

Research Article

INVESTIGATION OF THE FACTORS AFFECTING THE MICROBIAL POLLUTION WITH ANTIBIOTIC RESISTANT BACTERIA IN THE BURIGANGA RIVER OF DHAKA, BANGLADESH

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ABSTRACT

Dhaka, the capital of Bangladesh, is located on the bank of the Buriganga River. Pollution of Buriganga river water with different types of waste containing antibiotic-resistant (AR) bacteria poses a threat to human health. Therefore, this study aims to analyze the physicochemical parameters, total aerobic bacterial count (TBC), total coliform count (TC), and antibiotic-resistant bacteria from waste disposal points located in the river seasonally. We collected 59 water samples from sewage waste (SW), 31 from industrial waste (IW), and 9 from hospital waste (HW) disposal sites during the wet (November to April) and dry (May to September) seasons in the Buriganga river. We found that water temperature, pH, BOD, and COD decreased, but water total dissolved solids (TDS) increased significantly in the dry season compared to the wet season. Bacteriological analysis showed that total TBC and TC were significantly increased in the dry season compared to the wet. Further analysis showed that TBC and TC from the water of SW and HW disposal sites increased in the dry season compared to the wet. However, TBC and TC from the water at IW disposal sites were not changed. These results indicate that SW and HW are the main contributors to the bacterial coliform contamination in the Buriganga River. *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus*, *Streptococcus* spp., and *Salmonella* spp. isolates showed a high prevalence of resistance against commonly used antibiotics. It can be concluded that SW and HW might spread AR bacteria in the water of the Buriganga River, which causes a significant threat to humans and the environment.

Keywords: *Buriganga River, Hospital waste, Sewage waste, Industrial waste, Antibiotic-resistant bacteria.*

Introduction

The Buriganga River is located at the western and southern boundaries of Dhaka City, the capital of Bangladesh. This river is economically very important for Dhaka facilitating communication, culture, trade, and commerce. Once it was the main source of water supply, drainage outlet and

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flood control for Dhaka city inhabitants. However, nowadays this important river has already been declared by environmentalists as a dying river of Bangladesh due to severe water pollution (Kibria *et al.*, 2015). Common sources of this water pollution are the continuous addition of untreated waste effluents such as sewage waste (SW), industrial waste (IW), hospital waste (HW) effluents, surface runoff and leachate from waste disposal site directly and indirectly into the river water (Mallick *et al.*, 2018). Continuous additions of these pollutants result in the change of physicochemical parameters such as temperature, p^H , biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and biological properties like flora, fauna, microorganisms of river water system. The change of these parameters of river water depends on effluent types, quality and quantity discharged, seasonal water flow, and dilution capability by the river system (Ahmed *et al.*, 2010; Moniruzzaman *et al.*, 2012). For example, it was reported that untreated wastewater discharged into the Buriganga has risen steadily for last several decades with low water flow in dry season water quality falls posing a severe threat to the aquatic life and ecosystem (Mir *et al.*, 1999; Ahmad *et al.*, 2010). Therefore, it is necessary to monitor the change of physicochemical and biological parameters of the river water seasonally in a continuous manner.

Previous study suggested that Buriganga river water was heavily contaminated with different pathogenic bacteria like *Salmonella* spp., *Escherichia coli*, *Pseudomonas* spp., *Vibrio* spp. etc (Saha *et al.*, 2009). Our previous study reported that SW and HW effluent water as well as Buriganga river water harbored multi-drug resistant bacteria like *E.coli* (Mia *et al.*, 2017). About 50% wastewater samples of Dhaka city area were positive for NDM-1 producing organisms (NDM-1, New Delhi Metallo- β -Lactamase variant-1 enables drug resistance to move between communities & hospitals). Infact, Municipal wastewater, as well as fecal carriage of humans and animals, is a primary contributor of antimicrobial-resistant microorganisms to the aquatic environment, and this rate rises during rainfall and flooding seasons along with the liquid waste from hospitals, and pharmaceuticals industries (Linton, *et al.*, 1974; Kamruzzaman *et al.*, 2013). Thus, these results suggested that SW and HW effluent water as well as Buriganga river water contain huge amounts of pathogenic multi-drug resistant bacteria. Does these SW, HW and IW contribute to the development of antibiotic resistant bacteria in the Buriganga river water? There is no study showing that SW, HW and IW directly contribute to the development of antibiotic resistant bacteria in the Buriganga river water. Therefore, in this study, we collected water samples from the SW, HW and IW disposal sites located in the Buriganga river. Then we analyzed physicochemical parameters, such as pH, TDS, BOD, COD, and biological parameters like total aerobic bacterial count (TBC), total coliform count (TC) in different seasons. In addition, we assessed the resistance pattern and percentage of antibiotic-resistant bacteria isolated from these river water samples.

Materials and Methods

Sampling sites and sample collection

The sampling sites were in a 25 km long strip of Buriganga river, which started from Boshila, Dhaka following the river to Fatullah, Narayanganj. We selected three different types of sampling locations such as water from sewage waste (SW), industrial waste (IW) and hospital waste (HW)

disposing sites along the above mentioned Buriganga river area (Fig. 1). Bangladesh Inland Water Transport Authority (BIWTA) found that about a total of 68 sewerage drainage lines are directly connected to Buriganga rivers and about 27 establishments including hospitals and industries were adjacently located to the Buriganga river (The financial express, December 4, 2019). Hence a total of 94 samples consisting of 54 from SW, 31 from IW, 9 from HW disposing area were collected during the period of July 2017 to March 2018 covering both the dry and wet seasons. Water samples were collected in plastic bottles sterilized with alcohol.

Physicochemical parameters analysis

Physicochemical parameters of river water such as temperature, pH, total dissolved solids (TDS), biological oxygen demand (BOD) and chemical oxygen demand (COD) were measured following standard methods (APHA, 1998). In brief, temperature of water was measured at the time of water sampling with the help of a thermometer. The pH, TDS, BOD, COD of collected water samples were measured in the laboratory by electric pH meter, TDS meter probe, BOD meter, and COD meter, respectively.

Total aerobic bacterial count (TBC) analysis

Total aerobic bacterial count was determined by standard plate count method (Maturin *et al.*, 2001) on nutrient agar (NA) media with some modifications. Briefly, for the enumeration of TBC, serial dilution of each sample was done up to 10^{-5} dilutions with 9 mL normal saline. Then, 0.1 mL aliquot of each dilution was aseptically spread onto sterile nutrient agar plate and incubated at inverted position at 37°C for 24-48 hours. The following day, the plates having well separated colonies (30-300) were selected for counting and counted using digital colony counter J-2 (China). Total aerobic bacterial count (TBC) per mL of water samples was calculated by multiplying the average number of colonies per plate by reciprocal of the dilution and expressed as colony forming units (CFU) per mL (Collins *et al.*, 1984).

Total coliform count (TC) analysis

Total coliform count (TCC) test was performed following the standard plate count method (Baird RB *et al.*, 2015; Wohlsen *et al.*, 2006) with some modification using MacConkey agar (Himedia, UK, MH081). Briefly, original water samples were exposed to 10-fold serial dilutions such as 10^{-1} , 10^{-2} and 10^{-3} . Next, 0.1 mL water sample from each dilution was spread onto MacConkey agar [3] plate following standard spread technique and incubated at 37°C for 18-24 hours. Following day, bacterial colony was counted using digital colony counter J-2 (China). Total coliform bacterial count (TC) per mL of water samples was calculated by multiplying the average number of colonies per plate by reciprocal of the dilution and expressed as colony forming units CFU per mL (Collins *et al.*, 1984).

Isolation and identification of bacterial species

We selected and picked different colonies from NA, and MacConkey agar plates. Next, we streaked the selected colonies onto different selective media such as MacConkey agar, mannitol salt agar, xylose lysine deoxycholate (XLD) agar, blood agar media and presumptive bacterial species were isolated. Furthermore, these presumptive bacterial colonies were identified following Standard biochemical tests (Cappuccino JG, 2007).

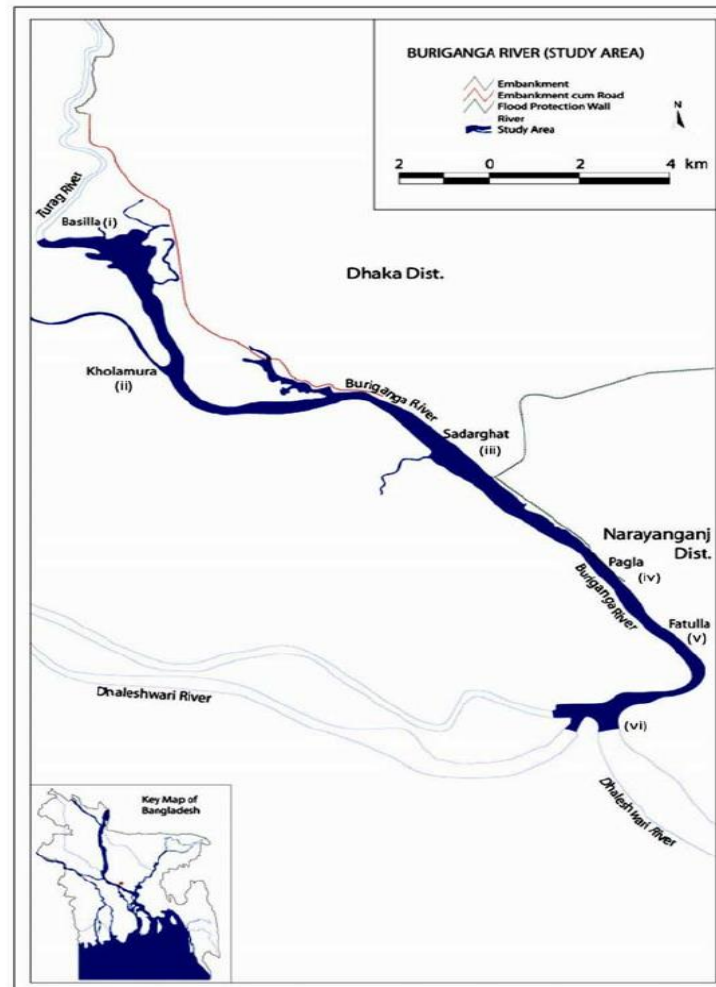


Fig. 1. Location of sampling site along the Buriganga River.

Antibiotic sensitivity assay

The antibiotic susceptibility test was performed by the standard agar disc diffusion method (Bauer *et al.*, 1966). On Mueller-Hinton agar (OXOID, England) using commercial discs like Amoxicillin, Gentamicin, Streptomycin, Methicillin, Rifamycin, Tetracycline, Clindamycin, Tigecycline, Chloramphenicol, Sulfamethoxazole Trimethoprim, Oxacillin, Vancomycin, Linezolid, according to the National Committee for Clinical Laboratory Standards Guideline (NCCLS, 2006). Briefly, selected bacterial colonies were cultured into 4 mL tryptic soy broth and incubated at 37 °C for 6 hours as the turbidity comply with 0.5 McFarland standards. After that, Mueller-Hinton agar plates were inoculated by swab over the entire surface following the placement of discs on the inoculated surface and incubated at 37 °C for 16-18 hours. The following day the diameter of the zones of complete inhibition were measured by using slide calipers. These measured diameters were interpreted with the zone diameter interpretive standards

from NCCLS, 2006 and organisms were reported as susceptible, intermediate in sensitivity or resistant to the agents that have been tested (CLSI, 2006).

Statistical analysis

The data presented in the tables and figures were the average value \pm standard deviation. The statistical analysis was done using the student's t-test. The percentage of resistant pathogens were calculated using Microsoft office software. The significance level was set at p value < 0.05 . The analysis was conducted with the Microsoft Excel program (Redmond, Washington DC, USA).

Results and Discussion

Results

Change of physical parameters in different seasons

Water physicochemical parameters such as temperature, pH, BOD, COD, TDS influence the number and species of bacteria. In this study, among the physicochemical parameters of Buriganga river water, wet season water temperature (32.54 ± 0.57) was significantly decreased at dry season (27.45 ± 0.40). Similarly, water pH (6.61 ± 0.24), BOD (35.78 ± 2.45), COD (34.80 ± 2.85) at wet season were significantly decreased compared to dry season (6.33 ± 0.16 , 32.47 ± 2.23 , 29.50 ± 2.41), respectively. However, TDS (146.95 ± 19.04) of river water at wet season was increased significantly compared to that of dry season (351.90 ± 26.73) (Fig. 2A, 2B, 2C, 2D and 2E).

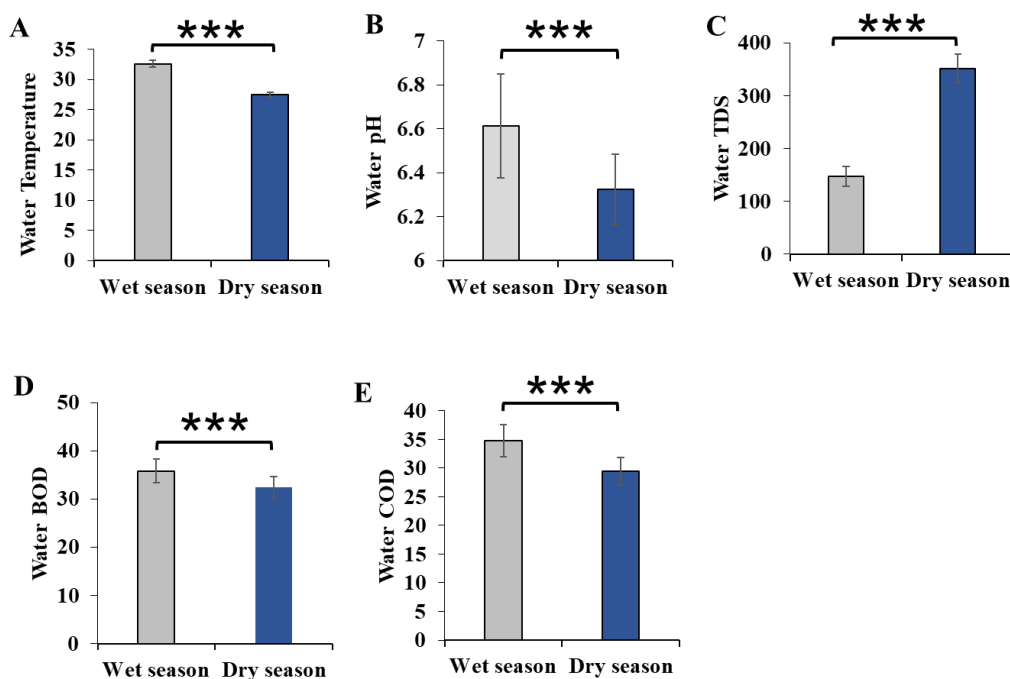


Fig. 2. Change of physicochemical parameters like temperature, p^H , TDS, BOD, COD of water samples in different seasons.

Physicochemical parameters like temperature, p^H , TDS, BOD, COD were measured following standard procedures described in detail in the methods and materials sections. Fig. 2 (A-E) indicates the change results of water temperature, p^H , TDS, BOD, COD, respectively.

Change of TBC in Buriganga river water in different seasons

Since, physicochemical parameters were found different between wet and dry seasons which might influence TBC. In this study, we found that total TBC $(16.83 \pm 10.54) \times 10^9$ CFU/mL at dry season was significantly increased compared to that $(8.02 \pm 6.98) \times 10^9$ CFU/mL at wet season. Further analysis showed that TBC from SW $(21.29 \pm 9.54) \times 10^9$ CFU/mL and HW $(32.88 \pm 3.34) \times 10^9$ CFU/mL disposal point at dry season were increased significantly compared to that $(7.04 \pm 6.90, 20.95 \pm 3.63) \times 10^9$ CFU/mL at wet season. But there was no change found for water from IW disposal sites between wet and dry season (Fig. 3A, 3B, 3C, 3D).

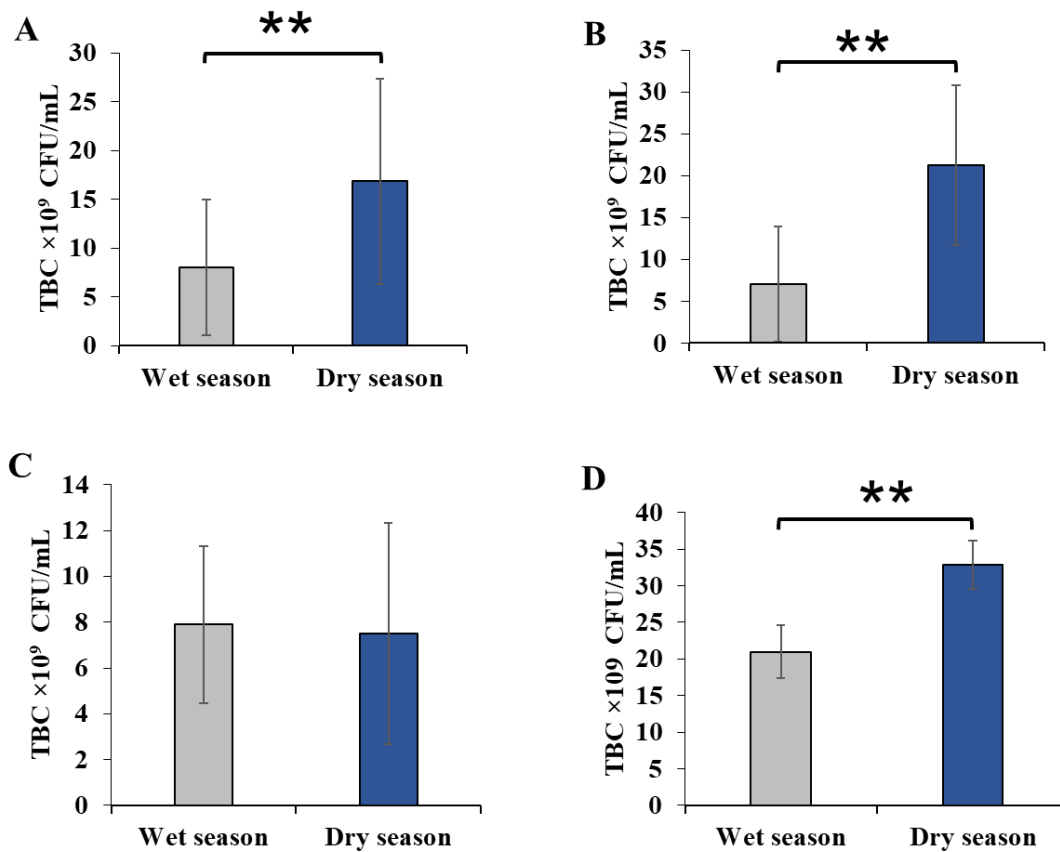


Fig. 3. Change of TBC in the Buriganga river water during different season.

Total aerobic bacterial count (TBC) was determined following the standard methods described in the methods and materials section. Fig. 3A, 3B, 3C, 3D indicates the results of water samples from total, SW disposal points, IW disposal points, HW disposal points.

Change of TC in Buriganga river water in different seasons

In this study, we found that total TC $(17.21 \pm 10.41) \times 10^7$ CFU/mL at dry season was significantly increased compared to that $(10.98 \pm 6.44) \times 10^7$ CFU/mL at wet season. Further analysis showed that samples from SW $(14.55 \pm 7.52) \times 10^7$ CFU/mL and HW $(34.67 \pm 2.67) \times 10^7$ CFU/mL disposal point at dry season were increased significantly compared to that $(9.04 \pm 6.25, 20.19 \pm 0.40) \times 10^7$ CFU/mL at wet season. But there was no change found for water from IW disposal sites between wet and dry season (Fig. 4A, 4B, 4C, 4D).

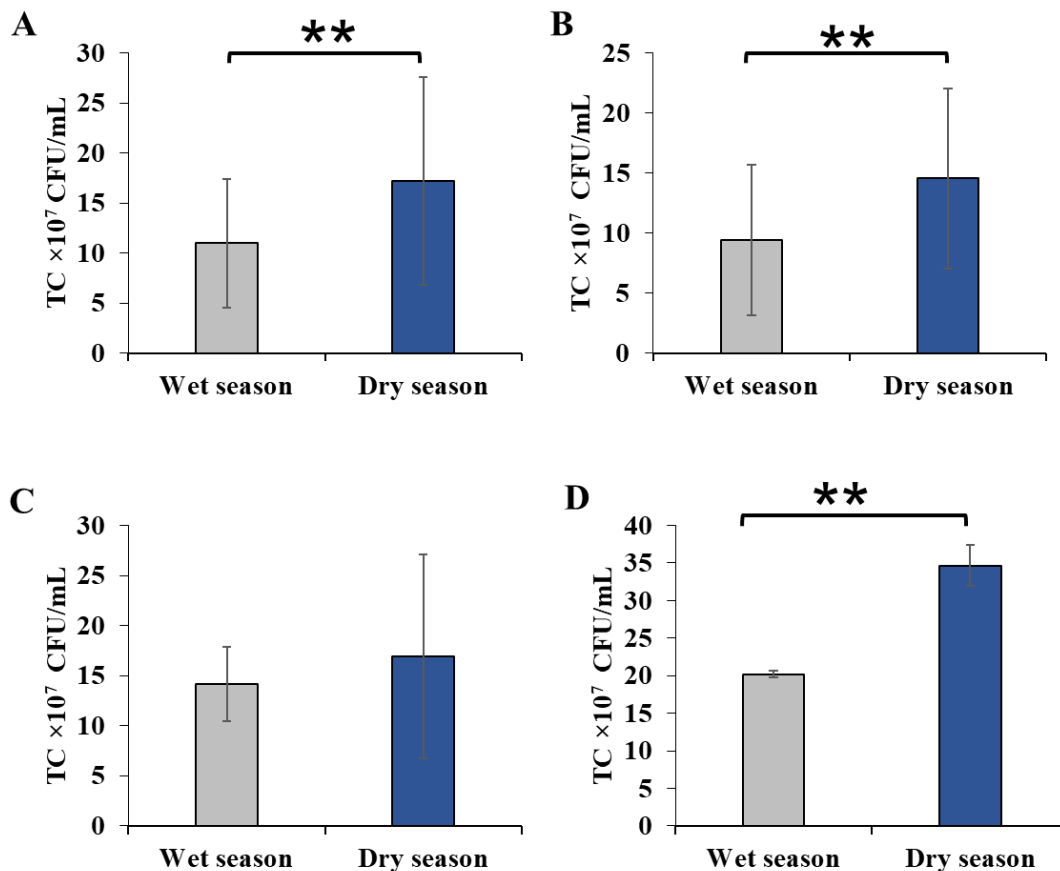


Fig. 4. Change of TC in the Buriganga river water due to seasonality.

Total coliform count (TC) was determined following the standard methods described in the methods and materials section. Fig. 4A, 4B, 4C, 4D indicates the results of water samples from total, SW disposal points, IW disposal points, HW disposal points.

Isolation and Identification of different bacterial species in the water of SW and HW disposal area

In our previous study, we found (Zakaria *et al.*, 2017) that sewage water, hospital wastewater and Buriganga river were contaminated with antibiotic resistance bacterial isolates (*E. coli*). Hence, in

this study, we isolated and identified different bacterial species like *Escherichia coli*, *Pseudomonas spp.*, *Staphylococcus aureus*, *Streptococcus spp.*, and *Salmonella spp.* isolates from the water of SW and HW disposal area.

Antibiotic resistant bacteria in the water of SW and HW disposal area

Since we isolated and identified different bacterial species like *Escherichia coli*, *Pseudomonas spp.*, *Staphylococcus aureus*, *Streptococcus spp.*, and *Salmonella spp.* isolates from the water of SW and HW disposal area. Then, we analyzed antibiotic resistant patterns of bacterial isolates. Our results showed that bacterial species such as *E. coli*, *Salmonella spp.*, *Pseudomonas spp.*, *Streptococcus aureus*, *Streptococcus spp.* isolated from water of these disposal sites showed 46%, 70%, 46%, 54%, and 46% resistance against different types of available antibiotics, respectively (Fig. 5).

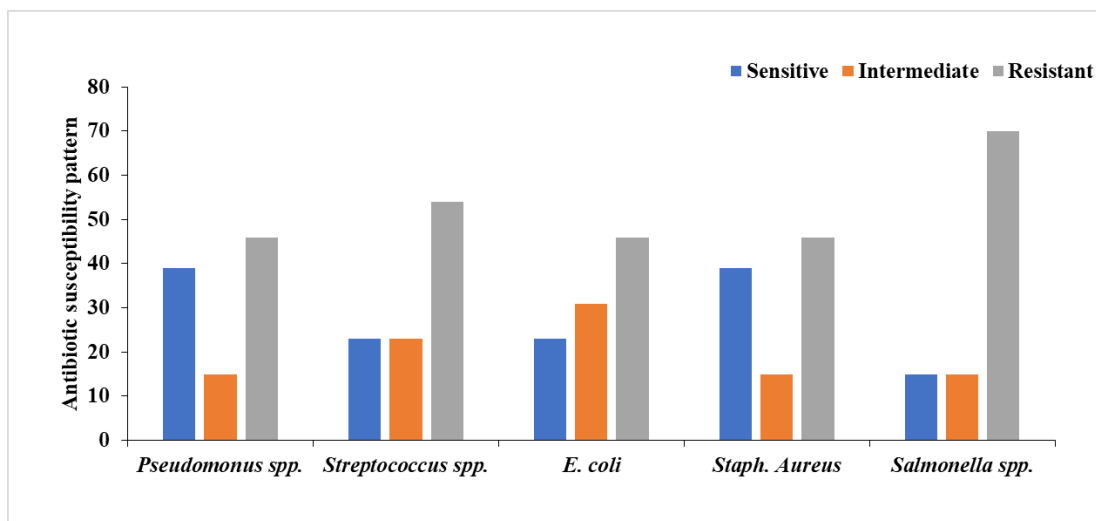


Fig. 5. Antibiotic susceptibility pattern of bacterial isolates from SW and HW disposal points. Antibiotic susceptibility test was done by the standard agar disc diffusion methods described in detail in methods and material sections.

Discussion

The major findings of this study are that SW and HW might spread antibiotic resistant bacteria like *E. coli*, *Salmonella spp.*, *Pseudomonas spp.*, *Streptococcus aureus*, *Streptococcus spp.* in the Buriganga river water. Seasonal variation influences physical parameters as well as TBC and TC of water from different disposal sites. In addition, only SW and HW might contribute to the change of TBC and TC in the Buriganga river of Bangladesh, while various disposed organic IW pollutants result in deoxygenation followed by release of ammonia and extra mineral nutrients in the water due to high microbial activity as shown in many other studies (Bhuiyan *et al.*, 2015; Uddin *et al.*, 2021).

Previous report (Saha *et al.*, 2009) found that Buriganga river water was heavily contaminated with inorganic, organic and bacterial population. Because Buriganga river receives millions of

litters of SW, IW and HW effluents through numerous outfalls which contain huge number of both pathogenic bacteria and organic matter (Islam *et al.*, 2019). Amount of organic material in the river water depends on the seasonal water flow and dilution capability by the river system (Ahmed *et al.*, 2010; Moniruzzaman *et al.*, 2018). Water quality deteriorates in dry seasons due to low water flow and pose a severe threat to the aquatic life and ecosystems (Mir *et al.*, 1999; Ahmad *et al.*, 2010). In dry seasons, water level and water flow are low. On the other hand, amount of disposed waste effluent in dry season is same compared to that in wet season. These properties of Buriganga river ecosystem might increase organic material in the disposing area and change the physical and microbiological properties of water in the disposal area (Al-amin H *et al.*, 2016). In consistent with this idea, TDS level was found higher in dry seasons compared to that in wet seasons. Also, we found pH, BOD, COD level was lower in dry season than that in wet season. A recent comparative study between developed like Japan and developing countries like Bangladesh showed that these physicochemical parameters were found to be higher in the river water of developing countries compared to developed countries (Sikder *et al.*, 2013).

Indeed, augmented organic material might encourage bacterial growth. In addition, low water flow is favorable for microbial growth (Saha ML *et al.*, 2009). In echoed with this statement, we also found that total TBC, TC level from SW, HW disposing area was higher in dry seasons compared to that in wet season. However, we did not find any change of TBC and TC from IW disposing area. These results indicate that SW and HW might contribute to the major microbiological change in Buriganga river system.

In this study, the results of physicochemical parameters like BOD, COD exceed the WHO (WHO,1998) or Department of Environment (DoE), Bangladesh standard limits (EQS, 1997). These results are consistent with national authority research reports (DoE report, 2016) and other studies reports (Al-amin H *et al.*, 2016; Akbor M *et al.*, 2017; Saifullah A *et al.*, 2013). Similarly, the bacteriological analysis like TBC, TC results also showed that these counts are far beyond the limits of WHO standards (WHO,1998).

Conclusion

The load of aerobic heterotrophic bacteria and the presence and abundance of Enterobacteriaceae like *E. coli*, *Salmonella* spp, *Pseudomonas* spp, *Streptococcus aureus*, *Streptococcus* spp in the water clearly showed significant level of microbial pollution of the river waste disposal site. AMR bacteria are contributed by SW and HW water, indicating that SW and HW might spread AMR bacteria in the water of Buriganga river and it cause a major threat to the environment as well as human. According to BOD and COD analysis result, disposal site's river water was polluted with organic and chemical pollutants polluting the whole river water. Therefore, a well-managed waste disposal system should be practiced saving the River Buriganga from the pollution.

Our study provides an important indication about the source of antibiotic resistant bacteria found in the water of Buriganga river. However, some limitations of this study should be noted. First, the number of samples specially IW, HW were small. Large number of samples could be important for better understanding about the source of antibiotic resistant bacteria. Secondly, the pathogenic bacteria and their resistance to antibiotic were identified and determined based on conventional methods rather than molecular identification. Further studies will be worthwhile to find

out the molecular based identification of pathogenic bacteria and their antibiotic resistance genes as well as pathways.

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