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Isolation and Characterization of Escherichia coli from Rivers of Trivandrum City and Assessment of its Antibiotic Sensitivity

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ABSTRACT

Water pollution is a major problem which arises due to different human activities and the availability of pure water for human consumption is decreasing gradually. One of the predominant causes of water pollution is the deposition of fecal matters and thereby spreading microorganisms which can cause serious water borne diseases. Enterobacteria like *E. coli* are the major indicators of fecal contamination in the water bodies. The present study was conducted to explore the presence of *E. coli* from the two major rivers, located in two different places of the Trivandrum city, which are highly depended by the citizens. The presence of *E. coli* in the water samples were confirmed by several biochemical tests and the molecular confirmation of *E. coli* was done through polymerase chain reaction for 16S rRNA. Antibiotic sensitivity of *E.coli* against the commonly used antibiotics was also determined in this study. This study of isolation and characterization of *E. coli* from the two major rivers in Trivandrum and comparing their antibiotic sensitivity is first of its kind. Although water quality analysis of the water samples from these rivers have been done to study the physio-chemical parameters and total coliforms present, no attempts have been made to assess the antibiotic sensitivity of *E. coli* isolated from the samples. Our study was carried out to see the antibiotic sensitivity of the *E. coli* isolated and future studies could confirm if these can act as the indicators of water quality. Through this analysis we have concluded the results which can support future research by acting as a credible milestone.

KEY WORDS: ANTIBIOTIC SENSITIVITY, DRINKING WATER, *E. COLI*, INDICATOR, MICROBIOLOGICAL QUALITY, TRIVANDRUM RIVER

INTRODUCTION

Water is an essential component consumed in the greatest quantity around the world. It is the most vital element for life, procured from natural sources such as rivers, underground water, and lake water. Consequently, large number of health risks are associated with consumption of contaminated water. Drinking water should be safe and free from chemical toxins and pathogenic microorganisms. Accessibility and availability of fresh clean water not only plays a crucial role in economic development and social welfare, but also it is an essential element in health, food production and poverty reduction (Eckner, 1998; Odonkor and Ampofo., 2013). Methods had been being methods have been developed since 1900s to assess water quality regarding public health by enumerating coliforms and *Escherichia coli* cells in water as indicators of water purity. *E. coli* are widely

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distributed in the gastro-intestinal tract of humans, pests, ruminants, and wild animals, where they are known to live as commensals (Eckner, 1998; Feng et al, 2009). In normal habitat, *E.coli* is beneficial for digestion. Beyond certain limit or by ingestion of contaminated food and water, toxin produced by the bacteria cause infection in the cells of intestinal tract, enter into the blood and finally leads to many diseases (Adzitey et al., 2015).

The presence of *E. coli* in food or water indicates that there is an elevated risk of the presence of other enteric bacteria and viruses, such as Salmonella spp. Shigella or hepatitis A virus, etc. Therefore *E. coli* is universally considered as an indicator organism of fecal contamination in food and water samples and to compare the degree of contamination (Odonkor and Ampofo., 2013). *E.coli* O157:H7 was first human pathogen. Some of the pathogenic strains includes Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC) etc (Ebomah et al., 2018; Rayasam et al., 2019). *Escherichia coli* is a useful enteric bacterium in the study of waterborne transfer of antibiotic resistance. A lot of studies have brought out the existence of antibiotic resistant microorganisms and their prevalence in aquatic bodies (Nahar et al., 2019). Among such antibiotic resistant organisms, *E. coli* is a major candidate. Antibiotic resistance is a worldwide obstacle in therapeutics and new forms of antibiotic resistance are arising which can spread all over the world easily (Dhawde et al., 2018; Bong et al, 2020).

The use of antibiotics to combat infections in humans and other animals is a common practice, but indiscriminate use of antibiotics leads to drug resistance in these microbes, which warrants the initiation of steps to prevent public health hazards (Rather et al., 2012). Antibiotic sensitivity test is used to help to choose the antibiotics effective against the specific types of bacteria. The disk diffusion method is the gold standard for confirming the susceptibility of bacteria to various antibiotics. Some types of bacteria are resistant to certain antibiotics because of their genetic material. Infection caused by the resistant bacteria is not cured by treatment with those antibiotics. Polymerase Chain Reaction (PCR) is a molecular biology technique used for enzymatically replicating DNA of organisms (Rahman et al., 2013).

Molecular methods for the detection of *E. coli* in food and water have mainly concentrated on the use of PCR gene probe technology. However, there are a few reports on the potential use of 16s rRNA gene target method for the detection of *E. coli* (Bej et al., 1991; Fattahi et al., 2013). Using conserved sequences, flanking variable region as primers, the sequence of the variable region of the 16s rRNA gene could be amplified. Several studies have been carried out to isolate and characterize *E. coli* from major rivers on a global scenario (Tsen et al., 1998; Bong et al., 2020; Praveenkumarreddy et al., 2020). Most of these studies would confirm the presence of *E. coli* as indicator of the water quality and thus an indicator organism. Some of these studies would also focus on analyzing the antibiotic sensitivity of the isolates as indexing antimicrobial resistance has significance in clinical domain (Dhawde et al., 2018; Odonkor and Addo., 2018; Nahar et al., 2019; Purohit et al., 2020).

MATERIAL AND METHODS

The water samples were collected from selected stations from Killiyar river and the Vamanapuram river located near to the college where the present study was conducted. The water samples were collected and stored in sterile screw capped containers and transported to lab. For the isolation and biochemical analysis for identification of *E. coli*, the water samples were serial diluted in lactose broth to reduce the density of the culture to more usable concentrations to carry out MPN technique to estimate the viable number of organisms in the sample. The diluted samples were incubated for 24 hours at room temperature. After incubation, a loopful of the enriched culture from lactose broth of the presumptive tests was streaked onto EMB Agar and incubated at 37°C for 24 h. For the completed test, the pure colonies from the incubated EMB plates were cultured in lactose broth or nutrient agar plates and, gram staining and motility of the organism was performed.

The microbial motility was checked by hanging drop method and agar stab method. Biochemical tests are performed for the further identification and confirmation of the organism. The biochemical test performed were: IMViC Test (Indole test, Methyl Red test, Voges-Proskauer test and citrate test), Catalase test, Urease test, Motility Indole Urease test (MIU), Triple sugar iron test. To determine the antibiotic sensitivity by disc diffusion method, the Kirby-Bauer disc diffusion method was used. It was performed in Muller Hinton agar plates. Six antibiotics were tested: Tetracycline (10 µg), Gentamycin (10 µg) Ciprofloxacin (5 µg), Amoxicillin (10 µg), Ampicillin (10 µg), Cefixime (5 µg). A Muller-Hinton medium plate was swabbed with LB broth inoculated with *E. coli* overnight. Sterile discs were impregnated with each of the antibiotics and later, the antibiotic impregnated discs were placed properly above the uniformly spread inoculum containing plates with sterile forceps under aseptic conditions. The plates were incubated for 48 hours at room temperature. By using a scale, the zone of inhibition was measured after incubation.

PCR was conducted after the isolation of DNA from the bacterial samples. The latter was performed using phenol: chloroform extraction method. The *E. coli* cells which were cultured overnight in LB broth was selected for DNA extraction. DNA was extracted from exponential cultures by alkaline lysis with 0.5% of sodium dodecyl sulphate treatment, followed by alkaline lysis. The impurities were removed by the treatment with phenyl chloroform - isoamyl alcohol (24:24:2) extraction. DNA was then precipitated by 2.5 volume of isopropyl alcohol and pelleted by centrifugation. The DNA pellet was washed

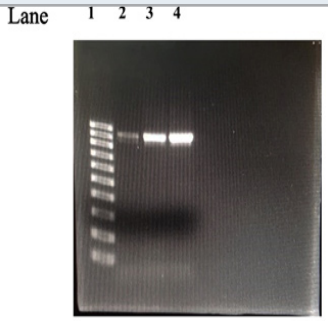
once with 70% alcohol and dried under vacuum. After centrifugation, the DNA was resuspended in TE buffer (Bej et al., 1991).

For the PCR reaction, the primers targeting variable regions of the *E. coli* 16S rRNA gene were developed by Indigenous DNA pvt. Ltd., and the primers were BGT24238 (*E. coli* forward -5'AGAGTTTGATCCTGGCTCAG3') and BGT24239 (*E. coli* reverse- 5'CTTGTGCGGGCCCCCGTCAATTC3'). The PCR solution contained 1X PCR buffer (10X PCR reaction buffer contains 500 µM KCl, 500 µM tris-HCl, (pH 8.3) and 25 µM $MgCl_2$), 200 µM each of the dNTPs, (Perkin- Elmer Cetus) 0.2-0.6 µM each of the primers, 2.5 U of DNA Taq polymerase and the template DNA. The total volume of PCR reaction was 100 µL. For each PCR cycle, the denaturation temperature was 94°C for 1 minute, annealing and extension temperatures 56°- 60°C and 70°C for 30 seconds respectively. The PCR products were examined by agarose gel electrophoresis using ethidium bromide dye.

Table 1. Characteristics of bacteria isolated from water samples

	TEST	OBSERVATION	RESULTS
	Indole production	Appearance of red color band at the junction of medium and reagent	Positive
Water samples from Killiyar and Vamanapuram rivers	Methyl Red	Appearance of red color	Positive
	Voges-Proskauer	No color change	Negative
	Simmon's citrate	No color change	Negative
	Catalase test	Production of gas bubbles	Positive
	Motility	Motile	Motile
	Gram's staining	Appearance of pink color	Gram negative
	MPN	Gas production	Positive
	Urease	No colour change	Negative
	TSI Agar	Yellow slant and yellow butt	A/A
	MIU Test	Bacterial growth occurs throughout the agar	Positive

Figure 1: Agarose gel electrophoresis image of PCR products after amplification of 16S rRNA for E. coli.
Lane 1 : 100 bp DNA ladder Lane 2 : positive control
Lane 3 : Amplicon from sample A (Killiyar river)
Lane 4 : Amplicon from sample A (Vamanapuram river)



Agarose gel electrophoresis (1.5%) of PCR products after amplification of 16S rRNA for E. coli. Lane 1: 100 bp DNA ladder, Lane 2: positive control Lane 3: E coli isolated from sample A Lane 4: E coli isolated from sample B

RESULTS AND DISCUSSION

Coliform such as *E. coli* have been widely used as indicator of the microbiological quality of surface and ground water. Thus, the presence of coliform is an index of bacteriological quality of water. So, in the present study, water samples were collected from two major rivers (i.e., Killiyar and Vamanapuram) in the Trivandrum City and analyzed for the presence of coliforms isolated and the antibiotic sensitivity of the samples were investigated. In the standard MPN test, the presumptive tests showed the presence of gas production in the tubes containing lactose broth with inverted Durham tube, inoculated with the water samples. It indicates the presence of lactose fermenting coliforms in both of the water samples. After incubation, the sample showed turbidity indicating the growth of coliforms. The confirmed test showed small colonies with green metallic sheen on EMB agar which confirms the presence of *E. coli* bacteria. The completed test gave final confirmation that the organism is Gram-negative, non-spore forming, rod shaped, lactose fermenting coliforms. Both hanging drop method and agar stab method showed high bacterial motility of the microbes in the sample (Sreelekshmi et al., 2020).

Upon Gram staining, the colonies showed pink coloration which is a characteristic of gram-negative bacteria. Biochemical analysis also helped to conclude that the water samples from the Killiyar river and the Vamanapuram river contained Coliform bacteria (Table 1). The isolates were confirmed to be *E. coli* by molecular analysis by the amplification of 16S rRNA. The antibiotic sensitivity of *E. coli* against some commonly used antibiotics such as Cefixime, Ciprofloxacin, Tetracycline, Gentamycin, Ampicillin and Amoxycillin was checked by the Kirby-Bauer disc diffusion method. The sensitivity range was observed by analyzing the diameter of Inhibition zone (in mm) on the 48 hours incubated MHA plates (Fig.1). The range of

inhibition zones are shown in table 2. The values clearly indicate that these *E. coli* isolates are highly sensitive to Cefixime, Ciprofloxacin, Gentamycin and least sensitive to Ampicillin and Amoxycillin. From these observations it was confirmed that the antibiotic sensitivity range of the isolated *E. coli* from both the rivers against the above antibiotics were almost similar indicating the strain similarities of both of the isolates (Sreelekshmi et al., 2020).

Figure 2: Antibiotic sensitivity test by Kirby-Bauer disk diffusion method using MHA Agar plates, showing antibiotic sensitivity pattern of *E. coli* isolates from Samples A and B (Killiyar and Vamanapuram rivers).

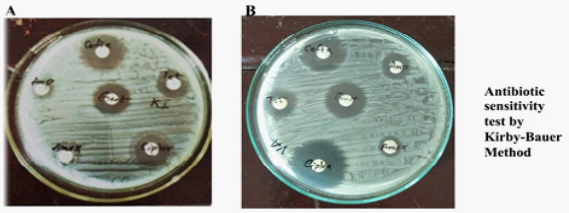


Table 2. The Zone of inhibition measurements

ANTIBIOTIC	ZONE OF INHIBITION	
	WATER SAMPLEFROM KILLIYAR RIVER	WATER SAMPLE FROM VAMANAPURAM RIVER
Cefixime (5µg)	22 mm	23 mm
Ciprofloxacin (5 µg)	20 mm	24 mm
Tetracycline (10 µg)	17 mm	16 mm
Gentamycin (10 µg)	16 mm	19 mm
Ampicillin (10 µg)	16 mm	15 mm
Amoxycillin (10 µg)	8 mm	10 mm

Previous studies have looked at the water quality of rivers like Vamanapuram and Karamana with emphasis to the physio-chemical parameters and presence of total coliforms, but no attempts were done to isolate or characterize *E. coli* from the samples and perform a comparative analysis. Although certain studies have shown the En-Antimicrobial resistance of bacteria isolated from various stations at Karamana river, the sites of present study were not included. Thus, the present study characterizes the *E. coli* isolated from two major rivers in Trivandrum city which is highly depended by the citizens and looks at the antibiotic resistance of the bacteria (Athira, 2019; Sreelekshmi et al., 2020).

CONCLUSION

The water samples were collected from selected stations on Killiyar and Vamanapuram rivers in Trivandrum city where anthropogenic activity is remarkably high. A major population in the city is depending upon these rivers for drinking water. Several biochemical analyses of the river samples revealed the presence of *E. coli.* which could be an indicator of poor water quality of the samples. The presence of *E. coli* was also confirmed by 16S analysis. Both the water samples tested had *E. coli* that were sensitive to the antibiotics with maximum sensitivity towards cefixime when compared to other antibiotics and most resistant to Amoxyllin, which is alarming as it is a commonly used antibiotic (Table 2). The antibiotic sensitivity range of the isolated *E. coli* from both the rivers against the tested antibiotics were almost similar indicating the possibility of strain similarities between the isolates. This study is the first of its kind which characterizes and compares the antibiotic sensitivity of *E. coli* in the heart of Trivandrum city isolated and characterized from these two major rivers. Future studies could confirm the possibility of choosing these strains as an indicator organism of water quality analysis. Further this study cautions the use of water from these rivers as they are subjected to contamination by coliforms

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Conflict of Interest: The authors declare no conflict of interest.

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