









Original Research

Observing the proliferation of *Salmonella* sp. in Karnaphuli River: Catastrophic for the Riverbank dwellers

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Abstract

The quality of Karnaphuli River water has an important role in human infection and disease sheltered near Karnaphuli River along with the people of Chittagong. The once-powerful Karnaphuli River is severely contaminated, which could lead to the spread of bacterial infections. However, there is very little information available regarding the diversity of bacterial pathogens in Karnaphuli, particularly in the estuary area. The present study was conducted to systematically examine the prevalence of indicator and pathogenic microorganism *Salmonella* sp. in water and sediment samples along with their relationship with some physiochemical parameters like pH, salinity, DO, temperature, etc. during September 2021. A total of 6 water samples from 6 different stations were taken and samples were examined by the pour plate culture method along with chemical and biological tests for detection of *Salmonella* sp. This study showed that all water and sediment samples were positive for *Salmonella* sp. There were significant differences between Station no. 2, referred to as Firingee Bazar, and the 6th station, known as Soltgola. The present research shows that pollution indicators like DO, salinity and the amount of cells of bacteria prevail in the unhygienic and polluted situation of the river which is a clear picture of uncontrolled hazardous activity.

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1. Introduction

The proliferation of *Salmonella* sp. in river water and soil exemplifies the contamination of aquatic environments. Indubitably, contaminated water bodies are major sources of infectious diseases, causing suffering among residents inhabiting riverside areas. The prevalent reservoir of *Salmonella* is the gastrointestinal tract of many animals, including birds, reptiles, livestock and humans. Once released from the gastrointestinal tract through feces or exudates, *Salmonella* can be conveyed to water bodies through rainfall and surface runoff, highlighting its persistence in the environment. As a significant human pathogen, *Salmonella* is responsible for salmonellosis, which encompasses acute diarrheal disease, enteric fever, gastroenteritis, abdominal pain and vomiting. The prevalence of *Salmonella* sp. is a major global public health concern, with an estimated 93.8 million cases of illness and 155,000 deaths annually due to salmonellosis (Liu *et al.*, 2018). Despite this alarming prevalence, the specific pathways through which *Salmonella* contaminates local water sources remain poorly understood, underscoring the urgent need for further investigation.

Salmonella is a rod-shaped, flagellated, gram-negative facultative anaerobe that belongs to the family Enterobacteriaceae. According to the Centers for Disease Control and Prevention (CDC) nomenclature system, the genus *Salmonella* contains only two species—*S. enterica* and *S. bongori*—based on differences in their 16S rRNA sequence analysis. Notably, *Salmonella enterica* subsp. *enterica* accounts for

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approximately 99% of *Salmonella* infections in humans and warm-blooded animals, with over 1,400 serotypes identified. Among these, *S. enterica* subsp. *enterica* serotypes *typhi* and *paratyphi* are predominantly transmitted through water, causing enteric fever, while *S. enterica* subsp. *enterica* serotype Typhimurium has a broad host range and primarily causes gastroenteritis. This longstanding disease, resulting from *S. typhi* and *Salmonella enterica* serovar *paratyphi* (*S. paratyphi*), was among the deadliest during the time before antibiotics were available (Saha *et al.*, 2018). Studies indicate that enteric fever results in 2.9 million cases and 95,000 deaths globally, with about 200 cases per 100,000 person-years during the period of 2003 to 2004 (Naheed *et al.*, 2010).

The lifeline and major watercourse of the Chattogram region, the largest port city on the southeastern coast of Bangladesh, the Karnaphuli River plays a vital role in agribusiness, industries, and the necessities of local communities and wildlife. Approximately 300 industries, including 100 ship-breaking operations, 19 tanneries and 26 textile factories, discharge waste near the Karnaphuli River (Uddin and Jeong, 2021; Hossain, 2006). The Department of Environment (DoE) has reported that more than 350 metric tons of toxic substances are being released into the Karnaphuli River daily (Hossain, 2006). Additionally, 5,000 tons of household wastes are discharged into the river every day through 36 canals of Chattogram city, containing pathogenic microorganisms, including *Salmonella* sp., thereby polluting surface water and sediment. This rampant contamination raises critical concerns regarding the persistence of *Salmonella* sp. in these waters, posing significant health risks to the local population dependent on this water source.

Moreover, industrial waste contains high concentrations of heavy metals, leading to stress factors that promote the formation of biofilms by *Salmonella* (Sallami *et al.*, 2022). Such biofilms, complex surface-associated communities, are implicated in approximately

80% of human bacterial infections (Borges et al., 2018). Pollution from industrial effluents and household waste destabilizes physiochemical parameters, with the DoE reporting that many characteristics of the Karnaphuli River fall below recommended standards, affecting the presence and persistence of *Salmonella* sp. (Uddin and Jeong, 2021). The use of contaminated water for drinking, household or recreational purposes, as well as the use of riverbank sediment for agriculture, poses severe health risks. The World Health Organization and the Environment Quality Standard for Bangladesh stipulate that the standard value for *Salmonella* sp. in drinking water should be nil/ml, yet the Karnaphuli River contains ~18,000 coliforms/100 ml near sewage disposal areas, indicating significant contamination (Dey et al., 2017).

The levels of *Salmonella* sp. in the water and sediment of the Karnaphuli River are expected to be significantly higher in areas with greater pollution loads, which may correlate with increased incidences of waterborne diseases among the local population. While existing research has addressed the contamination of the Karnaphuli River, comprehensive studies investigating the persistence and ecological dynamics of *Salmonella* sp. in both water and sediment are still lacking, particularly concerning industrial pollution and its impact on public health. This study aims to evaluate the persistence of *Salmonella* sp. in the water and sediment of the Karnaphuli River, providing critical insights into its environmental dynamics. The findings will enhance the understanding of *Salmonella's* role in waterborne disease transmission and may inform local health policies and pollution management strategies, ultimately promoting public health and environmental sustainability in the Chattogram region.

2. Materials and Methods

2.1 Ethical approval

No ethical approval is required for this study.

2.2 Sampling area

The sampling sites were selected right from the upstream to downstream of the river limited to the area dominated by pollution status (Figure 1; Table 1). The upstream locations are significant and abound in sewage activities from the drainage system of Chittagong city.

Table 1. Description and GPS coordinates of the sampling points.

Station names	Latitude	Longitude
1. Chaktai Khal Matha	22.32526	91.84862
2. Firingee Bazar	22.32628	91.83854
3. Avaymitra ghat	22.32568	91.83699
4. Sadar ghat	22.32349	91.83005
5. Banglabazar Ghat	22.31954	91.8177
6. Solt gola	22.30167	91.79741

The six selected sampling stations are known as Chaktai Khal Matha, Firingee Bazar Ghat, Avaymitra Ghat (which is close to the most polluted water channel in Chittagong city known as Rajakhal), Sadarghat, Bangla Bazar ghat and Saltgola respectively showed in Figure 1.

There are about 300 factories and industrial establishments along the 100-kilometer area of the Karnaphuli extending from Chandraghona to the mouth of the river at Patenga, including cement factories, fertilizer manufacturers, paper mills, rayon mills, tanneries, oil refineries, power plants, dyeing and washing plants (Bhuyan and Islam, 2017). Among 36 canals that are connected to the Karnaphuli river, some significant canals known as Mohesh Khal, Chaktai Khal, Murari Khal, Khondokia Khal, Boalkhali canal, BoRubi Gate Khal, Maizpara Khal, and Rajakhali Khal, dump raw and partially decomposed sewages, solid wastes, domestic organic wastes and untreated industrial discharge into the Karnaphuli river (Hossain et al., 2006).

Chaktai Khal, also known as the grief of Chittagong City, plays the most dominant role in the environment of the Karnaphuly River by dumping a large amount of solid and liquid wastes from the industrial,

commercial, and residential areas adjacent to it. Again, the Saltgola canal contains a high amount of chemical waste from CEPZ and port-based industries which contain many heavy mineral particles. Fish wastage, human wastage, oil spills of fishing vessels, market waste, etc. also caused pollution at Firingee Bazar Ghat and Sadarghat area of Karnaphuli River. Besides, there are 400 slaughterhouses in the Firingee Bazar and Dewanhat areas alone. The blood from the slaughtered animals directly finds its way into the river. Moreover, about 50,000 sanitary and 24,000 unhygienic traditional latrines of the port city are directly linked to the study area of this river (Islam et al., 2016).

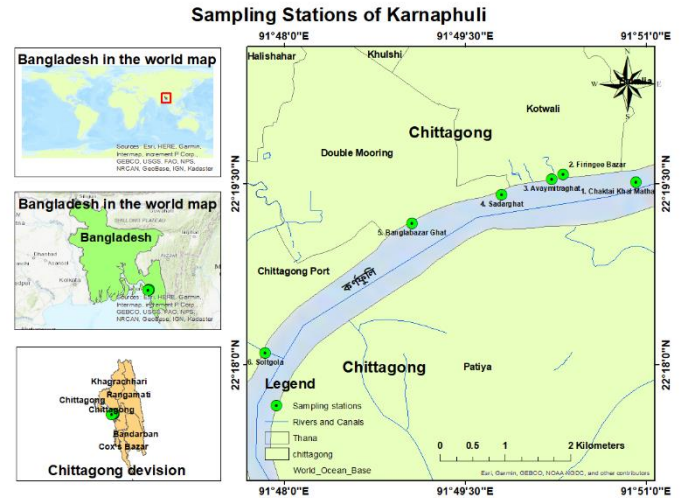


Figure 1. Study area map showing the sampling stations.

2.3 Sample collection and analysis with preservation

The present work was carried out in September 2021 just after heavy rain. Water and soil samples were collected from six different stations of the Karnaphuli River estuary after extreme high tide when low tide started during this study period. In situ measurement water temperature was measured by a centigrade thermometer where water pH and salinity were measured by a pen pH meter and refractometer respectively. Dissolve Oxygen (DO) concentration was measured in the laboratory by the standard method (APHA, 2017). Water samples from three different stations were collected from 500 ml. bottles from the Karnaphuli River estuary with screw-capped and sealed with aluminum foil paper to control the bacterial growth before proceeding in an ice box at about 4 °C temperature until brought to the laboratory of the Department of Oceanography, CU. Soil samples were also collected from six same stations with grab samplers and stored in 500 g jars. During the sampling, at first, the grab samplers were throughout to the bottom which carries a lot of soil filled with jars and then capped under an icebox and brought to the specified laboratory.

2.4 Bacterial isolation and enumeration

The diversity of pathogenic bacteria (*Salmonella* sp.) in water and soil was identified by standard plate count (SPC) techniques (Some et al., 2021). The above methods for enumeration of pathogenic bacteria were because each of them gives characteristics type of colony confirmed by further gram staining, biochemical, and fermentation tests for accuracy.

As we are searching for *Salmonella* sp., we used specific agar media Bismuth sulfite agar (BSA) particularly useful for the isolation of lactose-fermenting Salmonellae. *S. typhi*, *S. enteritidis*, and *S. typhimurium* typically grow as black colonies with a surrounding metallic sheen resulting from hydrogen sulfide production and reduction of sulfite to black ferric sulfide.

For isolation from the water sample, aseptically 1 ml suspension from diluted suspensions of 10⁻¹ and 10⁻² was poured into BSA agar plates for isolation of *Salmonella* sp. and gently rotated for spreading the suspension. Agar plates were incubated for 24 hours at 37 °C. In

the case of sediment, 1 g sediment from each sample was suspended into 9 ml sterile distilled water and serially diluted to suspensions of 10⁻², and 1 ml suspension from 10⁻¹ and 10⁻² dilution was poured into BSA agar plates for isolation and incubated for 48 hours in 37 °C. After 24 hours, all the black colonies with a surrounding metallic sheen are counted. The number of colonies is multiplied by the dilution factor and the mean result from dilution 10⁻¹ and 10⁻² plates is recorded. The cultures were identified according to the bacteriological analytical manual (BAM) of the USFDA. 2 colonies from Petri dishes are further tested (gram staining, Indole test, methyl red test, urease test Voges Proskauer test, starch hydrolysis test, catalase activity test, and fermentation test) for confirmation of *Salmonella* sp.

2.5 Data analysis

The relation between the physical parameters and *Salmonella* sp. from water and sediment are statistically analyzed with R programming and visualized with the correlogram showing similarity and dissimilarity among the parameters along with *Salmonella* sp. The result and the spatial distribution of *Salmonella* sp. are visualized with ArcGIS (10.8).

3. Results and Discussion

Assembled samples were collected from the surface water and sediment at the interval time prevailed ending of high tide and the beginning of low tide (Table 2).

Table 2. Different types of parameters, HT-LT= high tide to low tide.

Sample	Station	Time	Tidal Situation	Depth of sample taken (m)	Odor	Color	Pollution for visible eyes	Temperature(°C)	Salinity(ppt)	DO(mg/L)	pH	Ec (mS/m)
Water	S1	12:04 pm	HT-LT	Surface	Highly Pungent	Muddy	Extremely polluted	30	1.1	4.05	7.5	0.22
	S2	12:19 pm	HT-LT	Surface	Highly Pungent	Oily black	Extremely polluted	30.3	0.9	3.54	7.4	0.22
	S3	12:26 pm	HT-LT	Surface	Pungent	Turbid color	Highly polluted	28.9	1	4.05	7.6	0.22
	S4	12:38 pm	HT-LT	Surface	Extremely Pungent	Oily black	Extremely polluted	30.4	1.3	3.3	7.4	0.25
	S5	1:10 pm	Near LT	Surface	Extremely Pungent	Muddy	Less Polluted	30.4	2.6	4.5	7.4	0.29
	S6	1:23 pm	Near LT	Surface	Odorless	Turbid color	Highly polluted	30.6	5.8	5.06	7.1	8
Sediment	S1	12:07 pm	HT-LT	3.05	Highly Pungent	Blackish	Extremely polluted	30	0	0	6.65	0
	S2	12:21 pm	HT-LT	4.57	Extremely Pungent	Blackish	Extremely polluted	30.3	0	0	6.73	0
	S3	12:28 pm	HT-LT	2.45	Pungent	Blackish	Highly polluted	28.9	0	0	6.5	0
	S4	12:39 pm	HT-LT	2.13	Extremely Pungent	Black & Ash	Extremely polluted	30.4	0	0	6.76	0
	S5	1:11 pm	Near LT	4.26	Extremely Pungent	Black & Ash	Less Polluted	30.4	0	0	6.55	0
	S6	1:25 pm	Near LT	1.82	Odorless	Brownish	Highly polluted	30.6	0	0	6.2	0

In a detailed comparative assessment of water and sediment samples across six stations (St-1 to St-6), a consistent tidal situation of HT-LT (after extreme high tide when low tide started) was noted for most samples, with stations St-5 and St-6 presenting data near LT. Surface water samples predominantly exhibited alarming signals of environmental distress: the odor ranged from pungent to extremely pungent; visual inspection revealed colors from muddy to oily black; and all stations, except St-5, presented extremely to highly polluted conditions. Temperature values hovered around 30 °C, with salinity fluctuating between 0.9 to 5.8 ppt and DO levels varying from 3.3 to 5.06 mg/L. Notably, St-6 had a remarkably higher salinity of 5.8 ppt. The pH levels remained consistent, averaging around 7.4, and the Ec, intriguingly, soared to 8 mS/m at St-6. In the sedimentary analysis, all stations demonstrated profound pollution levels, with colors ranging from blackish to brownish. The depth of these sediment samples varied from 1.82 to 4.57 m, and all exhibited a lack of salinity, DO, and an average pH value lower than that of water samples, suggesting distinct yet alarming ecological disparities between water and sediment profiles (Bhuyan and Islam, 2017).

In an evaluation of water quality parameters across six distinct stations, temperature remained relatively consistent, ranging from 28.9 °C to 30.6 °C, suggesting minimal thermal variation across the sites. Salinity displayed a more diverse profile, with values ranging from 0.9 ppt at Firingee Bazar to 5.8 ppt at Soltgola. Dissolved oxygen levels fluctuated between 3.3 mg/L and 5.06 mg/L, but no discernible correlation with *Salmonella* sp. concentrations was evident. Notably, pH measurements were predominantly neutral, barring an outlier at Soltgola with an elevated pH of 8. Electrical conductivity was largely consistent, yet an anomalous value of 8 mS/m at Soltgola warrants further investigation. Preliminary observations do not indicate a direct

correlation between the assessed physicochemical parameters and *Salmonella* sp. Counts (Counts are given in supplementary file as table), necessitating a more expansive dataset for robust statistical analysis (Hossain et al., 2006).

In analyzing the interdependencies among environmental parameters positive correlations are depicted in shades of blue, while negative correlations are represented by shades of red (Figure 2).

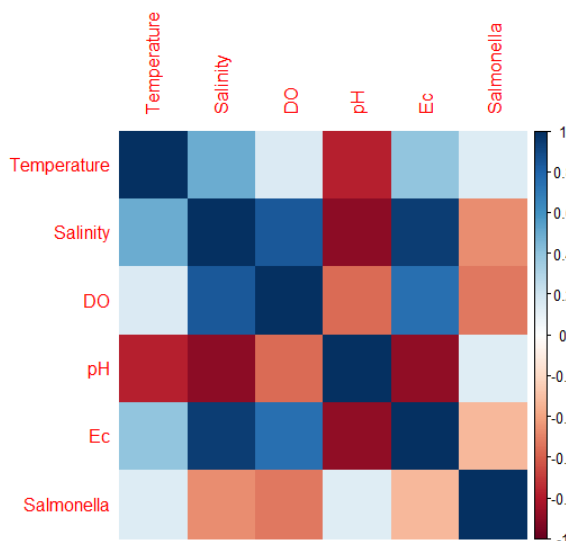


Figure 2. The correlogram represents the correlations for all pairs of physical parameters with *Salmonella* sp. distribution in water.

We observe the temperature exhibits a positive moderate correlation with salinity ($r=0.497$) suggesting that rising temperatures might be concomitant with increasing salinity levels. Additionally, there's a subtle positive association with both DO ($r=0.158$) and Ec ($r=0.399$), whereas a notably strong negative correlation is discerned with pH ($r=-0.772$). The relationship between temperature and *Salmonella* prevalence is weak yet positive ($r=0.147$). Salinity shows a particularly strong positive linkage with DO ($r=0.848$) and Ec ($r=0.752$), while manifesting a pronounced negative correlation with pH ($r=-0.895$) and a moderate one with *Salmonella* ($r=-0.467$). DO levels inversely correlate with pH ($r=-0.567$) and directly with Ec ($r=0.752$), and a decrease in *Salmonella* prevalence appears concomitant with increasing DO levels ($r=-0.527$). Furthermore, pH values and Ec illustrate a strong inverse relationship ($r=-0.881$) and only a marginal positive association between pH and *Salmonella* is observed ($r=0.133$). Lastly, an increase in Ec seems to be mildly associated with a decrease in *Salmonella* presence ($r=-0.327$). As expected, all parameters correlate perfectly with themselves ($r=1.0$). This comprehensive analysis provides critical insights into the intricate relationships among the evaluated environmental parameters (Some et al., 2021).

The status of *Salmonella* sp. in sediment and water sample is shown in Figures 3 and 4. *Salmonella* sp. is found more in water than in sediment. In the case of water, the 1st and 2nd stations Chaktai Khal matha and Firingee Bazar are mostly threatened by the colossal concentration of *Salmonella* sp. due to their locations and hazardous condition already described in sampling area. In between these two sites, the 2nd station, Firingee Bazar is the dominant reservoir of *Salmonella* sp. containing 1725 cells/ml. Among the six stations, the 5th and 6th stations comprise the lowest concentration of *Salmonella* sp. containing 250 cells/ml, significantly higher than the recommended standard level.

In the case of sediment, 1st and 2nd stations have a high concentration of bacteria whereas 2nd station holds 365 cells/ml of *Salmonella* sp. which is the highest concentration among the six stations.

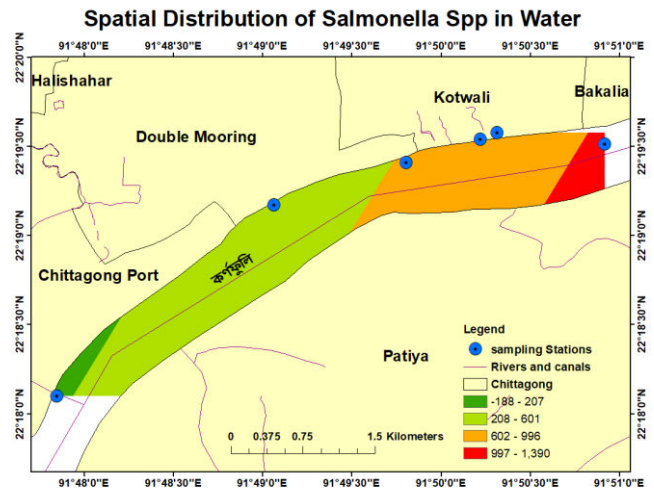


Figure 4. Spatial distribution of *Salmonella* sp. in water.

Table 1. Morphological tests and studies of *Salmonella* sp.

Sample	Growth on Bismuth Sulfite Agar	Growth	Sulfite	Gram staining		Identification of bacterial genus
				Shape	Arrangement	
Water	Black colonies	Good growth,	Very fine growth	Short rod	Single	<i>Salmonella</i> sp.
Soil	Black colonies	Growth is fine but abundant	Turbidity found	Short Rod	Single	<i>Salmonella</i> sp.

Table 2. Bio-chemical test results of bacteria colony.

	Water	Soil
Gram staining test	Negative	Negative
Indole test	-	-
Methyl red test	+	+
Voges Proskauer test	-	-
Urea test	-	-
Catalase test	-	-
Starch Hydrolysis test	-	-
Bacteria	<i>Salmonella</i> sp.	<i>Salmonella</i> sp.

*+ = positive reaction or result; - = negative reaction or result.

Fermentation tests showed variable gas and acid production, with both samples fermenting glucose, maltose, and mannitol, while lactose was not fermented (Table 4). These findings confirm the pathogenic nature of *Salmonella* sp. and its potential threat to public health due to the environmental contamination of the river (Liu et al., 2018).

Table 3. Fermentation test result of bacteria colony.

	Water	Soil
Glucose	G	A
Sucrose	G	A/G
Lactose	G	A
Maltose	A/G	A
Mannitol	A/G	A/G
Bacteria	<i>Salmonella</i> sp.	<i>Salmonella</i> sp.

*A= acid produced; G = gas produced; A/G =acid or gas produced

4. Conclusions

The once-mighty river Karnaphuli, which shaped the lives of its bank dwellers, now harbors life-threatening pathogenic *Salmonella* sp. This study demonstrated that high concentrations of *Salmonella* sp. were found in both the water and sediment of the Karnaphuli River, indicating a severe public health threat associated with the consumption of water and soil from this river. The health implications for the riverside residents are alarming, as many suffer from various *Salmonella* infections, including acute diarrhea, enteric fever and abdominal pain. Throughout the study period, detected bacterial concentrations of *Salmonella* sp. in both water and sediment exceeded standard levels at all sampling stations. The growth of

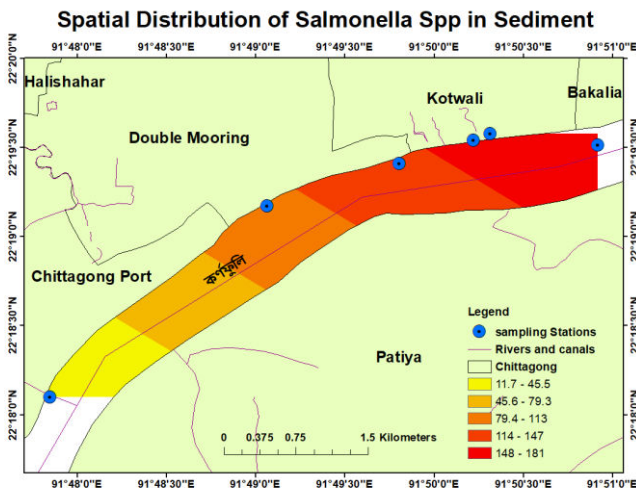


Figure 3. Spatial distribution of *Salmonella* sp. in sediment.

The morphological, biochemical, and fermentation studies conducted on *Salmonella* sp. from water and soil samples of the Karnaphuli River confirmed its presence in both environments. Morphological analysis using Bismuth Sulfite Agar showed characteristic black colonies, with Gram staining identifying the bacteria as short, rod-shaped, and arranged singly, indicative of *Salmonella* sp. (Table 2).

Biochemical tests revealed that the isolates were Gram-negative, methyl red-positive, and indole, Voges-Proskauer, urea, catalase, and starch hydrolysis-negative, consistent with the known traits of *Salmonella* (Table 3).

nutrient overflow and land runoff has contributed to a marked increase in *Salmonella* colony growth, reflecting the deteriorating status of the river. While several investigations have been conducted on various water bodies within the Karnaphuli system, further research is crucial to comprehensively assess the presence of diverse waterborne pathogens. If unaddressed, the situation in the Karnaphuli River area and broader Chattogram region will lead to substantial public health challenges due to salmonellosis, as the river water becomes increasingly unsuitable for consumption and other uses. Addressing these issues is essential for safeguarding the health of local communities and restoring the integrity of this vital waterway.

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Data availability

All data generated or analyzed during this study are included in this published article.

Informed consent statement

Not required.

Conflict of interest

The authors report there are no competing interests to declare.

Authors' contribution

Conceptualization: Md. Wahidul Alam and Md. Atiqul Islam Mondal; **Data collection:** All authors involved in data collection; **Data analysis:** Md. Jahin Khandakar; **Figure preparation:** Md. Jahin Khandakar and Fowzia Aktar; **Manuscript writing and editing:** Most. Israt Jahan, MD. Mosfiqur Rahman and Fowzia Aktar. All authors critically reviewed the manuscript and agreed to submit the final version of the manuscript.

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