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ORIGINAL RESEARCH ARTICLE

Current status of bacterial contamination in some fish species of Bakkhali river Estuary, Bangladesh

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ABSTRACT

The present study aims to investigate the isolation of human pathogenic bacteria (*Escherichia coli*, *Salmonella* spp. *Shigella* spp. and *Vibrio* spp.) and in gills, intestines, skin of fishes of Bakkhali River Estuary, Cox's Bazar. A total of 50 fish species (25 of *Pomadasys hasta* and 25 of *Glossogobius giuris*) were collected from two sampling stations namely Station-1 (Rumalia Chara) and Station-2 (Kusturi Ghat). Bacterial analyses were made by standard methods. Total heterotrophic bacterial load of the isolates was found to be lower than the recommended public health and standard. However, the highest pathogenic bacterial (*E. coli*, *Salmonella* spp. *Shigella* spp. and *Vibrio* spp.) count at Station-2 might be due to the contamination of municipal and domestic wastes and discharges from small industries that constitute the main pollution source of this estuarine river. Skin was found to be the most preferred organs for higher bacterial load compared to intestine and lower count was recorded in gills. Further research on the microbial quality assessment should be undertaken to prevent pollution of this river estuary.

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INTRODUCTION

Several researchers have reported the isolation of bacteria belonging to different genera from fish as an indicator of pollution study (Adewoye and Lateef, 2004; Hamed *et al.*, 2013; Kolawole *et al.*, 2011). Fish can harbor varieties of pathogens on or inside its body as they reside at the top of the food chain (Dahunsi *et al.*, 2012). Fishes are extremely susceptible to bacterial contamination due to their soft body organs. Use of fish as a bioindicator of bacterial pollution can provide cumulative effect of different pollutant in the ecosystem (Santos *et al.*, 2011). The presence of FC as *E. coli* serves as an indicator for the possible presence of other disease-causing pathogens (Rajkumar and Sharma, 2013). Currently, coliforms and *E. coli* are of great importance among bacterial indicators used in water quality definition and health risk (Giannoulis *et al.*, 2005). Pathogens are a serious concern for managers of water resources, because excessive amounts of faecal bacteria in sewage and urban run-off have been known to indicate risk of pathogen-induced illnesses in humans. The pathogens that may occur in surface water as a result of fecal contamination vary depending on the source of the contamination. Human fecal pollution may cause the greatest health risk because humans act as reservoirs for human enteric bacteria such as *Salmonella* sp. and *Shigella* sp. (McLellan and Salmore, 2003). Thus, detection and an enumeration of indicator organisms are of primary importance for the monitoring of sanitary and microbiological quality of water (Gunnison, 1999).

The Bakkhali river estuary located at the south-eastern part of Bangladesh serves an important harbor in the economics of local fishery of Cox's Bazar district (Hena *et al.*, 2007). It is enriched with about 490 species of fishes (Hossain, 1971) and 19 species of shrimps/prawn (Chowdhury and Sanaullah, 1991)

that ensure sustainable livelihood of a large number of fishing households. This estuarine river also possesses an extensive environment for different aquatic organisms, which serves as feeding, breeding and nursery grounds for a variety of animals. It is highly productive in terms of nutrient input from different sources that promotes other living resources (Kamal and Khan, 2009). However, continuous environmental disturbance like sedimentation, urban runoff and pollution are badly affecting the ecosystem of this estuary (Hena *et al.*, 2012). Bakkhali river is the main discharge point of the city waste of Cox's Bazar. Most of the indigenous effluents come from Cox's Bazar city and eventually flow into the Bakkhali river through a system of canals. As there are no potential industries in Cox's Bazar, municipal and domestic wastes and discharges from small industries constitute the main pollution source of this estuarine river. Waste discharge and chemical spills are mainly connected with boat repair industry that represents an additional source of pollutants to the water and sediments.

However, several studies have been conducted on pollution and other entities of Bakkhali river estuary (Siddique *et al.*, 2012; Rashed-Un-Nabi *et al.*, 2011; Hena *et al.*, 2012). But information regarding bacterial pollution of this estuarine system is lacking. The present study was therefore designed to evaluate the bacterial load in different organs of fishes to evaluate the current pollution status of this estuarine river.

MATERIALS AND METHODS

Collection of sample

A total of 50 fish species (25 of and 25 of) were collected from two sampling stations namely Station-1 (Rumalia chara) and Station-2 (Kusturi Ghat) (Figure 1). A total of 50 fish species (25 of species 1 and 25 of species 2) were collected from the selected two sampling stations. Species-1 was *Pomadasys hasta* and Species-2 was *Glossogobius giuris*. Station-1 was characterized by less polluted areas and Station-2 was contaminated with anthropogenic and industrial activities where large amount of organic and inorganic wastes are being discharged. The fishes were collected from local fisherman in the morning. Gill nets of about 12.192 m long and 1.828 m wide with a cork line at the top rope and metal line with the ground rope made locally of nylon were used for fishing. The collected fishes were transported with sterilized plastic bucket to the laboratory.

Preparation of samples

In the laboratory, fish samples were washed with phosphate buffer saline and the various organs were separated with sterilized knife. Twenty (20) gram of each part (skin, gill and intestine) was homogenized separately in 250 mL of 0.1% (w/v) PBS using vortex machine (Model iSwix VT) before serial dilution using the method of Odoli (2006). All the samples were collected in triplicates.

Bacterial analysis

Bacterial analysis was conducted in the microbiology laboratory of Bangladesh Fisheries Research Institute (BFRI), Marine Fisheries & Technology Station, Cox's Bazar, Bangladesh. Spread plate method was carried out to count the bacterial density. 0.1 ml of diluted samples was incubated in the petri-dish containing the culture media. Enumeration of Total heterotrophic bacteria (THB) was done on nutrient agar media (HiMedia) after incubating at 37° C for 18-24 h. MacConkey agar and EMB agar were used to the enumeration of *Escherichia coli*. Pink colonies that occur in MacConkey agar plate after the incubation period of 18-24 h at 37°C were recultured in EMB agar plate at 44-44.5° C for overnight. Colonies appearing as green metallic sheen in EMB agar were further subjected to biochemical test (iMVIC test) for the confirmation of the presence of *E. coli*. *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. were enumerated using TCBS agar and SS agar plate (HiMedia) after incubation at 37° C for 18-24 h. Yellow with opaque centers colonies were considered as *Vibrio* spp.; and colourless transparent and black centers colonies were considered as *Salmonella* spp. Colonies were counted on a digital colony counter as colony forming unit per gram (Cfu/g) of the sample.

Statistical analysis

The data were analyzed by the use of Statistical Package for Social Science (SPSS software Version 20.0). T-test was used to show the difference between the bacterial counting of two study sites at significance level of $p < 0.05$.

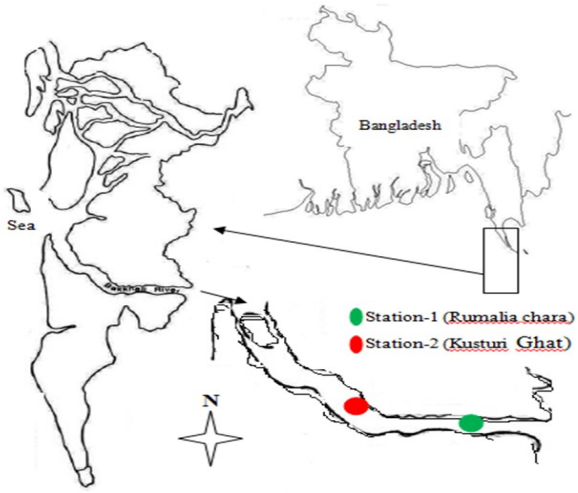

Figure 1. *Sampling stations in Bakkhali river estuary.*

RESULTS AND DISCUSSION

Mean bacterial count

Mean bacterial count of fishes at two different sampling stations is shown in Table 1. Counting showed a significant difference in THB, *E. coli*, *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. between the two study locations, whereas significantly higher bacterial count was found at Station-2 in both of the two fish species. However, irregular pattern in bacterial count was observed between the fish species. The maximum microbiological limit for the THB, which separates the good quality products from bad quality, is 500000 cfu/g (ICMSF, 1986). The THB of the studied samples was below the maximum acceptable limit. Pathogenic bacteria *i.e. E. coli*, *Salmonella* spp., *Shigella* spp. and *vibrio* spp. isolated from the studied fish species were also beyond the limit of microbiological guidelines for foodborne pathogens as cited by Gilbert *et al*. (2000). Zambuchini *et al*. (2008) reported that the Enterobacteriaceae count is considered as the index of fish quality. Claucas *et al*. (1996) showed that when pathogens such as *Salmonella spp*., *Shigella* spp., *Vibrio* spp. present in food they are most likely to cause foodborne diseases. Therefore, monitoring of these microorganisms has been suggested as a measure of fish quality as well as environmental quality. According to Sichewo *et al.* (2014), the presence of coliforms in fish demonstrates the level of pollution of their environment because coliforms are not the normal bacterial flora in fish. The highest pathogenic bacterial count at Station-2 might be due to the poor sanitation system in that surrounding area where latrines directly connected with the river discharging of municipal and domestic wastes and industrial pollution. Runoff from vegetable and fish market which carry animal wastes was also a significant source of higher bacterial count in the Bakkhali river estuary.

Bacterial counts in different organs

Bacterial count in different organs of the fish species at the two studied sites is shown in Table 2. THB count was found highest at Station-2 in the skin of Species-1 (147333.33±21361.96 cfu/g) and the lowest in the gill of Species-2 (1600.00±529.15 cfu/g) at Station -1. Skin of Species-1 (6260.00±1080.56 cfu/g) was found vastly contaminated with *E. coli* at Station-2; however, *Vibrio* spp. count was the highest at the same Station in Species-2 (1343.33±496.62 cfu/g). *Salmonella* spp. and *Shigella* spp. count were observed higher at Station-2 in the skin of Species- 2 (3346.67±423.36 cfu/g and 1536.67±272.27 cfu/g, respectively). Similarly, Sujatha *et al.* (2011) isolated *E. coli*, *Shigella* spp. and *Salmonella* spp. from the gills, intestines and skin of *Megalaspis cordyla* and muscles of *Priacanthus hamrur*, whereas they reported higher bacterial count in the skin of the fishes. The higher bacterial count in the skin of fishes in both Station-1 and Station-2 might by due to the higher bacterial load in the water. However, in the present study, higher bacterial count in the fishes of Station-2 might be due to more polluted water of this Station. Similar observation was also made by Latha and Mohan (2013), where they reported that in polluted water fish showed higher load of microorganisms count in skin due to the pollution of water body. In the present study, the bacterial load was lower in gills of both the fish species compared to other organs. That might be due to the nature of the gills, where micro flora was unlikely unable to alter significantly the physical and chemical environment of the gill (Latha and Mohan, 2013). In the present study, the pollution nature of the water body was found to determine the bacterial load of intestine of the fishes. The growth promoting and inhabiting substances were found to decrease the bacterial count in the intestine compared to the skin of the studied fishes as similar to the findings of Austin (2002). Therefore, the present study has displayed that Station-2 was more contaminated representing a potential hazard to the health of local people.

Table 1. Mean bacterial count (cfu/g) of fishes.

	Station-1	Station-2	*t-value*	*P-value*
Species -1				
THB	3366.67±1830.30	107066.67±42411.17	-7.329	0.000
E. coli	255.56±88.19	4217.78±1898.54	-6.254	0.000
Salmonell spp.	211.11±105.41	2537.78±1015.16	-6.839	0.000
Shigella spp.	15.56±18.10	847.78±604.89	-4.126	0.001
Vibrio spp.	17.78±17.16	883.33±581.98	-4.730	0.000
Species -2				
THB	2766.67±1072.38	61444.44±19626.46	-8.956	0.000
E. coli	300.00±239.79	3133.33±1268.52	-6.584	0.000
Salmonell spp.	233.33±150.00	2333.33±849.44	-4.460	0.000
Shigella spp.	31.11±34.08	1058.89±676.97	-4.549	0.000
Vibrio spp.	13.33±12.25	681.11±423.39	-7.304	0.000

Table 2. Bacterial counts (cfu/g) in different organs of fishes.

Bacteria		Organs	Station 1	Station 2
THB	Species-1	Skin	5300.00±953.94	147333.33±21361.96
		Gill	1466.67±642.91	60033.33±10758.41
		Intestine	3333.33±1026.32	113833.33±28350.72
	Species-2	Skin	3133.33±901.85	84366.67±7054.31
		Gill	1600.00±529.15	44166.67±11025.58
		Intestine	3566.67±550.76	55800.00±9260.13
E. Coli	Species-1	Skin	266.67±57.74	6260.00±1080.56
		Gill	233.33±57.74	2156.67±408.57
		Intestine	266.67±152.75	4236.67±674.19
	Species-2	Skin	366.67±305.51	4346.67±699.45
		Gill	133.33±57.74	1696.67±207.93
		Intestine	400.00±264.58	3356.67±724.59
Salmonella spp.	Species-1	Skin	200.00±100.00	3260.00±1080.56
		Gill	200.00±100.00	1856.67±142.95
		Intestine	233.33±152.75	2496.67±1205.54
	Species-2	Skin	300.00±200.00	3346.67±423.36
		Gill	133.33±57.74	1656.67±231.59
		Intestine	266.67±152.75	1996.67±506.19
Shigella spp.	Species-1	Skin	30.00±20.00	1210.00±115.33
		Gill	0.00±0.00	323.33±95.04
		Intestine	16.67±15.28	1010.00±890.17
	Species-2	Skin	53.33±51.32	1536.67±272.27
		Gill	26.67±25.17	220.00±105.36
		Intestine	13.33±11.55	1420.00±392.81
Vibrio spp.	Species-1	Skin	20.00±10.00	893.33±65.06
		Gill	3.33±5.77	323.33±95.04
		Intestine	16.67±15.28	826.67±642.21
	Species-2	Skin	13.33±15.28	1343.33±496.62
		Gill	13.33±11.55	220.00±105.36
		Intestine	26.67±25.17	1086.67±240.07

Conclusion

Presence of large amount of pathogenic bacteria i.e. *E. coli*, (4217.78±1898.54 cfu/g in Species-1 at Station-2) *Salmonella* spp. (2537.78±1015.16 cgu/g in Species-1 at Station-2), *Shigella* spp. (1058.89±676.97 cgu/g in Species-2 at Station-2) and *Vibrio* spp. (883.33±581.98 in Species-1 at Station-2) in the two fish species collected from Bakkhali river estuary river of Cox's bazar indicated high levels of faecal contamination in the river due to heavy load of improper sewage dumping, municipal waste and industrial waste water. There may be a potential hazard of infection from food borne diseases to the residents from the encircling inhabitants from consuming the fish. Further research work should be conducted on overall pollution status of Pasur river estuary.

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