Journal of the University of Ruhuna
Volume 13 No 2, December 2025. Pp 162-172
DOI: https://doi.org/10.4038/jur.v13i2.8113
ISSN 2659-2053

RESEARCH ARTICLE

Molecular detection of enteropathogenic *Escherichia coli* in urban canals of Colombo, Sri Lanka

K Rajan[1*] and YS Shelina[1]
[1]Faculty of Life and Medical Sciences, Business Management School (BMS) Campus, Colombo, Sri Lanka

Abstract: Enteropathogenic *Escherichia coli* is a major diarrheagenic pathogen. However, its significance is often overlooked unless there is an outbreak. Previous studies predominantly focused on freshwater systems rather than contaminated water bodies, which are primary sources of pathogenic *Escherichia coli*. Hence, the present study aims at detecting the presence of Enteropathogenic *Escherichia coli* in polluted water canals in Colombo, Sri Lanka. Water samples were collected from the Hamilton, Wellawatte, Dehiwala, and Norris canals within Colombo. Pure *Escherichia coli* isolates were obtained by culturing membrane-filtered samples on MacConkey agar, followed by Luria-Bertani broth. DNA was extracted using a modified boiling cell technique. Molecular identification was performed by amplifying two key virulence genes (*eaeA* and *bfpA*). The obtained results indicate the presence of Enteropathogenic *Escherichia coli* in the Hamilton, Wellawatte and Dehiwala canals. Isolates obtained from the Hamilton and Wellawatte canals were of typical pathotypes, which were positive for both *eaeA* and *bfpA* genes. Atypical pathotypes were detected only in the Dehiwala Canal, which were positive only for the *eaeA* gene. Despite yielding *Escherichia coli* colonies on the agar, isolates obtained from the Norris Canal were negative for both the tested genes. In conclusion, the results indicate microbial contamination of the Colombo Canals. The findings of this study prompt the need for continuous canal water monitoring and implementation of effective remediation measures to mitigate the evident microbial contamination. This is especially important in Colombo, considering that some communities continue to rely on these waterways despite the health risks associated with untreated water contamination.

Keywords: *Canal contamination*; *Diarrheagenic pathogen*; *Enteropathogenic*; *Escherichia coli*; *Water pollution*

Introduction

Water pollution is a common environmental challenge, accounting for one in six deaths worldwide (Lin *et al.*, 2022). Despite global attempts to reduce water contamination to secure safe water for all, approximately 1.7 billion people still drink polluted water with faecal matter. Ingestion of contaminated water with human and animal faeces facilitates the spread of pathogenic microorganisms, leading to waterborne gastrointestinal diseases such as cholera, diarrhoea, dysentery, hepatitis A, polio and typhoid (World Health Organization, 2023). Among the numerous physical, chemical and microbiological contaminants, microbes cause around 505,000 diarrhoeal deaths annually (Anindita *et al.*, 2025). Waterborne diseases contribute to 3.6% of the total disability-adjusted life year globally. Their prevalence is high in developing countries, particularly in Africa (Li *et al.*, 2024).

Sri Lanka, as one of the developing countries, also faces water pollution as a major ecological problem, particularly in rapidly urbanizing metropolitan areas due to inadequate waste management (Nuwanka and Gunathilaka, 2023). Colombo, the commercial centre of the country is home to 752,993 people (World Population Review, 2024). Since the city is low-lying, it heavily depends on a complex network of canals and marshes to prevent flooding. This interconnected canal system of 67 km drains surface flood water and directs it to the sea and the Kelani River (Moufar and Perera,

*corresponding author: kavinya.r@bms.ac.lk https://orcid.org/0009-0008-8027-5288

2018). The Colombo canal system is mainly divided into two, which are subdivided into multiple canals as shown in Figure 1a. These canals become easily polluted due to the direct discharge of domestic sewage and industrial effluents into the water along its course through densely populated areas. This contaminates canal water with pathogenic microorganisms leading to the spread of waterborne diseases within the community (Eriyagama and Ratnayake, 2008). Although projects such as the Greater Colombo Flood Control and Environment Improvement Project (GCFC&EIP) and the Colombo Canal System Water Quality Improvement Project focus on improving canal water quality, visible pollution can still be seen in most of its parts (Jayaweera and Samarakoon, 2020). Despite the severe pollution, many slum residents continue to rely on the canals for various domestic and recreational purposes. Hence, it is critically important to assess the microbial contamination of Colombo Canal to help mitigate health risks, particularly among the slum communities.

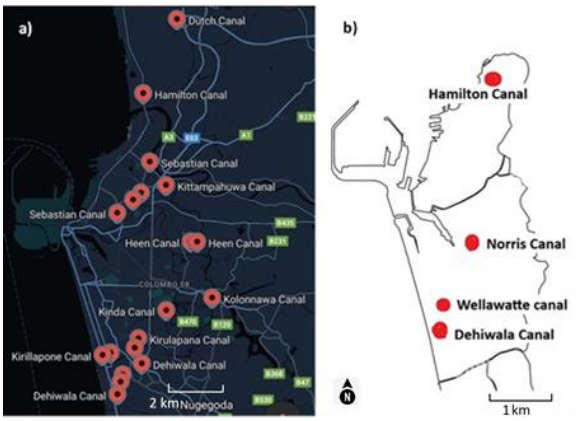

Figure 1: Colombo canal network; a) Branches of Colombo Canal b) Canals selected for the study.

Main pathogens causing waterborne diseases due to pollution include *Escherichia coli* (*E. coli*), *Shigella* species, *Vibrio cholerae* and *Salmonella typhi*. They cause symptoms such as diarrhoea, nausea, vomiting, fever and abdominal pain (Pandey *et al.*, 2014). Among these organisms, *E. coli* infection causes thousands of deaths annually, especially in children and the elderly. It was first isolated from the faeces of healthy infants and described by Theobald Escherich, a German paediatrician in 1885 as *Bacterium coli* commune. They are rod-shaped, gram-negative, facultative anaerobes of the family *Enterobacteriaceae* and class *Gammaproteobacteria* (Basavaraju and Gunashree, 2023). Both abiotic and biotic factors influence the growth and survival of *E. coli* in natural environments. These include temperature, moisture, nutrients, pH, sunlight, interactions with other microorganisms and the ability to form biofilms (van Elsas *et al.*, 2010). *E. coli* can enter water bodies such as rivers, streams, canals and groundwater by washing away of human and animal faecal matter, along with wastewater from treatment plants, leading to contamination (Hernández-Vásquez *et al.*, 2022). *E. coli* concentration in faeces varies depending on the host organism. For example, 10^7–10^9 Colony Forming Units (CFU) per gram are found in humans while domestic animal faeces contain 10^4–10^6 CFU per gram. *E. coli* is the indicator for faecal contamination in water sources, with the standard safety limit set to zero per 100 millilitres (ml) of drinking water (Jang *et al.*, 2017). Water guidelines published by the United States Environmental Protection Agency (USEPA) advise that a bacterial concentration above 235 CFU per 100 ml of freshwater is unsafe for consumption (Banach and Van Der Fels-Klerx, 2020). Most of the *E. coli* strains help in the digestion of foods thus, are harmless. However, 10-15% of the strains cause gastrointestinal, urinary and neuronal disorders due to the acquisition of virulence factors and antibiotic-resistance genes (Mueller and Tainter, 2023). Such pathogenic strains are classified into six major intestinal pathotypes based on the characteristics of their virulence and the infection caused. They are Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). Among these, STEC and EPEC have been reported frequently in many waterborne disease outbreaks, with the former accounting for 5% while the latter for 10% of paediatric fatalities worldwide (Hunter, 2003).

Neter and colleagues introduced the term EPEC in 1955. It was used to describe a faecal, coliform, intestinal infection-causing *E. coli* strain that is rarely found in the faeces of individuals negative for diarrhoeal diseases (Kralicek *et al.*, 2022). It is identified as the main cause of persistent, watery diarrhoea in infants in developing countries. Other common symptoms include dehydration, fever and vomiting (Ochoa and Contreras, 2011). Previous studies have detected EPEC in approximately 30% of diarrhoeal stool samples collected from individuals in developed and developing countries. EPEC infections are transmitted through the faecal-oral route, via contaminated hands, fomites, weaning foods and infant formula (Aslani and Alikhani, 2011). EPEC strains can enter water bodies through the disposal of faeces of symptomatic and asymptomatic infants, as well as adults who frequently interact with them (Ochoa and Contreras, 2011). EPEC primarily infects

the epithelial lining of the gastrointestinal tract, altering ion balance, fluid transport and the regulation of pro-inflammatory cytokines release. These alterations are mediated through the production of toxins, effector proteins and suppression of early inflammatory responses, enabling the bacteria to evade host immune mechanisms and survive longer within the host (Pearson and Frankel, 2016). The hallmark of EPEC infections is the formation of attaching and effacing (A/E) lesions on small intestinal enterocytes, mediated by the *eaeA* gene (Chen and Frankel, 2005). Based on the presence and absence of the *eaeA* gene and another important marker, the *bfpA* gene, EPEC strains can be classified into two subgroups. EPEC strains positive for both the *eaeA* and *bfpA* genes are known as typical EPEC (tEPEC) strains, which belong to specific O:H serotypes. EPEC strains possessing only the *eaeA* genes are classified as atypical EPEC (aEPEC) strains (Oh *et al.*, 2012). Another characteristic feature of EPEC is the inability to produce Shiga toxin. It was believed, that the aEPEC strains originated from STEC and tEPEC after the loss of *stx* and *bfpA* gene, respectively (Alikhani *et al.*, 2006). The *eaeA* gene is responsible for encoding intimin, a vital virulence factor for causing EPEC infections. Multiple evolutionary studies that have been conducted on the *eaeA* gene have identified 27 isotypes to date, each encoding for distinct intimin types (Oh *et al.*, 2012). The *bfpA* gene encodes for the bundle-forming pilus (BFP), an adherence factor. BFP plays an important role in the formation of EPEC microcolonies and tight junction disruption through the transport of effector proteins in tEPEC. In contrast, aEPEC acquire additional virulence factors to compensate for the lack of BFP. Due to the genetic diversity in virulence, aEPEC can impose heterogeneous pathogenicity (Carlino *et al.*, 2020). For example, although it is reported to cause severe diarrhoea, it is also found in 20% of healthy individuals in the US, China, Japan and Finland (Aslani and Alikhani, 2011, Hu and Torres, 2015, Snehaa *et al.*, 2021, Kralicek *et al.*, 2022). Unlike tEPEC, which causes infantile diarrhoea, aEPEC can cause a serious case of diarrhoea in both infants and adults. Diarrhoea caused by aEPEC infections can last for more than two weeks and result in death (Ochoa and Contreras, 2011).

Traditionally, *E. coli* is detected by culturing on selective media under appropriate incubation conditions (Nurliyana *et al.*, 2018). Biochemical tests that target enzymes such as tryptophanase, β-D-glucuronidase, β-galactosidase, β-lactamase and lysine decarboxylase can also be used for detection (Thavaththurai and Amarasekara, 2021). For rapid and cost-effective quantification of *E. coli* in water samples, commercial kits like Colilert-18 and the Quanti-Tray/2000 system have been developed (Kinzelman *et al.*, 2005). Molecular detection relies on amplification of genes such as 16S ribosomal Ribonucleic Acid (rRNA), uidA, lacY and virulence genes using polymerase chain reaction (PCR). Currently, phenotypic and genotypic methods are being introduced to replace the historical O:H serotyping for EPEC diagnosis (Fratamico *et al.*, 2016).

Over the past few decades, the significance of EPEC as a major gastrointestinal pathogen has diminished in scientific literature. It remains uncertain whether this decline is due to public health interventions, such as breastfeeding promotion, or if earlier studies overestimated its prevalence by relying on serotyping instead of molecular methods. However, recent meta-analyses have detected a severe underreporting of EPEC in developing countries, particularly among adults (Kralicek *et al.*, 2022). While extensive research has been done on the clinical aspects of EPEC infection, the environmental prevalence of the organism has not been thoroughly examined, particularly in non-intestinal environments such as water bodies. Notably, none of the available reports provide information on the prevalence of *E. coli* or EPEC infections and related deaths in Sri Lanka or Colombo, with existing studies primarily focusing on antimicrobial-resistant *E. coli*. Additionally, previous research in Sri Lanka has heavily relied on biochemical methods for *E. coli* detection, with limited application of molecular approach. Hence, this study has been designed to detect the presence of EPEC in four visibly polluted canals in Colombo city, given their importance in urban drainage using both microbiological and molecular techniques. The selected canals include Hamilton Canal, Wellawatte Canal, Dehiwala Canal and Norris Canal (Figure 1b). The study aims to assess microbial contamination of Colombo Canal, thereby helping to understand the prevalence of EPEC in Sri Lanka and Colombo City, where only a minority of similar studies have been conducted. Hence, the findings of the study would contribute to the improved surveillance and control of public health risks imposed by EPEC strains in urban water systems.

Materials and Methods

Sample collection

Sampling sites were selected based on their proximity to human settlements and domestic wastewater

discharge points to capture regions with faecal contamination. These areas had visible pollution indicators such as water discolouration, sewage debris and odour. Two surface water samples of 200 ml were collected from a selected site in Hamilton Canal, Wellawatte Canal, Dehiwala Canal and Norris Canal into sterile, labelled containers. All samples were collected on the same day (two days before bacterial isolation) within a period of 3 hours. Collected samples were filtered using a strainer to remove solid waste. Filtrates were stored in sterile, labelled containers at room temperature away from light until analysis. Storing samples in the dark helps to maintain the integrity of samples by preventing unwanted photochemical reactions.

Bacterial isolation and identification

Bacterial isolation was performed under sterile conditions. Initially, 100 ml of each sample was filtered through separate filter papers which were kept on a Porcelain funnel. Filtrates were secondly filtered through separate nitrocellulose membranes (pore size: 0.45 µm) which were kept on a Porcelain funnel. After filtration, nitrocellulose membranes were placed on separate, labelled Vancomycin-containing MacConkey agar plates. Similarly, autoclaved distilled water was prepared as the negative control using the same steps. *E. coli* (ATCC 25922) was streaked on a fresh MacConkey agar plate to be used as the positive control. All of the agar plates including the negative and positive controls were incubated at 37 ºC for 24 hours.

Following 24 hours, the incubated agar plates were observed for *E. coli* colonies. The morphology of the colonies from the sample plates was compared to that of the positive control for identification. Colonies were also compared with the evidence published in Barcella *et al.*, (2016) for further confirmation. Smears of the colonies that were morphologically similar to that of the positive control were heat-fixed on sterile, labelled slides. These smears were subjected to Gram staining and examined using a light microscope. Oil immersion (100x) microscopic images of the smears were compared to the positive control and the descriptions of *E. coli*, which are presented by Zahra *et al.*, (2024) and Sallam *et al.*, (2017). Following the morphological and microscopic confirmation, colonies resembling that of the *E. coli* were sub-cultured into Luria- Bertani (LB) broth to obtain pure isolates. Sub-culturing was done in duplicates to avoid any uncertainties. The inoculated LB broth tubes were incubated at 37 ºC for 24 hours.

Bacterial deoxyribonucleic acid (DNA) isolation and quantification

After incubation, bacterial DNA was isolated using the modified boiling cell extraction technique presented in Shafi *et al.*, (2019) under sterile conditions. Extracted DNA was stored at -20 ºC in Tris-Ethylenediaminetetraacetic acid (TE) buffer until PCR. The extracted DNA was visualized on 1% agarose gel using the spot gel technique. The gel image was visualized and captured using the gel documentation system. The concentration of the extracted DNA was calculated using the below Equation (Eq1) after measuring the absorbance of each sample at 260 nm using a spectrophotometer (Guha *et al.*, 2018).

$$[DNA\ extract] = A_{260}\ x\ Df\ x\ 50\ ng\mu l^{-1} \ldots\ldots Eq1$$

In Equation 1, [DNA extract] is the concentration of DNA extracted (in ng/ µl) and A_{260} is the absorbance measured at 260 nm in units of Optical Density, and Df is the dilution factor. The dilution factor is 100 and 50 ngµl^{-1} is the concentration of DNA extract with an A_{260} reading of 1.0.

Amplification of selected genes and visualization

Two sets of primers targeting important EPEC virulence genes that are tabulated under Table 1 were used for this study.

Table 1: Table of primer details (Adapted from (d'Auriac, 2009)[1]

Target gene		Sequences (5/ to 3/)	Product size in base pairs (bp)
eaeA	F	AGGCTTCGTCACAGTTG	570
	R	CCATCGTCACCAGAGGA	
bfpA	F	AATGGTGCTTGCGCTTGCTGC	747
	R	GCCGCTTTATCCAACCTGGTA	

[1]*Note: F = Forward primer; R = Reverse primer*

Two PCR master mixes were prepared separately for the amplification of both *eaeA* and *bfpA* genes. The volume of DNA required to be added in the master mix was calculated using Equation 2 (Eq2) (Guha *et al.*, 2018). Each of the master mixes was prepared to be

sufficient for nine samples, including a blank and eight DNA extracts. It was prepared by orderly adding specific volumes of each component, except DNA as listed in Table 2.

$$V_{DNA} = \frac{Desired\ [DNA]}{Extracted\ [DNA]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad Eq2$$

V_{DNA} is the volume of DNA required for PCR. The desired DNA concentration is considered as 50 ngµl^{-1}. [Extracted DNA] is the concentration of DNA extracted as calculated using Eq1.

Table 2: Table of PCR master mixture components (Adapted from Thavaththurai and Amarasekara, 2021)

Reagent	Working concentration	Volume per sample (µl)	Volume per sample x 9 (µl)
Autoclaved distilled water		9.75	87.75
PCR buffer	1x	5.00	45.00
Magnesium Chloride	1.5 mM	1.50	13.5
Deoxy-nucleoside triphosphate	0.2 mM	0.50	4.50
Forward primer	0.2 µM	2.50	22.50
Reverse primer	0.2 µM	2.50	22.50
Taq polymerase	0.05 U/µl	0.25	2.25
DNA		3.00	
Total		25	

A volume of 22 µl of the prepared master mixture was aliquoted into different, labelled PCR tubes. Subsequently, 3 µl of the respective DNA extract was added so that the final volume in each PCR tube is 25 µl. *eaeA* and *bfpA* genes present in the DNA samples were amplified separately in two amplification reactions, according to the protocols described in Table 3 using a programmable thermal cycler. For each amplification reaction, a blank solution was also included. Blank was prepared by adding autoclaved distilled water to the master mixture aliquot, instead of DNA.

Gel electrophoresis was performed at 75 Volts for 1 hour to visualize the resultant PCR products using 1% agarose gel. Generated bands were compared to 100 bp DNA ladder. Visualized gel images were captured using the gel documentation system. The presence or absence of EPEC in collected water samples was detected based on the bands of expected amplicon size that were obtained in the gel image.

Table 3: Table of optimized PCR protocol (Fröhlicher *et al.*, 2008, Pérez *et al.*, 2010)[2]

Process	*eaeA*			*bfpA*		
	°C	t	Cycles	°C	t	Cycles
Initial denaturation	94	5 min		94	3 min	
Denaturation	94	30 s		94	1 min	
Annealing	50	30 s		56	1 min	
Extension	72	30 s	35	72	1 min	35
Final extension	72	5 min		72	5 min	
Final hold	4	∞		4	∞	

[2]*Note: °C = Temperature in degree Celsius; t = Time; min = Minutes; sec = Seconds*

Data analysis

The association between the canal and the type of EPEC isolate (based on the presence of amplicons corresponding to *eaeA* or *bfpA* genes) was tested using a chi-square analysis. The chi-square test was performed using the 'CHISQ. TEST' function in Microsoft Excel (2021). A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

The contamination of water bodies with faecal matter imposes significant health risks to humans. Hence, the detection of waterborne bacteria is crucial to develop methods for the detection of these pathogens. Over the past decade, molecular diagnostics such as PCR have become valuable in detecting various pathogens by amplifying their virulence genes. Given the challenges of the identification of diarrheagenic *E. coli* using conventional techniques, PCR offers a more sensitive and specific alternative (Nurliyana *et al.*, 2018). However, its implementation in developing countries has been limited primarily due to financial constraints faced by local authorities (Fratamico *et al.*, 2016). This study used a microscopic and molecular analysis for the detection of EPEC strains from four polluted water canals in Colombo, Sri Lanka. EPEC was selected for this study due to its significant role in causing severe

and persistent diarrhoea, particularly in children of developing countries like Sri Lanka. Unlike the other pathogenic strains such as EHEC and ETEC, which produce toxins, EPEC causes intestinal damage and malabsorption by forming A/E lesions (Hunter, 2003). Additionally, the transmission of EPEC through contaminated water, makes it a key indicator of faecal contamination in water bodies.

The colony morphology of culture plates and the microscopic images of the colonies are represented in Figure 2.

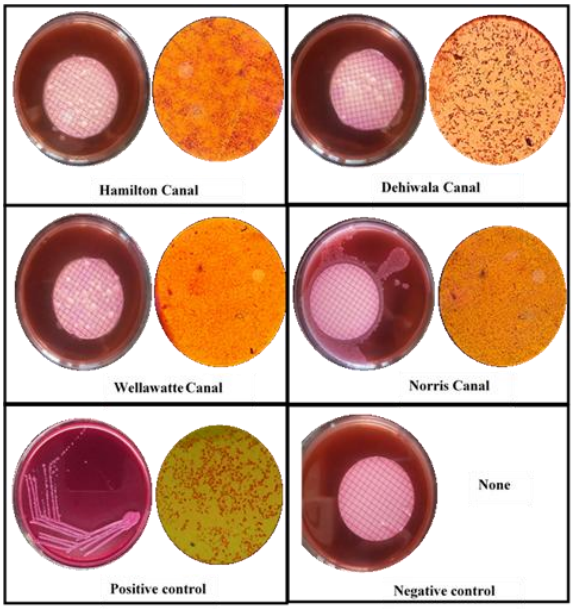

Figure 2: Colony morphology and microscopic view of the cultures observed on different sample plates

The results in Figure 2 represent the extent of microbial contamination in these canals. *E. coli* cannot colonize chlorinated water with 5 parts per million (ppm) chlorine content (Thavaththurai and Amarasekara, 2021) . Since the canal water is non-chlorinated, *E. coli* growth cannot be hindered. Further, the Colombo Canal water has an average temperature of 30 ºC, pH of 7.8 and Oxidation-Reduction Potential (ORP) of -44.8 millivolts (mV), which are all within the recommended ranges for *E. coli* growth (Jayaweera and Samarakoon, 2020) . These physicochemical parameters and a higher concentration of NO_3^- (5.8 ppm) and PO_4^{3-} (0.4 ppm) in the canal network could be because of the discharge of organic matter, which has led to the microbial contamination of the canals as observed in this study. However, similar microscopic and macroscopic morphology as in Figure 2 could also be presented by other gram-negative, water-borne bacteria such as *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia marcescens* (Jung and Hoilat, 2024). Hence, to confirm the presence of *E. coli*, molecular analysis was carried out.

Following DNA extraction, the concentration and purity of the extracted DNA were analysed using a spot gel and spectrophotometer analysis. This is essential as impurities present in the extracted DNA can interrupt the downstream PCR reactions (Thavaththurai and Amarasekara, 2021). Spot gel image (Figure 3) indicated a successful extraction of bacterial DNA from each sample. The intensity of the spots observed on the gel can be used as an estimation of the DNA concentration.

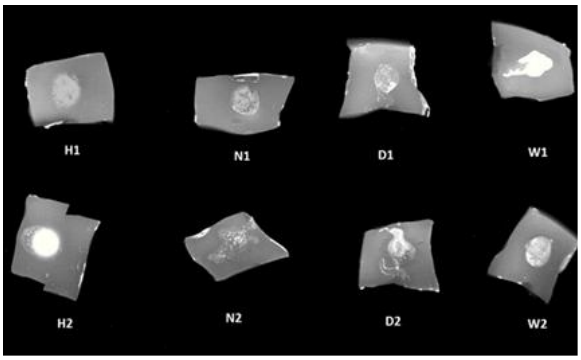

Figure 3: Spot gel image

Table 4 summarizes the absorbance at 260 nm and concentration of extracted DNA (Equation 1) from each sample. The concentration of all the extracted DNA was within the range of 2.85 to 3.35 ng/µl, which is suitable for a successful PCR. Table 4 also tabulates the desired volumes of DNA extracts for PCR, which is approximately calculated as 3 µl using Equation 2.

Table 4: Table of absorbance, concentration of DNA extracts and the required volume for PCR[3]

DNA extract	A_{260}	[DNA] ($ng\mu l^{-1}$)	Volume required for PCR (µl)
H1	0.0034	17.0	2.94 ~ 3.0
H2	0.0035	17.5	2.86 ~ 3.0
W1	0.0036	18.5	2.78 ~ 3.0
W2	0.0035	17.5	2.86 ~ 3.0
D1	0.0031	15.5	3.23 ~ 3.0
D2	0.0033	16.5	3.03 ~ 3.0
N1	0.0033	16.5	3.03 ~ 3.0
N2	0.0030	15.0	3.33 ~ 3.0

[3]*Note: H1, H2 = Hamilton Canal extracts; W1, W2 = Wellawatte Canal extracts; D1, D2 = Dehiwala Canal extracts; N1, N2 = Norris Canal extracts*

Concentration values calculated using absorbance (Table 4) correlate well with the intensity of DNA

samples observed in the spot gel image (Figure 3) thus, excluding errors in calculation. Differences in the amount of DNA extracted as in the above results (Table 4 and Figure 3), provide insight into bacterial load present in the samples. The highest concentration of DNA was extracted from the water sample from Norris Canal. This also correlates with the larger number of colonies observed on the culture plate and a denser population of *E. coli* observed using the microscope (Figure 2). Norris Canal is surrounded by a lot of pharmaceutical and metal industries compared to other canals leading to greater contamination and an increased number of isolates (Eriyagama and Ratnayake, 2008). The second highest concentration of DNA were extracted from the sample from the Dehiwala Canal. However, the culture plate and the microscopic view of the same sample show only fewer *E. coli* growth. In contrast, DNA extracts from the Hamilton Canal water, which is of the second lowest concentration show a denser growth of colonies than that of the Dehiwala Canal extracts. The lowest DNA concentration was extracted from the Wellawatte canal sample, which is also evident from the microscopic analysis. Results (Table 4) also indicate that the concentration of DNA extracts from Hamilton and Wellawatte canal samples are almost equal. While the observed differences in results could have a significant meaning in terms of bacterial load, several other factors could also lead to the difference in DNA yield. For example, the presence of contaminants such as proteins and ribonucleic acid (RNA) and ineffective lysis can prevent efficient extraction (Guha *et al.*, 2018). Hence, further analysis using quantitative PCR is required for more confirmation.

The *eaeA* gene is a characteristic virulence gene present in both tEPEC, aEPEC and EHEC (Hunter, 2003). Hence, the amplification of the *eaeA* gene alone is not sufficient for the detection of EPEC in the samples. *bfpA* gene, another virulence gene present only in tEPEC was also amplified to improve detection. Amplification of these two genes not only aids the detection of EPEC but also the pathotype of EPEC. Historically, tEPEC has been detected more frequently in industrialized countries. However, the recent outbreaks of diseases linked to the aEPEC strains necessitate the importance of similar studies (Aslani and Alikhani, 2011) . Differentiation of the type of EPEC is advantageous due to the differences in disease characteristics. Most of the similar studies have also amplified the 16S rRNA gene to confirm the presence of bacteria (Thavaththurai and Amarasekara, 2021). Since, this study uses a microscopic approach to detect the presence of *E. coli*, the 16S rRNA gene was not amplified.

Gel electrophoresis of the PCR products revealed the presence of the *eaeA* gene in three of the four tested samples as shown in Figure 4a. These include the water samples collected from the Dehiwala Canal, Hamilton Canal and Wellawatte Canal. Bands for the amplification of the *bfpA* gene were observed in two of the samples, namely Hamilton and Wellawatte Canal (Figure 4b). Hence, the strains isolated from these canal water samples are of tEPEC while that of Dehiwala canal is aEPEC.

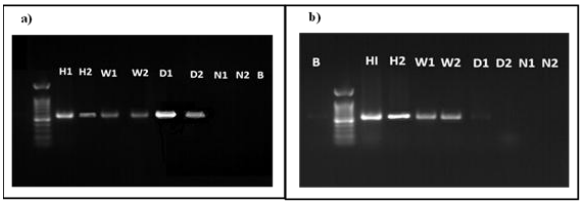

Figure 4: Gel electrophoresis results of PCR products; a) Amplification of *eaeA* gene b) Amplification of *bfpA* gene. B=Blank.

The detection of the same type of EPEC strain from Hamilton and Wellawatte canal samples could be because of the exchange of water between the two canals via Kelani River (Moufar and Perera, 2018). Gomes *et al.*, (2024) has reported the increased growth of *eaeA* gene-positive strains in beachy environments. A similar observation was seen in Wellawatte and Dehiwala canal samples, which are closer to the beach. However, despite the water exchange, two different pathotypes were identified from these two samples. Despite having a higher *E. coli* growth and DNA concentration, none of the tested genes were amplified from the Norris Canal sample. This could be because the isolated strains are non-pathogenic or of another pathogenic variety, which requires further testing. Flood water is exchanged from Norris Canal to Hamilton Canal via Beira Lake (Eriyagama and Ratnayake, 2008). This could indicate that the detection of tEPEC strain in the Hamilton Canal sample can be due to the contamination in Beira Lake. These results align with similar studies conducted in other developing countries, which have detected the presence of EPEC in drinking water sachets, tap water, rivers, bays and recreational water sources (Kinzelman *et al.*, 2005, Gemechu *et al.*, 2018, Nurliyana *et al.*, 2018, Ram *et al.*, 2024). All of these studies also detected tEPEC as the dominant strain in the tested samples. However, the same was not observed by Gomes *et al.*, (2024) in South Africa, where the presence of aEPEC was detected to be higher than tEPEC. This difference can be because of the geographical variations and the extent of anthropogenic activities. The blank (Figure 4a and

Figure 4b) has no bands indicating that there is no contamination.

The Chi-Square analysis generated a p-value of 0.0245, which is less than 0.05. This indicates a statistically significant association between the presence of EPEC strain and the canal, where some canals are more likely to harbour certain strains. It could be explained based on the vicinity of the canal to sources of faecal or industrial contamination. The main reason for the abundance of EPEC in water sources is the faecal contamination, as suggested by most of the studies conducted on diarrhoeal patients from Costa Rica, Indonesia, Iran and Southern Ethiopia (Harti *et al.*, 2006, Aslani and Alikhani, 2011, Lim *et al.*, 2020). In addition to human origin, faeces from animals such as sheep, cattle, chicken and pigs have been identified as the main sources for aEPEC (Fröhlicher et al., 2008; Oh et al., 2012). However, Byappanahalli *et al.*, (2012) also suggest that these strains could be environmentally adapted, disapproving of the use of EPEC or *E. coli* as indicators for faecal contamination. Environmentally derived strains in water sources originate from algae and beach sand. Hence, the use of techniques such as source-specific qPCR helps more in the prediction of health risks in humans (Jang *et al.*, 2017).

Previous studies have also identified the influence of seasonal variation in the type of EPEC strain detected in the water sources (Ram *et al.*, 2024). Samples for the present study were collected in August, which is a warmer month. Similar studies have indicated a higher prevalence of aEPEC in water sources during summer (Gomes *et al.*, 2024). However, this contradicts our findings, in which tEPEC were detected as the most prevalent pathotype. Studies have also indicated the inactivation of *E. coli* upon exposure to sunlight due to the increase in temperature, leading to less culturable bacteria in the sample (Edward *et al.*, 2020). Norris canal sample, which was collected at 9.00 am yielded a higher bacterial growth in comparison to those collected from Dehiwala and Wellawatte canals, which were collected after 11.00 am. The time difference in sample collection could have caused greater sun exposure to those collected later. Hence, the amount of bacterial growth cannot be truly attributed to the difference in the canal from which the sample is collected.

Although the presence of virulence factors alone cannot determine the pathogenicity of bacterial isolates, previous studies have explored the potential of these strains to cause diarrhoea in humans (Pearson and Frankel, 2016). Further analysis such as screening for additional virulence factors, serotyping and phylogenetic analysis could provide more evidence to support this hypothesis (Carlino *et al.*, 2020). While antimicrobial therapy is prescribed to lower the diarrhoeal symptoms, raising resistance to ampicillin, chloramphenicol, and trimethoprim is concerning (Thavaththurai and Amarasekara, 2021). Hence, further studies can be conducted on the antibiotic resistance of EPEC and the difference in resistance patterns among the two pathotypes. MacConkey agar, which is used in this study, is effective in identifying lactose-fermenting microbes. Hence, *E. coli* prevalence can be under- or overestimated as it does not provide species-level identification or differentiates coliforms like Eosin Methylene Blue (EMB) agar. Future studies are recommended to use EMB agar for accurate confirmation of *E. coli*. PCR-based detection is often affected by contamination and false negative results. Future research could integrate Next Generation Sequencing or immunological techniques to monitor water quality and pathogen detection (Fratamico *et al.*, 2016). The alarming presence of EPEC in canal water as shown in our findings, imposes a significant risk to communities that rely on it directly or indirectly. This highlights the urgent need for regular monitoring of canal water, policies and practice interventions to address pathogenic *E. coli* contamination.

Conclusion

This study detected the presence of EPEC in water samples collected from Hamilton, Wellawatte and Dehiwala canals. Samples from the Hamilton and Wellawatte canals were positive for both *eaeA* and *bfpA* genes, indicating the presence of tEPEC. Isolates obtained from the Dehiwala Canal were positive for only the *eaeA* gene thus, they were aEPEC. Despite producing *E. coli*-like colonies, samples from the Norris Canal were negative for both the tested genes, which requires further testing. These results indicate microbial contamination in the Colombo Canal network, calling for continual monitoring of canal water quality. This helps to mitigate the spread of water-borne diseases, particularly diarrhoea among children in the surrounding communities. Hence, it is evident that the screening for *eaeA* and *bfpA* genes helps in the detection of EPEC and differentiation of the EPEC pathotypes in polluted water canals.

Acknowledgement

The authors would like to thank BMS Campus, Faculty of Life and Medical Sciences, for providing the opportunity to conduct this research.

Declaration of Conflict of Interest

The authors declare no conflicts of interest.

Reference

Alikhani, M. Y., Mirsalehian, A., and Aslani, M. M. (2006). Detection of typical and atypical enteropathogenic Escherichia coli (EPEC) in Iranian children with and without diarrhoea. Journal of Medical Microbiology 55:1159-1163. https://doi.org/10.1099/jmm.0.46539-0.

Anindita, M., Rahman, A., Alim, M. A., Xiong, C., Hossain, S., and Sathasivan, A. (2025). Microbial ecology of harvested rainwater: Assessing quality, antimicrobial resistance and geographical variation. Journal of Cleaner Production 486:144439. https://doi.org/10.1016/j.jclepro.2024.144439.

Aslani, M. M., and Alikhani, M. Y. (2011). Molecular and phenotypic characterization of atypical enteropathogenic Escherichia coli serotypes isolated from children with and without diarrhea. Journal of Microbiology, Immunology and Infection 44:27-32. https://doi.org/10.1016/j.jmii.2011.01.006.

Banach, J. L., and Van Der Fels-Klerx, H. J. (2020). Microbiological Reduction Strategies of Irrigation Water for Fresh Produce. Journal of Food Protection 83:1072-1087. https://doi.org/10.4315/JFP-19-466.

Barcella, L., Barbaro, A. P., and Rogolino, S. B. (2016). Colonial morphology of Escherichia coli: impact of detection in clinical specimens. Microbiologia Medica 31:51-55.

Basavaraju, M., and Gunashree, B. (2023). Escherichia coli: an overview of main characteristics. Pages 1-21 *in* S. Marjanca, Erjavec, editor. Escherichia coli-old and new insights.

Byappanahalli, M. N. M. B. N., Korajkic, A., Staley, Z. R., and Harwood, V. J. (2012). Enterococci in the Environment. Microbiology and Molecular Biology Reviews 76:685-706. https://doi.org/10.1128/mmbr.00023-12.

Carlino, M. J., Kralicek, S. E., Santiago, S. A., Sitaraman, L. M., Harrington, A. T., and Hecht, G. A. (2020). Quantitative analysis and virulence phenotypes of atypical enteropathogenic Escherichia coli (EPEC) acquired from diarrheal stool samples from a Midwest US hospital. Gut Microbes 12:1824562. https://doi.org/10.1080/19490976.2020.1824562.

Chen, D., Huiwen, and Frankel, G. (2005). Enteropathogenic Escherichia coli: unravelling pathogenesis. FEMS Microbiology Reviews 29:83-98. https://doi.org/10.1016/j.femsre.2004.07.002.

d'Auriac, M. A. (2009). Development of microbiological molecular diagnostic techniques for the rapid screening and identification of selected human bacterial pathogens and indicators. University of Oslo, Oslo.

Edward, K., Ibekwe, V., Amadi, E., and Umeh, S. (2020). Prevalence and antibiotic susceptibility pattern of Escherichia coli isolated from abattoir wastewaters in Abia State, Nigeria. nternational Research Journal of Public and Environmental Health 7:140-148. https://doi.org/10.15739/irjpeh.20.020.

Eriyagama, N., and Ratnayake, N. (2008). Decision support tool for Colombo canal system water quality monitoring. Engineer: Journal of the Institution of Engineers, Sri Lanka 41:24-33. https://doi.org/10.4038/engineer.v41i1.7081.

Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., and Feng, P. (2016). Advances in Molecular Serotyping and Subtyping of Escherichia coli†. Frontiers in Microbiology Volume 7 - 2016. 10.3389/fmicb.2016.00644.

Fröhlicher, E., Krause, G., Zweifel, C., Beutin, L., and Stephan, R. (2008). Characterization of attaching and effacing Escherichia coli (AEEC) isolated from pigs and sheep. BMC Microbiology 8:144. https://doi.org/10.1186/1471-2180-8-144.

Gemechu, A., Zewdie, O., Mussema, A., Amante, A., and Asmera, B. (2018). Bacteriological quality of drinking water obtained from main sources, reservoirs and consumers tap in Arba Minch town, Southern Ethiopia. African Journal of Microbiology Research 12:567-573. https://doi.org/10.5897/AJMR2018.8871.

Gomes, L., Bordalo, A. A., and Machado, A. (2024). Characterization of Escherichia coli Isolates in Recreational Waters: Implications for Public Health and One Health Approach. Water 16:2695. https://doi.org/doi:10.3390/w16182695.

Guha, P., Das, A., Dutta, S., and Chaudhuri, T. K. (2018). A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. Journal of Clinical Laboratory Analysis 32:e22181. https://doi.org/10.1002/jcla.22181.

Harti, A. S., Iravati, S., and Asmara, W. (2006). Detection of eae, bfpA, espA genes on Diarrhoeagenic Strains of Escherichia coli Isolates. Indonesian Journal of Biotechnology 11:889-893.

Hernández-Vásquez, A., Visconti-Lopez, F. J., and Vargas-Fernández, R. (2022). Escherichia coli Contamination of Water for Human Consumption and Its Associated Factors in Peru: A Cross-Sectional Study. Am J Trop Med Hyg 108:187-194. https://doi.org/10.4269/ajtmh.22-0240.

Hu, J., and Torres, A. G. (2015). Enteropathogenic Escherichia coli: foe or innocent bystander? Clinical Microbiology and Infection 21:729-734. https://doi.org/10.1016/j.cmi.2015.01.015.

Hunter, P. R. (2003). Drinking water and diarrhoeal disease due to *Escherichia coli*. Journal of Water and Health 1:65-72. https://doi.org/10.2166/wh.2003.0008.

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., and Ishii, S. (2017). Environmental Escherichia coli: ecology and public health implications—a review. Journal of Applied Microbiology 123:570-581. https://doi.org/10.1111/jam.13468.

Jayaweera, J., and Samarakoon, M. (2020). Effect on Water Quality due to Residential Development–A Case Study on Kolonnawa Canal 13th International Research Conference. General Sir John Kotelawala Defence University 27-35

Jung, B., and Hoilat, G. J. (2024). MacConkey medium StatPearls [internet]. StatPearls Publishing

Kinzelman, J. L., Singh, A., Ng, C., Pond, K. R., Bagley, R. C., and Gradus, S. (2005). Use of IDEXX Colilert-18® and Quanti-Tray/2000 as a Rapid and Simple Enumeration Method for the Implementation of Recreational Water Monitoring and Notification Programs. Lake and Reservoir Management 21:73-77. https://doi.org/10.1080/07438140509354414.

Kralicek, S. E., Sitaraman, L. M., Kuprys, P. V., Harrington, A. T., Ramakrishna, B., Osman, M., and Hecht, G. A. (2022). Clinical Manifestations and Stool Load of Atypical Enteropathogenic Escherichia coli Infections in United States Children and Adults. Gastroenterology 163:1321-1333. https://doi.org/10.1053/j.gastro.2022.07.064.

Li, T., Qiang, N., Bao, Y., Li, Y., Zhao, S., Chong, K. C., Deng, X., Zhang, X., Ran, J., and Han, L. (2024). Global burden of enteric infections related foodborne diseases, 1990–2021: findings from the Global Burden of Disease Study 2021. Science in One Health 3:100075. https://doi.org/10.1016/j.soh.2024.100075.

Lim, M.-A., Kim, J.-Y., Acharya, D., Bajgain, B. B., Park, J.-H., Yoo, S.-J., and Lee, K. (2020). A Diarrhoeagenic Enteropathogenic Escherichia coli (EPEC) Infection Outbreak That Occurred among Elementary School Children in Gyeongsangbuk-Do Province of South Korea Was Associated with Consumption of Water-Contaminated Food Items. International Journal of Environmental Research and Public Health 17:3149. https://doi.org/doi:10.3390/ijerph17093149.

Lin, L., Yang, H., and Xu, X. (2022). Effects of Water Pollution on Human Health and Disease Heterogeneity: A Review. Frontiers in Environmental Science Volume 10 - 2022. 10.3389/fenvs.2022.880246.

Moufar, M. M. M., and Perera, E. D. P. (2018). Floods and Countermeasures Impact Assessment for the Metro Colombo Canal System, Sri Lanka. Hydrology 5:11.

Mueller, M., and Tainter, C. R. (2023). Escherichia coli infection. StatPearls Publishing, Treasure Island (FL).

Nurliyana, M. R., Sahdan, M. Z., Wibowo, K. M., Muslihati, A., Saim, H., Ahmad, S. A., Sari, Y., and Mansor, Z. (2018). The Detection Method

of Escherichia coli in Water Resources: A Review. Journal of Physics: Conference Series 995:012065. https://doi.org/10.1088/1742-6596/995/1/012065.

Nuwanka, M., and Gunathilaka, M. (2023). Complexities of water pollution: A review of surface water contamination in Sri Lanka. International Journal of Environment, Engineering and Education 5:72-78. https://doi.org/10.55151/ijeedu.v5i2.97.

Ochoa, T. J., and Contreras, C. A. (2011). Enteropathogenic escherichia coli infection in children. Current Opinion in Infectious Diseases 24:478-483. https://doi.org/10.1097/QCO.0b013e32834a8b8b.

Oh, J. Y., Kang, M. S., An, B. K., Shin, E. G., Kim, M. J., Kim, Y. J., and Kwon, Y. K. (2012). Prevalence and characteristics of intimin-producing Escherichia coli strains isolated from healthy chickens in Korea. Poultry Science 91:2438-2443. https://doi.org/10.3382/ps.2012-02301.

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., and Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. AMB Express 4:51. https://doi.org/10.1186/s13568-014-0051-x.

Pearson, J. S., and Frankel, G. (2016). Immunity to Enteropathogenic Escherichia coli. Pages 43-51 *in* M. J. H. Ratcliffe, editor. Encyclopedia of Immunobiology. Academic Press, Oxford.

Pérez, C., Gómez-Duarte, O. G., and Arias, M. L. (2010). Diarrheagenic Escherichia coli in children from Costa Rica. Am J Trop Med Hyg 83:292-297. https://doi.org/10.4269/ajtmh.2010.09-0412.

Ram, S., Gautam, R. L., Singh, S., Singh, D., Naraian, R., and Arif, N. (2024). Prevalence of multidrug-resistant enteropathogenic Escherichia coli (EPEC) in river Gomti at Jaunpur city. Cleaner Water 2:100048. https://doi.org/10.1016/j.clwat.2024.100048.

Sallam, A., A. , Ahlam, E. Y., and Saad, E. A. K. G. (2017). Some studies on immunoglobulin A, complement 3 and nitric oxide on the level of Escherichia coli enteritis in broiler with pathological changes in Bohera Province Animal Health Research Journal 5:184-195.

Shafi, N., Piyasena, T., and Weerasooriya, P. (2019). Identification of Lactobacillus in fermented cheese and the analysis of their antibiotic resistance. GARI International Journal of Multidisciplinary Research 5.

Snehaa, K., Singh, T., Dar, S. A., Haque, S., Ramachandran, V. G., Saha, R., Shah, D., and Das, S. (2021). Typical and atypical enteropathogenic Escherichia coli in children with acute diarrhoea: Changing trend in East Delhi. Biomedical Journal 44:471-478. https://doi.org/10.1016/j.bj.2020.03.011.

Thavaththurai, M., and Amarasekara, U. (2021). Identification of E. Coli in drinking water samples in Jaffna (Valikamam, West) area and detection of antibiotic resistance. GARI International Journal of Multidisciplinary Research 7:61-84.

van Elsas, J. D., Semenov, A. V., Costa, R., and Trevors, J. T. (2010). Survival of Escherichia coli in the environment: fundamental and public health aspects. The ISME Journal 5:173-183. https://doi.org/10.1038/ismej.2010.80.

World Health Organization (2023). Drinking Water

World Population Review (2024). Colombo Population. Retrieved November 20, 2025, from https://worldpopulationreview.com/cities/brazil/colombo

Zahra, R. L. A., Effendi, M. H., Suprayogi, T. W., Prayudi, S. K. A., Kurniawan, S. C., Wardhana, D. K., and Khairullah, A. R. (2024). Identification of the Genotypes of Extended-spectrum β-lactamase Producing Escherichia coli in the Small Intestine of Broiler Chickens at a Traditional Market in Surabaya, Indonesia. Asian Journal of Dairy and Food Research:1-8. https://doi.org/10.18805/ajdfr.drf-376.