**Research Article**

# Conditions of total coliform in the Aur River, Palembang City, South Sumatra, Indonesia

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## Abstract

Pathogenic microorganisms present in river water pollution are a serious health risk. *Escherichia coli* is a pathogen commonly found in water. *Escherichia coli* is an indicator of water contaminated with feces. These pathogens have an impact on human health. Aur River is one of the tidal rivers in Palembang City. From the observations, many people dispose of domestic waste into river bodies, so the river is at risk of being polluted by pathogenic microorganisms. When it recedes, the water looks pitch black, and when it is high, many people bathe, brush their teeth, and wash their clothes in this river. Thus, this study aimed to monitor water quality, including total coliform, of the Aur River Palembang City. The results of this monitoring can be used as the basis for making a policy and strategy to control the quality of this river water. This study was carried out on the Aur River, which is 2.5 km long. Measurements were made at three stations, namely at the upstream, middle, and downstream. Based on measurements of the pH, BOD, COD, DO, and total coliform concentrations, it is concluded that the Aur River has been polluted because the BOD, COD, DO, and total coliform were above the quality standards. The pH is still at the quality standard.

## Introduction

Humans really need water in their lives. This is because water is needed for household needs, recreation, and crop irrigation. Currently, surface water pollution caused by pathogenic microorganisms is a serious health risk, thus impacting access to clean water (Amirat et al., 2012; Khan, 2020). Water pollution, such as rivers, can be caused by various kinds of residual activities (garbage), ranging from organic and household waste to hazardous and toxic industrial waste. Meanwhile, groundwater is not safe for consumption because it has been contaminated by seepage from septic tanks or surface water (Pou et al., 2022; Raj et al., 2023). Seepage from septic tanks can cause fecal pollution. Fecal pollution damages water quality worldwide (Manini et al., 2022). At least 2 billion people worldwide use drinking water sources contaminated by feces (World Health Organization and UNICEF, 2017). This has an impact on diseases such as diarrhea, typhoid, cholera, and dysentery (Troeger et al., 2018). Water that has been indicated for fecal pollution contains fecal coliform bacteria. *Escherichia coli* is an indicator of water contaminated with feces from humans or animals (Anggara et al., 2020). Therefore, fecal bacteria quantification is

important for both basic and applied research in aquatic microbial ecology and the development of parameter-based technologies for drinking water quality assessment (Some et al., 2021). *Escherichia coli*, better known as *E. coli*, is a bacterium that lives and grows naturally in the digestive tract of humans and animals. It is very small in size; therefore, this bacterium is difficult to control. These bacteria are excreted in feces (Rock et al., 2018).

In Palembang, many houses are located on the banks of rivers. They throw domestic waste directly into the river (Oktriyedi et al., 2022). The Aur River is one of the tidal rivers in Palembang City, and it flows into the Musi River. The results of monitoring the quality of the Aur River in 2017 showed that the pollution status was predominantly moderately polluted with a pollutant index of 5.02-8.61 (Jauhari, 2018). In the Musi River, many industries have the potential to contaminate the river (Oktriyedi et al., 2021). From observations, many people throw domestic waste into river bodies, so the river is at risk of being polluted by pathogens. When it recedes, the water looks pitch black, and when it is high, many people bathe, brush their teeth, and wash clothes in this river. In the upstream part, the population density is high, and poor community behavior patterns exist. Apart from that, people living on the Aur River banks directly channel their waste into the river because they do not have septic tanks. In the middle part of the Aur River, the population density level is almost the same as in the upstream part; it is just that in the middle part,

most of them already have septic tanks. However, the septic tank has not been maintained optimally, so when it rains, the water in the septic tank overflows and flows into the river. The population density level is lower in the downstream part than in the upstream and middle parts. However, downstream, there are many public facilities such as schools, markets, and other buildings whose waste is also directly discharged into this river, especially waste originating from market activities.

This study aimed to identify the water quality, including total coliform, of the Aur River, Palembang City.

## Materials and Methods

### Sampling location

Each station was taken in 5 points. Total coliform analysis was carried out in Sungai Aur, 9-10 Ulu Village, Jakabaring District, Palembang City. Overall, the Aur River that is located in 9-10 Ulu Village has a flow length of 949.18 m, and for the 9-10 Ulu Sub-district area, it reaches 60.27 hectares. This figure was obtained from air capture or Kosmosnimki Satellite imagery in 2022 with a resolution of 2.39 m/pixel at a height of 500 meters. With this area, the 9-10 Ulu Sub-district has a total population of 12,380 people spread across 8 RWs and 34 RTs, with a high population density of 28,791/km$^2$. The condition of the Aur River is shown in Figure 2.



Figure 1. The condition of the Aur River at low tide.

Samples were taken in the Aur River, Palembang City. Samples were taken at three stations, namely station 1 in the upstream section (ordinate point -2.99143, 104.76671), station 2 in the middle (ordinate point - 2.99509, 104.76784, 107.76293), and station 3 in the downstream section (ordinate point -2.99743, 104.76964,107.76707). The sampling points can be seen in Figure 2.
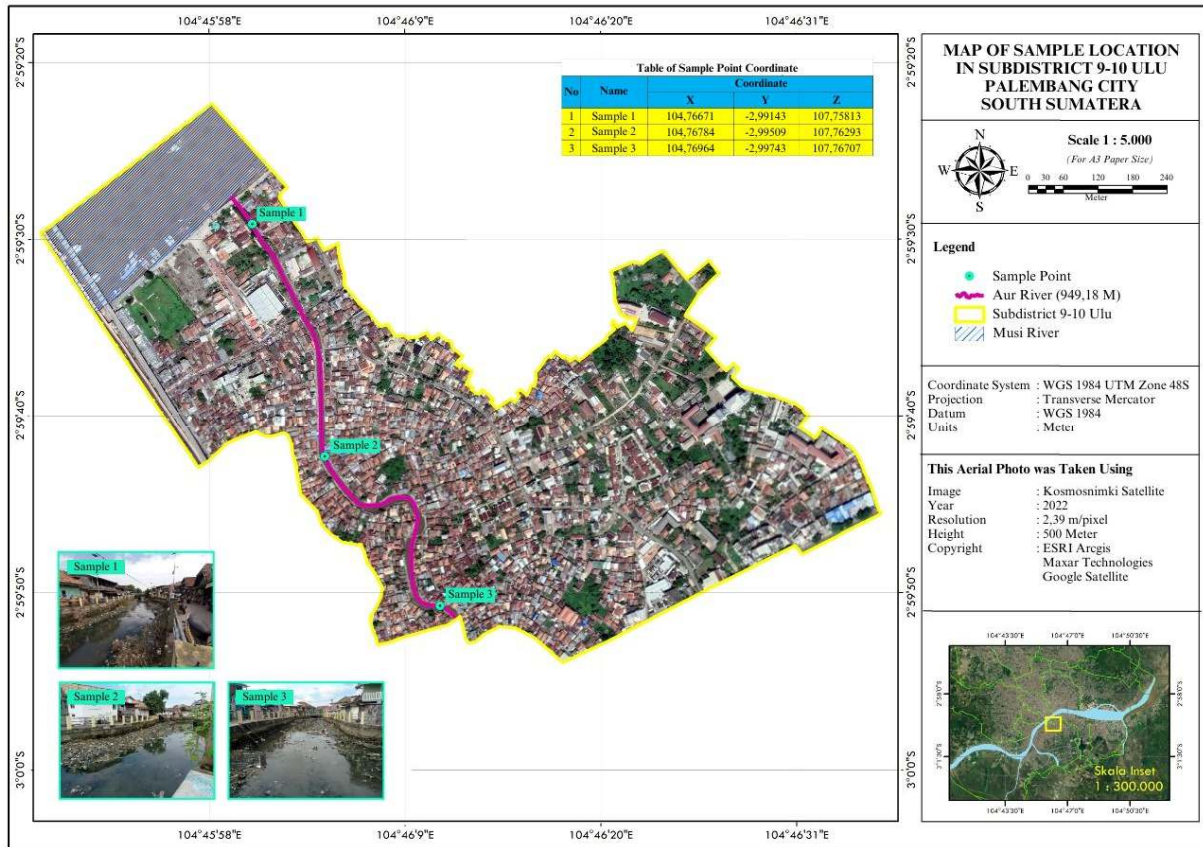


Figure 2. Sampling points.

## Tools and sampling methods

Samples were taken using a polyethylene plastic bucket. The bucket was cleaned using water before use. The water sample was put into the prepared plastic conductor. Sterilized glass containers were used for total coliform samples (National Standardization Agency, 2008).

## Samples test

### pH

The sample was placed in a 1,000 mL volumetric flask. The pH meter electrode was rinsed with mineral-free water and then dried with soft tissue paper. The electrode was then dipped into the test sample until the pH meter showed a stable reading. The scale reading results or numbers on the display of the pH meter were recorded. The electrodes were rinsed again with mineral-free water after measurements (National Standardization Agency, 2019).

### BOD (biological oxygen demand)

Two DO (dissolved oxygen) bottles were prepared, each bottle marked with the notation A1 and A2. The sample solution was put into each DO A1 and DO A2 bottle until it overflowed. Then, each bottle was slowly closed; after that, it was shaken several times, and then mineral-free water was added around the mouth of the closed DO bottle. Bottle A1 was stored in an incubator at $20°C \pm 1°C$ for five days. Dissolved oxygen in the solution in bottle A1 was measured using a DO meter. This was also done on bottles B1 and B2. The BOD5 value of the test sample was calculated using Equation 1 (National Standardization Agency, 2009b).

$$BOD_5 = \frac{(A_1 - A_2) - \left(\frac{B_1 - B_2}{V_B}\right)V_S}{P} \quad \text{................................} (1)$$

where:

| | | |
|---|---|---|
| BOD5 | = | BOD value of 5 test samples (mg/L) |
| A1 | = | dissolved oxygen level of the test sample before incubation (0 days) (mg/L) |
| A2 | = | dissolved oxygen level of the test sample after five days of incubation (mg/L) |
| B1 | = | blank dissolved oxygen level before incubation (0 days) (mg/L) |

B2 = blank dissolved oxygen level after five days of incubation (mg/L)

VB = the volume of microbial suspension (mL) in a blank DO bottle

Vc = volume of microbial suspension in the test sample bottle (mL)

P = ratio of test sample volume (V1) per total volume (V2)

### COD (chemical oxygen demand)

The samples were refluxed and then cooled to room temperature. The suspension was allowed to settle, and the sample measured was a completely clear portion. The absorbance of the test sample was measured at a wavelength of 600 nm. COD levels were calculated based on the linear equation of the calibration curve. The COD value of the test sample was calculated using Equation 2 (National Standardization Agency, 2009a).

COD content (mg $O_2$/L) = C x f ……….…………(2)

where: C = COD value of test sample, expressed in milligrams per liter (mg/L)

### DO (dissolved oxygen)

The prepared samples were taken 1 mL of $MnSO_4$, and 1 mL of alkali iodide azide was added with a pipette tip just above the surface of the solution. The sample was immediately covered and homogenized until a perfect lump was formed. The clots were allowed to settle for 5 minutes. 1 mL of concentrated $H_2SO_4$ was added, covered, and homogenized until the precipitate was completely dissolved. A 50 mL pipette was inserted into a 150 mL Erlenmeyer flask and was then titrated with $Na_2S_2O_3$ with starch/starch indicator until the blue color disappeared. The DO value of the test sample was calculated using Equation 3 (National Standardization Agency, 2004).

$$DO = \frac{V \times N \times 8000 \times F}{50}$$ …………………………….....(3)

where:

V = $Na_2S_2O_3$ (mL)

N = normality of $Na_2S_2O_3$

F = factor (bottle volume divided by bottle volume minus the volume of $MnSO_4$ reagent and alkali iodide azide)

### Total coliform

Samples were stored at 3°C for 24 hours before testing. After 24 hours, the sample was filtered using a membrane filter, and then the membrane filter was placed on the surface of a chromogenic coliform agar plate. The samples were incubated on membrane filters at 37°C for 22 hours. Next, the number of colonies was counted, which showed positive β-D-galactosidase (pink to red), which was suspected to be colonies of coliform bacteria that were not E. coli. The suspected colony was confirmed by showing a negative oxidase reaction. Counting the number of colonies that show positive reactions for β-D-glucuronidase and β-D-galactosidase as indicated by the presence of dark blue to purple colonies, which are E. coli colonies. Total coliform bacteria is the number of all pink to red colonies that are negative for oxidase, and the total number of colonies is dark blue to purple. The total number of colonies was calculated using Equation 4 (National Standardization Agency, 2015).

$$C_s = \frac{Z}{V_{tot}} x V_s$$ ……………………………………..(4)

where:

Cs = the estimated number of colonies in the Vs volume of the sample;

Z = the number of colonies counted on the plate or on the membrane obtained from dilutions d1, d2, ..., di, or obtained from a certain volume of the test portion (sample or dilution);

Vs = the reference volume chosen to express the concentration of microorganisms in the sample;

Vtot = the total volume of the calculated initial sample used in the enumerated cup. Vtot is calculated using the Equation 5

$$V_{tot} = (n_1 V_1 d_1) + (n_2 V_2 d_2) + \cdots + (n_i V_i d_i)$$ ……(5)

## Results and Discussion

### Station 1 in the upstream section

The measurement results of Station 1 in the upstream section presented in Table 1 show that The concentrations of BOD (34-36 mg/L), COD (141-160 mg/L), and DO (0.70-0.83 mg/L) are above the quality standard, while the pH is still at the quality standard, namely 8.51-8.66. The average total coliform concentration of Station 1 (upstream section) was 5.24 x $10^6$ ± 1.1 x $10^5$/100 mL.

The concentration of the measurement results has exceeded the class II water quality standard threshold of 5 x $10^3$/100 mL (Governor of South Sumatra, 2005). River water pollution is caused by the discharge of domestic waste into the river (Andriani and Ariesyady, 2013). Some major health risks are caused by microorganisms such as bacteria, pathogens, etc. because these microorganisms can live, reproduce, and spread in water systems (Khan, 2020). Fecal microorganisms are carried into the aquatic environment primarily through wastewater discharges surface and subsurface runoff. Escherichia coli is an accepted indicator for quantifying fecal contamination because Escherichia coli is present in large quantities in the feces of warm-blooded animals (Amirat et al., 2012).

Bacteriophages were identified from human fecal metagenomes, as they are highly abundant in the human intestine (Karkman et al., 2019). Commonly affected areas include soil, sediment, agricultural land, wastewater treatment plants (WWTP), and many water

bodies such as drinking water sources, recreational water, and groundwater (Li et al., 2021). Fecal pollution can occur from untreated waste. In developing and underdeveloped countries, untreated waste is sometimes discharged directly into surface water bodies (Abd-Elhamid et al., 2021). Polluted water can seep into the ground, causing groundwater

to no longer be safe for drinking and agricultural purposes (Kuroda et al., 2012). The concentration of major ions in untreated wastewater is much higher than in treated wastewater, except for bicarbonate. Untreated wastewater is not suitable for irrigation purposes, while treated wastewater is suitable for irrigation (Rehman et al., 2020).

Table 1. pH, BOD, COD, DO, and total coliform concentration at Station 1 in the upstream section.

| Parameters | Unit | Sampling Point | | | | | Quality Standard |
|---|---|---|---|---|---|---|---|
| | | Point 1 | Point 2 | Point 3 | Point 4 | Point 5 | |
| pH | - | 8.58 | 8.54 | 8.60 | 8.66 | 8.51 | 6-9 |
| BOD | mg/L | **36*** | **34*** | **36*** | **35*** | **34*** | 2 |
| COD | mg/L | **145*** | **141*** | **143*** | **159*** | **160*** | 10 |
| DO | mg/L | **0.75*** | **0.83*** | **0.75*** | **0.74*** | **0.70*** | 6 |
| Total coliform | 100 mL | $5.4 \times 10^6$ | $5.1 \times 10^6$ | $5.2 \times 10^6$ | $5.3 \times 10^6$ | $5.2 \times 10^6$ | $5 \times 10^3/100$ |

In the Upstream part, the population density is high, and poor community behavior patterns exist. Most people said they channeled waste directly into the Aur River from septic tanks. Apart from that, people living on the Aur River banks directly channel their waste into the river because they do not have septic tanks. Many people there also do not have private toilets, so

they carry out toilets in the river (Figure 3). This is thought to be the leading cause of the high total coliform. *Escherichia coli* usually resides in the intestinal flora of warm-blooded animals, including humans. Environmental water sources are vulnerable to bacterial pollution from humans and animals (Cho et al., 2018).
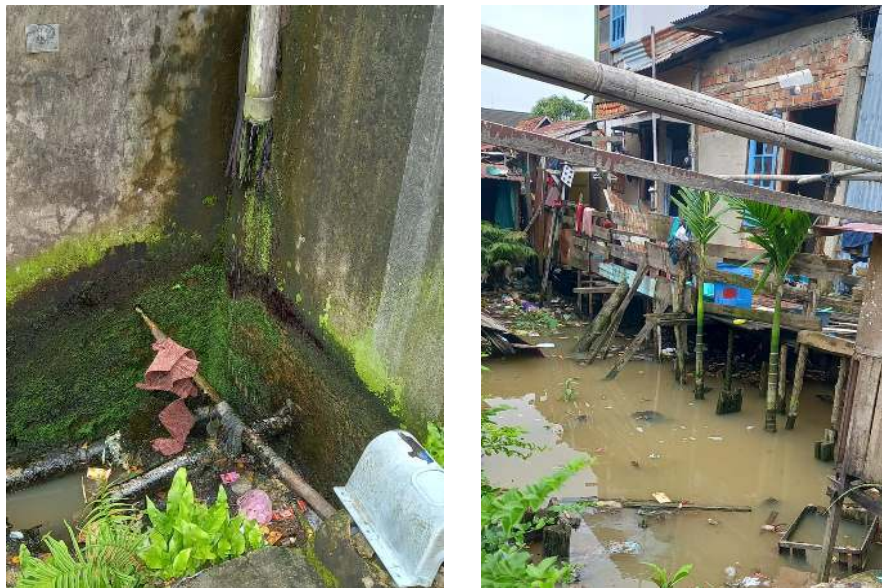


Figure 3. Domestic household waste is disposed of directly into the ditch, which flows into the Station 1 river in the upstream section.

*Escherichia coli* is a bacterium commonly found in water. *Escherichia coli* is an indicator of water contaminated with feces from humans or animals (Anggara et al., 2020). Water pollution, such as rivers, is caused by domestic waste disposal (Andriani and Ariesyady, 2013). *E. coli* in surface water systems is determined by several physical factors: nutrients, sulfates, and nitrates, as well as biological factors. The relationship between these factors and the number of *E. coli* is also influenced by flow rate and turbidity (Dwivedi et al., 2013).

### Station 2 in the middle

The measurement results of Station 2 in the middle section presented in Table 2 show that the concentrations of BOD (17-19 mg/L), COD (158-168 mg/L), and DO (0.78-0.91 mg/L) are above the quality standard, while the pH is still at the quality standard, namely 8.51-8.66 and the total coliform concentration of Station 2 (central section) averages $1.1 \times 10^4 \pm 5.5 \times 10^2/100$ mL. The concentration of the measurement results has exceeded the class II water quality standard threshold of $5 \times 10^3/100$ mL (Pergub Sumsel, 2005).

Table 2. pH, BOD, COD, DO, and total coliform concentration at Station 2 in the middle part of the Aur River.

| Parameters | Unit | Sampling Point | | | | | Quality Standard |
|---|---|---|---|---|---|---|---|
| | | Point 1 | Point 2 | Point 3 | Point 4 | Point 5 | |
| pH | - | 8.60 | 8.66 | 8.86 | 8.79 | 8,53 | 6-9 |
| BOD | mg/L | **18*** | **17*** | **17*** | **19*** | **18*** | 2 |
| COD | mg/L | **167*** | **165*** | **168*** | **160*** | **158*** | 10 |
| DO | mg/L | **0.84*** | **0.89*** | **0.78*** | **0.91*** | **0.82*** | 6 |
| Total coliform | 100 mL | 1.1 x $10^4$ | 1.1 x $10^4$ | 1.2 x $10^4$ | 1.1 x $10^4$ | 1.2 x $10^4$ | 5x$10^3$/100 |

In the middle part of the Aur River, the population density level is almost the same as in the upstream part; in the middle part, most of them already have septic tanks (Figure 4). However, the septic tank has not been maintained optimally, so when it rains, the water in the septic tank overflows and flows into the river. During the rainy season, fecal pollution increases due to overflowing septic tanks (Li et al., 2021). Possible sources are wastewater discharges, sewage leaks, damaged septic tanks, and municipal, residential, medical, and industrial waste facilities (Cho et al., 2018). The highest numbers of *E. coli* were found in urban river streams compared to rural rivers (Paruch et al., 2019).



Figure 4. Domestic household waste is disposed of directly into the ditch, which flows into the middle section of the river Station 2.

### Station 3 in the downstream

The measurement results of Station 3 in the downstream section presented in Table 3 show that the concentrations of BOD (34-37 mg/L), COD (147-163 mg/L) and DO (0.75-0.79 mg/L) are above the quality standard, while the pH is still at the quality standard, namely 8.51-8.66 and the total coliform concentration of Station 3 (downstream section) averages 9.2 x $10^6$ ± 1.1 x $10^5$/100 mL. The concentration of the measurement results has exceeded the class II water quality standard threshold of 5 x 103/100 mL (Pergub Sumsel, 2005). Meanwhile, the population density level is lower in the downstream part than in the upstream and middle parts. However, downstream, there are many public facilities such as schools, markets, and other buildings whose waste is also directly discharged into this river, especially waste originating from market activities (Figure 5). Besides that, many public toilets do not have septic tanks, and the waste is dumped directly into the river. In markets, many traders do not directly throw vegetable waste, fish water, or meat into the river. This is also thought to be the cause of the high total coliform in the river compared to other parts. The presence of coliforms in water bodies clearly indicates pollution by disposing of waste or rotting materials, especially organic waste (Fernández et al., 2022). Market conditions like this provide opportunities for cross-contamination; vegetables are usually sold alongside raw meat and often in unhealthy market environments, and basic good food hygiene practices are often not followed by market traders and visitors (Michaels et al., 2004).

Table 3. pH, BOD, COD, DO, and total coliform concentration at Station 3 downstream.

| Parameters | Unit | Sampling Point | | | | | Quality Standard |
|---|---|---|---|---|---|---|---|
| | | Point 1 | Point 2 | Point 3 | Point 4 | Point 5 | |
| pH | - | 8.98 | 8.9 | 8.92 | 8.89 | 8.94 | 6-9 |
| BOD | mg/L | **37*** | **34*** | **35*** | **36*** | **37*** | 2 |
| COD | mg/L | **151*** | **147*** | **153*** | **163*** | **163*** | 10 |
| DO | mg/L | **0.76*** | **0.75*** | **0.76*** | **0.76*** | **0.79*** | 6 |
| Total coliform | 100 mL | $9.2 \times 10^6$ | $9.1 \times 10^6$ | $9.2 \times 10^6$ | $9.4 \times 10^6$ | $9.3 \times 10^6$ | $5 \times 10^3/100$ |



Figure 5. Domestic waste from the market is discharged directly into the river downstream.

Apart from the total concentration of coliforms, types of coliforms were also identified based on shape, elevation, color, and edges (Table 4). Data presented in Table 4 show that there are four types of coliforms, namely *Klebsiela pneumoniae*, *Proteus mirabilis, Escherichia coli,* and *Enterobacter*. Most are rod-shaped, the elevations are convex, with colors varying from white, red, and pink, and some have flat edges. *Klebsiella pneumoniae* is one of the clinically significant organisms that has caused many public health problems. *Klebsiella pneumoniae* is an important *Enterobacteriaceae* that is considered one of the opportunistic pathogens that causes a wide spectrum of diseases and increasingly shows resistance to antibiotics (Effah et al., 2020). Proteus mirabilis belongs to the class *Gammaproteobacteria* and has long been known as a member of *Enterobacteriales*, family *Enterobacteriaceae* (Armbruster et al., 2018).

Table 4. Identified coliform types based on shape, elevation, color, and edge.

| Type of Coliform | Shape | Elevation | Color | Edge |
|---|---|---|---|---|
| *Klebsiela pneumoniae* | Stem | Convex | Pink | Flat |
| *Proteus mirabilis* | Stem | Convex | White | Flat |
| *Escherichia coli* | Stem | Convex | Red | Real |
| *Enterobacter* | circular | Convex | White | Flat |

*Proteus mirabilis* is a common cause of urinary tract infections (UTIs). UTI caused by *P. mirabilis* requires medical treatment because it can persist and cause secondary complications such as urolithiasis, obstruction, and pyelonephritis (Marques et al., 2022).

*Escherichia coli* is a rod-shaped gram-negative bacterium classified as a member of *the Enterobacteriaceae* family in the *Gammaproteobacteria* class (Jang et al., 2017). Pathogenic *E. coli* strains include those that cause disease in the human gastrointestinal tract, namely diarrheagenic *E. coli* (DEC) and extra-intestinal *E. coli* (ExPEC), which cause infections of the urinary tract (uropathogenic *E. coli*, UPEC), septicemia. Associated E. *coli* (SEPEC) and meningitis-associated *E. coli* (MNEC). Infections that occur in other parts of the body include those that cause blood infections (bacteremia) (Aijuka and Buys, 2019).

*Enterococcus* species are not believed to be harmful to humans and are not medically important. Even though this bacterium does not produce toxins, it has virulence factors in the form of aggregation substances, so it can cause disease based on how it attaches to host tissue. *Enterococcus* species are now considered emerging pathogens based on the production of virulence factors and high antibiotic resistance. The medical importance of enterococci in nosocomial, iatrogenic infections, and bacteremia has increased; they are the cause of urinary tract infections, endocarditis, meningitis, wound infections, and abdominal and pelvic infections (Soares et al., 2023).

The five reported types of DEC are enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC) (Chique et al., 2021). *Escherichia coli* is a rod-shaped, anaerobic, gram-negative bacterium belonging to the Enterobacteriaceae family. The

bacterium is found in human intestines, warm-blooded animals, cold-blooded animals, and different environments (Ramos et al., 2019). Apart from that, *Salmonella typhimurium, Salmonella typhi, Salmonella enteritidis, Shigella* spp., and *Proteus* spp. are the dominant bacterial flora in waste samples (Korajkic et al., 2018).

## Conclusion

Based on measurements of the pH, BOD, COD, DO, and total coliform concentrations, it is concluded that the Aur River is polluted because the BOD, COD, DO, and total coliform are above the quality standards. The pH is still at the quality standard. In addition, there are also four types of coliforms that are dominant in Aur river water, namely *Klebsiela pneumoniae, Proteus mirabilis, Escherichia coli,* and *Enterobacter.* From the above data, Aur River water has been polluted with coliform, so it is necessary to analyze the causes of high concentrations and intervene to reduce total coliform concentrations.

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