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# Microbiological analysis of drinking water quality of Bihar River of Rewa district (M.P.) India

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**Abstract**
Bacteriological analyses were carried out on Bihar river water of Rewa, Madhya Pradesh, India. The Bihar river was selected in this study because this river runs about nearly 97 km and supplies water for many villages for drinking and bathing purposes. The standard membrane filtration technique and multiple tube technique were employed to conduct fecal and total coliform counts. The obtained results were then compared to the reports of the esteemed All India Institute of Medical Sciences Standards for Drinking and Recreational Water. *Faecal coliform* counts varied from 12 to 180 MPN/100 ml while *Escherichia coli* counts ranged from 6 to 161 MPN/100 ml for all the sampled sites. Among the total coliform *Pseudomonas aeruginosa*, *Shewanella putrefaciens*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Proteus mirabilis* are reported. The presence of excessive *Faecal coliform* and *E. coli* levels signifies the contamination caused by untreated household waste originating from numerous informal settlements situated along the river's edge. A comprehensive examination of water utilization in the region revealed its primary purpose to be domestic and recreational. The river's alarming pollution endangers the health of the local populace, who heavily rely on it as their primary water source.

**Keywords:** *Escherichia coli*, *Pseudomonas aeruginosa*, *Shewanella putrefaciens*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus mirabilis*

## 1. Introductions
India's bountiful water resources, adorned with a splendid tapestry of rivers and bestowed with a heavenly mantel of snow in the majestic Himalayan range, possess the capability to fulfill an array of water necessitations within the nation (Bhardwaj, 2005) [1]. The rivers of India hold a profound significance in the lives of its people, serving as a vital resource for drinking water, irrigation, aquaculture, and power generation. With the increasing exploitation of freshwater resources, there is a growing recognition of the need for in-depth hydrological studies to effectively manage these invaluable water sources. A recent report from the esteemed All India Institute of Medical Sciences (AIIMS) in New Delhi reveals a distressing prevalence of disease-causing microorganisms in both drinking water and recreational water, emphasizing the potential for severe and even fatal illnesses resulting from the use of such contaminated water. Different authors also reported that Indian River system is polluted mainly because of the human impact (Goel and Bhosale, 2001; Patil *et al*., 2003; Maity *et al*., 2004) [2, 4]. The true importance of water as a powerful ecological force can only be fully understood through an examination of its physical, chemical, and microbial properties.
The microbiological quality of surface waters is greatly influenced by the discharges from sewage works and the runoff from informal settlements. In order to evaluate this quality, indicator organisms are frequently employed, with faecal coliforms (FC) being the predominant bacterial indicator for faecal pollution (South Africa, 1998) [5]. They can be found in water that has been contaminated with human and animal waste. Total coliforms, which include bacteria from feces as well as other bacterial groups found in soil, serve as indicators of water hygiene and the potential for infectious diseases. High levels of fecal coliforms and total coliforms in water often lead to symptoms such as diarrhea, fever, and other complications. It is common for children and adults in the local community to bathe and swim in streams and rivers. There is a high likelihood of ingesting a harmful amount of disease-causing microorganisms, as waterborne pathogens typically have a low infectious dose.

It is one of the most important river of Rewa district. Bihar river is south to North flowing river of Rewa district and is about 97 kilometers long. The river originates in the Kaimore hills of Kharamkheda village (Satna district) at the elevation of 600 meters above sea level in the Satna district (M.P.). After its origin in Kharamkheda, it flows through the hilly tract of Amarpatan, courses through plateau of Huzur and Sirmour tehsil, reaches the edges of plateau of Chachai village, where with its other tributaries, it forms a water fall, known as "Chachai fall". The river descends about 115 meters below its normal level and flow through a plain, to join the tons rivers, which is one of the important tributaries of Ganga river. Its catchment covers an area of about 1685 sq.km. out of which 636 sq.km. is in Satna and rest 1049 sq.km. in Rewa district. The objective of this work is to evaluate the general bacteriological parameters of the Bihar river of Rewa District, source of water used for drinking and bathing purposes.

## 2. Materials and Methods

### 2.1 Sample collection

The collection of samples is a vital aspect of studying rivers, as the conclusions drawn rely solely on the analysis of these collected samples. The objective of obtaining samples is to acquire information that represents the aquatic system from which they are taken. Following the recommended Standard Method for microbiological analysis, the grab sampling technique was employed. Over a period of five months, from February 2022 to June 2023, water samples were collected on a monthly basis during the lean season. Specifically, samples were gathered from three different locations along the river: Uprahti, Nipania, and Ghoghar. To ensure accurate microbiological examination, the water samples were carefully collected in 500 ml borosilicate glass bottles that had undergone thorough cleansing, rinsing, and sterilization. The process involved submerging the bottle, with the neck pointing downwards, into the river and then turning it until the neck slightly tilted upwards, allowing the mouth to face the current. The sampling bottle was not filled up to the brim and 20 mm to 30 mm space was left for effective shaking of the bottle (APHA, 1998) [6]. Microbiological analysis of water samples was started as soon as possible after collection to avoid unpredictable changes in the microbial population (Gaudy, 1998) [7].

### 2.2 Microbiological analysis

Fecal and total coliform counts were performed using the standard membrane filtration technique. The 100 ml water sample was filtered using 0.45 mm pore size, 47 mm diameter filter membrane as described by APHA (1998) [6]. The enumeration of coliform bacteria was conducted using the renowned multiple tube technique. To determine enteric bacteria, a combination of nutrient agar (NA) as a basal medium, MacConkey agar as a differential medium, and Blood agar as a special medium was employed. The isolation of *Escherichia coli* was achieved through the inoculation of the sample in Bismuth green bile broth. Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colonial, morphological and Biochemical properties (Table 1) following Bergey's Manual of Determinative Bacteriology, (Holt, 1994) [19].

**Table 1:** Biochemical analysis

| S. No. | Biochemical Testing | Inferences | Type of Bacteria |
|---|---|---|---|
| 1. | Kovacs' reagent: (Paradimethyl amino bezaldehyde + Isoamyl alcohol + Sulphuric acid) | Appearance of pink coloured ring | Presence of *E. coli.* |
| 2. | Methyl red test: | Appearance of pink coloured ring in methyl red. | Presence of *E. coli* and *Citrobacter freundii* |
| 3. | Citrase utilization test: (Simmon's citrate medium + Bromo thymol indicator | Appearance of green colour or blue colour in the medium Green Negative. Blue- Posit | Absence or presence of *Citrobacter freundii*. |
| 4. | Urease test: (Urease is digested by urease enzyme resulted in release of ammonia) | Appearance of yellow colour shows negative. Appearance of pink colour positive. | Presence of *Citrobacter freundii*. and *Klebsiella pneumoniae* |
| 5. | Oxidase reaction: (Tetra methyl parapholene diamine dihydro chloride) | Appearance of purple colour within 30 minutes. | Presence of bacteria contains cytochrome oxidase like |
| 6. | Fermentation and gas production test: (Glucose, lactose, sucrose, mannose | Change of colour from blue to yellow. | Presence of fermenting and gas producing bacteria |
| 7. | Triple sugar iron test (Glucose, lactose and sucrose 1:10:10). | Black colour change in the media. | Presence of hydrogen sulphide gas producing bacteria |

*Escherichia coli* were identified using MacConkey and Brilliant green blue broth as total coliform units in the samples. These two media types support growth of coliforms. *Citrobacter freundii* is capable of using citrate as carbon source for metabolic energy in the absence of fermentable glucose or lactose (Cappuccino and Sherman, 1996; Ashbolt, 2004; Hörman, 2005) [8, 10]. In Simmon's citrate medium, the absence of glucose and lactose inhibits the growth of E. coli, as it lacks the ability to metabolize citrate. However, *Citrobacter freundii* possesses citrate permease, a feature that enables the transportation of citrate into the cell, thus allowing it to thrive in this medium.

## 3. Results and Discussion

In the examination of microbial composition, the total coliform count and *E. coli* count were observed over a period of 24 and 48 hours, respectively. The figure depicted in Figure 1 showcases the bacteriological characteristics of various water samples. Notably, in the Uprahti station, the presence of *Faecal coliform* and *E. coli* was found to be relatively lower when compared to the Nipania and Ghoghar stations (Fig. 1). Additionally, Table 2 reveals the identification of diverse pathogenic coliform in all the water samples that were collected.

**Table 2:** Microbial Isolates from Water Samples

| S. No. | Name of the bacteria | Stations | | |
|---|---|---|---|---|
| | | Uprahti | Nipania | Ghoghar |
| 1. | *Pseudomonas aeruginosa* | + | + | + |
| 2. | *Shewanella putrefaciens* | + | + | + |
| 3. | *Klebsiella pneumonia* | + | + | + |
| 4. | *Citrobacter freundii* | + | + | + |
| 5. | *Proteus mirabilis* | + | + | + |

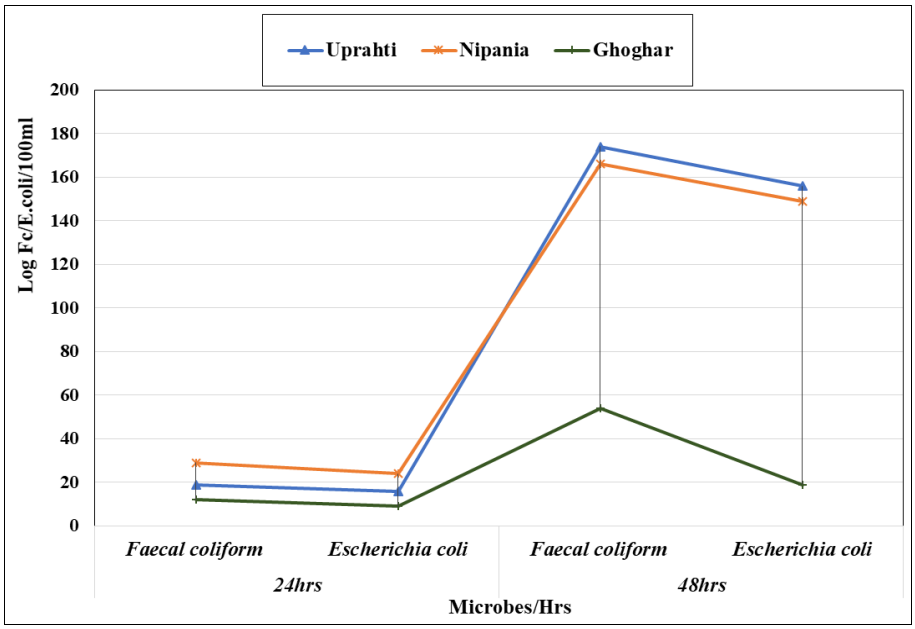


**Fig 1:** Graph analysis of Coliform and *E. coli* bacteria in different stations

The evaluation of water through bacteriological analysis serves as a determinant of its suitability for consumption. As outlined by the esteemed Indian standard (BIS, 1981) [11], a remarkable 95% of samples ought to exhibit an absence of coliform organisms, undetectable in 100 ml of any two consecutive samples, while no sample should contain *E. coli* within the same volume. The desirable limit of coliform in water is 10 MPN/100ml (ISI). The analysis reveals that the water samples collected from Uprahti, Nipania, and Ghoghar all exhibited contamination levels exceeding the acceptable limit set by Indian standards. This elevated bacterial population can be attributed to the insufficient maintenance of water reservoirs and the direct introduction of sewage into these reservoirs or nearby rivers. In the case of Uprahti, the exceptionally high count of total coliforms may indicate the presence of significant organic compounds in the water, likely stemming from the proximity to the origin site and the numerous rubber processing industries in the vicinity. As for Nipania and Ghoghar, the primary sources of bacterial contamination in the water are animal and human waste. These sources, including surface runoff, pastures, and land areas where animal waste is deposited, contribute to the proliferation of bacteria in the samples from these stations. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria (EPA, 2003) [12]. The same results of the high number of total coliforms were observed by different authors in different water bodies in India during pre-monsoon and post monsoon seasons (Rajurkar *et al.*, 2003; Radha Krishnan *et al.*, 2007) [13, 14].

Table 2 revealed an intriguing discovery: the water samples contained an impressive array of five distinct types of bacteria. By utilizing the biochemical analysis outlined in table 1, it became possible to discern unique colonies. A comprehensive examination of Bihar river's drinking water unveiled worrisome findings in three specific regions - Uprahti, Nipania, and Ghoghar. These areas were found to be contaminated with coliforms and pathogenic bacteria. The bacterial species responsible for this contamination were identified as belonging to the esteemed Enterobacteriaceae family, as detailed in Table 2. Strikingly, Uprahti exhibited a lower count of bacterial presence compared to the other regions, potentially attributed to reduced human interference. However, it is crucial to acknowledge that this limited presence of bacteria may be accredited to the prevalence of coliform bacteria in nature, which do not necessarily indicate fecal pollution (Binnie *et al.*, 2002; Griffith *et al.*, 2003) [15, 16].

## 4. Conclusion

The current research reveals the alarming state of the water supply, which will have severe consequences. According to the World Health Organization (1996) [18], enteric pathogens typically do not thrive in water, thus it is not a common mode of transmission to humans. However, the presence of enterobacteriae in sufficient infective doses can pose a significant risk to individuals with weakened local or general natural defense mechanisms. The most vulnerable groups include the elderly, young children, and those undergoing immunosuppressive therapy. Additionally, individuals with compromised immune systems due to AIDS are also at risk. Furthermore, when water contaminated with bacteria contaminates food, it allows for the proliferation of pathogens to reach dangerously high levels.

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