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EXPLORATION OF BACTERIAL POPULATION PRESENT IN GROUND WATER SAMPLE

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ABSTRACT

Water is a basic need for daily life, and its safety directly affects human health. This study focused on Hapania (Women's Polytechnic) and nearby residential houses to evaluate the microbiological quality of drinking and utility water sources. Water samples were cultured using broth and nutrient agar and incubated at 37°C for 24 hours to detect microbial growth. The presence of colonies indicated microbial contamination. Colony Forming Units (CFU) were calculated to estimate the number of viable bacteria per 5µl of each sample. Gram staining was performed to identify the shape and type of bacteria, and the catalase biochemical test was carried out for further identification. The results revealed varying levels of contamination among different locations. Drinking water samples generally showed lower bacterial counts, with Sample 1(House 1) recording the lowest count of 0.4 CFU/5µl and Hapania showing 3.2 CFU/5µl. However, Sample 3(House 3) drinking water showed significantly high contamination with 216 CFU/5µl. Utility water samples consistently exhibited higher bacterial counts compared to drinking water, ranging from 9.6 CFU/5µl at Hapania to a maximum of 376 CFU/5µl in (Sample 3) House 3. (Sample 1) House 1 and (Sample 2)House 2 utility water samples also demonstrated elevated counts of 147.4 CFU/5µl and 42.6 CFU/5µl, respectively, indicating considerable microbial presence. Microscopic examination showed both Gram-positive cocci and rods, along with Gram-negative comma-shaped bacteria, including *Escherichia coli,* detected in the Hapania utility water sample. The catalase test indicated that most isolates were catalase-positive, suggesting the probable presence of *Staphylococcus* species, while catalase-negative results in certain samples pointed toward possible *Streptococcus* species. Overall, the findings highlight that although some drinking water sources showed relatively low contamination, several utility water samples showed high microbial counts. The presence of potential pathogenic bacteria highlights the urgent need for regular monitoring, better sanitation practices, and effective water treatment to reduce the risk of waterborne infections and ensure safe water consumption within the Hapania (Women's Polytechnic) and nearby areas.

1. INTRODUCTION

Safe and clean water is essential for human health and environmental sustainability. However, microbial contamination of water sources remains a major public health concern, especially in developing regions where sanitation and water treatment systems may be inadequate. Contaminated water can act as a vehicle for many pathogenic microorganisms that cause waterborne diseases such as diarrhoea, cholera, dysentery, and typhoid fever. According to the World Health Organization, millions of people worldwide are affected every year due to the consumption of unsafe drinking water (Organization, 2022).Bacteria are among the most common microbial contaminants found in natural and domestic water sources. The presence of indicator organisms such as *Escherichia coli* suggests faecal contamination and poor hygienic conditions of the water supply. *E. coli* is a Gram- negative, rod-shaped

bacterium commonly found in the intestinal tract of humans and animals, and its detection in water is widely used as an indicator of microbial pollution (Edberg et al., 2000). Monitoring bacterial populations in water is therefore an important step in evaluating water quality and assessing potential health risks. Microbiological analysis of water often includes bacterial culture, colony-forming unit (CFU) enumeration, Gram staining, and biochemical tests to identify bacterial species. Among these biochemical tests, the catalase test is widely used to differentiate catalase-producing bacteria such as *Staphylococcus* species from catalase-negative organisms like *Streptococcus*. The catalase enzyme breaks down hydrogen peroxide into water and oxygen, producing visible bubbles that indicate a positive reaction (Madigan et al., 1997).In addition to biochemical characterization, enumeration of bacteria using CFU counts provides a quantitative estimate of viable microbial populations present in water samples. High CFU counts generally indicate poor water quality and possible contamination from environmental or domestic sources (Som et al., 2023). Therefore, microbiological assessment of water sources is essential for determining the safety of drinking water and for identifying potential risks associated with microbial contamination.

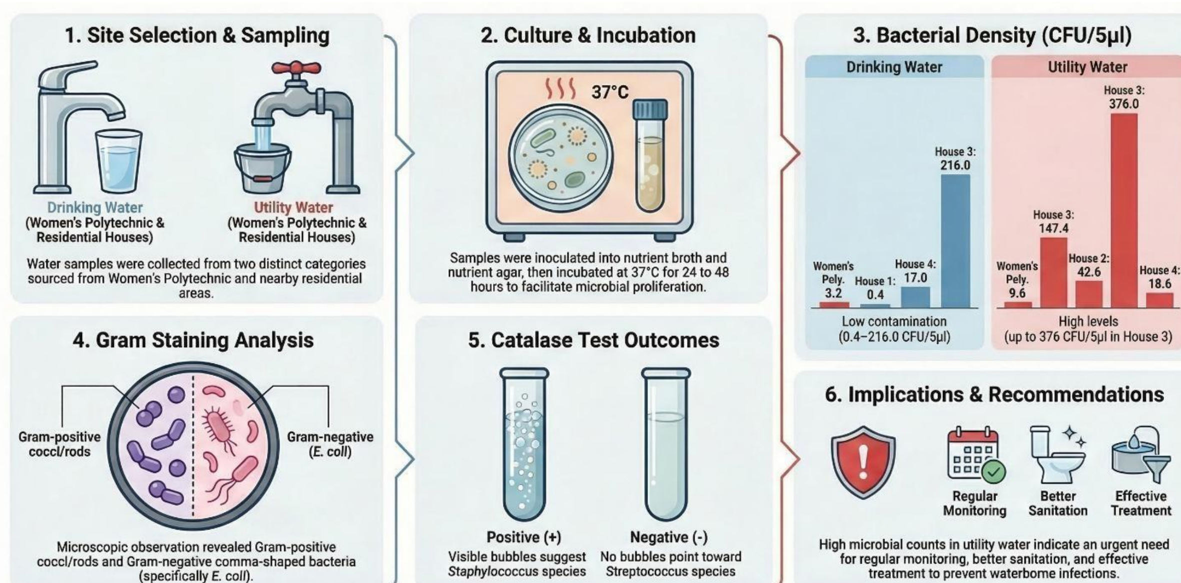

Figure 1: Graphical abstract showing the study workflow, including water sample collection, culture and incubation at 37 °C, bacterial density analysis (CFU/5 µl), Gram staining, and catalase testing. Results indicate higher microbial contamination in utility water than in drinking water, highlighting the need for improved monitoring and sanitation.

The present study aims to investigate the bacterial contamination present in drinking and utility water samples collected from Hapania and surrounding residential areas. Microbial growth was evaluated through nutrient broth culture, CFU enumeration on nutrient agar, Gram staining, and catalase biochemical testing. The findings of this study help assess the microbiological quality of local water sources and highlight the importance of regular monitoring and proper sanitation practices to ensure safe water consumption.

2. MATERIALS AND METHODS

2.1 Preparation of nutrient broth

Nutrient broth is a liquid culture medium used to grow and maintain bacteria. It has a similar composition to nutrient agar, but without the agar powder that makes the medium solidify. It is made by dissolving a mixture of Tryptone or Peptone and beef or yeast extract along with sodium chloride (NaCl) in distilled water. These ingredients together provide carbohydrates, proteins, lipids, minerals, and water — all of which are necessary for the growth of microorganisms. Sodium chloride helps maintain the proper osmotic pressure in the medium, which keeps bacterial cells healthy. A total of 1.5 g of nutrient broth powder was weighed and dissolved in 100 mL of distilled water in a clean conical flask. The solution was mixed thoroughly until the powder was completely dissolved. The flask was plugged with cotton and sterilized by autoclaving at 121°C under 15 psi pressure for 15–20 minutes. After sterilization, the medium was allowed to cool to room temperature before use.

2.2 Preparation of nutrient agar

Nutrient agar is a solid culture medium that allows a wide variety of microorganisms to grow, including those that do not need any special nutrients. It is made from the same base as nutrient broth, peptone and beef extract with the addition of agar-agar powder, which causes the medium to solidify at room temperature. Besides these basic nutrients, trace elements and some vitamins are also included in the medium to support better bacterial growth. Once prepared and poured into petri plates, it

provides a firm surface on which bacterial colonies can form and be counted.

1.5 g of nutrient broth powder and 3 g of agar-agar powder in 100 ml of distilled water dissolved. Stired the mixture well to fully combine all components. Autoclave at 121°C and 15 psi for 15– 20 minutes. After sterilization, poured the hot liquid agar into petri plates under sterile conditions and leave them on a flat surface until the agar solidifies. These plates are then ready for culturing bacteria.

2.3 Preparation of nutrient agar slants

An agar slant is a test tube partially filled with nutrient agar that has been allowed to solidify at an angle. This angled surface provides a large area for bacteria to grow and be stored. Slants are commonly used for maintaining pure cultures and for long-term storage of bacterial strains. Take 6 test tubes in a test tube rack and add the prepared nutrient agar mixture into each. Plug the test tubes with cotton and autoclave them at 121°C and 15 psi for 15–20 minutes. After autoclaving, remove the test tubes and immediately place them at an angle in the laminar airflow unit so the agar solidifies as a slant. Once solidified, the slants are ready to be inoculated and can be stored in an incubator for up to 48 hours.

2.4 Subculture of bacteria in nutrient broth

Subculturing is the process of transferring a small number of microorganisms from an existing culture into a fresh medium to allow continued growth. Nutrient broth is one of the simplest and most commonly used media for this purpose. It is a non-selective medium, meaning it can support the growth of most bacteria that do not have very specific nutritional requirements.

When enriched with biological fluids like blood or serum, the broth can also support the growth of more delicate (fastidious) organisms. The growth of bacteria in broth is measured by checking the optical density (OD) of the liquid using a spectrophotometer at 600 nm. A higher OD reading indicates greater bacterial growth. Sterilized nutrient broth was allowed to cool under aseptic conditions inside a laminar airflow cabinet. A small volume (100 µL) of the bacterial culture was transferred aseptically into the fresh nutrient broth using a sterile micropipette. The inoculated broth culture was incubated at 37°C for 24–48 hours. After incubation, bacterial growth was assessed by observing turbidity in the broth medium. The optical density (OD) of the culture was measured using a spectrophotometer at 600 nm to estimate bacterial growth.

2.5 Subculture in nutrient agar and measurement of colony forming units (CFU)

Colony Forming Units (CFU) is a measurement used to estimate the number of living, active bacterial cells in a sample. Each visible colony on an agar plate is assumed to have grown from a single viable cell or a group of cells. CFU gives a more accurate count of live cells compared to total cell counts, as it only counts cells capable of growing and multiplying.

The formula used to calculate CFU is:

$$\text{CFU/mL} = (\text{Number of Colonies} \times \text{Dilution Factor}) \div \text{Volume of Culture Plated}$$

Serial dilutions are performed to reduce the number of bacteria to a countable range (ideally 30– 300 colonies per plate). The dilution that produces a countable number of colonies is then used for the calculation. Sterile nutrient agar plates were prepared under aseptic conditions. A small aliquot (5 µL) of each water sample was aseptically transferred onto the surface of the agar plate using a sterile micropipette. The sample was spread evenly across the agar surface using a sterile cotton swab. The inoculated plates were incubated at 37°C for 24–48 hours. After incubation, visible bacterial colonies were counted manually. Each colony was considered to have originated from a single viable bacterial cell.

2.6 Gram staining of bacteria

Gram staining is one of the most important techniques in microbiology. It is used to classify bacteria into two broad groups — gram-positive and gram-negative — based on the structure of their cell walls. This classification helps in identifying bacteria and choosing the right treatment or antibiotics. In the procedure, the primary stain Crystal Violet is applied first. A mordant called Gram's Iodine is then added, which fixes the stain inside the cells. After this, alcohol or acetone is used as a decolorizer. Gram-positive bacteria have a thick layer of peptidoglycan (a protein- sugar complex) in their cell wall with very little fat. When decolorized, this thick layer shrinks and traps the Crystal Violet inside the cell. As a result, gram-positive bacteria retain the purple stain and appear blue-purple under the microscope. Gram-negative bacteria, on the other hand, have a thin peptidoglycan layer covered by a thick outer layer made of lipids (fats). When alcohol is applied, it dissolves this fatty outer layer and allows the Crystal Violet to wash out. These bacteria then absorb the secondary stain called Safranin and appear pink or red under the microscope. A thin smear of the bacterial culture was prepared on a clean, grease-free glass slide using a sterile inoculating loop. The smear was allowed to air-dry and subsequently heat-fixed by gently passing the slide over a flame. The smear was stained with Crystal Violet for 1 minute and rinsed gently with distilled water. Gram's iodine was then applied as a mordant for 1 minute, followed by rinsing with water. The slide was decolourized using ethanol or acetone for 10–20 seconds and immediately washed with water. Finally, the smear was counterstained with Safranin for 1 minute, rinsed, and allowed to air-dry. The stained slides were examined under a microscope at different magnifications. Gram-positive bacteria appeared purple, whereas Gram- negative bacteria appeared pink or red (Hadano et al., 2018).

2.7 Catalase test

The catalase test was performed to determine the presence of the catalase enzyme in bacterial isolates. This enzyme catalyzes the breakdown of hydrogen peroxide into water and oxygen, resulting in the formation of visible bubbles. A small amount of bacterial culture was transferred onto a clean glass slide using a sterile inoculating loop. A drop of 3% hydrogen peroxide (H_2O_2) was added onto the bacterial smear. The immediate formation of bubbles indicated a catalase-positive reaction, whereas the absence of bubbles indicated a catalase-negative reaction(Das et al., 2025).

3. RESULTS

Table 1: Staining data of water collected from Hapania.

Types of Sample			
Hapania Drinking water		**Hapania Utility Water**	
Shape	**Organism Type**	**Shape**	**Organism Type**
Rod	Gram Positive	Coma	Gram Negative
Coma	Gram Negative	Rod	Gram Positive
Round	Gram Positive	Round	Gram Positive

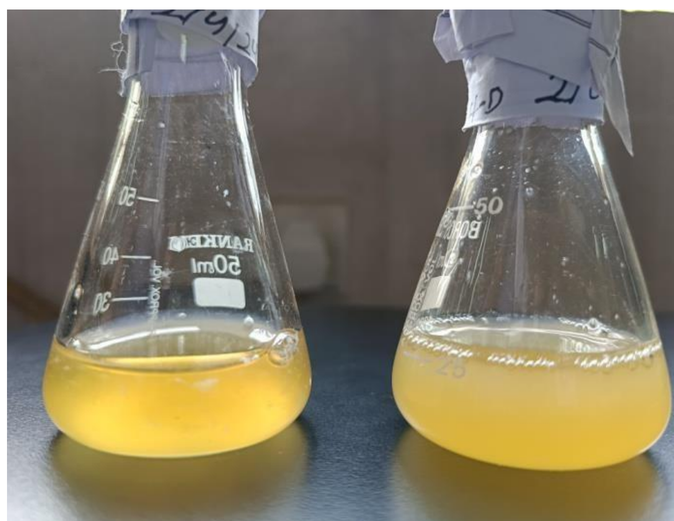

Figure 2: In this Figure Growth of bacteria in water collected from the Hapania area. Here the collected water (i) Drinking Water (ii) Utility Water were grown in broth culture for 24 hrs at 37 °C.

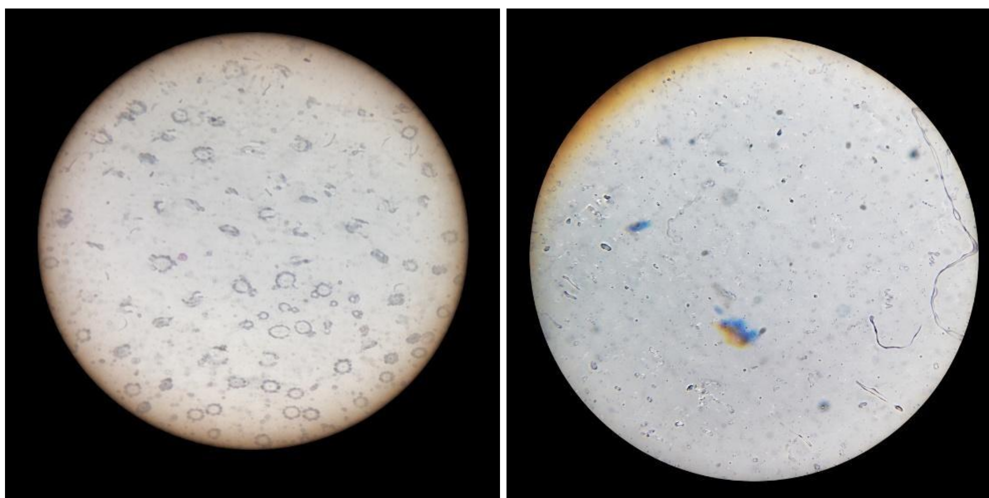

Figure 3: Shows the microscopic observation of the staining of water sample collected from Hapania area.

Table 2: CFU count of water collected from Hapania.

Types of Sample	CFU Count
Hapania Drinking Water	16/5µl =3.2
Hapania Utility Water	48/5µl=9.6

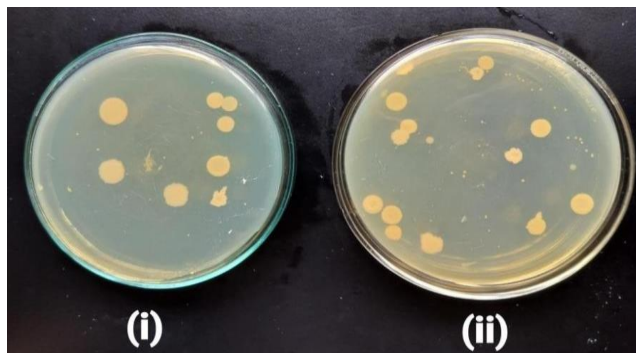

Figure 4: Growth of bacteria in water collected from Hapania. Here the collected water (i) Drinking Water (ii) Utility Water were grown in broth culture for 24 hrs at 37 °C.

Table 3: Staining data of water collected from Sample-1.

Types of Sample			
Sample-1 Drinking water		**Sample-1 Utility Water**	
Shape	**Organism Type**	**Shape**	**Organism Type**
Round	Gram Positive	Round	Gram Positive
Coma	Gram Negative	Coma	Gram Negative
		Rod	Gram Positive

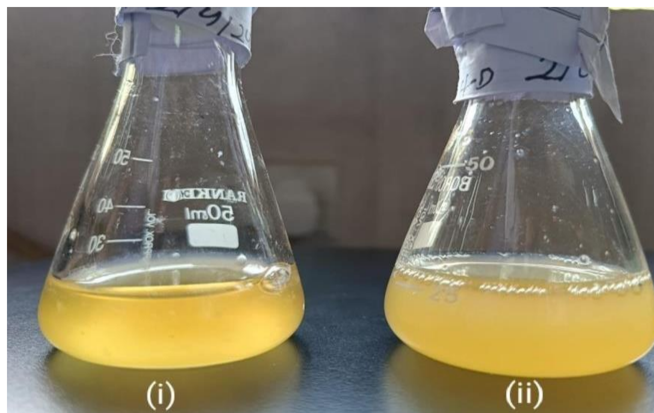

Figure 5: Growth of bacteria in water collected from Sample-1. Here collected drinking water (i) and utility water (ii) were grown in broth culture for 24 hrs at 37^{0}C.

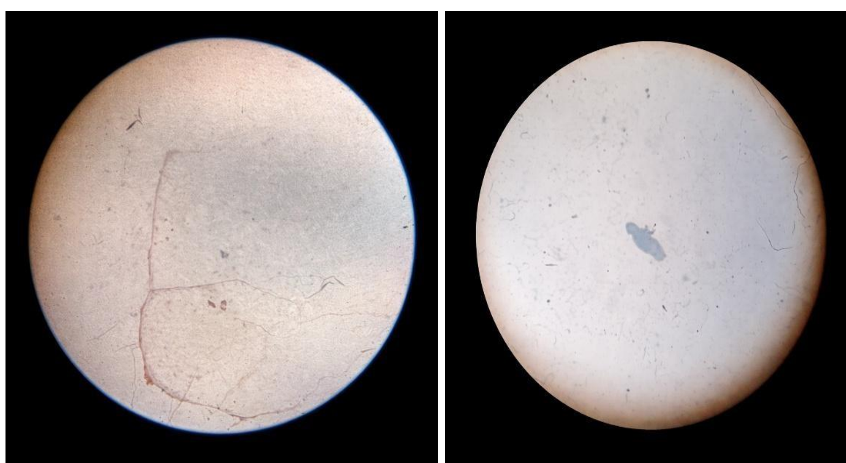

Figure 6: Shows the microscopic observation of the staining of water sample collected from Sample-1.

Table 4: CFU count of water collected from Sample-1.

Sample-1	**Sample-1 CFU Count**
Sample1-Drinking water	2/5µl= 0.4
Sample1-Utility water	737/5µl= 147.4

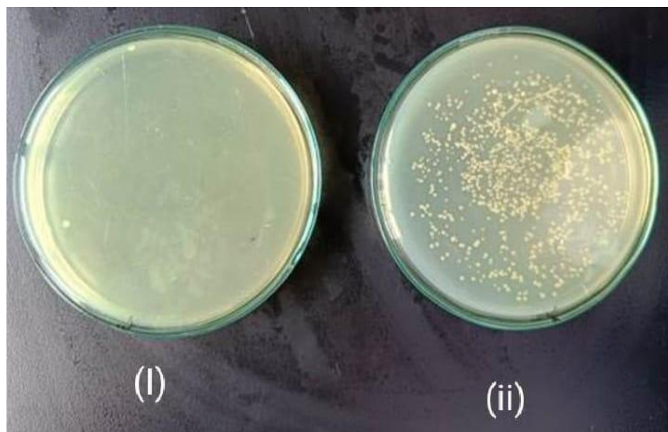

Figure 7: Growth of bacteria in water collected from Sample-1. Here collected drinking water (i) and utility water (ii) were grown in Nutrient Agar culture for 24 hrs at 37 ^{0}C.

Table 5: Staining data of water collected from Sample-2.

Types of Sample			
Sample-2 Drinking water		**Sample-2 Utility Water**	
Shape	**Organism Type**	**Shape**	**Organism Type**
Rod	Gram Positive	Round	Gram Positive
Coma	Gram Negative	Coma	Gram Negative

Figure 8: Growth of bacteria in water collected from Sample-2. Here collected drinking water (i) and utility water (ii) were grown in broth culture for 24 hrs at 37^{0}C.

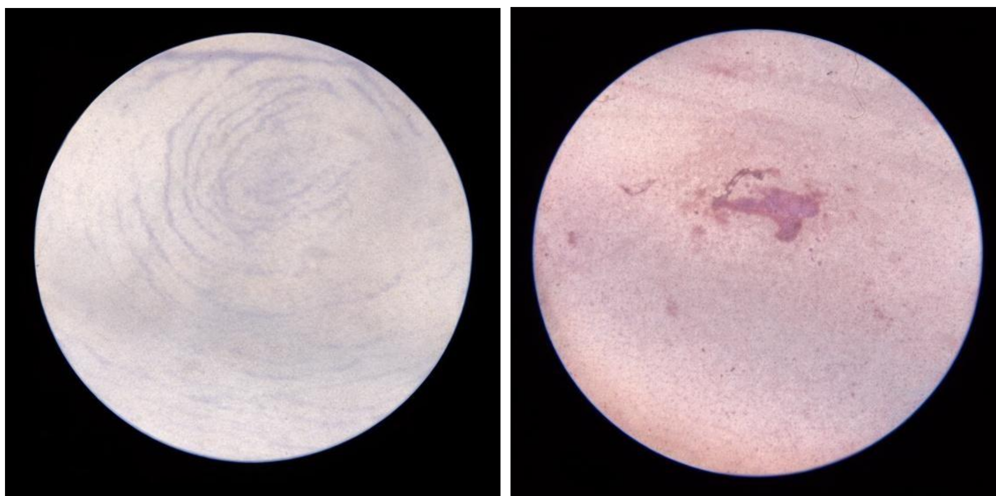

Figure 9: Shows the microscopic observation of the staining of water sample collected from Sample-2.

Table 6: CFU count of water collected from Sample-2.

Sample-2	Sample-2 CFU Count
House2– Utility Water	213/5µl= 42.6
House2 – Drinking Water	28/5µl= 5.6

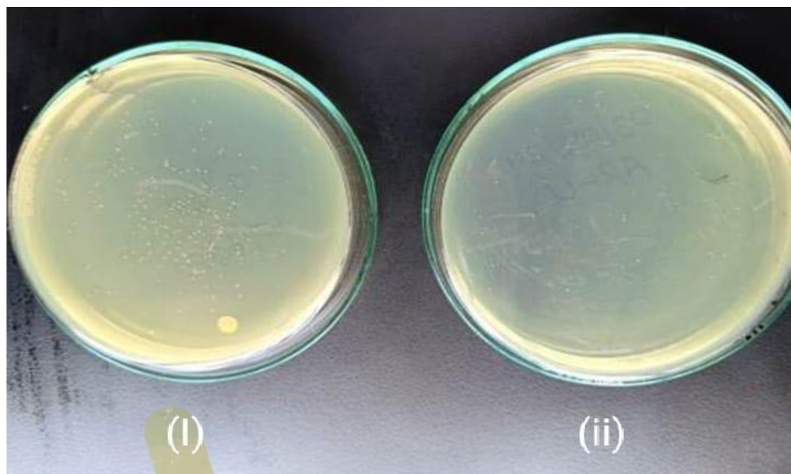

Figure 10: Growth of bacteria in water collected from Sample-2. Here collected drinking water (i) and utility water (ii) were grown in Nutrient Agar culture for 24 hrs at 37^0C.

Table 7: Staining data of water collected from Sample-3.

Types of Sample			
Sample-3 Drinking water		**Sample-3 Utility Water**	
Shape	**Organism Type**	**Shape**	**Organism Type**
Round	Gram Positive	Rod	Gram Positive
Rod	Gram Positive		

Figure 11: Growth of bacteria in water collected from Sample-3. Here collected drinking water (i) and utility water (ii) were grown in broth culture for 24 hrs at 37^0C.

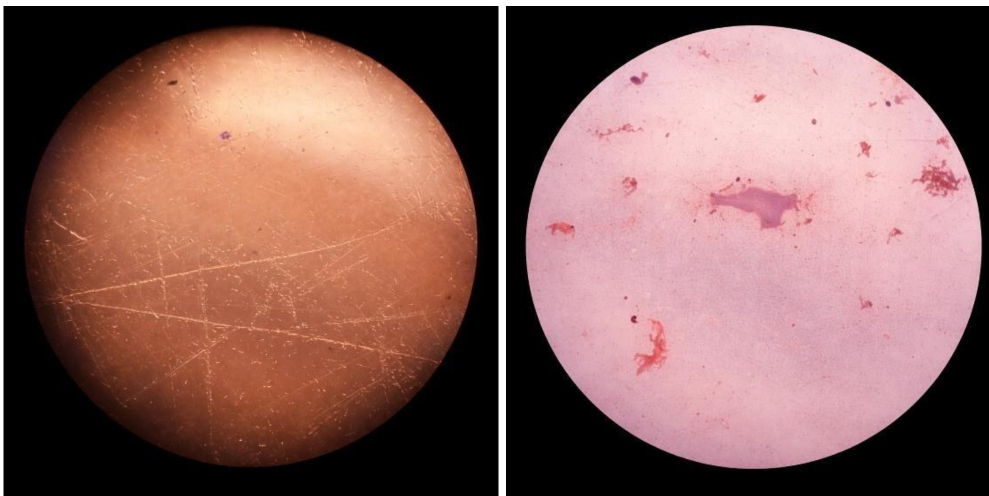

Figure 12: Shows the microscopic observation of the staining of water sample collected from Sample- 3.

Table 8: CFU count of water collected from Sample-3.

Sample-3	Sample-3 CFU Count
House3-Drinking water	1080/5µl=216
House3-Utility water	1880/5µl=376

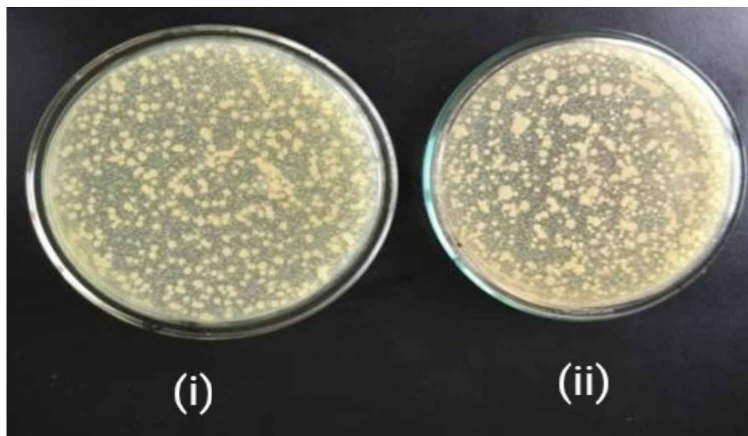

Figure 13: Growth of bacteria in water collected from Sample-3. Here, collected drinking water (i) and utility water (ii) were grown in Nutrient Agar culture for 24 hrs at 37^0C.

Table 9: Staining data of water collected from Sample- 4.

Types Of Sample			
Sample-4 Drinking water		**Sample-4 Utility Water**	
Shape	**Organism Type**	**Shape**	**Organism Type**
Comma	Gram Negative	Rod	Gram Positive
		Round	Gram Positive
		Comma	Gram Negative

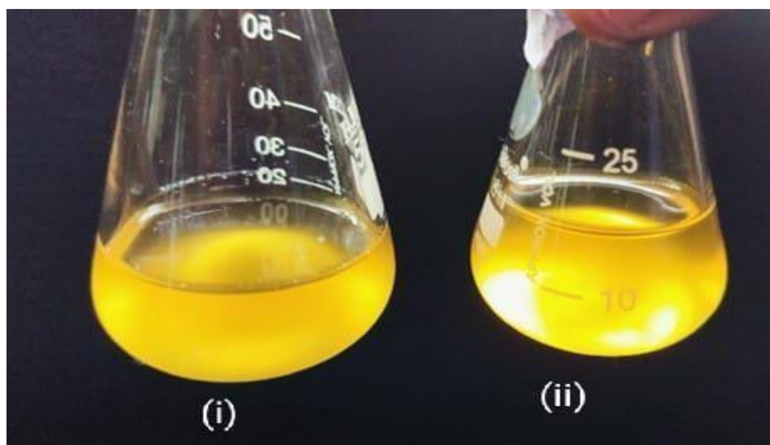

Figure 14: Growth of bacteria in water collected from Sample-4. Here collected drinking water (i) and utility water (ii) were grown in broth culture for 24 hrs at 37^0C.

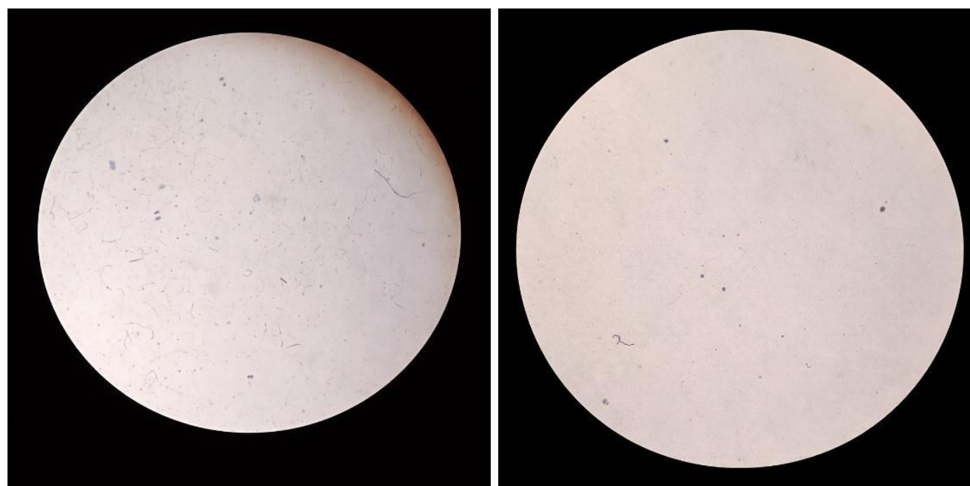

Figure 15: Shows the microscopic observation of the staining of water sample collected from Sample-4.

Table 10: CFU count of water collected from Sample-4.

Sample-4	Sample-4 CFU Count
Sample-4-Drinking water	85/5μl=17
Sample-4-Utility water	93/5μl=18.6

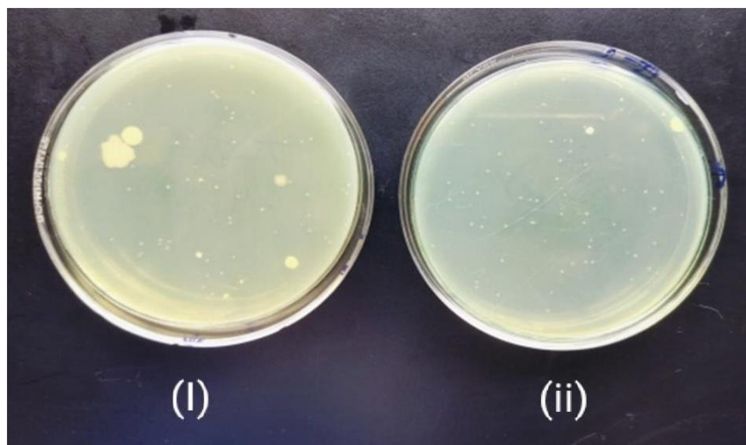

Figure 16: Growth of bacteria in water collected from Sample-4. Here collected drinking water (i) and utility water (ii) were grown in Nutrient Agar culture for 24 hrs at 37^0C.

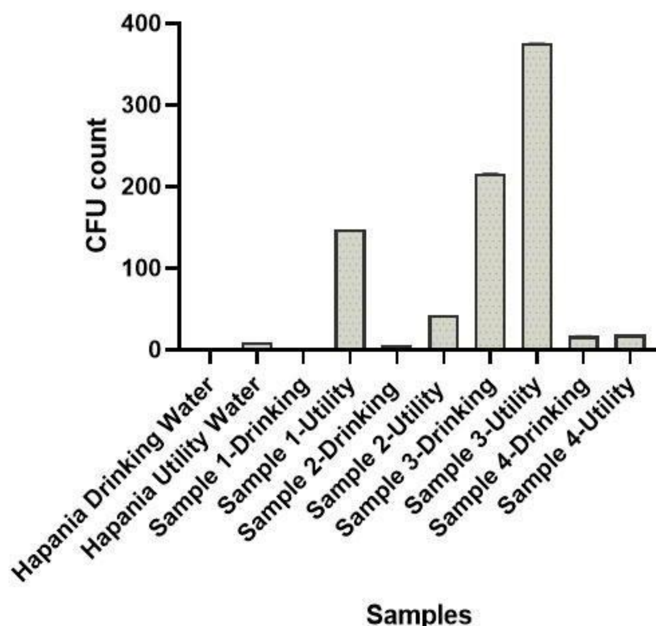

Figure 17: Bacterial growth (CFU count) in drinking and utility water samples collected from different locations. Samples were cultured on nutrient agar and incubated at 37 °C for 24 hours, showing higher bacterial counts in several utility water samples compared to drinking water.

BIOCHEMICAL TESTS

Table 11: Catalase sensitivity test for collected water samples.

Sample	Organism Type	Catalase Sensitivity
Hapania-Utility water	*E. coli*	Positive
Hapania-Drinking water	*Streptococcus*	Negative
Sample-1-Drinking water	*Staphylococcus*	Positive
Sample-1-Utility water	*Streptococcus*	Negative
Sample-2-Drinking water	*E. coli*	Positive
Sample-2-Utility water	*E. coli*	Positive
Sample-3-Drinking water	*Staphylococcus*	Positive
Sample-3-Utility water	*Staphylococcus*	Positive
Sample-4-Drinking water	*E. coli*	Positive
Sample-4-Utility water	*Staphylococcus*	Positive

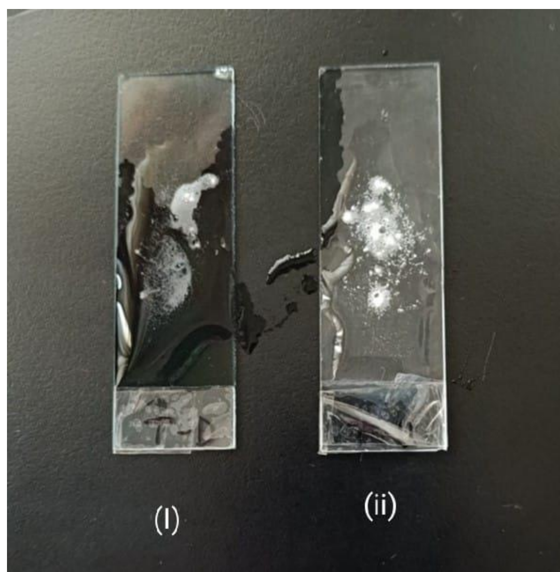

Figure-18:

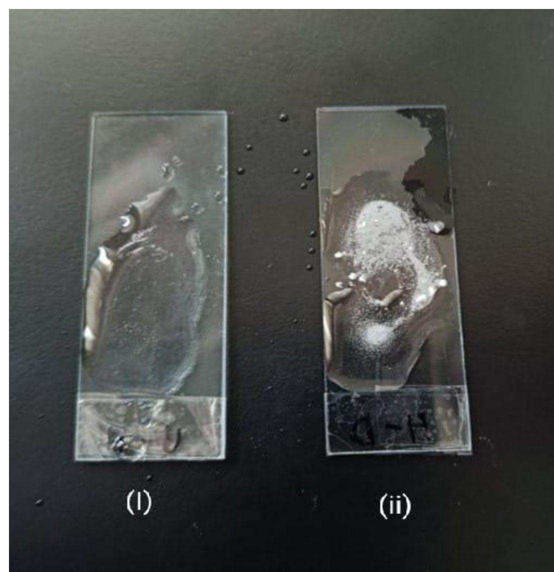

Figure-19:

Figure 18 and 19: The above pictures show the results of the catalase test. The appearance of bubbles in the slides indicate catalase positive organisms. Whereas no bubbles indicate catalase-negative organism.

4 DISCUSSION

Escherichia coli is a gram-negative bacterium and stains pink during Gram staining. Under the microscope, the organism appears as a pink or red comma-shaped cell. Nutrient broth is a versatile medium used for the cultivation and subculture of *E. coli*, allowing bacteria to grow to significant populations while maintaining their physiological traits. Nutrient agar contains essential nutrients suitable for subculturing *E. coli* and serves as an excellent solid medium to assess purity before conducting biochemical or serological tests. Agar slants are prepared to facilitate the growth of *E. coli* over a large surface area. After 48 hours of incubation, the media becomes turbid, appearing as cloudy white to yellowish, indicating bacterial proliferation and increased cell count. A colony-forming unit (CFU) estimates the number of viable microbial cells in a sample capable of multiplying through binary fission under controlled conditions. CFU counts of *E. coli* reflect this cellular increase. When stained, bacteria may remain purple or turn pink or red. Gram-positive bacteria retain the purple stain, while Gram-negative bacteria appear pink or red. In this test, *E. coli*, being Gram-negative, would typically stain pink, but the observation noted was purple, suggesting possible experimental error or atypical characteristics. Water and utility samples were collected from Hapania and surrounding areas. These samples were cultured on agar plates, revealing bacterial colonies that indicate the presence of bacteria in the water. CFU counts were determined for each sample. Bacteria were also identified based on shape, size, and Gram stain characteristics. Most bacteria observed were Gram-positive cocci, with some Gram-positive rods and a few Gram-negative comma-shaped bacteria. Catalase tests showed these bacteria were catalase-positive, suggesting that the Gram-positive cocci could be *Staphylococci*. In summary, bacterial strains were detected in water samples from Hapania and nearby areas.

5 CONCLUSION

Water samples from Hapania and nearby houses were tested for bacterial contamination in drinking and utility water. Gram Staining revealed three bacterial types: Gram-positive cocci, Gram-positive rods, and Gram-negative comma-shaped bacteria. Some samples showed bacteria resembling *E. coli*, indicating possible faecal contamination from poor sanitation. CFU Analysis showed that utility water had higher bacterial counts than drinking water. Sample-3 had the highest CFU in both water types, suggesting poor storage or inadequate treatment. Catalase Testing of Gram-positive cocci indicated the presence of *Staphylococcus* (catalase-positive) and *Streptococcus* (catalase-negative) species, likely entering water through soil or human contact. All water sources showed bacterial contamination, signalling poor water quality and the need for proper treatment and hygiene practices.

6 Competing Interest

All authors declare that they have no competing interest.

7 Authors' Contributions

GD, PD and RS collected data from experimental sets and compiled data. GD and RS contributed to synchronize and organize collected information. SA and MCD contributed to the study's design and critically analyse the entire work and prepared the manuscript, GD, PD, MG and RS have analysed data and prepared all figures. All authors mentioned in the manuscript have reviewed and approved the final manuscript.

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9 Financial grants
None to declare.

10 Data availability
All datasets generated or analyzed in this study are included in the manuscript.

11 Ethics statement
Not applicable for this study.

REFERENCES

1. Das, B. K., Das, M. C., Ghosh, S., Debnath, R., Gomes, A., & De, U. C. Spondias pinnata mediated silver nanoparticles with antibiofilm and catalytic potential. *Sci Rep.*, 2025; *15*(1): 34502. https://doi.org/10.1038/s41598-025-17558-1
2. Edberg, S. C., Rice, E. W., Karlin, R. J., & Allen, M. J. Escherichia coli: the best biological drinking water indicator for public health protection. *Symp Ser Soc Appl Microbiol,* 2000; 29: 106s-116s. https://doi.org/10.1111/j.1365-2672.2000.tb05338.x
3. Hadano, Y., Isoda, M., Ishibashi, K., & Kakuma, T. Validation of blood culture gram staining for the detection of Staphylococcus aureus by the 'oozing sign' surrounding clustered gram- positive cocci: a prospective observational study. *BMC Infect Dis*, 2018; *18*(1): 490. https://doi.org/10.1186/s12879-018-3412-2
4. Madigan, M. T., Martinko, J. M., & Parker, J. *Brock biology of microorganisms,* 1997; 11.
5. Prentice hall Upper Saddle River, NJ.
6. Organization, W. H. (2022). *Guidelines for drinking-water quality: incorporating the first and second addenda*. World Health Organization.
7. Som, S. V., Wieringa, F. T., Campos Ponce, M., Polman, K., Dakurah, P., Duncan, D., Blomberg, J., Rasphone, S., & Hoeven, M. V. Association of both Water, Sanitation and Hygiene (WASH) and Infant and Young Child Feeding (IYCF) practices with childhood malnutrition in Lao PDR: a cross-sectional study of the 2017 Lao Social Indicator Survey II. *BMJ Open*, 2023; *13*(10): e073974. https://doi.org/10.1136/bmjopen-2023-073974