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Monitoring Antibiotic-Resistant Bacteria in Tapak River Estuary, Semarang City, Indonesia

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Abstract

The estuary of the Tapak River in the Tugu Subdistrict, Semarang City, has been classified as lightly polluted by domestic and industrial waste from Tambak Aji Village. Waste discharges flow into ponds and the open coast, potentially increasing bacterial resistance and impacting aquatic life. This study aims to identify antibiotic-resistant bacterial isolates in the area. The research was conducted from January to April 2024 using an exploratory descriptive method, with sediment samples taken from three stations. Two Gram-positive bacterial isolates resistant to Ciprofloxacin were found: DI.C_10-5/2 (with a 15.39 mm inhibition zone) and DI.C_10-5/3 (with a 15.19 mm inhibition zone). One of the isolates, DI.C_10-5/3, was identified as closely related to Bacillus licheniformis (99.75% similarity) using 16S rRNA gene sequencing. These findings highlight the need for regular monitoring of resistant bacteria in aquatic environments to prevent the uncontrolled spread of antibiotic resistance.

Keywords: antibiotics, bacterial isolates, resistance, river estuary, sediment

1. Introduction

Bacterial pollution in water bodies is a growing concern. Rivers, in particular, collect various types of waste, including domestic, industrial, livestock, and agricultural runoff. Improper river management, such as the direct discharge of industrial waste, can proliferate antibiotic-resistant bacteria. These bacteria can survive in harsh environments with low oxygen levels and other unstable conditions (Rompis *et al*., 2018).

Urban and village wastewater discharges are causes of bacterial water pollution. Wastewater not only promotes the growth of coliform bacteria but also raises the number of pathogenic bacteria. Resistant microbes may emerge as a result of spontaneous mutations, the transfer of resistance genes, contact with resistant bacteria from animals and the environment, and global human travel (Dongoran *et al*., 2022). For instance, sediment from the Haihe River in China contains antibiotic resistance genes (ARGs) that encode sulfonamide resistance (sul1 and sul2), with concentrations of $(7.8 \pm 1.0) \times 10^9$ copies/g for *sul1* and $(1.7 \pm 0.2) \times 10^{11}$ copies/g for *sul2* (Luo *et al.,* 2010). In the Tinto River Estuary, Spain, 92.7% of bacterial isolates were resistant to four or more antibiotic classes (Eduardo-Correia *et al*., 2020). However, there is a paucity of information regarding the bacterial resistance of river estuaries in Indonesia.

The Tapak River, located in the Tugu subdistrict of Semarang City, Indonesia, is contaminated with domestic, aquaculture, and industrial waste. The Tapak River Estuary has been classified as lightly polluted, with a Water Quality Index (WQI) of 1,627-1,710 and a Pollution Index (PI) of 1,787-1,975 (Larasati *et al*., 2021). Industrial waste often contains antibiotics and pesticides, which can increase bacterial resistance (Morrissey *et al*., 2014).

Waste from the Tapak River Estuary flows into the open sea and affects nearby ponds. Pathogenic bacteria such as *Vibrio* sp., *Aeromonas* sp., and *Pseudomonas* sp. pose a risk to aquaculture, potentially causing disease in cultured fish (Fitri *et al*., 2020). Sabdaningsih *et al.* (2024) reported that both *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, found in the gills of fish and in the sediment of a traditional pond, exhibited resistance to both Erythromycin and Ciprofloxacin. This contamination necessitates preventive measures as it threatens aquatic biota.

Bacteria thrive in various environments, including sediments. Unlike water, which flows downstream and mixes with rain runoff, sediment accumulates in estuarine waters due to slower circulation. Human activities, environmental conditions, and the dynamics of microbial communities shape the occurrence and spread of ARGs in estuarine rivers. ARGs enter water systems through urban runoff, aquaculture, agriculture, livestock farming, and effluents from hospitals and treatment plants (Chauhan and Punia, 2023). These resistant bacteria can pass their genes to aquatic microbes with similar resistance traits. Effective monitoring and management strategies are crucial to curb the proliferation of antibiotic resistance and safeguard both environmental and public health. This study aims to monitor bacterial resistance in the sediments of the Tapak River Estuary to multiple commercial antibiotics.

2. Methodology

2.1 Materials

Sampling tools included a GPS (Garmin, US), sediment core (local modified), cool box (Lion Star, Indonesia), thermometer, refractometer (ATAGO, Japan), DO meter (YSI Pro, US), pH meter (Lutron, Taiwan), soil pH meter (Soiltech, US), ziplock plastic bags, and stationery. Sample analysis tools included an autoclave (Hirayama, Japan), Erlenmeyer flasks (IWAKI, Japan), beakers (IWAKI, Japan), a hot plate magnetic stirrer (Corning, US), vortex mixer (Thermo Scientific, Finland), Laminar Air Flow (LAF) (Airtech, China), incubator, microscope (Olympus, Japan), inoculation loop, analytical scales (Ohaus, US), Bunsen burner, petri dishes, test tubes, measuring cylinders, forceps, pipettes, microtips, aluminium foil, and glass preparation clamps. The materials used in the study included sediment samples, sterile seawater, 70% alcohol, distilled water, Nutrient Agar (NA) (Himedia, India), Gram stain kit (Himedia, India), 0.5 McFarland standard solution (Himedia, India), blank paper discs, and the antibiotics nystatin, erythromycin, ciprofloxacin, tetracycline, and chloramphenicol (Oxoid, UK).

2.2 Methods

This research employed an exploratory descriptive approach, using sediment samples from three stations: Station I (near industry), Station II (near pond), and Station III (near sea), with triplicates collected from each station. Exploratory research is pertinent to this study due to the lack of information on resistant bacteria in the Tapak River Estuary. As this topic is in its early stages of investigation, the descriptive aim is to outline the pattern of bacterial resistance in this area.

2.2.1 Sampling

Samples were collected using purposive sampling. The study's sampling locations comprise three observation stations, each featuring three sampling points. The stations are spaced approximately 600 meters apart. The selection of these sampling points aims to compare the prevalence of resistant bacteria near the industry, the shrimp farm, and the sea (Figure 1). Sediment samples from each station were taken at a depth of 50 cm using sediment cores, stored in zip-lock bags, and placed in a cool box. Observing sediment texture is achieved through the texture by feel technique. This approach relies on the sensitivity of the thumb and index finger. By taking sediment samples and kneading them into a ball or bolus, one can add water or sediment until the mixture no longer adheres to the fingers. It is important to note the sediment's feel during kneading: sandy, smooth (silty), or sticky (clay) (Saputro *et al.*, 2017). *In situ* environmental parameters, such as temperature, salinity, dissolved oxygen (DO), and water and soil pH, were also measured.

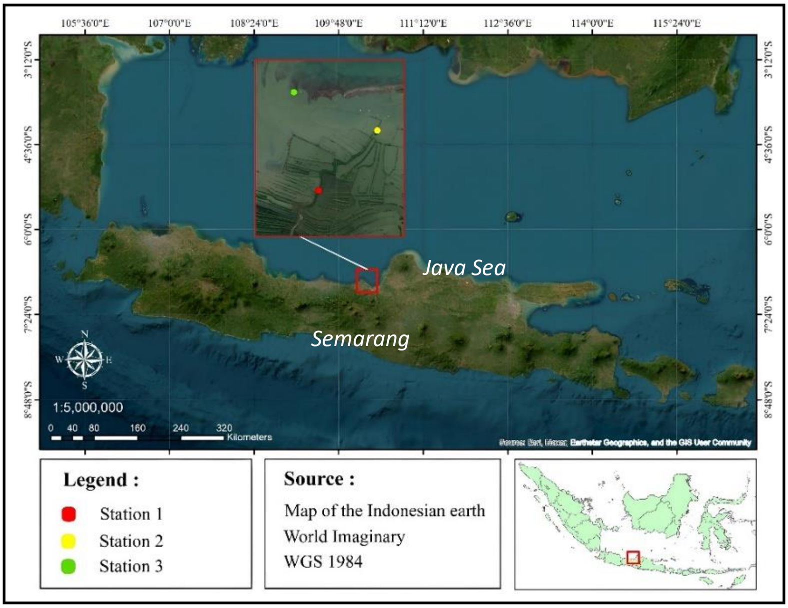

Figure 1. Map of sampling locations

2.2.2 Preparation of culture media

NA was prepared by dissolving 20 grams of NA in 1 L of seawater, with salinity adjusted to match conditions at each sampling station (Rosmania and Yuniar, 2021). The medium was homogenized using a hot plate stirrer and sterilized by autoclaving at 121°C for 20 minutes. After sterilization, 0.1% nystatin (antifungal) was added (Wulansari *et al.,* 2019).

2.2.3 Serial dilution

Sediment samples (1 g) were placed in a test tube with 9 mL of sterile seawater and homogenized using a vortex mixer to obtain a 10^{-1} dilution. A 1 mL aliquot was then transferred to another test tube containing 9 mL of sterile seawater and vortexed, creating a 10^{-2} dilution (Safriana *et al.,* 2019). This process continued until a 10^{-5} dilution was achieved for all samples.

2.2.4 Isolation and purification

Bacterial isolation was performed using the pour plate method on Nutrient Agar (NA) media. A series of 10^{-4} and 10^{-5} dilutions was prepared, from which 1 mL of each dilution was inoculated onto a petri dish. The bacterial suspensions were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were purified by streak plate method. Based on their distinct macroscopic characteristics, the colonies were transferred to fresh NA media using an inoculating loop and incubated at 37°C for 24 hours.

2.2.5 Bacterial resistance test against antibiotics

Purified bacterial colonies were subcultured onto NA medium in test tubes and incubated at 37°C for 24 hours. After incubation, the bacterial suspension was standardized to McFarland 0.5 turbidity (Rosmania and Yanti, 2020). The antibiotics tested in this study were Erythromycin, Ciprofloxacin, Tetracycline, and Chloramphenicol. These antibiotics were selected due to their widespread use in human therapy and fish medicine (Government of Republic of Indonesia, 2019). Bacterial suspensions with a density of 1.5×10^8 CFU (Colony Forming Unit)/mL were swabbed onto NA plates using cotton swabs, and tested with antibiotic discs. The Duplo plates were incubated at 37°C for 24 hours, and the inhibition zones around the antibiotic discs were measured to assess bacterial resistance (Murray *et al.,* 2022). The results were averaged from Duplo plates and interpreted following the 34th edition of Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024). Bacterial resistance was categorized as Sensitive (S), Intermediate (I), or Resistant (R) based on the size of the inhibition zones. Bacteria classified as "Sensitive" formed clear zones around the discs, while "Intermediate" and "Resistant" bacteria exhibited progressively smaller or absent inhibition zones (Artati *et al.,* 2018).

2.2.6 Microscopic characterization

Microscopic characterization was done with Gram staining. This method employs four reagents: crystal violet (Gram A), iodine (Gram B), a decolourizer (Gram C), and safranin (Gram D). Stained bacterial samples were examined under a microscope at 1000x magnification. Gram-positive bacteria appeared purple-blue, while Gram-negative bacteria appeared red (Ismail *et al.,* 2017).

2.2.7 Molecular Identification of Bacterial Isolates

Molecular identification was conducted on bacterial isolates showing antibiotic resistance. DNA extraction was performed using the Chelex method. The extracted DNA was amplified through PCR using the following cycling conditions: initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation (95°C for 1 minute), annealing (55°C for 1 minute), and extension (72°C for 1 minute). A final extension was carried out at 72°C for 7 minutes. Universal 16S rRNA primers (27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-GGTTACCTTGTTACGACTT-3') were used (Van Pelt-Verkuil *et al.,* 2008). PCR products were run on 1% agarose gel, stained with ethidium bromide, and visualized under UV light. The PCR products were sent for Sanger sequencing at PT Genetika Science Indonesia. Sequence alignment was performed using MEGA XI software, and Basic Local Alignment Search Tool (BLAST) analysis was conducted against the GenBank database for species identification found at the National Center for Biotechnology Information.

3. Results and Discussion

3.1 Environmental conditions of the study

Environmental parameters at the sampling sites are presented in Table 1, based on data collected from three stations: Station I, Station II, and Station III.

The temperature ranged from 31.7°C to 34.4°C, a suitable range for bacterial growth, as optimal bacterial incubation occurs between 27°C and 37°C (Mahrus *et al.,* 2020). The sampling sites were near mangroves, where soil pH ranged from 5.3 to 6.7. This pH range aligns with the optimal conditions for sediment bacteria in mangrove estuaries, which grow best at pH levels between 5 and 7 (Fajar *et al.,* 2022). Dissolved oxygen (DO) levels ranged from 5.24 to 6.13 mg/L, which meets the Seawater Quality Standards (Government Regulation No. 22, 2021) requiring a minimum of 5 mg/L for rivers. Higher DO values are generally observed farther from land, likely due to increased oxygen diffusion in open water (Turnip *et al.,* 2021).

Table 1. Results of environmental parameters

Station	Sampling point	Salinity (°/₀₀)	pH		DO (mg/L)	Temp (°C)	Sediment texture
			Soil pH	Water pH			
I Near industry	Point 1	27	5.00	9.38	5.12	34.40	mud
	Point 2	27	5.00	9.80	5.33	33.00	
	Point 3	27	6.50	9.14	5.29	33.70	
Average		27	5.50	9.44	5.24	33.70	
II Near pond	Point 1	26	7.00	8.92	5.27	31.70	mud
	Point 2	26	6.50	8.90	5.37	32.40	
	Point 3	26	6.50	8.55	5.29	32.60	
Average		26	6.67	8.79	5.31	32.23	
III Near sea	Point 1	32	6.00	8.95	6.02	32.00	sand
	Point 2	32	5.00	8.90	6.15	33.70	
	Point 3	32	5.00	8.64	6.23	33.00	
Average		32	5.30	8.83	6.13	32.90	

Salinity, a critical factor for bacterial growth, ranged between 26-32 ‰, which is within the optimal range for marine bacteria (25-40 ‰) (Lubis *et al.,* 2021). Higher salinity levels are generally found farther from land, making the water more alkaline due to increased carbonate ion concentrations (Mukanthi *et al.,* 2021). Overall, the environmental conditions were suitable for mangrove vegetation and aquatic biota in the Tapak River estuary.

3.2 Isolation and Purification

Nine samples from the three stations were diluted from 10^{-1} to 10^{-5}, with 18 samples isolated from dilutions 10^{-4} and 10^{-5}. From the purification process, 23 bacterial isolates were obtained, including eight from Station I, nine from Station II, and six from Station III.

Morphological observations revealed varied bacterial colony colours, including milky white, cloudy white, off-white, yellow, and orange (Table 2). Colony margins ranged from entire to lobate and rhizoid, while colony elevations were convex, flat, or umbonate. Some isolates from different stations exhibited similar morphological features, suggesting they may belong to the same species. However, bacteria of the same type can exhibit different species-level characteristics depending on environmental conditions (Handayani *et al.,* 2023). Morphological diversity may result from adaptation to culture media, temperature, incubation time, and the age of the culture (Rizqoh *et al.,* 2021).

Table 2. Results of observations of the morphological characteristics of bacteria

Station	Bacterial isolate	Colour	Form colonies	Margins	Elevation
I Near industry	DI.A_10^{-4}/1	yellow	*circular*	*Entire*	*convex*
	DI.A_10^{-5}/1	milky white	*circular*	*Entire*	*convex*
	DI.B_10^{-4}/1	cloudy white	*circular*	*Entire*	*convex*
	DI.B_10^{-5}/1	cloudy white	*circular*	*Entire*	*umbonate*
	DI.C_10^{-4}/1	cloudy white	*circular*	*Entire*	*convex*
	DI.C_10^{-5}/1	off white	*rhizoid*	*Rhizoid*	*flat*
	DI.C_10^{-5}/2	off white	*circular*	*Entire*	*convex*
	DI.C_10^{-5}/3	off white	*irregular*	*Entire*	*flat*
II Near pond	DT.A_10^{-4}/1	milky white	*circular*	*Entire*	*convex*
	DT.A_10^{-5}/1	cloudy white	*circular*	*Entire*	*flat*
	DT.A_10^{-5}/2	cloudy white	*circular*	*Entire*	*convex*
	DT.B_10^{-4}/1	orange	*circular*	*Entire*	*convex*
	DT.B_10^{-4}/2	milky white	*circular*	*Entire*	*convex*
	DT.B_10^{-4}/3	cloudy white	*irregular*	*Entire*	*umbonate*
	DT.C_10^{-4}/1	cloudy white	*circular*	*Entire*	*convex*
	DT.C_10^{-5}/1	milky white	*circular*	*Entire*	*convex*
	DT.C_10^{-5}/2	orange	*circular*	*Entire*	*convex*
III Near sea	DL.A_10^{-4}/1	milky white	*circular*	*Entire*	*convex*
	DL.A_10^{-5}/1	milky white	*circular*	*Entire*	*convex*
	DL.B_10^{-4}/1	cloudy white	*irregular*	*Lobate*	*umbonate*
	DL.B_10^{-4}/2	cloudy white	*circular*	*Entire*	*convex*
	DL.C_10^{-4}/3	cloudy white	*circular*	*Entire*	*convex*
	DL.C_10^{-5}/1	milky white	*circular*	*Entire*	*convex*

Information: DI: Near industry, DT: Near pond, DL: Near sea, A: Point 1, B: Point 2, C: Point 3, Numbers (1,2,3): Pure bacterial isolate, (10^{-4}; 10^{-5}): Dilution rate.

3.3 Resistance testing and bacterial characterization

This study examined the resistance of bacterial isolates to four antibiotics: chloramphenicol (30 mcg/disc), tetracycline (30 mcg/disc), ciprofloxacin (5 mcg/disc), and erythromycin (15 mcg/disc). The study's location near residential areas, industrial zones, and aquaculture ponds influenced the selection of these antibiotics. The research accommodates waste from both domestic and aquaculture sources. Specifically, erythromycin and tetracycline are commonly used in aquaculture, while ciprofloxacin and chloramphenicol are frequently prescribed to humans. The average diameter of the antibiotic inhibition zones was measured per the standards set by the Clinical and Laboratory Standards Institute (CLSI) in 2024.

Table 3. Resistance test results and bacterial Gram staining

Station	Bacterial isolate	Inhibition Zone Diameter (mm) C [1] (30 mcg)	TE [2] (30 mcg)	CIP [3] (5 mcg)	E [4] (15 mcg)	Gram Identification Form	Color
I Near industry	DI.A_10^{-4}/1	20.19	36.21	33.44	27.06	*Staphylococci*	Purple (+)
	DI.A_10^{-5}/1	32.55	26.20	19.50	28.62	*Bacill*	Red (-)
	DI.B_10^{-4}/1	31.73	23.36	17.26	31.08	*Bacill*	Purple (+)
	DI.B_10^{-5}/1	35.03	24.26	19.30	31.92	*Staphylococci*	Purple (+)
	DI.C_10^{-4}/1	32.02	26.76	18.02	31.20	*Diplobacill*	Purple (+)
	DI.C_10^{-5}/1	35.59	22.97	21.75	32.48	*Diplobacill*	Red (-)
	DI.C_10^{-5}/2	25.55	23.53	15.39	29.20	*Streptobacill*	Purple (+)
	DI.C_10^{-5}/3	27.49	23.39	15.19	29.55	*Diplobacill*	Purple (+)
	Average ± stdev	30.02 ± 5.24	25.84 ± 4.42	20.07 ± 5.86	30.14 ± 1.84		
II Near pond	DT.A_10^{-4}/1	29.66	26.70	20.80	31.60	*Staphylococci*	Purple (+)
	DT.A_10^{-5}/1	26.90	25.57	19.79	29.99	*Bacill*	Red (-)
	DT.A_10^{-5}/2	32.91	27.16	23.75	29.28	*Staphylococci*	Purple (+)
	DT.B_10^{-4}/1	31.30	29.11	25.07	35.53	*Bacill*	Purple (+)
	DT.B_10^{-4}/2	32.73	28.17	19.84	29.48	*Staphylococci*	Red (-)
	DT.B_10^{-4}/3	31.69	26.25	23.59	29.42	*Streptobacill*	Purple (+)
	DT.C_10^{-4}/1	33.40	31.94	21.57	31.15	*Diplobacill*	Purple (+)
	DT.C_10^{-5}/1	31.79	23.97	18.58	29.52	*Staphylococci*	Purple (+)
	DT.C_10^{-5}/2	32.73	30.92	21.64	29.75	*Diplobacill*	Purple (+)
	Average ± stdev	31.46 ± 2.04	27.75 ± 2.56	21.63 ± 2.14	30.64 ± 2.01		
III Near the sea	DL.A_10^{-4}/1	26.76	35.02	28.15	33.32	*Coccobacill*	Purple (+)
	DL.A_10^{-5}/1	26.10	30.19	26.52	29.34	*Bacill*	Red (-)
	DL.B_10^{-4}/1	31.10	28.54	28.04	30.70	*Diplobacill*	Purple (+)
	DL.B_10^{-4}/2	31.45	29.21	25.17	35.73	*Coccobacill*	Purple (+)
	DL.C_10^{-4}/3	33.06	25.28	18.61	30.80	*Diplococci*	Purple (+)
	DL.C_10^{-5}/1	35.55	30.72	17.63	32.29	*Staphylococci*	Purple (+)
	Average ± stdev	30.67 ± 3,65	29.83 ± 3.18	24.02 ± 4.71	32.03 ± 2.28		

Information: DI: Near industry, DT: Near the pond, DL: Near the sea, A: Point 1, B: Point 2, C: Point 3, Numbers (1,2,3): Pure bacterial isolate, (10^{-4}; 10^{-5}): Dilution rate, C [1]: Chloramphenicol ($S \geq 18$; $I = 13\text{-}17$; $R \leq 12$), TE [2]: Tetracycline ($S \geq 19$; $I = 15\text{-}18$; $R \leq 14$), CIP [3]: Ciprofloxacin ($S \geq 21$; $I = 16\text{-}20$; $R \leq 15$), E [4]: Erythromycin ($S \geq 23$; $I = 14\text{-}22$; $R \leq 13$): Resistant isolate

Among the bacterial isolates from Station 1 (near industry), two exhibited resistances to ciprofloxacin: isolate DI.C_10^{-5}/2, with an inhibition zone diameter of 15.39 mm, and isolate DI.C_10^{-5}/3, with a diameter of 15.19 mm (Table 3). According to CLSI guidelines, ciprofloxacin resistance is indicated by an inhibition zone of ≤15 mm. Both resistant isolates displayed characteristics of purple bacteria, specifically streptobacilli and diplobacilli, categorizing them as Gram-positive.

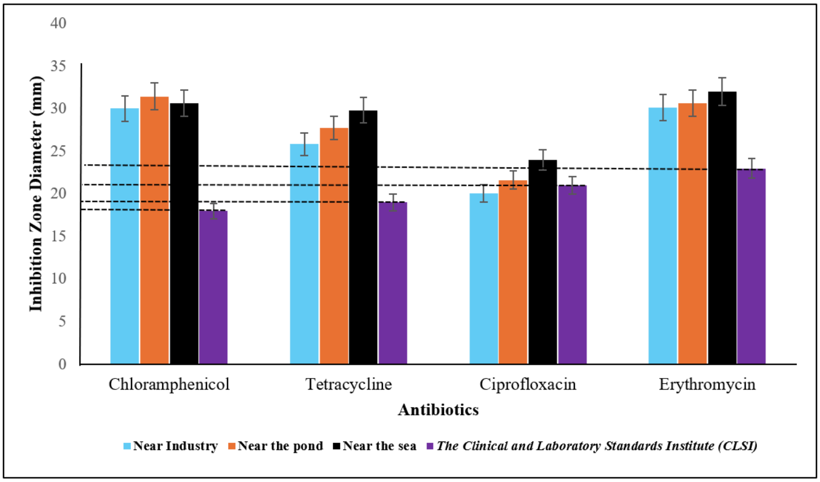

Figure 2. Average zone of inhibition bacterial isolates at each station from sediment of Tapak River, Semarang

As illustrated in Figure 2, the average inhibition zone of sediment bacterial isolates from the Tapak River Estuary demonstrated the highest sensitivity to chloramphenicol compared to erythromycin, tetracycline, and ciprofloxacin. The average inhibition zones for chloramphenicol were as follows: Station I (near industry) recorded 30.02 mm ± 5.24, Station II (near pond) 31.64 mm ± 2.04, and Station III (near sea) 30.67 mm ± 3,65. In contrast, ciprofloxacin showed a trend towards bacterial resistance, with average inhibition zones measuring 20.07 mm ± 5.86 at Station I, 21.63 mm ± 2.14 at Station II, and 24.02 mm ± 4.71 at Station III. Overall, ciprofloxacin exhibited the lowest inhibition effect among the tested antibiotics. However, these findings indicate that 91.30% isolates were sensitive to antibiotics. In contrast to findings from the Tinto River Estuary in Spain, it was discovered that 92.7% of bacterial isolates showed resistance to four or more classes of antibiotics (Eduardo-Correia *et al*., 2020). The variation in results between Tapak and the Tinto River Estuary might be attributed to the differing numbers of isolates and antibiotics tested. Thus, the limitations of this study include the number of bacterial isolates, the antibiotics tested, and the exclusive focus on sediment.

Antibiotic resistance in aquatic bacteria is often attributed to wastewater discharge. The estuarine waters of the Tapak River in Semarang serve as a final receptacle for the Jumbleng River, Silandak River, Tapak River, and Tugurejo River, leading to the accumulation of pollutants in the estuary. The high population density contributes to the inflow of both domestic and industrial waste. Domestic waste results from the activities of local residents, while industrial waste is discharged by companies in the food processing and chemical sectors. Notably, the food processing and preservation industry accounts for 31.43% of the industrial activities in the Tugurejo District, followed by the chemical and pharmaceutical industry (20%), textile industry (11.43%), packaging (5.71%), wood and furniture (8.75%), and metal or machinery industries (17.41%) (Astrini *et al.,* 2014). These industries produce organic and inorganic waste released into the river systems.

The relatively low prevalence of resistant bacteria in the waters of the Tapak River Estuary may be influenced by rainfall. Sampling conducted in January 2024 coincided with the onset of the rainy season, which may dilute the concentration of wastewater in the water body. Furthermore, seawater can act as a natural coagulant (Permana *et al.,* 2014; Liang *et al.,* 2013). Applying this coagulant during the coagulation/flocculation process has effectively reduced turbidity, colour, and natural organic matter from wastewater and leachate, producing clean potable water (Soedjono *et al.,* 2021). Moreover, as reported by Liang *et al*. (2013), as salinity levels rose, the concentration of antibiotics in the water noticeably diminished, implying that the introduction of seawater significantly diluted the antibiotics. When the pH increases to a range of 7-9, it can convert the cationic form of an antibiotic into its non-ionized form in an aqueous solution, leading to an increase in its log K_{ow} (Wunder *et al.,* 2011). A higher log K_{ow} value enhances the adsorption of pollutants onto sediments (Baker *et al.,* 1997). This aligns with the study's findings that the waters exhibit high salinity (26-32‰) and low sediment pH (5-7), reducing the resistance of bacteria collected from the sampling site. In addition, antibiotics that accumulate in sediments can exert selective pressure on antibiotic-resistant microbes (Luo *et al*., 2010). The limited presence of resistant bacteria in the Tapak River Estuary suggests that antibiotics such as erythromycin, ciprofloxacin, tetracycline, and chloramphenicol remain viable options for treating bacterial infections originating from these waters.

3.4 Molecular identification of bacterial samples

Bacterial identification focused on isolates exhibiting the highest resistance among all tested samples. The most resistant isolates to ciprofloxacin, DI.C_10^{-5}/3, recorded an inhibition zone of 15.19 mm. BLAST search results revealed that the highest similarity score corresponded to *Bacillus licheniformis*, with a percent identity of 99.75% (Table 4). Query coverage refers to the percentage of the nucleotide sequence that aligns with entries in the BLAST database.

Table 4. BLAST Search Result on DI.C_10^{-5}/3 Isolate

Sample Code	Closest Family	Query Cover	E-Value	Per cent Identity	Access Number
DI.C_10^{-5}/3	*Bacillus licheniformis*	99%	0.0	99.75%	PP718307.1

The resistant bacterial isolates are believed to produce endospores, as endospore formation is typically associated with Gram-positive bacteria (Schmidth, 2019). Endospores are dormant, resilient structures that can withstand extreme environmental conditions, including ultraviolet radiation, desiccation, high temperatures, extreme cold, and exposure to toxic chemicals (Wahyuni *et al.,* 2023). Endospore-forming bacteria have been isolated from diverse environments, including soil, water, sediment, air, ice, and the intestines of humans and animals (Fauzaan *et al.,* 2022). The inherent resistance of endospores is significant as it confers substantial chemical and enzymatic resilience. Notable examples of endospore-forming bacteria include *Bacillus* and *Clostridium* species.

4. Conclusion and Recommendation

This research identified two bacterial isolates from the sediment of the Tapak River Estuary that are resistant to ciprofloxacin. The resistant isolates, classified as Gram-positive, include DI.C_10^{-5}/2, which exhibited an inhibition zone diameter of 15.39 mm, and DI.C_10^{-5}/3, with a diameter of 15.19 mm. The latter isolate was identified through universal 16S rRNA primers and showed a 99.75% similarity to *Bacillus licheniformis*. These findings underscore the importance of regularly monitoring resistant bacteria in aquatic environments to prevent the uncontrolled spread of antibiotic resistance. Additionally, monitoring water quality for aquatic organisms provides valuable information regarding public health awareness. Consequently, to gain a deeper understanding of their resistance mechanisms and concentrations in aquatic environments, as well as to evaluate their potential risks to aquatic ecosystems, further investigation into the microbial communities within these sediments is necessary. Furthermore, this study's limitations encompass the number of bacterial isolates, the range of antibiotics tested, and the sole focus on sediment.

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