

## Research

# Investigation and detection of multiple antibiotic-resistant pathogenic bacteria in municipal wastewater of Dhaka city

Abu Bakkar Siddique<sup>1</sup> · Atia Munni<sup>1</sup> · Maruf Hasan<sup>1</sup> · Rayhan Raj<sup>1</sup> · Md. Abdul Mutalib<sup>1</sup> · Md. Tajuddin Sikder<sup>1</sup> · Tatsufumi Okino<sup>2</sup> · Ayesha Ahmed<sup>1</sup> · Md. Shakhaoat Hossain<sup>1,3</sup> 

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## Abstract

**Background** Water pollution in densely populated urban areas, mainly from municipal wastewater, poses a significant threat. Pathogenic bacteria, such as *Vibrio* spp. and fecal coliform, endanger public health and the environment. Additionally, antibiotic-resistant bacteria in wastewater complicate treatment and heighten public health concerns.

**Methods** The study sampled municipal wastewater from ten Dhaka neighborhoods, selecting treatment plants, sewage outlets, and various collection points using meticulous techniques for representative samples. Bacteriological and biochemical analyses were conducted using standardized methods. Antimicrobial susceptibility testing (AST) was performed with the disk diffusion method against 13 widely used antibiotics.

**Results** All sampled areas exhibited positive results for *Vibrio* spp., fecal coliform, *E. coli*, and *Salmonella* spp. Varying bacterial concentrations were observed, with the highest concentration of TVC, total *vibrio* spp., and total fecal coliform, total *E. coli* count, and total *Salmonella* spp. were found in Uttara ( $1.9 \times 10^4$  CFU/ml), Bangshal ( $1.8 \times 10^2$  CFU/ml), and Lalbag ( $2.1 \times 10^3$  CFU/ml), Mirpur ( $3.70 \times 10^2$  CFU/ml), and Lalbag ( $6 \times 10^2$  CFU/ml) respectively. AST results revealed significant resistance among all bacterial species to various antibiotics. Specifically, *Vibrio* spp. showed 100% resistance to cefuroxime, fecal coliform exhibited 90% resistance to cephadrine, *E. coli* demonstrated 60% resistance to cephadrine, and *Salmonella* spp. displayed 90% resistance to ampicillin.

**Conclusion** The study highlights the existence of multiple antibiotic-resistant bacteria in Dhaka's wastewater. Addressing antibiotic resistance is essential to manage the risks of multiple antibiotic-resistant infections and maintain antibiotic effectiveness. These implications are critical for various stakeholders, including public health officials, policymakers, environmentalists, and urban planners.

**Keywords** Multiple antibiotic-resistant pathogenic bacteria · *Vibrio* spp. · Fecal coliform · *E. coli* · *Salmonella* spp. · Municipal wastewater · Dhaka city

## Abbreviations

AST Antimicrobial susceptibility testing

CFU/ml Colony-forming units per milliliter

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✉ Ayesha Ahmed, [ayasha@juniv.edu](mailto:ayasha@juniv.edu); ✉ Md. Shakhaoat Hossain, [shakhaoat@juniv.edu](mailto:shakhaoat@juniv.edu) | <sup>1</sup>Department of Public Health and Informatics, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh. <sup>2</sup>Faculty of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan. <sup>3</sup>Air Quality, Climate Change and Health (ACH) Lab, Department of Public Health and Informatics, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.



CIP	Ciprofloxacin
CL	Colistin
COT	Cotrimoxazole
CTX	Cefotaxime
CXM	Cefuroxime
<i>E. coli</i>	<i>Escherichia coli</i>
MAR	Multiple antibiotic-resistant
MEM	Meropenem
NMAR	Non-multiple antibiotic-resistant
Spp.	Species (plural)
TVC	Total viable count

## 1 Introduction

Water pollution continues to be a critical global issue, especially in densely populated urban areas where municipal wastewater serves as a major source of contamination [1]. Furthermore, waterborne diseases pose a substantial threat to public health in these urban settings, as municipal wastewater can become a breeding ground for pathogenic bacteria [2]. Among the various pathogens found in wastewater, *Vibrio* spp. and fecal coliform bacteria stand out due to their ability to cause severe illnesses, presenting challenges to both environmental safety and public health [3]. *Vibrio* is a genus of Gram-negative bacteria characterized by a curved-rod structure, some of which can cause foodborne infections [4, 5]. Certain species of *Vibrio* are known to be pathogenic, primarily causing gastroenteritis, but they can also infect open wounds and lead to sepsis [6]. Pathogenic *Vibrio* species include *V. cholera*, *V. parahaemolyticus*, and *V. vulnificus* [4].

Fecal coliforms are facultatively anaerobic, rod-shaped, gram-negative, and non-sporulating bacteria [7]. Fecal coliforms are widely found in large amounts in the lower digestive tracts and feces of humans and other warm-blooded animals [8]. If they are found in water, it usually means the water has been recently contaminated by feces, which suggests a higher risk of harmful germs being present compared to just finding total coliform bacteria [9, 10]. Fecal coliforms serve as markers for enteric pathogenic pathogens [11, 12]. Some fecal coliforms, such as *E. coli*, can cause severe gastrointestinal illnesses, including diarrhea, abdominal cramps, and vomiting [13]. Pathogenic strains may also lead to more serious conditions like urinary tract infections, respiratory illnesses, and even life-threatening conditions such as septicemia [14]. The pathogenic forms of *E. coli* can cause severe intra- and extraintestinal infections in mammals and birds [15, 16].

*Salmonella* is a genus of rod-shaped gram-negative bacteria of Enterobacteriaceae [17]. The two known species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori* [18]. *Salmonella* species are non-spore-forming, predominantly motile enterobacteria. *Salmonella* species are intracellular pathogens, of which specific serotypes cause illness. Most infections result from ingesting food contaminated with feces and from drinking water contaminated with feces [19].

Dhaka city, home to over 22.4 million of residents, faces a dual challenge: ensuring adequate sanitation facilities for its citizens while grappling with escalating water pollution in its municipal wastewater [20, 21]. Rapid urbanization, inadequate sewage treatment, and improper disposal practices have contributed to the contamination of water bodies, creating an environment conducive to the proliferation of pathogenic bacteria [20, 22]. The emergence of these multiple antibiotic-resistant bacteria further complicates the situation, raising concerns about the effectiveness of conventional treatment methods and the potential implications for public health [23, 24].

Moreover, studying antibiotic resistance in municipal wastewater is crucial due to its direct connection with human households, serving as a primary source of contamination [25]. As the population grows and antibiotic usage increases, resistant bacteria enter wastewater, creating a reservoir of antibiotic-resistant strains [26]. Understanding these resistance patterns in municipal wastewater is vital because it reflects the antibiotic resistance landscape within communities. Monitoring wastewater helps identify emerging antibiotic-resistant strains, enabling proactive measures in healthcare and environmental policies [27]. By studying resistance in this context, scientists gain insights into the prevalence and dissemination of antibiotic-resistant genes, guiding the development of effective strategies to curb the spread of antibiotic-resistant bacteria and safeguard public health [27].

Additionally, our capacity to cure common diseases is still in danger due to the creation and spread of antibiotic-resistant organisms that have developed new resistance mechanisms, or antimicrobial resistance [28]. With the increasing

global development of multi- and pan-resistant bacteria, it is quite impossible to tackle these in a limited-resourced country like Bangladesh.

Bacteria with multiple antibiotic resistance (MAR) is a major global public health concern [29]. Therefore, research on multiple antibiotic-resistant (MAR) microorganisms is crucial. The purpose of this study is to examine and identify several antibiotic-resistant pathogenic bacteria (fecal coliform and *Vibrio* spp.) in Dhaka city's municipal wastewater. To date, no study has studied multiple antibiotic-resistant pathogenic bacteria (*Vibrio* spp. and fecal coliform) in municipal wastewater of Dhaka city. Additionally, this research is essential for multiple stakeholders, including public health officials, policymakers, environmentalists, and urban planners [30]. By comprehending the prevalence and antibiotic resistance patterns of *Vibrio* spp. and fecal coliform bacteria in Dhaka's municipal wastewater, tailored interventions can be developed. The findings will aid in refining sewage treatment methods, enhancing water quality monitoring systems, and shaping evidence-based public health policies.

## 2 Materials and methods

### 2.1 Sampling area and period of study

The study area for our research comprises ten neighborhoods in Dhaka (five samples from Dhaka north region- Kallayanpur, Rampura, Mohammadpur, Mirpur, Uttara, five samples from Dhaka south region- Dhanmondi, Sayedabad, Lalbag, Sadarghat, Bangshal) which were identified to represent the municipal wastewater system scattered throughout whole Dhaka city (see Fig. 1). Locations such as wastewater treatment plants, sewage outlets, and other points of municipal wastewater collection or discharge were included in the selection. A meticulous sampling technique was used. This study is cross sectional in nature and samples were collected in August 2023.

### 2.2 Sample collection

Before sample collection, all necessary equipment was sterilized, including obtaining sterile containers with tight-fitting lids, wearing gloves, and having disinfectant (70% ethanol solution), labels, and data collection sheets ready. Precautions included wearing disposable gloves and additional personal protective equipment like lab coats. Sampling points near inflow/outflow pipes and areas with significant wastewater flow were chosen. Equipment was disinfected by autoclaving, and samples were collected by submerging sterile containers into the wastewater stream, ensuring no air bubbles. The sampling process was conducted at multiple locations within each study area (composite sampling), ensuring the samples were representative and accounted for any potential spatial variations in antimicrobial resistance [31]. From each composite sample, 40 ml was collected and transferred to sterile Falcon tubes, providing more than enough quantity for laboratory analyses. Samples were immediately stored in ice-filled cooler/ice box at 4 °C and transported to the laboratory within 12 h. Samples were tightly sealed, labeled, and transported with care [32, 33]. After reaching the lab, samples were immediately subjected to bacteriological analyses using validated protocols [34, 35]. Detailed records were maintained, ensuring each sample's clear chain of custody. These meticulous techniques were crucial for providing reliable and representative wastewater samples from Dhaka city for studying antibiotic-resistant pathogenic bacteria.

### 2.3 Sample processing

To prepare the water samples, 1 ml of the sample was mixed with 9 ml of normal saline solution in a test tube. This mixture underwent initial dilution, followed by a series of dilutions from 1 to 5. Each serial dilute was marked in different test tubes.

### 2.4 Bacteriological analyses

The tests were conducted following the International Organization for Standardization (ISO) 2023 guidelines for all the species [36]. In the procedure, selective agar plates were prepared according to the manufacturer's instructions. After dilution, using a glass spreader (spread plate method), the diluted sample was spread over Oxoid UK's Thiosulfate Citrate Bile Sucrose agar (TCBS) and incubated at 37 °C for 24 h. TCBS-agar served as a selective medium for *Vibrio* spp., and plates were inspected for typical colonies, usually yellow and green [37]. For fecal coliform isolation, diluted water

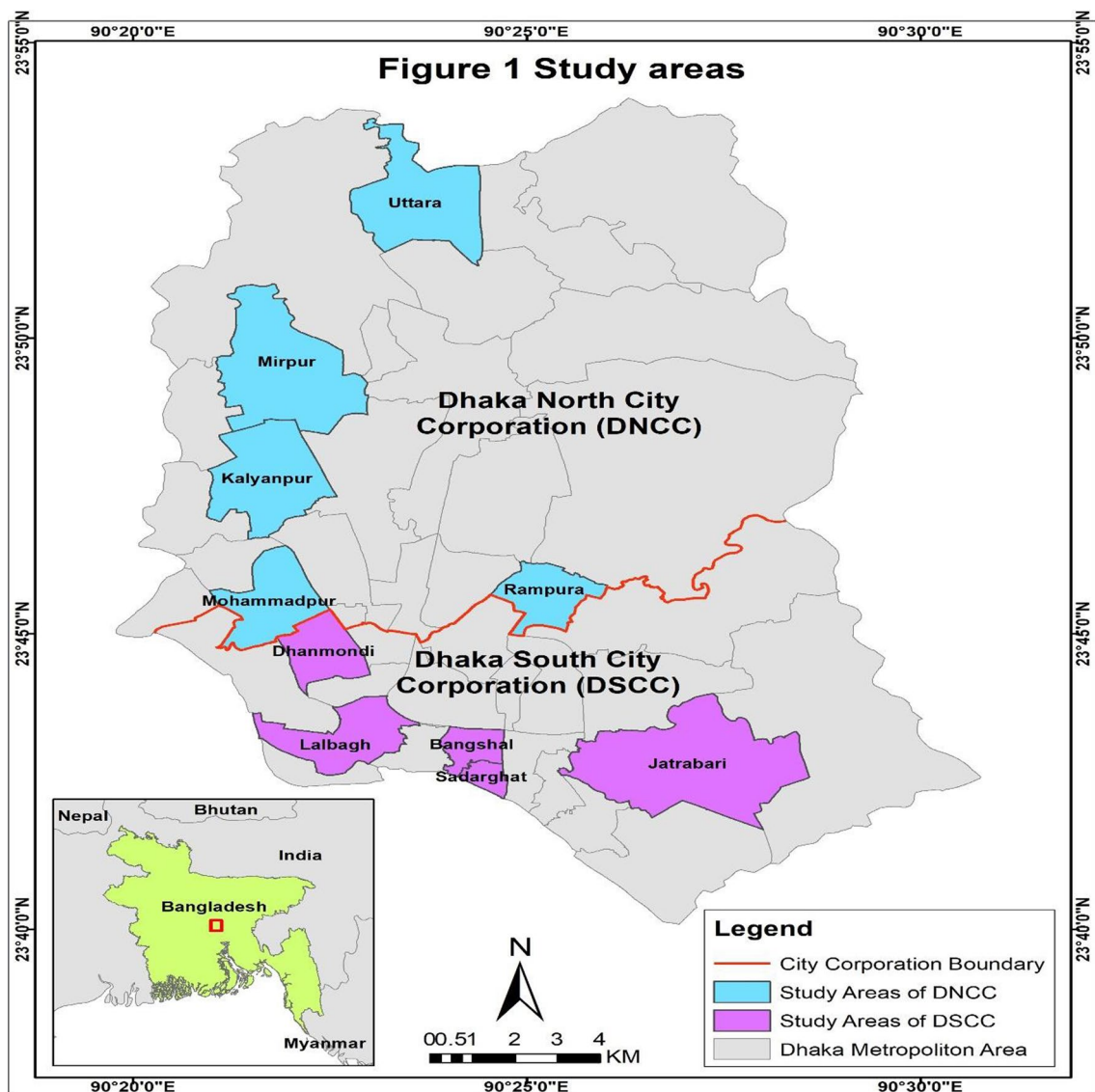


Fig. 1 Study areas

samples were spread evenly onto the surface of Oxoid UK's m-FC agar plate using a glass spreader. The plate was then placed in an incubator at 44 °C for 24–48 h, allowing fecal coliforms to grow and produce characteristic colonies [35]. Following incubation, colonies on the m-FC agar plate were observed. Total viable count (TVC), total *Vibrio* count, total fecal coliform count, total *E. coli* count, and total *Salmonella* count from wastewater samples were counted using colony counter machine. These procedures ensured adherence to standardized testing methods as outlined in validated studies [34, 35]. The same procedure was followed for the *E. coli* and *Salmonella* spp. Isolates. EMB (Eosin methylene blue agar) and SS (Salmonella-Shigella) agar were used for selective media of *E. coli* and *Salmonella* spp., respectively [38]. See the figures of isolates plates at supplementary files.

## 2.5 Biochemical confirmation

In the wastewater analysis, a battery of tests was performed to identify the bacterial species. For *Vibrio* spp., fecal coliforms, *E. coli*, and *Salmonella* spp., gram staining revealed a pink/red color, confirming their gram-negative nature [39]. Catalase activity was detected in all isolates (*Vibrio* spp., fecal coliforms, *E. coli*, and *Salmonella* spp.), as indicated by the formation of bubbles upon the addition of hydrogen peroxide [39]. Positive results for oxidase were observed, signified by the development of a purple color when the oxidase reagent was applied to *Vibrio* spp [40]. On the other hand, fecal

coliforms, *E. coli*, and *Salmonella* spp. were negative for oxidase, as evidenced by the absence of a color change with the oxidase reagent. Moreover, each bacterial isolate was examined under a microscope for detecting the motility and morphological characteristics. These tests conclusively confirmed the presence of *Vibrio* spp. and fecal coliforms in our wastewater samples, offering valuable insights for our study. Our principal investigator, a prominent authority in this field, ensured the quality of this work throughout the study.

## 2.6 Antimicrobial susceptibility testing (AST)

Several subcultures were performed to enhance the purification of specific bacteria. Bacterial susceptibility to antimicrobial agents was determined using the disk diffusion method, following the guidelines established by Bauer et al. [41]. Thirteen antibiotic discs (Oxoid Ltd., Basingstoke, Hampshire, UK) containing Meropenem (10 g), Cefuroxime (30 g), Chloramphenicol (30 g), Cefotaxime (30 g), Tetracycline (30 g), Colistin (30 g), Nalidixic Acid (30 g), Azithromycin (15 g), Erythromycin (15 g), Ampicillin (10 g), Cephadrine (30 g), Ciprofloxacin (5 g), and Cotrimoxazole (25 g) were utilized. Mueller–Hinton agar (Merck®) was employed according to the manufacturer's instructions for the antimicrobial susceptibility testing medium. Within 15 min of the application of the discs, the plates were inverted and incubated at 37 °C. After 16–18 h of incubation, the plates were examined and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The antimicrobial susceptibility testing (AST) was conducted three times, and the average zone diameters were calculated for final categorization. The mean zone diameters for each antimicrobial agent were then classified as susceptible, intermediate, or resistant based on the interpretation table provided by following CLSI guidelines in 2020 [42]. See the figures of the AST plates in the supplementary section.

## 2.7 Grouping of the multiple antibiotic resistant (MAR) isolates

Based on the occurrence of resistance to more than three antibiotics, the isolates of each sampling site were grouped as multiple antibiotic-resistant isolates [43].

## 2.8 Ethics declarations

We affirm that this manuscript is original and has not been previously presented or published within this university or institution. Furthermore, all methods outlined in the manuscript were conducted in accordance with relevant guidelines and regulations.

## 2.9 Statistical analysis

The analysis involved Microsoft Excel (version 2021) and the Statistical Package for Social Sciences (SPSS version 26.0). Microsoft Excel was utilized for data cleaning, coding, and organization, following which the processed data was imported into SPSS software to compute descriptive statistics including frequencies, percentages, and correlations.

# 3 Result

## 3.1 Bacterial concentration

All the samples (10 out of 10) were positive for *Vibrio* spp., fecal coliform, *E. coli*, and *Salmonella* spp. Table 1 presents the concentration of different bacteria in various areas. The highest concentration of TVC, total *vibrio* spp., and total fecal coliform, total *E. coli* count, and total *Salmonella* spp. were found in Uttara ( $1.9 \times 10^4$  CFU/ml), Bangshal ( $1.8 \times 10^2$  CFU/ml), and Lalbag ( $2.1 \times 10^3$  CFU/ml), Mirpur ( $3.70 \times 10^2$  CFU/ml), and Lalbag ( $6 \times 10^2$  CFU/ml) respectively.

**Table 1** Enumeration of Total viable count, total fecal coliform count, and total vibrio count from municipal wastewater

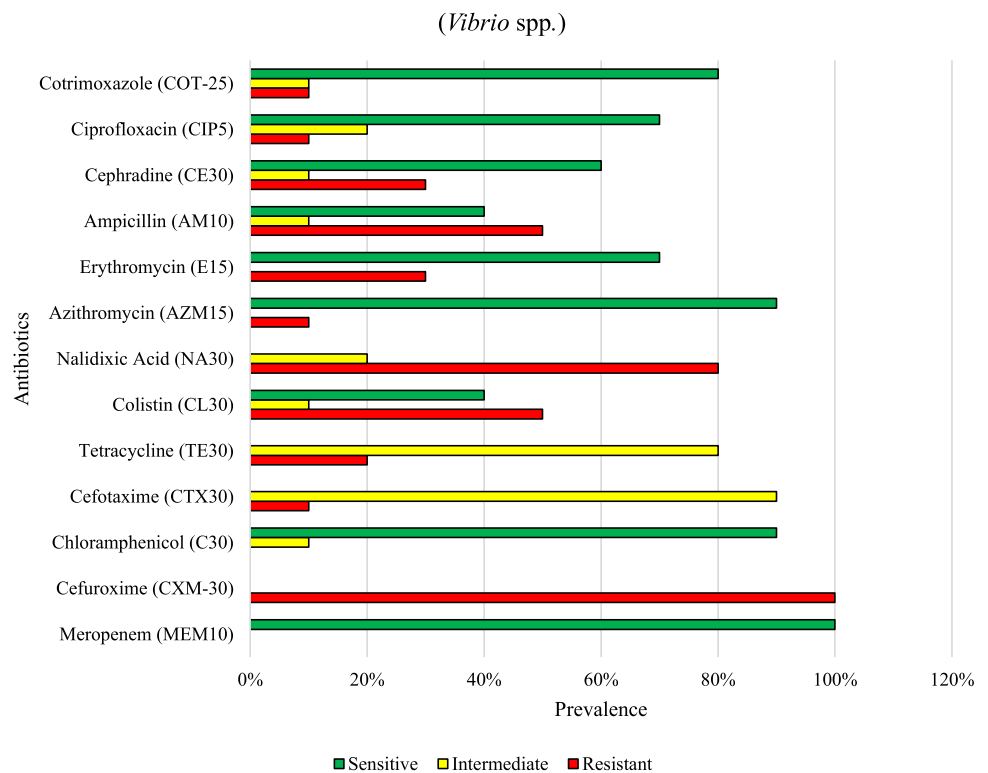
Sample ID	Samples	Total viable count (CFU/ml)	<i>Vibrio</i> spp. count (CFU/ml)	Fecal coliform count (CFU/ml)	Total <i>Salmonella</i> spp. count (CFU/ml)	Total <i>Escherichia coli</i> count (CFU/ml)
1	Kallyanpur	$1.2 \times 10^4$	$3.1 \times 10^1$	$2.8 \times 10^2$	$4 \times 10^1$	$8 \times 10^1$
2	Rampura	$1.2 \times 10^3$	$1.06 \times 10^1$	$6 \times 10^1$	$6.3 \times 10^1$	$2 \times 10^1$
3	Mohammadpur	$1.19 \times 10^4$	$3.06 \times 10^1$	$4.35 \times 10^2$	$4.8 \times 10^1$	$7. \times 10^1$
4	Mirpur	$1.7 \times 10^4$	$6.32 \times 10^1$	$4.5 \times 10^2$	<b><math>3.70 \times 10^2</math></b>	$4.5 \times 10^1$
5	Uttara	<b><math>1.9 \times 10^4</math></b>	$5.06 \times 10^1$	$5 \times 10^1$	$2.28 \times 10^2$	$6 \times 10^1$
6	Dhanmondi	$1.8 \times 10^4$	$1.27 \times 10^2$	$2.5 \times 10^2$	$4.5 \times 10^1$	$4 \times 10^1$
7	Sayedabad	$1.6 \times 10^4$	$4.75 \times 10^1$	$2.9 \times 10^1$	$7.5 \times 10^1$	$2 \times 10^1$
8	Lalbag	$1.6 \times 10^4$	$1.1 \times 10^2$	<b><math>2.1 \times 10^3</math></b>	$1.5 \times 10^2$	<b><math>6 \times 10^2</math></b>
9	Sadarghat	$1.5 \times 10^3$	$1.0 \times 10^2$	$2.6 \times 10^1$	$2.6 \times 10^1$	$3 \times 10^1$
10	Bangshal	$1.74 \times 10^4$	<b><math>1.8 \times 10^2</math></b>	$3.65 \times 10^1$	$3.5 \times 10^1$	$5 \times 10^1$

Bold indicates the highest bacterial concentration for each bacteria  
 CFU Colony forming unit

### 3.2 AST results

Figure 2 The prevalence of resistance among the tested *Vibrio* spp. strains was observed for selected antibiotics. Cefuroxime exhibited complete resistance (100%), while Cefotaxime faced intermediate in 90% of cases. Tetracycline showed an 80% intermediate rate, and Colistin was resistant in 50% of instances. Nalidixic Acid exhibited an 80% resistance rate, Ampicillin was resistant in 50% of cases, and Cephradine showed 30% resistance. Ciprofloxacin demonstrated 10% resistance, and Cotrimoxazole displayed a 10% resistance rate. These results highlight the presence of

**Fig. 2** Antibiotic resistance to the selected isolates (*Vibrio* spp.)



resistant *Vibrio* spp. strains, emphasizing the need for careful antibiotic selection to manage infections and mitigate antibiotic resistance effectively.

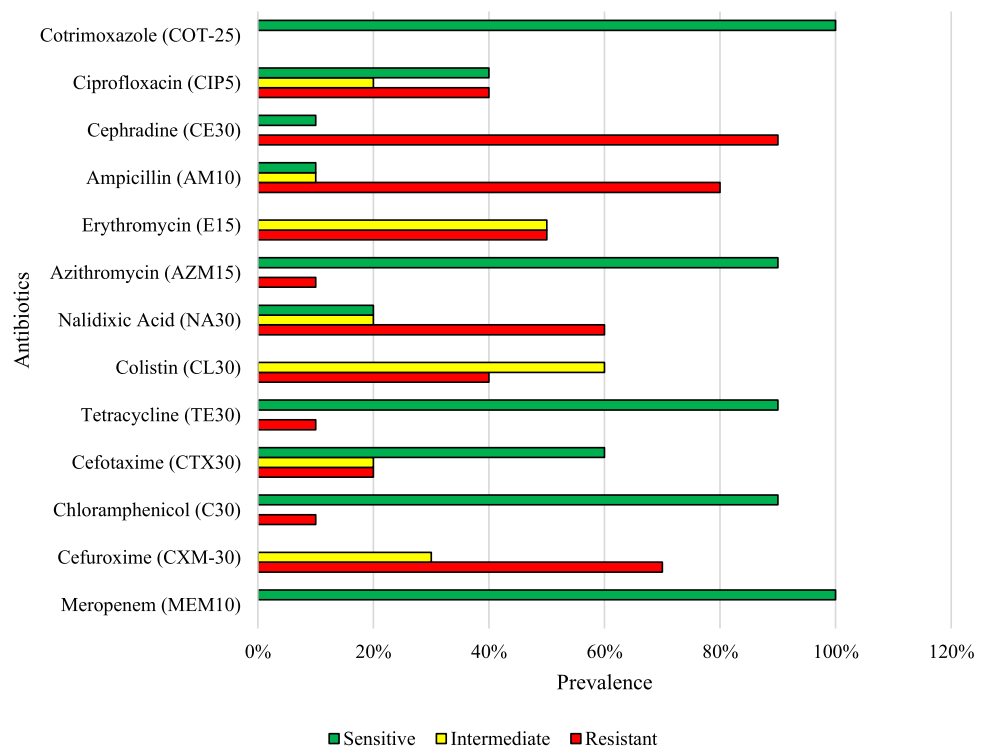
Figure 3 demonstrates the prevalence of resistance among the tested fecal coliform bacteria varied for the antibiotics. Cefuroxime (CXM-30) exhibited significant resistance, with 70% of isolates being resistant. Chloramphenicol (C30) encountered resistance in 10% of isolates, while Colistin (CL30) faced resistance in 40% of isolates. Ciprofloxacin (CIP5) displayed a resistance rate of 40%. Erythromycin (E15) exhibited 50% resistance, and Ampicillin (AM10) faced resistance in 80% of isolates. Cephradine (CE30) showed a high resistance rate of 90%. Conversely, Meropenem (MEM10), Cotrimoxazole (COT-25), and Tetracycline (TE30), Azithromycin (AZM15) displayed no or least resistance among the tested isolates.

The antibiotic resistance profile of the selected *Escherichia coli* (*E. coli*) isolates was assessed for various antibiotics, as depicted in Fig. 4. Notably, the isolates showed high sensitivity to Meropenem (MEM10) and Cefotaxime (CTX 30), with 80% and 60% susceptibility, respectively. Conversely, significant resistance was observed against certain antibiotics, including Cefuroxime (CXM-30) and Ampicillin (AM10), with 50% and 50% resistance rates, respectively. Intermediate resistance was noted for some antibiotics, such as Cefotaxime (CTX30) and Cotrimoxazole (COT-25), indicating a moderate level of susceptibility. The isolates exhibited an exceptionally high sensitivity to Erythromycin (E15) and Azithromycin (AZM15), with resistance rates of only 10% for both antibiotics.

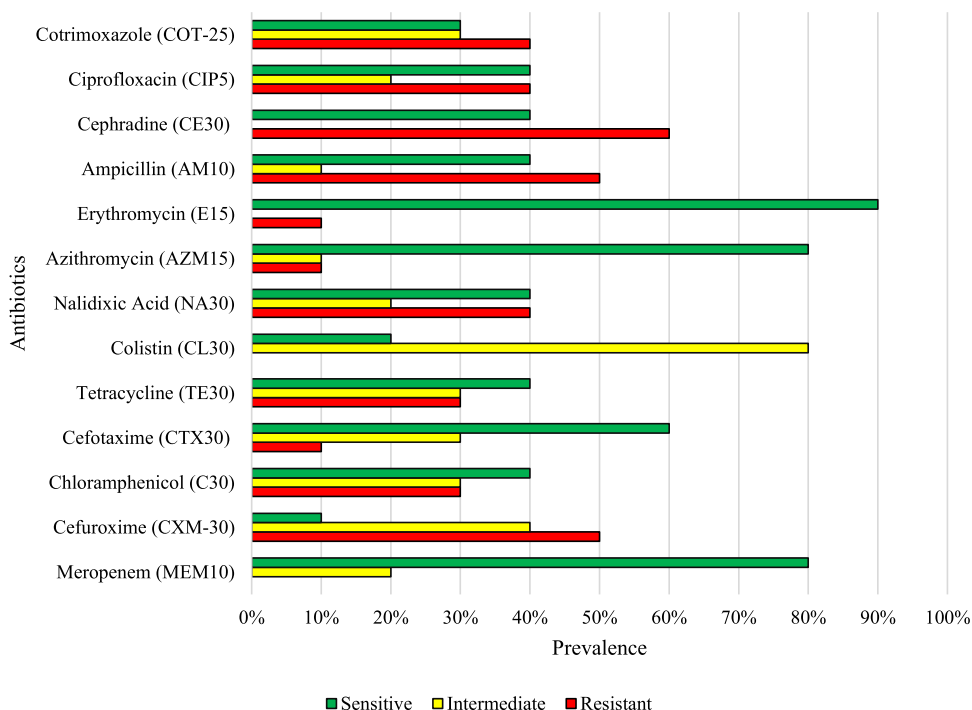
Figure 5 demonstrates the antibiotic resistance profile of the selected *Salmonella* spp. isolates is presented in. The isolates showed varying degrees of resistance to the tested antibiotics. Notably, meropenem (MEM10) exhibited 100% sensitivity, while cefotaxime (CTX30) and ampicillin (AM10) showed high resistance rates of 80% and 90%, respectively. Ciprofloxacin (CIP5) displayed a balanced resistance pattern, with 20% resistance, 40% intermediate susceptibility, and 40% sensitivity. Colistin (CL30) revealed 50% resistance and 50% intermediate susceptibility. Overall, the isolates exhibited diverse antibiotic resistance patterns, underscoring the importance of prudent antibiotic use and continuous monitoring to address emerging resistance trends in *Salmonella* spp.

These findings emphasize the challenges of antibiotic resistance in microbial infections and underscore the importance of careful antibiotic use to combat resistance effectively [44]. See details in Figs. 2, 3, 4, and 5. *The supplementary section* provides the correlation matrixes for the antibiotics used among all four types of microbes.

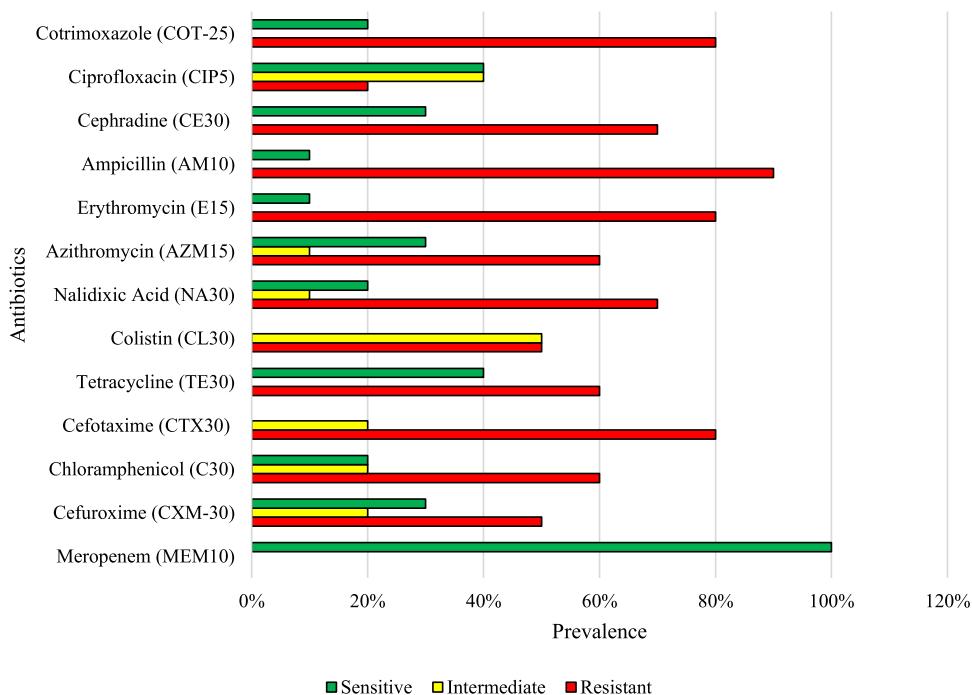
**Fig. 3** Antibiotic resistance to the selected isolates (fecal coliform)



**Fig. 4** Antibiotic resistance to the selected isolates (*E. coli*)



**Fig. 5** Antibiotic resistance to the selected isolates (*Salmonella* spp.)



### 3.3 MAR

Table 2 reveals the antibiotic resistance profiles of *Vibrio* spp. strains from different sources. Among them, Kallayanpur, Rampura, Mohammadpur, Dhanmondi, Sayedabad, Lalbag, Sadarghat, and Bangshal exhibit multiple antibiotic-resistant (MAR) patterns, indicating resistance to multiple antibiotic classes, posing significant challenges for effective treatment. In contrast, Mirpur and Uttara display non-multiple antibiotic-resistant (NMAR) patterns, denoting resistance to specific antibiotics. Mohammadpur, Lalbag, and Sadarghat strains have particularly high MAR Indices



**Table 2** Antibiotic resistance patterns of selected isolates (*Vibrio* spp.) from wastewater

Sample ID Vibrio	Sources	Phenotypic resistance	MAR index	Resistance classification
1	Kallayanpur	CXM-CL-E-AM-CE	0.38	MAR
2	Rampura	CXM-NA-E-AM	0.31	MAR
3	Mohammadpur	CXM-TE-NA-AZM-E-AM	0.46	MAR
4	Mirpur	CXM	0.08	NMAR
5	Uttara	CXM-NA	0.15	NMAR
6	Dhanmondi	CXM-NA-CE	0.23	MAR
7	Sayedabad	CXM-CL-NA	0.23	MAR
8	Lalbag	CXM-CTX-TE-CL-NA-AM	0.46	MAR
9	Sadarghat	CXM-CL-NA-AM-CE-CIP-COT	0.54	MAR
10	Bangshal	CXM-CL-NA	0.23	MAR

*MAR Index*: number of resistant antibiotics/total number of antibiotics tested, *MEM* Meropenem (10 g), *CXM* Cefuroxime (30 g), *C* Chloramphenicol (30 g), *CTX* Cefotaxime (30 g), *TE* Tetracycline (30 g), *CL* Colistin (30 g), *NA* Nalidixic Acid (30 g), *AZM* Azithromycin (15 g), *E* Erythromycin (15 g), *AM* Ampicillin (10 g), *CE* Cephadrine (30 g), *CIP* Ciprofloxacin (5 g), *COT* Cotrimoxazole (25 g), *MAR* multiple antibiotic resistant, *NMAR* non multiple antibiotic resistant

(0.46 and 0.54), suggesting widespread antibiotic resistance. Additionally, Table 3 demonstrates the antibiotic resistance profiles of fecal coliform. Strains from same sources all the samples exhibit multiple antibiotic-resistant (MAR) patterns, indicating resistance to multiple antibiotic classes, posing significant challenges for effective treatment. Kallayanpur, Uttara, and Lalbag strains have particularly high MAR Indices (0.46), suggesting widespread antibiotic resistance. This emphasizes the urgent need for vigilant antibiotic use and continuous surveillance to curb the escalation of multiple antibiotic-resistant bacterial infections and preserve the effectiveness of available antibiotics [45].

Table 4 outlines the antibiotic resistance patterns of selected *E. coli* isolates from various wastewater sources. The isolates from different locations exhibited diverse phenotypic resistance profiles, indicating resistance to specific antibiotics. The multiple antibiotic resistance (MAR) Index, a measure of the number of antibiotics an isolate is resistant to, ranged from 0.08 to 0.77. Notably, isolates from Kallayanpur, Rampura, Mohammadpur, Mirpur, Dhanmondi, Sayedabad, Lalbag, Sadarghat, and Bangshal were classified as multiple antibiotic-resistant (MAR) based on their resistance to multiple antibiotic classes. In contrast, the Uttara isolate demonstrated a relatively low MAR Index of 0.08, categorizing it as non-multiple antibiotic-resistant (NMAR). This data underscores the prevalence of multiple

**Table 3** Antibiotic resistance patterns of selected isolates (fecal coliform) from wastewater

Sample ID Fecal coliform	Sources	Phenotypic resistance	MAR index	Resistance classification
1	Kallayanpur	CXM-CL-NA-AM-CIP-CE	0.46	MAR
2	Rampura	CXM-C-TE-CL-E	0.38	MAR
3	Mohammadpur	CXM-CL-E-AM-CE	0.38	MAR
4	Mirpur	CXM-E-AM-CE	0.31	MAR
5	Uttara	CXM-CTX-CL-AZM-CIP-CE	0.46	MAR
6	Dhanmondi	NA-AM-CE	0.23	MAR
7	Sayedabad	NA-E-AM-CE-CIP	0.38	MAR
8	Lalbag	CXM-CTX-NA-E-AM-CE	0.46	MAR
9	Sadarghat	NA-AM-CIP-CE	0.31	MAR
10	Bangshal	NA-AM-CIP-CE	0.31	MAR

*MAR Index*: number of resistant antibiotics/total number of antibiotics tested, *MEM* Meropenem (10 g), *CXM* Cefuroxime (30 g), *C* Chloramphenicol (30 g), *CTX* Cefotaxime (30 g), *TE* Tetracycline (30 g), *CL* Colistin (30 g), *NA* Nalidixic Acid (30 g), *AZM* Azithromycin (15 g), *E* Erythromycin (15 g), *AM* Ampicillin (10 g), *CE* Cephadrine (30 g), *CIP* Ciprofloxacin (5 g), *COT* Cotrimoxazole (25 g), *MAR* multiple antibiotic resistant, *NMAR* non multiple antibiotic resistant

**Table 4** Antibiotic resistance patterns of selected isolates (*E. coli*) from wastewater

Sample ID <i>E. coli</i>	Sources	Phenotypic resistance	MAR index	Resistance classification
1	Kallayanpur	CXM-E-AM	0.23	MAR
2	Rampura	NA-AM-CE-COT	0.31	MAR
3	Mohammadpur	CXM-TE-NA-AZM-E-CIP	0.46	MAR
4	Mirpur	CXM-E-AM-CE	0.31	MAR
5	Uttara	E	0.08	NMAR
6	Dhanmondi	E-AM-CE-CIP-COT	0.38	MAR
7	Sayedabad	CXM-C-CTX-TE-NA-E-AM-CE-CIP-COT	0.77	MAR
8	Lalbag	CXM-E-AM-CE	0.31	MAR
9	Sadarghat	C-E-AM	0.23	MAR
10	Bangshal	C-TE-NA-E-AM-CE-CIP-COT	0.62	MAR

*MAR Index*: number of resistant antibiotics/total number of antibiotics tested, *MEM* Meropenem (10 g), *CXM* Cefuroxime (30 g), *C* Chloramphenicol (30 g), *CTX* Cefotaxime (30 g), *TE* Tetracycline (30 g), *CL* Colistin (30 g), *NA* Nalidixic Acid (30 g), *AZM* Azithromycin (15 g), *E* Erythromycin (15 g), *AM* Ampicillin (10 g), *CE* Cephadrine (30 g), *CIP* Ciprofloxacin (5 g), *COT* Cotrimoxazole (25 g), *MAR* multiple antibiotic resistant, *NMAR* non multiple antibiotic resistant

antibiotic resistance among *E. coli* isolates in wastewater, emphasizing the need for effective surveillance and control measures to mitigate the spread of antibiotic resistance.

Table 5 presents the antibiotic resistance patterns of *Salmonella* spp. isolates from various wastewater sources. The phenotypic resistance profiles reveal multiple antibiotic resistances in these isolates. The multiple antibiotic resistance (MAR) Index, calculated based on resistance to different antibiotics, ranges from 0.23 to 0.85. All sources demonstrate a high MAR Index, suggesting exposure to various antibiotics. The Resistance Classification indicates that all isolates from different locations are classified as multiple antibiotic-resistant (MAR), emphasizing the widespread and concerning resistance patterns observed across these environmental samples. The findings underscore the urgency of monitoring and addressing antibiotic resistance in wastewater to mitigate potential public health risks associated with resistant strains of *Salmonella* spp.

**Table 5** Antibiotic resistance patterns of selected isolates (*Salmonella* spp.) from wastewater

Sample ID <i>Salmonella</i>	Sources	Phenotypic resistance	MAR index	Resistance classification
1	Kallayanpur	CXM-C-CTX-TE-CL-AM-CE-COT	0.62	MAR
2	Rampura	C-CTX-TE-CL-NA-AZM-E-AM-CE-CIP-COT	0.85	MAR
3	Mohammadpur	C-CTX-CL-NA-AZM-E-CIP-COT	0.62	MAR
4	Mirpur	CXM-CTX-NA-AZM-E-AM-COT	0.54	MAR
5	Uttara	CTX-TE-NA-AZM-E-AM-CE-COT	0.62	MAR
6	Dhanmondi	CXM-CTX-TE-CL-AZM-E-AM-CE-COT	0.69	MAR
7	Sayedabad	C-E-AM	0.23	MAR
8	Lalbag	CXM-CTX-TE-NA-AZM-E-AM-CE-COT	0.69	MAR
9	Sadarghat	C-NA-E-AM-CE	0.38	MAR
10	Bangshal	CXM-C-CTX-TE-CL-NA-E-AM-CE-COT	0.77	MAR

*MAR Index*: number of resistant antibiotics/total number of antibiotics tested, *MEM* Meropenem (10 g), *CXM* Cefuroxime (30 g), *C* Chloramphenicol (30 g), *CTX* Cefotaxime (30 g), *TE* Tetracycline (30 g), *CL* Colistin (30 g), *NA* Nalidixic Acid (30 g), *AZM* Azithromycin (15 g), *E* Erythromycin (15 g), *AM* Ampicillin (10 g), *CE* Cephadrine (30 g), *CIP* Ciprofloxacin (5 g), *COT* Cotrimoxazole (25 g), *MAR* multiple antibiotic resistant, *NMAR* non multiple antibiotic resistant

## 4 Discussion

The findings of this study underscore the concern for the presence of multiple antibiotic-resistant pathogenic bacteria, namely *Vibrio* spp. and fecal coliform, within the municipal wastewater of Dhaka city. All the collected samples tested positive for *Vibrio* spp. and fecal coliform, signifying a pervasive contamination across the various locations sampled, including Kallyanpur, Rampura, Mohammadpur, Mirpur, Uttara, Dhanmondi, Sayedabad, Lalbag, Sadarghat, and Bangshal.

The enumeration of bacterial concentrations in these samples revealed varying levels of total viable count (TVC), total *Vibrio* count, and total fecal coliform count. The highest concentrations of TVC, *Vibrio* spp., and fecal coliform were notably found in Uttara, Bangshal, and Lalbag, respectively, suggesting diverse contamination levels at these specific locations. Similar findings were found in separate studies investigating antimicrobial resistance in Bangladesh [46, 47]. The high bacterial concentration in Bangshal and Lalbag may be primarily due to their higher population densities and associated increased wastewater production, inadequate sanitation, improper waste disposal, and poorly managed sewage systems in these areas. These factors collectively contribute to elevated bacterial counts in the wastewater [48, 49].

The Antibiotic Susceptibility Testing (AST) results unveiled significant resistance among the tested *Vibrio* spp. and fecal coliform strains to various antibiotics. *Vibrio* spp. strains exhibited high resistance to antibiotics such as Cefuroxime, Cefotaxime, Tetracycline, Colistin, Nalidixic Acid, Ampicillin, and Cephadrine, highlighting the urgent need for a cautious and precise selection of antibiotics for effective infection management and combating the rise of antibiotic resistance [50, 51]. A study conducted in South Africa found quite similar results to *Vibrio* isolates from wastewater [52]. The high resistance levels observed in bacteria can be attributed to several factors, including the overuse and misuse of antibiotics in human health, animal treatment, and environmental contamination resulting from the release of antibiotics [53–55].

Similarly, fecal coliform strains displayed resistance to antibiotics like Cefuroxime, Chloramphenicol, Colistin, Ciprofloxacin, Erythromycin, Ampicillin, and Cephadrine, emphasizing the challenges posed by antibiotic resistance in microbial infections and the critical role of judicious antibiotic use to counteract this resistance effectively [12, 56].

In comparison to the findings of previous studies, our results revealed both similarities and notable differences. Among the *E. coli* isolates high sensitivity to Meropenem (MEM10) and Cefotaxime (CTX 30) was consistent with some studies [57, 58], emphasizing the efficacy of these antibiotics. However, significant resistance against Cefuroxime (CXM-30) and Ampicillin (AM10) was observed, aligning with reports highlighting the emergence of resistance to commonly used antibiotics [58, 59]. The intermediate resistance observed for Cefotaxime (CTX30) and Cotrimoxazole (COT-25) suggests a moderate level of susceptibility, contrasting with studies reporting lower resistance rates. Notably, the high sensitivity to Erythromycin (E15) and Azithromycin (AZM15) implies potential alternative treatment options [60, 61].

The *Salmonella* spp. isolates displayed diverse antibiotic resistance patterns, with 100% sensitivity to Meropenem (MEM10) and high resistance rates to Cefotaxime (CTX30) and Ampicillin (AM10), which aligns with previous literature [62, 63]. The varying degrees of resistance observed in this study are consistent with findings from other studies, suggesting a global challenge in combating antibiotic resistance among *Salmonella* spp. strains [64, 65]. Ciprofloxacin (CIP5) displayed a balanced resistance pattern, with 20% resistance, 40% intermediate susceptibility, and 40% sensitivity. Colistin (CL30) revealed 50% resistance and 50% intermediate susceptibility, emphasizing the need for cautious use of these antibiotics to prevent further development of resistance [66, 67].

The observed multiple antibiotic-resistant (MAR) classification of *E. coli* isolates from various locations, with MAR Indices ranging from 0.08 to 0.77, aligns with global concerns about the widespread dissemination of multiple antibiotic resistance in environmental samples. The urgency of addressing antibiotic resistance in wastewater is evident, emphasizing the need for ongoing surveillance, prudent antibiotic use, and the development of strategies to mitigate the public health risks associated with resistant strains.

The detection of multiple antibiotic-Resistant (MAR) patterns in several sources signify resistance to multiple antibiotic classes, presenting significant challenges in treating bacterial infections. Particularly, locations like Kallyanpur, Mohammadpur, Lalbag, and Sadarghat exhibited high MAR Indices, indicating widespread resistance to various antibiotics, demanding immediate attention, and stringent surveillance to mitigate the escalation of multiple antibiotic-resistant bacterial infections [46, 47].

Overall, these findings emphasize the urgent need for heightened vigilance in antibiotic use, continual surveillance, and the development of precise treatment strategies to address the widespread antibiotic resistance observed in pathogenic bacteria within Dhaka's municipal wastewater. Taking immediate steps to address this issue is crucial in preserving the effectiveness of available antibiotics and managing the risks associated with multiple antibiotic-resistant bacterial infections.

## 4.1 Strength and limitations of the study

The study addresses a critical public health concern by investigating the prevalence of antibiotic-resistant pathogenic bacteria in municipal wastewater, focusing on *Vibrio* spp., fecal coliform, *E. coli*, and *Salmonella* spp. Additionally, this is the first study investigating these bacteria simultaneously from wastewater in Bangladesh. While offering valuable insights into Dhaka's municipal wastewater antibiotic resistance, this study has limitations. It focused on specific bacteria, excluding potential contributors to overall resistance. The study's snapshot in August 2023 may not represent evolving resistance patterns. Reliance on phenotypic profiles might miss genetic diversity; genomic studies could enhance understanding. The study concentrated on wastewater, lacking a holistic approach to antibiotic-resistant gene transmission among environmental, human, and animal sources. Specific sources of antibiotic contamination were not explored, hindering targeted interventions for pollution reduction. Additionally, the cross-sectional design hinders the establishment of causal relationships and a nuanced understanding of changes over time. A longitudinal or prospective study would be beneficial in this regard.

## 5 Conclusion

This study uncovers significant antibiotic-resistant bacteria, *Vibrio* spp., fecal coliform, *E. coli* and *Salmonella* spp. in Dhaka's municipal wastewater. Widespread contamination in neighborhoods like Kallyanpur, Rampura, Mohammadpur, Mirpur, Uttara, Dhanmondi, Sayedabad, Lalbag, Sadarghat, and Bangshal necessitates urgent interventions. Variable bacterial concentrations, particularly in Uttara, Bangshal, and Lalbag, reflect the impact of population density, wastewater production, and sanitation practices. Antibiotic Susceptibility Testing (AST) reveals considerable resistance in *Vibrio* spp. and fecal coliform, urging cautious antibiotic use. Challenges in *E. coli* and *Salmonella* spp. highlight the need for tailored treatments and continuous surveillance. The multiple antibiotic-resistant (MAR) classification, notably in Kallyanpur, Mohammadpur, Lalbag, and Sadarghat, poses a pressing challenge, requiring immediate attention, stringent surveillance, and targeted interventions to address widespread antibiotic resistance. The findings underscore the complexity of factors contributing to resistance, urging comprehensive measures to safeguard public health.

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**Data availability** Upon reasonable request, the corresponding author will provide access to all the data supporting this article.

## Declarations

**Ethics approval and consent to participate** No human or animal sample was used in this study.

**Consent for publication** Not applicable.

**Competing interests** The authors assert that there are no potential competing interests associated with the publication of this paper.

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