I	I	S	S	I	I	S	I	S	I
Prawn	P27	I	S	S	S	S	S	S	I	I	I
Prawn	P29	I	I	S	S	R	R	S	I	S	I
Prawn	P34	S	S	S	S	S	S	S	S	S	I
Prawn	P35	I	S	S	S	S	I	S	I	S	I
Prawn	P37	S	S	S	S	S	S	I	I	S	I
Prawn	P41	I	S	S	R	I	I	S	I	I	R
Prawn	P43	I	S	S	S	S	S	S	I	S	I
Prawn	P45	R	S	S	S	S	I	S	I	S	R
Prawn	P50	R	S	S	I	I	I	S	R	S	R
Prawn	P52	S	S	S	S	S	S	S	I	S	S
Prawn	P54	S	S	S	R	I	S	S	R	S	I
Prawn	P56	R	S	S	S	S	I	I	R	S	S
Prawn	P58	I	S	S	I	I	S	I	I	I	S
Prawn	P59	R	S	S	S	I	I	S	R	S	I
Prawn	P60	S	S	S	S	S	S	S	I	I	I
Soil	S4	S	I	S	S	S	S	S	I	S	S
Soil	S5	I	S	S	S	S	I	S	I	I	S
Soil	S6	S	S	S	S	S	I	S	I	I	I
Soil	S9	S	S	S	S	S	R	I	I	I	I
Soil	S11	S	S	R	S	S	S	S	I	S	S
Soil	S12	S	S	S	S	S	S	S	I	S	I
Soil	S20	S	I	R	R	S	S	S	I	S	R
Soil	S23	S	S	S	S	S	S	S	I	I	S
Soil	S31	S	S	S	S	S	S	S	I	I	S
Soil	S33	S	S	S	S	S	S	S	I	S	I
Soil	S48	S	S	S	S	S	S	S	I	S	I
Soil	S49	S	S	R	R	I	I	S	R	S	R
Soil	S51	S	S	S	S	S	R	S	I	S	S
Soil	S56	S	S	S	S	S	S	S	S	S	R
Soil	S65	S	S	R	R	S	R	S	I	R	I
Soil	S74	S	S	S	S	S	S	R	S	S	S
Soil	S76	S	S	R	S	S	S	R	I	S	I
Soil	S78	S	S	S	S	S	S	S	I	S	S
Soil	S79	S	S	S	S	S	S	S	I	S	S
Soil	S80	S	S	R	R	S	S	R	I	S	S
Soil	S82	S	S	S	S	S	S	R	I	S	R
Soil	S84	S	S	S	S	S	S	S	I	R	S
Soil	S85	S	S	S	S	S	S	I	I	S	I

Water	W13	I	S	R	S	S	R	R	R	S	R
Water	W14	S	S	S	S	I	S	S	R	S	S
Water	W27	S	S	S	S	S	S	S	I	S	S
Water	W31	S	S	S	S	S	S	R	I	S	I
Water	W42	S	S	R	R	S	S	S	I	S	S
Water	W46	S	S	R	S	S	S	S	I	S	R
Water	W49	S	S	S	S	S	S	I	I	S	S
Water	W50	I	S	R	S	R	R	R	R	R	R
Water	W55	S	S	R	R	S	S	S	I	S	S
Water	W56	I	R	R	S	R	R	S	R	R	R
Water	W81	S	S	S	S	S	S	R	I	S	S
Water	W84	I	I	R	S	R	R	R	R	R	R
Water	W85	S	S	R	S	S	S	S	I	S	I
Water	W86	S	S	S	S	S	S	S	I	S	I
Water	W88	S	S	S	S	S	S	S	I	S	S
Water	W89	S	S	S	S	I	S	I	I	S	I
Water	W90	S	S	R	S	S	R	I	I	S	R
Water	W91	R	I	S	I	I	R	R	I	I	S
Water	W92	S	S	R	S	S	S	R	I	S	I
Water	W93	S	S	S	S	S	S	S	I	R	S
Street Food	SF-6	I	S	S	S	S	S	S	I	I	R
Street Food	SF-V(5)	S	R	S	S	S	S	I	R	R	R
Clinical	ME 3	S	S	S	S	S	S	I	I	S	S
Clinical	ME 4	I	S	S	S	S	S	I	R	S	S
Clinical	ME 5	S	S	R	R	R	S	R	R	R	R
Clinical	ME 6	S	S	S	S	S	S	S	I	I	S
Clinical	ME 7	S	S	S	S	S	S	I	I	I	S
Clinical	ME 8	S	S	S	S	S	S	I	I	S	S
Clinical	ME 9	S	S	S	S	S	S	R	I	R	S
Clinical	ME 10	S	S	S	S	S	S	I	I	I	S
Positive Control	ATCC 25922	S	S	S	S	S	S	S	S	S	S

Percentage of antibiotic sensitivity and resistance pattern of *E. coli* isolated from different environments

Sample ID	Antibiotic Sensitive Bacteria (%)	Antibiotic Resistant Bacteria (%)	Intermediately Resistant Bacteria (%)
394	50	40	10
496	20	80	0
585	40	40	20
464	50	40	10
47990	50	50	0
47697	80	0	20
47509	100	0	0
O38	50	30	20
3491	30	50	20
689	60	40	0
647	70	20	10
595	80	10	10
168	90	0	10
564	40	50	10
47770	70	20	10
25733	60	20	20
407	10	10	80
779	20	80	0
534	70	10	20
685	80	0	20
544	60	10	30
265	30	60	10
774	20	60	20
180	20	30	50
397	10	50	40

940	50	50	0
938	40	20	40
30955	30	50	20
657	70	10	20
30987	90	0	10
26170	20	50	30
588	10	70	20
O75	0	90	10
O32	40	50	10
176	30	70	0
521	60	10	30
340	20	70	10
8996	30	40	30
755	0	70	30
C/O Saidul	20	50	30
9312	30	70	0
343	40	60	0
425	80	10	10
823	50	20	30
914	20	70	10
380	60	20	20
O64	80	10	10
47508	90	0	10
9	40	60	0
13	40	50	10
14	40	60	0
68	20	80	0
ME 3	80	0	20
ME 4	70	10	20
ME 5	30	70	0

ME 6	80	0	20
ME 7	70	0	30
ME 8	80	0	20
ME 9	70	20	10
ME 10	70	0	30
A1	20	50	30
A2	60	30	10
A6	50	40	10
A7	50	40	10
A8	30	60	10
A9	70	20	10
A10	70	20	10
A12	20	70	10
A13	20	60	10
A14	10	70	20
A18	20	70	10
A19	40	60	0
A20	20	70	10
A21	30	70	0
A23	40	30	30
A26	20	70	10
A27	20	80	0
A28	20	80	0
A30	50	30	20
A31	60	40	0
P0	40	30	30
P7	30	20	50
P10	50	30	20
P11	30	20	50
P12	20	60	20

P20	70	0	30
P21	50	20	30
P22	30	40	30
P24	60	0	40
P26	40	0	60
P27	60	0	40
P29	40	20	40
P34	90	0	10
P35	60	0	40
P37	70	0	30
P41	30	20	50
P43	70	0	30
P45	60	20	20
P50	40	30	30
P52	90	0	10
P54	60	20	20
P56	60	20	20
P58	40	0	60
P59	50	20	30
P60	70	0	30
S4	80	0	20
S5	60	0	40
S6	60	0	40
S9	50	10	40
S11	80	10	10
S12	80	0	20
S20	50	30	20
S23	80	0	20
S31	80	0	20
S33	80	0	20

S48	80	0	20
S49	40	40	20
S51	80	10	10
S56	90	10	0
S65	40	40	20
W13	40	50	10
W14	80	10	10
W27	90	0	10
W31	70	10	20
W42	70	20	10
W46	70	20	10
W49	80	0	20
W50	20	70	10
W55	70	20	10
W56	20	70	10
SF-6	60	10	30
SF-V(5)	50	40	10
S74	90	10	0
S76	60	20	20
S78	90	0	10
S79	90	0	10
S80	60	30	10
S82	70	20	10
S84	80	10	10
S85	70	0	30
W81	80	10	10
W84	10	70	20
W85	70	20	10
W86	80	0	20
W88	90	0	10

W89	60	0	40
W90	50	30	20
W91	20	30	50
W92	60	20	20
W93	80	10	10

Appendix V

Calculation formula of Similarity and Diversity indices

Calculation of Shannon Diversity Index (H) by using Microsoft Excel

Shannon Diversity Index calculation for Human Host *E .coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	6	0.1	-2.3025851	-0.2302585		5.3018981	0.530189811	
B1	30	0.5	-0.6931472	-0.3465736		0.480453	0.240226507	
B2	3	0.05	-2.9957323	-0.1497866		8.9744119	0.448720593	
C	0	0	N/A	N/A		N/A	N/A	
D	16	0.2666667	-1.3217558	-0.3524682		1.7470385	0.465876933	
E	1	0.0166667	-4.0943446	-0.0682391		16.763657	0.27939429	
F	4	0.0666667	-2.7080502	-0.1805367		7.3335359	0.488902393	
Total	60			-1.3278627	1.7632193		2.453310527	0.012196
Richness	6							
H	1.33							

Shannon Diversity Index calculation for Non Human source *E. coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	46	0.370968	-0.99164	-0.36787		0.983350225	0.364791213	
B1	56	0.451613	-0.79493	-0.359		0.631913506	0.285380293	
B2	0	0	N/A	N/A		N/A	N/A	
C	15	0.120968	-2.11223	-0.25551		4.461521337	0.539700162	
D	2	0.016129	-4.12713	-0.06657		17.03323823	0.274729649	
E	5	0.040323	-3.21084	-0.12947		10.30951697	0.415706329	
F	0	0	N/A	N/A		N/A	N/A	
Total	124			-1.17842	1.38866226		1.880307645	0.004095
Richness	5							
H	1.18							

Shannon Diversity Index calculation for Animal Host *E. coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	∑ pi.lnpi)^	(ln pi)^2	pi*(ln pi)^2	Variance
A	30	0.55556	-0.5878	-0.3265		0.34549	0.191940646	
B1	4	0.07407	-2.6027	-0.1928		6.77399	0.501777304	
B2	0	0	N/A	N/A		N/A	N/A	
C	13	0.24074	-1.424	-0.3428		2.02787	0.48819208	
D	2	0.03704	-3.2958	-0.1221		10.8625	0.40231632	
E	5	0.09259	-2.3795	-0.2203		5.66224	0.524281463	
F	0	0	N/A	N/A		N/A	N/A	
Total	54			-1.2046	1.45096		2.108507814	0.01286
Richness	5							
H	1.2							

Shannon Diversity Index calculation for Cow *E. coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	18	0.78261	-0.2451	-0.1918		0.06009	0.047023059	
B1	3	0.13043	-2.0369	-0.2657		4.14889	0.541159302	
B2	0	0	N/A	N/A		N/A	N/A	
C	0	0	N/A	N/A		N/A	N/A	
D	1	0.04348	-3.1355	-0.1363		9.83132	0.427448869	
E	1	0.04348	-3.1355	-0.1363		9.83132	0.427448869	
F	0	0	N/A	N/A		N/A	N/A	
Total	23			-0.7302	0.533143687		1.443080098	0.0424
Richness	4							
H	0.73							

Shannon Diversity Index calculation for Chicken *E. coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	8	0.38095	-0.9651	-0.3676		0.93138	0.354811861	
B1	0	0	N/A	N/A		N/A	N/A	
B2	0	0	N/A	N/A		N/A	N/A	
C	11	0.52381	-0.6466	-0.3387		N/A	N/A	
D	0	0	N/A	N/A		N/A	N/A	
E	2	0.09524	-2.3514	-0.2239		5.52897	0.526568152	
F	0	0	N/A	N/A		N/A	N/A	
Total	21			-0.9303	0.86545778		0.881380014	0.00303
Richness	3							
H	0.93							

Shannon Diversity Index calculation for Water *E. coli* Phylogroups

Phylogroup	Total Number	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	10	0.5	-0.6931	-0.3466		0.48045	0.240226507	
B1	9	0.45	-0.7985	-0.3593		0.63761	0.286926543	
C	1	0.05	-2.9957	-0.1498		8.97441	0.448720593	
Total	20			-0.85569	0.732203095		0.975873643	0.014684
Richness	3							
H	0.85							

Shannon Diversity Index calculation for Soil *E. coli* Phylogroups

Phylogrou	otal Numb	Pi	ln pi	pi*lnpi	(∑ pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2	Variance
A	1	0.04348	-3.1355	-0.1363		9.83132	0.427449	
B1	22	0.95652	-0.0445	-0.0425		0.00198	0.00189	
Total	23			-0.1788	0.0319855		0.429339	0.01822
Richness	2							
H	0.17							

Calculation of antibiotic resistance diversity by using Shannon Diversity Index (H) among Human host *E. coli* isolates

Antibiotic	Resistant Isolates	pi	lnpi	pi*lnpi	∑(pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2		Variance
F-300	2	0.00935	-4.6728	-0.0437		21.8353	0.204068		
C	2	0.00935	-4.6728	-0.0437		21.8353	0.204068		
COT	30	0.14019	-1.9648	-0.2754		3.86036	0.541171		
TE	22	0.1028	-2.2749	-0.2339		5.17532	0.532043		
AMC	13	0.06075	-2.801	-0.1702		7.84575	0.476611		
CRO	23	0.10748	-2.2305	-0.2397		4.97505	0.534702		
CFM	31	0.14486	-1.932	-0.2799		3.73258	0.540701		
CIP	34	0.15888	-1.8396	-0.2923		3.38419	0.537674		
Gen	21	0.09813	-2.3215	-0.2278		5.38915	0.528841		
AZM	36	0.16822	-1.7825	-0.2999		3.17715	0.534474		
	Total=214			-2.1063	4.4366375		4.634354		0.00103
				H=2.11					df=233.43

Calculation of antibiotic resistance diversity by using Shannon Diversity Index (H) among Non- Human source *E. coli* isolates

Antibiotic	sistant Isolates I	pi	lnpi	pi*lnpi	∑(pi.lnpi)^	(ln pi)^2	pi*(ln pi)^2		Variance	
F-300	15	0.06912	-2.6718	-0.1847		7.13877	0.493463			
C	14	0.06452	-2.7408	-0.1768		7.5122	0.484658			
COT	29	0.13364	-2.0126	-0.269		4.05056	0.54132			
TE	27	0.12442	-2.0841	-0.2593		4.34331	0.540412			
AMC	24	0.1106	-2.2018	-0.2435		4.84811	0.536197			
CRO	10	0.04608	-3.0773	-0.1418		9.46985	0.436399			
CFM	36	0.1659	-1.7964	-0.298		3.22698	0.535351			
CIP	23	0.10599	-2.2444	-0.2379		5.03735	0.533912			
Gen	13	0.05991	-2.8149	-0.1686		7.92393	0.474706			
AZM	26	0.11982	-2.1218	-0.2542			4.576417			
	Total=217			-2.2339	4.9903		9.152834		0.01928	
				H=2.23						
									t value=0.86	
									df=241.28	

Calculation of antibiotic resistance diversity by using Shannon Diversity Index (H) among animal *E. coli* isolates

Antibiotic	animal resistant isolates	pi	lnpi	pi*lnpi	∑(pi.lnpi)^2	(ln pi)^2	pi*(ln pi)^2		Variance	
F-300	8	0.07619	-2.5745	-0.1962		6.62815	0.505001683			
C	12	0.11429	-2.1691	-0.2479		4.70479	0.537690738			
COT	12	0.11429	-2.1691	-0.2479		4.70479	0.537690738			
TE	16	0.15238	-1.8814	-0.2867		3.53956	0.539361402			
AMC	20	0.19048	-1.6582	-0.3159		2.74972	0.523756258			
CRO	0	0.10798	-2.2258	-0.2403		4.95418	0.534958027			
CFM	20	0	N/A	N/A		N/A	N/A			
CIP	11	0.10476	-2.2561	-0.2363		5.08983	0.533220247			
Gen	0	0	N/A	N/A		N/A	N/A			
AZM	6	0.05714	-2.8622	-0.1636		8.19219	0.468125365			
	Total=105			-1.9347	3.7431572		4.179804458		0.00457	
				H=1.93						
									t value=0.58	
									df=233.43	

Calculation of Simpson Diversity Index (D) by using Microsoft Excel

Simpson Diversity Index calculation for Human Host *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)				
A	4	3540	3	12				
B1	32		31	992				
B2	3		2	6				
	0		-1	0				
D	16		15	240				
E	1		0	0	D=1-{∑n(n-1)/N(N-1)}			
F	4		3	12		0.643503		
Total Number(N)	60			1262				

Simpson Diversity Index calculation for Non-Human source *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)				
A	47	15500	46	2162				
B1	56		55	3080				
B2	0		-1	0				
C	15		16	240				
D	2		1	2				
E	5		4	20	D=1-{∑n(n-1)/N(N-1)}			
F	0		-1	0		0.644903		
otal Number(N	125			5504				

Simpson Diversity Index calculation for animal *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)				
A	30	2862	29	870				
B1	4		3	12				
B2	0		-1	0				
C	13		12	156				
D	2		1	2				
E	5		4	20	D=1-{∑n(n-1)/N(N-1)}			
F	0		-1	0		0.62963		
Total Number(N)	54			1060				

Simpson Diversity Index calculation for prawn *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)			
A	5	600	4	20			
B1	19		18	342			
B2	N/A		N/A	N/A			
C	1		0	0			
D	N/A		N/A	N/A			
E	N/A		N/A	N/A		D=1-{∑n(n-1)/N(N-1)}	
F	N/A		N/A	N/A		**0.396667**	
otal Number(	25			362			

Simpson Diversity Index calculation for water *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)				
A	10	380	9	90				
B1	9		8	72				
B2	N/A		N/A	N/A				
C	1		0	0				
D	N/A		N/A	N/A				
E	N/A		N/A	N/A		D=1-{∑n(n-1)/N(N-1)}		
F	N/A		N/A	N/A		**0.573684**		
Total Number(N)	20			162				

Simpson Diversity Index calculation for soil *E. coli* Phylogroups

phylogroups	individual number(n)	N(N-1)	(n-1)	n(n-1)			
A	1	506	0	0			
B1	22		21	462			
B2	N/A		N/A	N/A			
C	N/A		N/A	N/A			
D	N/A		N/A	N/A			
E	N/A		N/A	N/A		D=1-{∑n(n-1)/N(N-1)}	
F	N/A		N/A	N/A		**0.086957**	
Total Number(N)	23			462			

Calculation of Pianka's index of similarity by using Microsoft Excel

Pianka's index of similarity for (Human VS Non-Human)

Phylogroups	Non human	pj	p^2	HUMAN	pk	p^2	pj*pk
A	46	0.370968	0.137617	6	0.1	0.01	0.037097
B1	56	0.451613	0.203954	30	0	0	0
B2	0	0	0	3	0.05	0.0025	0
C	15	0.120968	0.014633	0	0	0	0
D	2	0.008065	6.5E-05	16	0	0	0
E	5	0.040323	0.001626	1	0.016667	0.000278	0.000672
F	0	0	0	4	0.066667	0.004444	0
	124		**0.357895**	60		**0.017222**	**0.037769**
						pj2pk2	**0.006164**
						SQRT	**0.07851**
						O	**0.481073**
						%	**48.1**

Pianka's index of similarity for (Human VS Animal)

Phylogroups	ANIMAL	pj	p^2	HUMAN	pk	p^2	pj*pk
A	30	0.555556	0.308642	6	0.1	0.01	0.055556
B1	4	0.074074	0.005487	30	0	0	0
B2	0	0	0	3	0.05	0.0025	0
C	13	0	0	0	0	0	0
D	2	0.018519	0.000343	16	0	0	0
E	5	0.092593	0.008573	1	0.016667	0.000278	0.001543
F	0	0	0	4	0.066667	0.004444	0
	54		**0.323045**	60		**0.017222**	**0.057099**
						pj2pk2	**0.005564**
						SQRT	**0.074589**
						O	**0.765509**
						%	**76.55**

Pianka's index of similarity for (Cow VS Chicken)

phylogroups	Cow	pj	p^2	Chicken	pk	p^2	pj*pk
A	18	0.782609	0.612476	8	0.380952	0.145125	0.298137
B1	3	0.130435	0.017013	0	0	0	0
C	0	0	0	11	0.52381	0.274376	0
D	1	0.043478	0.00189	0	0	0	0
E	1	0.043478	0.00189	2	0.095238	0.00907	0.004141
	23		**0.63327**	21		**0.428571**	**0.302277**
						pj2pk2	**0.271402**
						SQRT	**0.520962**
						O	**0.580229**
						%	**58.02**

Pianka's index of similarity for (Water VS Soil)

phylogroups	Water	pj	p^2	Soil	pk	p^2	pj*pk
A	10	0.5	0.25	1	0.043478	0.00189	0.021739
B1	9	0.45	0.2025	22	0.956522	0.914934	0.430435
C	1	0.05	0.0025	0	0	0	0
D	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0
	20		**0.455**	23		**0.916824**	**0.452174**
						pj2pk2	**0.417155**
						SQRT	**0.645875**
						O	**0.700095**
						%	**70**

Pianka's index of similarity for (Water VS Prawn)

phylogroups	Water	pj	p^2	Prawn	pk	p^2	pj*pk
A	10	0.5	0.25	5	0.2	0.04	0.1
B1	9	0.45	0.2025	19	0.76	0.5776	0.342
C	1	0.05	0.0025	1	0.04	0.0016	0.002
D	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0
	20		**0.455**	25		**0.6192**	**0.444**
						pj2pk2	**0.281736**
						SQRT	**0.530788**
						O	**0.836492**
						%	**83**