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Analysis of microbial contamination by MPN method from the different sources of drinking water of North Anandanagar, West Tripura

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ABSTRACT
Water pollution is a key global concern and a major cause of illness, accounting for 1.8 million deaths in 2015, according to The Lancet Commission on Pollution and Health. Unsafe drinking water contaminated with faecal microorganisms remains a major concern in rural regions, including villages in North Anandanagar, Dukli block, West Tripura. The purpose of this study is to analyse microbial contamination in drinking water sources using a laboratory-based qualitative analysis of the Most Probable Number (MPN) approach, with a focus on detecting faecal pathogens that pose health hazards. A total of 24 drinking water samples were collected from various families in North Anandanagar and tested for pH and turbidity to evaluate water acidity and clarity, respectively. The MPN approach discovered that 12.5% of the samples were satisfactory, 29.16% were suspicious, and 58.33% were unsatisfactory, indicating considerable contaminants. Several Gram-negative bacteria, including coliforms, Bacillus subtilis, Micrococcus, Serratia, Alcaligenes faecalis, Aeromonas, and Acinetobacter, were found in the samples, with coliform bacteria being detected in sample 22, indicating their importance as a faecal contamination indicator. These findings highlight the importance of targeted interventions to improve water quality, as well as a complete development program to assure access to safe drinking water. To lessen health hazards related with polluted water in the Dukli block and neighbouring areas, public awareness campaigns on waterborne diseases and preventive actions should be prioritised.

Keywords: Contamination, Drinking Water Samples, Faecal Pathogen, Indicator bacteria, MPN method

I. INTRODUCTION

Water is the emblematic inheritance of the Indian continent, represented by the Ganga and Brahmaputra rivers[1]. This resource is of utmost importance, and its use is imperative. Its necessity in daily life impacts the welfare and health of every individual. Most governments worldwide have succeeded in providing clean water, and advanced nations have already ensured tap water meets basic drinking standards[1]. Groundwater is the main source used for irrigating crops, landscapes, lawns, and industrial grounds. Various land and water-related activities primarily contribute to water pollution, and overuse is causing contamination in some instances[2]. Thus, for supporting and nourishing public health, economic growth, and productivity, safe drinking water is a fundamental provision.

Microbial contaminants are often found in significant amounts in areas with high water demand[3]. In crowded states like Tripura, groundwater contamination is a common issue, resulting from various factors such as natural chemicals, inadequate sanitation, improper sewage disposal, excessive use of fertilizers and pesticides, and groundwater over-extraction[4-6]. Ineffective water management sometimes leads to severe waterborne diseases such as cholera, diarrhoea, and dysentery, caused by pathogens like E. coli, Salmonella, and Vibrio[7,8]. The presence of coliforms and other pathogens in drinking water mainly arises from infectious agents or poor water treatment methods[6,9-13]. Coliforms, indicating bacterial contamination, signal faecal pollution in water, responsible for acute waterborne infections. Further detection of coliforms in drinking water is essential and can be easily conducted using the Most Probable Number (MPN) technique, a quantitative method for detecting coliforms and assessing water safety and purity [12,13].

The MPN test includes three steps: presumptive, confirmed, and completed tests[14]. Lactose fermentation, producing acid and gas, indicates a positive coliform test, with E. coli presence confirmed by a greenish metallic sheen on Eosin Methylene Blue (EMB) agar, followed by

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Gram staining and other confirmatory tests. The MPN technique can identify coliform pathogens but does not quantify their levels in water[15,16]. This comprehensive study identifies contamination sites to protect public health and conserve natural resources[17]. This paper explores the current microbial contamination levels in various water sources, such as filtered, tap, and pond water, in the North Anandanagar area of Dukli block.

II. MATERIALS AND METHODS

2.1 STUDY AREA AND SAMPLING

During this experiment, some of the methods tested for treatment after collecting water samples from different houses in various areas of North Anandanagar, Agartala, included filtration, boiling, and sieving. A total of 24 water samples were collected through random sampling from the North Anandanagar area, Dukli block, West Tripura district. These samples were taken between October and December 2023 and February and April 2024 from tap, filter, and pond water.Sterilised 100 ml plastic vials were used to collect the samples. Using a pH meter and a turbidity meter, the physical parameters, pH and turbidity, were promptly measured in the laboratory at the time of collection.

2.2 MPN ANALYSIS

2.2.1 PRESUMPTIVE METHOD

The MPN test's first section is known as the presumptive method. Coliform bacteria in water samples can be easily detected through this presumptive test. For this method, three sets of five test tubes containing 10 ml of Mac Conkey broth are prepared. Water samples of 10 ml, 1 ml, and 0.1 ml are added serially to each of the five tubes in one set of double-strength medium and two single-strength mediums [Table 1]. Each tube in all three sets is fitted with a Durham's tube to capture gas production. The tubes are further incubated at 37°C for 48 hours. Using the probability table, the tubes demonstrating acid and gas generation are used to calculate the presumed coliform count per 100 millilitres of drinking water [Table 2].

Table 1: Number of tubes and sample volumes collected for the MPN test.

Volume and strength of Macconkey broth	10 ml double strength	5 ml single strength	5 ml single strength
Number of tubes are taken	5	5	5
Volume of water samples added	10 ml	1 ml	0.1 ml

Table 2: Interpretation of results categorized as excellent, satisfactory, suspicious, or unsatisfactory.

Class	Grade	Presumptive coliform count(per 100ml)
I	Excellent	0
II	Satisfactory	1-3
III	Suspicious	4-10
IV	Unsatisfactory	>10

2.2.2 CONFIRMED TEST

These test tubes, which showed positive results through the accumulation of acid and gas, were selected for the confirmed test to check for the presence of E. coli and other organisms in the water samples. Samples from the broths that yielded positive results in the presumptive test were inoculated onto EMB agar. By sub-culturing on EMB agar, E. colican be differentiated from other coliform bacteria.

2.2.3 COMPLETEDTEST

In this final stage of the MPN test procedure, the suspicious colony(E. coli or atypical) from the EMB agar plate is transferred to nutrient agar slants. The plates were then incubated at 37°C for 24 hours to observe gas production in

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the tube. Gram staining was also performed to identify the colony grown on the plate.

III. RESULTS

The contamination of drinking water has made waterborne diseases a serious issue in most developed countries[6,7,25,26]. The MPN technique was employed to assess the quality of drinking water, as it is the simplest method for identifying coliform bacteria compared to other conventional methods. The physico-chemical characteristics and biochemical analysis of all drinking water samples taken from various households in the North Anandanagar area, Dukli block, are presented in Table 3 and Table 4.

The pH levels of all water samples collected from different sources in North Anandanagar ranged from 3.97 to 7.10 [Table 3]. The pH of some filtered and tap water samples was slightly acidic, primarily in sources used for drinking in North Anandanagar, while the pH of other collected drinking water samples remained within the permissible limits set by BIS guidelines. Turbidity levels were found to be within the permissible range set by WHO guidelines, between 0.56 NTU and 1.54 NTU. However, the turbidity of tap water samples collected from various sources exceeded permissible limits, likely due to microbial and other types of contamination.

Table 3: Physical properties of water samples in different areas of North Anandanagar. Turbidity ranged from 0.56 to 1.54 NTU, and pH values ranged from 3.97 to 7.10.

Serial no.	Type of the sample	pH	Turbidity(NTU)
1	Filter water	6.0	0.79
2	Tap water	5.4	0.56
3	Tap water	5.98	4.21
4	Tap water	6.7	0.68
5	Tap water	6.04	0.76
6	Tap water	3.97	1.54
7	Filter water	6.95	0.66
8	Tap water	7.6	42.4
9	Tap water	7.5	7.36
10	Tap water	7.10	25.5
11	Filter water	7.25	0.88
12	Tap water	4.20	1.23
13	Filter water	7.35	2.35
14	Tap water	6.80	0.98
15	Tap water	5.8	0.86
16	Filter water	5.6	0.58
17	Filter water	6.0	2.45
18	Filter water	5.5	4.45

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19	Tap water	7.10	0.76
20	Filter water	6.90	1.50
21	Filter water	5.90	4.95
22	Filter water	6.5	8.38
23	Tap water	7.10	1.27
24	Tap water	5.2	1.12

MPN ANALYSIS OF THE WATER SAMPLES

Gas production in the inverted Durham tubes was observed in varying numbers across all 24 tested samples, indicating the possible presence of coliform bacteria in the presumptive coliform test. The lowest MPN/100 ml counts, specifically 4, 6, and 7, were recorded in samples 4, 9, and 15 [Table 4]. The majority of samples from different sources in North Anandanagar showed the highest counts. While indicator bacteria were found in the water samples, this alone does not confirm fecal contamination but suggests its potential presence[Figure 1].

Figure 1: MacConkey broth (10 ml double strength) showing acid and gas production.Water samples were added to MacConkey broth for processing. While most of the tubes showed alkaline production, only a few showed acidic productions.

Out of the presumptive positive samples, only 11 were confirmed by the Eijkman test. In some of these positive samples, the presence of E. coli was indicated by a green metallic sheen on the EMB agar surface. Further confirmation was done through Gram staining [Figure 2] and additional biochemical tests, establishing a comprehensive microbiological profile of the drinking water, including E. coli.

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Table4: The MPN per 100 ml, 95% confidence limits, and positive tubes at three dilutions (10 ml, 1 ml, and 0.1 ml) are listed. Variable water quality between samples is reflected in the results, which range from minimal contamination (e.g., MPN 2) to high contamination (e.g., MPN 110).

Sample	5 of 10 ml each	5 of 1ml each	5 of 0.1 ml each	MPN/100ml	Lower limit of 95% confidence	Upper limit of 95% confidence
1	3	2	0	14	35	6.0
2	0	1	0	2	10	1.0
3	4	2	1	26	65	12
4	1	2	0	6	18	2.0
5	3	0	1	11	55	9.0
6	2	2	0	9	25	3.0
7	2	1	1	9	80	16
8	5	3	1	110	300	40
9	4	1	1	21	29	4.0
10	3	1	1	14	35	6.0
11	1	2	0	6	18	2.0
12	2	3	0	12	29	5.0
13	4	2	1	26	29	4.0
14	3	1	0	11	65	12
15	1	0	0	2	11	1.0
16	2	1	1	9	24	3.0
17	3	2	0	14	35	6.0
18	4	3	1	33	77	15
19	2	0	1	7	20	2.0
20	3	1	0	11	29	4.0
21	4	3	0	27	67	12
22	4	3	1	33	77	15
23	1	0	0	2	11	1.0
24	2	2	0	9	63	12.0

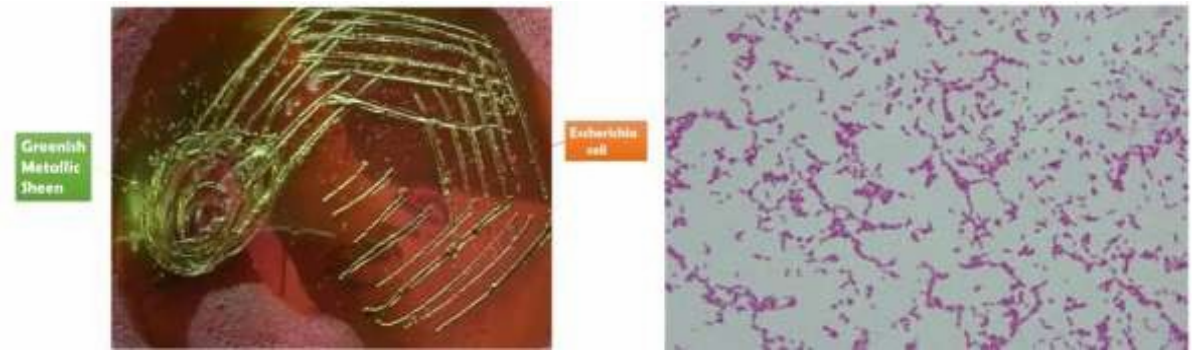

Figure2: Culture of E.Coli by the production of (a) greenish metallic sheen and (b) gram negative bacilli by gram staining.
(a) Greenish Metallic Sheen (b) Gram Negative Bacilli

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Table 5: The confirmed test results of culture using EMB agar results depicts the potability of the water. Majority of samples were potable, however, Samples 8 (no growth) and 22 (green metallic sheen) were non-potable, indicating contamination in both instances.

Sample	Growth on EMB	Green metallic sheen	Result
1	+	-	potable
2	-	-	potable
3	-	-	potable
4	+	-	potable
5	-	-	potable
6	+	-	potable
7	+	-	potable
8	-	-	Non-potable
9	-	-	potable
10	+	-	potable
11	-	-	potable
12	-	-	potable
13	-	-	potable
14	-	-	potable
15	-	-	potable
16	-	-	potable
17	+	-	potable
18	+	-	potable
19	-	-	potable
20	-	-	potable
21	-	-	potable
22	+	+	Non-potable
23	-	-	potable
24	-	-	potable

IV. DISCUSSION

Studies on bacteriological analysis of water using the MPN method have been predominant, aligning closely with findings from similar research. Sample number 22 showed a greenish metallic sheen on EMB agar, indicating the presence of faecal coliforms, specifically E. coli, rendering the sample non-potable [Table 5]. Waterborne diseases tend to rise in poor-quality water, as bacteria thrive in contaminated, stagnant water. Bacterial infections from water contamination often present with symptoms such as diarrhoea, vomiting, fever, and abdominal pain. These diseases are particularly common in developing regions due to poor drinking water quality.

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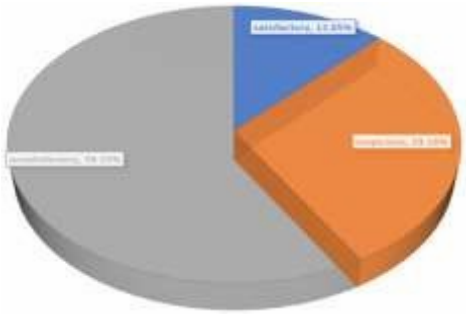

Figure3: Distribution of water samples collected from the different sources of Anandanagar.Among the 24 samples tested, 12.05% were deemed satisfactory, 29.16% were suspicious, and 58.33% were unsatisfactory

Globally, poor water quality, sanitation, and hygiene contribute to approximately 2 million deaths each year, mainly from infectious waterborne diseases[18-20]. Among the 24 samples tested, 12.05% were deemed satisfactory, 29.16% were suspicious, and 58.33% were unsatisfactory [Figure 3].The results align with findings from similar studies using the same methodology. In previous research, 64.61% of samples were found unsatisfactory for human consumption, while 33.55% were rated as excellent[20]. Concordant findings have also been reported in studies from different regions of Northern India and parts of Bangladesh[18,21,22].

In a study by Balaraman et al. on bacteriological analysis of drinking water using the MPN method, 85% of the 20 samples were unsatisfactory, 15% were satisfactory, and none were suspicious[23,24]. Another study on water microbiology found that out of six samples, five (83.34%) were unsatisfactory, one (16.67%) was satisfactory, and none were suspicious[19].

Improving drinking water quality can be achieved through more advanced treatment methods and by monitoring contamination levels to help reduce health-related risks. Key causes of drinking water contamination include environmental pollution, inadequate treatment processes, and improper handling. A practical, cost-effective approach to providing safe drinking water should be part of comprehensive development programs to lower the risk of waterborne diseases[20].

V. CONCLUSION

The results of our microbiological investigation indicated contamination in the drinking water samples studied. As a hilly state, Tripura faces various diseases due to poor sanitation and unhygienic water sources. The present study includes microbiological testing of water in the northern part of Anandanagar area in Dukli, West Tripura. Although the sample size is small and may not fully represent the broader situation, most drinking water samples collected were unsatisfactory. This finding reflects issues with the water distribution system and indicates a heightened risk of waterborne diseases.

Water distribution systems can suffer from issues such as unpleasant taste and odours, higher chlorine demand, bacterial growth, and microorganism development. These problems may worsen in Anandanagar and other villages in the Dukli block if immediate steps are not taken by authorities to improve the quality of drinking water within the distribution system.

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