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Article

Salmonella and *Escherichia coli* contamination in wild catfish and rivers at northern part of Bangladesh

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Abstract: A variety of fishes consumed regularly are prone to pathogenic spoilage especially by different microbes. It is important to find out microbiological quality of fish that we consume regularly to protect the public health. Current research was conducted to assess the incidence of *Salmonella* and *Escherichia coli* (*E. coli*) contamination in three selected catfish species namely, Ayer (*Sperata seenghala*), Pabda (Ompok *pabda*) and Magur (*Clarias batrachus*) *of* five rivers (Dhepa, Atrai, Punorvoba, Kakra, and Ghorveshori) of Dinajpur district. The study showed that *Salmonella* and *E. coli* positive isolates were 33.33% and 55.55% in Punorvoba river, 31.03% and 50.00% in Atrai river, 30.19% and 56.60% in Dhepa river, 29.63% and 59.26% in Ghorveshori river and 27.08% and 56.25% in Kakra river, respectively. Highest percentage of *Salmonella* and *E. coli* were found in *Ompok pabda* (39.40%) and in *Clarias batrachus (*63.84%), respectively. Lowest percentage of *Salmonella and E. coli* were found in *Sperata seenghala (*26.50% and 41.07%), respectively. The percentage of *E. coli* was greater than *Salmonella for* each of the experimental samples that are very alarming for consumption of wild catfish. At the same time, the microbial quality of water of Dhepa, Atrai, Punorvoba, Kakra, and Ghorveshori rivers are not satisfactory regarding fish consumption and public health.

Keywords: *Salmonella; Escherichia coli;* contamination; catfish; rivers; public health

1. Introduction

Bangladesh is a riverine country located in South Asia. The open water resources of Bangladesh are estimated 3927142 hectares of which 853863-hectare river and coastal area, 68800-hectare Kaptai lakes, 114161 hectares (FRSS, 2016) beels and 2712618 hectares floodplains (DoF, 2019). Microorganisms are widely distributed in inland closed and open water bodies. Microbiological quality of open water is frequently threatened by the contamination with untreated domestic wastewater particularly in urban areas. Physical, chemical and biological pollutants have a potent negative influence on the microbial flora in water and sediments of any water bodies (Filimon *et al*., 2010). As a significant part of the aquatic environment, the fishes have attracted great interest and emphases by many scientists. The bacteria living in the aquatic environment play key roles in the biogeochemical cycles of elements (Brune *et al*., 2000; Spring *et al*., 2000) and can cause perceptible changes in the surrounding environment by their uptake and release of chemicals (Wetzel, 2000; Borsodi *et al*., 2003).

An attempt was made in this report to investigate and identify the occurrence of bacterial populations in water and fish samples collected from different rivers of the northern part of Bangladesh. Three different fish species namely *Sperata seenghala*, *Ompok pabda* and *Clarias batrachus* are available in the open water bodies of Dinajpur and Rangpur (Ava *et al*., 2020). For the assessment of microbial quality these fishes were chosen because these species are available and have high demand in market. Microbiological quality assessment of water and fish is very essential as it is closely related to public health and also beneficial for both domestic consumption and export of fish and fishery products (Faridullah *et al*., 2016). *Salmonella* and *E. coli* are pathogenic bacteria and cause different types of problems like food poisoning. On the other hand, the importing countries always much aware about these hazardous pathogens. Thus, identification of these bacterial species and find out possible sources of contamination is very much important for producing good aquaculture products.

2. Materials and Methods

2.1. Sample collection

Samples (water, scum and fish) were collected from five rivers (Dhepa, Atrai, Punorvoba, Kakra, and Ghorveshori rivers) of Dinajpur district (Figure 1) of Bangladesh for the detection of *Salmonella* sp. and *E. coli*. Samples were shifted to the laboratory within 24 hours of collection and incubated. Bacteriological analysis was performed for the detection of *Salmonella* sp. and *E. coli* according to the method of Association of Official Analytical Chemist (AOAC, 1984).

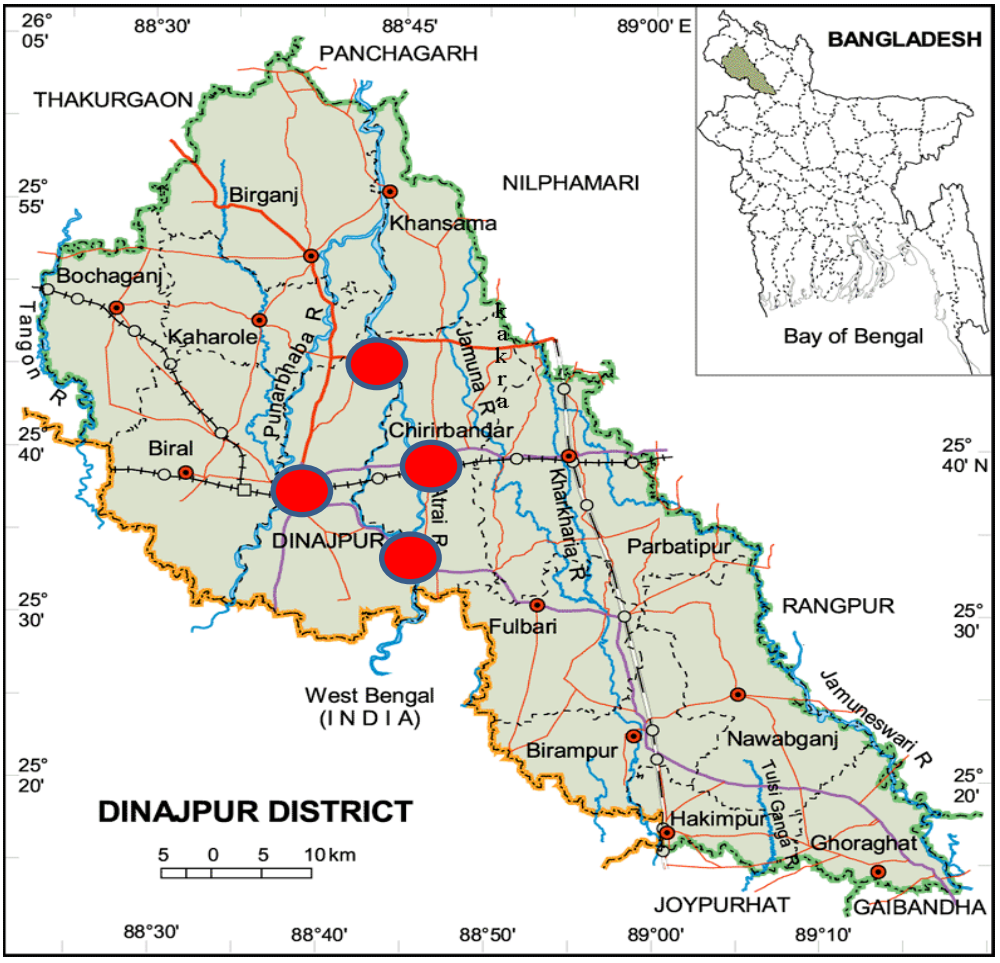

Figure 1. Sampling areas.

2.2. Bacteriological analysis

2.2.1. Pre-enrichment and Enrichment of samples

All of the collected samples were pre-enriched in ordinary nutrient broth (mixture of 0.5% peptone, 0.3% meat extract, 0.2% NaCl and distilled water) after that inoculated into tetrathionate broth, selenite broth (Hi Media, India) for selective enrichment and incubated at 35°C for 24 hours in an incubator (Binder, Germany) at the

department of Fisheries Technology laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

2.2.2. Streaking on to the Selective media

Samples were streaking carefully into the different selective media such as S-S agar (Hi Media, India), XLD agar (Hi Media, India) and DCA agar (Hi Media, India). After that incubated at 35^0C for 24 hours and well isolated bacterial colonies were observed.

2.2.3. Sample preservation

Agar slant were used for the purpose of sample preservation which is prepared from ordinary nutrient agar medium (mixture of 0.5% peptone, 0.3% meat extract, 0.2% NaCl, 1.5% bacteriological agar and distilled water). Well isolated bacterial colonies were streaking carefully on to the surface of agar slant and kept into the refrigerator (Model: WNJ-5A2-0101; Walton, Bangladesh) at 4±1°C for further characterization of *Salmonella* and *E. coli.*

2.2.4. Screening and biochemical tests for characterization of *Salmonella* and *E. coli*

Previously preserved samples having well isolated bacterial colonies were used for the screening and characterization of *Salmonella* and *E. coli.* TSI agar medium (Hi Media, India) was used to perform characterization of *Salmonella* and *E. coli*. After inoculation the slants were incubated at 35^oC for 24 hours. *Salmonella* cultures typically produced an alkaline (red) slant and *E. coli* cultures typically produced an acidic slant and all isolates suspected to be *Salmonella* and *E. coli* were inoculated to the EMB agar medium (Hi Media, India) from previously preserved samples. Inoculation was done with the help of an inoculating wire by streaking into the medium and finally incubated at 35^oC for 24 hours.

3. Results and Discussion

3.1. Level of contamination at Atrai river

Total number of suspected isolates were 58, among them 18 isolates were *Salmonella* positive; 29 isolates were *E. coli* positive, and rest of the isolates were unidentified (Table 1). The study showed that the samples of water, scum and fish were contaminated with *Salmonella* and *E. coli*. The percentage of *Salmonella positive* and *E. coli* positive isolates were found 31.03 and 50.00%, respectively (Figure 2). In ayer fish, the percentage of *Salmonella* positive and *E. coli* positive isolates were found 28.57 and 35.71%, respectively. In pabda fish, the percentage of *Salmonella* positive and *E. coli* positive isolates were found 33.3 and 58.33%, respectively. In magur fish, percentage of *Salmonella* positive and *E. coli* positive isolates were found 35.71 and 57.14%, respectively The highest percentage of *Salmonella* and *E. coli* positive isolates were found 35.71% in magur and 58.33% in pabda fish, respectively. On the other hand, lowest percentage of *Salmonella* and *E. coli* positive isolates were found 28.57 and 35.71% respectively in ayer fish (Figure 3). A similar study by Ava *et al.* (2020) found that the incidence of *Salmonella* was greater in scum samples (93.8%) and for *E. coli* the higher contamination estimates in open water samples (81.3%), and water and fish samples were also *Salmonella* positive and the percentage was 87.5 and 57.8% respectively which is in the line of our present findings. Similar drop of result showed by Faridullah *et al*. (2016) who observed that the prevalence of *Salmonella* positive samples was 43.7, 62.5 and 20% in water, scum and shrimp samples, respectively. The trend of previous study more or less similar with the current research findings.

3.2. Level of contamination at Dhepa river

Among the isolates, 16 isolates were *Salmonella* positive; and 30 were *E. coli* positive (Table 1). The percentage of *Salmonella* and *E. coli* positive isolates were found 30.19 and 56.60%, respectively (Figure 2). The percentage of *Salmonella and E. coli* positive isolates in ayer fish were 25.00 and 50.00%, respectively. In pabda fish, the percentage of *Salmonella* and *E. coli* positive isolates were found 33.33 and 55.55%, respectively. The percentage of *Salmonella* and *E. coli* positive isolates in magur were found 29.41 percent and 58.82 percent, respectively. The highest percentage of *Salmonella* and *Escherichia coli* positive isolates were found at 33.33% in pabda and 58.82% in magur fish, respectively. On the other hand, lowest percentage of *Salmonella and E. coli* were found 25.00 and 50.00%, respectively in ayer fish (Figure 3). Faridullah *et al.*, (2016) reported that incidence of *Salmonella* positive samples was 43.7 and 62.5% in water and pond scum samples. Simultaneously, they showed that the prevalence of *E. coli* positive samples was 62.5, 43.7 and 60% for water, pond scum and shrimp samples, respectively at the farm of Dinajpur district.

3.3. Level of contamination at Punorvoba river

Total number of suspected isolates were 45, among them 15 were *Salmonella positive* and 25 were *E. coli* positive (Table 1). The percentage of *Salmonella* and *E. coli* positive isolates were found 33.33 and 55.56%, respectively (Figure 2). In case of ayer fish, the percentage of *Salmonella* and *E. coli* positive isolates were 27.27 and 45.45 %, respectively. In pabda fish, the percentage of *Salmonella and E. coli* positive isolates were 50.00 and 66.67%, respectively. In magur, the percentage of *Salmonella* positive and *Escherichia coli* positive isolates were found 26.67 percent and 60.00 percent, respectively. The highest percentage of *Salmonella* and *E. coli* positive isolates were found at 50.00 and 66.67%, respectively in pabda fish. On the other hand, lowest percentage of *Salmonella* was found 26.67% in magur and *E. coli* positive 45.45% in ayer fish (Figure 3). Similar type of research conducted by Faridullah *et al*. in 2016 who stated that the *Salmonella* positive samples was 43.7%, 62.5%, 20% and 0.0% for water, pond scum, shrimp and basket samples, respectively and prevalence of *E. coli* positive samples was 62.5%, 43.7%, 60% and 60% for water, pond scum, shrimp and basket samples, respectively at farm level. The level of incidence of *Salmonella* was greater in scum samples (93.8%) and for *E. coli* the higher contamination (Pamuk *et al.,* 2011) estimates in open water samples (81.3%), and water and fish samples were also *Salmonella* positive and the percentage was 87.5 and 57.8% respectively (Ava *et al*., 2020).

3.4. Level of contamination at Ghorveshori river

Suspected isolates were 54 among them 16 isolates were *Salmonella* positive and 32 isolates were *E. coli* positive (Table 1). The percentage of *Salmonella* and *E. coli* positive isolates were found 29.63 and 59.26%, respectively (Figure 2). In ayer fish, the percentage of *Salmonella* positive and *E. coli* positive isolates were found 28.57 and 35.71%, respectively. The percentage of *Salmonella* positive and *E. coli* positive isolates at pabda fish were 37.50 and 62.50%, respectively. In magur fish, the percentage of *Salmonella* and *E. coli* positive isolates were 23.53 and 82.53%, respectively. The highest percentage of *Salmonella* and *E. coli* positive isolates were found in pabda and magur fish, respectively (Figure 3). Maxine *et al*. (2000) found the overall incidence of *Salmonella* was 1.3% for domestic seafood whereas, around 10% of import raw seafood and 2.8% of domestic raw seafood were positive for *Salmonella*. Water, pond scum and fish samples are contaminated with *Salmonella* and *E. coli*, *Salmonella* positive samples were 43.7, 62.5 and 20% for water, pond scum, shrimp respectively Meanwhile, the prevalence of E. coli positive samples was 62.5%, 43.7%, 60% and 60% for water, pond scum, shrimp respectively observed by Faridullah *et al*. (2016). Ava *et al*. (2020) who found that the level of incidence of *Salmonella was* greater in scum samples (93.8%) and for *E. coli* the higher contamination (Pamuk *et al*., 2011) estimates in open water samples (81.3%), and water and fish samples were also *Salmonella* positive and the percentage was 87.5 and 57.8% respectively.

3.5. Level of contamination at Kakra river

Total number of suspected isolates were 48, among them 13 isolates were *Salmonella* positive; and 27 isolates were *E. coli* positive (Table 1). The percentage of *Salmonella and E. coli* positive isolates were found 27.08 and 56.25%, respectively (Figure 2). In pabda fish, the percentage of *Salmonella* positive and *E. coli* positive isolates were determined at 42.86 and 71.43%, respectively. In magur, percentage of *Salmonella* positive and *E. coli* positive isolates were found at 25.00 and 68.75%, respectively. The highest percentage of *Salmonella* and *E. coli* positive isolates were found in 42.86 and 71.43%, respectively in pabda fish. On the other hand, lowest percentage of *Salmonella* and *E. coli* isolates were found at 23.08 and 38.46% in ayer fish, respectively (Figure 3). The present study revealed that the average highest percentage of *Salmonella* isolates 39.40% were found in pabda, and *E. coli* isolates were 63.84% in magur fish among three selected catfish, respectively.

Bartolomeu *et al.* (2011), reported that total coliform and *Escherichia coli*, coagulase-positive *Staphylococcus* and *Salmonella* in tilapia fish fillets. A study by Faridullah *et al*. (2016) pointed out that *Salmonella* positive samples was 43.7, 62.4 and 20% for water, pond scum and shrimp samples, respectively which is in the line with the present findings. Ava *et al*. (2020) conducted similar types of work and found that the level of incidence of *Salmonella* was greater in scum samples (93.8%) and for *E. coli* the higher contamination (Pamuk *et al*., 2011) estimates in open water samples (81.3%), and water and fish samples were also *Salmonella* positive and the percentage was 87.5 and 57.8%, respectively.

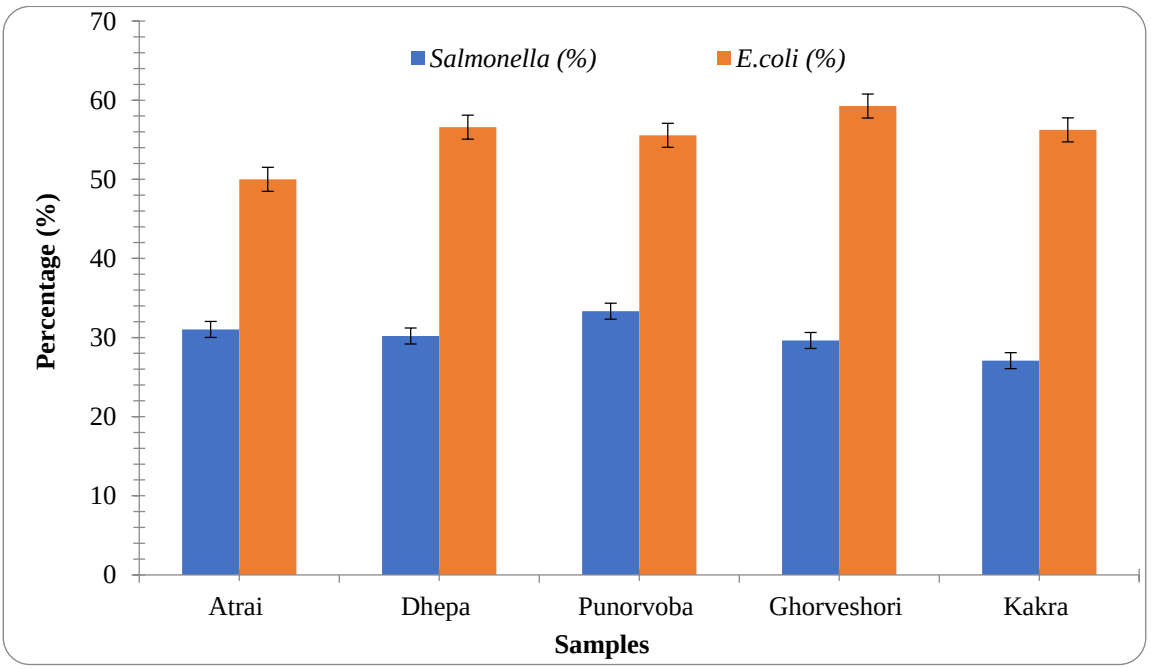

Figure 2. *Salmonella* and *E. coli* percentage at five rivers.

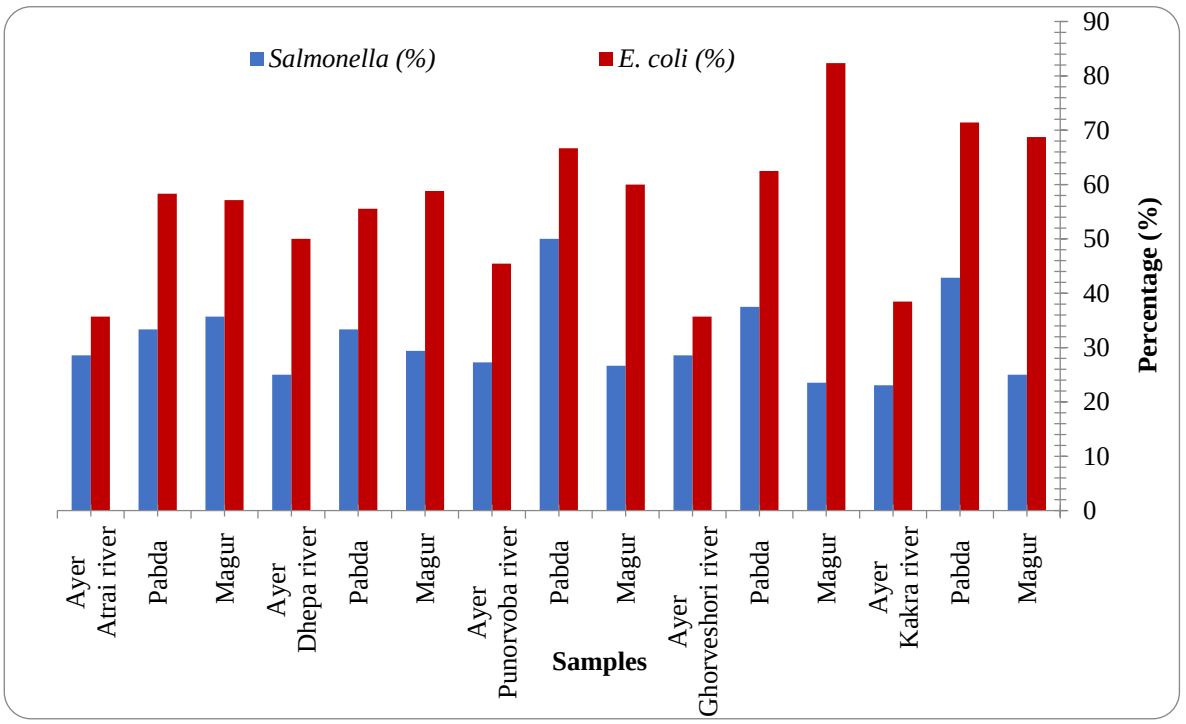

Figure 3. *Salmonella* and *Escherichia coli* positive percentage in three catfish of five rivers.

Table 1. Level of incidence of *Salmonella* and *E. coli* contamination at Atrai, Dhepa, Punorvoba, Ghorveshori and Kakra river in Dinajpur district.

Site	**Atrai river**			**Total**	**Percentage of positive isolates (%)**
Sample	Water	Scum	Fish	21	
No. of isolates	9	9	40	58	
Salmonella positive	3	2	13	18	31.03
E. coli positive	5	4	20	29	50.00
Dhepa river					**Percentage of positive isolates (%)**
Sample	Water	Scum	Fish	Total	
No. of isolates	8	7	38	53	
Salmonella positive	3	2	11	16	30.19
E. coli positive	5	4	21	30	56.60
Punorvoba river					**Percentage of positive isolates (%)**
Sample	Water	Scum	Fish	Total	
No. of isolates	8	5	32	45	
Salmonella positive	3	2	10	15	30.19
E. coli positive	4	3	18	25	56.60
Ghorveshori river					**Percentage of positive isolates (%)**
Sample	Water	Scum	Fish	Total	
No. of isolates	9	6	39	54	
Salmonella positive	3	2	11	16	29.63
E. coli positive	5	3	24	32	59.26
Kakra river					**Percentage of positive isolates (%)**
Sample	Water	Scum	Fish	Total	
No. of isolates	7	5	36	48	
Salmonella positive	2	1	10	13	27.08
E. coli positive	3	3	21	27	56.25

4. Conclusions

The present study revealed that the bacteriological condition of the water and catfish samples of the selected five rivers were not so good. The study results showed that almost all of the collected samples were contaminated with *Salmonella* and *Escherichia coli* bacteria that might be occurred through aquatic environment, humans' activity, poultry manure, flood, sewage pollution, lack of consciousness of people about personal hygiene and sanitation Therefore, it is crucial to improve the awareness of the people about water quality management of rivers in order to avoid public health risks.

Conflict of interest

None to declare.

Authors' contribution

Md. Faridullah: Conceptualizing, methodology, interpretation of data, and manuscript writing. Banya Rani: Performing research work. Md. Reazul Islam: Supervising and reviewing. Md. Masud Rana: Reviewing and editing. All authors have read and approved the final manuscript.

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