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# Molecular Characterization of *Escherichia coli* Pathotypes Isolated from Water Sources in the Northern Union Territory of India

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**ABSTRACT**

Pathogenic *Escherichia coli* is transmitted to humans via the fecal–oral route. This study assessed the safety of water sources with respect to the presence of pathogenic *E. coli* in Jammu and Kashmir, a Union Territory in northern India. A total of 141 water samples were collected from natural sources (81 samples: 43 river, 28 pond, and 10 lake) and drinking water sources (60 samples: 40 municipal supplies and 20 wells). These samples were analyzed for *E. coli* isolation, and pathotyping for ETEC, EPEC, and EHEC was done using PCR. Of the 81 samples collected from natural water sources, 56 (27 rivers, 20 ponds, and 9 lakes) tested positive for *E. coli.* None of these 56 isolates were positive for ETEC or EPEC, while 03 river and 02 pond samples tested positive for EHEC. Among the 60 drinking water samples, 15 (13 from municipal supplies and 2 from wells) were positive for *E. coli*. None of these isolates were positive for ETEC, EPEC, or EHEC. *E. coli* is widely used as an indicator organism for fecal contamination. The findings of this study reveal the extent of contamination of different water sources and emphasize the need for improved water quality monitoring to protect public health.

**HIGHLIGHTS**

- The study revealed high prevalence of *E. coli* in natural and drinking water sources.
- Presence of *E. coli* in water sources is clear indication of fecal contamination of water.
- Fecal contamination of water sources poses significant health risks to both humans and animals of the area.

**Keywords**: *E. coli*, Coliforms, ETEC EHEC, EPEC, India, Jammu, water

Waterborne illnesses are significant contributors to global morbidity and mortality. It is estimated that 4% of all deaths and 5.7% of the global disease burden are attributable to infectious agents associated with unsafe water, inadequate sanitation, and poor hygiene (WASH).

Approximately 37.7 million Indians are affected by waterborne diseases each year; 1.5 million children die from diarrhea alone, and an estimated 73 million working days are lost, resulting in an economic burden of $600 million annually (India Water Portal, 2019). A report indicates that Uttar Pradesh has the highest incidence of diarrheal deaths in India, followed by West Bengal, Assam, Odisha, and Madhya Pradesh (Central Bureau of Health Intelligence, 2018).

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*Escherichia coli* is a Gram-negative, rod-shaped bacterium that exists as a commensal organism in the intestines of warm-blooded animals, including humans. It is present in large numbers in the gut and can survive outside the intestinal tract for a limited period (Feng *et al.*, 2002). Therefore, their presence in environmental samples, food, or water typically indicates recent fecal contamination or poor sanitation and detection of *E. coli* in drinking water is commonly used to assess fecal pollution (Odonkor *et al.*, 2013). The identification of *E. coli* to detect fecal contamination has been a fundamental principle in water quality assessment and monitoring, with significant implications for evaluating the potability of water.

*Escherichia coli*, being a commensal bacterium, generally does not cause disease in the host. However, certain *E. coli* pathotypes have emerged that can cause gastrointestinal and systemic infections. The pathotypes of *E. coli* associated with intestinal disease are collectively classified as Diarrheagenic *E. coli* (DEC) and include six pathotypes *viz*., Enterotoxigenic *E. coli* (ETEC), Verocytotoxigenic *E. coli* (VTEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), and Diffusely adherent *E. coli* (DAEC). Each pathotype is defined by the presence of one or more specific virulence markers. Unlike commensal *Escherichia coli*, these pathotypes produce toxins and other virulence factors that enable them to colonize body sites that are not typically inhabited by *E. coli*, leading to host cell damage (Feng *et al.*, 2002). Diarrheagenic *E. coli* has been implicated in numerous waterborne outbreaks worldwide, including in Ghana (Adzitey *et al.*, 2015), Tanzania (Lyimo *et al.*, 2016), California (Probert *et al.*, 2017), and India (Hamner *et al.*, 2007; Batabyal *et al.*, 2013). In June 2015, a waterborne outbreak of *E. coli* associated with contaminated drinking water occurred at a school camp in Korea (Park *et al.*, 2018). In developing countries, ETEC is the most commonly isolated bacterial enteropathogen in children below five years of age, leading to millions of diarrheal cases and thousands of deaths annually (WHO, 2017).

In India, numerous studies have reported the detection of *E. coli* and pathogenic *E. coli* in various water sources (Dhawde *et al.*, 2018; Ambili and Sebastian, 2019; Ambili *et al.*, 2019), and the safety of these water sources has been assessed. However, there are very few reports available from Jammu region of Jammu and Kashmir (Wani *et al.*, 2014; Mahajan *et al.*, 2018). Therefore, to strengthen the existing data on water source contamination in this region, the present study was undertaken.

## MATERIALS AND METHODS

### Sample collection and processing

A total of 141 water samples were collected over a one-year period (October 2019 to November 2020) from various water sources in the Jammu division of Jammu and Kashmir, India. The samples were broadly categorized into two main groups: water from natural sources and water directly intended for human consumption. The water samples from natural sources ($n$ = 81) included 43 river samples, 28 pond samples, and 10 lake samples. The samples from sources intended for human consumption ($n$ = 60) included 40 municipal supply samples and 20 well samples. The samples were georeferenced, with details provided in the supplementary material. Water samples were collected aseptically in autoclaved bottles, with a volume of 250 mL per sample.For the sampling of municipal supply, sodium thiosulfate solution (3% w/v in water) was added to each sampling bottle at a concentration of 0.25 mL per 250 mL of sample prior to autoclaving, in order to neutralize residual chlorine present in municipal water. The samples were transported to the laboratory in an icebox and processed immediately upon arrival.

### Isolation and identification of *E. coli*

The samples were swabbed onto MacConkey agar plates (HiMedia, India) and incubated at 37°C for 24 hours. Plates were screened for pink, circular, convex colonies indicative of lactose fermenters. Five lactose-fermenting colonies from each plate were streaked onto eosin methylene blue (EMB) agar (HiMedia, India) and incubated at 37°C for 24 hours. Colonies exhibiting a greenish metallic sheen on EMB agar were further purified on nutrient agar. The purified colonies were then subjected to phenotypic and biochemical characterization. The isolates with greenish metallic sheen, gram-negative rods, positive indole test, positive methyl red test, negative Vogues Proskauer test, and negative citrate utilization were confirmed as *Escherichia coli*.

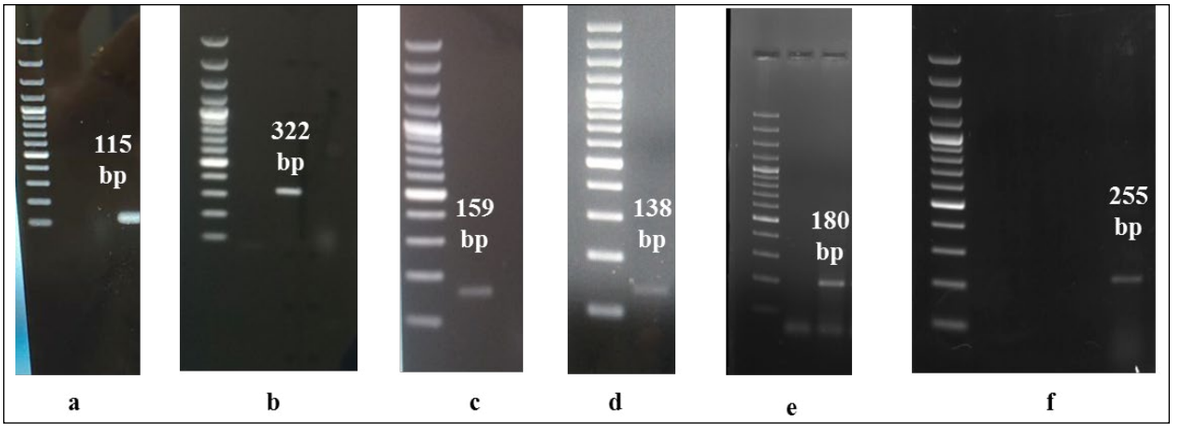


**Fig. 1:** PCR amplification of **(a)** *lt*, **(b)** *stIa*, **(c)** *stIb*, **(d)** *stx1* and **(e)** *stx2* genes

**Detection of *E. coli* pathotypes**

Phenotypically confirmed *E. coli* isolates were subjected to molecular detection of three pathotypes: Enterotoxigenic (*ETEC*), Enterohemorrhagic (*EHEC*), and Enteropathogenic *E. coli* (*EPEC*). The primers, reaction components, and PCR conditions used are listed in Table 1. The amplified products (Fig. 1) were analyzed using agarose gel electrophoresis. Agarose gel was prepared as 1.5% (for > 200 bp size) or 2% (for < 200 bp size) in 1x TAE buffer with ethidium bromide @ 0.5 µg/ml gel. The agarose gel was electrophoresed in 1x TAE buffer at 8V/cm using a Hoefer electrophoresis system with a 100 bp gene ruler as a marker. Agarose gels were photographed using an Eppendorf gel documentation system.

## RESULTS AND DISCUSSION

To assess fecal contamination of natural water sources, 81 samples—including 43 from rivers, 28 from ponds, and 10 from lakes—were processed for the isolation of *E. coli*. Out of these, *E. coli* was detected in 27 river samples (62.79%), 20 pond samples (71.4%), and 9 lake samples (90%) (Table 2). A total of 146 *E. coli* isolates were obtained from the 56 positive samples (Table 2). Upon pathotyping by PCR, none of the 146 *E. coli* isolates tested positive for the *lt*, *stIa*, *stIb*, or *eaeA* genes. However, five isolates—three from river samples and two from pond samples—were positive for the *stx1* gene. Additionally, one isolate from a river sample tested positive for the *stx2* gene (Table 3). Two major lakes in the region, Surinsar Lake and Mansar Lake, were sampled, and water samples from both were found to be positive for *E. coli*. The high abundance of fish in these lakes may explain the highest percentage of *E. coli*. Among the available reports, *E. coli* in lakes was reported by various other researchers (Mehboob *et al.,* 2015; Bhumbla *et al.*, 2020; Krishna *et al.*, 2020). Water sampling from rivers focused on Chenab River and its main left-bank tributary, Tawi River. It was observed that river water was used for human activities including bathing, washing and drinking for livestock at various sampling sites. *E.coli* isolation from rivers have been widely reported in literature by Hamner *et al.* (2007), Mulamattathil *et al.* (2014), Dhiman *et al.* (2018), Kaushik *et al.* (2019), Ogura *et al.* (2020) and Purohit *et al.* (2020). The water samples from ponds were primarily collected from villages in the region. A higher prevalence of *E. coli* was observed in areas inhabited by both humans and livestock. Among the available reports, *E. coli* in ponds was reported by Batabyal *et al.* (2013), Gogoi *et al.* (2013) and Krishna *et al.* (2020).

To assess the contamination of drinking water sources, 60 samples were processed, comprising 40 from municipal supplies (households) and 20 from wells. *E. coli* was detected in 13 municipal supply samples (32.5%) and 2 well samples (10%) (Table 2), yielding a total of 43 isolates. Upon pathotyping by PCR, none of 43 *E. coli* isolates were positive for *lt*, *stIa*, *stIb*, *eaeA*, *stx1*, or*stx2* genes. Municipal supply samples were collected from various locations in Jammu and its adjoining areas. Water samples were obtained directly from household taps,

**Table 1:** PCR details for detection of pathogenic *E. coli*

| Gene | Primers (Reference) | PCR reaction mixture (25 µl) | PCR conditions |
|---|---|---|---|
| *lt* | Forward<br>5'– TCTCTATGTGCATACGGAGC –3'<br>Reverse<br>5'– CCATACTGATTGCCGCAAT –3'<br>(Guion *et al.*, 2008) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.2 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 30 cycles of denaturation – 95ºC for 30 sec, annealing - 56ºC for 45 sec and extension 72ºC for 30 sec<br>• Final extension – 72ºC for 5 min |
| *stIa* | Forward<br>5'– TTTCCCCTCTTTTAGTCAGTCAA –3'<br>Reverse<br>5'– CAGGATTACAACACAATTCACAGCAG–3'<br>(Guion *et al.*, 2008) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.2 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 30 cycles of denaturation – 95ºC for 30 sec, annealing - 55ºC for 45 sec and extension 72ºC for 45 sec<br>• Final extension – 72ºC for 5 min |
| *stIb* | Forward<br>5'– TGCTAAACCAGTAGAGTCTTCAAAA –3'<br>Reverse<br>5'– GCAGGATTACAACACAATTCACAGCAG–3'<br>(Guion *et al.*, 2008) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.2 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 30 cycles of denaturation – 95ºC for 30 sec, annealing - 55ºC for 45 sec and extension 72ºC for 45 sec<br>• Final extension – 72ºC for 5 min |
| *stx1* | Forward<br>5'– TTGAAAATGGTCTGCTGCTG –3'<br>Reverse<br>5'– TATTGGCTTCATCCACCACA –3'<br>(Paton *et al.*, 1998) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.4 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 35 cycles of denaturation – 95ºC for 60 sec, annealing - 62ºC for 2 min and extension 72ºC for 90 sec<br>• Final extension – 72ºC for 5 min |
| *stx2* | Forward<br>5'– GGCACTGTCTGAAACTGCTCC –3'<br>Reverse<br>5'– TCGCCAGTTATCTGACATTCTG –3'<br>(Paton *et al.*, 1998) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.4 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 35 cycles of denaturation – 95ºC for 60 sec, annealing - 62ºC for 2 min and extension 72ºC for 90 sec<br>• Final extension – 72ºC for 5 min |
| *eaeA* | Forward<br>5'– ATGCTTAGTGCTGGTTTAGG –3'<br>Reverse<br>5'– GCCTTCATCATTTCGCTTTC –3'<br>(Guion *et al.*, 2008) | 1x PCR Buffer<br>$MgCl_2$ – 1.5mM<br>dNTP– 200 µM each<br>Primer – 0.4 µM each<br>Taq polymerase – 1 U<br>DNA Template – 2 µl | • Initial denaturation – 95ºC for 2 min<br>• 35 cycles of denaturation – 95ºC for 30 sec, annealing - 55ºC for 45 sec and extension 72ºC for 45 sec<br>• Final extension – 72ºC for 5 min |

**Table 2:** *E. coli* isolation results among water sources

| Sample category | Sample type | Samples Analyzed | Samples Positive for *E. coli* (%) | No. of *E. coli* isolates obtained |
|---|---|---|---|---|
| Natural water sources | River | 43 | 27 (62.79%) | 69 |
| | Pond | 28 | 20 (71.4%) | 35 |
| | Lake | 10 | 9 (90%) | 42 |
| | **Total** | **81** | **56(69.13%)** | **146** |
| Sources intended for human drinking | Municipal supply | 40 | 13 (32.5%) | 35 |
| | Wells | 20 | 2 (10%) | 8 |
| | **Total** | **60** | **15(25%)** | **43** |

**Table 3:** Pathotyping of *E. coli* isolated from Natural water sources

| Sl. No. | Source | No. of *E. coli* isolates tested | ETEC | | | EHEC | | EPEC |
|---|---|---|---|---|---|---|---|---|
| | | | *lt* | *stIa* | *stIb* | *stx1* | *stx2* | *eaeA* |
| 1 | River | 69 | 0 | 0 | 0 | 3 | 1 | 0 |
| 2 | Pond | 35 | 0 | 0 | 0 | 2 | 0 | 0 |
| 3 | Lake | 42 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Total** | **146** | **0** | **0** | **0** | **5** | **1** | **0** |

without any prior filtration or disinfection at the household level. The percentage of *E. coli*-positive samples was higher in areas of Jammu North compared to other regions. Notably, these areas primarily receive their water supply from a water filtration plant (Asian Development Bank, 2012). This likely indicates that contamination occurred in the water distribution system in these areas. Among the 20 well water samples, the majority were negative for *E. coli* but tested positive for other coliforms such as *Klebsiella*, indicating environmental contamination rather than fecal contamination. The two wells that tested positive for *E. coli* were located on sites with poultry farms, suggesting possible contamination of the well water with poultry fecal material.

In the present study, river and pond samples were found to be positive for pathogenic *E. coli*, specifically the EHEC pathotype, whereas municipal supplies, wells, and lakes tested negative. Although numerous studies have reported the isolation of *E. coli* from water sources across different regions of India, pathotyping data remain limited. Among the available studies, a high prevalence of EHEC has been reported, particularly in major rivers such as the Ganga and Gomati. However, the findings of the present study contrast with these reports. A probable explanation for this discrepancy could be the geographic course of the rivers and the season during which samples were collected. The Tawi and Chenab rivers, sampled in this study, originate from higher mountainous regions and may carry fewer contaminants compared to rivers like the Ganga and Gomati, which flow through densely populated and industrial areas. When compared with a previous study conducted in the Jammu region by Wani *et al.* (2014), the percentage of *E. coli* positive municipal supply samples (7/25) was found to be comparable to that observed in the present study. However, the earlier study also reported the detection of three EHEC isolates, which were not observed in our findings. Contrary to our expectations, in the present study, a very low occurrence of pathogenic *E. coli* was reported. Similar findings have been reported in other studies. In Brazil, an analysis of 1,185 water samples from municipal supplies identified only 12 pathogenic *E. coli* isolates (6 ETEC, 4 EHEC, and 2 EPEC) (Moreira *et al.*, 2020). In Turkey, Gumus *et al.* (2015) isolated a single *E. coli* strain from 50 drinking spring water samples, which tested positive for the *lt* gene.

The geographical coordinates of the sampling sites, along with their *E. coli* isolation results, are provided in the Supplementary Material. Additionally, the results have been mapped using Google Maps and can be accessed via the links included in the Supplementary Material.

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## SUPPLEMENTARY MATERIAL

Supplementary_material_1 (excel file) indicating geo-coordinates of sampling sites and their *E. coli* isolation results

Google map of rivers sampled and their *E. coli* isolation results: https://www.google.com/maps/d/edit?mid=18gAmnllZUzGEK2BtYu788MNQ--ZxOufG&usp=sharing

Google map of ponds sampled and their *E. coli* isolation results: https://www.google.com/maps/d/edit?mid=1Fotr5MsXf6i8wUUEayhH0rKllWd6fsGW&usp=sharing

Google map of lakes sampled and their *E. coli* isolation results: https://www.google.com/maps/d/edit?mid=1IIbeMHVYaRpCCqj1cWy9ZglwbAU-a4U3&usp=sharing

Google map of Municipal supply sampled and their *E. coli* isolation results: https://www.google.com/maps/d/edit?mid=1yBetWWdLVcej-PYkhHF05-EVkAMCYlbU&usp=sharing

Google map of wells sampled and their *E. coli* isolation results: https://www.google.com/maps/d/edit?mid=1Fotr5MsXf6i8wUUEayhH0rKllWd6fsGW&usp=sharing