Studies on Enterotoxigenic *Escherichia coli* in slums of Jammu

By

Nikhil Sharma
(J-20-MV-644)

Thesis submitted to Faculty of Veterinary Sciences and Animal Husbandry
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CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on Enterotoxigenic *Escherichia coli* in slums of Jammu"** submitted in partial fulfilment of the requirements for the degree of **Masters of Veterinary Science**, in subject of **Veterinary Public Health and Epidemiology**, to the Faculty of **Veterinary Sciences and Animal Husbandry**, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with URKUND software provided by the University.

The work has been carried out by **Mr. Nikhil Sharma**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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ABSTRACT

Title of Thesis	:	**STUDIES ON ENTEROTOXIGENIC *Escherichia coli* IN SLUMS OF JAMMU**
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Abstract

The current investigation analysed diverse sources of samples for *Escherichia coli* and Enterotoxigenic *Escherichia coli* (ETEC). A total of 120 samples were analysed (75 drinking water, 25 waste water, and 20 dog faeces). A total of 71 samples (26 drinking water, 25 waste water, and 20 dog faeces samples) tested positive for *E. coli*, yielding 142 *E. coli* isolates. On analysis of 142 isolates by PCR for *lt*, *stIa*, and *stIb* genes to detect, none was positive. Isolates were analysed for their antibiotic resistance pattern against 9 antibiotics. The isolates yielded moderate to low resistance to antibiotics with highest resistance to norfloxacin (28.16%) followed by ofloxacin (16.91%), ampicillin/sulbactam (11.27%), ciprofloxacin (9.15%), ampicillin (7.74%), cefoperazone (3.53%) and cefixime (1.41%). Isolates were sensitive to amikacin and gentamicin. There were similarities regarding antibiotic resistance pattern among *E. coli* isolated from different sources, probably indicating about the contamination of water with faecal matter occurring in the study areas. For assessing the WASH (water, sanitation and hygiene) conditions in the study areas, data by questionnaire was collected. All households have piped water supply with fifty five percent (55%) using public taps and forty five percent (45%) using household supplies. Fifty percent (50%) of households do nothing to make water safer to drink, while thirty percent (30%) strain it with a cloth, eleven point six percent (11.6%) use a filter, and eight point three three percent (8.33%) boil it. Fifty five percent (55%) of households practise open defecation, twenty three point three percent (23.3%) have open drain toilet facilities, and twenty one point six seven percent (21.67%) have proper toilet facilities with septic tank. After defecation, all households used water and soap/detergent for hand washing. The study generated the information on water, sanitation and hygiene in the slums of Jammu.

Keywords: *E. coli*, ETEC, PCR, WASH, *lt*, *stIa*, *stIb*

Signature of Major Advisor **Signature of Student**

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ABBREVIATIONS AND SYMBOLS USED

°C	**:**	Degrees Celsius
%	**:**	Percentage
µg	**:**	Microgram
µl	**:**	Microlitre
<	**:**	Less than
>	**:**	Greater than
ADI	**:**	Acute diarrheal disease
BIS	**:**	Bureau of Indian Standards
B.C	**:**	Before Christ
Bp	**:**	Base pairs
CF	**:**	Colonization factors
CLSI	**:**	Clinical and laboratory standards institute
cm	**:**	Centimeter
DAEC	**:**	Diffusely adherent *E. coli*
DEC	**:**	Diarrhoeagenic *E. coli*
DNA	**:**	Deoxyribonucleic acid
dNTPs	**:**	Deoxyribonucleotide triphosphates
EAEC	**:**	Enteroaggregative *E. coli*
E. coli	**:**	*Escherichia coli*
EHEC	**:**	Enterohemorrhagic *E. coli*
EIEC	**:**	Enteroinvasive *E. coli*
ELIZA	**:**	Enzyme-linked immunosorbent Assay
EMB	**:**	Eosin Methylene Blue
EPEC	**:**	Enteropathogenic *E. coli*
ETEC	**:**	Enterotoxigenic *E. coli*
et al.,	**:**	Any other people
GDP	**:**	Gross Domestic Product
GIT	**:**	Gastrointestinal tract
HC	**:**	Haemorrhagic colitis
Hrs	**:**	Hours

JMP	**:**	Joint Monitoring Programme
JMP	**:**	Jammu Master Plan
LT	**:**	Labile toxin
MDR	**:**	Multidrug resistant
Mg_2Cl_2	**:**	Magnesium chloride
N/A	**:**	Not applicable
PCR	**:**	Polymerase Chain Reaction
PFGE	**:**	Pulsed field gel electrophoresis
RFLP	**:**	Restriction fragment polymorphism
RPLA	**:**	Reversed passive latex Agglutination
RIA	**:**	Radioimmunoassay
SDG	**:**	Sustainable Development Goal
STEC	**:**	Shiga toxin producing E. coli
Stx	**:**	Shiga toxin
ST	**:**	Heat stable toxin
Taq	**:**	Thermophilus aquaticus
UPEC	**:**	Uropathogenic E. coli
UN	**:**	United Nations
UNICEF	**:**	United Nations International Children's Emergency Fund
V	**:**	Volts
VTEC	**:**	Vero toxin producing E. coli
WASH	**:**	Water Sanitation and Hygiene
WHO	**:**	World Health Organization

Chapter-1

Introduction

CHAPTER-1

INTRODUCTION

Diarrhea is a serious health issue that affects people all over the world. Globally, 4 billion episodes of diarrhea are predicted to occur each year. As a result, the implications of this important healthcare issue affect 2-3 million children and 0.5-1 million adults. Children are particularly vulnerable to the negative effects of acute diarrheal disease, with each child aged 5 years having an average of 3.2 episodes of diarrhea per year (Kosek *et al*., 2003). According to recent data, diarrhea accounts for 8% of all fatalities among children under the age of five (WHO, 2019) and is the second largest cause of death in this age range (WHO, 2019).

Diarrheal diseases are primarily faecal in origin and are transmitted by the consumption of contaminated foods and drinks. This type of transmission is dependent on the availability of a asufficient and healthy water supply, as well as sanitation and hygiene, which is linked to food safety. According to the World Health Organization, poor drinking water (35%), sanitation (31%), and hygiene (12%) account for 60% of all diarrheal deaths in low- and middle-income countries, resulting in 829000 deaths each year (Pruss-Uston *et al*., 2019).

WASH (Water, sanitation, and hygiene) is defined as widespread, inexpensive, and long-term access to safe water, sanitation facilities, and soap and water for hand washing, all of which act as preventative measures to restrict the spread of illnesses and infections (UNICEF Report, 2015). WASH is a critical public health concern in international development, and it is the subject of the Sustainable Development Goal 6 (SDG 6)'s first two targets (Sustainable Development knowledge platform). According to the WHO, around 2.4 billion people still do not have access to sanitation facilities, and 663 million people do not have access to safe and clean drinking water (Joint Monitoring Programme 2015). It is predicted that increasing WASH might prevent around a tenth of the world's disease burden (Pruss-Uston *et al*., 2002). Waterborne infections afflict around 37.7 million Indians each year, with 1.5 million children dying from diarrhea alone (Parsai and Rokade, 2019).

Diarrhea can be caused by a variety of infections. Many bacterial pathogens (*Escherichia coli*, *Vibrio cholerae*, *Shigella*, *Salmonella Typhi*, *Campylobacter*, and others), viral pathogens (Rotavirus and Norovirus) and parasitic pathogens (*Cryptosporidium*, etc.) are on the list. In underdeveloped countries, bacterial infections such as *E. coli*, *Shigella*, and *Salmonella typhi* represent a major public health concern (Cabral, 2010). Furthermore, in developing nations, only two microorganisms, *E. coli* and Rotavirus, are responsible for the majority of episodes of mild to severe diarrhea (WHO, 2017).

The Gram-negative, rod-shaped commensal bacteria *Escherichia coli* resides in the intestines of warm-blooded animals and humans. It is extremely unusual that it causes illness (Singleton, 1999). When the bacterium invades the intestine, however, some pathotypes induce diarrhea. *E. coli* pathotypes associated with intestinal disorders include enterotoxigenic E. coli (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC). One or more distinct virulence markers identify each pathotype.

In underdeveloped and developing nations, ETEC infection is one of the most common causes of diarrhea. Children under the age of five are particularly vulnerable to intestinal infection, with 400 million diarrheal cases recorded annually, 280 million of which are children (Wenneras and Erling, 2004). ETEC is also responsible for 50-60% of all instances of traveler’s diarrhea from industrialized to developing nations, particularly in areas with poor sanitation, with an estimated 60 million cases per year (Gascon *et al*., 1998; Qadri *et al*., 2005; Jiang *et al*., 2002). According to a study, ETEC infection alone causes an estimated 3,70,000 deaths every year, the bulk of which are youngsters (Wenneras and Erling, 2004).

Two types of plasmid-encoded virulence factors are involved in the pathogenesis of ETEC: a. CFs (colonization factors): It's an adhesion molecule found on bacteria's surfaces that helps them colonize the small intestine by binding to enterocytes (Gaastra and Svennerholm 1996; Nataro and Kaper 1998), b. Toxins: ETEC makes two types of enterotoxins: heat-labile (LT) and heat-stable (ST). In humans, diarrhea is induced when the enterotoxins LT and ST bind to receptors on the intestinal epithelium and activate signaling pathways, causing substantial volumes of water and electrolytes to be secreted into the intestinal lumen (Field *et al*., 1978; Sack 1980; Sixma *et al*., 1993). ETEC strains can produce one or both toxins at the same time. ETEC is carried in the intestines of both animals and people, and transmission of the disease occurs when water is contaminated with faeces. ETEC has been found to survive for lengthy periods of time in fresh water (Lothigius *et al*., 2010), suggesting that water is one of ETEC's reservoirs. Pathogenic *E. coli* has also been found in the intestines of pet dogs (VegaManriquez *et al*., 2020); however, there are few studies on pathogenic *E. coli* in dogs. Additionally, the majority of ETEC isolated from diarrheic canines are ST-positive (Hammermueller *et al*., 1995).

Slums are residential areas where dwellings are in any way unfit for human habitation due to dilapidation, overcrowding, faulty arrangements and designs of such buildings, narrowness or faulty arrangement of streets, lack of ventilation, light, sanitation facilities, or any combination of these factors that are detrimental to safety, health, and morals, as defined by the Slum Area Improvement and Clearance Act (1956). A slum, according to UN Habitat, is defined by a lack of long-term housing, insufficient living space, a lack of access to clean water, poor sanitation, and insecure tenure.

There are numerous reports on the assessment of WASH conditions and diarrheal cases in slums around the world. Furthermore, some research have looked into the role of ETEC in causing diarrhea in such neighborhoods. Some research analyzing WASH conditions in slums and the association between WASH conditions and diarrheal cases in these regions are available at the country level (Pati *et al*., 2014; Sangra *et al*., 2020). The link between diarrhea in children under the age of five and contaminated drinking water has been discovered in slum studies (Patil *et al*., 2012; Gupta *et al*., 2015; Bhar *et al*., 2017). However, there is still a need to examine the risk of ETEC through slum drinking water. Furthermore, there is no information on the transmission of ETEC by humans and dogs in poor regions. At both the global and national levels, such data is scarce.

According to the 2011 Indian Census, Jammu and Kashmir contains less than 1% of the country's total slum population. Nonetheless, the region is immensely popular among the working class, particularly those from Uttar Pradesh, Bihar, and Madhya

Pradesh, because J&K has a greater per capita income than these states (India GDP per capita by state, 2020). These low-wage employees in Jammu and its environs live in overcrowded neighborhoods with inadequate sanitation. These labourers and other communities live in squalid conditions in slums and other regions that have been designated as slums. Jammu city and its outgrowths contain eleven notified slums, according to the Jammu Master Plan 2032. Overcrowding in these places may exacerbate the lack of safe drinking water and insufficient sanitation, as well as cause diarrheal outbreaks. Only one study on WASH conditions in slums in Jammu, Jammu and Kashmir is accessible (Sangra *et al*., 2020), but no studies on water potability or risk assessment of ETEC in drinking water or waste water are available. Nonetheless, there are limited research on *E. coli* identification in the Jammu region. *E.coli* has been found in river samples (Mahajan *et al*., 2018), while EHEC and EPEC have been found in drinking water in one investigation (Najimaana *et al*., 2012). EHEC was found in river samples from the Jammu region by Padha (2021). Water samples gathered from several districts of Jammu failed to meet BIS criteria, according to a story published in the newspaper Daily Excelsior on November 20th, 2019.

The emergence and spread of bacterial resistance to frequently used antibiotics has sparked widespread alarm. In this regard, many governments have created resistance monitoring systems in order to protect the health of both humans and animals. Antimicrobial therapy is frequently required to treat infections caused by this bacterium. Antibiotic resistant genes have spread rapidly, aided by mobile genetic elements such as plasmids and transposons, resulting in the establishment of multidrug resistant (MDR) strains of many therapeutically relevant species, leaving doctors with few treatment options.

As ETEC is one of the most important diarrheal agents and slum area conditions facilitate transmission of such diarrheal agents, this study was conducted with the following objectives:

1. To assess the drinking water and waste water of slums of Jammu for Enterotoxigenic *Escherichia coli.*

2. To assess the dog faecal samples of slums of Jammu for Enterotoxigenic *Escherichia coli*

3. To determine the antibiotic resistance of *Escherichia coli* isolates.

Chapter-2

Review of Literature

CHAPTER-2

REVIEW OF LITERATURE

Because water covers the majority of the earth's surface, it is the most precious resource. Fresh water makes up only 2.5% of total water, and the majority of it is frozen and inaccessible, with less than 1% of fresh water, such as lake water, river channels, and underground water, being accessible. World Water Day was founded on March 22nd, 1992, following the Earth Summit in Rio de Janeiro, Brazil, to highlight the importance of water as a valuable resource that is vital for living. Health is a valuable commodity that demands utmost care. Water and health are two invaluable resources that, when combined, boost the chances of progress.

Waterborne illnesses have been documented from ancient times, with descriptions occurring in the 'Sushruta Samhita,' a Sanskrit literature produced in India between 500 and 400 B.C. (Colwell,1996). Waterborne illnesses can harm a significant number of individuals in a short amount of time if contaminated water is used (Mellou *et al*., 2014).

Pathogens can contaminate a variety of water sources, including natural and manmade sources used by humans and animals (Adzitey *et al*., 2015). In the underdeveloped world, children and adults suffer from infectious diarrheal disease on a regular basis, with water being the most common source of pathogen exposure (Sobsey *et al*., 2003). According to the World Health Organization (WHO), 2 million deaths worldwide are caused by waterborne infections each year, with the majority of these deaths occurring in children under the age of five (WHO, 2017). In India, diarrheal illnesses are the third greatest cause of pediatrics mortality, accounting for 13% of all deaths in children under the age of 5 (Lakshminarayanan and Jayalakshmy, 2015). Due to faecal contamination of waterbodies, which renders them microbiologically unsafe for consumption, waterborne infections are the most common cause of morbidity and mortality in both underdeveloped and developing countries, with 80% of infectious diseases being waterborne in India (Saxena *et al*., 2015). Untreated waste is thought to account for a quarter of all waste for contaminating three-quarters of India's surface water resources, contributing to 75-80% of water pollution (Anonymous, 2016).

While studying the intestinal flora of newborns, Dr. Theodor Escherich, a German-Austrian doctor, isolated *E. coli* and named it Bacterium coli commune in 1885. (Shulman *et al*., 2007). Bacterium coli commune was called *Escherichia coli* by Castellani and Chalmer in 1919. (Castellani and Chalmer, 1919).

Shardinger proposed *E. coli* as a marker organism for faecal pollution in water in 1892, citing the bacterium's origins in human and animal faeces and its absence from other specializations. As a result, the presence of *E. coli* in water suggests recent faecal contamination and is a component of the coliform index, which includes bacterial species that are used to determine sanitary hygiene (Leclerc *et al*., 2001). Approx. 109 *E. coli* germs have been found in one gram of faeces (Payment *et al*., 2003). Coliform bacteria, which include the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*, were once thought to signify faecal pollution in water; however, *Citrobacter*, *Enterobacter*, and *Klebsiella* have been discovered to be extensively distributed in nature and are not usually connected with the gastrointestinal tract of warm-blooded animals (Martin *et al*., 2016). This gave rise to the idea of 'faecal coliforms,' a subtype of coliform bacteria that can grow at a higher temperature (44.5°C) (thermotolerant coliforms) and is a better diagnostic of faecal contamination than coliforms. *E. coli* is the most significant bacterial genera of the 'faecal coliforms' group, as it is exclusively linked with the gastrointestinal tract of warm-blooded animals, and its presence in water always implies faecal contamination of water (Cabral, 2010). *E. coli* shall not be detectable in any 100 ml sample of drinking water, according to WHO bacteriological drinking water recommendations (WHO, 2017).

Despite the fact that *E. coli* is a commensal bacteria found in the intestines that rarely causes disease in its host, distinct *E. coli* pathotypes have emerged that cause gastrointestinal and systemic infections in humans. Diarrheagenic *E. coli* is a phrase that refers to *E. coli* pathotypes that cause intestinal disease (DEC). The six pathotypes discovered in DEC are ETEC, VTEC, EIEC, EPEC, EAEC, and DAEC. Depending on the type of infection, DEC can cause anything from mild diarrhea to severe hemorrhagic colitis in humans (Park *et al*., 2018). The infectious dose of DEC varies by pathotype, however it can be as low as 10 bacteria in the case of VTEC. The most common DEC pathotypes that cause infectious diarrhea in young children in most poor countries are EPEC, ETEC, and EAEC (Rajendran *et al.*, 2010). Because routine microbiological and biochemical analysis of *E. coli* isolates cannot identify and differentiate six DEC pathotypes, identification techniques such as Polymerase Chain Reaction (PCR), Radioimmunoassay (RIA), Enzyme-linked immunosorbent Assay (ELISA), Reversed passive latex Agglutination (RPLA), RFLP (Restriction Fragment Polymorphism), and Pulsed field gel electrophoresis (PFGE) are used.

ETEC

Traveler's diarrhea is caused by the ETEC bacteria. There are two types of enterotoxins produced by it: heat-labile (LTs) and heat-stable (STs). ETEC strains can have one or both of them. Increased chlorine secretion from secretory crypt cells is caused by LT, resulting in diarrhea. STs are single-peptide toxins that fall into two distinct categories: STa and STb enterotoxins, which differ in structure and mechanism of action. Human disease has been linked to the STa class, which is a 2-kDa peptide with 18 or 19 amino acid residues. It stimulates secretion via increasing intracellular cGMP, which activates cGMP-dependent and/or cAMP-dependent kinases. STb toxin is a 48-amino-acid peptide with two disulphide linkages that is linked to animal illness (Nataro and Kaper, 1998). STb increases cytosolic Ca2+ levels and stimulates the release of prostaglandin E2 and serotonin, all of which can lead to enhanced ion secretion. ETEC has a high infectious dose (106 organisms) and an incubation period of 12–72 hours. It is the leading cause of death in children under the age of five in poor countries (Percival *et al.*,2004). ETEC is usually detected using PCR for the *lt* and *st* genes, which encode the LT and ST toxins, respectively.

Studies on WASH conditions and diarrheic cases in slums

For Jammu area of Jammu and Kashmir, one study by Sangra *et al.* (2020) is reported. Study was conducted in urban slums of Trikuta Nagar, Jammu to assess WASH conditions. Study reported that 60% of household members were getting water from tube well/bore well, 82.5% of household members were using improved source of water, 21.5% of individuals treat their water through boiling, 49.5% of individuals used improved sanitation facilities, 90% of households were using shared toilets and stool of 51.72% of children under the age of 3 years who were not using toilets were disposed off safely.

Mathur *et al.* (2019) conducted a study related to WASH programme in rural as well as slums of Ujjain, India. Randomly 2830 households were selected and 1181 households meet eligibility criteria of having children less than 5 years. A total of 1571 children participated including 858 children from nine rural villages and 713 children from four urban slums. 60% of households used hand-pump as source of drinking water and 71% of households filtered water by simple cloth before storing. 93% households had a toilet in their houses but only 23% children used them. From total, 521(33%) children were suffering with acute diarrhea.

Adane *et al.* (2017) carried out a community based cross-sectional household survey on sanitation facilities, hygienic conditions and prevalence of acute diarrhea on children under five years age in 2014 in Addis Ababa. 697 children of age group 0-50 months from two districts were selected. The survey reported that the prevalence of acute diarrhea in children of age group 0-50 months was 11.9% and 94.6% of sanitation facilities were unimproved. This study observed that the environment of slum was at high risk for diarrhea because of close proximity of sanitation facilities to homes, sharing of sanitation facilities and poor hygienic condition of the sanitation facilities and housing compounds.

Melo *et al.* (2008) conducted a study on diarrhea incidence in children of two urban slums of Salvador in Brazil. 42 children younger than 40 months from each slum were selected and information from their parents about the occurrence of diarrhea on present day or on previous day was collected. The findings revealed that the prevalence of diarrhea at the moment of first visit was 3.6%, about 2.8 episodes per child per year. 25% of children did not experience any episode of diarrhea during whole observation period. On an average 5.9% children were affected per month. About 11.1 days of diarrhea per child per year was reported.

Studies on ETEC in human diarrheic cases

Nycz *et al.* (2020) conducted a study on enteropathogenic *E. coli* in pediatric patients in which 5692 stool samples of children were collected from October 2015 to October 2017. They took samples for testing with the Bio Fire FilmArray Gastrointestinal Pathogen Panel (GIP). Among 5692 stool samples, 58(1%) were positive for ETEC. Out of 58 ETEC positive patients, 49 (85%) were coinfected with other pathogens also.

Singh *et al.* (2019) carried out a cross-sectional study on DEC between January 2008 and December 2012 in two institutions at Belgaum, Karnataka. 300 stool samples from diarrheic patients were analyzed. 198(66%) *E. coli* isolates were detected. On virulence gene typing, ETEC (13.6%) was third predominant DEC pathotype.

Moharana *et al.* (2019) carried out a study on 320 diarrheic children under five years of age. From 320 cases, 77 were positive for DEC including 40 (51.9%) ETEC. More than 60% of DEC were resistant to 3rd generation cephalosporins and fluoroquinolones.

Trainor *et al.* (2016) carried out a study of detection of ETEC in hospitalized acute gastroenteritis children under 5 years of age at Queen Elizabeth Central Hospital (QECH), Blantyre Malawi in between July 1997 and June 2007. Other Children of same age group admitted in QECH for acute medical illness other than gastroenteritis were also selected (July 1997-June 1999). The total number of diarrheal samples collected was 1941. QIA symphony (Qiagen) was used to detect ETEC by extracting nucleic acid from 10% faecal suspension for detecting ETEC heat labile (LT) and heat stable (STh and STp) enterotoxins. Out of 1941 diarrheal samples, ETEC were detected in 205 (10.5%) samples. During 1997-1999, ETEC prevalence was higher in children with diarrhea rather than without diarrhea. The most common ETEC toxin in diarrheic cases was STh (6.6%) followed by LT (2.1%) and then STp (0.9%).

Bonkoungou *et al.* (2013) conducted a study on childhood diarrhea between January 2009 and January 2010 at Central Mediacl Antenne Chiruicale. They collected stool samples of 283 children suffering from diarrhea and 60 non-diarrheic children (control); all children were under 5 years of age. For the detection of DEC, the stool samples were cultured on Sorbitol MacConkey Agar and followed by PCR for identification of ETEC. DEC were identified in 67 (24%) diarrheic cases and 4 (7%) non-diarrheic cases with ETEC in 10 (4%) and 0 cases, respectively. Among DEC, ETEC was at 3rd in causing diarrhea among children under 5 years of age.

Dutta *et al.* (2013) conducted a study on prevalence of DEC in which they collected 3826 stool samples of all age group patients suffering from acute diarrhea from January 2008-December 2011 in hospitals of Kolkata. Out of 3826 patients, 863 were children less than 2 years of age. For isolation and identification of DEC, stool samples were plated on MacConkey agar and identification of DEC was done using PCR (*eltB* and *estB* for ETEC). 452 patients were positive for DEC. 164 patients out of 452 were positive for ETEC including 27 children below 2 years of age.

Rodas *et al.* (2011) conducted a study on ETEC on hospitalized children under 5 years of age with diarrhea from 2002 to 2006 in La Paz, Bolivia. 853 samples from non-bloody diarrhea affected children were analyzed. For detection of ETEC, the study used GM1 ELISA, multiplex PCR and dot blot. Out of 853 samples, 79 were positive for ETEC. Among ETEC strains, *lt* toxin was most common. Antimicrobial resistance was highest for ampicillin (53.5%) followed by ampicillin-sulbactam (46.5%), trimethoprim-sulfamethoxazole (32.5%), tetracycline (28%), and chloramphenicol (14%).

Rajendran *et al.* (2010) carried out a study on pathotypes of DEC in children in Vellore, in which they selected 394 children less than 5 years suffering with acute diarrhea and 198 children as control. From 394 diarrheic samples, 203 (52%) DEC isolates were found. Out of 198 control children, 126 (63%) DEC pathotypes were found. Among cases and controls, ETEC was detected as 16 (4.1%) and 8 (4%) respectively.

Moyo *et al.* (2007) conducted a cross-sectional study on identification of DEC among children of age less than 5 years (n=280) admitted to hospitals due to diarrhea from December 2005 to February 2006. Out of 280 children, 10 (3.6%) were infected with ETEC. The detected strains of ETEC had either toxins *st1a* or *st1b* but not both.

Nguyen *et al.* (2006) started a study on etiology and epidemiology of diarrhea in children from March 2001 to April 2002 in Hanoi Vietnam. 836 children of age group 0-

60 months were selected including 587 children suffering from diarrhea and 249 children having no any diarrheal episode since last one month. For identification of pathogens, they carried out conventional methods in combination with ELISA, immunosuppression, and PCR. In this study, the second most prevalent pathogen for causing diarrhea is Diarrheagenic *E. coli* (The first most prevalent pathogen was Group A rotavirus). Among DEC, ETEC was isolated from 13 (2.2%) diarrheic cases while 1 sample from non-diarrheic children.

Studies on ETEC in water sources and waste water

Padha *et al*. (2021) investigated the various water sources in the Jammu region. A total of 141 water samples were tested (43 rivers, 28 ponds, 10 lakes, 40 municipal supplies, and 20 wells). Following PCR, 56 samples (27 river, 20 pond, and 9 lake samples) were found to be positive for *E. coli* and 146 *E. coli* isolates were collected from 81 natural water sources. None of the 146 isolates tested positive for the *lt*, *stIa*, *stIb*, or *eaeA* genes, but five (3 river and two pond samples) tested positive for *stx1*, and one isolate tested positive for *stx2*. 15 samples (13 municipal supply and 2 wells) were positive for *E. coli*, and 43 *E. coli* isolates were recovered from 60 samples taken from sources meant for human consumption. The *lt*, *stIa*, *stIb*, *eaeA*, *stx1* and *stx2* genes were not found in any of the 43 isolates.

Roman *et al.* (2019) conducted a study on DEC from January to December 2015. 472 samples including 429 samples from irrigation canal, 29 from river water, 9 from dike water and 5 from dam water were analyzed. 43.6% samples were positive for *E. coli.* Of 43.6% *E. coli* isolates, DEC was isolated only in 14% samples and among 14% of DEC, 6.89% were ETEC. In study, half of strains were resistant to cefotaxime (48%), followed by ampicillin (44%), tetracycline (38%), sulfamethoxazole-trimethoprim (14%), ceftazidime (10%) and gentamicin (7%). The ETEC isolates strains presented resistance to tetracycline ampicillin (7%), and trimethoprim-sulfamethoxazole (4%).

Harada *et al.* (2018) conducted a study in Bangladesh investigating the occurrence of *E. coli* pathotypes in sanitary wastewater as well in drinking water (tube well and stored drinking water) in an urban slum. From the samples of deep tube well water, no *E. coli* were detected, but 17 out of 18 stored drinking water samples were positive for *E. coli*. The study showed that during storage the drinking water gets contaminated. Through multiplex PCR, and dual index sequencing, 14 virulence genes from the 621 *E. coli* isolates were analyzed. ETEC was not detected in any stored water sample while sanitary wastewater was positive for ETEC. *StIb* was the dominant pathotype among sanitary wastewater.

Too *et al.* (2016) conducted a study on faecal contamination of drinking water between 2013 and 2014 in Kericho district in Western Kenya. During this study, water samples were collected from 103 households. Samples were incubated on lauryl sulphate agar at 44-46^{O}C for 18-24 hours for thermotolerant coliform (TTCs). *E. coli* were confirmed using lactose pentose water and indole test. ETEC were identified using multiplex PCR. Out of 103 households, 48(46.6%) households were positive for TTCs and 5 (10.4%) were positive for pathogenic *E. coli*. Out of 5 pathogenic *E. coli,* 2 (40%) were ETEC. The pathogenic *E. coli* in this study were resistant to commonly used antibiotics in Kenya i.e., sulfamethoxazole/trimethoprim, ampicillin, tetracycline and sulbactam.

Gumus *et al*. (2015) investigated the presence of several *E. coli* pathotypes in Istanbul, Turkey. A total of 134 water samples were collected and evaluated for ETEC, EHEC, and EPEC from the taps and water tanks of two separate medical hospitals, as well as fifty different branded drinking water spring samples. *E. coli* was found in two water tank samples and one spring water sample. None of them tested positive for EHEC or EPEC, however one *E. coli* sample from drinking water tested positive for the *lt* gene.

Nontongana *et al.* (2014) conducted a study on the prevalence of *Escherichia coli* pathotypes from the Kat river in South Africa. Samples were collected from five sites once a month for three months. The membrane filtration technique was used. From study, a total of 278 *E. coli* isolates were analyzed for the presence of pathotypes using PCR. ETEC was the most prevalent pathotype accounting for 47% of the isolates. Approx. 98% of isolates were 100% susceptible to norfloxacin followed by amikacin (97%), then ciprofloxacin (93%), streptomycin (77%), tetracycline (75%) and chloramphenicol (73%).

Akter *et al.* (2013) conducted a study on major water bodies in Bangladesh. They collected 46 water samples including 35 from fresh water sites and 11 from marine/brackish sites. ETEC was the most prevalent pathotype among DEC. ETEC prevalence during winter, summer and rainy season was 63%, 61% and 76%, respectively. In case of marine samples, ETEC was most prevalent in winter (46%) as well as in rainy season (37%) while in case of fresh water sites, ETEC was the most prevalent during summer (86%) and rainy season (72%).

Taneja *et al.* (2012) conducted a study on the relationship between drinking water and acute gastroenteritis due to ETEC in Chandigarh. They collected 364 water samples (251 semi urban, 72 urban and 41 from rural area). 116 (31.8%) samples were contaminated with faecal coliforms (58.5% rural, 33.4% semiurban and 11.1% of samples from urban areas). 92 isolates of *E. coli* were tested for detection of *lt* through reverse passive agglutination method and *st* through ELISA. Out of 92, 8 and 24 were positive for *lt* and *st*, respectively.

Taneja *et al.* (2011) conducted a study on faecal contamination of drinking water supplies in and around Chandigarh. They collected 364 water samples from semi urban area (n=251) followed by urban (n=72) and rural area (n=41) from different sources viz., water pumps (n=12), water stand posts (n=47), public taps (n=206), stored water (n=37), mineral water bottles (n=6), tube wells (n=13), hand pumps (n=35), village wells and ponds (n=8). Out of 364 samples, 116 (31.8%) samples were positive for faecal coliforms, out of which 58 samples were used for isolating *E. coli*. 92 *E. coli* were obtained which were screened for ETEC and 8 were positive for *lt*, 24 were positive for *st* and 4 ETEC strains produced both *lt* and *st*. ETEC were found to be maximum for stored water samples followed by ponds and public taps.

Ram *et al.* (2008) investigated to determine the virulence gene in surface water isolates of *Escherichia coli* from Gomti river. A total of six samples from different sites of river were collected three times. From this study, a total 90 isolates of *E. coli* were obtained and subjected to PCR to detect virulence genes. Results shows that *lt* and *st* genes were positive in 21.2% of isolates.

Pathak and Gopal (2008) conducted a study in Lucknow for ETEC in treated drinking water from 11 residential, 6 commercial and 3 industrial areas of Lucknow. The study revealed that the water contamination of residential area with *E. coli* was highest (59%). For detection of entero toxigenicity of *E. coli* isolates (n=10), suckling mouse bioassay was used. 60% of test isolates were found to be enterotoxigenic.

Begum *et al.* (2007) carried out a study for comparison of ETEC isolated from surface water and diarrheal stool samples. They collected water samples from February 2001 to September 2001 twice every month from Dhaka city and Matlab rural field. They collected 94 water samples around Dhaka city and 56 samples from Matlab area. They also collected 1156 stool samples from patients of Matlab area and 1544 from Dhaka city. Then fresh stool and water samples were plated on MacConkey agar. For confirmation of ETEC freshly cultured colonies were tested for presence of toxins by GM1 ELISA procedure. From 94 water samples from the Dhaka and 56 samples from Matlab, ETEC was isolated from 29 samples and 16 samples, respectively. From total 45 ETEC strains, 30 strains were positive for *st*, 11 for both *lt* and *st*, and 4 for *lt*. From 1156 stool samples of Matlab patients, 212 ETEC strains were isolated while from 1544 stool samples of Dhaka 306 ETEC strains were isolated.

Begum *et al.* (2005) conducted a study for isolating ETEC from surface water in Bangladesh. 107 samples were obtained from seven water sources in and around Dhaka (five ponds and two rivers) as well as four sources in Matlab (one river, one lake, and two ponds). The technology utilized was membrane filtration. ELISA was used to assess *E.coli* isolates for heat-labile toxin (LT) and heat-stable toxin (ST). ETEC was isolated from 32% of the samples (34/107), and it was discovered that 65 percent of ETEC produced only heat stable toxin (ST), 9% produced only heat-labile toxin (LT), and 26% produced both heat-labile and heat-stable toxins.

Studies on DEC in dogs

Vega-Manriquez *et al.* (2020) conducted a study on transmission of pathogenic *E. coli* through dogs in Mexican city of San Luis Potosi. They analyzed 30 dog samples including 13 diarrheal and 17 non-diarrheal dogs. 6 diarrheic and 7 healthy dog samples yielded *E. coli*. From 13 positive samples, 85 *E. coli* isolates were obtained. Among 46 isolates from healthy dogs, 39 isolates were positive for any of *eaeA*, *st* or *stx1* genes while 7 isolates have combination of virulence genes (*eaeA*/*st*, *eaeA*/*stx1* and *st*/*stx1*). Among 39 strains from diarrheic dogs, 24 were possessing *eaeA*. 30% of *E. coli* strains were resistant to penicillins, parenteral cephalosporins and folate inhibitors while all *E. coli* strains were sensitive to carbapenems, nitrofurantoins, monobactams, fluoroquinolones and phosphomiocins.

Askari *et al*. (2020) undertook a study in Kerman Province, in the southeast of Iran, to identify the virulence genes linked to diarrheagenic *E. coli* strains detected from healthy pet dogs, and their owners. A total of 49 healthy domestic dogs and their owners produced 168 *E. coli* isolates. As a control group, 70 isolates were gathered from non-pet owners. The most prevalent pathotypes identified in dogs, their owners and controls by PCR were EIEC (6.1%), EHEC (4.08%) and EPEC (8.5%), respectively. Both the dog and its owner from one of the houses under the study had *E. coli* strains with the same pathotype EHEC. Even people who don't own pets seemed to be a significant source of intestinal pathotypes for their environment, particularly EPEC.

Hammermueller *et al*. (1995) conducted a study at Ontario Veterinary College in Canada, in this study faeces samples from dogs with and without diarrhea were analyzed. The results showed that 20 of the 45 dogs with diarrhea had *stIa* (26.7%) and *stIb* (4.4%) in their *E. coli* isolates, *stIa* and *stIb* genes were not found in non-diarrheic dogs. Heat labile (*lt*) gene was not found in faecal samples from dogs with or without diarrhea.

Chapter-3

Materials and Methods

CHAPTER-3

MATERIALS AND METHODS

3.1 Sampling

One hundred twenty samples comprised of drinking water (n=75), waste water (n=25) and dog faecal samples (n=20) were collected from six slums of Jammu district. Samples were collected aseptically in sterilized sampling bottles with proper ice packs and were processed at Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S.Pura, Jammu. Table 1 shows number of samples with respective areas. Samples were collected from September 2021 to May 2022.

Table 1: Location, type and number of samples for isolation and identification of E. *coli*

S.No.	Site Name	No. of Drinking water samples	No. of Waste water samples	No. of Dog faecal samples	Latitude	Longitude
1.	Dhounthly	10	5	4	32.743226	74.873580
2.	Rajiv nagar	13	4	3	32.719258	74.890138
3.	Qasim nagar	12	3	3	32.719445	74.889909
4.	Sheikh nagar	12	3	3	32.719441	74.889920
5.	Kalka colony	14	5	3	32.720292	74.875578
6.	Pata paloura	14	5	4	32.751621	74.815281
	Total	75	25	20		

3.2 Media, Reagents and Biologicals

3.2.1 MacConkey Broth (Double strength) (HiMedia) (Catalogue No. M539-500G)

Ingredients Gms/litre of media

Peptone	40.00
Lactose	20.00
Bile salts	10.00
Sodium chloride	10.00
Neutral red	0.15

Heat was applied to completely dissolve the 80.15 grams of dehydrated media that was suspended in 1000ml of distilled water. The media was autoclaved for 15 minutes at 121^0C and 15lbs of pressure to sterilise it.

3.2.2 MacConkey Agar (HiMedia) (Catalogue No. M008E-500G)

Ingredients Gms/litre of media

Peptone	20.00
Sodium chloride	5.00
Lactose	10.00
Bile salts	5.00
Neutral red	0.07
Agar	15.30

Heat was applied to completely dissolve the 55.37 grams of dehydrated media that was suspended in 1000ml of distilled water. The media was autoclaved for 15 minutes at 121^0C and 15lbs of pressure to sterilise it.

3.2.3 EMB Agar (HiMedia) (Catalogue No. M317-500G)

Ingredients Gms/litre of media

Peptone	10.00
Dipotassium hydrogen phosphate	2.00

(a)

(b)

Plate 1: Collection of drinking water samples

(a)

(b)

Plate 2: Collection of waste water samples

Plate 3: Collection of dog faecal sample

Plate 4: Data collection related to WASH

Lactose	5.00
Saccharose (Sucrose)	5.00
Eosin Y	0.40
Methylene blue	0.065
Agar	13.50

Heat was applied to completely dissolve the 35.96 grams of dehydrated media that was suspended in 1000ml of distilled water. The media was autoclaved for 15 minutes at 121^0C and 15lbs of pressure to sterilise it.

3.2.4 Nutrient Agar (HiMedia) (Catalogue No. M001-500G)

Ingredients Gms/litre of media

Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	1.50
Sodium chloride	5.00
Agar	15.00

Heat was applied to completely dissolve the 28.0 grams of dehydrated media that was suspended in 1000ml of distilled water. The media was autoclaved for 15 minutes at 121^0C and 15lbs of pressure to sterilise it.

3.3 Isolation of *Escherichia coli*

For isolation of *E. coli* from waste water samples, 1ml sample was added in single strength MacConkey's broth (HiMedia, India) followed by incubation for 24 hrs at 37°C. For drinking water samples, samples were subjected to membrane filtration method by using sterile Gridded cellulose nitrate membrane having diameter 47mm and pore size 0.22 micron (HiMedia, India). The membrane was put into the double strength MacConkey's broth and incubated for 24 hrs at 37°C. For dog faecal samples, faecal samples were swabbed in single strength MacConkey's broth and incubated for 24 hrs at 37°C. After incubation, the broths were analysed for change in colour to identify growth (yellow colour) and presence of gas bubbles in durham tubes (durham tubes were present in all big tubes) which shows the presence of lactose fermenter. The tubes showing yellow colour growth and gas, were picked for further isolation and identification of *E. coli*. From each positive, the inoculum was streaked on eosin methylene blue (EMB) agar (HiMedia, India) and incubated at 37°C for 24 hours. The dark purple colonies having greenish metallic sheen were presumed to be *E. coli*. From each EMB plate, 2-3 presumed colonies were picked and purified on nutrient agar plates for further confirmation by gram staining and biochemical tests.

FLOW DIAGRAM FOR *E. coli* ISOLATION FROM DIFFERENT SAMPLES

Samples

↓

Drinking water samples were first subjected to membrane filtration method, membrane added into double strength MacConkey broth/1ml Waste water samples directly added into single strength broth/Dog faecal samples were also directly added into single strength MacConkey broth and incubated at 37ºC for 24 hrs

↓

Observed for change in colour (yellow) and gas bubbles captured inside durham tubes

↓

Tubes giving yellow colour and presence of gas bubbles inside durham tubes were selected

↓

Selected tubes were streaked on EMB agar plates and incubated at 37ºC for 24 hrs

↓

On EMB agar plates, colonies with a metallic sheen or a dark purple colour were chosen for further investigation

↓

On nutrient agar plates, suspected colonies were streaked, and they were then incubated at 37ºC for 24 hrs

↓

Further Gram’s staining and biochemical characterization
Gram (-), Catalase test (+), Oxidase test (-), IMViC (+ + - -)

↓

Molecular analysis (PCR)

Plate 5: Growth of lactose fermenter in MacConkey broth

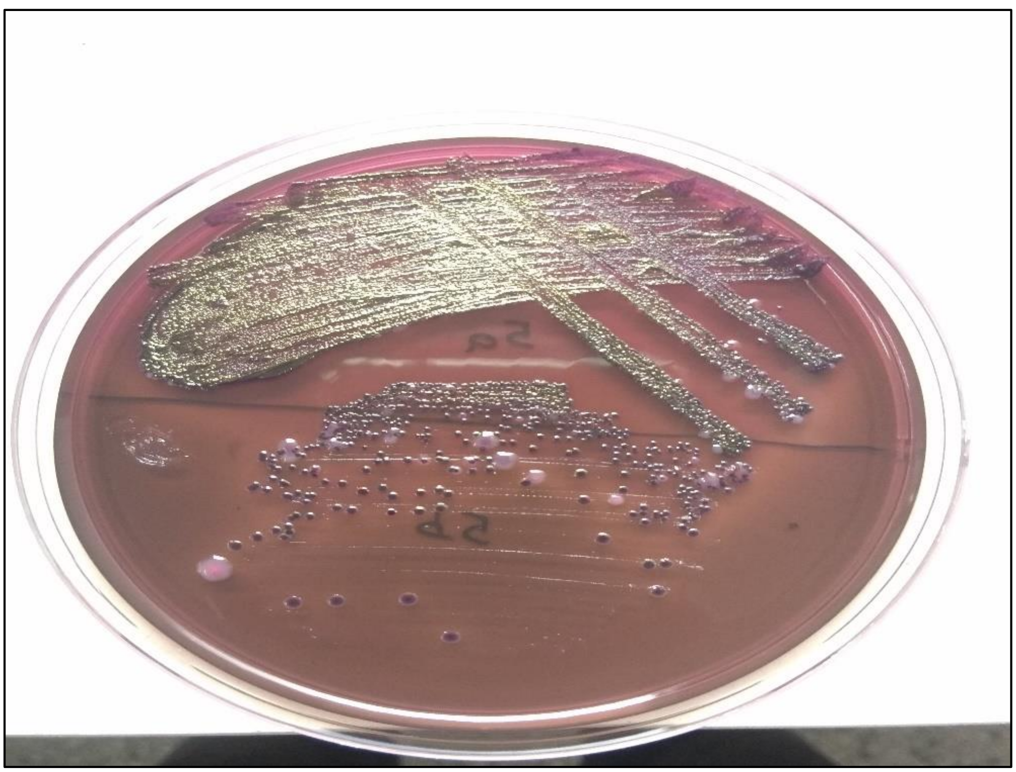

Plate 6: Growth of *E. coli* on EMB agar

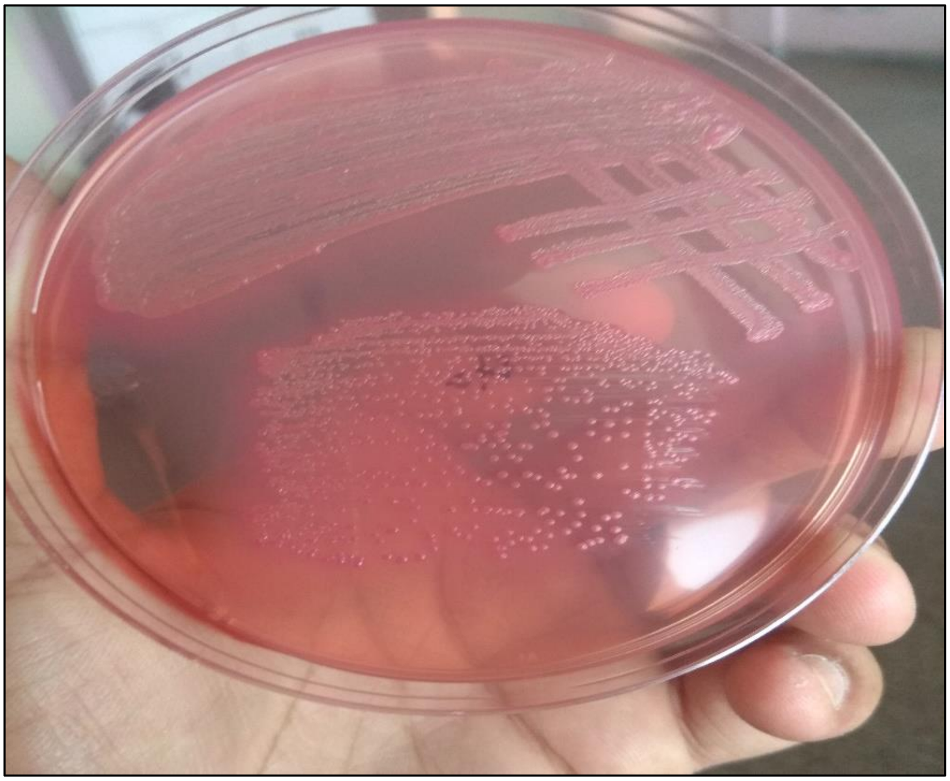

Plate 7: Growth of *E. coli* on MacConkey agar

Plate 8: Growth of *E. coli* on Nutrient agar

3.4 Morphological and Biochemical analysis

For confirmation, Gram's staining and biochemical assays were performed on suspected *E. coli* isolates purified on nutrient agar.

3.4.1 Gram’s staining

A thin smear in sterilised normal saline solution was made and heat glued on a grease-free slide. On a fixed smear, crystal violet stain (HiMedia) was allowed to sit for 1 minute before being washed with tap water, and then Gram's iodine (HiMedia) was allowed to sit for 1 minute before being washed with water. The smears were decolorized with acid alcohol for 15-20 seconds, then counterstained with safranin (HiMedia) for 30 seconds before being washed under tap water. Air dried smears were examined under a microscope. Short rods and coccobacilli in Gram negative reactions were presumptively positive for *E. coli*.

3.4.2 Biochemical analysis

Biochemical reactions were determined by picking colonies from nutrient agar plates and testing them as follows:

3.4.2.1 Catalase test

On a clean, grease-free glass slide, a drop of 3 percent (v/v) hydrogen peroxide was placed. With the use of a wooden toothpick, the suspected colony was chosen and added. The development of gas bubbles within 30 seconds indicates a positive test. *E. coli* is catalase positive.

3.4.2.2 Oxidase test

Using an oxidase disc (Himedia), the isolates were picked up and examined for the oxidase reaction. A tiny amount of culture was smeared over the oxidase disc with a wooden toothpick. The appearance of violet colour within 30 seconds indicates a favourable reaction. *E. coli* is oxidase negative.

3.4.2.3 Indole test

A loopful of suspected bacterial colony was cultured for 24 hours at 37°C in test tubes containing 5 mL tryptone broth (Himedia), then a few drops of Kovac's reagent were added (Hi-media, India). The presence of a red ring indicates that indole is being produced. *E. coli* is indole positive.

3.4.2.4 Methyl-Red (MR) test

A loopful of suspected isolates were inoculated in tubes containing 5 ml glucose phosphate peptone broth (Hi-media) and cultured at 37°C for 48 hours, after which 5 drops of methyl red solution were added from the test tube's side. The immediate appearance of a red colour ring was regarded as a positive MR test result. For the methyl red test, *E. coli* is positive.

3.4.2.5 Voges-Proskauer (VP) test

Suspected isolates were inoculated into tubes containing 5 mL of glucose phosphate peptone broth (Hi-media) and cultured for 48 hours at 37°C. Barrit's reagent solution A and B (Hi-media) were added after incubation. For the VP test, the appearance of cherry red colour was positive. The VP test, which produces a yellow-brown colour, is negative for *E. coli*.

3.4.2.6 Citrate utilization test

Slants of Simmon's citrate medium (Hi-media, India) were created for citrate use. For 24 hours, cultures were streaked on slants and incubated at 37°C. A colour transition from green to blue is considered positive, whereas no colour change is considered negative. Citrate utilisation is negative in *E. coli* strains.

Finally, *E. coli* isolates with a greenish metallic sheen on the EMB, Gram negative rods, positive Indole test, positive methyl red test, negative VP-test, and negative citrate utilisation were identified. *E. coli* isolates were also treated to a polymerase chain reaction (PCR) molecular analysis to determine that they were *E. coli*.

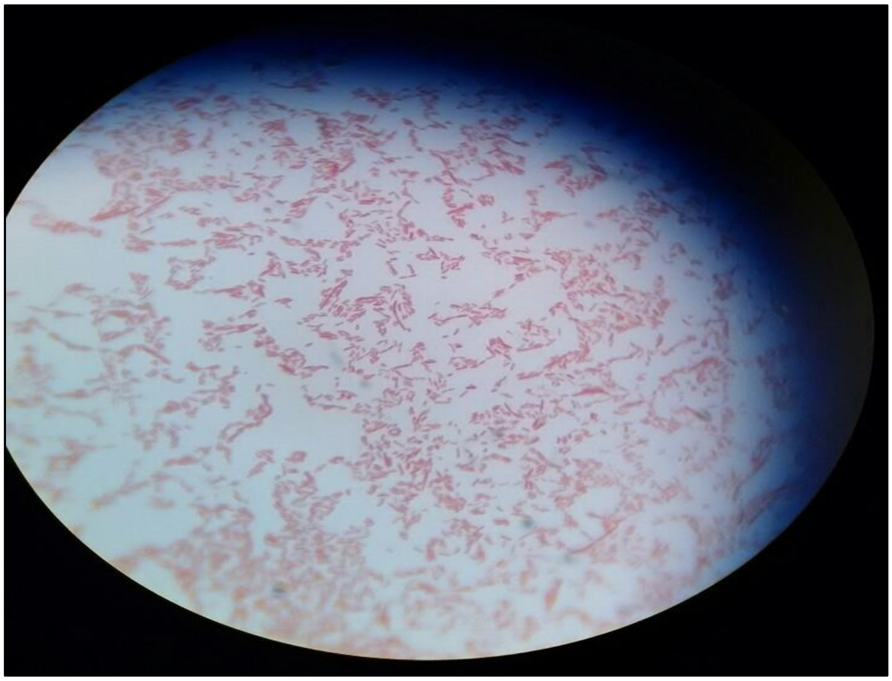

Plate 9: Gram staining of *E. coli* (1000X)

Plate 10: Catalase test (*E. coli* are catalase positive)

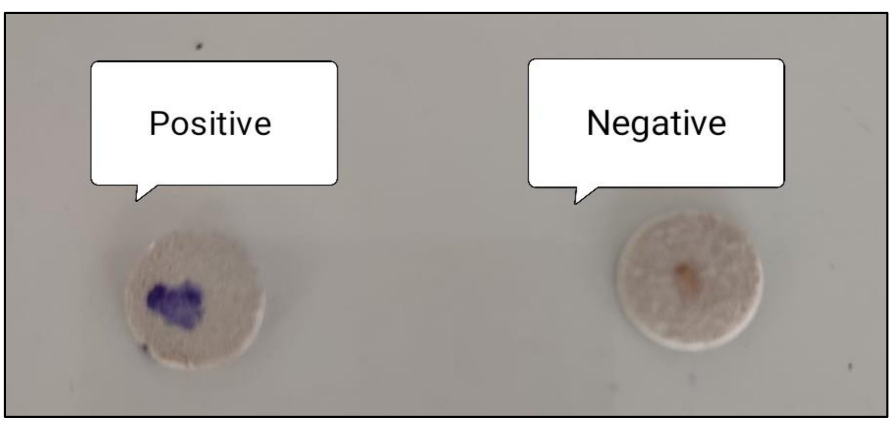

Plate 11: Oxidase test (*E. coli* are oxidase negative)

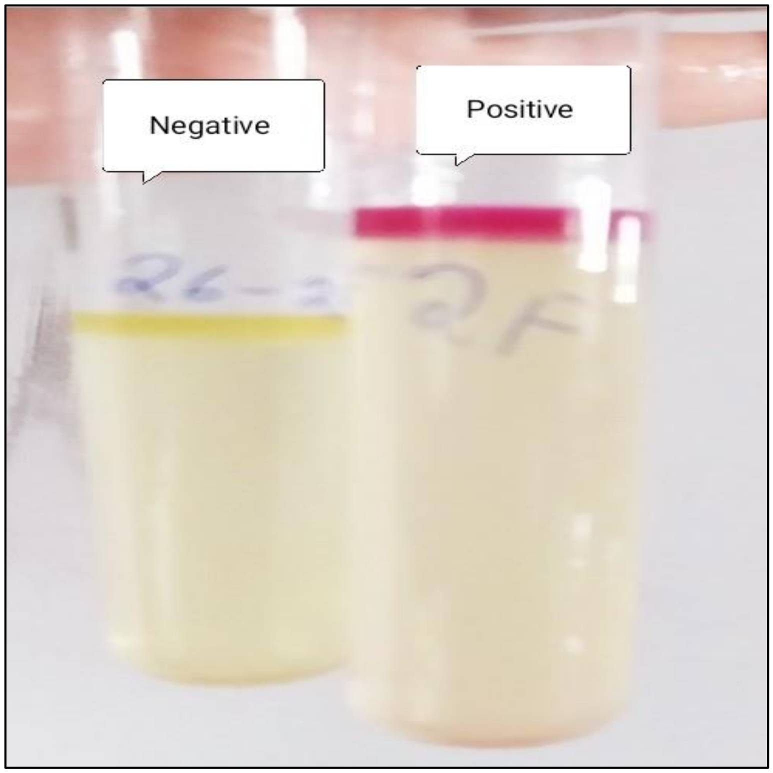

Plate 12: Indole test (*E. coli* are Indole positive)

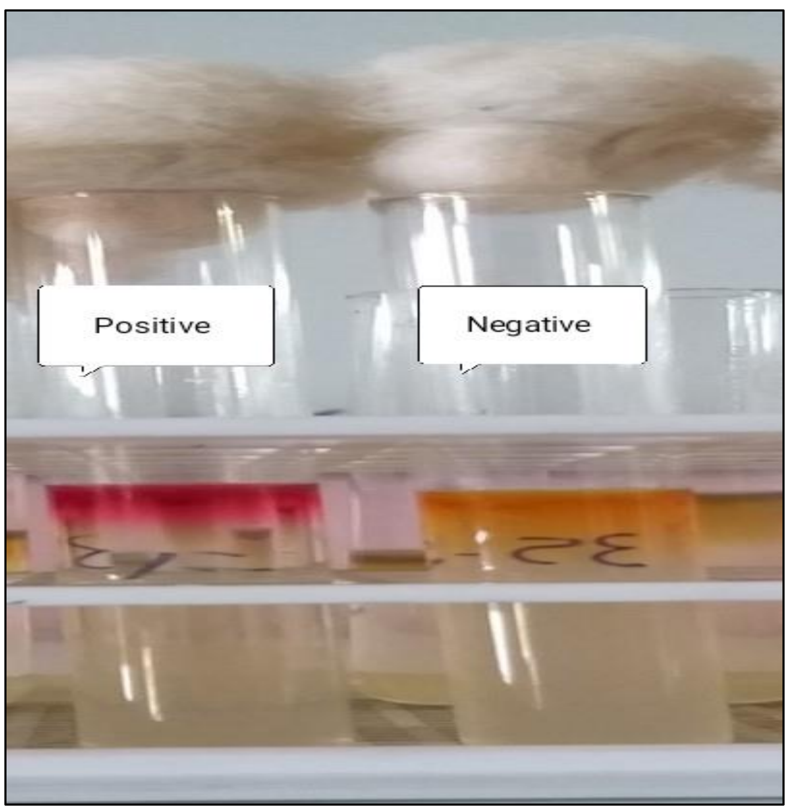

Plate 13: Methyl Red test (*E. coli* are Methyl red positive)

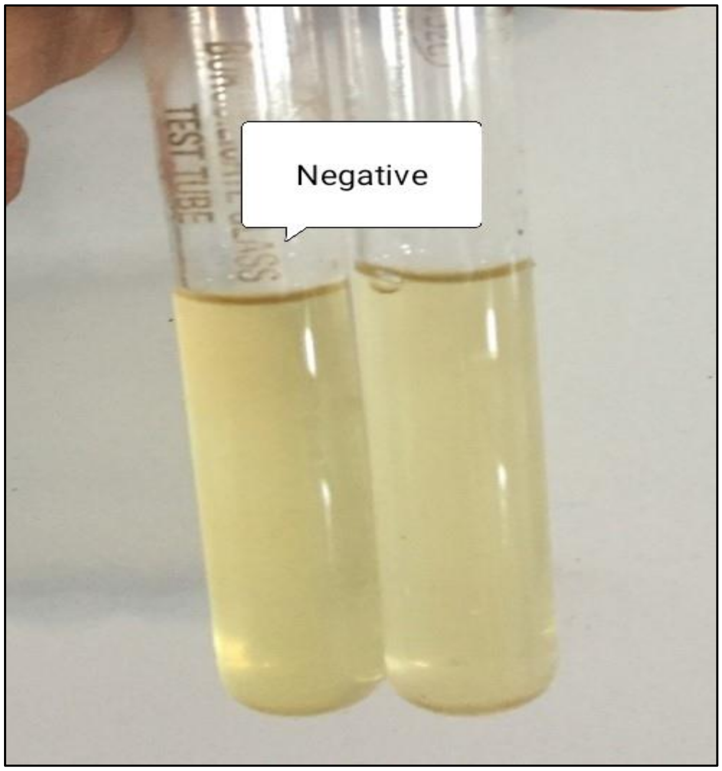

Plate 14: Voges Proskauer test (*E. coli* are Voges Proskauer negative)

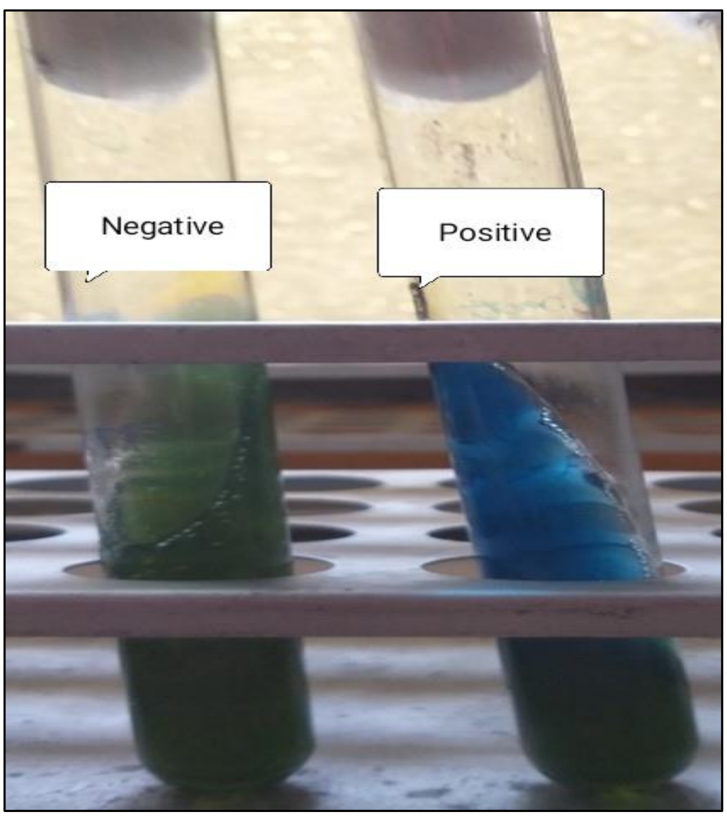

Plate 15: Citrate utilization test (*E. coli* are Citrate negative)

3.5 Molecular confirmation of *E. coli* isolates

Uniplex-PCRs were used to detect the *lt*, *st1a* and *st1b* genes in all *E. coli* isolates. The Gene Amp PCR System 9700 thermal cycler was used to do uniplex-PCR. Table 2-4 lists the primers and expected lengths of PCR amplification products.

3.5.1 Extraction of DNA

Boiling-snap chilling method was used to extract DNA. *E. coli* isolates were streaked on nutrient agar and cultured for 24 hours at 37°C. Next day, a sterile microcentrifuge tube was filled with 1 ml NSS and a peanut-sized inoculum from a growing colony was added. Then centrifuge it for 5 minutes at 5000 rpm. The resulting supernatant was discarded and 500 µl molecular grade water was added in microcentrifuge tube. Microcentrifuge tubes were placed in a hot water bath (95°C) for 10 minutes. From hot water bath, tubes were immediately shifted into deep freezer (-20°C) for 10 minutes followed by centrifugation for 5 minutes at 10,000 rpm. The resulting supernatant was stored at -20°C and utilised as template DNA in PCR experiments.

Table 2: PCR details for detection of *lt* gene

Primers (Guion *et al*., 2008)	Forward primer (*lt* forward) 5'– TCTCTATGTGCATACGGAGC –3' Reverse Primer (*lt* reverse) 5'– CCATACTGATTGCCGCAAT –3'
PCR reaction mixture (25µl)	1x PCR Buffer (Promega, USA) $MgCl_2$ – 1.5mM dNTP each – 200µM Primer each – 0.2µM Taq polymerase – 1 unit Template – 2µl
PCR conditions	Initial denaturation – 95°C for 2 min 30 cycles of denaturation - 95°C for 30 sec, annealing - 56°C for 45 sec and extension 72°C for 30 sec, Final extension – 72°C for 5 min
Specific base pair	322bp

Table 3: PCR details for detection of *stIa* gene

Primers (Guion *et al*., 2008)	Forward primer (*stIa* forward) 5'– TTTCCCCTCTTTTAGTCAGTCAA –3' Reverse Primer (*stIa* reverse) 5'– CAGGATTACAACACAATTCACAGCAG –3'
PCR reaction mixture (25µl)	1x PCR Buffer (Promega, USA) $MgCl_2$ – 1.5mM dNTP each – 200µM Primer each – 0.2µM Taq polymerase – 1 unit Template – 2µl
PCR conditions	Initial denaturation – 95°C for 2 min 30 cycles of denaturation - 95°C for 30 sec, annealing - 55°C for 45 sec and extension 72°C for 45 sec, Final extension – 72°C for 5 min
Specific base pair	159bp

Table 4: PCR details for detection of *stIb* gene

Primers (Guion *et al*., 2008)	Forward primer (*stIb* forward) 5'– TGCTAAACCAGTAGAGTCTTCAAAA –3' Reverse Primer (*stIb* reverse) 5'– GCAGGATTACAACACAATTCACAGCAG –3'
PCR reaction mixture (25µl)	1x PCR Buffer (Promega, USA) $MgCl_2$ – 1.5mM dNTP each – 200µM Primer each – 0.2µM Taq polymerase – 1 unit Template – 2µl
PCR conditions	Initial denaturation – 95°C for 2 min 30 cycles of denaturation - 95°C for 30 sec, annealing - 55°C for 45 sec and extension 72°C for 45 sec, Final extension – 72°C for 5 min
Specific base pair	138bp

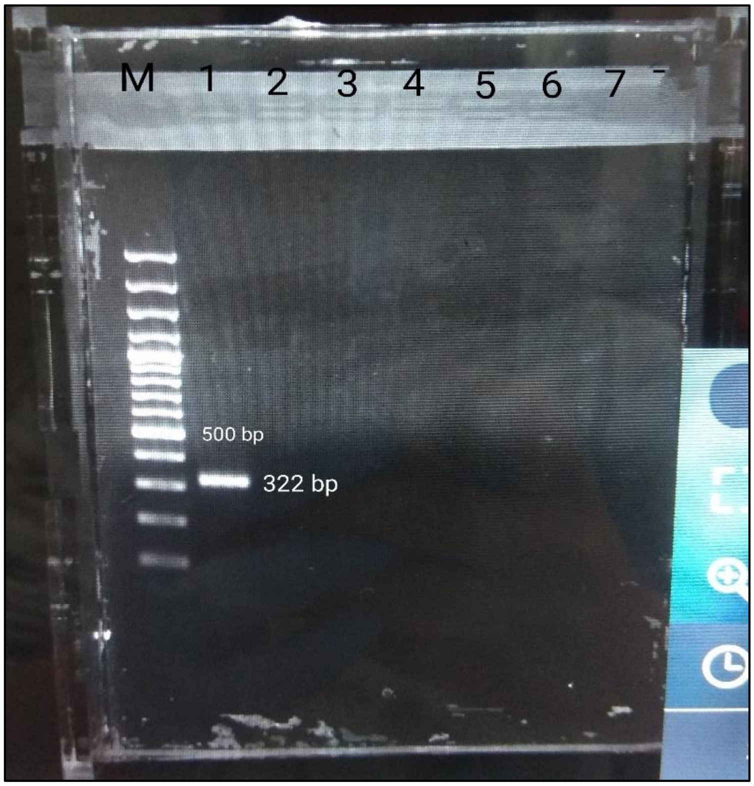

Plate 16: PCR amplification of *lt* gene
(Lane M: Marker, 1: Positive control, 2: Negative control, 3-7 Sample isolates)

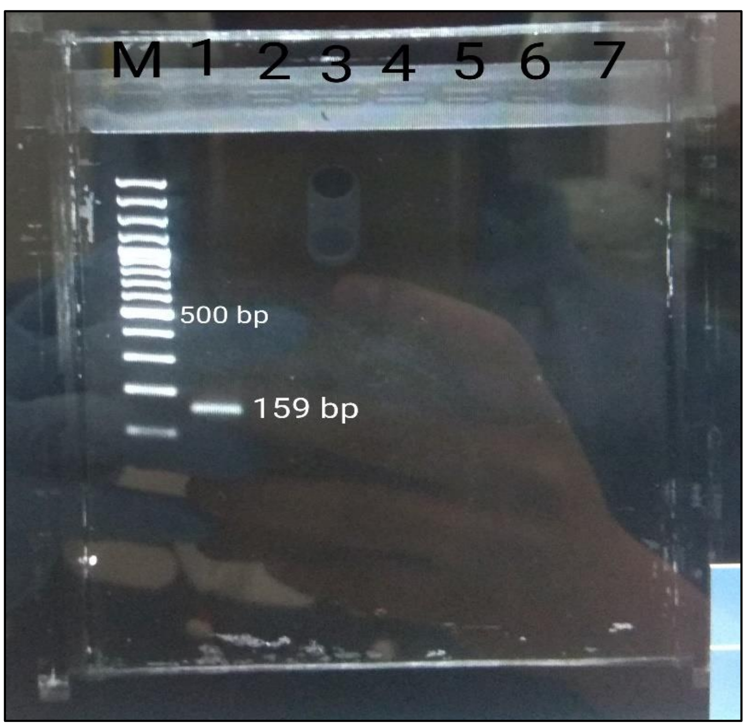

Plate 17: PCR amplification of *stIa* gene
(Lane M: Marker, 1: Positive control, 2: Negative control, 3-7 Sample isolates)

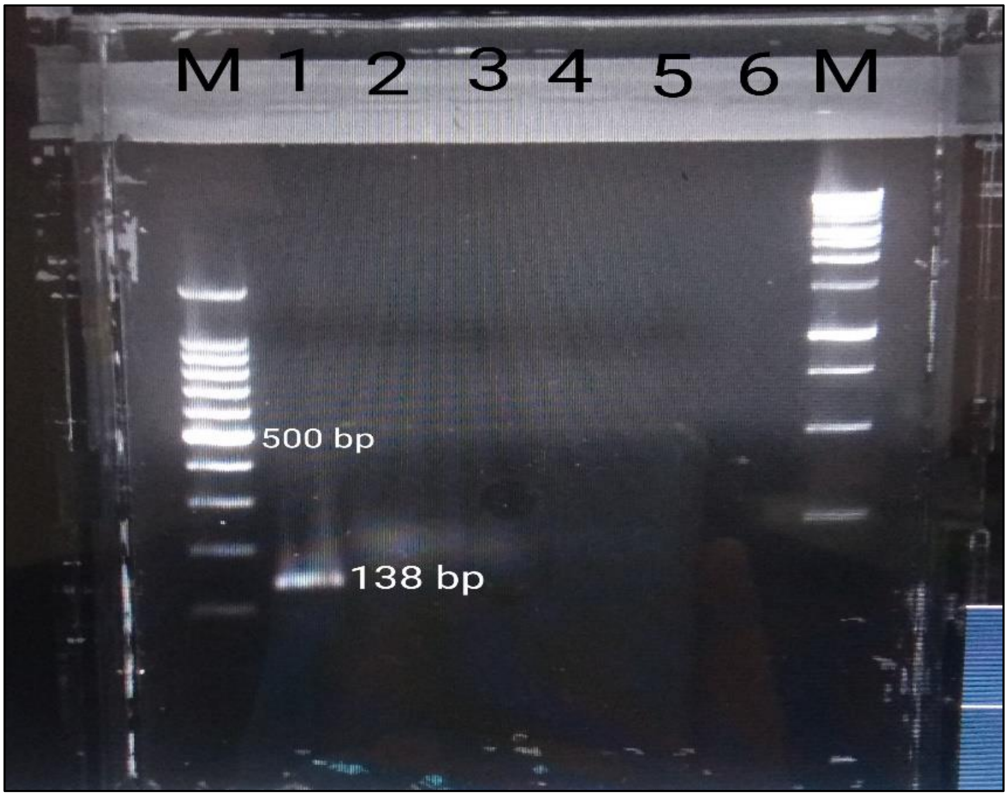

Plate 18: PCR amplification for *stIb* gene
(Lane M & M: Markers, 1: Positive control, 2: Negative control, 3-6: Sample isolates)

3.5.2 Analysis of PCR products

Agarose gel electrophoresis was used to examine the amplified products. 1.5% (for >200bp size) or 2% (for <200bp size) agarose gel in 1X TAE buffer with ethidium bromide @ 0.5g/ml of gel were used. In a Hoefer electrophoresis machine, the agarose gel was electrophoresed in 1X TAE buffer at 8V/cm using a 100 bp gene ruler as a marker. The Eppendorf gel documentation machine was used to photograph the agarose gels.

3.6 Antibiotic sensitivity assay of bacterial isolates

Antibiotic sensitivity testing was performed on E. coli isolates obtained in the study using disc diffusion method as per standards of Clinical and Laboratory Standards Institute (CLSI, 2012). Total 9 antibiotics viz., Amikacin (30mcg), Ampicillin (10mcg), Ampicillin/Sulbactam (10/10mcg), Cefixime (5mcg), Ciprofloxacin (5mcg), Gentamicin (10mcg), Norfloxacin (10mcg), Ofloxacin (5mcg) and Cefoperazone (75mcg) were used.

Briefly, confirmed *E. coli* colony was inoculated in Muller-Hilton broth for 4-6 hours at 37°C, till the turbidity equates to 0.5 McFarland standard. Using a sterile swab, the growth was evenly swabbed on Muller-Hinton agar plates. The antibiotic discs (Hi-Media) were placed on the agar at equal spaces. The plates were incubated for 24 hours at 37°C and zone of inhibition were measured. The results were interpreted as per standards of Clinical and Laboratory Standards Institute (CLSI, 2012).

Table 5: Antibiotics used in the study and their zone diameter interpretive standards for Enterobacteriaceae as per CLSI,2021

S.No.	Antimicrobial Agent	Disc content	Zone Diameter Breakpoints (Nearest whole mm)		
			S (>)	I	R (<)
1.	Amikacin (AK)	30mcg	17	15-16	16
2.	Ampicillin (AMP)	10mcg	17	14-16	13
3.	Ampicillin/Sulbactam (A/S)	10/10mcg	15	12-14	11
4.	Cefixime (CFM)	5mcg	19	16-18	15
5.	Cefoperazone (CPZ)	75mcg	21	16-20	15
6.	Ciprofloxacin (CIP)	5mcg	26	22-25	21
7.	Gentamicin (GEN)	10mcg	15	13-14	12
8.	Norfloxacin (NX)	10mcg	17	13-16	12
9.	Ofloxacin (OF)	5mcg	16	13-15	12

3.7 Data collection of water supply, sanitation and hygiene from study areas

To assess the water supply, sanitation and hygiene in study areas, the questionnaire was developed from the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply, Sanitation, and Hygiene, 2018. The questions relevant to study were selected from JMP. The questionnaire had ten questions, four of which were related to water, four were related to sanitation and the remaining two were related to hygiene. From each slum area, ten houses were chosen at random to acquire the data necessary for the current study.

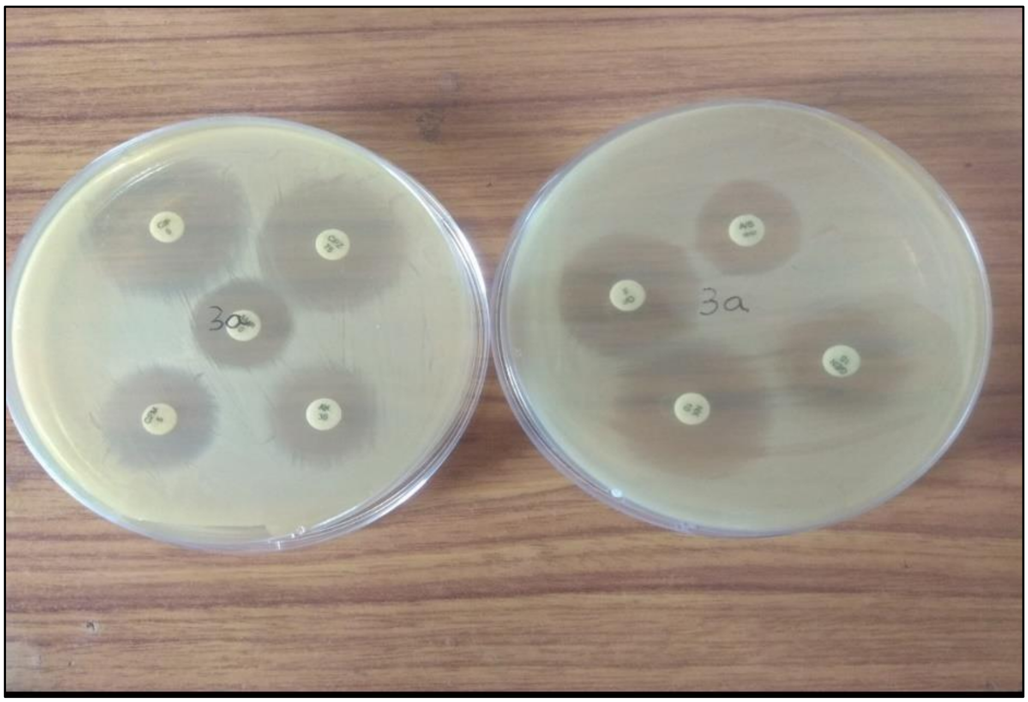

Plate 19: Antibiotic resistance of *E. coli* on Mueller Hinton Agar

Table 6: Questionnaire to assess Water Supply, Sanitation and Hygiene (WASH) in study areas (WHO/UNICEF JMP, 2018)

S.No.	Questions	Answers
Questions related to Water:		
1.	What is the main source of drinking water for members of your household?	• Piped water • Dug well • Water from spring • Rainwater collection • Delivered water • Water kiosk • Packaged water • Surface water (river, stream, dam, lake, pond, canal, irrigation channel)
2.	How long does it take to go there, get water, and come back?	• Members do not collect • Number of minutes • Household supply
3.	Is water always available from your main water source?	• Yes, water is always available • No, water is available most of the time • No, water is available some of the time • No, water is rarely available
4.	What do you usually do to the water to make it safer to drink?	• Boil • Add bleach/chlorine • Strain it through a cloth • Let it stand and settle • Use water filters
Questions related to Sanitation:		
5.	What kind of toilet facility do members of your household usually use?	• Flush/pour flush Flush to piped sewer system Flush to septic tank Flush to open drain • Dry pit latrines • Container based sanitation • No facility/Bush/Field
6.	Do you share this facility with others who are not members of your family?	• Yes • No
7.	The last time your child passed stool, what was done to dispose off the stool?	• Child used toilet/latrine • Put/rinsed into toilet or latrine • Put/rinsed into drain or ditch • Thrown into garbage • Buried • Left in the open
8.	Does your sanitation facility leak or overflow wastes at any time of year?	• No, never • Yes, sometimes • Yes frequently
Questions related to Hygiene:		
9.	Can you please show me where members of your household most often wash their hands?	• Fixed facility observed (sink/tap) • Mobile object observed (bucket/jug/kettle) • No handwashing place
10.	Observe availability of water and soap or detergent at the place for hand washing?	• Water & soap/detergent available • Water & soap/detergent not available

Chapter-4

Results

CHAPTER-4

RESULTS

4.1 Objective 1: To assess the drinking water and waste water of slums of Jammu for Enterotoxigenic *Escherichia coli*.

75 samples of drinking water and 25 samples of waste water were collected from 6 different slums of Jammu and processed for *E. coli* isolation to analyse the contamination of drinking and waste water with ETEC. *E. coli* was found in 26 (34.6 %) and 25 (100 %) drinking water samples and waste water samples, respectively. This information is summarised in Table 7, 8 and 9.

Table 7: Occurrence of *E. coli* among drinking water and waste water samples

Sample type	No. of samples analysed	No. of samples positive	Positive %	No. of *E. coli* isolates
Drinking water	75	26	34.67%	52
Waste water	25	25	100%	50
Total	**100**	**51**	**51%**	**102**

Table 8: *E. coli* isolation from drinking water samples in different slums

S.No.	Site name	No. of samples analysed	No. of samples positive	Positive %	No. of *E. coli* isolates
1.	Dhounthly	10	6	60%	12
2.	Rajiv nagar	13	4	30.77%	8
3.	Qasim nagar	12	3	25%	6
4.	Sheikh nagar	12	2	16.67%	4
5.	Kalka colony	14	3	21.43%	6
6.	Pata paloura	14	8	57.14%	16
	Total	**75**	**26**	**34.67%**	**52**

Table 9: *E. coli* isolation from waste water samples in different slums

S.No.	Site name	No. of samples analysed	No. of samples positive	Positive %	No. of *E. coli* isolates
1.	Dhounthly	5	5	100%	10
2.	Rajiv nagar	4	4	100%	8
3.	Qasim nagar	3	3	100%	6
4.	Sheikh nagar	3	3	100%	6
5.	Kalka colony	5	5	100%	10
6.	Pata paloura	5	5	100%	10
	Total	**25**	**25**	**100%**	**50**

A total of 102 *E. coli* isolates were isolated from 51 positive samples. These isolates were subjected to uniplex PCRs for *lt*, *stIa* and *stIb* genes to detect ETEC. None of the 102 isolates were positive for *lt*, *stIa* and *stIb* genes. In Table 10, the results are tabulated and detailed.

Table 10: Results of *E. coli* isolates from water sources on pathotyping for ETEC

S No.	Sample type	No. of *E. coli* isolates tested	ETEC		
			lt	***stIa***	***stIb***
1.	Drinking water	52	0	0	0
2.	Waste water	50	0	0	0
	Total	**102**	**0**	**0**	**0**

Objective 2: To assess the dog faecal samples of slums of Jammu for Enterotoxigenic *Escherichia coli*

To determine whether dogs of the slums are harboring ETEC, 20 dog faecal samples from slums were collected and processed for *E. coli* isolation; all 20 samples (100%) tested were positive for *E. coli*.

Table 11: Occurrence of *E. coli* among dog faecal samples

Sample type	No. of samples analysed	No. of samples positive	Positive %	No. of *E. coli* isolates
Dog faeces	20	20	100%	40
Total	**20**	**20**	**100%**	**40**

20 positive samples yielded a total of 40 *E. coli* isolates. PCR was used to test these isolates. None of the 40 isolates were positive for *lt*, *stIa*, or *stIb* genes of ETEC. In Table 12, the results are tabulated and detailed.

Table 12: Results of *E. coli* isolates from dog faecal samples on pathotyping for ETEC

Sample type	No. of *E. coli* isolates tested	ETEC		
		lt	***stIa***	***stIb***
Dog faeces	40	0	0	0
Total	**40**	**0**	**0**	**0**

Objective 3: To determine the antibiotic resistance of *Escherichia coli* isolates.

Isolates of *E. coli* obtained from water and dog faecal samples were analysed by *in vitro* antibiotic sensitivity test for 9 antibiotics. In the overall antibiotic sensitivity test, *E. coli* isolates were sensitive to majority of all nine antibiotics. Overall highest resistance was seen to norfloxacin (28.17%) and ofloxacin (16.9%), both of which are commonly used by humans during diarrhoea (Table 13). The results for *E. coli* isolates from different sample categories are tabulated in Table 14 to 16. Generally, isolates from different categories have similar antibiotic resistance pattern.

Table 13: Results of antibiotic sensitivity assay of *E. coli* isolates (n=142)

S.No.	Antibiotic (Abbreviation)	Concentration	Sensitive	Intermediate	Resistant
1.	Amikacin (AK)	30mcg	119 (83.80%)	23 (16.20%)	0.0%
2.	Ampicillin (AMP)	10mcg	126 (88.73%)	5 (3.52%)	11 (7.75%)
3.	Ampicillin/Sulbactam (A/S)	10/10mcg	119 (83.80%)	7 (4.93%)	16 (11.27%)
4.	Cefixime (CFM)	5mcg	140 (98.59%)	0.0%	2 (1.41%)
5.	Cefoperazone (CPZ)	75mcg	133 (93.66%)	4 (2.82%)	5 (3.52%)
6.	Ciprofloxacin (CIP)	5mcg	98 (69.01%)	31 (21.83%)	13 (9.15%)
7.	Gentamicin (GEN)	10mcg	142 (100%)	0.0%	0.0%
8.	Norfloxacin (NX)	10mcg	75 (52.82%)	27 (19.01%)	**40 (28.17%)**
9.	Ofloxacin (OF)	5mcg	103 (72.53%)	15 (10.56%)	**24 (16.90%)**

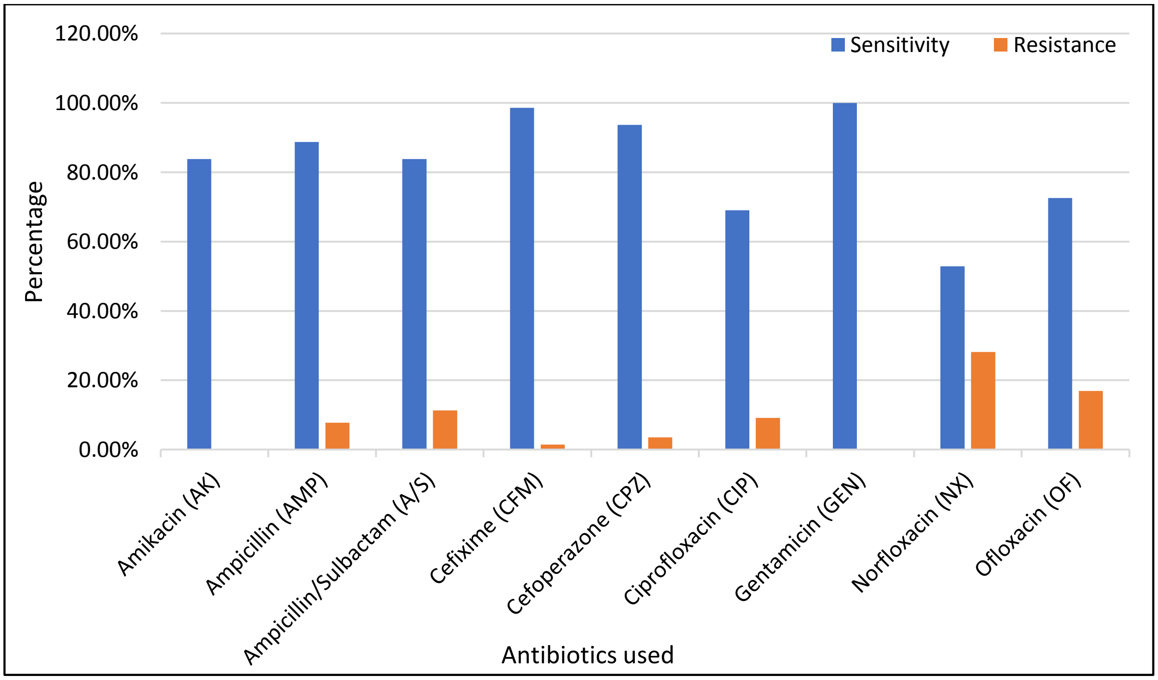

Fig. No. 1: Bar diagram showing results of antibiotic sensitivity assay of overall *E. coli* isolates (n=142)

Table 14: Results of antibiotic sensitivity assay of *E. coli* isolates from drinking water samples (n=52)

S.No.	Antibiotic (Abbreviation)	Concentration	Sensitive	Intermediate	Resistant
1.	Amikacin (AK)	30mcg	44 (84.62%)	8 (15.38%)	0.0%
2.	Ampicillin (AMP)	10mcg	48 (92.31%)	1 (1.92%)	3 (5.77%)
3.	Ampicillin/Sulbactam (A/S)	10/10mcg	42 (80.77%)	3 (5.77%)	**7 (13.46%)**
4.	Cefixime (CFM)	5mcg	52 (100%)	0.0%	0.0%
5.	Cefoperazone (CPZ)	75mcg	49 (94.23%)	1 (1.92%)	2 (3.85%)
6.	Ciprofloxacin (CIP)	5mcg	34 (65.38%)	11 (21.15%)	**7 (13.46%)**
7.	Gentamicin (GEN)	10mcg	52 (100%)	0.0%	0.0%
8.	Norfloxacin (NX)	10mcg	29 (55.77%)	12 (23.08%)	**11 (21.15%)**
9.	Ofloxacin (OF)	5mcg	41 (78.85%)	4 (7.69%)	**7 (13.46%)**

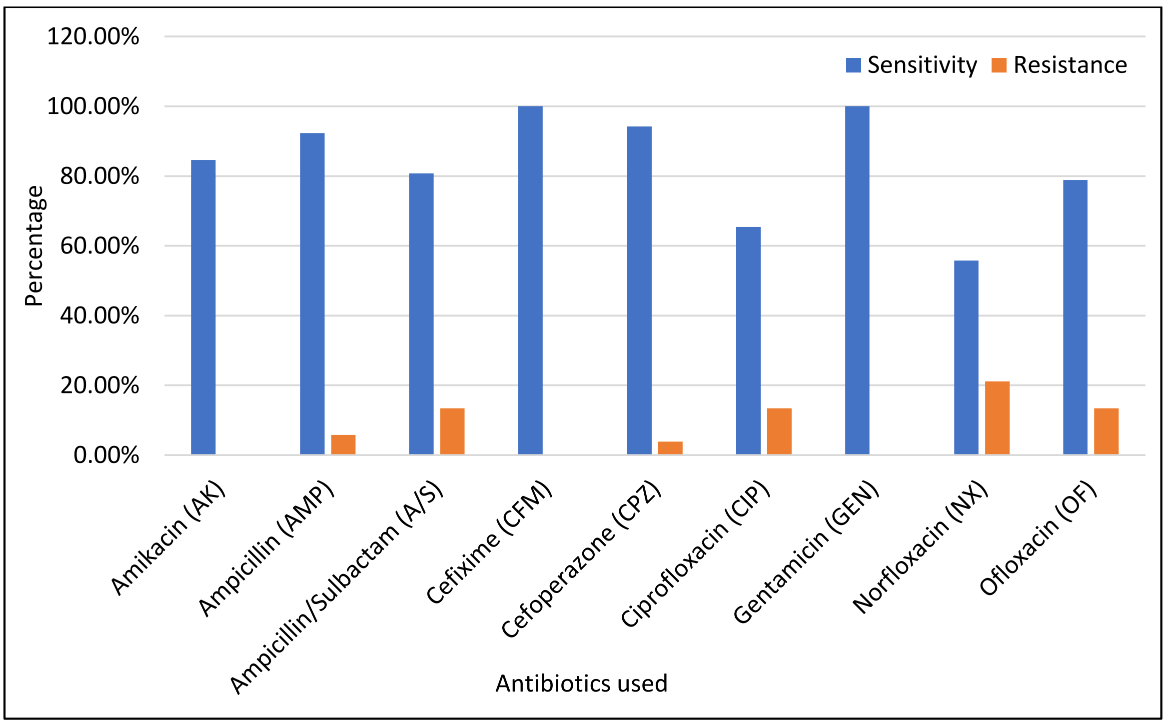

Fig. No. 2: Bar diagram showing results of antibiotic sensitivity assay of *E. coli* isolates from drinking water samples (n=52)

Table 15: Results of antibiotic sensitivity assay of *E. coli* isolates from waste water samples (n=50)

S.No.	Antibiotic (Abbreviation)	Concentration	Sensitive	Intermediate	Resistant
1.	Amikacin (AK)	30mcg	41 (82%)	9 (18%)	0.0%
2.	Ampicillin (AMP)	10mcg	41 (82%)	3 (6%)	6 (12%)
3.	Ampicillin/Sulbactam (A/S)	10/10mcg	42 (84%)	2 (4%)	6 (12%)
4.	Cefixime (CFM)	5mcg	48 (96%)	0.0%	2 (4%)
5.	Cefoperazone (CPZ)	75mcg	46 (92%)	1 (2%)	3 (6%)
6.	Ciprofloxacin (CIP)	5mcg	33 (66%)	12 (24%)	5 (10%)
7.	Gentamicin (GEN)	10mcg	50 (100%)	0.0%	0.0%
8.	Norfloxacin (NX)	10mcg	17 (34%)	10 (20%)	**23 (46%)**
9.	Ofloxacin (OF)	5mcg	30 (60%)	7 (14%)	**13 (26%)**

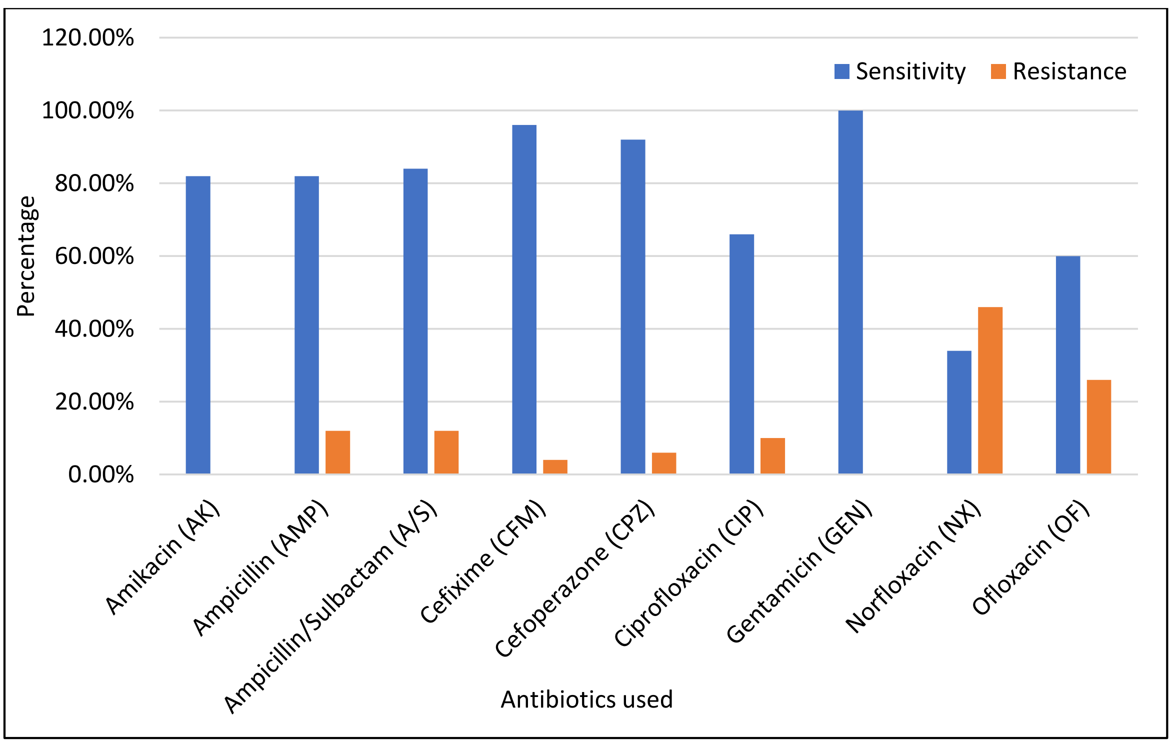

Fig. No. 3: Bar diagram showing results of antibiotic sensitivity assay of *E. coli* isolates from waste water samples (n=50)

Table 16: Results of antibiotic sensitivity assay of *E. coli* isolates from dog faecal samples (n=40)

S.No.	Antibiotic (Abbreviation)	Concentration	Sensitive	Intermediate	Resistant
1.	Amikacin (AK)	30mcg	34 (85%)	6 (15%)	0.0%
2.	Ampicillin (AMP)	10mcg	37 (92.5%)	1 (2.5%)	2 (5%)
3.	Ampicillin/Sulbactam (A/S)	10/10mcg	35 (87.5%)	2 (5%)	3 (7.5%)
4.	Cefixime (CFM)	5mcg	40 (100%)	0.0%	0.0%
5.	Cefoperazone (CPZ)	75mcg	38 (95%)	2 (5%)	0.0%
6.	Ciprofloxacin (CIP)	5mcg	31 (77.5%)	8 (20%)	1 (2.5%)
7.	Gentamicin (GEN)	10mcg	40 (100%)	0.0%	0.0%
8.	Norfloxacin (NX)	10mcg	39 (97.5%)	5 (12.5%)	**6 (15%)**
9.	Ofloxacin (OF)	5mcg	32 (80%)	4 (10%)	**4 (10%)**

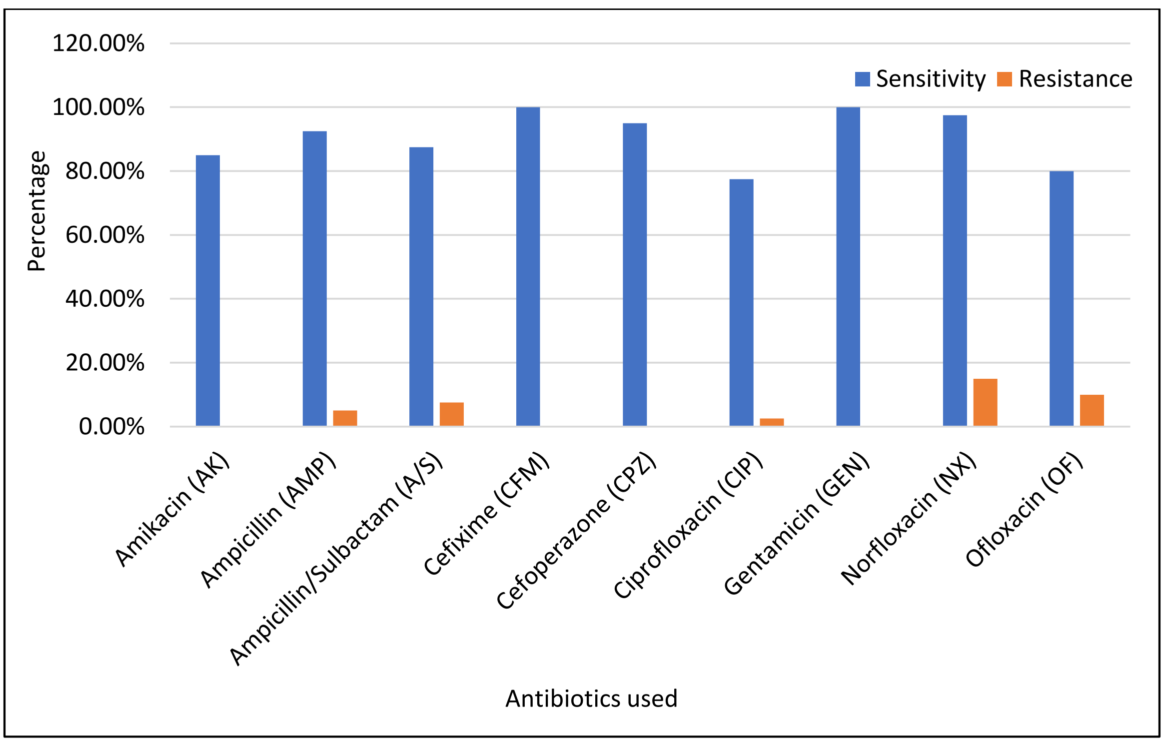

Fig. No. 4: Bar diagram showing results of antibiotic sensitivity assay of *E. coli* isolates from dog faecal samples (n=40)

4.2 Assessment of water supply, sanitation and hygiene (WASH) in study areas

On collecting information through questionnaire from six slum areas, it was found that all residents of six slums have access to piped water. Among them 55% of households use public tap water that is located at a short distance from their homes while 45% have household supplies. Of the households studied, 50% do nothing to make the water safer to drink, while 30% strain the water through a cloth, 11.67% use water filters, and 8.33% boil the water.

On the sanitation front, 55% of households did not have proper toilet facilities; instead, they practise open defecation and share this open system with the rest of the population of their slum; 23.33% of households had toilet facilities and their sewage went into an open drain; this open drain occasionally overflowed during rainy season, but is walled from all sides, preventing waste water from coming into contact with the population; and 21.67% of households had toilet facilities with proper septic tank.

In the hygienic section, all households in all study areas used water and soap or detergent after defecation for washing hands. For handwashing, 55% of households have moveable objects such as jugs/buckets/kettles, while the remaining 45% have proper fixed facilities such as a sink/tap.

Table 17: Data gathered via Questionnaire to assess WASH in study areas

S.No.	Questions	Answers
Questions related to Water:		
1.	What is the main source of drinking water for members of your household?	• **Piped water (100%)** • Dug well* • Water from spring* • Rainwater collection* • Delivered water* • Water kiosk* • Packaged water* • Surface water* (river, stream, dam, lake, pond, canal, irrigation channel)
2.	How long does it take to go there, get water, and come back?	• Members do not collect* • **Number of minutes (55%)** • **Household supply (45%)**
3.	Is water always available from your main water source?	• Yes, water is always available* • **No, water is available most of the time (100%)** • No, water is available some of the time* • No, water is rarely available*
4.	What do you usually do to the water to make it safer to drink?	• **Boil (8.33%)** • Add bleach/chlorine* • **Strain it through a cloth (30%)** • Let it stand and settle* • **Use water filters (11.67%)** • **Do nothing (50%)**
Questions related to Sanitation:		
5.	What kind of toilet facility do members of your household usually use?	• Flush/pour flush Flush to piped sewer system* **Flush to septic tank (21.67%)** **Flush to open drain (23.33%)** • Dry pit latrines* • Container based sanitation* • **No facility/Bush/Field (55%)**
6.	Do you share this facility with others who are not members of your family?	• **Yes (55%)** • **No (45%)**
7.	The last time your child passed stool, what was done to dispose off the stool?	• **Child used toilet/latrine (33.33%)** • Put/rinsed into toilet or latrine* • Put/rinsed into drain or ditch* • Thrown into garbage* • Buried* • **Left in the open (43.3%)**
8.	Does your sanitation facility leak or overflow wastes at any time of year?	• No, never* • **Yes, sometimes (78.3%)** • Yes frequently*
Questions related to Hygiene:		
9.	Can you please show me where members of your household most often wash their hands?	• **Fixed facility observed (sink/tap) (45%)** • **Mobile object observed (bucket/jug/kettle) (55%)** • No handwashing places*
10.	Observe availability of water and soap or detergent at the place for hand washing?	• **Water & soap/detergent available (100%)** • Water & soap/detergent not available*

(Note: *options were N/A)

Chapter-5

Discussion

CHAPTER-5

DISCUSSION

Unsafe drinking water is a major source of morbidity and mortality. Drinking water that is free of contaminants is essential for good health. Diseases associated with water, sanitation, and hygiene are estimated to be responsible for 4% of global fatalities and 5.7 % of disease burden (Pruss-Uston *et al*., 2002). According to a survey, just 19% of the global population washes their hands after coming into contact with faeces and 26% consume water contaminated with faecal bacteria (Anonymous, 2019).

According to the World Health Organization, nearly 1.8 million people die each year from diarrheal diseases, the majority of which are caused by consumption of contaminated drinking water. Every year, approximately 37.7 million Indians are affected by waterborne illnesses, 1.5 million children die of diarrhoea, and 73 million working days are lost, resulting in a financial burden of $600 million (Anonymous, 2019). According to a 2018 survey in India, Uttar Pradesh state of the country has the greatest rate of diarrhea-related mortality followed by West Bengal, Assam, Odisha and Madhya Pradesh (Anonymous, 2018).

Jammu, the winter capital of Jammu and Kashmir, is situated on the banks of the Tawi River, which is a main left bank of the Chenab River. According to a research published in November 2020, almost two million people in the Jammu region are constantly exposed to water-borne ailments as a result of water contamination. (Anonymous, 2020).

Waterborne diseases are transmitted through faecal contamination of water, which emphasises the need of detecting faecal contamination in water. One of the most commonly used bacteria to confirm faecal contamination is *E. coli.* The abundance of *E. coli* in the gastrointestinal tracts of animals and humans is one of the main causes for this. Furthermore, *E. coli* detection technologies are widely available, simple to use, and interpret. This makes *E. coli* detection ideal for developing countries like India and locations with low resources.

E. coli has progressed from commensal to pathogen status, making it one of the most serious aquatic and foodborne infections. Diarrheagenic *E. coli* (DEC) is a pathogen that primarily infects the gastrointestinal system and is categorised into six pathotypes: ETEC, EHEC, EIEC, EPEC, EAEC, and DAEC. Depending on the type of infection, DEC can cause anything from mild diarrhoea to severe haemorrhagic colitis in humans (Park *et al*., 2018). The six DEC pathotypes ETEC, EHEC, EIEC, EPEC, EAEC, and DAEC have been associated to diarrhoea in most developing countries, particularly in young children (Rajendran *et al*., 2010). DEC has been linked to waterborne outbreaks in Ghana (Adzitey *et al*., 2015), Tanzania (Lyimo *et al*., 2016), California (Probert *et al*., 2017) and India (Adzitey *et al*., 2015, Hamner *et al*., 2007; Batabyal *et al*., 2013). As a result, the isolated *E. coli* strains were tested for ETEC.

The most typical cause of diarrhoea in children in developing nations and in travellers to these regions is ETEC. In the small intestine, where few bacteria typically establish, the bacteria colonise. They must defeat several host defences, such as mucus production, bile salts, secretory IgA (S-IgA), and epithelial shedding, in order to accomplish this. ETEC produces the enterotoxins that cause the symptoms once they established themselves in the small intestine. Colonization factors are proteinaceous surface structures that ETEC use to cling to epithelial surfaces (CFs). About 35% of the ETEC strains isolated globally express both heat-labile (LT) and heat-stable (ST) enterotoxins, 35% express only ST, and remaining strains express only LT (Gaastra and Svennerholm 1996). By avoiding or properly preparing foods and drinks that might contain the bacteria, as well as by frequently washing our hands with soap, we can avoid getting sick.

In this investigation, 71 samples were found to be positive for *E. coli* after screening 120 samples including drinking water (n=75), waste water (n=25), and dog faeces (n=20). In both dog faeces and waste water samples, the positive proportion for *E. coli* was 100%, followed by 34.6% in drinking water. 142 *E. coli* isolates were isolated from positive samples. However, no ETEC were discovered on pathotyping of 142 *E. coli* isolates. Similarly, Harada *et al*. (2018) in urban slums in Bangladesh didn’t detect ETEC in drinking water samples.

When comparing the current study to previous research conducted in urban slums in Kolkata, study reported 28% (56/200) of stored drinking water samples to be positive for faecal coliforms (Patil *et al*., 2012). In Chandigarh, *E. coli* was found in 31.8% (116/364) water samples, with 8 ETEC strains producing only *lt* gene, 24 ETEC strains producing only *st* gene, and 4 ETEC strains producing both *lt* and *st* genes (Taneja *et al*., 2011). In Turkey, from 50 drinking water samples, only one sample was positive for ETEC the (Gumus *et al*., 2015). When compared to a previous study conducted in Jammu by Padha (2021), 60 samples of drinking water were analysed, yielding 43 *E. coli* isolates, none of which were positive for *lt*, *stIa* and *stIb* genes.

E. coli was also isolated from waste water in present study. All waste water samples were positive for *E. coli*. However, none of the isolates was positive for ETEC. When compared to a previous study conducted in urban slums of Bangladesh, 16.3% of 264 *E. coli* isolates from sanitary waste water were positive for ETEC, with *stIb* being the most frequently detected (Harada *et al*., 2018). In a previous study conducted by Padha (2021) in Jammu, where 81 surface water samples (river, lake and pond) were analysed, yielding 146 *E. coli* isolates, none of which were positive for *lt*, *stIa* and *stIb* genes.

In the present investigation, all dog faeces samples tested positive for *E. coli*, however ETEC was not detected after pathotyping. Although collection of stray dog faeces was difficult due to the fact that it dried quickly and it was impossible to tell whether the faeces came from the same dog or from a different one. Globally, there have been very few studies on ETEC from dog faeces. In contrast to a previous study carried out at Ontario Veterinary College in Canada, where *stIa* (26.7%) and *stIb* (4.4%) were found in *E. coli* isolates from the faeces of 20 out of 45 dogs with diarrhea but no *lt* gene was found, *lt*, *stIa* and *stIb* genes were not found in normal dogs (Hammermueller *et al*., 1995). The significance of dog as carrier of *E. coli* has been clearly shown by Askari *et al*. (2020) who detected same phenotype and strains of *E. coli* in both dog and owner in a family.

During the study, nine antibiotics were used to investigate antibiotic resistance in *E. coli*. The overall pattern of resistance in *E. coli* was norfloxacin (28.17%) followed by ofloxacin (16.90%), ampicillin/sulbactam (11.27%), ciprofloxacin (9.15%), ampicillin

(7.75%), cefoperazone (3.52%), cefixime (1.41%). None of the isolates were resistant to amikacin and gentamicin (0.0%).

Antibiotic resistance is a phenomenon driven by numerous factors. It can be due to overuse of antibiotics, a lack of knowledge, and the fact that the majority of people prefer chemist shop prescriptions over doctor consultations as per information. Without determining the true cause of the diarrhea, norfloxacin and/or ofloxacin are administered in the study areas. The three basic mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) modification of antimicrobial target bacterial proteins, and (3) changes in membrane permeability to antibiotics. Antibiotic resistance can be transmitted via plasmids and/or maintained on the bacterial chromosomes.

As *E. coli* isolates from drinking water, waste water and dog faecal sample have similar antibiotic pattern, it probably indicates the drinking water is being contaminated with waste water and dog faeces samples. Dog faeces samples were taken from stray dogs, and assumption was made that no antibiotics had ever been given to these animals for any reason. However, canines also demonstrated resistance to norfloxacin and ofloxacin, which were commonly used for treating humans. This indicates that *E. coli* must have been transferred from people to dogs and vice versa.

During the study, relevant data on water, sanitation and hygiene were also obtained via a questionnaire derived from Joint Monitoring Programme (JMP) WHO/UNICEF, 2018, consisting of ten questions. Ten households from each slum were selected randomly. It was found that all six slum households had access to piped water supply, with the majority of households (55%) using public tap water located a short distance from their homes. Majority of households (50%) do nothing to make their drinking water safer.

All residents of the Dhounthly and Pata paloura slums use open defecation and mobile sanitary facilities, but the majority of residents of the remaining four slums have toilet facilities, with some having septic tanks and the rest draining their sewage into open drainage that eventually joins the Tawi River, and all of them having fixed sanitary facilities in their homes. After defecation, the majority of the population in the slums uses sanitary amenities (mobile or fixed) such as water and soap.

The majority of the population in Dhounthly and Pata Paloura slums lived in kutcha houses with no toilet facilities, instead performing open defecation and using public tap water; in the present study the highest number of *E. coli* isolates from drinking water samples were obtained from these two slum areas. The factors affecting drinking water contamination are lack of knowledge, faulty water storage arrangements or mishandling during collection and transportation back to the home. The majority of the people in the other four slums were aware of the importance of hygiene and sanitation. In these slums, majority of people use water filter, strain water with cloth and boil water to make it safer to drink. When compared to the results from Dhounthly and Pata paloura, these four slums had a lower number of *E. coli* isolates in drinking water samples.

In the current study, contrary to our expectations, no ETEC pathotype was detected in any of the samples collected during the study. Despite the fact that *E. coli* was isolated from all types of samples (drinking water, waste water and dog faeces), this demonstrates faecal contamination of drinking water. Further antibiotic sensitivity tests on *E. coli* isolates revealed high resistance to norfloxacin and ofloxacin due to indiscriminate use of these two antibiotics during diarrhoea without knowing the cause of the diarrhoea. The sensitivity and resistance pattern of all nine antibiotics shown by *E. coli* isolates from drinking water, waste water and dog faeces were similar, indicating that *E. coli* strains circulated in the study areas.

Chapter-6

Summary and Conclusions

CHAPTER-6

SUMMARY AND CONCLUSIONS

As a commensal bacterium, *Escherichia coli* is present in the intestines of warm-blooded animals and humans. Their presence in water usually indicates recent faecal contamination or poor sanitation, and their presence in drinking water is frequently used to assess water faecal pollution. *E. coli* is a commensal bacterium that lives in the intestines and causes sickness in its host only rarely. However, particular *E. coli* pathotypes have evolved as sources of gastrointestinal and systemic infections in the host, including ETEC, EHEC, EIEC, EPEC, EAEC, and DAEC.

The current investigation looked for *E. coli* and its pathogenic strain ETEC in water and dog faeces samples. A total of 120 samples were obtained from six slum areas of Jammu city. 75 drinking water samples, 25 waste water samples, and 20 dog faecal samples were among the samples collected. Each sample was collected in an autoclaved sampling bottle aseptically.

Drinking water samples were filtered before being placed in MacConkey broth for *E. coli* isolation, whereas waste water and dog faeces samples were placed directly in MacConkey broth and cultured for 24 hours at 37°C. The tubes were examined after incubation to see if the broth colour had changed and if there were any gas bubbles inside the durhum tubes. The tubes that showed a change in colour and the presence of gas bubbles inside the durhum tubes were chosen for further analysis. To observe the typical green metallic sheen of *E. coli,* inoculum from suspicious tubes was streaked on EMB agar and incubated at 37°C for 24 hours. To purify the colonies, they were streaked on nutrient agar plates with a green metallic sheen or a dark purple colour. Phenotypic characterization of pure colonies included morphological and biochemical characterisation, such as Gram staining and biochemical assays such as catalase, oxidase, indole, methyl red, Voges Proskauer test, and citrate utilisation test. PCR was used to confirm phenotypically identified *E. coli* isolates.

ETEC was also evaluated on *E. coli* isolates. To detect ETEC strains, three uniplex PCR for three genes were used: the *lt* gene for heat labile toxin, and the *stIA* and *stIB* genes for heat stable toxin.

From 120 samples, 71 were positive for *E. coli*, with 142 isolates recovered [26 drinking water (34.6%), 25 waste water (100%), and 20 dog faeces (100%)]. None of the 142 isolates tested positive for the *lt*, *stIa*, or *stIb* genes when pathotyping them with PCR. Antibiotic resistance of 9 antibiotics on *E. coli* isolates (n=142) collected during the study is also included in this investigation. Norfloxacin (28.16%) was the most resistant antibiotic, followed by Ofloxacin (16.91%), Ampicillin/Sulbactam (11.27%), Ciprofloxacin (9.15%), Ampicillin (7.74%), Cefoperazone (3.53%), Cefixime (1.41%), Amikacin (0.0%) and Gentamicin (0.0%). The pattern of antibiotic resistance from different sample isolates was similar.

To assess the wash condition of slums, a questionnaire containing ten questions was obtained from JMP WHO/UNICEF, 2018, and ten households were randomly selected from each slum and information was gathered. All six slum households had access to piped water, with 55% using public tap water and 45% having household supply. Following water collection, 50% of households do nothing to make water safer to drink, while the remaining households do a variety of activities to keep water safe, such as 30% strain with cloth, 11.67% use water filters and 8.33% boil it. Open defecation was practised by 55% of households, while the remainder have toilet facilities. After defecation, all households used water and soap/detergent to wash their hands.

As a result of the current study, the following conclusions were made:

- The study identified the faecal contamination of water sources in all the six slum areas of Jammu by isolating *E. coli*.
- No ETEC pathotype detected from *E. coli* isolates probably indicating absence of circulation of ETEC in all the six slum areas.

- *E. coli* isolates from different sample categories had similar antibiotic resistance pattern, probably indicating the circulation of similar *E. coli* strains in the studied area.

- The data collected from the study areas indicates variations in drinking water, sanitation and hygiene conditions.

- Further studies on circulation of other pathotypes of *E. coli* and other diarrheagenic microorganisms should be conducted in future in the region.

- Urban authorities must take strict action against open defecation practice by providing community toilet facilities to the people of slums for their welfare.

References

REFERENCES

Adane, M., Mengistie, B., Kloos H., Medhin, G. and Mulat, W. 2017. Sanitation facilities, hygienic conditions, and prevalence of acute diarrhea among under five children in slums of Addis Ababa, Ethiopia: Baseline survey of a longitudinal study. *PLoS One Journal,* **12(8)**:e0182783.

Adzitey, F., Sumaila, N. and Saba, C.K.S. 2015. Isolation of *E. coli* from drinking water sources for humans and farm animals in Nyankpala Community of Ghana. *Research Journal of Microbiology*, **10(3)**: 126-131.

Akter, S., Islam, M., Afreen, K.S., Khan, S.I. and Birkeland, N.K. 2013. Prevalence and distribution of different diarrhoeagenic *Escherichia coli* virulotypes in major water bodies in Bangladesh. *Epidemiology & Infection,* **141(12):** 2516-2525.

Anonymous. 2016. Report 70% Of Urban India's Waste Is Untreated, IndiaSpend.

Anonymous. 2018. Report CBHI, National Health Profile, 13th Issue.

Anonymous. 2019. Report Water Quality Branch, PHE Department, Jammu.

Anonymous. 2019. Report Water Quality, Indian Water Portal.

Anonymous. 2020. Report Jammu Express (http://jammuexpress.in/newsdet.aspx?q=361)

Askari, A., Ghanbarpour, R., Akhtardanesh, B., Aflatoonian, M.R., Sharifi, H., Jajarmi, M. and Molaei, R. 2020. Detection of zoonotic diarrheagenic pathotypes of *Escherichia coli* in healthy household dogs. *Iranian Journal of Microbiology*, **12(6)**: 522-530.

Batabyal, P., Mookerjee, S., Sur, D. and Palit, A. 2013. Diarrheogenic *Escherchia coli* in potable water sources of West Bengal, India. *Acta Tropica,* **127(3):** 153-157.

Begum, Y.A., Talukder, K.A., Nair, G.B., Qadri, F., Sack, R.B. and Svennerholm, A.M. 2005. Enterotoxigenic *Escherichia coli* isolated from surface water in urban and rural areas of Bangladesh. *Journal of Clinical Microbiology,* **43(7):** 3582-3583.

Begum,Y.A., Talukder,k.a., Nair,G.B., Khan,S.I., Svennerholm,A.M., Sack,R.B. and 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.

Bhar, D., Bhattacherjee, S., Mukherjee, A., Sarkar, T.K. and Dasgupta, S. 2017. Utilization of safe drinking water and sanitary facilities in slum households of Siliguri, West Bengal. *Indian Journal of Public Health,* **61(4)**:248-253.

Bonkoungou, I.J.O., Haukka, K., Osterblad, M., Hakanen, A.J., Traore, A.S., Barro, N. and Sittonen, A. 2013. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. *BMC Pediatrics* Journal, **13**:36.

Cabral, J.P. 2010. Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health,* **7(10)**:3657-3703.

Castellani, A. and Chalmers, AJ. 1919. *Manual of Tropical Medicine* (3rd ed.). Williams Wood and Co, New York.

Colwell, R.R. 1996. Global climate and infectious disease: The Cholera Paradigm. *Science,* **4(5295):**2025–2031.

Dutta, S., Guin, S., Ghosh, S., Pazhani, G.P., Rajendran, K., Bhattacharya, M.K., Takeda, Y., Nair, G.B. and Ramamurthy, T. 2013. Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. *PLoS One Journal,* **8(2)**:e56068.

Field, M., Graf, L.H.Jr., Laird, W.J. and Smith, P.L. 1978. Heat-stable enterotoxin of *Esherichia coli*: in vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. *Proceedings of the National Academy of Sciences USA,* **75(6)**:2800-2804.

Gaastra, W. and Svennerholm, A.M. 1996. Colonization factors of human Enterotoxigenic *Escherichia coli* (ETEC). *Trends Microbiology,* **4(11)**:444-452.

Gascon, J., Vargas, M., Quinto, L., Corachan, M., Jimenez de Anta, M.T. and Vila, J. 1998. Enteroaggregative *Escherichia coli* strains as a cause of travelers diarrhea: A cross-control study. *The Journal of Infectious Diseases,* **177(5)**:1409-1412.

Guion, C.E., Ochoa, T.J., Walker, C.M., Barletta, F. and Cleary, T.G. 2008. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *Journal of Clinical Microbiology,* **46(5):** 1752-1757.

Gümüş, D. and Küçüker, M.A., 2015. EHEC, EPEC, and ETEC strains and their antibiotic resistance in drinking and tap water samples. *Clinical Laboratory*, **61(12)**:113-121.

Gupta, A., Sarker, G., Rout, A.P., Mondal, T. and Pal, R. 2015. Risk correlates of diarrhea under 5 years of age in slums of Bankura, West Bengal. *Journal of Global Infectious Disease*, **7(1)**:23-29.

Hammermueller, J., Kruth, S., Prescott, J. and Gyles, C. 1995. Detection of toxin genes in *Escherichia coli* isolated from normal dogs and dogs with diarrhea. *Canadian Journal of Veterinary Research,* **59(4)**:265-270.

Hamner, S., Broadaway, S.C., Mishra, V.B., Tripathi, A., Mishra, R.K., Pulcini, E., Pyle, B.H. and Ford, T.E. 2007. Isolation of potentially pathogenic *Escherichia coli* O157: H7 from the Ganges River. *Applied and Environmental Microbiology,* **73(7):** 2369-2372.

Harada, H., Fujimori, Y., Gomi, R., Ahsan, Md.N., Fuji, S., Sakai, A. and Matsuda, T. 2018. Pathotyping of Escherichia coli isolated from community toilet wastewater and stored drinking water in a slum in Bangladesh. *Letters in Applied Microbiology,* **66(6)**:542-548.

India GDP per capita by state, 2020 – knoema.com *Knoema.* Retrieved 2 August 2021.

Jiang, Z.D., Lowe, B., Verenkar, M.P., Ashley, D., Steffen, R., Tornieporth, N., von Sonnenburg, F., Waiyaki, P. and DuPont, H.L. 2002. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). *The Journal of Infectious Diseases*, **185(4)**:497-502.

Joint Monitoring Programme for Water Supply, Sanitation and Hygiene WHO/UNICEF, 2018.

Kosek, M., Bern, C. and Guerrant, R.L. 2003. The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. *Bulletin World Health Organization*, **81(3)**:197-204.

Lakshminarayanan, S. and Jayalakshmy, R. 2015. Diarrheal diseases among children in India: Current scenario and future perspectives. *Journal of Natural Science, Biology, and Medicine*, **6(1):**24.

Leclerc, H.D.A.A., Mossel, D.A.A., Edberg, S.C. and Struijk, C.B. 2001. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. *Annual Reviews in Microbiology*, **55(1):**201-234.

Lothigius, A., Sjoling, A., Svennerholm, A.M. and Bolin, I. 2010. Survival and gene expression of Enterotoxigenic *Escherichia coli* during long term incubation in sea and fresh water. *Journal of Applied Microbiology*, **108(4)**:1441-1449.

Lyimo, B., Buza, J., Subbiah, M., Smith, W. and Call, D.R. 2016. Comparison of antibiotic resistant in *Escherichia coli* obtained from drinking water sources in northern Tanzania: a cross-sectional study. *BMC Microbiology*, **16(1):** 254.

Mahajan, A., Sharma, H., Haq, Z., Jeelani, R., Ganai, A.W. and Sharma, R. 2018. Microbial examination of drinking water in district Jammu of J&K, India. *Journal of Entomology and Zoology Studies*, **6(3)**:1532-1535.

Martin, N.H., Trmčić, A., Hsieh, T.H., Boor, K.J. and Wiedmann, M. 2016. The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology*, **7**:1549. https://doi.org/10.3389/fmicb.2016.01549.

Mathur, A., Baghel, D., Jaat, J., Diwan, V. and Pathak, A. 2019. Community-based participatory research to improve childhood diarrhea case management in Ujjain, India: A cross-sectional survey. *International Journal of Environmental Research and Public Health,* **16(9)**:1646.

Mellou, K., Katsioulis, A., Potamiti-Komi, M., Pournaras, S., Kyritsi, M., Katsiaflaka, A., Kallimani, A., Kokkinos, P., Petinaki, E., Sideroglou, T. and Georgakopoulou, T. 2014. A large waterborne gastroenteritis outbreak in central Greece, March 2012: challenges for the investigation and management. *Epidemiology & Infection*, **142(1):**40-50.

Melo, M.C.N.de., Taddei, J.A.A.C., Santos, D.R.D., Vieira, C., Carneiro, N.B., Melo, R.F. and Silva, L.R. 2008. Incidence of diarrhea in children living in urban slums in Salvador, Brazil. *The Brazilian Journal of Infectious Diseases,* **12(1)**:89-93.

Moharana, S.S., Panda, R.K., Dash, M., Chayani, N., Bokade, P., Pati, S. and Bhattacharya, D. 2019. Etiology of childhood diarrhea among under five children and molecular analysis of antibiotic resistance in isolated enteric bacterial pathogens from a tertiary care hospital, Eastern Odisha, India. *BMC Infectious Diseases Journal,* **19(1)**:1018.

Moyo, S.J., Maselle, S.M., Matee, M.I., Langeland, N. and Mylvaganam, H. 2007. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infectious Diseases Journal,* **9(7)**:92.

Najimaana, S.K., Mawia, K., Sambyal, N., Lone, J.A., Iqbal, A., Kumar, Y. and Singh, M. 2102. Indicator organisms based hygienic assessment of water sources of filtration plants of Jammu. *Journal of Veterinary Public Health,* **10(2)**:125-129.

Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews Journal*, **11(1)**:142-201.

Nguyen, T.V., Van, P.L., Huy, C.L., Gia, K.N. and Weintraub, A. 2006. Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. *International Journal of Infectious Diseases,* **10(4)**:298-308.

Nontongana, N., Sibanda, T., Ngwenya, E. and Okoh, A.I. 2014. Prevalence and antibiogram profiling of *Escherichia coli* pathotypes isolated from the Kat river and Fort Beaufort abstraction water. *International Journal on Environmental Research and Public Health,* **11(8)**:8213-8227.

Nycz, B.T., Pretty, K., Trujillo, A.G., Sanchez, B. and Dominguez, S.R. 2020. Description of enteropathic *Escherichia coli* species in pediatric patients at a quaternary children hospital. *Journal of the Pediatric Infectious Diseases Society,* **9(5)** :573-579.

Padha, S. 2021. *Risk assessment of water sources for pathogenic Escherichia coli*. MVSc Thesis, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, India.

Park, J., Kim, J.S., Kim, S., Shin, E., Oh, K.H., Kim, Y., Kim, C.H., Hwang, M.A., Jin, C.M., Na, K. and Lee, J. 2018. A waterborne outbreak of multiple diarrhoeagenic *Escherichia coli* infections associated with drinking water at a school camp. *International Journal of Infectious Diseases,* **66**:45-50.

Parsai, A. and Rokade, V. 2019. Water quality monitoring infrastructures for tackling water-borne diseases in the state of Madhya Pradesh, India, and its implication on the Sustainable Development Goals (SDGs). *Paripex-Indian Journal of Research,* **8(7)**:1-13.

Pathak, S.P. and Gopal, K. 2008. Prevalence of bacterial contamination with antibiotic resistant and enterotoxigenic faecal coliforms in treated drinking water. *Journal of Toxicology and Environmental Health, Part A,* **71(7)**:427-433.

Patil, A., Batabyal, P., Kanungo, S. and Sur, D. 2012. In-house contamination of potable water in urban slum of Kolkata, India: a possible transmission route of diarrhea. *Water Science and Technology Journal,* **66(2)**:299-303.

Pati, S., Kadam, S.S. and Chauhan, A.S. 2014. Hand hygiene behavior among urban slum children and their care takers in Odisha, India. *Journal of Preventive Medicine and Hygiene*, **55(2)**:65-68.

Payment, P., Waite, M. and Dufour, A. 2003. Introducing parameters for the assessment of drinking water quality. *Assessing microbial safety of drinking water*, **4** :47-77.

Percival, S.L., Chalmers, R., Embrey, M., Hunter, P.R., Sellwood, J. and Wyn-Jones, P. 2004. *Escherichia coli.* In: Percival,S.L. and Williams, D.W.(eds) *Microbiology of Waterborne Diseases*. Pp 89-117. Academic Press, United States.

Probert, W.S., Miller, G.M. and Ledin, K.E. 2017. Contaminated stream water as source for *Escherichia coli* O157 illness in children. *Emerging Infectious Diseases,* **23(7):** 1216.

Pruss-Uston, A., Kay, D., Fewtrell, L. and Bartram, J. 2002. Estimating the burden of disease from water, sanitation, and hygiene at a global level. *Environmental Health Perspectives Journal*, **110(5)**:537-542.

Pruss-Uston, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M.C., Gordon, B., Hunter, P.R., Medlicott, K. and Johnston, R. 2019. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low and middle income countries. *International Journal of Hygiene and Environmental Health*, **222(5)**:765-777.

Qadri, F., Svennerholm, A.M., Faruque, A.S. and Sack, R.B. 2005. Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical Microbiology Reviews Journal,* **18(3)**:465-483.

Rajendran, P., Ajjampur, S.S.R., Chidambaram, D., Chandrabose, G., Thangaraj, B., Sarkar, R., Samuel, P., Rajan, D.P. and Kang, G. 2010. Pathotypes of diarrheagenic *Escherichia coli* in children attending a tertiary care hospital in South India. *Diagnostic Microbiology and Infectious Disease Journal*, **68(2)**:117-122.

Ram, S., Vajpayee, P., Tripathi, U., Singh, R.L., Seth, P.K. and Shanker, R. 2008. Determination of antimicrobial resistance and virulence gene signatures in surface water isolates of *Escherichia coli. Journal of Applied Microbiology,* **105(6)**:1899-1908.

Rodas, C., Mamani, R., Blanco, J., Blanco, J.E., Wiklund, G., Svennerholm, A.M., Sjoling, A. and Iniguez, V. 2011. Enterotoxins, colonization factors, serotypes and antimicrobial resistance of Enterotoxigenic *Escherichia coli* (ETEC) strains isolated from hospitalized children with diarrhea in Bolivia. *The Brazilian Journal of Infectious Diseases,* **15(2)**:132-137.

Roman, A.D., Roman, J.V., Flores, M.A.V., Villasenor, H.F., Vidal, J.E., Amador, S.M., Llanos, A.M.G., Nunez, E.G., Serrano, J.M., Pastrana, G.T. and Sicairos, N.L. 2019. Detection of antimicrobial-resistance diarrheagenic *Escherichia coli* strains in surface water used to irrigate food products in the northwest of Mexico. *International Journal of Food Microbiology,* **2;304**:1-10.

Sack, R.B. 1980. Enterotoxigenic *Escherichia coli*: identification and characterization. *The Journal of Infectious Diseases,* **142(2)**:279-286.

Sangra, S., Choudhary, N. and Narangyal, A. 2020. Reporting of the core indicators on drinking water and sanitation from urban slums of Jammu: A cross-sectional study. *Journal of Family Medicine and Primary Care,* **9(6)**:2747-2750.

Saxena, T., Kaushik, P. and Mohan, M.K. 2015. Prevalence of *E. coli* O157:H7 in water sources: an overview on associated diseases, outbreaks and detection methods. *Diagnostic Microbiology and Infectious Disease,* **82(3):**249-264.

Shulman, S. T.; Friedmann, H. C.; Sims, R. H. 2007. "Theodor Escherich: The First Pediatric Infectious Diseases Physician?". *Clinical Infectious Diseases*, **45 (8):**1025–1029.

Singh, P., Metgud, S.C., Roy, S. and Purwar, S. 2019. Evolution of diarrheagenic *Escherichia coli* pathotypes in India. *Journal of Laboratory Physicians,* **11(4)**:346-351.

Singleton, P. 1999. *Bacteria in Biology, Biotechnology and Medicine*, 5th Edition, pp. 444-454. Wiley, Hoboken.

Sixma, T.K., Kalk, K.H., van Zanten, B.A., Dauter, Z., Kingma, J., Witholt, B. and Hol, W.G. 1993. Refined structure of *Escherichia coli* heat-labile enterotoxin, a close relative of cholera toxin. *Journal of Molecular Biology*, **230(3)**:890-918.

Sobsey, M.D., Handzel, T. and Venczel, L. 2003. Chlorination and safe storage of household drinking water in developing countries to reduce waterborne disease. *Water science and Technology*, **47(3)**: 221-228.

Taneja, N., Singh, M., Rao, P., Biswal, M., Priya, S., Chander, R. and Sharma, M. 2011. Faecal contamination of drinking water supplies in and around Chandigarh and correlation with acute gastroenteritis. *Journal of Communicable Diseases,* **43(3)**:193-199.

Taneja, N., Singh, M., Rao, P., Biswal, M., Priya, S., Chander, R. and Sharma, M. 2012. Faecal contamination of drinking water supplies in and around Chandigarh and correlation with acute gastroenteritis. *Journal of Communicable Diseases,* **44(4):**201-209.

Too, J.K., Sang, W.K., Ng ang a, Z. and Ngayo, M.O. 2016. Faecal contamination of drinking water in Kericho district, western Kenya: role of source and household water handling and hygiene practices. *Journal of Water and Health,* **14(4)**:662-671.

Trainor, E., Gomara, M.I., Ngwira, B. and Cunliffe, N. 2016. Detection of enterotoxigenic *E. coli* in hospitalized children with and without diarrhea in Blantyre, Malawi. *Journal of Pediatrics and Child Health,* **36(2)**:102-105.

UNICEF (2015) *Water, sanitation and hygiene. The case for support.* Available at: https://www.unicef.org/publicparternships/files/WASHTheCaseForSupport.pdf (accessed 5 September 2019).

UNICEF (2017) Water, environment and sanitation. Available at: https://unicef.in/story/1125/water-environment-and-sanitation (accessed 29 April 2019).

Vega-Manriquez, X.D., Ubiarco-Lopez, A., Verdugo-Rodriguez, A., Hernandez-Chinas, U., Navarro-Ocana, A., Ahumada-Cota, R.E., Ramirez-Badillo, D., Hernandez-Diaz de Leon, N. and Eslava, C.A. 2020. Pet dogs potential transmitters of pathogenic *Escherichia coli* with resistance to antimicrobials. *Archives of Microbiology,* **202(5)**:1173-1179.

Wenneras, C. and Erling, V. 2004. Prevalence of enterotoxigenic *Escherichia coli* associated diarrhea and carrier state in the developing world. *Journal of Health, Population and Nutrition,* **22(4)**:370-382.

World Health Organization (WHO), 2019. Safer water, better health. 2019 update. Geneva, Switzerland.

CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Studies on Enterotoxigenic *Escherichia coli* in slums of Jammu"**, submitted by **Mr. Nikhil Sharma**, Registration No. **J-20-MV-644**.

Dr. H.K. Sharma
(Major Advisor)

Place: R.S. Pura

Date: 22-09-2022

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EDUCATIONAL QUALIFICATION

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OGPA	7.96 /10
Title of Thesis	STUDIES ON ENTEROTOXIGENIC *Escherichia coli* IN SLUMS OF JAMMU

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