

Microbiological Quality And Environmental Determinants Of Drinking Water Sources: A Culture-Based And Quantitative Assessment Of Fecal Indicators And Enteric Pathogens

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Abstract

Background: Microbial contamination of drinking water remains a significant public health challenge in developing regions, contributing to waterborne infections and gastrointestinal diseases. This study assessed the microbiological quality and environmental determinants of drinking water sources in Moradabad district, Uttar Pradesh, India.

Materials and Methods: A cross-sectional study was conducted on 246 drinking water samples collected from urban (n=118) and rural (n=128) areas, including well water, municipal tap water, rainwater harvesting systems, and bottled water. Samples were processed using standard culture-based microbiological techniques. Quantitative bacterial load was determined by colony-forming unit (CFU/mL) estimation, and isolates were identified using Gram staining and selective biochemical methods.

Results: Overall, 40.7% of samples showed bacterial growth. Rural samples had significantly higher contamination (53.1%) than urban samples (27.1%) ($p < 0.001$). Well water showed the highest contamination (64.7%), while bottled water had the lowest (14.8%). Gram-negative bacteria predominated (68%). Moderate bacterial load (10^2 – 10^3 CFU/mL) was most common. Total coliforms (23.6%) and *Escherichia coli* (16.7%) indicated fecal contamination. Enteric pathogens identified included *Salmonella* spp. (7.3%), *Shigella* spp. (4.9%), and *Vibrio* spp. (3.7%).

Conclusion: Drinking water sources, particularly in rural and groundwater-based settings, show significant microbial contamination, highlighting the need for improved surveillance, source protection, and water treatment interventions.

Keywords: Drinking Water; Water Microbiology; Coliform Bacteria; *Escherichia coli*; Waterborne Diseases

Highlights

- 40.7% of drinking water samples showed microbial contamination.
- Rural and well water sources had significantly higher contamination rates.
- Fecal indicators and enteric pathogens pose substantial public health risk.

Date of Submission: 28-02-2026

Date of Acceptance: 08-03-2026

I. Introduction

Safe drinking water is fundamental to public health, yet microbial contamination remains a major concern in developing regions (1). Waterborne diseases continue to contribute significantly to gastrointestinal morbidity and mortality, particularly in rural and environmentally vulnerable settings (2). Fecal contamination of drinking water sources facilitates the transmission of enteric pathogens such as *Salmonella*, *Shigella*, and *Vibrio* species, posing serious health risks (3). Indicator organisms, particularly total coliforms and *Escherichia coli*, are widely used to assess microbiological safety of drinking water (4). While molecular approaches are increasingly utilized, culture-based microbiological assessment remains the standard reference method in routine surveillance due to its cost-effectiveness and reliability in pathogen detection. Environmental determinants such as rural habitation, industrial exposure, and type of water source may influence contamination levels (5). However, comprehensive analyses integrating culture-based identification, biochemical characterization, quantitative bacterial load estimation, and statistical risk evaluation remain limited (6). The present study was conducted to evaluate the microbiological quality of drinking water sources using culture-based techniques, quantify bacterial load, detect fecal indicators and specific enteric pathogens, and assess the association of contamination with geographical and environmental determinants.

II. Material And Methods

The present cross-sectional study was conducted in Moradabad district, Uttar Pradesh, India. Drinking water samples were collected from various locations across Moradabad and nearby adjoining areas to ensure representative geographical coverage. Sampling was carried out from both urban and rural regions of the district. A total of 246 drinking water samples were collected during the study period. Of these, 118 samples were obtained from urban areas and 128 samples from rural areas. Water samples were collected from multiple sources including well water, municipal tap water, rainwater harvesting systems, and bottled drinking water. Sterile, screw-capped sampling bottles were used for collection. Prior to sampling, taps were allowed to run for 2–3 minutes where applicable. All samples were labeled appropriately with details of location (urban/rural), source type, and date of collection. Samples were transported to the microbiology laboratory within 2–4 hours of collection under aseptic and temperature-controlled conditions to prevent external contamination or microbial overgrowth.

Microbiological analysis was performed in the Department of Microbiology, College of Paramedical Sciences, Teerthanker Mahaveer University (TMU), Moradabad, Uttar Pradesh, India. All samples were processed using standard culture-based microbiological techniques. All microbiological procedures were performed following standard laboratory protocols and quality control measures to ensure reliability and reproducibility of results.

III. Result

A total of 246 drinking water samples collected from urban and rural areas of Moradabad district and nearby regions were analyzed. The microbiological findings are presented below.

Overall microbiological contamination

Out of 246 samples, 100 (40.7%) showed bacterial growth, while 146 (59.3%) showed no growth (Table 1 and illustrated in Figure 1).

Table 1. Overall culture positivity among drinking water samples (n=246)

Culture result	Number (n)	Percentage (%)
Culture positive	100	40.7
No growth	146	59.3

Values expressed as number and percentage of total samples analyzed.

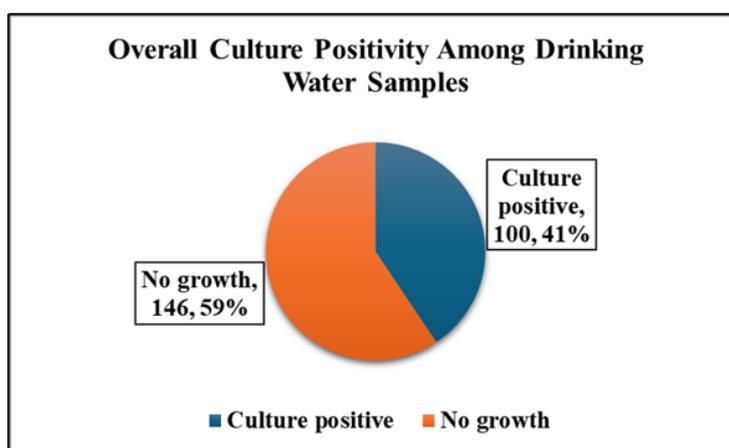


Figure 1. Overall Culture Positivity Among Drinking Water Samples (n = 246)

Distribution according to geographical area

Rural samples demonstrated significantly higher contamination compared to urban samples (Table 2). Among rural samples (n=128), 53.1% were culture positive, whereas only 27.1% of urban samples (n=118) showed contamination.

Table 2. Distribution of culture positivity according to geographical area

Area	Total samples (n)	Culture positive n (%)	Culture negative n (%)
Rural	128	68 (53.1)	60 (46.9)
Urban	118	32 (27.1)	86 (72.9)
Total	246	100 (40.7)	146 (59.3)

Chi-square = 15.84; p < 0.001; OR = 3.04 (95% CI: 1.75–5.29). Rural areas showed significantly higher odds of contamination.

Distribution according to water source

Contamination rates varied according to source type (Table 3). Well water demonstrated the highest contamination (64.7%), whereas bottled water showed the lowest (14.8%) as illustrated in Figure 2.

Table 3. Microbial Contamination According to Water Source Type

Water source	Total (n)	Culture positive n (%)
Well water	68	44 (64.7)
Rainwater harvesting	52	28 (53.8)
Municipal tap water	72	20 (27.8)
Bottled water	54	8 (14.8)
Total	246	100 (40.7)

Percentages calculated within each water source category.

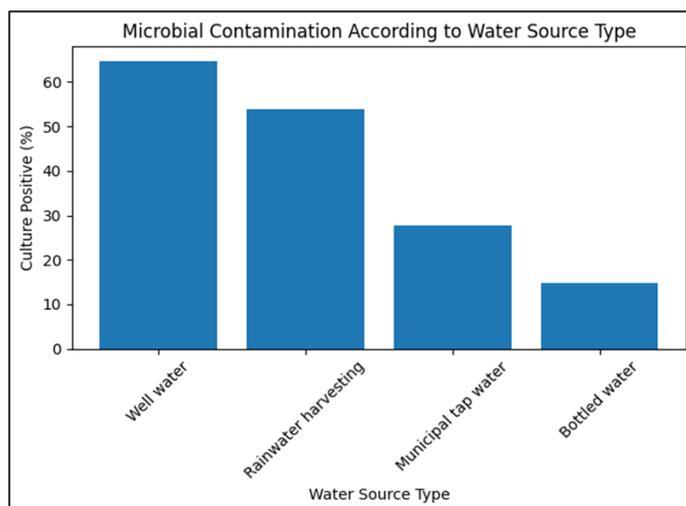


Figure 2. Microbial Contamination According to Water Source Type.

Growth on different culture media

Growth was observed most frequently on nutrient agar, followed by MacConkey agar as given in Table 4 and illustrated in Figure 3.

Table 4. Growth Distribution on Different Culture Media

Culture medium	Positive samples (n)	Percentage (%)
Nutrient agar	98	39.8
MacConkey agar	64	26.0
XLD agar	34	13.8
TCBS agar	28	11.4

Multiple media growth may occur in the same sample.

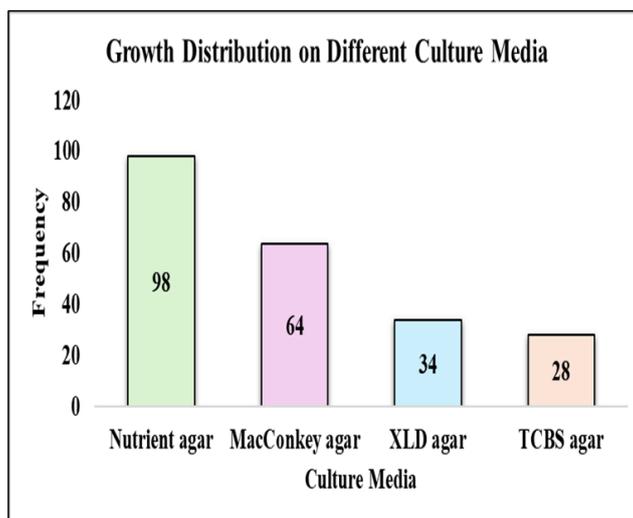


Figure 3. Growth Distribution on Different Culture Media.

Gram staining characteristics

Gram-negative organisms predominated among culture-positive isolates mention in Table 5 and illustrated in Figure 4.

Table 5. Gram staining distribution among culture-positive isolates (n=100)

Gram reaction	Number (n)	Percentage (%)
Gram-negative	68	68
Gram-positive	32	32

Percentages calculated among culture-positive isolates only.

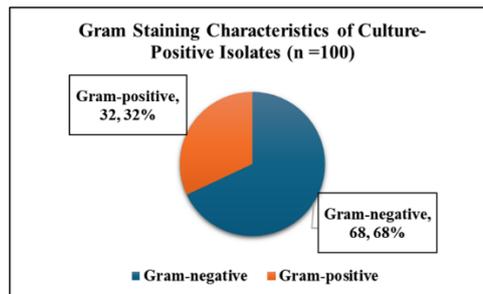


Figure 4. Gram Staining Characteristics of Culture-Positive Isolates (n = 100)

Quantitative bacterial load (CFU/ml distribution)

Quantitative analysis revealed varying degrees of contamination (Table 6). Moderate bacterial load (10^2 – 10^3 CFU/ml) was the most common among positive samples as illustrated in Figure 5.

Table 6. Quantitative bacterial load distribution (CFU/ml)

Bacterial load (CFU/ml)	Number (n)	Percentage (%)
No growth	146	59.3
$<10^2$	32	13.0
10^2 – 10^3	38	15.4
10^3 – 10^4	20	8.1
$>10^4$	10	4.1
Total	246	100

CFU/ml = Colony, = Colony-forming units per milliliter.

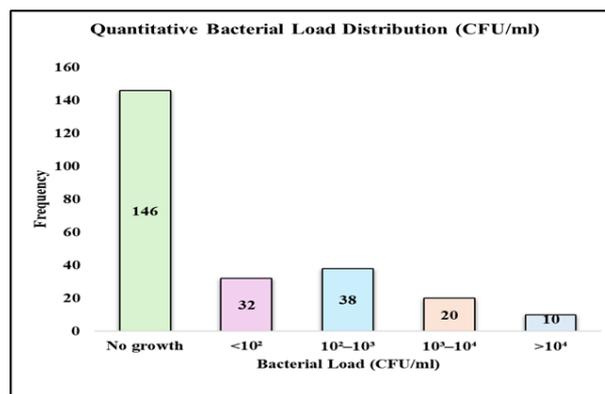


Figure 5. Quantitative Bacterial Load Distribution (CFU/ml).

Detection of fecal indicator organisms

Total coliforms were detected in 23.6% of samples, while Escherichia coli was confirmed in 16.7% (Table 7 and Figure 6).

Table 7. Detection of fecal indicator organisms

Organism detected	Number (n)	Percentage (%)
Total coliforms	58	23.6
<i>Escherichia coli</i>	41	16.7

Presence of *E. coli* indicates fecal contamination.

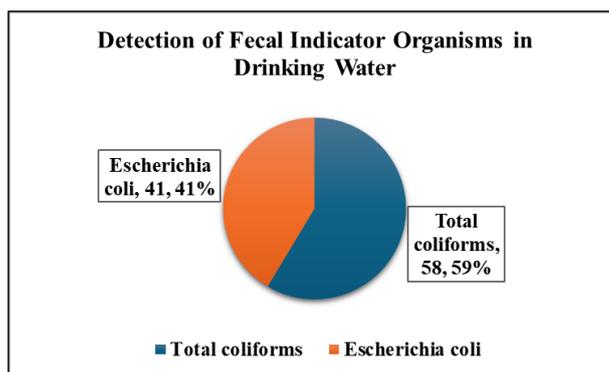


Figure 6. Detection of Fecal Indicator Organisms in Drinking Water.

Isolation of enteric pathogens

Selective culture identified enteric pathogens in a subset of samples (Table 8 and Figure 8).

Table 8. Isolation of enteric pathogens

Pathogen	Number (n)	Percentage (%)
<i>Salmonella</i> spp.	18	7.3
<i>Shigella</i> spp.	12	4.9
<i>Vibrio</i> spp.	9	3.7

Identification based on selective culture and biochemical characterization.

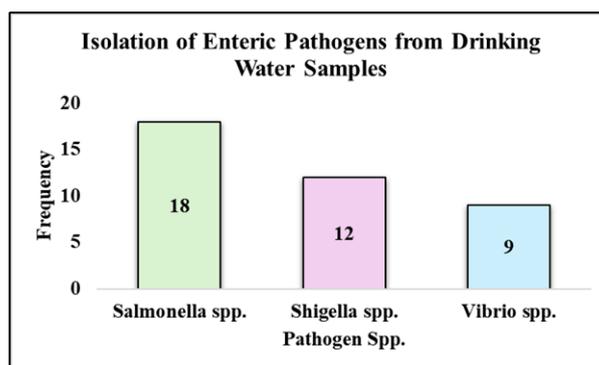


Figure 7. Isolation of Enteric Pathogens from Drinking Water Samples

IV. Discussion

The present study demonstrates substantial microbiological contamination of drinking water sources in Moradabad district, with an overall culture positivity rate of 40.7%. This finding exceeds the permissible microbiological limits recommended by the WHO (2017), which stipulate zero detectable *Escherichia coli* per 100 mL in safe drinking water (WHO Guidelines for Drinking-water Quality, 2017; 2022 update). The observed contamination burden indicates compromised water safety and inadequate treatment or post-treatment handling practices in the study region (7). The significantly higher contamination in rural areas (53.1%) compared to urban areas (27.1%) ($p < 0.001$; OR = 3.04) aligns with previous Indian and international studies reporting increased vulnerability of rural water systems due to poor infrastructure, shallow wells, open defecation, agricultural runoff, and lack of routine chlorination. Bain et al. (2014) reported that rural water sources in low- and middle-income countries are disproportionately affected by fecal contamination, particularly at the point of use. (8) Similarly, a national assessment by Mukherjee et al, (2019) highlighted higher coliform contamination rates in rural groundwater compared to piped municipal supplies (9). Our findings are also consistent with a recent systematic review by Li et al. (2026), which demonstrated a significantly greater likelihood of microbial contamination in non-piped rural sources (10). Analysis by water source revealed the highest contamination in well water (64.7%), followed by rainwater harvesting (53.8%), municipal tap water (27.8%), and bottled water (14.8%). This pattern agrees with global evidence showing that unimproved groundwater and surface water sources are more susceptible to microbial contamination than treated or packaged water Li et al., (2021) (11). Kostyla et al. (2015) found that shallow wells without secure linings frequently harbor fecal bacteria (12). Source-wise analysis revealed the highest contamination in well water (64.7%), followed by rainwater harvesting systems (53.8%), municipal tap water (27.8%), and bottled water (14.8%). These results corroborate findings by Wright et al.

(2004) and Kostyla et al. (2015), who reported that unimproved and groundwater-based sources are more susceptible to fecal contamination due to surface infiltration and inadequate protection (12,13). Although bottled water showed the lowest contamination, the presence of 14.8% positivity indicates potential lapses in storage, handling, or regulatory compliance, echoing concerns raised by Venieri et al. (2006) regarding microbiological safety in packaged drinking water in developing regions (14). Gram-negative organisms predominated among isolates in this study (68%), a pattern supported by recent evidence of Gram-negative *E. coli* and other enteric bacteria dominating drinking water contamination worldwide (Gaihre et al., 2024; Alawi et al., 2024) (15,16). Contamination with *E. coli*, coliforms, and other Enterobacteriaceae has been documented in tap and well water, often with antimicrobial resistance profiles indicative of fecal pollution (Kassa et al., 2026; Nadeem et al., 2025) (17,18). Multidrug-resistant *E. coli* and related organisms continue to be isolated from wastewater environments, underscoring the ongoing public health challenge posed by Gram-negative pathogens in water systems (Abdelgalel et al., 2025) (19). Additionally, studies on bottled water have found biofilm-forming and resistant Gram-negative bacteria, reinforcing the need for improved handling and regulatory compliance (Shrestha et al., 2024) (20). The quantitative bacterial load in this study (10^2 – 10^3 CFU/mL) aligns with evidence that moderate CFU counts in fecally contaminated drinking water represent intermediate to high risk according to WHO microbial water quality guidelines, highlighting a persistent global public health concern. Detection of total coliforms (23.6%) and *Escherichia coli* (16.7%) in the present study indicates significant fecal contamination and potential pathogen transmission risk. Similar prevalence has been reported in rural drinking water sources in North India by Gautam et al. (2025) (21). A global meta-analysis by Bain et al. (2014) further estimated that nearly 25% of improved water sources in low-income settings are contaminated with fecal indicator bacteria (8). More recent studies also report comparable findings, including contamination of drinking and packaged water with *E. coli* and coliforms (Shrestha et al., 2024; Alawi et al., 2024; Kassa et al., 2026) (15,17,20). These studies collectively support the magnitude of fecal contamination observed in the present investigation.

Importantly, selective culture identified enteric pathogens including *Salmonella* spp. (7.3%), *Shigella* spp. (4.9%), and *Vibrio* spp. (3.7%), indicating a direct public health threat from contaminated drinking water. These organisms are recognized as significant waterborne pathogens associated with gastrointestinal illness in unsafe water supplies WHO, (2025) (22). The detection of *Vibrio* species is particularly concerning given its documented prevalence in water environments and association with cholera and other diarrheal outbreaks in endemic regions Awere-Duodu et al., (2025) (23). Recent environmental microbiology studies also continue to report the occurrence of *Salmonella* and *Shigella* in contaminated water sources, underscoring their ongoing public health relevance (24). Overall, the findings of this study align with national and global evidence demonstrating that rural and groundwater-based sources are at significantly higher risk of fecal contamination. The combined qualitative and quantitative culture-based assessment strengthens the evidence for microbial risk stratification in the study area. Compared with prior studies, the relatively high prevalence of enteric pathogens in our samples underscores the urgent need for improved source protection, routine microbial surveillance, chlorination practices, and community-level hygiene interventions

V. Conclusion

The present study demonstrates a substantial burden of microbiological contamination in drinking water sources of Moradabad district, with an overall culture positivity rate of 40.7%. Rural areas exhibited significantly higher contamination compared to urban regions, and well water and rainwater harvesting systems were identified as high-risk sources. Gram-negative bacteria predominated among isolates, and fecal indicator organisms were detected in nearly one-fourth of samples. The presence of *Salmonella*, *Shigella*, and *Vibrio* species further indicates potential public health risk. These findings highlight the need for strengthened water quality monitoring, improved sanitation infrastructure, and regular microbiological surveillance, particularly in rural and environmentally vulnerable areas. Implementation of preventive strategies and community awareness programs is essential to reduce the risk of waterborne diseases in the region.

Declarations:

- **Ethical Approval:** Not Applicable
- **Informed Consent:** Not Applicable.
- **Conflict of Interest:** The authors declare no conflict of interest.
- **Funding:** The authors received no external funding for this study.
- **Data Availability:** Data supporting the findings of this study are available from the corresponding author upon reasonable request.
- **Author Contributions:** SB, conceptualized the study, conducted laboratory analysis, performed data interpretation, and drafted the manuscript. MF, supervised the research work, contributed to study design, critically revised the manuscript, and approved the final version for publication.

Acknowledgment: The authors acknowledge the support of the Microbiology Laboratory, College of Paramedical Sciences, Teerthanker Mahaveer University, for providing facilities and technical assistance. We also thank the faculty members for their guidance and support.

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