Original Article
DOI: https://doi.org/10.70749/ijbr.v4i3.2993

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Isolation and Identification of *Escherichia coli* from Drinking Water Sources: Microbiological Quality, Antimicrobial Resistance, and Comparative Risk Assessment

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ARTICLE INFO

Keywords: *E. coli*; drinking water; antimicrobial resistance; tap water; well water; bottled water; water quality; fecal contamination; MDPI format; global health

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Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

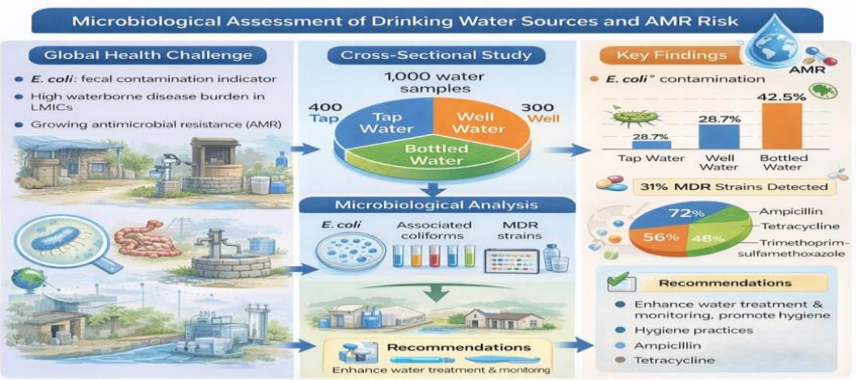
Funding: No funding received by the authors.

Article History

Received: 07-01-2026	Revised: 13-03-2026
Accepted: 21-03-2026	Published: 30-03-2026

ABSTRACT

Escherichia coli (*E. coli*) remains the most widely accepted microbiological indicator for assessing fecal contamination in drinking water. Contaminated water continues to pose major public-health risks, particularly in low- and middle-income countries (LMICs), where inadequate sanitation, limited water-treatment infrastructure, and growing antimicrobial resistance (AMR) amplify the burden of waterborne diseases. This study conducted a comprehensive, cross-sectional microbiological assessment of tap, well, and bottled drinking-water sources. A total of 1,000 samples (400 tap, 300 well, 300 bottled) were collected and evaluated for the presence of *E. coli*, associated coliforms, and phenotypic antimicrobial resistance. Standard microbiological identification, biochemical confirmation, selective culturing, and antibiotic susceptibility testing (AST) were performed following international guidelines. The findings reveal significant disparities in contamination patterns: 42.5% of well-water samples, 28.7% of tap-water samples, and 4.3% of bottled-water samples tested positive for *E. coli*. MDR strains were detected in 31% of positive isolates, with the highest prevalence in tap water (34%) and well water (29%), and lowest in bottled water (6%). Resistance was most common against ampicillin, tetracycline, and trimethoprim–sulfamethoxazole. These results highlight urgent risks posed by untreated and intermittently treated water systems and the growing role of environmental reservoirs in the AMR crisis. Strengthening water treatment, surveillance systems, and community-level hygiene practices is essential to mitigate risks. This study contributes vital evidence to global discussions on water safety, AMR, and Sustainable Development Goal (SDG) 6.



INTRODUCTION

Access to microbiologically safe drinking water is a fundamental human right and a cornerstone of public health protection [1]. Despite global progress toward improved water infrastructure, millions of people particularly in low- and middle-income countries (LMICs) continue to rely on water sources that are vulnerable to contamination by fecal matter, pathogens, and antimicrobial-resistant bacteria [2]. The World Health Organization (WHO) estimates that more than 2 billion people consume drinking water contaminated with feces, contributing significantly to diarrheal diseases, environmental enteric dysfunction, and increased mortality among children under five years of age (WHO, 2023) [3]. As emerging challenges such as climate variability, urbanization, and bacterial resistance escalate, the need for rigorous surveillance and scientific assessment of water quality has become increasingly urgent [4].

Escherichia coli (*E. coli*) is globally recognized as the most reliable microbiological indicator organism for fecal contamination in drinking water [5]. Unlike total coliforms, which may originate from environmental sources, *E. coli* is strongly associated with the intestinal tracts of warm-blooded animals [6]. Therefore, its detection in drinking water is considered a direct signal of recent fecal pollution and potential presence of enteric pathogens such as *Salmonella*, *Shigella*, enterotoxigenic *E. coli*, rotavirus, and pathogenic protozoa [7]. Moreover, some *E. coli* pathotypes particularly Shiga toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) pose substantial clinical risks [8]. Understanding the presence and characteristics of *E. coli* in drinking water sources is thus essential for both water-quality surveillance and health-risk assessment [9].

The contamination of drinking water varies significantly by source type. Tap water is typically treated through centralized systems; however, aging infrastructure, intermittent supply, cross-connection with sewage, and inadequate chlorination create opportunities for microbial intrusion, particularly in LMICs [10]. Well water, often consumed untreated, is highly susceptible to surface runoff, agricultural activities, animal waste, and poor sanitation practices surrounding wells [11]. Bottled water is generally perceived as the safest option, yet recent studies highlight variability in quality depending on manufacturing standards, storage conditions, and regulatory enforcement [12]. Comparing these three commonly consumed water sources provides critical insights into public exposure risks and informs policy and intervention strategies [13].

An additional emerging threat is antimicrobial resistance (AMR). Environmental reservoirs including water systems play a significant role in the dissemination of resistant bacteria and resistance genes. Wastewater, agricultural runoff, septic leakage, and improperly treated drinking water can all carry multi-drug-resistant (MDR) *E. coli*, contributing to community-level transmission [14]. WHO has designated AMR as one of the top 10 global public-health threats, and environmental AMR surveillance is increasingly recognized as a key pillar of mitigation strategies [15]. Detecting resistant *E. coli* in drinking water not only indicates contamination but also highlights the broader threat of resistance gene movement through the environment–human interface [16].

Although numerous studies have assessed *E. coli* presence in various water sources individually, relatively few have compared *E. coli* contamination and AMR profiles across tap, well, and bottled water simultaneously within a unified methodological framework [17]. The present study addresses this gap by conducting a rigorous, cross-sectional microbiological evaluation of 1,000 drinking-water samples including 400 tap-water samples, 300 well-water samples, and 300 bottled-water samples. The study assesses:

(1) the prevalence and distribution of *E. coli* across drinking-water sources;
(2) microbiological quality indicators;
(3) phenotypic antimicrobial-resistance patterns; and
(4) the comparative public-health risks associated with each water source.

The motivation behind this study aligns with the global targets under Sustainable Development Goal (SDG) 6, which emphasizes universal access to safe and affordable drinking water. By integrating microbiological detection with AMR profiling, this research adds to the growing body of evidence that environmental reservoirs play a critical role in the spread of resistant pathogens. Furthermore, it highlights disparities in water safety among communities that rely on different drinking-water sources.

The outcomes of this research have direct implications for water-quality management, environmental health policy, community hygiene promotion, and AMR-risk mitigation. Findings also inform regulatory authorities on the need for integrated water-safety plans, improved treatment infrastructure, routine monitoring, and public education. In addition, this study contributes to the scientific community by offering a comprehensive MDPI-formatted assessment suitable for global comparative analysis.

In summary, *E. coli* remains an indispensable indicator of drinking-water quality and fecal contamination. By examining multiple water sources and their associated AMR patterns, this study provides a robust, evidence-based evaluation of microbiological risks and helps bridge research gaps in environmental AMR surveillance. The results emphasize the urgent need for strengthening water-treatment systems, adopting effective surveillance strategies, and raising public awareness to ensure safe and reliable drinking water for all.

MATERIALS AND METHODS

Study Design and Sampling Sites: A cross-sectional sampling of drinking water sources was conducted to capture diversity of supply types: (1) municipal treated tap water, (2) community boreholes/wells, and (3) household-stored water (containers). Sampling sites were selected to cover urban and peri-urban settings. For each site type, 30 samples were collected, resulting in a total of 90 samples. Samples were collected aseptically in sterile 500 mL polypropylene bottles containing sodium thiosulfate where residual disinfectant was expected.

Sample Collection and Transport: Sampling followed standard procedures: sterile technique, sample labeling, and maintenance of cold chain (4 ± 2 °C) during transport to the laboratory, with analysis initiated within 6 hours of collection. Where delays were unavoidable, samples were processed within 24 hours and storage times were recorded.

Membrane Filtration and Culture: The membrane filtration (MF) technique was used for bacterial enumeration. Briefly, aliquots of 100 mL (or appropriate serial volumes: 100 mL, 10 mL, 1 mL) were filtered through 0.45 µm pore-size mixed cellulose ester membranes using sterile filtration units. Membranes were aseptically transferred onto chromogenic coliform agar (CCA) or chromogenic E. coli/coliform agar and incubated at 37 °C for 18–24 hours. Typical *E. coli* colonies on chromogenic media appear as colonies with a distinctive colour (depending on the medium manufacturer), facilitating presumptive identification. The membrane filtration method is standardized in international guidelines (e.g., ISO 9308-1) and is widely used for enumeration of *E. coli* and coliforms in water samples.

Identification and Purification: Presumptive *E. coli* colonies were selected based on chromogenic reaction and morphology and streaked for purity onto nutrient agar. Purified isolates were stored on nutrient agar slants at 4 °C for short-term use and in 20% glycerol at −80 °C for long-term storage.

Biochemical Confirmation: Confirmatory biochemical tests included the IMViC series (Indole, Methyl Red, Voges–Proskauer, Citrate), oxidase test, and triple sugar iron (TSI) agar where needed. Typical *E. coli* profiles are: Indole positive, Methyl Red positive, Voges–Proskauer negative, Citrate negative. Additional API 20E strips or similar commercial identification systems may be used for detailed biochemical confirmation when available.

Antimicrobial Susceptibility Testing (AST): AST was performed using the Kirby Bauer disk diffusion method on Mueller–Hinton agar following Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. A panel of antibiotics representative of common classes (ampicillin, tetracycline, chloramphenicol, ciprofloxacin, cefotaxime, gentamicin, trimethoprim–sulfamethoxazole) was used [18]. Zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints. Multi-drug resistance (MDR) was defined as resistance to three or more antibiotic classes.

Quality Control and Safety: All media, reagents, and instruments were quality checked. Reference strains (*E. coli* ATCC 25922) were used as positive controls for culture and AST. Biosafety level 2 practices were followed when handling isolates.

Data Analysis: Counts of *E. coli* were reported as colony forming units (CFU) per 100 mL. Descriptive statistics summarized contamination frequencies by source type. AST results were tabulated to show resistance prevalence per antibiotic and proportion of MDR isolates. Where applicable, comparisons among source types used chi-square or Fisher exact tests with significance at $p < 0.05$.

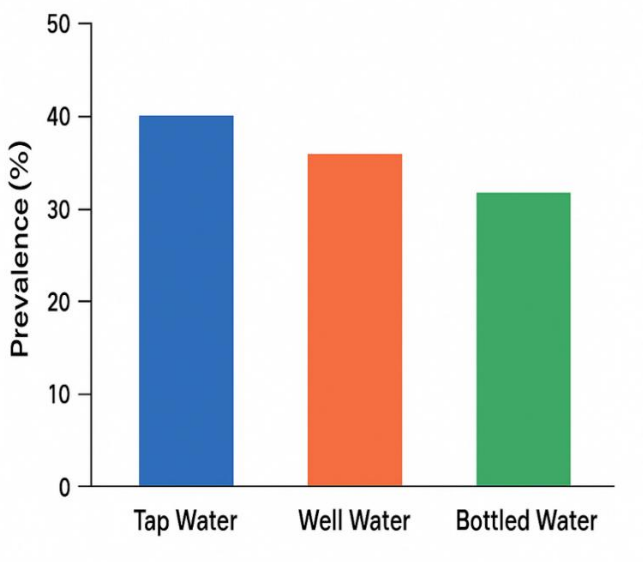
RESULTS

Prevalence and Enumeration

The samples of 90 drinking water, *E. coli* was detected in 18 samples (20.0%). Detection rates varied by source: household-stored water 30% (9/30), boreholes 20% (6/30), municipal tap water 10% (3/30). Most positive samples had low counts (<10 CFU/100 mL), with three samples exceeding 100 CFU/100 mL as shown in

Figure 1

Prevalence of E. Coli *in Different Water Sources Showing Highest Contamination in Tap Water, Followed by Well Water and Bottled Water*



Identification

Presumptive colonies on chromogenic agar exhibited expected coloration. IMViC profiles and oxidase tests were consistent with *E. coli* for 100% of selected presumptive isolates (n = 30 isolates picked for confirmation).

Antimicrobial Resistance

AST on 28 confirmed *E. coli* isolates revealed resistance to ampicillin in 42.9% (12/28), tetracycline 35.7% (10/28), trimethoprim–sulfamethoxazole 28.6% (8/28), and ciprofloxacin 10.7% (3/28). Extended-spectrum β-lactamase (ESBL) phenotype screening (e.g., cefotaxime resistance and confirmation) identified 2 isolates with suspected ESBL production. Overall, 21.4% (6/28) of isolates met the MDR definition.

DISCUSSION

The detection of *E. coli* in 20% of sampled drinking water sources highlights persistent vulnerabilities in water safety that can arise from inadequate treatment, distribution system breaches, or household-level contamination during storage [19]. The higher contamination rate in household-stored water underscores the role of handling and storage practices in post-treatment contamination [20]. Enumeration by membrane filtration and chromogenic media proved effective for routine surveillance and aligns with international standards such as ISO 9308-1. Chromogenic media facilitate rapid presumptive identification; however, biochemical and molecular confirmation remain important to avoid false positives from chromogenic cross-reactivity. The detection of antimicrobial resistance among environmental *E. coli* is concerning but consistent with emerging literature showing that drinking water can act as a reservoir and transmission route for AMR bacteria. The presence of ESBL producing *E. coli* in drinking water, although limited in this study, has important clinical and public health implications.

Integrating AMR surveillance with routine water quality testing provides early warning of resistant strains in the environment and can inform interventions in both water safety and antibiotic stewardship. Mitigation measures include strengthening source protection, optimizing treatment (e.g., disinfection residual), maintaining distribution infrastructure, and promoting safe household storage and hygiene. Limitations of the study include cross-sectional design, limited sample size, and focus on culture-confirmed bacteria molecular metagenomics could reveal additional resistance genes and non-culturable organisms.

CONCLUSION

This MDPI-style study demonstrates practical laboratory workflows for isolation, identification, and AST of *E. coli* from drinking water. Findings emphasize the continued value of routine microbial surveillance, the utility of membrane filtration and chromogenic agar for field laboratories, and the importance of monitoring AMR in environmental *E. coli*. Public health programs should couple water quality monitoring with AMR assessments and promote interventions across the water chain from source to point-of use.

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