BIODIVERSITAS
Volume 26, Number 10, October 2025
Pages: 4923-4931

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d261008

Fecal contamination and antimicrobial susceptibility profiles of *Escherichia coli* in rural water sources of Bali, Indonesia

I WAYAN AGUS GEDE MANIK SAPUTRA[1,2,♥], I PUTU BAYU MAYURA[3], MADE AGUS HENDRAYANA[3], I WAYAN SUARDANA[4], NI NYOMAN SRI BUDAYANTI[3,5]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Udayana. Jl. P.B. Sudirman, Denpasar 80114, Bali, Indonesia.
Tel./fax.: +62-361-222510, ♥email: gedemaniksaputra@gmail.com
[2]Bali Mandara Regional General Hospital. Jl. Bypass Ngurah Rai No. 548, Denpasar 80227, Bali, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Universitas Udayana. Jl. P.B. Sudirman, Denpasar 80234, Bali, Indonesia
[4]Faculty of Veterinary Medicine, Universitas Udayana. Jl. Jend. Sudirman, Denpasar 80234, Bali, Indonesia
[5]Udayana One Health Collaborating Centre, Faculty of Medicine, Universitas Udayana. Jl. P.B. Sudirman, Denpasar 80234, Bali, Indonesia

Manuscript received: 19 July 2025. Revision accepted: 3 October 2025.

Abstract. *Saputra IWAGM, Mayura IPB, Hendrayana MA, Suardana IW, Budayanti NNS. 2025. Fecal contamination and antimicrobial susceptibility profiles of* Escherichia coli *in rural water sources of Bali, Indonesia. Biodiversitas 26: 4923-4931.* *Escherichia coli* is a key indicator of human fecal contamination in environmental waters, providing insight into sanitary conditions and potential health risks, including the presence of diarrheagenic genotypes and antimicrobial-resistant strains such as extended-spectrum β-lactamase (ESBL) producers. This study assessed fecal coliform contamination in rural water sources and characterized *E. coli* isolates for pathogenic potential and antimicrobial susceptibility within the context of the SAJAKA (Antibiotic-Wise Village) Project in Bali, Indonesia. A cross-sectional survey was conducted in August 2024 across 24 sites, including 16 river points (upstream, midstream, downstream) and 8 springs, in four rural villages. Single 250 mL water samples were analyzed using the Most Probable Number (MPN) method, cultured in Lactose Broth at 37°C, and confirmed in Brilliant Green Lactose Bile Broth at 44.5°C. Positive cultures were transferred to eosin methylene blue agar, colonies with a metallic green sheen were purified and identified using the VITEK 2 Compact system, and confirmed *E. coli* isolates were subjected to PCR to detect virulence-associated genes. Fecal coliforms were detected in 41.7% of sites (10 of 24, n=24) of sites, with higher prevalence in rivers (8 of 16, 50%) compared to springs (10 of 24, 25%), and spatial clustering was observed in downstream segments of the Yeh Ge and Yeh Dati Rivers. Eight isolates were identified as *E. coli* and two as *Klebsiella pneumoniae* subsp. *pneumoniae*. None of the *E. coli* isolates carried virulence genes of diarrheagenic pathotypes, and all were susceptible to third-generation cephalosporins, indicating the absence of ESBL production. Although ESBL and diarrheagenic *E. coli* were not detected, the presence of *E. coli* indicates contamination and therefore the water is nonpotable and calls for corrective action. Continued monitoring, particularly for antimicrobial resistance, is essential to support water safety and public health strategies within a One Health framework.

Keywords: *Escherichia coli*, fecal indicators, groundwater contamination, *Klebsiella pneumoniae*, rural sanitation

INTRODUCTION

Access to clean and safe water is fundamental to safeguarding public health and achieving long-term environmental sustainability, yet microbial contamination of surface water remains a persistent and widespread threat, particularly in low-resource and rural settings where water treatment infrastructure is lacking (Tintaya et al. 2022; Ejiohuo et al. 2025). Among microbial indicators, *Escherichia coli* is globally recognized as the most reliable marker of recent fecal contamination due to its strong ecological association with the intestinal tracts of humans and warm-blooded animals (Boulgueraguer et al. 2022), as well as its well-documented correlation with the risk of waterborne diseases (Abualtayef et al. 2014; Wen et al. 2020). Detection of *E. coli* not only indicates sanitary breaches but also reflects systemic vulnerabilities in water safety management that may predispose communities to disease outbreaks (Deblais et al. 2024).

Although many *E. coli* strains are harmless commensals, the emergence of diarrheagenic *E. coli* and extended-spectrum β-lactamase (ESBL)-producing strains has raised substantial public health concerns worldwide (Han et al. 2024). These pathogenic and drug-resistant variants contribute to the dual challenges of enteric disease transmission and the spread of antimicrobial resistance (AMR), underscoring the need for robust microbial water quality surveillance (Some et al. 2021). Environmental water bodies such as rivers, streams, and springs can act as reservoirs for such strains, facilitating persistence in aquatic habitats and enabling horizontal gene transfer within complex microbial communities (Michaelis and Grohmann 2023). This environmental persistence allows resistant and pathogenic bacteria to move between ecosystems, livestock, and human populations, amplifying One Health risks.

In Southeast Asia, the use of untreated surface and groundwater for domestic purposes is common, particularly in rural areas where alternative safe water sources are unavailable or unaffordable. These water sources are highly susceptible to fecal contamination from both diffuse and point sources, including agricultural runoff, livestock grazing and watering points, wastewater discharge, and inadequate sanitation infrastructure (Holcomb and Stewart 2020; Li et al. 2021b; Fatokun et al. 2024). Recent research in tropical regions has revealed that microbial contamination levels often fluctuate with seasonal rainfall patterns, hydrological conditions, and land-use practices, emphasizing the importance of long-term and spatially explicit monitoring strategies (Delpy et al. 2024). However, despite these advances, there remain substantial gaps in data regarding the spatial distribution of microbial contamination and the antimicrobial resistance traits of environmental *E. coli* in rural water systems, limiting the ability to design targeted water safety interventions (Rose et al. 2023).

Indonesia faces similar challenges, with rural communities in provinces such as Bali depending heavily on rivers and springs as their primary sources of water for drinking, cooking, bathing, and other domestic activities, often without any form of treatment (Carrard et al. 2019; Widianingtias et al. 2023). These sources are increasingly vulnerable to microbial pollution due to land-use change, agricultural intensification, and expanding human settlements. The situation is further complicated by the potential for AMR dissemination through environmental pathways, making rural water safety an urgent priority for integrated public health and environmental management (Moretto et al. 2021; Adenaya et al. 2025).

In response to these risks, the SAJAKA (Antibiotic-Wise Village) Project was initiated as an academic-community partnership led by Universitas Udayana in Bali. The project integrates health promotion, rational antibiotic use campaigns, and environmental health interventions to foster sustainable community practices. Water quality monitoring is a central component of SAJAKA's strategy, aligning with the One Health approach that recognizes the interconnectedness of human, animal, and environmental health (Saputra et al. 2025). By coupling microbiological assessments with community engagement, SAJAKA aims to strengthen local capacity for early detection and response to waterborne and AMR-related threats.

The aims of this study were to investigate the occurrence and distribution of fecal coliforms in rural water sources and to characterize *E. coli* isolates for pathogenic potential and antimicrobial susceptibility in the context of SAJAKA in Bali, Indonesia. This work underscores the importance of spatially informed, community-linked water quality surveillance systems as an essential component of integrated rural health strategies in Indonesia and comparable contexts.

MATERIALS AND METHODS

Study area

This cross-sectional study was conducted as part of the SAJAKA (Antibiotic-Wise Village) Project in four rural villages in Tabanan District, Bali, Indonesia: Belalang, Nyitdah, Pejaten, and Buwit. GPS coordinates were recorded for all sampling points, as shown in Figure 1, and additional site characteristics are detailed in Table 1. Although altitude was not measured in this study, detailed descriptions were recorded for each sampling point regarding conditions and activities around the sites, including both human and animal aspects.

Ethics approval

This study was approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia, No: 0882/UN14.2.2.VII.14/LT/2024 dated March 18, 2024.

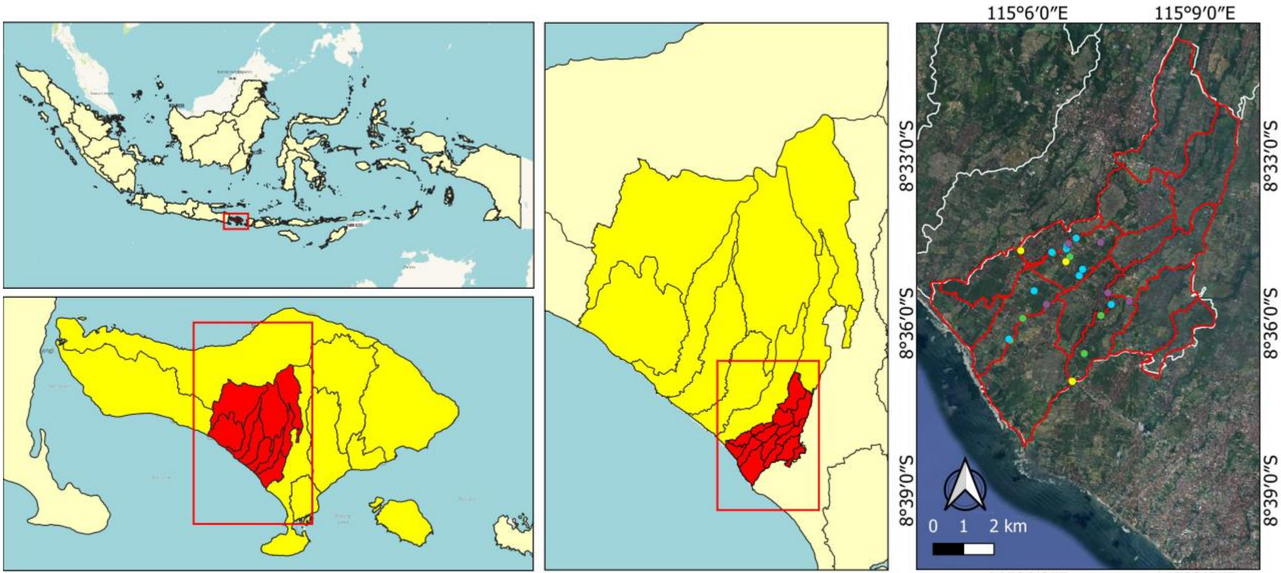

Figure 1. Geographic distribution of sampling sites for water quality assessment in Kediri Sub-district, Tabanan Regency, Bali, Indonesia (Geospasial 2025) (●: downstream; ●: middlestream; ●: upstream; ●: spring water sources)

Table 1. Distribution of fecal coliform contamination in four rural villages, Bali, Indonesia

Villages	Water sources name	Types	Coordinates	Human activity	Poultry farm	Health facility
Belalang	Yeh Kutikan	River (upstream)	8.59430°S, 115.10575°E	No	No	No
Belalang	Yeh Kutikan	River (midstream)	8.59845°S, 115.09866°E	Yes	No	No
Belalang	Yeh Kutikan	River (downstream)	8.60488°S, 115.09476°E	Yes	Yes	No
Nyitdah	Yeh Ge	River (upstream)	8.57573°S, 115.12192°E	Yes	Yes	No
Nyitdah	Yeh Ge	River (midstream)	8.58006°S, 115.11274°E	Yes	No	No
Nyitdah	Yeh Ge	River (downstream)	8.58152°S, 115.11155°E	Yes	No	No
Pejaten	Yeh Dati	River (upstream)	8.576111°S, 115.112306°E	No	Yes	Yes
Pejaten	Yeh Dati	River (midstream)	8.57890°S, 115.10760°E	Yes	Yes	No
Pejaten	Yeh Dati	River (downstream)	8.57820°S, 115.09809°E	No	Yes	No
Buwit	Yeh Poh	River (upstream)	8.590806°S, 115.124111°E	Yes	Yes	No
Buwit	Yeh Poh	River (midstream)	8.597611°S, 115.122028°E	Yes	Yes	No
Buwit	Yeh Poh	River (downstream)	8.6171481°S, 115.11332°E	Yes	No	No
Buwit	Yeh Ulam	River (upstream)	8.593361°S, 115.130472°E	Yes	No	No
Buwit	Yeh Ulam	River (midstream)	8.608972°S, 115.117056°E	Yes	Yes	No
Buwit	Yeh Ulam	River (downstream)	8.617281°S, 115.11351°E	Yes	No	No
Belalang	Beji Ratu Ngurah Sakti	Spring	8.59022°S, 115.10205°E	Yes	No	No
Belalang	Beji Banjar Kedungu	Spring	8.60465°S, 115.09451°E	Yes	No	No
Nyitdah	Beji Sengara	Spring	8.57445°S, 115.11464°E	Yes	No	No
Nyitdah	Beji Sengguan	Spring	8.57769°S, 115.11171°E	Yes	No	No
Nyitdah	Beji Mengening	Spring	8.58570°S, 115.11553°E	Yes	No	No
Nyitdah	Beji Babakan	Spring	8.58378°S, 115.11655°E	Yes	No	No
Pejaten	Beji Baleran	Spring	8.576500°S, 115.111889°E	Yes	No	No
Pejaten	Beji Penimbangan	Spring	8.578694°S, 115.107361°E	Yes	No	No
Buwit	Beji Golok	Spring	8.5942840°S, 115.1251030°E	Yes	No	No

Sampling design

A total of 24 water sources were selected using purposive sampling to identify potential hotspots of fecal contamination. Sampling included both river sites (upstream, midstream, downstream) and spring water sources, with site selection guided by known or suspected sanitation risks. These included proximity to human settlements, agricultural activity (particularly poultry farms), and nearby healthcare facilities. The aim was to capture a diverse range of contamination scenarios influenced by varying degrees of anthropogenic pressure.

Sample collection and processing

Sample collection was conducted in early August 2024, which corresponds to the local dry season in Bali. A 250 mL of single-point water sample was collected from both river and spring water using sterile glass bottles. For river sites, surface water samples were collected at a depth of approximately 0.5 m to minimize surface debris and ensure representative sampling. The total number of samples collected was 24, without replication, representing point prevalence at each location. Samples were kept in an ice-cooled box and immediately transported within six hours to the microbiology laboratory. All water samples were analyzed following standard procedures for bacteriological water quality testing (Cappuccino and Sherman 2005; APHA 2023). Cooler temperatures were checked at dispatch and on receipt with a calibrated thermometer and logged on the chain of custody form. Collection and receipt times were recorded for all samples to calculate holding time. Field blanks were processed alongside samples at representative sites, and trip blanks accompanied each cool box. For each specimen, we recorded bottle integrity, sample ID, and transport conditions.

Microbiological analysis and bacterial identification

Bacteriological water analysis was performed following the standard three-step Most Probable Number (MPN) procedure, consisting of presumptive, confirmed, and completed tests (Cappucino and Welsh 2018; APHA 2023). The presumptive test, serving as an initial screening for the presence of coliform bacteria, was conducted by inoculating water samples into Lactose Broth (LB; Oxoid® CM0137) tubes, each containing 5 mL of LB broth, and incubating them at 37°C for 48 hours. Serial dilutions of 10, 1, and 0.1 mL of the water sample were inoculated across the tubes. Tubes showing gas production were considered presumptively positive for coliforms. The number of coliform bacteria present in the sample was estimated using the MPN method by analyzing the pattern of positive tubes across these serial dilutions. Subsequently, confirmatory tests were performed by transferring one calibrated loop (10 µL) from each gas-producing tube into 10 mL Brilliant Green Lactose Bile Broth (BGLB; Oxoid® CM0031), followed by incubation at 44.5°C for 48 hours. Tubes that remained positive and produced gas under these thermotolerant conditions were interpreted as containing fecal coliforms.

Coliforms that were presumptively positive in Lactose Broth at 37°C but negative in BGLB at 44.5°C were classified as non-fecal (non-thermotolerant) coliforms, and species-level identification was not undertaken for these isolates. For the completed test, positive BGLB tubes were sub-cultured onto eosin methylene blue agar (EMBA; Oxoid® CM0069) and incubated at 37°C for 24 hours to observe colony morphology. Colonies exhibiting a characteristic green metallic sheen, typical for strong fermenters such as *E. coli*, a primary indicator of fecal contamination, were further sub-cultured onto MacConkey agar for purification and then tested using the VITEK 2 Compact system (bioMérieux, France) for species-level identification.

Each batch included uninoculated media to verify sterility of LB, BGLB, EMBA, and MacConkey. Incubator temperatures at 37 and 44.5°C were checked daily with a calibrated thermometer and logged. An *E. coli* control confirmed expected growth and morphology on EMBA and MacConkey, and negative controls showed no growth.

Antimicrobial susceptibility testing

Isolates confirmed as *E. coli* or other coliforms were subjected to automated antimicrobial susceptibility testing using the VITEK 2 system (bioMérieux, France). Instrument and card performance were verified according to manufacturer guidance with routine control strains, including *E. coli* ATCC 25922, and control results were within acceptable ranges before interpreting study isolates. ESBL production was inferred from third-generation cephalosporin resistance profiles and confirmatory susceptibility patterns. All isolates from fecal coliform-positive samples were recorded with their ESBL status.

Molecular detection

Eight *E. coli* isolates were subjected to PCR to detect the diarrheagenic *E. coli* virulence genes using primers targeting EAEC (*CVD432*), ETEC (*elt*, *est*), DAEC (*daaD*), EPEC (*bfpA*), and EHEC (*stx1*, *stx2*). The PCR conditions corresponding to each primer pair are detailed in Table 2.

Bacterial DNA isolation

Subjected colonies were picked from McConkey Agar using a 10 µL loop, then resuspended in 200 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The cell suspension was then subjected to boiling at 100°C for 10 minutes. The mixture was centrifuged at 8000 rpm for 1 minute. The DNA-containing upper aqueous phase was transferred into a separate 1.5 mL Eppendorf tube.

Polymerase chain reaction (PCR) condition

The PCR was performed with Go Taq® Green Master Mix (Promega, Madison, USA) with primer concentrations of 0.3 M were utilized. Then, the amplicons were electrophoresed for 35 min on a 1.5% Agarose (1st BASE, Singapore) gel in TBE buffer at 100 volts. The DNA was visualized using FloroSafe DNA Stain (1st BASE, Singapore) and then documented using Gel Doc (Bio-Rad).

Data analysis

Descriptive analysis was conducted to evaluate the frequency and spatial distribution of fecal and non-fecal coliforms, identify bacterial species, and determine the presence of virulence genes and antimicrobial resistance profiles across different water sources and villages. Total coliform levels were summarized as means with standard deviations (SD) and compared between river and spring water within each village. The relationship between anthropogenic activities and total coliform occurrence was assessed using Chi-square test. Differences in total coliform counts between spring and river water were evaluated using the Mann-Whitney U test, a non-parametric alternative to the independent t-test, with all analyses performed using SPSS version 20 (IBM Corp., Armonk, NY, USA).

Table 2. Primer sequences, target genes, amplicon sizes, and annealing conditions for diarrheagenic *Escherichia coli* pathotype detection

Diarrheagenic *E. coli* pathotypes	**Genes**	**Amplicon length (bp)**	**Annealing (°C)**	**Primer direction**	**Primer sequences**	**Sources**
EAEC	CVD432	194 bp	50	Forward Reverse	AGACTCTGGCGAAAGACTGTATC ATGGCTGTCTGTAATAGATGAGAAC	(Toma et al. 2003)
ETEC						
Labile toxin	*elt*	322 bp	54	Forward Reverse	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT	
Stabile toxin	*est*	147 bp	54	Forward Reverse	TTAATAGCACCCGGTACAAGCAGG CCTGACTCTTCAAAAGAGAAAATTAC	
DAEC	*daaD*	371 bp	52	Forward Reverse	TGAACGGGAGTATAAGGAAGATG GTCCGCCATCACATCAAAA	(Abbasi et al. 2017)
EPEC	*bfpA*	326 bp	56	Forward Reverse	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	(Gunzburg et al. 1995)
EHEC						
Shiga toxin-1	*stx1*	1168 bp	52	Forward Reverse	TCAACGAAAAATAACTTCGCTGAATCCC CAGTTAATGTGGTTGCGAAGGAATTACC	(Lee et al. 2007)
Shiga toxin-2	*stx2*	1241 bp	52	Forward Reverse	ATGAAGTGTATATTATTTAAATGGGTACTGTG TCAGTCATTATTAAACTGCACTTCAG	(Lee et al. 2007)

Note: EAEC: Enteroaggregative *E. coli*, ETEC: Enterotoxigenic *E. coli*, DAEC: Diffusely Adherent *E. coli*, EPEC: Enteropathogenic *E. coli*, EHEC: Enterohaemorrhagic *E. coli*

RESULTS AND DISCUSSION

Isolation and identification of bacteria

The microbiological surveillance conducted across four villages in Bali, including Belalang, Nyitdah, Pejaten, and Buwit, revealed notable spatial and source-based variation in the presence of fecal coliforms and *E. coli* in community water sources. Among the 24 water source sites assessed, 10 sites (41.7%) were positive for fecal coliforms, whereas the remaining 14 samples yielded non-fecal coliforms. Non-fecal coliforms were not speciated, consistent with our a priori focus on fecal coliform positive isolates for downstream testing. The detailed distribution of fecal and non-fecal coliform-positive sites by location and water source type is presented in Table 3.

Total coliform levels were consistently higher in river water than in spring water across most villages (Figure 2). In Belalang and Nyitdah River samples averaged approximately 2,400 MPN/100 mL, whereas spring samples were notably lower at around 1,200 MPN/100 mL and 600 MPN/100 mL, respectively. In Pejaten, river water showed similarly high levels (~2,400 MPN/100 mL), while spring water was near zero, suggesting effective protection of spring sources. In contrast, Buwit exhibited comparable contamination levels in both river and spring water (~2,400 MPN/100 mL), indicating widespread fecal contamination across all water sources in this village. These patterns highlight rivers as consistently high-risk sources and reveal variability in spring water vulnerability between villages.

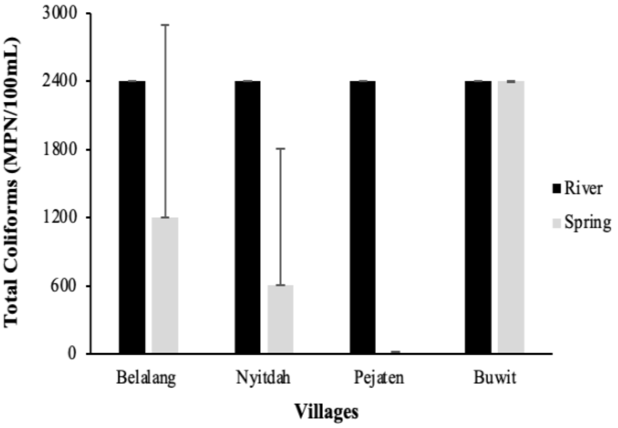

Figure 2. Bar chart comparing total coliform levels (MPN/100 mL) in river and spring water across four SAJAKA villages in Bali

Table 3. Bacterial identification and ESBL status across water sources in four rural villages, Bali

Villages	Water sources name	Types	Microbiology result	Bacterial identification	ESBL status	MPN/ 100 mL
Belalang	Yeh Kutikan	River (upstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Belalang	Yeh Kutikan	River (midstream)	Non-fecal coliform	NT	NT	≥2400
Belalang	Yeh Kutikan	River (downstream)	Non-fecal coliform	NT	NT	≥2400
Nyitdah	Yeh Ge	River (upstream)	Non-fecal coliform	NT	NT	≥2400
Nyitdah	Yeh Ge	River (midstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Nyitdah	Yeh Ge	River (downstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Pejaten	Yeh Dati	River (upstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Pejaten	Yeh Dati	River (midstream)	Non-fecal coliform	NT	NT	≥2400
Pejaten	Yeh Dati	River (downstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Buwit	Yeh Poh	River (upstream)	Fecal coliform	*Klebsiella pneumoniae* subsp. *pneumoniae*	Non-ESBL	≥2400
Buwit	Yeh Poh	River (midstream)	Non-fecal coliform	NT	NT	≥2400
Buwit	Yeh Poh	River (downstream)	Fecal coliform	*Klebsiella pneumoniae* subsp. *pneumoniae*	Non-ESBL	≥2400
Buwit	Yeh Ulam	River (upstream)	Non-fecal coliform	NT	NT	≥2400
Buwit	Yeh Ulam	River (midstream)	Non-fecal coliform	NT	NT	≥2400
Buwit	Yeh Ulam	River (downstream)	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400
Belalang	Beji Ratu Ngurah Sakti	Spring	Non-fecal coliform	NT	NT	≥2400
Belalang	Beji Banjar Kedungu	Spring	Non-fecal coliform	NT	NT	9
Nyitdah	Beji Sengara	Spring	Non-fecal coliform	NT	NT	7
Nyitdah	Beji Sengguan	Spring	Non-fecal coliform	NT	NT	0
Nyitdah	Beji Mengening	Spring	Non-fecal coliform	NT	NT	≥2400
Nyitdah	Beji Babakan	Spring	Fecal coliform	*Escherichia coli*	Non-ESBL	14
Pejaten	Beji Baleran	Spring	Non-fecal coliform	NT	NT	17
Pejaten	Beji Penimbangan	Spring	Non-fecal coliform	NT	NT	2
Buwit	Beji Golok	Spring	Fecal coliform	*Escherichia coli*	Non-ESBL	≥2400

Note: MPN/100 mL indicate total coliform count. NT: Not Tested. For non-fecal coliform results, species identification and ESBL testing were not performed; only fecal coliform-positive isolates proceeded to identification and ESBL assessment

The majority of fecal contamination occurred in river water sources, particularly in downstream segments, such as Yeh Kutikan in Belalang, Yeh Ge in Nyitdah, and Yeh Ulam and Yeh Poh in Buwit. These downstream sections often represent hydrological convergence points with increased exposure to human settlements, runoff, and diffuse pollution sources. Although river systems were more frequently contaminated, fecal coliforms were also identified in two spring sources: Beji Babakan in Nyitdah and Beji Golok in Buwit. This finding raises important concerns about the vulnerability of groundwater-fed systems, which are traditionally perceived as protected from surface contamination.

Contrary to expectations, the presence of fecal coliforms did not display a consistent association with the proximity of potential risk factors such as human activity, poultry farming, or health facilities. Yeh Dati in Pejaten exhibited fecal contamination both upstream and downstream, despite the absence of direct human settlement at the latter site. Similarly, Yeh Ge in Nyitdah showed upstream non-contamination, but midstream and downstream sections tested positive, suggesting the influence of localized inputs or hydrological dispersion.

Notably, spatial clustering of fecal contamination was observed in several river systems. In Nyitdah, the Yeh Ge river exhibited contamination in both midstream and downstream sections, with non-fecal coliforms present upstream. Similarly, in Pejaten, Yeh Dati showed fecal contamination in the upstream and downstream points, while the midstream site was free of fecal indicators. This pattern may reflect dilution effects, natural attenuation processes, or intermittent contaminant influxes along the river course. In Buwit, Yeh Poh River showed fecal contamination at both extremities but not in the midstream, suggesting episodic or point-source contamination events.

Among the 10 fecal coliform-positive samples, bacterial identification revealed *E. coli* as the predominant isolate in eight samples, while the remaining two, both from Buwit's Yeh Poh River (upstream and downstream), harbored *Klebsiella pneumoniae* subsp. *pneumoniae*. All *E. coli* and *K. pneumoniae* isolates were confirmed to be non-ESBL producers based on phenotypic antimicrobial susceptibility profiles, indicating susceptibility to third-generation cephalosporins. This finding is consistent with the relatively low levels of antibiotic resistance typically observed in rural environmental reservoirs, though it warrants ongoing monitoring due to the potential for resistance gene acquisition via horizontal gene transfer.

Molecular analysis

Molecular characterization using PCR assays targeting virulence genes associated with five major diarrheagenic *E. coli* pathotypes (EAEC, ETEC, DAEC, EPEC, and EHEC) yielded negative results for all eight *E. coli* isolates, with detailed findings presented in Table 4. This suggests that detected strains were likely commensal in nature and did not harbor genes associated with enteric pathogenicity. From public health perspective, the absence of diarrheagenic markers, combined with the non-ESBL phenotype, implies a currently low-risk profile for enteric disease transmission via these sources.

Significant relationship were identified by Chi-square test between human settlements and both fecal coliform counts ($p=0.049$) and *E. coli* counts ($p=0.028$), suggesting that human habitation may contribute to fecal contamination. No significant relationship were found for poultry farms or healthcare facilities, with all $p>0.05$. Total coliforms showed no significant relationship with any anthropogenic activity, indicating that their occurrence may be driven by diffuse or environmental sources. Comparison of mean MPN values revealed significantly higher total coliform counts in rivers than in springs ($p=0.047$), supporting the view that surface waters are more susceptible to contamination from runoff, direct human and animal access, and reduced natural filtration compared to protected spring sources (Table 5). Overall, these findings illustrate the ongoing vulnerability of rural water sources to fecal contamination, particularly in open and unprotected riverine environments. While the detected strains of *E. coli* and *K. pneumoniae* subsp. *pneumoniae* were non ESBL producers, their presence signifies lapses in environmental sanitation and the potential for future colonization by more virulent or antibiotic-resistant strains. The detection of fecal indicators in spring sources also underscores the need to reassess assumptions regarding their intrinsic safety. Routine microbial monitoring, integrated with land-use management and community-level education, remains essential to safeguarding public health, in alignment with One Health framework that recognize the interconnectedness of environmental, human, and animal health systems.

Table 4. PCR detection results for diarrheagenic *Escherichia coli* pathotypes (EAEC, ETEC, DAEC, EPEC, EHEC) in water samples from four rural villages, Bali

Villages	Water sources name	Types	EAEC	ETEC	DAEC	EPEC	EHEC
Belalang	Yeh Kutikan	River (upstream)	Neg	Neg	Neg	Neg	Neg
Nyitdah	Yeh Ge	River (midstream)	Neg	Neg	Neg	Neg	Neg
Nyitdah	Yeh Ge	River (downstream)	Neg	Neg	Neg	Neg	Neg
Nyitdah	Beji Babakan	Spring	Neg	Neg	Neg	Neg	Neg
Pejaten	Yeh Dati	River (upstream)	Neg	Neg	Neg	Neg	Neg
Pejaten	Yeh Dati	River (midstream)	Neg	Neg	Neg	Neg	Neg
Buwit	Yeh Ulam	River (downstream)	Neg	Neg	Neg	Neg	Neg
Buwit	Beji Golok	Spring	Neg	Neg	Neg	Neg	Neg

Note: Neg: Negative

Table 5. Association of total coliform counts with anthropogenic activities and water source type

Indicators	Anthropogenic activity			Comparison MPN between spring and river (Sig.)
	Human (Sig.)	Poultry far (Sig.)	Health care (Sig.)	
Fecal coliform	0.049	0.561	0.417	N/A
Escherichia coli	0.028	0.447	0.333	N/A
Total coliform	0.087	0.667	0.958	0.047

Note: N/A: Not Applicable, p≤0.05 indicate statistical significance

Discussion

This study provides critical baseline data on the microbiological quality of rural water sources in four villages involved in the SAJAKA Project in Bali, Indonesia. Fecal coliforms were detected in 41.7% of samples, with higher contamination in rivers than in springs. This pattern is consistent with findings from other tropical regions, where hydrological dynamics and anthropogenic pressures exacerbate microbial pollution (Wen et al. 2020; Díaz-Gavidia et al. 2022). Notably, the presence of fecal indicators in two spring sources, Beji Babakan and Beji Golok, challenges the assumption that spring water is inherently safe, suggesting contamination via shallow aquifers or unsealed collection structures (Bagordo et al. 2024).

The susceptibility of these spring sources can be attributed to hydrogeological characteristics typical of volcanic island settings such as Bali, where fractured bedrock and thin soil cover facilitate rapid infiltration of surface contaminants into groundwater (Fenta et al. 2020; Race et al. 2024). In tropical groundwater systems, microbial persistence is further promoted by high temperatures and organic-rich environments, which can extend bacterial survival (Petersen and Hubbart 2020; Li et al. 2021a). Shallow groundwater tables, particularly in upland areas with permeable substrates, heighten the risk of bacterial transport during rainfall events or surface runoff (Chen et al. 2023; Bagordo et al. 2024). The proximity of household drainage channels and livestock pens can exacerbate this vulnerability by enabling direct contamination of recharge zones, a pattern also observed in other tropical island settings (Nayebare et al. 2022; Wu et al. 2025). Collectively, these findings underscore that ensuring microbial safety in rural water systems requires not only consideration of source type but also careful evaluation of local hydrogeological conditions and sanitation practices.

The results of present study revealed that total coliform levels were significantly higher in river water than in spring water across most villages, with rivers averaging around 2,400 MPN/100 mL. Spring water contamination varied: Belalang exhibited ~1,200 MPN/100 mL, Nyitdah ~600 MPN/100 mL, Pejaten was near zero, and Buwit matched river contamination (~2,400 MPN/100 mL). In Pejaten, very low MPN values in springs likely reflect protective measures observed on site, including sealed intake structures, controlled access, runoff diversion away from the spring head, sanitary setbacks from latrines and livestock, and tap-based collection. These patterns are consistent with broader evidence. A study in 2020 reported that 25% of protected springs still tested positive for fecal coliforms, compared to much higher rates in shallow wells and hand-dug sources, underscoring the partial efficacy of protective measures in mitigating contamination (Gizachew et al. 2020). Similar patterns have been reported in Indonesia, where total coliform concentrations in the Bedadung River, East Java, consistently exceeded national standards (>1,600 MPN/100 mL), due to domestic and agricultural inputs, indicating that both surface and groundwater in rural areas remain vulnerable without effective protection and monitoring (Novita et al. 2020).

In accordance with Ministry of Health Regulation No. 2 of 2023 on Environmental Health, the permissible limit for total coliform in drinking water is 0 CFU/100 mL. All river samples and some spring sources exceeded this threshold, posing significant health risks if consumed untreated. Rivers thus represent consistently high-risk water sources. In contrast, the safety of springs sources was village-specific: Pejaten's springs remained well protected, Belalang and Nyitdah exhibited moderate contamination, and Buwit's springs posed risks comparable to rivers. These findings call for targeted interventions, including enhanced protection of spring sources, routine microbial monitoring, and community sanitation measures to reduce fecal contamination and ensure compliance with national standards. In contrast to contaminated sources, one uncontaminated spring in Nyitdah, Beji Sengguan, located within a Hindu temple compound, recorded 0 MPN/100 mL and displayed several protective features. These included a sealed masonry collection box with an intact cover, controlled access through fencing and temple stewardship, a paved apron with drainage that diverts runoff from the spring head, sanitary setbacks from latrines and livestock, collection from taps rather than container dipping, and the absence of bathing or laundry at the spring head.

A notable spatial clustering was observed in rivers such as Yeh Dati and Yeh Ge, where upstream and downstream segments tested positive for *E. coli* while midstream segments were negative, suggesting episodic contamination from diffuse sources like agricultural runoff or informal sanitation systems. Similar patterns in Southeast Asian watersheds link seasonal rainfall and land-use practices to fluctuating contamination risks (Deblais et al. 2024; Saldaña Almazán et al. 2025), underscoring the need for integrated land-use planning and localized protection measures such as spring sealing and controlled drainage. While *E. coli* was the predominant fecal indicator, detection of *Klebsiella pneumoniae* subsp. *pneumoniae* in the Yeh Poh River highlights environmental reservoirs of opportunistic pathogens and potential antimicrobial resistance (AMR) gene sources (Maes et al. 2022). No isolates exhibited extended-spectrum β-lactamase (ESBL) activity, aligning with regional reports of lower resistance rates in environmental and community isolates compared to hospital settings (Moretto et al. 2021; Fatokun et al. 2024). Nevertheless, given the potential for horizontal gene transfer in aquatic environments, particularly within biofilms, ongoing surveillance is essential to detect emerging resistance threats (Abe et al. 2021).

Molecular analysis using PCR targeting diarrheagenic *E. coli* pathotypes, including EAEC, ETEC, DAEC, EPEC, and EHEC, confirmed the absence of virulence genes among the eight *E. coli* isolates tested. These results show that the tested isolates lacked the screened diarrheagenic markers. However, risk assessment for water quality is determined by fecal indicator evidence. Fecal indicator bacteria such as *E. coli* constitute the primary standard for assessing fecal contamination and public health risk. Pathogen-specific PCR provides supplementary context and does not determine potability. Accordingly detection of *E. coli* indicates contamination and the water should not be considered potable. Detection in domestic or recreational water sources highlights ongoing exposure risks and supports continued surveillance within a One Health framework (Belete et al. 2022; Lau et al. 2024).

The results highlight the need for integrated water safety plans in rural Bali, combining source protection, improved sanitation, and participatory water quality monitoring. Such measures align with SDG 6 (Clean Water and Sanitation) and the One Health framework, addressing the interconnected risks of waterborne pathogens, environmental contamination, and human-livestock interactions (Rose et al. 2023; Onwudiegwu and Chibueze 2025). Proactive surveillance is critical to anticipate and mitigate emerging threats, particularly under pressures from climate change and land-use intensification in tropical island settings.

In summary, these findings emphasize the critical of proactive microbial water quality surveillance in rural settings, where untreated water remains a primary source for household use. The detection of fecal indicators in both surface and spring sources underscores the need for integrated water safety plans, which combine technical improvements with community-based sanitation interventions (Li et al. 2021b). Although no virulent or resistant strains were identified in this study, sustained environmental surveillance within a One Health framework remains essential to anticipate and prevent the potential emergence of antimicrobial-resistant or pathogenic bacteria in these vulnerable ecosystems.

Study limitations

This cross-sectional study, based on single-time sampling in four villages, cannot assess seasonal or climatic variation and may overlook broader spatial trends in water quality. Reliance on culture-based methods and targeted PCR may have resulted in missing non-culturable microbes, other pathogens, or resistance genes not included in the screening. The absence of virulence genes does not exclude other pathogenic determinants. Antibiotic concentrations and other environmental factors influencing microbial persistence were not measured. Future studies should adopt high-resolution spatiotemporal sampling and metagenomic approaches for comprehensive risk assessment.

In conclusion, the results of this study highlight ongoing fecal contamination in rural Balinese water sources, particularly rivers. The detection of *E. coli* and other fecal indicators reflects persistent sanitary vulnerabilities that warrant attention. Although ESBL and diarrheagenic *E. coli* were not detected, any *E. coli* indicates contamination, the water is nonpotable, and requires corrective action. The SAJAKA framework, integrating culture-based and molecular methods, provides a practical approach for community-based water quality surveillance in low-resource settings. These findings underscore the need for sustained, One Health-aligned environmental health initiatives to reduce the risk of antimicrobial resistance and thereby safeguard human health. This cross-sectional study, based on single-time sampling in four villages, cannot assess seasonal or climatic variation and may overlook broader spatial trends in water quality. Future studies should adopt high-resolution spatiotemporal sampling and metagenomic approaches for comprehensive risk assessment.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to all colleagues who contributed to the successful completion of this research. Special thanks are extended to the laboratory team, particularly Wahyu Hidayati, Tatik, and Mahendra, for their invaluable technical assistance and support throughout the study.

REFERENCES

Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani-Dalini S, Dehyadegari MA. 2017. Molecular detection of diffusely adherent *Escherichia coli* strains associated with diarrhea in Shiraz, Iran. Arch Pediatic Infect Dis 5 (2): e37629. DOI: 10.5812/pedinfect.37629.

Abe K, Nomura N, Suzuki S. 2021. Biofilms: Hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. Oxford University Press, Oxford. DOI: 10.1093/FEMSEC/FIAA031.

Abualtayef MT, Abd Rabou AN, Abu Foul AA, Ghabayen SM, Elsinwar HM. 2014. Microbial water quality of coastal recreational water in the Gaza Strip, Palestine. Nusantara Bioscience 6: 26-32. DOI: 10.13057/nusbiosci/n060105.

Adenaya A, Adeniran AA, Ugwuoke CL, Saliu K, Raji MA, Rakshit A, Ribas-Ribas M, Könneke M. 2025. Environmental risk factors contributing to the spread of antibiotic resistance in West Africa. Microorganisms 13: 951. DOI: 10.3390/microorganisms13040951.

APHA [American Public Health Association]. 2023. Standard Methods for the Examination of Water and Wastewater 24th Edition 24 (American Public Health Association).

Bagordo F, Brigida S, Grassi T, Caputo MC, Apollonio F, De Carlo L, Savino AF, Triggiano F, Turturro AC, De Donno A, Montagna MT, De Giglio O. 2024. Factors influencing microbial contamination of groundwater: A systematic review of field-scale studies. Microorganisms 12: 1-18. DOI: 10.3390/microorganisms12050913.

Belete MA, Demlie TB, Chekole WS, Tessema TS. 2022. Molecular identification of diarrheagenic *Escherichia coli* pathotypes and their antibiotic resistance patterns among diarrheic children and in contact calves in Bahir Dar City, Northwest Ethiopia. PLoS One 17 (9): e0275229. DOI: 10.1371/journal.pone.0275229.

Boulgueraguer Z, Chaffai H, Gasmi L, Djabri L, Hani A. 2022. Impact of organic and microbiological pollution on the quality of water in

Boussellam Valley and its environment, Setif City, Northeast Algeria. Biodiversitas 23: 2621-2629. DOI: 10.13057/biodiv/d230545.

Cappuccino JG, Sherman N. 2018. Microbiology: A laboratory Manual. Pearson/Benjamin Cummings, San Francisco.

Carrard N, Foster T, Willetts J. 2019. Groundwater as a source of drinking water in southeast Asia and the Pacific: A multi-country review of current reliance and resource concerns. Water 11 (8): 1605. DOI: 10.3390/w11081605.

Chen F, Zhou B, Yang L, Chen X, Zhuang J. 2023. Predicting bacterial transport through saturated porous media using an automated machine learning model. Front Microbiol 14: 1152059. DOI: 10.3389/fmicb.2023.1152059.

Deblais L, Ahmedo BU, Ojeda A, Mummed B, Wang Y, Mekonnen YT, Weldesenbet YD, Hassen KA, Brhane M, Mckune SL, Havelaar AH, Liang S, Rajashekara G. 2024. Assessing fecal contamination from human and environmental sources using *Escherichia coli* as an indicator in rural eastern Ethiopian households - a study from the EXCAM project. Front Public Health 12: 1484808. DOI: 10.3389/fpubh.2024.1484808.

Delpy L, Astbury CC, Aenishaenslin C, Ruckert A, Penney TL, Wiktorowicz M, Ciss M, Benko R, Bordier M. 2024. Integrated surveillance systems for antibiotic resistance in a One Health context: A scoping review. BMC Public Health 24: 1717. DOI: 10.1186/s12889-024-19158-6.

Díaz-Gavidia C, Barría C, Weller DL, Salgado-Caxito M, Estrada EM, Araya A, Vera L, Smith W, Kim M, Moreno-Switt AI, Olivares-Pacheco J, Adell AD. 2022. Humans and hoofed livestock are the main sources of fecal contamination of rivers used for crop irrigation: A microbial source tracking approach. Front Microbiol 13: 768527. DOI: 10.3389/fmicb.2022.768527.

Ejiohuo O, Onyeaka H, Akinsemolu A, Nwabor OF, Siyanbola KF, Tamasiga P, Al-Sharify ZT. 2025. Ensuring water purity: Mitigating environmental risks and safeguarding human health. Water Biol Secur 4: 100341. DOI: 10.1016/j.watbs.2024.100341.

Fatokun O, Selvaraja M, Anuar H, Jamaluddin TZMT, Ismail SNS, Mansor R, Shah SA, Oranye N. 2024. Antimicrobial resistance at the human-animal-environment interface: A focus on antimicrobial-resistant *Escherichia coli* transmission dynamics, clinical implications, and future directions. Intl J One Health 10: 161-171. DOI: 10.14202/IJOH.2024.161-171.

Fenta MC, Anteneh ZL, Szanyi J, Walker D. 2020. Hydrogeological framework of the volcanic aquifers and groundwater quality in Dangila town and the surrounding area, Northwest Ethiopia. Groundwater Sustain Dev 11: 100408. DOI: 10.1016/j.gsd.2020.100408.

Geospasial. 2025. Badan Informasi Geospasial Tanah Air Indonesia. Available at https://tanahair.indonesia.go.id/portal-web/. [August 13, 2025] [Indonesian]

Gizachew M, Admasie A, Wegi C, Assefa E. 2020. Bacteriological contamination of drinking water supply from protected water sources to point of use and water handling practices among beneficiary households of Boloso Sore Woreda, Wolaita Zone, Ethiopia. Intl J Microbiol 2020 (1): 5340202. DOI: 10.1155/2020/5340202.

Gunzburg ST, Tornieporth NG, Riley LW. 1995. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. J Clin Microbiol 33: 1375-1377. DOI: 10.1128/jcm.33.5.1375-1377.1995.

Han H, Li W, Liu J, Zhang X, Huo X, Sun Y, Chen J, Fan R, Zhang J, Chen Y, Yang H, Chen S, Li Y, Tan D, Ma X. 2024. Seven - year overview of antimicrobial resistance in diarrheagenic *Escherichia coli* from sporadic human diarrhea cases in 20 Chinese provinces. One Health Adv 2 (1): 29. DOI: 10.1186/s44280-024-00064-w.

Holcomb DA, Stewart JR. 2020. Microbial indicators of fecal pollution: Recent progress and challenges in assessing water quality. Curr Environ Health Rep 7: 311-324. DOI: 10.1007/s40572-020-00278-1.

Lau M, Monis PT, King BJ. 2024. The efficacy of current treatment processes to remove, inactivate, or reduce environmental bloom-forming *Escherichia coli*. Microbiol Spectr 12: e0085624. DOI: 10.1128/spectrum.00856-24.

Lee JE, Reed J, Shields MS, Spiegel KM, Farrell LD, Sheridan PP. 2007. Phylogenetic analysis of Shiga toxin 1 and Shiga toxin 2 genes associated with disease outbreaks. BMC Microbiol 7: 109. DOI: 10.1186/1471-2180-7-109.

Li P, Karunanidhi D, Subramani T, Srinivasamoorthy K. 2021a. Sources and consequences of groundwater contamination. Arch Environ Contam Toxicol 80: 1-10. DOI: 10.1007/s00244-020-00805-z.

Li E, Saleem F, Edge TA, Schellhorn HE. 2021b. Biological indicators for fecal pollution detection and source tracking: A review. Processes 9 (11): 2058. DOI: 10.3390/pr9112058.

Maes S, Odlare M, Jonsson A. 2022. Fecal indicator organisms in northern oligotrophic rivers: An explorative study on *Escherichia coli* prevalence in a mountain region with intense tourism and reindeer herding. Environ Monit Assess 194 (4): 264. DOI: 10.1007/s10661-022-09865-1.

Michaelis C, Grohmann E. 2023. Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics 12 (2): 328. DOI: 10.3390/antibiotics12020328.

Moretto VT, Cordeiro SM, Bartley PS, Silva LK, Ponce-Terashima R, Reis MG, Blanton RE, Barbosa LM. 2021. Antimicrobial-resistant enterobacteria in surface waters with fecal contamination from urban and rural communities. Revista da Sociedade Brasileira de Medicina Tropical 54: 1-6. DOI: 10.1590/0037-8682-0724-2020.

Nayebare JG, Owor MM, Kulabako R, Taylor RG. 2022. Fecal contamination pathways of shallow groundwater in low-income urban areas: mplications for water resource planning and management. Water Pract Technol 17: 285-296. DOI: 10.2166/wpt.2021.110.

Novita E, Pradana HA, Purnomo BH, Puspitasari AI. 2020. River water quality assessment in East Java, Indonesia. J Water Land Dev 47: 135-141. DOI: 10.24425/jwld.2020.135040.

Onwudiegwu C, Chibueze IS. 2025. Integrated one health strategies for sustainable potable water systems and ecosystem protection. Greener J Environ Manag Public Saf 13: 1-18. DOI: 10.15580/gjemps.2025.1.020425018.

Petersen F, Hubbart JA. 2020. Physical factors impacting the survival and occurrence of *Escherichia coli* in secondary habitats. Water 12: 1796. DOI: 10.3390/w12061796.

Race AS, Spoelstra J, Parker BL. 2024. Wastewater contaminants in a fractured bedrock aquifer and their potential use as enteric virus indicators. Appl Environ Microbiol 90 (2): e0121323. DOI: 10.1128/aem.01213-23.

Rose JB, Hofstra N, Hollmann E, Katsivelis P, Medema GJ, Murphy HM, Naughton CC, Verbyla ME. 2023. Global microbial water quality data and predictive analytics: Key to health and meeting SDG 6. PLOS Water 2: e0000166. DOI: 10.1371/journal.pwat.0000166.

Saldaña Almazán M, Leyva Zuñiga AP, Moreno Mendoza E, Calderón Arellanes MP, Suastegui Cruz S. 2025. Community management and sustainability of water supply in a rural area of Guerrero, Mexico. Sustainability 17: 4633. DOI: 10.3390/su17104633.

Saputra IWAGM, Yanti NKS, Lesmana CBJ, Nasrum MM, Weta IW, Suardana IW, Sawitri AAS, Hendrayana MA, Wirawan IMA, Budayanti NNS. 2025. The antibiotic-wise village: A multisectoral one health approach for local AMR mitigation. Universal J Public Health 13 (5): 1121-1134. DOI: 10.13189/ujph.2025.130504.

Some S, Mondal R, Mitra D, Jain D, Verma D, Das S. 2021. Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. Energy Nexus 1: 100008. DOI: 10.1016/j.nexus.2021.100008.

Tintaya PAA, Martínez EMV, Vicent BM, Garcia JL, Torregrosa-López JI, Lo-Iacono-ferreira VG. 2022. On the road to sustainable water supply: Reducing public health risks and preserving surface water resources in the milluni micro-basin, Bolivia. Environments 9 (1): 4. DOI: 10.3390/environments9010004.

Toma C, Lu Y, Higa N, Nakasone N, Chinen I, Baschkier A, Rivas M, Iwanaga M. 2003. Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. J Clin Microbiol 41: 2669-2671. DOI: 10.1128/JCM.41.6.2669-2671.2003.

Wen X, Chen F, Lin Y, Zhu H, Yuan F, Kuang D, Jia Z, Yuan Z. 2020. Microbial indicators and their use for monitoring drinking water quality-A review. Sustainability 12: 2249. DOI: 10.3390/su12062249.

Widianingtias M, Kazama S, Benyapa S, Takizawa S. 2023. Assessment of water reclamation and reuse potential in Bali Province, Indonesia. Water 15 (14): 2642. DOI: 10.3390/w15142642.

Wu J, Cao Y, Islam MS, Emch M. 2025. Application of machine learning to identify influential factors for fecal contamination of shallow groundwater. Water 17 (2): 160. DOI: 10.3390/w17020160.