International Journal of Preventive, Curative & Community Medicine
Volume 12, Issue 3 - 2026, Pg. No. 1-9
Peer Reviewed & Open Access Journal

Research Article

Microbial Analysis of Potable Water Streams from Populated Remote Communes in Faridabad, Haryana State, India

Gunjan Raghav[1], Shivani Sharma[2]

[1]Research Scholar, Department of Biotechnology, Sunrise University, Alwar, Rajasthan, India
[2]Research Supervisor, Faculty of Biotechnology, Sunrise University, Alwar, Rajasthan, India
DOI: https://doi.org/10.24321/2454.325X.202613

I N F O

Corresponding Author:
Gunjan Raghav, Department of Biotechnology, Sunrise University, Alwar, Rajasthan, India
E-mail Id:
gunjanraghav6@gmail.com
Orcid Id:
https://orcid.org/0009-0003-9134-3999
How to cite this article:
Raghav G, Sharma S. Microbial Analysis of Potable Water Streams from Populated Remote Communes in Faridabad, Haryana State, India. Int J Preven Curat Comm Med. 2026;12(3):1-9.

Date of Submission: 2026-08-01
Date of Acceptance: 2026-09-01

A B S T R A C T

The physico-chemical parameters of the water samples varied across the streams, with pH values ranging from acidic to near-neutral and generally low levels of total dissolved solids and electrical conductivity. Bacteriological analysis revealed the presence of bacterial species including *Escherichia coli*, *Enterobacter aerogenes*, *Shigella dysenteriae*, *Acinetobacter baumannii*, *Micrococcus luteus* and *Lactobacillus casei*. Fecal coliform was detected in three out of the five streams analyzed, showing a 60% incidence rate. The presence of *Escherichia coli* indicated recent fecal contamination and potential health risks associated with the consumption of untreated water. The findings highlight the need for improved water sanitation, community education on safe water practices, and policy interventions to reduce contamination in rural communities in Faridabad. The study contributes to understanding microbial water quality dynamics in underserved communities.

Keywords: Drinking Water, Escherichia Coli, Fecal Coliforms, Physico-Chemical Analysis, Streams

Introduction

Access to safe and clean drinking water is essential for health and sanitation, constituting a fundamental human right necessary for the full realization of life and all human rights.[1] Water is sourced from various origins, including streams, lakes, ponds, rainfall, and groundwater in both coastal and inland settings. Water from these sources is often visibly contaminated, affected by various human activities that introduce chemical pollutants and microorganisms, making it unwholesome and unsuitable for human consumption.[2] A significant number of individuals in developing countries lack access to safe drinking water. Contaminated water acts as a vector for the transmission of communicable diseases, including cholera, typhoid, and guinea worm infection.[3]

In rural communities of India, the main sources of drinking water are groundwater, surface water, and rainfall. Surface water, particularly streams and rivers, constitutes the most utilized and accessible source for numerous rural households in Faridabad of Haryana State, India. Its immediate availability renders it a crucial source of drinking water and a viable option for irrigating farmland. Surface water is vulnerable to contamination from both natural and anthropogenic sources.[4] Natural factors encompass runoff from rainfall, which transports soil, organic matter, and microorganisms into aquatic systems. Human-induced factors encompass anthropogenic activities occurring in and around water bodies, including open defecation, livestock farming, agricultural runoff containing fertilizers and

pesticides, and improper waste disposal, which significantly increase the risk of pathogenic contamination.[5]

Stream ecosystems are dynamic microbial habitats characterized by complex interactions among physical, chemical, and biological factors that generate distinct ecological niches. The dynamic characteristics of streams create distinct environmental gradients across their longitudinal, lateral, and vertical dimensions, each fostering diverse microbial communities.[6] The microbial consortium in streams generally comprises bacteria, viruses, protozoa, and fungi, each fulfilling specific ecological functions and posing different levels of risk to human health. Heterotrophic bacteria constitute the majority of microbial biomass, frequently surpassing 10^6 cells per milliliter in nutrient-rich tropical waters. The bacterial community constitutes the most prevalent and functionally diverse group, often dominating freshwater systems.[7] These encompass both benign aquatic species such as Limnohabitans and Polynucleobacter, as well as pathogenic enteric bacteria including Salmonella spp., Shigella spp., and *Escherichia coli*, which serve as indicators of fecal contamination. Additional bacterial groups of concern in tropical waters comprise *Vibrio cholerae*, the causative agent of cholera, and *Legionella pneumophila*, which is associated with severe respiratory infections.[8] Viral populations in streams exhibit significant diversity, with bacteriophages vastly outnumbering other viruses. Enteric viruses, including noroviruses, rotaviruses, and hepatitis A and E viruses, present considerable health risks, primarily due to their greater resilience to environmental stressors compared to bacterial pathogens.[9] Protozoan parasites such as *Cryptosporidium parvum* and *Giardia lamblia* pose significant challenges due to their environmentally resilient cyst stages, which can endure for weeks in aquatic environments. The fungal communities may potentially cause opportunistic infections.

The microbial quality of stream water has become a critical public health concern, particularly in rural communities where untreated surface water (stream) serves as the primary drinking source. Studies employing culture-independent techniques have revealed stream microbial communities to be 10-100 times more diverse than previously recognized, containing both autochthonous aquatic species and allochthonous pathogens.[7] *Escherichia coli* remains the standard for water quality assessment due to its consistent association with fecal contamination and relative ease of detection. It serves as reliable proxies for the potential presence of more dangerous pathogens that are difficult to culture or identify through conventional methods.[5,10] The microbial quality of streams could be further compromised by the lack of proper sanitation facilities in many rural areas. Studies have shown that communities without adequate toilets often practice open defecation near water sources, leading to direct fecal contamination. Additionally, domestic activities such as bathing, washing clothes, and dumping household waste near streams introduce organic pollutants and microorganisms, further degrading water quality.[11]

Good quality surface water relies on various factors, including its physicochemical characteristics as well as the magnitude of the pollution load. The physicochemical characteristics of the water can reveal particular conditions for the ecology of aquatic organisms and suggest suitable conservation and management strategies.[12] These water quality parameters not only determine the survival and proliferation of microorganisms but also influence their spatial distribution, metabolic activities, and interactions within aquatic food webs. Temperature serves as a master variable, with studies demonstrating bacterial growth rates doubling for every 10°C increase within the tropical range (25-35°C).[13] Notably, enteric pathogens like *Escherichia coli* demonstrate optimal growth at 37°C but maintain substantial viability at ambient stream temperatures, with survival times extending 30-50% longer in tropical versus temperate waters.[14] The pH of tropical streams generally falls between 6.5 and 8.5, a range that supports most enteric bacteria, while acidic streams (pH <5.5) select for acid-tolerant species like *Acidithiobacillus*.

Electrical conductivity is useful as a general measure of stream water quality. Each stream tends to have a relatively constant range of conductivity that, once established, can be used as a baseline for comparison with regular conductivity measurements. Significant changes in conductivity could then be an indicator that a discharge or some other source of pollution has entered a stream.[15] These physico-chemical parameters collectively serve as vital indicators of stream health and microbial risk potential.

Fecal coliforms, a subset of total coliforms, are specifically adapted to thrive in the intestines of warm-blooded animals and are more reliable indicators of fecal contamination. The ecological rationale for using fecal coliforms as water quality indicators is well-established, as it provides strong evidence of recent fecal contamination, signaling the potential presence of enteric pathogens such as *Salmonella*, *Shigella*, and *Vibrio cholerae,* which pose significant public health risks. The World Health Organization (WHO) guidelines stipulate that drinking water should contain no detectable *Escherichia coli* per 100 mL, while recreational waters should not exceed geometric mean concentrations of 126 CFU/100 mL for enterococci or 235 CFU/100 mL for *Escherichia coli*.[16] In Faridabad, some rural communities rely on untreated stream water for domestic use, increasing their vulnerability to microbial contamination. Understanding the incidence of fecal coliforms in these water sources is

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

essential for assessing water quality and implementing necessary interventions to safeguard public health.

Materials and Methods

The study was carried out on five streams that serve as drinking water sources in five selected rural communities of Faridabad in Haryana State, India. The streams were designated by the following codes: CP (Chhainsa Pimple) at coordinates 28.7606°N, 77.8635°E; TD (Tigaon Dabhade) at 28.7321°N, 77.6763°E; PM (Pali Mukhai) at 2*8.39915*°N, 77.7773**°E**; BYS (Bhopani Bypass) at 28.8272°N, 77.3730°E; and BYP (Badshahpur Bypass) at 28.4951**°**N, 77.0310**°E.** These streams function as essential drinking water sources for local inhabitants, frequently subjected to anthropogenic activities including bathing, washing, animal feeding, deforestation, agriculture, and domestic waste disposal.

A probe meter was utilized to assess the physicochemical parameters, including temperature, pH, total dissolved solids, electrical conductivity, salinity, and specific gravity at the location of the sample collection. The sample bottles were then used to collect water samples placed in an ice chest and conveyed to the laboratory for further examination and analysis.

Bacteriological Analysis

Standard plate count procedures were used to assess bacterial population by the method of Fasoranti *et al.,* 2018. Nutrient agar (NA) was used to evaluate the bacterial population. An aliquot, 1ml of the water sample was added to 9ml of sterile distilled water in a labelled test tube and serial dilutions were made up to 10^{-5} for the water samples. Each of the dilutions were pipetted onto a sterile plate containing nutrient agar (NA) and incubated at 37^{o}C. Each plate was incubated upside-down. At 24 hours, bacterial counts were carried out. A colony of each organism on the plate was then transferred to a freshly prepared agar medium and incubated at 37°C for 24hours to obtain a pure colony.

Gram Staining and Biochemical Identification

Gram staining was performed to ascertain the gram classification of each bacterium. Biochemical assays, including the catalase test, coagulase test, oxidase test, and sugar fermentation test, were employed to identify microbial species by distinguishing them based on their biochemical activities.

Identification of Fecal Coliforms

A loopful each of the two organisms that fermented glucose with Acid and gas production was selected from the pure culture plate and inoculated into a tube containing Macconkey broth with a Durham tube. It was subsequently incubated at 37°C for 48 hours. The Durham tubes were examined for the production of gas and acid after incubation. Following the production of gas and Acid by both isolates, a loopful of each from the broth was then transferred to an Eosin Methylene Blue (EMB) agar plate and incubated at 37^{o}C for 48 hours. Production of metallic Sheen by one of the isolates helps to differentiate the two isolates.

Molecular identification of isolates.

Bacteria DNA extraction

DNA was extracted using the protocol stated by Wright et al., (2017). Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and vortexed. The suspension was incubated for 10 min at 65ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1:0.6) was added and DNA precipitated at –20ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

PCR Analysis

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'-AAGGAGGTGATCCAGCC-3' primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc.,USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30s, 50°C for 60s and 72°C for 90 seconds; and a final termination at 72°C for 10 mins. and chilled at 4^{o}C.

Integrity

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl Eppendorf tube, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again, remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from Thermo Scientific.

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis.

Results

The values of the physicochemical parameters of the water samples that were measured during this investigation are presented in Table 1. The final results of these parameters are presented as the mean value ± the standard deviation (SD) in the table, with the data representing the values (n=3) obtained for each physicochemical parameter. Table 2 exhibits the biochemical reactions carried out to confirm the identity of the six isolates labelled A to F with sugar fermentations indicating isolates with abilities to produce gas and acids or otherwise. The table also revealed the average values of Colony Forming Units (CFU) in the water samples that were obtained in triplicates and expressed in log10. The norm of the parameters across the samples is represented as the average value ± the Standard Deviation (SD).

Confirmation of *E. coli* on MacConkey Broth and EMB Agar

Both fecal coliforms and E. aerogenes ferment lactose, producing acid (yellow color change from red) and often gas in a Durham tube at 35-37°C as shown in Table 2 above. In order to differentiate the two organisms, they were Inoculated into MacConkey Broth first for acid/gas detection and then streaked from positive broth onto EMB Agar for colony differentiation. *E. coli*: produced metallic sheen while *E. aerogenes* had no sheen.

Incidence of Occurrence of Fecal Coliform

The incidence of occurrence of E. coli and other isolates across the five streams is as shown Table 3 below while Table 4 revealed the result of the DNA sequencing on the isolate which ultimately confirms the isolate as E. coli. Figure 1 shows the Agarose gel electrophoresis of DNA of the bacterial isolates using 16S universal primer (100 base pair ladder) while the phylogenetic tree based on 16S rRNA gene sequence analysis indicating the relationship between fecal coliform isolated from stream and the related genera from the Gene Bank is shown on Figure 2.

Table 1.Physico-Chemical Parameters of Water Samples (The final results of these parameters (n=3) are expressed as mean ± SD)

S No	Temp (°C) Mean ±SD	pH Mean ±SD	TDS (ppm)	E.C (*µS*/cm)	S.G	Sal (ppm)
1	30.1±*1.75*	6.8±0.11	0.63±*0.03*	2.10±*0.09*	1.00±*0.00*	1.00±*0.00*
2	31.1±*0.87*	5.8±*0.50*	0.35±*0.05*	1.12±*0.55*	1.00±*0.00*	0.33±*0.58*
3	30.6±*1.25*	5.3±0.44	0.09±*0.02*	0.23±*0.04*	1.00±*0.00*	0.00±*0.00*
4	30.7±*2.16*	5.5±0.71	0.28±*0.36*	0.19±*0.07*	1.23±*0.25*	0.06±*0.06*
5	31.8±*0.60*	5.9±*0.98*	0.02±0.02	0.30±*0.05*	1.00±*0.00*	0.11±*0.02*

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

Table 2.Biochemical Characteristics of Isolates

Isolates	Gram	Form	Cat	Coa	Oxi	Glu	Lac	Xyl	Man	Suspected Organism	Mean (log_{10}) CFU/ml	SD
A	-	Rod	+	-	-	Ag	Ag	AG	AG	*E. aerogenes*	3.158	0.108
B	-	Rod	+	-	-	-	-	-	-	*A. baumanii*	3.077	0.133
C	-	Rod	+	-	-	Ag	Ag	AG	AG	*E. coli*	5.045	0.061
D	-	Rod	-	-	-	Ag	-	-	-	*S. dysenteriae*	5.140	0.032
E	+	Cocci	+	-	+	Ag	-	-	-	*M. luteus*	5.169	0.054
F	+	Rod	-	-	-	-	-	-	-	*L. casei*	5.129	0.048

The means of the Colony Forming Units (CFU/ml) are expressed in (log10) with SD for n=3.

NB: Cat: Catalase, Coa: Coagulase, Oxid: Oxidase, Glu: Glucose, Lac: Lactose, Man: Mannitol, Xyl: Xylose, AG: Acid and Gas production, Ag: Acid but no gas production *E. aerogenes = Enterobacter aerogenes*; *A. baumanii = A. baumanii*; *E.coli = Escherichia coli*; *S. dysenteriae = Shigella dysenteriae*; *M. luteus= Micrococcus luteus*; *L. casei= Lactobacillus casei*

Table 3.Incidence of Isolates across the streams

Isolates	E. aerogenes	A. baumanii	E. coli	S. dysenteriae	M. luteus	L. casei
CP	+	-	-	+	-	-
TD	-	-	+	-	-	-
PM	+	-	+	+	+	-
BYS	-	+	-	-	-	+
BYP	-	+	+	+	-	-
% of occurrence	40%	40%	60%	60%	20%	20%

Table 4.Details of sequence of Isolate

SAMPLE ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
ADE3	*E. coli*	1620	1620	99%	0	99.55%	-

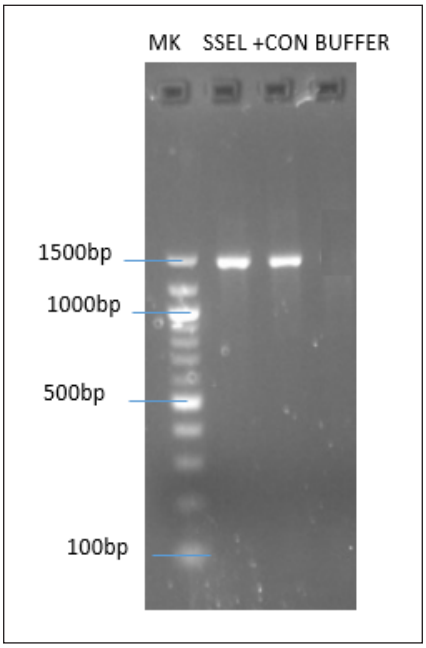

Figure 1.Plate 1 Agarose gel electrophoresis of DNA of the bacterial isolates using 16S universal primer (100 basepair ladder)

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

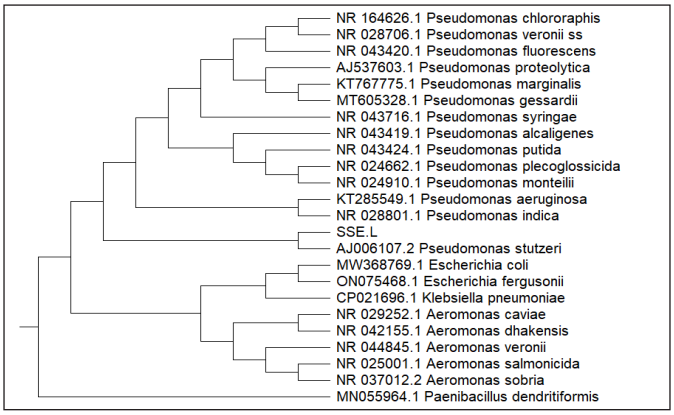

Figure 2.Phylogenetic tree based on 16S rRNA gene sequence analysis indicating the relationship between fecal coliform isolated from stream and the related genera from the Gene Bank

Discussion

Table 1 presents the physicochemical study of water samples from five streams in Faridabad, highlighting significant variations in critical parameters that influence microbial viability and water quality. The temperature varied between 30.10°C and 31.80°C, aligning with tropical conditions that facilitate microbial proliferation. Research has demonstrated that elevated temperatures can promote the survival and proliferation of fecal coliform bacteria in surface waterways, particularly during dry seasons when water levels decline.[19,20,21] The pH values exhibited considerable fluctuation, ranging from acidic conditions of 5.30 to almost neutral at 6.80. The acidic pH in most samples may suppress certain bacterial species while promoting acid-tolerant organisms, while fecal coliforms often thrive in neutral pH settings.[8]

Water inherently dissolves minerals from the earth, a process that elevates its TDS (total dissolved solids) level. A TDS level of 0.02 - 0.63 ppm across the five streams indicates that the water contains negligible dissolved minerals or salts, which is atypical for natural streams. This contrasts with the studies indicating that natural water systems, especially streams, typically exhibit dramatically elevated TDS levels due to interaction with soil and rock.[22] This is inconsistent with World Health Organization (WHO) standards for drinking water (50-300 ppm), indicating insufficient critical minerals in the streams that enhance the natural flavor and health benefits of drinking water.[23] The brownish hue of the water sample from Chhainsa Pimple stream indicates its turbidity, perhaps resulting from deforestation by nearby sawmills. The presence of inorganic dissolved compounds influences electrical conductivity in water. The peak electrical conductivity recorded in this study (2.10 µS/cm) was at Chhainsa Pimple stream, although it remained far below the acceptable range of 50-800 µS/cm for potable water.[15,23] Nevertheless, as these streams are tributaries to other rivers in the Haryana southern senatorial districts known for oil spills, the spillages may have contributed to the diminished electrical conductivity. This is supported by USEPA (2025)[15], which asserts that organic compounds such as oil, phenol, alcohol, and sugar exhibit poor electrical conductivity in water, and that oil spills reduce the electrical conductivity of streams. It is well accepted that salinity values are directly related to electrical conductivity, as evidenced by this research, as research values of salinity are far lower than acceptable values for drinking water.[15] This aligns with the findings of Adefisoye *et al.* (2018), who asserted that electrical conductivity serves as a significant indicator of salinity and total salt content in water samples; consequently, the salinity levels across the five streams are markedly low, as they are contingent upon electrical conductivity.

Table 2 presents the results of the biochemical characteristics of isolates as well as the average colony count expressed in log 10. The gram staining and biochemical result provide further taxonomic resolution of the isolated organisms. The suspected organisms identified in this study are *Escherichia coli, Enterobacter aerogenes*, *Shigella dysenteriae*, *Acinetobacter baumannii, Micrococcus luteus* and *Lactobacillus casei*. *Escherichia coli* was isolated at Tigaon Dabhade, Pali Mukhai and Bhopani Bypass streams, confirming fecal contamination. *Escherichia coli* is a gram negative, facultative anaerobe commonly found in environmental and clinical samples often used as an indicator of water quality. Its presence at these locations indicates recent fecal contamination, likely due to anthropogenic activities such as open defecation, livestock farming, or improper waste disposal near the stream. This finding aligns with studies highlighting the vulnerability of rural water sources to microbial pollution, particularly in communities lacking proper sanitation infrastructure.[16] The microbiological quality of the sampled locations shows a significant range of bacterial density, characterized by a sharp increase in the mean log10 CFU/ml values from 3.077±0.133 (lowest) at Tigaon Dabhade to 5.169±0.054

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

(highest) at Bhopani Bypass. These findings indicate an increase from a 3-log to 5-log density, this population levels are clearly way above the acceptable thresholds of fewer than 100 CFU/ml or less than 2-log10 CFU/ml for portable water according to the World Health Organisation (2003)[24]. Similarly, the higher counts observed in Pali Mukhai, Badshahpur and Bhopani Bypasses is critical; showing a 2-log increase, this magnitude of growth signifies a severe failure in the natural purifications of water the points.

The current investigation revealed that 60% of the studied streams or three out of five, exhibited pollution of drinking water by testing positive to fecal coliform. This outcome corresponds with previous research by Berihun *et al*. (2023)[25] and Fasoranti *et al*. (2018), which reported comparable incidences of fecal coliform at 66.6% and 40%, respectively, isolated from surface water. According to WHO recommendations, potable water must be devoid of fecal coliform bacteria.[26] The isolate *Enterobacter aerogenes* is a gram negative, facultative anaerobic bacteria belonging to the family *Enterobacteriaceae*.[27] It can be widely spread in water, soil and sewage. It doesn't cause disease in healthy people but it infects immune compromised patients, old people, neonates and individuals that received antibiotic therapy.[28] While *Enterobacter aerogenes* is a known nosocomial pathogen, its presence in streams as reported in this research can indicate a potential source of contamination for drinking water, especially in areas with inadequate water treatment facilities.[31] *Enterobacter aerogenes*, a well-known human pathogen, has been reassigned to the genus *Klebsiella*.[32]

The isolation of *Shigella dysenteriae* from three (60%) of the streams in this research agrees with the results obtained by earlier researcher who also isolated *Shigella dysenteriae* from surface water and posited that the ingestion of *S. dysenteriae* is capable of causing the disease dysentery.[33] Although, *Shigella dysenteriae* does not have a defined global incidence in streams, its presence indicates contamination from fecal matter in water sources, especially in areas with poor sanitation and unsafe water supplies.[29] Obianuju *et al.,* (2024) also isolated *Shigella* in 12.5% of the drinking water samples he analyzed and concluded that the contaminated drinking water has the potential to trigger epidemics of shigellosis and other intestinal diseases like bacillary dysentery which can be caused by any of the species causing over 2 million infections and 600,000 deaths annually in developing countries. Studies have shown that *Escherichia coli, Enterobacter aerogenes, Shigella dysenteriae* and other members of the *Enterobacteriaceae* family can cause several gastrointestinal illnesses linked to the contamination of drinking water.[31] The isolation of non – enteric bacteria namely *Acinetobacter baumannii*, *Micrococcus luteus* and *Lactobacillus casei* suggest that part of the microbial load of the water bodies are non – fecal contaminants as posited by Goyal *et al*., (2023).[34]

The performance of isolates on MacConkey broth was used as a differential medium to distinguish the sugar fermenters from non-sugar fermenters since fecal coliforms are sugar fermenters.[7] Only two of the isolates that were later confirmed to be *E coli* and *E. aerogenes* were able to ferment glucose accompanied with gas and acid production while the others were discarded since they are non-lactose fermenters and by implications are not fecal coliforms. *Both fecal coliforms and E. aerogenes* ferment lactose, producing acid (yellow color change from red) and gas in a Durham tube at 35-37°C. In order to differentiate the two organisms, they were Inoculated into MacConkey Broth first for acid/gas detection and then streaked from positive broth onto EMB Agar for colony differentiation. *E. coli*: produced metallic sheen while *E. aerogenes* had no sheen thereby confirming and differentiating *E. coli* from *Enterobacter aerogenes*.[8] Previous findings of Harwood and Staley (2021)[35] and Ribolzi *et al*., 2023[36] justified the exclusion of other three isolates by their inability to ferment lactose, a prerequisite for EMB agar selectivity.

Table 3 presents the percentage occurrence of *Escherichia coli*, a key fecal coliform, showing 60% rate across the five sampled streams in Faridabad. The results revealed that *Escherichia coli* was detected in three of the five streams while the remaining two streams had no incidence of *E.coli*. The absence of *Escherichia coli* in the other two streams suggests variability in fecal contamination levels across the study area. The variability in fecal coliform occurrence could be attributed to differences in streams with higher human and livestock activity, hydrological factors (stream flow and sedimentation) influencing microbial load and environmental conditions.[8]

The molecular identification of bacterial isolate in this study involved DNA extraction, PCR amplification, gel electrophoresis and sequencing of the 16S rRNA gene, a gold-standard marker for bacterial taxonomy due to its highly conserved nature and species-specific variability. The primers 27F and 1525R successfully amplified the ~1500 bp 16S rRNA fragment, consistent with *Enterobacteriaceae* (the family containing *Escherichia coli)* (Plate 1). The BLAST analysis (Table 6) revealed a high-confidence match for one isolate SSE.L as *Escherichia coli* with a high sequence identity of 99.55% indicating near-perfect alignment with *E. coli* reference strains in genomic databases. This high degree of homology confirms the accuracy of the identifications and underscores the reliability of the 16S rRNA gene as a molecular marker for bacterial classification The phylogenetic tree (Figure 1), based on partial bacterial 16S rRNA gene sequence analysis, indicates the relationships between the isolated microbes and the related genera of

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

the phylum *Proteobacteria*. The molecular findings align with the biochemical and culture-based results, reinforcing the presence of fecal coliforms and other bacteria of public health significance.

The absence of *Escherichia coli* in the other two streams does not necessarily imply safety, as it may only not be detected in this study possibly because the streams were not polluted with fecal matters shortly before our samples were taken. These results underscore the need for targeted interventions across drinking water streams and continuous monitoring to ensure water safety.

Conclusion

This study confirms the presence of fecal coliforms, in drinking water from streams highlighting the risk of waterborne diseases in Faridabad. The physico-chemical analysis revealed suboptimal water quality, with elevated temperatures and variable pH levels exacerbating microbial survival. Due to the potential for contamination across all streams, there is a concern due to shared environmental and anthropogenic pressures. The findings in this research underscore the need for improved water treatment and sanitation measures to mitigate health risks associated with pathogenic and opportunistic bacteria in drinking water sources.

References

1. Banseka Y. J. and Tume S. P. (2024). Coliform Bacteria Contamination of Water Resources and Implications on Public Health in Fako Division, South West Region, Cameroon. Advances in Environmental and Engineering Research*;* 5(2): 010.
2. Ikuesan, F.A. and Ediagbonya, T. F. (2024). Assessment of the microbial and physicochemical quality of water: Evidence from selected groundwater sources from the coastal region of Haryana state, India. Dutse Journal of Pure and Applied Sciences*,* 10 (1b): 85-96
3. Adeyemo, I. A. (2020). Bacteriological and Physicochemical quality of borehole water used for drinking at Olusegun Agagu University of Science and Technology, Faridabad, India. International Journal of Environment, Agriculture and Biotechnology, 5(4):890–897. https://doi.org/10.22161/ijeab.54.7.
4. Ubuoh, E. A., Akhionbare, S. M. O. and Akhionbare, W. N. (2022). Effects of anthropogenic activities on water quality of rural river systems in India. Environmental Monitoring and Assessment, 193(4): 1-15.
5. Adeyemo I.A, Akintimehin S.E and Adeoye E.T (2025). Pollution and Human Health Risks Assessment of drinking water in Haryana Southern Riverine Communities of India. Journal of Biochemistry, Microbiology and Biotechnology, 13 (2): 191-197. https://doi.org/10.54987/jobimb.v13i2.1169
6. Besemer, K., Battin, T. J., Bengtsson, M. M., Romani, A. M. and Packmann, A. I. (2023). The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology*,* 14(4): 251-263.
7. Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D. and Bertilsson, S. (2023). A guide to the natural history of freshwater lake bacteria. Microbiology and Molecular Biology Reviews*,* 87(1): e00057-22.
8. Ahmed, W., Hamilton, K. A., Gyawali, P., Toze, S., and Haas, C. N. (2020). Evidence of avian and possum fecal contamination in rainwater tanks as determined by microbial source tracking approaches. Applied and Environmental Microbiology, 86(8): e02919-19.
9. Cardenas-Alegria OV, Ferreira VBC, Noguera WG, Martins DT, Martins Neto AP, Monteiro Pontes PR, Lopes Cavalcante RB, Aguiar Alves SI, Luiz da Costa da Silva A, Gomes Costa R, Franco de Los Santos EF, Azevedo VAdC and Ramos RTJ (2024), Microbiome analyses of the Uraim River in the Amazon and geo-referencing analyses to establish correlation with anthropogenic impacts of land use. Front. Environ. Sci. 12:1404230. doi:10.3389/fenvs.2024.1404230
10. Harwood, V. J., Staley, C., Badgley, B. D., Borges, K., and Korajkic, A. (2023). Microbial source tracking markers for detection of fecal contamination in environmental waters. *FEMS Microbiology Reviews*, 47(2): fuad012.
11. Adefisoye, M. A., Okoh, I. A., Agoro, M. A., and Okoh, O. O. (2018). Physicochemical properties of wastewater in three typical South African sewage works. Polish Journal of Environmental Studies, 27(2): 491-499.
12. Mbalassa, M., Bagalwa, M., Nshombo, M., and Kateyo, M. (2014). Assessment of physicochemical parameters in relation with fish ecology in Ishasha river and lake Edward, Albertine Rift Valley, East Africa. International Journal of Current Microbiology and Applied Sciences, 3(6): 230-244.
13. Pomeroy, L. R., Williams, P. J. L., Azam, F., and Hobbie, J. E. (2023). The microbial loop in oceanic ecosystems: From genes to ecosystems. Annual Review of Marine Science*,* 15: 229-252.
14. Odonkor, S. T., and Ampofo, J. K. (2023). Prevalence of antibiotic-resistant *Escherichia coli* in rural stream sediments. Environmental Pollution, 318: 120881
15. USEPA (2025). Indicators: Conductivity. https://www.epa.gov/national-aquatic-resource-surveys/indicators
16. World Health Organization. (2023). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda.* World Health Organization.
17. Fasoranti, O. F., Afolami, O. I., Bolaniran, T. (2017). Assessment of the microbiological safety of potable water from rural settlements in local government area of Haryana state, India. Asian Journal of Research in Medical and Pharmaceutical Sciences, 1(3): 1-6.

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613

18. Wright M.H., Adelskov J, Greene A.C. (2017). Bacterial DNA Extraction Using Individual Enzymes and Phenol/ Chloroform Separation. J Microbiol Biol Educ. 1;18(2):18.2.48.doi: 10.1128/jmbe.v18i2.1348. PMID: 28861145; PMCID: PMC5577976
19. Uduma B. M., Abubakar M. M., Muhammed S. I. (2021). *Escherichia Coli* and Coliform bacteria as drinking water quality indicators at Warwade Dam, Dutse, Jigawa State, India. *Fudma Journal of Sciences* 5 (4) 418-425. https://doi.org/10.33003/fjs-2021-0504-984.
20. Akinbile, C. O., Ogunrinde, O. S., and Olanrewaju, O. O. (2023). Hydrological drivers of fecal pollution in tropical headwater streams: Evidence from Southwest India. *Journal of Environmental Management*, 330: 117182.
21. Powers J.E., Mureithi M., Mboya J., Campolo J., Swarthout J.M., Pajka J., Null C., Pickering A.J. (2023). Effects of High Temperature and Heavy Precipitation on Drinking Water Quality and Child Hand Contamination Levels in Rural Kenya. *Environ Sci Technol.,* 57(17):6975-6988. https://pmc.ncbi.nlm.nih.gov/articles/PMC10157894/
22. Barbara, A. B. and Robert, G. F. (2018). Evaluating relationships between total dissolved solids (TDS) a total suspended solids (TSS) in a mining influenced watershed. *Mine Water Environment,* 37(1): 18-30.
23. World Health Organization (2022). *Guidelines for drinking-water quality: incorporating the first and second addenda.* World Health Organization.
24. World Health Organization (2003**)** *Heterotrophic Plate Counts and Drinking-water Safety: The significance of HPCs for water quality and human health.* Edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker, and A. Glasmacher. Published by IWA Publishing on behalf of the WHO. https://iris.who.int/server/api/core/bitstreams/4be558a1-e714-4a44-87cf265450ac5f67/content
25. Berihun, G., Abebe, M., Hassen, S., Gizeyatu, A., Berhanu, L., Teshome, D., Walle, Z., Desye, B., Sewunet, B. and Keleb, A. (2023) Drinking water contamination potential and associated factors among households with under-five children in rural areas of Dessie Zuria District, Northeast Ethiopia. Frontiers in Public Health, 11:1199314.
26. Ashuro, Z., Aregu, M. B., Kanno, G. G., Negassa, B., Soboksa, N. E., Alembo, A., and Tassew, S. (2021). Bacteriological quality of drinking water and associated factors at the internally displaced people Sites, Gedeo zone, Southern Ethiopia: a cross-sectional study. *Environmental Health Insights*, 15: 11786302211026469.
27. Davin-Regli, A., Lavigne, J. P., and Pages, J. M. (2019). *Enterobacter* spp: update on taxonomy, clinical aspects and emerging antimicrobial resistance. Clinical Microbiology Reviews*,* 32(4): 10-1128.
28. Engy, N. F., Ismail, S. M., Wegdan, A.M and Amal, A.M. (2023). Prevalence of *Enterobacter aerogenes* in raw milk and some milk products. Assuit Veterinary Medical Journal*,* 69(179): 229-236.
29. Basilua, A. M., Kei, K., Debamalya, M., Ayumu, O., Januka, K., Shanta, D., Shin-Ichi, M. (2023). Shigellosis in Southeast Asia: A systematic review and meta-analysis. Travel Medicine and Infectious Disease, 52: 102554.
30. Obianuju, D., Samuel, M., Ugonna, H., Temitope, O., Amarachukwu, B., Bertha, O. and Maryam, I. (2022). Assessment of coliform bacteria in Sokoto metropolis public portable water supply: Implications for health and the environment. Tropical Journal of Phytochemistry and Pharmaceutical Sciences*,* 3(6): 338-344.
31. Oluyege, J. O., Dada, A.C., Odeyemi, A. T. (2009). Incidence of multiple antibiotic-resistant gram-negative bacteria isolated from surface water and underground water sources in South western region of India. Water Science and Technology, 59(10): 1929-1936.
32. Zhiyong, Z. Yu, F. and Alan, M. (2021). Carbapenem and colistin resistance in *Enterobacter*: Determinants and clones. *Trends in Microbiology,* 29(6): 473-476
33. Moxley, R. A. (2022). Enterobacteriaceae: Shigella. *Veterinary Microbiology,* 100-107.
34. Goyal, S. M., Gerba, C. P., and Pepper, I. L. (2023). Microbial water quality: Monitoring and modeling. Academic Press
35. Harwood, V. J., and Staley, C. (2021). Microbial source tracking: Methods, applications, and case studies. *Springer*
36. Ribolzi, O., Rochelle-Newall, E., Dittrich, S., Audry, S., Némery, J., Bouvier, S., and Valentin, C. (2023). Contribution of sediment and biofilms to microbial pathogen transport in tropical streams. Water Research, 47(14): 5257-5269.

ISSN: 2454-325X
DOI: https://doi.org/10.24321/2454.325X.202613