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Research Article
Antibiotic Resistance Profile of *Escherichia coli* and *Vibrio cholerae* in Water and Sediment of Padma River, Bangladesh

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Abstract
Background and Objective: Intrusion of wide range of antibiotics through the pollutants in river water makes the bacteria resistance to some specific antibiotics over a short period of time. Thus, the objective of this study was to determine antibiotic resistant pattern of *Escherichia coli* and *Vibrio cholerae* in water and sediment of Padma river to commonly used antibiotics. **Materials and Methods:** Multiple tube fermentation technique (MPN) and spread plate technique were used to the enumeration of *E. coli* and *V. cholerae* from water and sediment samples. Antimicrobial susceptibility testing was done by using Kirby-Bauer disc-diffusion method according to Clinical and Laboratory Standards Institute. **Results:** About 47 *Escherichia coli* and 38 *Vibrio cholerae* isolates were collected from water and sediment. The *E. coli* isolates were found highly resistant to ampicillin in water and to ciprofloxacin in sediment samples. The *V. cholerae* isolates showed the highest resistance to tetracycline and ciprofloxacin in water and sediment samples, respectively during summer season. During winter season, the highest resistance was obtained for ampicillin and for ciprofloxacin in water and sediment samples, respectively. The value of multiple antibiotic resistances (MAR) index crossed the high risk line at Site-2 (Padma garden) for both *E. coli* and *V. choleare*. **Conclusion:** It was concluded that the presence of multiple antibiotic resistant bacteria in Padma river is a serious concern for health status of the people inhabiting along the river.

Key words: Antibiotic resistance, *Escherichia coli*, *Vbrio cholerae*, MAR index, padma river, pollutants in river

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

No doubt that the problem of antimicrobial resistance of micro-organisms has become a significant public health concern worldwide[1,2] and especially in countries like Bangladesh where the antibiotic resistance pattern of its hundreds of river water is remain unrevealed. The river Padma (one of the largest river of Bangladesh) is situated along the Rajshahi city of Bangladesh, considered a very vital source of drinking water and irrigation, as well as for recreation along the region of Rajshahi city. It also serve as a reservoir for many micro-organisms from municipal waste as well as illegal dumping from industries, hospitals, households and from agricultural pollutants[3]. Therefore, use of this water for recreational activities together with fishing, drinking, bathing and irrigation of crops sometimes causes serious infectious disease. The indiscriminate use of antibacterial agent in fish feed and other agricultural products, household products and their uses in hospitals increase the level of antimicrobial resistant bacteria in surface water. Therefore, water pollution with multi drug resistant (MDR) bacteria requires evaluation[4]. *Escherichia coli,* a well-known resident of vertebrate's digestive tract, sometimes used as an indicator of fecal contamination in water[5]. The *E. coli* are found as normal flora in the human intestine. The *E. coli* and related bacteria constitute about 0.1% of gut flora and fecal-oral transmission is the major route for causing disease in human[6]. On the other hands, *Vibrio cholerae* is well known as the relevant agent for the human disease cholera that sometimes causes significant mortality. Generally, *V. cholerae* is diffused through contaminated food and water to the people who do not have proper sewage and water treatment systems. However, little is known about the level of microbial contamination and antimicrobial susceptibility patterns from Padma river. Therefore, the aim of this study was to determine antimicrobial susceptibility profiles among *E. coli* and *V. cholerae* isolated from this river.

MATERIALS AND METHODS

Selection of study locations: The present study was conducted along the Padma river from T-dam to Talaimari covering most of the part of Rajshshi city Corporation along the bank of the Padma river. Sampling was done on two, respective seasons namely summer and winter in the years 2016. Location and description of sampling sites is shown in Fig. 1 and Table 1.

Sampling: For the bacteriological analysis, 500 mL of water samples and required 500 g of sediment samples were collected from the selected sampling points. The materials used in sampling were sterile glass bottle, ice box and marker[7]. The samples were labeled and transported to the laboratory. Ice during transportation was used to prevent the reproduction of bacteria as well to ensure better survivability at low temperature. The time gap between sampling and analysis was maintained below 3 h.

Isolation, identification and confirmation of *E. coli* and *V. cholera* isolates: Multiple tube fermentation technique was used to enumerate the fecal coliforms from water sample, while spread plate method was used for the enumeration of fecal and *V. cholerae* from sediment samples. For isolation of *E. coli* from the confirmed fecal coliform collected from water and sediment samples, Eosine methylene blue agar (EMB) was used. Individual colonies showing a green metallic sheen on EMB agar were further confirmed using biochemical tests. The biochemical tests that were used for further identification of *E. coli* from other fecal coliform bacteria were indole, methyl-red, voges-proskauer and citrate test[8]. Antibiotic sensitivity testing was done for the isolates that showed positive results for biochemical tests. For the isolation of *V. cholerae*, 25 g of the sample was added with approximately 225 mL of alkaline peptone water in a warring blender flask and blended for 1 min. The sample was than incubated at 37°C for 6-8 h. After the incubation, a loopful of the alkaline peptone water was streaked on Thiosulphate Citrate Bile Salts (TCBS) agar plates. At the end of the incubation period TCBS agar plate was checked for growth of typical yellow or blue-green colonies. Confirmation of isolates of *V. cholerae* was done by Voges-Proskauer test and motility test.

Antibiotic sensitivity testing: Antimicrobial susceptibility testing was done by using Kirby-Bauer disc-diffusion method and using Mueller-Hinton agar (Difco, MI, USA) according to Clinical and Laboratory Standards Institute[9]. The standardized antibiotic discs of ampicillin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), streptomycin (10 µg), tetracycline (30 µg) and azithromycin (15 µg) (Hi media) were used for antibiotic sensitivity testing. The disc was distributed evenly at a distance of 24 mm from center to center of each disc. The 3 discs were used for a 100 mm plate. After an overnight incubation, the diameters of the zones of complete inhibition were measured to the nearest mm using slide calipers. The sizes of the zones of inhibition were interpreted according to protocols standardized for the assay of antibiotic compounds as guided by Clinical Laboratory Standards Institute[10].

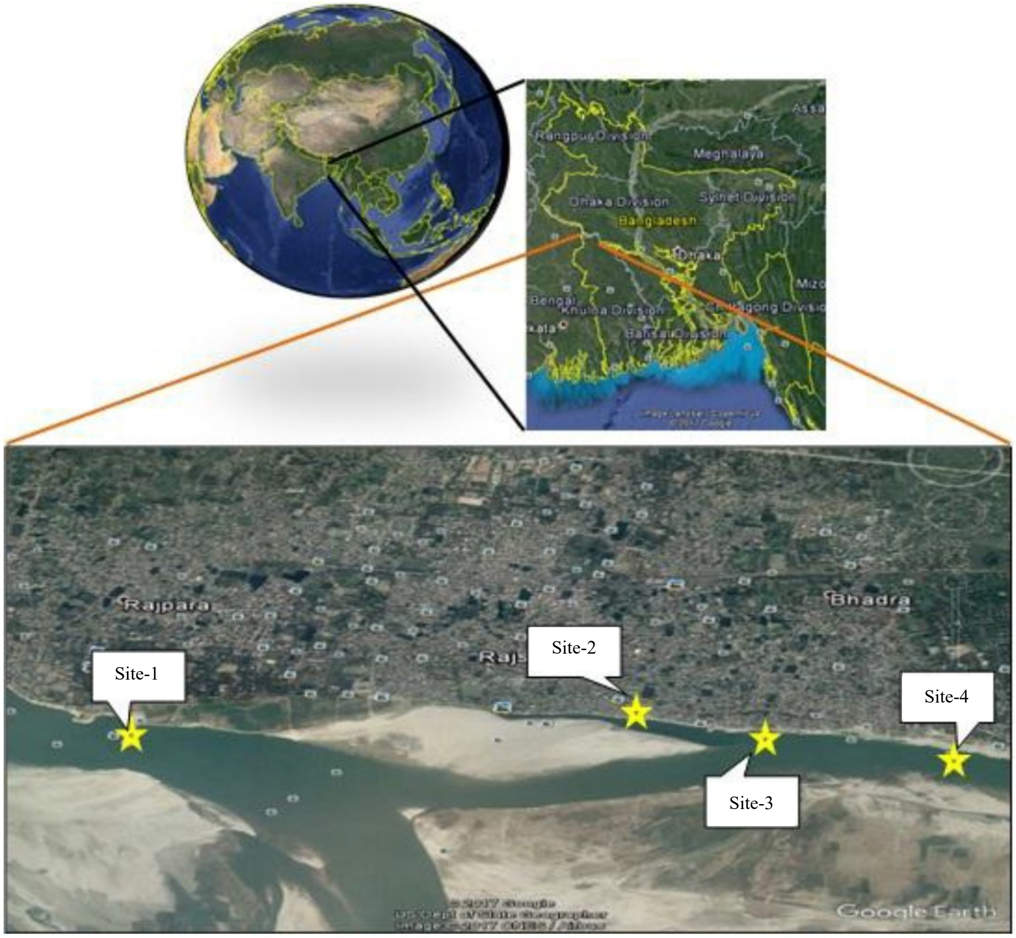

Fig. 1: Location of the study area (indicated with yellow stars). Modified from Google Earth-2017

Table 1: Sampling sites, sampling code and observation

Sampling station	Sampling code	Coordinates	Observations
T-dam	Site-1	Latitude: N-24°21' 42.41" Longitude: E-88°34' 31.18"	Discharge of effluent from some household garbage, no human activities except recreational activities
Padma garden	Site-2	Latitude: N-24°21' 42.30" Longitude: E-88°35' 52.44"	Direct discharges of effluent from vegetable markets and slaughter discharges; discharge from household septic tanks, more human activities as recreational site
I-dam	Site-3	Latitude: N-24°21' 34.95" Longitude: E-88°36' 39.92"	Direct discharges of effluent from household septic tanks, more human activities as recreational site
Talaimari point	Site-4	Latitude: N-24°21' 29.30" Longitude: E-88°37' 30.55"	No human activities and no source of discharge into the river

Multiple antibiotic resistance (MAR) indexing: The MAR index was performed to evaluate the health risk of the environments. Multiple antibiotic resistance index (MAR) for each test isolate was calculated as recommended by Krumperman[11]. The equation is as follows:

$$\text{MAR index} = \frac{\text{Number of antibiotics to which test isolate displayed resistance}}{\text{Total number of antibiotic to which the rest organisms evaluated}}$$

Statistical analysis: Values were represented as the mean (without standard deviation) of the replicates by using SPSS (Statistical Package for Social Science) version 20.0 (IBM Corporation, Armonk, NY, USA). Microsoft Excel (version 10) was used to represent the data in figures.

RESULTS

Antibiotic sensitivity testing of *E. coli* isolates: Antibiotic susceptibility test was carried out for 47 *E. coli* and 38

V. cholerae isolates collected from water and sediment samples. Antibiotic resistance profile of *E. coli* isolates of water samples is shown in Table 2. About 75% of the isolates were found to show resistance to ampicillin, 50% to ciprofloxacin and 25% to streptomycin during summer season at Site-2. During winter season, 16.67% isolates showed resistance to both gentamicin and streptomycin. At Site-3, 33.33% of the isolates showed resistance to streptomycin and azithromycin during summer, while 50% of the isolates showed resistance to ampicillin and streptomycin (Table 2). In sediment samples, isolates of *E. coli* did not show resistant to the studied antibiotics at Site-1 during summer and at Site-4 during both summer and winter season, respectively (Table 3). Only 50% isolates of *E. coli* showed resistance to ampicillin during winter season in Site-1. At Site-2, 40% isolates showed resistance to tetracycline and ciprofloxacin and 20% showed resistance to gentamicin in summer season. At Site-3, 16.67% isolates of *E. coli* showed resistance to ampicillin, gentamicin and ciprofloxacin, while 33.33% isolates showed resistance against azithromycin during summer season. During winter season, 50% of isolates showed resistance to ciprofloxacin and 25% to gentamicin at Site-3, respectively (Table 3).

Antibiotic sensitivity testing of *V. cholerae* isolates: Isolates of *V. cholerae* from water sample did not show any resistance to studied antibiotics at Site-1 and Site-4 during both summer and winter season (Table 4). While at Site-2, 16.67% of isolates showed resistance to tetracycline, streptomycin and ciprofloxacin and 33.33% isolates to azithromycin during summer season. During winter season, 33.33% isolates showed resistance to ampicillin, tetracycline and ciprofloxacin and 66.67% to azithromycin at Site-2. At Site-3, 66.67% isolates of *V. cholerae* showed resistance to tetracycline and 33.33% isolates to gentamicin and streptomycin during summer season and in winter season only 50% of isolates of *V. cholerae* showed resistance to ampicillin (Table 4). Antibiotic resistance profile of *V. cholerae* isolates in sediment samples, collected from the studied locations of Padma river, is shown in Table 5. Here isolates from Site-1 during both summer and winter season and from Site-4 during summer season did not show resistance to any types of antibiotics used. While at Site-2, 25% of the isolates showed resistance to tetracycline, gentamicin, ciprofloxacin and azithromycin during summer season and 50% of isolates to ampicillin and ciprofloxacin during winter season, respectively. At Site-3, 66.67% isolates were resistant to only azithromycin during summer season and 33.33% isolates showed resistance to ampicillin, gentamicin and ciprofloxacin during winter season. However, at Site-4, 50% of isolates of *V. cholera* from sediment sample showed resistance to only azithromycin during winter season (Table 5).

Table 2: Antibiotic resistance (%) profile of *E. coli* isolates in water samples collected from the studied locations of Padma river

	Site-1		Site-2		Site-3		Site-4	
	Su	Wi	Su	Wi	Su	Wi	Su	Wi
Antibiotics	(n = 2)	(n = 1)	(n = 4)	(n = 6)	(n = 3)	(n = 2)	(n = 1)	(n = 1)
Ampicillin (AMP)	0	0	75	50.00	0.00	50	0	0
Tetracycline (TE)	0	0	0	0.00	0.00	0	0	0
Gentamicin (CN)	0	0	0	16.67	0.00	0	0	0
Streptomycin (S)	0	0	25	16.67	33.33	50	0	0
Ciprofloxacin (CIP)	0	0	50	0.00	0.00	0	0	0
Azithromycin (AZM)	0	0	0	0.00	33.33	0	0	0

Su: Summer, Wi: Winter

Table 3: Antibiotic resistance (%) profile of *E. coli* isolates in sediment samples collected from the studied locations of Padma river

	Site-1		Site-2		Site-3		Site-4	
	Su	Wi	Su	Wi	Su	Wi	Su	Wi
Antibiotics	(n = 1)	(n = 2)	(n = 5)	(n = 5)	(n = 6)	(n = 4)	(n = 2)	(n = 2)
Ampicillin (AMP)	0	50	0	0	16.67	0	0	0
Tetracycline (TE)	0	0	40	20	0.00	0	0	0
Gentamicin (CN)	0	0	20	20	16.67	25	0	0
Streptomycin (S)	0	0	0	0	0.00	0	0	0
Ciprofloxacin (CIP)	0	0	40	40	16.67	50	0	0
Azithromycin (AZM)	0	0	0	20	33.33	0	0	0

Su: Summer, Wi: Winter

Table 4: Antibiotic resistance (%) profile of *V. cholerae* isolates in water samples collected from the studied locations of Padma river

	Site-1		Site-2		Site-3		Site-4	
Antibiotics	Su (n = 2)	Wi (n = 1)	Su (n = 6)	Wi (n = 3)	Su (n = 3)	Wi (n = 2)	Su (n = 1)	Wi (n = 1)
Ampicillin (AMP)	0	0	0.00	33.33	0.00	50	0	0
Tetracycline (TE)	0	0	16.67	33.33	66.67	0	0	0
Gentamicin (CN)	0	0	0.00	0.00	33.33	0	0	0
Streptomycin (S)	0	0	16.67	0.00	33.33	0	0	0
Ciprofloxacin (CIP)	0	0	16.67	33.33	0.00	0	0	0
Azithromycin (AZM)	0	0	33.33	66.67	0.00	0	0	0

Su: Summer, Wi: Winter

Table 5: Antibiotic resistance (%) profile of *V. cholerae* isolates in sediment samples collected from the studied locations of Padma river

	Site-1		Site-2		Site-3		Site-4	
Antibiotics	Su (n = 2)	Wi (n = 1)	Su (n = 4)	Wi (n = 2)	Su (n = 3)	Wi (n = 3)	Su (n = 2)	Wi (n = 2)
Ampicillin (AMP)	0	0	0	50	0	33.33	0	0
Tetracycline (TE)	0	0	25	0	0	0.00	0	0
Gentamicin (CN)	0	0	25	0	0	33.33	0	0
Streptomycin (S)	0	0	0	0	0	0.00	0	0
Ciprofloxacin (CIP)	0	0	25	50	0	33.33	0	0
Azithromycin (AZM)	0	0	25	0	66.67	0.00	0	50

Su: Summer, Wi : Winter

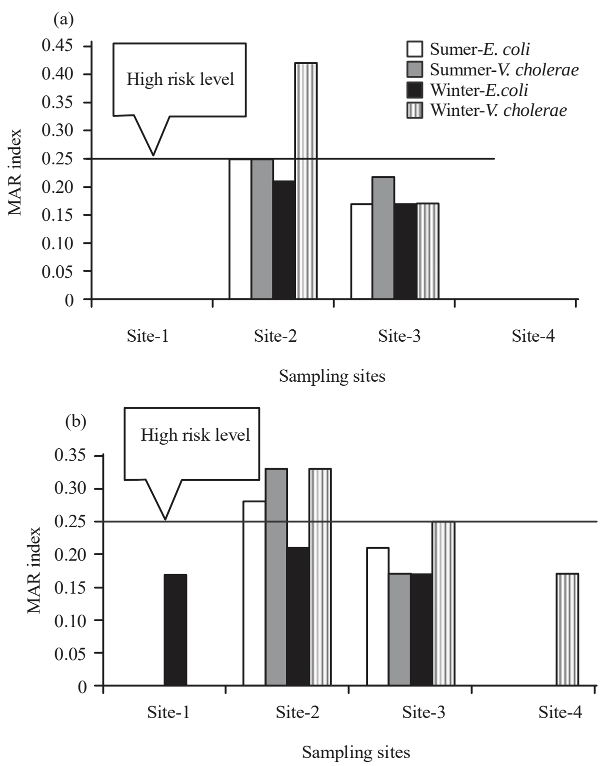

Fig. 2(a-b): Calculated MAR index for the study locations during summer and winter seasons based on *E. coli* in (a) Water and (b) Sediment samples of Padma river

Multiple antibiotic resistance (MAR) indexing: The MAR indexing of different study locations is shown in Fig. 2. The MAR index value of maximum 0.25 was considered high risk level in terms of resistance of multiple antibiotics. Resistance of *E. coli* isolates, collected from water samples, to antibiotics crossed the high risk value of MAR index during summer season at Site-2 (Fig. 2a). However, resistance of *V. cholerae* isolates collected from water samples crossed the high risk value of MAR index during winter season at Site-2 (Fig. 2a). In case of sediment sample, isolates of *E. coli* and *V. cholerae* showed multiple resistances to selected antibiotics and crossed the high risk value of MAR index at Site-2 except for *E. coli* isolates of winter season. However, isolates of *V. cholerae* during winter season crossed the high risk value of MAR index at Site-3 (Fig. 2b).

DISCUSSION

Contamination of river water through the microbes of vertebrate's digestive tract is a major threat to the people drinks river water because it may be responsible for numerous waterborne disease outbreaks. The presence of drug resistant bacteria in surface water contributed to the spread and persistence of antibiotic resistance bacteria in environment[12]. Antibiotic sensitivity testing was done during summer and winter season, as because isolates of *E. coli* and *V. cholerae* were not found during monsoon season. Although Kistemann *et al.*[13] stated that microbial contamination is increased following rainfall and runoff events, but a different result was observed in the present study. However, as the major factors affecting the microbial quality of surface water and underground waters are sewage runoff water, seepage from nearby sewage or septic tank[14], the presence of studied pathogenic bacteria during summer and winter might be due to the low level of water in the river that might be easily contaminated by sewage and septic tank disposal and other domestic waste, which is in agreement with an earlier study by Kenyon *et al.*[15]. The *E. coli* isolates exhibited highly resistant to ampicillin in water and to ciprofloxacin in sediment samples. Ampicillin is a broad spectrum penicillin and Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic which are frequently added to the poultry feed. Both of these antibiotics are effective against gram negative bacteria. Therefore, Padma river was considered a natural reservoir of these Gram-negative bacteria as because 12.5% of the isolates that were resistant could represent a natural reservoir of antibiotic resistance[16]. Resistance to Ampicillin is worrying because this antibiotic is in World Health Organization list of agents that are frequently used for treatment of many infections. On the other hand, *V. cholerae* isolates showed their highest resistance to tetracycline in water and ciprofloxacin in sediment samples during summer season. While during winter season, the highest resistance was obtained for ampicillin in water and for ciprofloxacin in sediment samples, respectively. The relatively high level of resistance to the studied antibiotics was an indicative of adverse use of these agents in the environment. Similar observation was also reported by Lateef[17], who stated that high resistance of antimicrobial agents represents misuse and abuse of these agents in environment. The value of MAR index crossed the high risk line during both summer and winter season, respectively for *E. coli* and *V. choleare* Site-2. This indicated that the phenomenon of multiple antibiotic resistant bacteria in aquatic environment was a serious concern for health status of the people inhabiting at Site-2 along the Padma river. The increasing resistance and multiple resistances of the microbial strains in this area might be due to the inefficiency of the water treatment plant. The fecal residues discharged from house-holding activities and hospital wastes sometimes also overflowed during monsoon and caused severe pollution in the Padma river at Site-2. Similar to the findings of the present study, a related study conducted by Armstrong *et al.*[18] showed that high multiple drug resistance (MDR) strains are more common from water contaminated with runoffs from land occupied by animal and human and those with urban and industrial sewerage. Presence of MDR in enteric bacteria isolates from aquatic environment has also been reported by Olaniran *et al.*[19], Abdo *et al.*[20] and Emmanuel *et al.*[21]. Findings of the study indicated that the *E. coli* and *V. cholera* recovered in this study most often expressed resistance to the antibiotics that are commonly used in clinical medicine it may limit the availability of antimicrobials for clinical management of waterborne outbreaks of the people living in this locality in future. This could contribute to the spread and persistence of antimicrobial resistant bacteria.

CONCLUSION

It was concluded that the presence of *E. coli* and *V. cholera* pathogens and the multiple antibiotic resistance tendencies among the isolates in the surface water and sediment of Padma river highlighted the human health risk associated with exposure to fishing, recreational and other household activities. Therefore, adequate risk prevention strategies need to be undertaken to protect the water and sediment and consequently to protect public health from these infectious pathogens.

SIGNIFICANCE STATEMENTS

The present study has established understanding on the antibiotic resistance of isolates of *E. coli* and *V. cholerae*. To detect any changing patterns in bacterial contamination, there is a need to monitor antibiotic sensitivity at regular intervals and knowledge of local presence of bacterial organisms and antibiotic sensitivities of the present study will provide the direction of antibiotic selection. These results would assist the authority to manage further bacterial contamination of water through proper guidelines.

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