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).

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 tetrahionate 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°C 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°C 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. Incubation was done with the help of an inoculating wire by streaking into the medium and finally incubated at 35°C 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 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 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.

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 positive 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          | Sample | Atrai river | Total | Percentage of positive isolates (%) |
|---------------|--------|-------------|-------|-------------------------------------|
|               |        | Water       | Scum  | Fish                               |                                     |
| 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 |       |                                    |                                     |
| 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   |       |                                    |                                     |
| 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 |       |                                    |                                     |
| 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  |       |                                    |                                     |
| 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.

References
AOAC, 1984. Association of Official Analytical Chemist. Official methods of analysis. Association of Official Analytical Chemist. 13th edition, Washington, D.C.
Ava A, M Faridullah, UJ Lithi and VC Roy, 2020. Incidence of *Salmonella* and *Escherichia coli* in fish farms and markets in Dinajpur, Bangladesh. Bangladesh J. Sci. Ind. Res., 55: 65-72.
Bartolomeu DAFS, BR Dallabona, REPFD Macedon and PG Kirschnik, 2011. Contaminacao microbiologica durante as etapas de processamento de file de tilapia (*Oreochromis niloticus*). Arch. Vet. Sci., 16: 21-30.
Borsodi A, P Vladar, G Ceck, G Gedeon and A Micsinai, 2003. Bacterial activities in the sediment of Lake Velencei. Hungary Hydrobiologia. 721-728.

Brune A, P Frenzel and H Cypionka, 2000. Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiol. Rev., 24: 691-700.

DoF, 2019. National fish week, compendium (In Bengali). Dhaka: Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

Faridullah M, VC Roy and UJ Lithi, 2016. Prevalence of Salmonella and Escherichia coli contamination in shrimp (Penaeus monodon) farms, depots and processing plants in different areas of Bangladesh. Asian J. Med. Biol. Res., 2: 171-176.

Filimon MN, A Borozan, A Sinitean, R Popescu, R Torok-Oance, and D Verdes, 2010. Bacteriological studies on timis and bega rivers with a role in appreciating pollution. Ann. Romanian Soc. Cell Biol., 15: 217-219.

FRSS, 2016. Fisheries resources survey system (FRSS), fisheries statistical report of Bangladesh. 32: 1e5.

MacConkey AT, 1908. Bile salt media and their advantages in some bacteriological examinations. J. Hyg. (Lond.), 8: 322-334.

Maxine L, Heinitz, D Ramona, Ruble, E Dean, Wagner and RT Sita, 2000. Incidence of Salmonella in fish and seafood. J. Food Prot., 63: 579-592.

Pamuk S, Z Gurler, Y Yildrim and B Siriken, 2011. Detection of microbiological quality of common carp (Cyprinus carpio) sold in public bazaar in Afyonkarahisar. J. Anim. Vet. Adv., 10: 1012-1018.

Spring S, R Schulze, J Overmann and KH Schleifer, 2000. Identification and characterization of ecologically significant prokaryotes in the sediment of freshwater lakes: molecular and cultivation studies. FEMS Microbiol. Rev., 24: 573-590.

Wetzel RG, 2000. Freshwater ecology; changes, requirements and future demands Limnology. 1: 3-9.