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Fecal Coliform Analysis of Hospital Wastewater in X City

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Abstract: Hospital wastewater may contain fecal indicator microorganisms that pose potential risks to public health and the environment if inadequately managed. Examination of fecal coliforms at wastewater treatment plant (WWTP) outlets is necessary to assess the microbiological quality of wastewater before its discharge into the environment. This study aimed to analyze fecal coliform concentrations in wastewater collected from hospital WWTP outlets in City X and compare the results with the wastewater quality benchmark used in the study. A descriptive observational design with a quantitative approach was employed. Wastewater samples were collected from WWTP outlets using the grab sampling technique in accordance with SNI 9063:2022. Laboratory examination was conducted *in vitro* using the Most Probable Number (MPN) method, comprising a presumptive test using Lauryl Tryptose Broth (LTB) and a confirmatory test using EC Broth. The examination of four samples showed that RS.A and RS.B had fecal coliform concentrations exceeding 160,000 MPN/100 mL, RS.C had a concentration of less than 180 MPN/100 mL, and RS.D had a concentration of 350 MPN/100 mL. Compared with the reference limit of 1,000 MPN/100 mL, samples RS.A and RS.B exceeded the limit, whereas RS.C and RS.D were below it. These findings demonstrate variations in the microbiological quality of wastewater at the investigated hospital WWTP outlets. Routine fecal coliform monitoring is therefore necessary to support the evaluation of wastewater quality before environmental discharge.

Keywords: Fecal coliform; Hospital Wastewater; MPN Method; SNI.

Pendahuluan

A hospital is a healthcare facility that provides health services encompassing promotive, preventive, curative, rehabilitative, and palliative efforts (Indonesian Ministry of Health, 2023). In conducting all these healthcare activities, hospitals not only deliver patient care but also generate waste as a consequence of their operational activities (Wula *et al*., 2022). This waste comprises solid waste, liquid waste, gaseous emissions, and hazardous and toxic material waste (Ganefati *et al*., 2025).

Hospital wastewater is also an environmental health issue that warrants attention because it contains various pollutants, such as pathogenic microorganisms, chemical compounds, pharmaceutical residues, and antibiotic resistance genes (Zhang *et al*., 2020). These constituents give hospital wastewater the potential to adversely affect public health and the environment if it is not subjected to adequate treatment prior to environmental discharge (Yuan & Pian, 2023). Effective management of hospital wastewater is a crucial component in preventing the release of various pollutants, particularly microbiological contaminants, from healthcare activities into the environment.

Hospital wastewater is a source of environmental pollution that potentially contains pathogenic microorganisms, high organic matter, and medical chemical residues (Habibah & Utami, 2025). Pursuant to the Regulation of the Minister of Environment of the Republic of Indonesia No. 11 of 2025 concerning Wastewater Quality Standards, every healthcare facility is

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required to manage both domestic wastewater and hazardous and toxic material waste, as well as ensure that the generated wastewater meets established quality standards (Winarti, C., 2020). This provision emphasizes that wastewater treatment is a mandatory obligation that must be fulfilled prior to discharging wastewater into the environment. A study by Amin et al. (2024) systematically evaluated the effectiveness of microbiological treatment of hospital wastewater in low- and middle-income countries. The review demonstrated that achieving the microbiological quality of treated wastewater remains a challenge; thus, effluent monitoring is necessary to assess treatment efficacy before wastewater is discharged into the environment.

An effective treatment process ensures that the treated wastewater complies with environmental quality standards and minimizes adverse impacts on surrounding ecosystems and communities. Several bacteria of concern include *Escherichia coli*, *Enterococcus*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Salmonella* sp., and other enteric bacteria. The presence of microorganisms in hospital wastewater is influenced by wastewater characteristics, operational conditions of the treatment system, biological processes, and the disinfection stage (Yuan & Pian, 2023). A study by Kristanto and Koven (2019) noted the presence of antibiotic-resistant *Escherichia coli* in hospital wastewater treatment plants in Indonesia. This study is relevant in demonstrating that hospital wastewater warrants attention not only regarding general pollution aspects but also concerning microorganisms that potentially pose health risks.

In this study, fecal coliform was selected as the examination parameter because it serves as an indicator bacterial group used to demonstrate fecal contamination in wastewater. The presence of fecal coliforms is associated with contamination originating from human or animal feces, making it a reliable indicator of the microbiological quality of wastewater. Coliforms are a group of rod-shaped, non-spore-forming bacteria capable of fermenting lactose to produce acid and gas within 48 hours at 35–37°C (Dewi & Gusnita, 2019). Based on their origin, coliform bacteria are classified into fecal coliforms and non-fecal coliforms. Fecal coliforms, particularly *Escherichia coli*, originate from the digestive tracts of humans and warm-blooded animals and are widely used as indicators of fecal pollution in water and wastewater.

Therefore, high concentrations of fecal coliforms may indicate inadequate wastewater treatment and suggest the potential presence of pathogenic microorganisms that pose risks to human health (Azahra & Irdawati, 2024). Fecal coliform measurements in hospital wastewater were also conducted by Guo et al. (2026), who evaluated the performance of wastewater treatment systems with a focus on removal efficiency and concentration dynamics of these bacteria. This study reinforces the importance of fecal coliform examination in obtaining a comprehensive understanding of the microbiological quality of treated wastewater.

In addition to threatening public health, fecal pollution can also exert adverse impacts on aquatic ecosystems and environmental sustainability. To mitigate these impacts, the Indonesian government has established maximum allowable concentration limits for fecal coliforms in domestic wastewater (Sebayang et al., 2025).

Quantitative analysis of fecal coliform bacteria at the discharge point (outlet) serves as an essential approach to evaluating the effectiveness of hospital wastewater treatment systems. Monitoring bacterial concentrations provides useful insights into treatment performance and ensures compliance with environmental regulations. One widely used technique for estimating coliform bacterial density is the Most Probable Number (MPN) method. This statistical method estimates the concentration of viable bacteria based on their ability to ferment lactose and produce gas in Durham tubes (Yoantrista & Yuniarti, 2025). The MPN method is particularly suitable for wastewater samples because it can analyze turbid samples containing high concentrations of suspended solids a characteristic commonly found in hospital wastewater (Anggraeni & Irdawati, 2025)

The thermotolerant coliform group is generally dominated by the genus *Escherichia*, although certain bacteria belonging to the genera *Citrobacter*, *Klebsiella*, and *Enterobacter* can also exhibit thermotolerant properties. *Escherichia coli* is the most specific member serving as an indicator of fecal pollution, as it is

present in high numbers in human and animal feces and is rarely detected in the absence of fecal contamination (Richiardi et al., 2023).

The MPN procedure comprises two sequential stages: the presumptive test and the confirmed test. These stages enhance the selectivity and reliability of coliform detection while minimizing false-positive results caused by non-coliform bacteria with similar biochemical characteristics (Mayangsari et al., 2023). As an indicator organism, fecal coliform provides critical information regarding microbiological contamination and the potential presence of enteric pathogens in wastewater, rendering it an essential parameter in wastewater quality assessment.

The Most Probable Number (MPN) is a microbiological method used to estimate bacterial populations in samples based on growth patterns or positive reactions across a series of test tubes (Nurrusyda et al., 2024). This method can be utilized to examine fecal pollution indicator bacteria, including fecal coliforms, particularly in water and wastewater samples (Nabila & Gultom, 2026). Microbiological water testing using the MPN method remains a classic approach widely employed in public health laboratories due to its quantitative, straightforward, and effective nature in detecting fecal pollution indicators such as *Escherichia coli*. The effectiveness of hospital wastewater treatment can also be assessed through the reduction of indicator bacterial counts. Chiemchaisri et al. (2022) examined the removal of coliform bacteria through treatment processes utilizing membrane bioreactors and disinfection in hospital wastewater. This highlights that treatment processes and disinfection are crucial aspects of controlling microbiological pollution in hospital wastewater

Several previous studies have reinforced the validity of this method in water quality monitoring (Aulya et al., 2020). The MPN method was chosen because of its ability to estimate bacterial counts based on lactose fermentation reactions, which are indicated by gas production in Durham tubes. The principle of the MPN method is based on the use of a series of sample dilutions inoculated into growth media. Tubes showing a positive reaction are then used to determine the combination of results based on the number of positive tubes at each dilution level (MacIntyre et al., 2019). These results are compared with the MPN table to derive the estimated bacterial count in the sample, which is generally expressed in units of MPN/100 mL.

Despite the establishment of national wastewater quality standards, information regarding fecal coliform concentrations in hospital wastewater in City X remains limited. Furthermore, data evaluating the efficacy of hospital WWTPs in reducing microbiological pollution are scarce. Therefore, continuous monitoring and assessment are required to determine whether treated wastewater complies with environmental regulations and to evaluate the operational performance of wastewater treatment systems in healthcare facilities.

Based on this background, this study aims to analyze fecal coliform concentrations at the inlet and outlet points of the hospital wastewater treatment system in City X using the Most Probable Number (MPN) method and to evaluate its compliance with applicable environmental quality standards. The findings of this study are expected to contribute to environmental quality monitoring efforts and provide an empirical basis for improving the effectiveness of hospital wastewater treatment systems in the region.

Materials and Methods

Research Time and Location

This study was conducted at the Environmental Laboratory of the Department of Housing, Settlement Areas, and Environment of City X. Sampling was performed on wastewater from the Wastewater Treatment Plant (WWTP) of a hospital in City X, while the fecal coliform analysis was carried out *in vitro* in the laboratory using the Most Probable Number (MPN) method.

Research Design

This study employed a descriptive observational design with a quantitative approach. The study population comprised all wastewater generated by the hospital WWTP system in City X, while the study samples consisted of WWTP wastewater collected at the outlet points from the designated study hospitals. The sample size was determined based on the number of sampling points at each hospital meeting the inclusion criteria. Sampling was

performed using a grab sampling technique in accordance with SNI 9063:2022 regarding procedures for water sample collection for microbiological analysis. The study variable was the concentration of fecal coliforms in the WWTP wastewater, expressed in MPN/100 mL. Data were obtained through direct examination of wastewater samples using the MPN method. Samples were collected in 250–500 mL sterile bottles, stored in a cool box at approximately 4°C, and transported to the laboratory for analysis. Equipment utilized included sterile sample bottles, a cool box, test tubes, Durham tubes, 5 mL and 10 mL pipettes, a pipette bulb, a vortex mixer, an inoculating loop, an incubator, a water bath, an autoclave, beakers, and a spirit lamp. Reagents and materials included wastewater samples, Lauryl Tryptose Broth (LTB) medium, and EC Broth medium.

Research Procedures

Sampling

Sampling was conducted directly at the WWTP outlet using a grab sampling technique in accordance with SNI 9063:2022. Sterile sample bottles were not rinsed prior to use. Each bottle was opened during sample collection, filled aseptically, and immediately recapped. Every sample was labeled with a sample code, location, sampling point, date, and time of collection. Samples were subsequently placed into a cool box maintained at approximately 4°C and immediately transported to the laboratory for analysis.

Media Preparation

Lauryl Tryptose Broth (LTB) and EC Broth media were prepared according to the manufacturer's instructions. The media were dissolved in distilled water at specified concentrations and transferred into test tubes equipped with Durham tubes. The prepared media were sterilized using an autoclave at 121°C for 15 minutes. Prior to use, the Durham tubes were inspected to ensure the complete absence of air bubbles.

Presumptive Test

Wastewater samples were inoculated into a series of tubes containing LTB medium across different sample volumes or dilution levels according to the MPN experimental design. Tubes were incubated at 35 ± 0.5°C for 24 ± 2 hours. If no positive results were observed, incubation was extended up to 48 ± 3 hours. Tubes exhibiting bacterial growth and gas production within the Durham tubes were recorded as positive results.

Confirmatory Test for Fecal Coliforms

Cultures from LTB-positive tubes were inoculated into EC Broth medium using a sterile inoculating loop. The tubes were then incubated in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours. Gas production inside the Durham tubes following the incubation period served as a positive indicator for fecal coliforms.

Determination of MPN Values

The number of positive tubes across each dilution series was recorded. The combination of positive results was used to determine the fecal coliform concentration based on the corresponding MPN index table. Test results were expressed in MPN/100 mL. If a specific combination of positive tubes was not listed in the standard MPN table, the bacterial density was calculated using the appropriate MPN formula.

Data Analysis

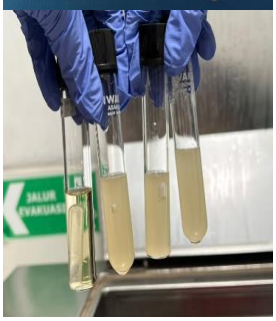
Fecal coliform test data were analyzed using quantitative descriptive methods. Data were derived from the count of positive tubes obtained during the presumptive and confirmatory tests using EC Broth medium. MPN values were subsequently determined based on the combination of positive tubes across each dilution series. Test results were expressed in MPN/100 mL and presented in tabular form. Fecal coliform concentrations for each sample were then evaluated against applicable wastewater effluent standards to characterize the microbiological quality of hospital wastewater at WWTPs in City X.

Results

Based on the analysis using the Most Probable Number (MPN) method, wastewater samples coded RS.A, RS.B, RS.C, and RS.D underwent serial dilutions to 10^{-1}, 10^{-2}, and 10^{-3}, with a maximum analyte volume of 1 mL pipetted at each dilution level. Serial dilution was performed to obtain the combination of positive

tubes used to determine the fecal coliform value based on the MPN table.

Table 1. Results of Presumptive and Confirmatory Tests for Fecal Coliform

	The presumptive test, conducted utilizing Lactose Broth (LB) medium, was characterized by gas production within the Durham tubes, serving as a positive indicator for the presence of coliform bacteria.
	The confirmation stage, performed utilizing selective media, was characterized by distinct media color changes that signified the proliferation of coliform bacteria within the analyzed samples.

Sample RS.A showed a positive tube combination of 0–0–0 with a fecal coliform value of <180 MPN/100 mL. Sample RS.B showed a combination of 5–5–0 with a value of 33 MPN/100 mL, while sample RS.C showed a combination of 5–5–2 with a value of 540 MPN/100 mL. Sample RS.D showed a combination of 5–1–0 with a value of 33 MPN/100 mL. Quality control was performed using positive control strains, negative control strains, and uninoculated LB and EC Broth media. Positive control strains yielded positive results, whereas negative control strains and uninoculated media yielded negative results. These results indicate that the testing process proceeded under the expected conditions and showed no contamination in the control media.

In the presumptive test stage, Lactose Broth (LB) medium was used to detect the presence of indicator bacteria based on gas formation in Durham tubes. Tubes showing gas formation were recorded as positive and proceeded to the confirmation stage. The confirmation stage was conducted using EC Broth medium. Positive tubes from the presumptive stage were inoculated into EC Broth and incubated under appropriate conditions. A positive result was indicated by bacterial growth accompanied by gas formation in the Durham tube. The combination of positive tubes obtained was then used to determine the fecal coliform value based on the MPN table.

Table 2. Results of Fecal Coliform Analysis Using the Most Probable Number (MPN) Method

Analyte Type (Blank/Sample No.)	Experimental Results	Fecal Coli form nsity (MPN/100mL)	Environmental Quality Standards (MPN/100 mL)
Strain positif	Positif	Positif	
Strain negatif	Negatif	Negatif	
LB	Negatif	Negatif	
BPW	Negatif	Negatif	
RS.A	5 5 5	> 160.000	1000
RS.B	5 5 5	> 160.000	1000
RS.C	0 0 0	<180	1000
RS.D	5 5 1	350	1000

Discussion

The high fecal coliform concentrations observed in samples RS.A and RS.B indicate that substantial numbers of fecal indicator bacteria remained detectable in the wastewater at the outlet sampling points. The presence of these bacteria suggests that the treated wastewater may still contain microorganisms associated with fecal contamination. However, fecal coliforms are indicator organisms, and their detection does not necessarily demonstrate the presence of a specific pathogenic microorganism. The elevated concentrations observed in these samples therefore indicate a potential microbiological concern that warrants further investigation and appropriate evaluation of the wastewater treatment process.

Differences in fecal coliform concentrations among the four samples may reflect variations in the microbiological characteristics of the wastewater and the performance of the respective treatment systems. Wastewater treatment effectiveness can be influenced by several factors, including the operational condition of treatment units, hydraulic retention time, disinfection practices, wastewater characteristics, and the maintenance of treatment facilities. Nevertheless, because this study examined wastewater exclusively at the WWTP outlets, the contribution of each factor cannot be determined directly. Furthermore, without corresponding inlet measurements, the reduction in fecal coliform concentrations during treatment cannot be calculated. Therefore, the observed differences should be interpreted as variations in the microbiological quality of outlet wastewater rather than direct measurements of treatment efficiency.

Sample RS.C exhibited the lowest fecal coliform concentration, with an MPN value of less than 180 MPN/100 mL. This result indicates that fecal coliforms were not detected in the tested tube combinations under the applied analytical conditions. However, a result of 0–0–0 should not be interpreted as proof that the wastewater was completely free of fecal coliforms. Rather, it indicates that the target organisms were not detected in the analyzed test portions. The actual concentration may be below the detection capability of the method used. This distinction is important when interpreting microbiological examination results, particularly when the findings are used to evaluate wastewater quality against an established standard.

Similarly, sample RS.D showed a fecal coliform concentration of 350 MPN/100 mL, which was below the 1,000 MPN/100 mL benchmark specified in the study. This finding indicates that the measured concentration in this sample was within the stated numerical limit. Nevertheless, compliance based on a single microbiological parameter does not establish that the wastewater meets all applicable discharge requirements. Other physical, chemical, and microbiological parameters may also be required for a comprehensive assessment of hospital wastewater quality. In addition, the applicable regulatory standard and its requirements should be verified before making a formal compliance determination.

The MPN method was used to estimate fecal coliform concentrations based on the combination of positive and negative tubes across the tested sample volumes or dilution levels. Gas production in Durham tubes during the presumptive and confirmatory stages was used as an indicator of bacterial activity consistent with the analytical procedure. The resulting tube combinations were then interpreted using the appropriate MPN reference table. This approach provides an estimate of the concentration of target indicator bacteria in a sample without requiring direct colony enumeration on solid agar media. However, the reliability of the resulting estimates depends on appropriate sample handling, correct incubation conditions, valid positive and negative controls, and the use of an MPN table that matches the number of tubes and sample volumes employed.

Quality-control results were also included to evaluate the validity of the analytical procedure. The positive controls reportedly produced positive results, whereas the negative controls and uninoculated media produced negative results. These findings are consistent with the expected control responses and support the validity of the testing process, provided that the control organisms and procedures were appropriate for the target analysis. Nevertheless, quality-control results should be interpreted alongside the consistency of the recorded sample results and the accuracy of the MPN calculations.

Overall, the findings indicate that the microbiological quality of hospital wastewater at the investigated WWTP outlets was not uniform. Two samples, RS.A and RS.B, exhibited concentrations substantially above the stated benchmark, while RS.C and RS.D showed lower concentrations. These findings highlight the importance of routine microbiological monitoring of hospital wastewater outlets and the need to evaluate treatment-system operation where elevated indicator-bacteria concentrations are identified. Because this study was limited to outlet sampling, the findings describe the microbiological conditions of the sampled discharge points and do not establish the degree of bacterial removal achieved by the treatment systems. Further investigations incorporating repeated sampling, inlet–outlet comparisons, and

additional wastewater quality parameters would provide a more comprehensive assessment of WWTP performance.

Conclusion

Based on the examination of fecal coliforms using the Most Probable Number (MPN) method in wastewater samples collected from hospital wastewater treatment plant (WWTP) outlets in City X, variations in fecal coliform concentrations were observed among the four samples. Samples RS.A and RS.B exhibited concentrations exceeding 160,000 MPN/100 mL, sample RS.C had a concentration of less than 180 MPN/100 mL, and sample RS.D showed a concentration of 350 MPN/100 mL. Compared with the reference limit of 1,000 MPN/100 mL used in this study, samples RS.A and RS.B exceeded the limit, whereas samples RS.C and RS.D were below it. These findings indicate variations in the microbiological quality of wastewater discharged from the investigated hospital WWTP outlets. Therefore, routine fecal coliform monitoring is necessary to evaluate wastewater microbiological quality before discharge into the environment. However, because this study was limited to outlet sampling, it could not determine the effectiveness of fecal coliform removal during the wastewater treatment process.

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