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Water quality assessment and characterization of pathogenic *Escherichia coli* in the Bangkahulu River, Bengkulu, Indonesia

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Abstract

The Bangkahulu River is one of the main rivers that is very important in Bengkulu Province, which is currently facing significant human-induced pressures. This study aims to evaluate the water quality of the Bangkahulu River, as well as to isolate, characterize, and profile the hemolytic Escherichia coli isolated from the upstream to downstream areas. Water samples were taken from five stations using a purposive sampling method. Physical, chemical, and microbiological parameters were analyzed according to the Indonesian national standards (SNI), while E. coli was isolated and identified through morphological characterization, Gram staining, and biochemical tests. Hemolytic activity of the isolates was tested on blood agar. The results showed that Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and Total Dissolved Solids (TDS) exceeded the Class I water quality standards as regulated by Government Regulation Number 22 of 2021. The Pollution Index values range from 2.15 to 6.32, indicating a light to moderate level of contamination. Out of 35 identified E. coli isolates, 18 showed ß-hemolytic activity, 12 showed γ-hemolytic activity, and 2 had hemolytic activity. The prevalence of ß-hemolytic isolates suggests the presence of potentially active phenotypic strains in the aquatic environment. The dominance of β-hemolytic isolates points to strains with phenotypic traits related to potential virulence, although hemolytic activity alone is not enough to determine pathogenicity. This finding provides basic information about the physical and chemical pollution levels of the Bangkahulu River and serves as a foundation for further knowledge and research on the importance of continuously monitoring water quality and implementing effective pollution control strategies to reduce potential public health risks from microbial contamination and physical and chemical pollution, ensuring the sustainable use of river water resources.

Introduction

Escherichia coli is a Gram-negative bacterium commonly found in the gastrointestinal tract of humans and animals. Although most *E. coli* strains are non-pathogenic and even beneficial to gut health, several pathogenic strains such as *E. coli* O157:H7, ETEC (Enterotoxigenic *E. coli*), and EPEC (Enteropathogenic

E. coli) can cause serious illnesses, including diarrhea, gastroenteritis, and urinary tract infections (Ghernaout *et al.*, 2022). On a global scale, the World Health Organization (2022) reported that pathogenic *E. coli* infections cause millions of illness cases and contribute to approximately 40% of AMR-related deaths. Antimicrobial Resistance (AMR) has become a global health crisis, claiming more than 1.27 million lives annually, and is projected to continually rise, causing 10 million deaths per year by 2050. In Indonesia, cases of gastrointestinal infections due to water contamination are frequently reported, with *E. coli* serving as one of the primary pathogens.

Water contamination by pathogenic *E. coli* frequently occurs through domestic, agricultural, and industrial wastewater, which increases the risk of disease transmission through the food chain and drinking water (Horecha and Mekonen, 2025). The pollution of pathogenic *E. coli* in aquatic environments carries serious consequences for both human health and ecosystem sustainability (Tarigan *et al.*, 2019). This contamination degrades water quality, directly impacting the health of communities that rely on river water for consumption, bathing, and daily activities, as these bacteria can cause severe conditions such as gastrointestinal illnesses, diarrhea, and other infections. The prevalence of *E. coli* in Indonesian aquatic environments reaches 33.33%–100%, exhibiting high resistance rates to the beta lactam group (Amoxicillin 66.7%–100%, Ampicillin 100%, and Penicillin 100%) and Aminoglycosides (Streptomycin 73.3%–86.7%, Tobramycin 100%, Gentamicin 100%, and Kanamycin 33.3%) (Mauwalan, 2022).

One of the polluted rivers in Indonesia is the Bangkahulu River, located in Bengkulu Province. This river is a vital watercourse flowing through rural, urban, and agricultural areas, making it highly vulnerable to contamination from domestic, agricultural, and industrial waste. Data from the Badan Standardisasi Nasional (2023) indicates that the Bangkahulu River water frequently exceeds water quality standards due to high levels of *E. coli* or coliform bacteria. Furthermore, a previous study conducted by Gazali and Widada (2021) analyzed *E. coli* pollution across five sampling points along the river. All locations exceeded the quality standards established by Government Regulation No. 82 of 2001, with *E. coli* levels ranging from 265 MPN to >979 MPN against the standard threshold of 100 MPN. This indicates that the Bangkahulu River is polluted from upstream to downstream and cannot be used as a raw water source for clean water treatment (such as municipal waterworks/PDAM), agriculture, or other activities. Gazali and Widada (2021) noted that this high, widespread pollution occurs because the downstream areas are used by residents for bathing, washing clothes, and defecation, alongside effluents from ponds. Additionally, rubber, palm oil, and coal mining industries operating at several locations are likely discharging their waste into the river. According to data from the Badan Standardisasi Nasional (2024) diarrhea remains the highest incidence of disease. In 2024, the diarrhea case rate across all age groups reached 73.0% of the 3,737 target, while diarrhea cases in toddlers reached 133.4% of the 686 target. Ensuring both microbiological and physical water quality is crucial, given that residents continue to rely on the Bangkahulu River for daily needs. Moreover, the river plays a critical role in supplying clean water for Bengkulu City residents, as PDAM Bengkulu utilizes the Bangkahulu River as its raw water source for clean water provision (Gazali and Widada, 2021).

Currently, there have been no reports regarding the prevalence of pathogenic bacteria originating from the Bangkahulu River in Bengkulu. This study aims to evaluate the water quality of the Bangkahulu River as well as to isolate and characterize, and profile the hemolytic Escherichia coli isolated from the upstream to the downstream parts. The current lack of data hinders the assessment of public health risks associated with microbiological water pollution. The findings of this study are expected to provide scientific insights into potential microbiological hazards in aquatic environments, particularly those resulting from pathogenic *E. coli* contamination, given that many rivers in Bengkulu are still heavily utilized by local communities for daily needs. Furthermore, this research aims to support sustainable river water quality management in Bengkulu while educating and raising awareness among the public regarding pathogenic *E. coli* contamination, thereby encouraging residents to be more cautious when using the Bangkahulu River water.

Materials and methods

This research was conducted from Februari to June 2026 at the Microbiology Laboratory, Biotechnology Laboratory, and Basic Biology Laboratory, Basic Science Building, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu.

1. Water Sampling and River Pollution Index Assessment

Samples were collected along the Bangkahulu River, covering Bengkulu Tengah Regency and Bengkulu City, at five locations from upstream to downstream: Station 1 (Rindu Hati Village), Station 2 (Lubuk Sini Village), Station 3 (Kembang Seri Village), Station 4 (Surabaya Sub-district), and Station 5 (Rawamakmur Sub-district), as illustrated in Figure 1. Surface water sampling was conducted across a total of 15 sampling points using a purposive sampling method, where samples were retrieved from the left bank, center, and right bank of the river section to ensure a representative sampling location. The samples were collected using sterile bottles at a depth of 20–30 cm below the water surface, in accordance with SNI 6989.57:2008, and were subsequently placed in a cooler box at 4 °C prior to laboratory delivery. The water quality of the samples was evaluated based on physical, chemical, and biological parameters. Measurements of pH, temperature, and dissolved oxygen (DO) were performed directly in the field (*in situ*). The analyzed chemical variables included pH levels, biochemical oxygen demand (BOD) in mg/L (SNI 6989.72:2009), chemical oxygen demand (COD) in mg/L (SNI 6989.2:2019), total suspended solids (TSS) in mg/L (SNI 6989.3:2019), and total dissolved solids (TDS) in mg/L (SNI 6989.27:2019). The analysis results of the water quality status were compared against the Class 1 water quality criteria pursuant to Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management. Furthermore, for the biological parameter, total microbial testing was conducted using the Total Plate Count (TPC) method. The coordinate points and sampling maps can be seen in Figure 1 and Table 1.

Table 1. Coordinates of sampling locations

Location	Coordinates		Sampling Representation
	Lat	Long	
RHR	-3.711469°	102.527996 °	Located in an upstream area with low anthropogenic activity and serving as an indicator of uncontaminated water bodies
LSR	-3.714445°	102.478447°	Located in the upper-middle transition zone, it is beginning to be affected by waste from agricultural activities, domestic waste and low-density rural settlements.
KSR	-3.790552°	102.363182°	Located in the middle of the river that runs through a residential area, it is the confluence of several small rivers and is situated close to industrial sites, mining operations, livestock farms, hospitals and the community's domestic activities
SBR	-3.779612°	102.298383°	Located in a densely populated residential area and close to one of the water supply points operated by the Bengkulu Municipal Water Supply Authority (PDAM)
RMR	-3772332°	102.263525°	Located in the lower reaches or estuary of a river, which serves as the final point of accumulation for all pollutants from the upper and middle reaches, and in close proximity to densely populated urban areas

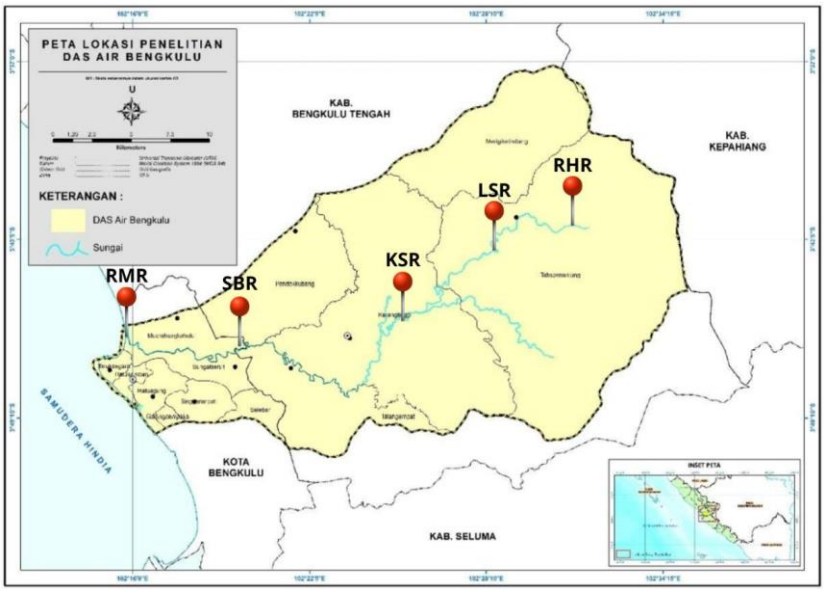


Figure 1. Sampling locations along the Bangkahulu River (Izzatuddinillah *et al.*, 2023)

2. Isolation and Purification of *Escherichia coli*

E. coli was isolated using the serial dilution and plate spread methods. River water samples were first vortexed using a vortex mixer (Thermo Scientific). Serial dilutions were then carried out at concentrations ranging from 10^{-1} to 10^{-5}. A total of 1 ml of filtered tepache drink was added to a test tube containing 9 ml of sterile distilled water. The mixture was then vortexed until homogeneous, producing a dilution solution with a concentration of 10^{-1}. This process was repeated up to a concentration of 10^{-5}. From each dilution, 100 µl was pipetted and poured into a Petri dish (90 × 15 mm) containing Eosin Methylene Blue Agar (EMBA) medium (Himedia) and spread using a spreader. The dishes were then left at room temperature for 15 minutes to allow the inoculum to be absorbed by the EMBA medium. They were subsequently incubated at 37 °C for 24 hours with the dishes inverted. Afterwards, the number of colonies that had grown was counted using a colony counter (B-one). The colonies counted were those characterised by a metallic green colour and a diameter of 0.5–3.0 mm. Purification was then carried out using the quadrant streak plate method (Cappucino and Sherman, 2021).

3. Macroscopic and Microscopic Identification of E. coli Isolates

E. coli isolates were identified by observing the morphology of the bacterial colonies, Gram staining and biochemical testing. The purified E. coli isolates were then identified macroscopically by observing colony morphology, including colony shape, colony surface, colony edges, colony colour and colony elevation. Gram staining was then carried out in accordance with the procedure described (Cappuccino and Welsh, 2021). A loopful of the purified test bacterial isolate, which had been incubated for 24 hours, was placed on a microscope slide onto which a single drop of distilled water had already been applied. The preparation was fixed by passing the slide over a spirit lamp flame until all the water on the slide had evaporated. Next, a drop of crystal violet solution was applied and left for 1 minute, then rinsed with distilled water. Subsequently, a drop of Lugol's solution was applied to the slide and left for 2 minutes, followed by a 10-second rinse with distilled water; this was followed by a 1-minute application of 96% alcohol and a 10-second rinse with distilled water. After that, safranin solution is added to the slide for 30 seconds and the slide is rinsed with distilled water. Next, immersion oil is added to the slide and it is observed under a microscope at 10×100 magnification. The shape and arrangement of the bacteria are then observed, and they are classified as Gram-positive or Gram-negative.

4. Biochemical Assay of E. coli

Biochemical tests were carried out to determine the physiological properties of the E. coli bacterial isolates by conducting various tests, namely the citrate test, the urea test, the catalase test, the carbohydrate fermentation test, and the motility test, in accordance with the procedures described by

Cappuccino and Welsh (2021). The citrate test was carried out using Simon Citrate Agar (SCA) (Himedia), and the urea test was carried out using Urea Base Agar (Himedia), prepared as slants in test tubes; these tests were performed by inoculating a single loopful of the test bacterial isolate onto the medium using a continuous streak. The motility test was carried out using semi-solid Nutrient Agar (NA) (Himedia) (0.7% w/v); the test bacterial isolates were inoculated by being streaked into the medium. Sugar fermentation tests were carried out using glucose (I), sucrose (II) and lactose (III) media. The media were prepared in test tubes that had previously been fitted with Durham tubes, and a single loop of the test bacterial isolate was then inoculated into the media. All tests were incubated at 37 °C for 24 hours. Changes in the test media were then observed. The catalase test was carried out by applying a drop of hydrogen peroxide reagent (H_2O_2 3% v/v) onto the bacterial isolates. A positive result was indicated by the formation of gas bubbles on the test bacteria.

5. Hemolytic Activity Assay

The haemolytic activity test was carried out using Blood Agar (BA) medium, prepared by mixing Blood Agar Base (Himedia) with sterile goat's blood to a concentration of 7% (v/v) at a temperature of 45–50 °C under aseptic conditions. Test isolates were inoculated using the spot inoculation method onto the agar surface, then incubated at 37 °C for 24 hours. The haemolysis patterns formed around the bacterial colonies were observed and categorised into three types: α-haemolysis (green zone/partial haemolysis), β-haemolysis (completely clear zone), and γ-haemolysis (no zone/non-haemolytic) (Rahmi *et al.*, 2015).

6. Data Analysis

Data analysis was conducted using a mixed descriptive approach, with findings presented in tables and percentages. The evaluation of the river water quality status was based on the Pollution Index (PI) as stipulated by the Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management. The identification of the isolated *E. coli* bacteria was performed with reference to *Bergey's Manual of Determinative Bacteriology* (9th edition).

Results and discussion

1. Rivers Pollution Index

The results of the water quality analysis of the Bangkahulu River for physical and chemical parameters were compared with the Class I water quality standards for rivers, as stipulated in Government Regulation of the Republic of Indonesia No. 22 of 2021. These parameters include temperature (Dev 3), pH (6–9), TSS (≤40 mg/L), TDS (≤1000 mg/L), BOD (≤2 mg/L), COD (≤10 mg/L), and DO (>6). This comparison was carried out to assess the extent to which water quality complies with the applicable standards. These parameters are key indicators for assessing the condition of water bodies and the level of river pollution. The results of the measurements of the physical and chemical factors of the Bangkahulu River are shown in (Table 2).

Table 2. Physical and Chemical Water Quality Parameters of the Bangkahulu River

Kode Sampel	Parameter Kualitas Air							
	DO (mg/L)	TDS (mg/L)	TSS (mg/L)	COD (mg/L)	BOD (mg/L)	Suhu (°C)	Kekeruhan (NTU)	pH
RHR	15,70	516	333,33	24	0.45	Dev3	1,80	7,61
LSR	20,77	400	266,67	54,67	1.05	Dev3	3,89	7,77
KSR	19,17	2700	133,33	57,33	0.85	Dev3	32,30	6,91
SBR	17,47	5300	366,67	70,67	5.25	Dev3	34,23	6,57
RMR	11,87	5767	433,33	153,33	12,39	Dev3	41,47	6,51

Note: Rinduhati river (RHR), Lubuksini river (LSR), Kembangseri river (KSR), Surabaya river (SBR), Rawamakmur river (RMR), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Dissolved Oxygen (DO)

Measurements of physical and chemical quality indicate that several water quality parameters still meet the quality standards, namely temperature, pH and dissolved oxygen (DO) at all sampling points; total dissolved solids (TDS) at the upstream points (RHR and LSR); biochemical oxygen demand (BOD) at the upstream to mid-river points (RHR, LSR, KSR); and turbidity at the upstream points only. Meanwhile, the TSS and COD parameters showed values exceeding the quality standards at all sampling points.

The temperature parameter values at all sampling points (RHR, LSR, KSR, SBR and RMR) fell within a deviation range of 3 (Dev 3), and thus still met the quality standards; the pH values at the five sampling points ranged from 6.51 to 7.77, all of which remained within the quality standard range (6–9). According to Quellet *et al.* (2020), temperature is a key factor controlling physical, chemical and biological processes in aquatic environments, whilst a pH within the neutral range plays a role in maintaining nutrient availability and suppressing the increased toxicity of various compounds in the water (Pinheiro *et al.*, 2021). These conditions indicate that the water of the Bangkahulu River is generally still neutral and shows no signs of pollution in terms of temperature and pH.

Turbidity parameter values at all observation points ranged from 1.80 to 41.47 NTU and increased from the upstream to the downstream sections of the river. Furthermore, the Total Suspended Solids (TSS) values at all observation points ranged from 133.33 to 433.33 mg/L, which exceeded the established quality standard of 40 mg/L. According to Islam *et al.* (2025), high TSS values indicate a large number of suspended particles originating from soil erosion, sedimentation, domestic waste and human activities along the river catchment area. These conditions can increase water turbidity, reduce light penetration and disrupt the photosynthetic processes of aquatic organisms.

The TDS parameter values differed at each location. The TDS values at the RHR and LSR sampling points still met the quality standard (<1,000 mg/L), at 516 mg/L and 400 mg/L respectively. Meanwhile, at the KSR, SBR and RMR points, TDS values have exceeded the quality standard, ranging from 2,700 to 5,767 mg/L. According to Jones *et al.* (2019), high TSS concentrations can disrupt the habitats of aquatic organisms and reduce the ecological quality of rivers. Furthermore, an increase in TSS can also lead to increased water turbidity, reduce the penetration of sunlight into the water column, and inhibit the photosynthetic processes of aquatic organisms (Islam *et al.*, 2025; Adjovu et al., 2023). Consequently, the rise in TDS values indicates an increase in pollution or the influx of dissolved substances from the middle to the lower reaches of the river; based on observations, these points are located near residential areas and sites of mining and domestic waste discharge, and many are situated downstream of small streams that flow into the main river.

The Biochemical Oxygen Demand (BOD) parameter values ranged from 0.45 to 12.39 mg/L, however based on the Class I quality standard (≤2 mg/L), therefore, only the RHR, LSR and KSR sampling points still met the standard, whilst the points exceeding the quality standard were SBR at 5.25 mg/L and RMR at 12.39 mg/L. Meanwhile, the Dissolved Oxygen (DO) values at all sampling points met the Class I quality standard (>6 mg/L), ranging from 11.87 to 20.77 mg/L. According to Vigiak *et al.* (2019), high BOD values indicate the accumulation of large quantities of organic matter from various sources, such as domestic waste from communities living along the riverbanks, industrial activities, and livestock farms that discharge their waste into water bodies. Furthermore, high BOD values indicate a high demand for oxygen by microbes to break down organic matter, which can consequently reduce dissolved oxygen levels (Sunarti *et al.*, 2022). Based on the DO values from the upstream to the downstream sections of the river, a decrease is observed, which correlates with the increase in BOD and COD values from the upstream to the downstream sections of the river.

The Chemical Oxygen Demand (COD) parameter values at all observation sites showed levels exceeding Class I quality standards (10 mg/L). COD values ranged from 24.00–153.33 mg/L. According to Sunarti *et al.* (2022), high COD values indicate a large amount of organic and inorganic pollutants that require oxygen in the chemical oxidation process. The increasing COD values from upstream to downstream show the growing pollutant load along the river flow. According to Ha *et al.* (2024), high BOD and COD levels are indicators of the accumulation of organic matter and pollutant loads that can damage water quality.

Based on the results of the Pollution Index (IP) calculation developed according to the Minister of Environment Decree No. 115 of 2003, with adjustments to the quality standards under Government Regulation No. 22 of 2021, it is known that the water quality status of the Bangkahulu River from upstream to downstream ranges from lightly polluted to moderately polluted. This indicates that the water quality of the Bangkahulu River cannot be used according to the class I water use designation under Government Regulation No. 22 of 2021. The pollution index (IP) values and the water quality status of the river can be seen in (Table 3).

Table 3. Pollution Index (PI) Values and Water Quality Status

Kode Sampel	Indeks Pencemaran (IP)		
	Nilai PI_{ij}	Status Mutu Air	Indikator Pencemar
RHR	2.15	Tercemar ringan	TSS, COD
LSR	2.78	Tercemar ringan	TSS, COD, Kekeruhan
KSR	4.82	Tercemar ringan	TDS, TSS, COD, Kekeruhan
SBR	5,64	Tercemar sedang	TDS, TSS, BOD, COD, Kekeruhan
RMR	6.32	Tercemar sedang	TDS, TSS, BOD, COD, Kekeruhan

The calculation results of the Pollution Index (IP) show that the water quality status at five sampling points in the Bangkahulu River varies, ranging from lightly to moderately polluted (Table 3). The IP values at the RHR, LSR, and KSR points are 2.15, 2.78, and 4.82, respectively, which fall into the lightly polluted category, while the SBR and RMR points have IP values of 5.64 and 6.32, which are classified as moderately polluted. According to the Minister of Environment Decree Number 115 of 2003, IP values between 1–5 indicate that the water has experienced light pollution, while IP values between 5–10 indicate moderate pollution. The results of this study are in line with previous research conducted by Gazali and Widada (2021), which stated that the water quality status of the Bangkahulu River from upstream to downstream has a moderate pollution index with values ranging from 5.22 to 6.51 and cannot be used as raw water for PDAM Bengkulu City's drinking water treatment. The dissolved residue (TSS) and pH parameters are below the standard quality, while BOD, COD, and E. coli content are above the class I river standards.

The Pollution Index (IP) values show an increase from the upstream to the downstream of the Bangkahulu River. This aligns with research conducted by Novianti *et al.* (2022), on the Cidurian River, which reported that pollution index values tend to rise in river segments that receive more pollutant loads from domestic activities and land use in downstream areas. The study shows that the more sources of pollution entering the river, the higher the IP value, leading to a decline in water quality.

2. Isolation and Characterization of E. coli from the Bangkahulu River

Based on the *E. coli* isolation results from the Bangkahulu River, the RHR and LSR stations showed no evidence of *E. coli* contamination, whereas the KSR, SBR, and RMR stations exhibited high levels of *E. coli*. Subsequent colony purification was conducted randomly on colonies displaying a metallic green sheen on EMBA medium (presumed to be *E. coli*), yielding a total of 35 distinct isolates for further characterization. Purification was also performed on Tryptic Soy Agar (TSA) medium for the rejuvenation of the isolates for subsequent use (Figure 2). On the EMBA medium (Figure 2A), the colonies appeared dark with a distinctive green metallic sheen. According to Reynolds (2021), this characteristic is a hallmark of vigorous lactose-fermenting coliform bacteria, particularly *E. coli*. The metallic green sheen is formed due to rapid lactose fermentation, which produces high amounts of acid, leading to the precipitation of the eosin and methylene blue dye complex onto the colony surface. Therefore, EMBA medium is widely

utilized as a selective and differential medium for the initial isolation and identification of *E. coli*. Morphological observations of the 35 bacterial isolates revealed that all colonies were circular, smooth, with entire margins, convex elevations, and a dark purple-black coloration that reflects the metallic green appearance. Meanwhile, on the TSA medium, the isolates exhibited identical morphological characteristics, differing only in colony color, which appeared creamy white.

Microscopic observations of the cell shape and arrangement of the *E. coli* isolates revealed short, rod-shaped bacterial cells with varying arrangements, specifically monobacillus and diplobacillus. Under Gram staining, the cells appeared red, which is characteristic of the Gram-negative bacterial group (Figure 2). According to Mueller *et al.* (2025), *Escherichia coli* is a Gram-negative, rod-shaped (bacillus) bacterium belonging to the Enterobacteriaceae family. Based on the morphological characteristics, cellular arrangement, and Gram reaction across all isolates, the results indicate that all identified isolates share consistent characteristics with the *E. coli* bacterial group.

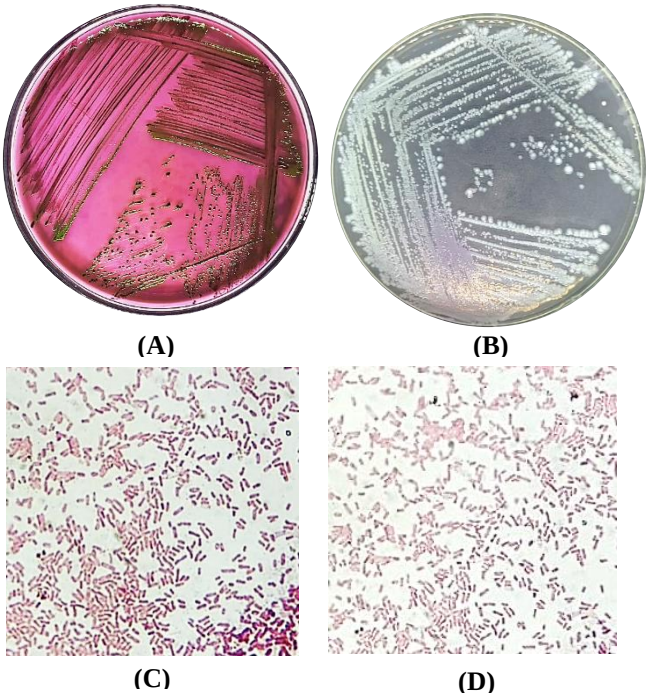


Figure 2. Purification of Escherichia coli isolates on Eosin Methylene Blue Agar (EMBA) (A) and Tryptic Soy Agar (TSA) (B). Gram staining and cellular morphology of representative E. coli isolates (C) and (D).

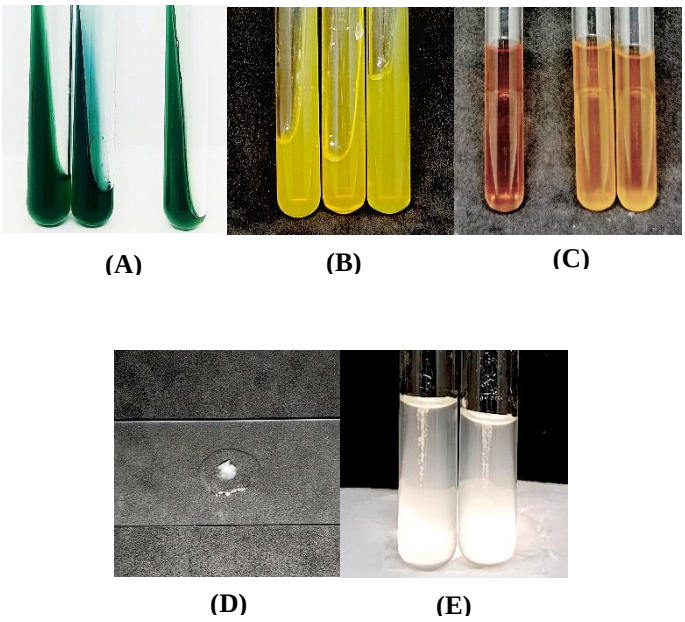
Biochemical tests on 35 isolates that had metallic green colony traits (suspected to be E. coli group) confirmed that all 35 isolates showed the same characteristics as E. coli (Table 3) and (Figure 3). Based on the biochemical test results, generally the bacterial isolates from the Bangkahulu River had characteristics such as negative citrate and urea tests, positive motility, positive catalase, and positive carbohydrate fermentation tests. According to Cappuccino and Welsh (2021), Escherichia coli has typical IMViC traits, which are positive indole, positive methyl red, negative Voges-Proskauer, and negative citrate. Additionally, according to Leimbach *et al.* (2013), E. coli is a facultative anaerobic bacterium that has a broad carbohydrate metabolism and quickly ferments lactose.

Table 3. Biochemical Test Characterization of E. coli Isolates

Kode Sampel		Sitrat		Urea		Motilitas		Katalase		Fermentasi Karbohidrat					
										Glukosa		Sukrosa		Laktosa	
		-	+	-	+	-	+	+	-	+	-	+	-	+	-
SBR	Kiri	10	0	10	0	10	0	7	3	10	0	10	0	10	0
	Tengah	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	Kanan	7	0	7	0	7	0	6	1	7	0	7	0	7	0
RMR	Kiri	4	0	4	0	4	0	4	4	4	0	4	0	4	0
	Tengah	6	0	6	0	6	0	3	3	6	0	6	0	6	0

Kanan	3	0	3	0	3	0	3	3	3	0	3	0	3	0		

Note: Rinduhati river (RHR), Lubuksini river (LSR), Kembangseri river (KSR), Surabaya river (SBR), Rawamakmur river (RMR), + (positive), - (negative)



Gambar 3. Figure 3. Biochemical identification of *E. coli* isolates: (A) Citrate utilization test; (B) Urease test; (C) Carbohydrate fermentation test; (D) Catalase test; (E) Motility test.

3. Hemolytic Activity

Based on the results of the hemolysis test on Blood Agar media, a total of 35 E. coli isolates showed hemolytic activity, consisting of α-hemolytic, β-hemolytic, and γ-hemolytic types. Beta-hemolytic activity is marked by complete red blood cell breakdown (seen as a very clear and wide transparent zone around the bacterial colony), while alpha-hemolytic is marked by the bacteria's ability to partially break down red blood cells on the blood agar (seen as a greenish zone due to partial lysis). The observations from the blood agar hemolytic activity test can be seen in Table 4 and Figure 3.

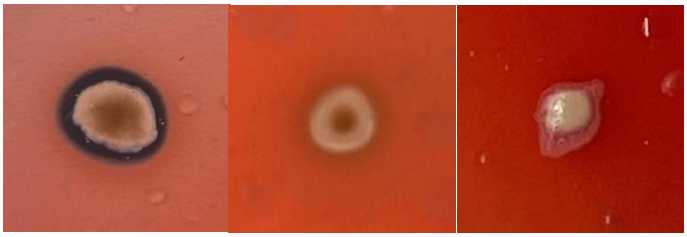
The observation results show that the E. coli isolates taken from the Bangkahulu River exhibit characteristics of pathogenic bacteria. Based on the observations, the most dominant type of hemolysis was β-hemolytic, with 18 isolates, γ-hemolytic with 15 isolates, and α-hemolytic with only 2 isolates. β-hemolytic activity was observed in isolates ERS4, ERS5, ERS6, ECS2, ECS3, ECS4, ECS5, ELS1, ELS2, ELS3, ELS4, ELS7, ERR2, ECR4, ECR5, ECR6, and ELR3, marked by the formation of a clear zone around the colonies. According to Mainil (2013), this clear zone indicates complete lysis of red blood cells due to hemolysin activity secreted by the bacteria. Hemolysin is a toxic protein that can form pores in the host cell membrane, causing cell damage and releasing nutrients that the bacteria can use to support their growth. According to Sora *et al.* (2021), the ability to produce β-hemolysis is related to the production of α-hemolysin (HlyA), a toxin that belongs to the Repeat-in-Toxin (RTX) group. Besides breaking down red blood cells, α-hemolysin can also damage epithelial cells, white blood cells, and endothelial cells, causing tissue damage and helping the bacteria evade the host's immune response.

Based on Table 4, α-hemolysis was only found in two isolates, namely ELS8 and ELR1. α-hemolysis activity is marked by the formation of a greenish or brownish zone around the colony. According to Jang *et al.* (2017), the formation of a greenish or brownish zone around the colony is due to partial lysis of red blood cells. This color change happens because the hemoglobin released from the red blood cells is oxidized into methemoglobin. Meanwhile, 15 isolates showed γ-hemolysis, namely ERS1, ERS2, ERS3, ERS7, ECS1, ELS5, ELS6, ELS9, ELS10, ERR1, ERR3, ECR1, ECR2, ECR3, and ELR2. γ-hemolysis is marked by the absence of any color change or clear zone around the colony.

Table 4. Hemolysis test *E. coli*

Sumber Isolat	Kode Isolat *E. coli* yang diuji Aktivitas Hemolitik		
	α-hemolitik	ß-hemolitik	γ-hemolisis
ERS	-	ERS4, ERS5, ERS6	ERS1, ERS2, ERS3, ERS7
ECS	-	ECS2, ECS3, ECS4, ECS5	ECS1
ELS	ELS8	ELS1, ELS2, ELS3, ELS4, ELS4, ELS7	ELS5, ELS6, ELS9. ELS10
ERR	-	ERR2	ERR1, ERR3
ECR	-	ECR4, ECR5, ECR6	ECR1, ECR2, ECR3
ELR	ELR1	ELR3	ELR2
TOTAL	2	18	15

Note: ERS= *Escherichia coli* Right Surabaya; ECS= *Escherichia coli* Central Surabaya; ELS= *Escherichia coli* Left Surabaya; ERR= *Escherichia coli* Right Rawamakmur; ECR= *Escherichia coli* Central Rawamakamur; ELR= *Escherichia coli* Left Rawamakmur;



(A) **(B)** **(C)**

Figure 3. Hemolytic activity of E. coli isolates on blood agar. Isolate ERS4: β-hemolytic (A); isolate ELS10: γ-hemolytic (B); isolate ELS8: α-hemolytic (C).

Conclusion

The results of this study show that the water quality of the Bangkahulu River does not meet the Class I water quality standards set by Indonesian Government Regulation No. 22 of 2021 on Environmental Protection and Management. Some water quality parameters, such as Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and Total Dissolved Solids (TDS), exceed the allowed limits. E. coli samples from various points showed β-hemolytic, α-hemolytic, and γ-hemolytic activity. Although hemolytic activity alone is not enough to determine its pathogenic nature, these findings provide basic information about the contamination levels of the Bangkahulu River in terms of physical and chemical properties. They also serve as a foundation for knowledge and further research on the importance of sustainable water quality monitoring and implementing effective pollution control strategies to reduce potential public health risks from microbial contamination and physical and chemical pollution, ensuring the sustainable use of river water resources.

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Data availability: The raw data supporting the conclusion of this article, including serial dilatation, IP calculation will be made available to any qualified researcher.

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