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Incidence of *Salmonella* and *Escherichia coli* in fish farms and markets in Dinajpur, Bangladesh

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Abstract

The research work was conducted to assess the incidence of *Salmonella* and *Escherichia coli* (*E. coli*) contamination in different fish farms and fish markets of Dinajpur district of Bangladesh. The level of incidence of *Salmonella* contamination was greater in scum samples (93.8%) and for *E. coli* the higher contamination found in water samples (81.3%). The study showed that, water and fish samples were also *Salmonella* positive and the percentage was 87.5% and 57.8% respectively and for *E. coli* contamination about 75% of scum and 75.6% of fish samples were *E. coli* positive among fish farms samples. In case of market, both basket and fish sample were tested *Salmonella* positive, the level of incidence of *Salmonella* contamination was higher for basket samples (100%) and lower in fish samples (48.9%). Meanwhile, *E. coli* contamination for fish, basket, and mat samples were 75.6%, 68.8% and 75% respectively. Therefore, the results are very much alarming.

Keywords: Contamination; Fish farms; Fish markets; *Salmonella; Escherichia coli*

Introduction

Fish is considered one of the most nutritive and highly desirable foodstuffs because of its high nutritional value being rich in proteins, vitamins and unsaturated fatty acids. Fish contains n-3 polyunsaturated fatty acids that are extremely important aspect for health-conscious people particularly in affluent countries where mortality due to cardiovascular disease is high. Fish contains higher amount of myofibrillar protein which is easily digestible compared to protein found from other sources. Fish are of great concern for export earnings because of their higher nutritive value, low cholesterol level and presence of essential amino acids (Emikpe *et al*., 2011). Bangladesh was the 5th in world aquaculture production, which accounted for half of the country's total fish production in 2015–2016 (DoF, 2016). This sector contributes 3.65% to GDP and 1.97% to foreign exchange earnings through export (DoF, 2016). It is the second largest export industry in Bangladesh and produces 2.5 percent of the global production of shrimp (DoF, 2016).

Export of shrimp accounts 92% of the total export earning of fish and fishery products from Bangladesh (DoF, 2016). Bangladesh is exporting frozen shrimp to about 40 countries of the world of which USA, Belgium, UK, Netherlands, Germany, Japan, France, Denmark, Vietnam, Russia and Italy are the top 10 buying countries. About 18.02 million people were directly and indirectly involved in fisheries sector as the source of earning in 2014-2015 (DoF, 2016). In the year 2015-2016, total fish production was 38 million mt. ton and export earning was 42.82 billion tk. The safe consumption of fish and fishery products requires adequate sanitary conditions from harvest to consumption (Alghabban, 2014). Consumption of fish and shell fish may also cause illness due to infection or intoxication. Most of the food borne illness is caused by *Salmonella* spp., *Staphylococcus* spp. and *Escherichia coli*, usually related to fish consumption infected by those bacterial species especially *Salmonella* and *E. coli* (Yagoub, 2009). *Salmonella* infections in humans include mainly typhoid fever and these infections are commonly called as enteric fever which continues to be one of the most serious public health problems worldwide. The presence of higher level of *Salmonella* in fish causes several symptoms in human body like diarrhoea, nausea, vomiting and abdominal

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pain (Wyatt *et al.*, 1979). *Escherichia coli* infects the intestine and causes several symptoms these can include-abdominal pain, watery diarrhoea, bright red bloody stools, nausea, in some cases fever, fatigue etc. (Wyatt *et al.,* 1979). Every year the fish and fishery products of Bangladesh had been rejected by the importing countries due to presence of *Salmonella* spp. and *E. coli,* though it is one of the most important sectors for earning foreign currency (Okonko *et al.,* 2008). So, *Salmonella* and *E. coli* contamination in fish and fishery products is very alarming for human health as well as for export earnings. The present study was set mainly to assess the incidence level of *Salmonella* and *E. coli* contamination in fish farms and fish markets of Dinajpur district of Bangladesh.

Materials and methods

Isolation and identification of *Salmonella* and *E. coli* were done by the conventional method that includes screening and biochemical tests.

Study area and period

Samples were collected from different fish farms and fish markets of Dinajpur districts of Bangladesh. Fish farms and markets samples were collected from Dinajpur Sadar, Kaharol, Bochagong, Cirrirbondar, Khansama, Parbotipur, Birol and Fulbari Upazila of Dinajpur districts of Bangladesh for isolation and identification of *Salmonella* and *E. coli*. The study was conducted for a period of March, 2017 to September, 2017.

Sample collection

The samples were collected from two fish farms and markets of each Upazilla of Dinajpur districts of Bangladesh for the detection of *Salmonella* sp. and *Escherichia coli*. Pond water, scum and fish samples (body surface, gills, and peritoneal cavity) were collected from fish farms and samples taken from fish markets were fish (body surface, gill and peritoneal cavity), basket/plastic drums/bowl, and polythene sheet/mat. During sampling, some important information were also collected such as status of sanitation system, culture system, water source etc. of each farm and market through the survey questionnaire.

Swab samples of fish (body surface, gill and peritoneal cavity), basket, polythene sheet and mat were aseptically taken by cotton bud and directly streaked on the surface of Salmonella-Shigella agar (S-S agar) plates and pond water

Table I. Total number of samples collected and number of samples found *Salmonella* and *E. coli* positive in fish farms and fish markets of Dinajpur district of Bangladesh

Bacteria	Type of sample	Fish Farms			Fish markets		
		No. of sample	No. of sample positive	Percent of positive	No. of sample	No. of sample positive	Percent of positive
Salmonella	Water	16	14	87.5	-	-	-
	Scum	16	15	93.8	-	-	-
	Fish	45	26	57.8	45	22	48.9
	Basket	-	-	-	16	16	100
	Mat	-	-	-	16	9	56.3
E. coli	Water	16	13	81.3	-	-	-
	Scu	16	12	75	-	-	-
	Fish	45	34	75.6	45	34	75.6
	Basket	-	-	-	16	11	68.8
	Mat	-	-	-	16	12	75

Table II. Biochemical tests for phenotypic characterization of *Salmonella* and *E. coli*

Tests (*Salmonella*)	Results	Tests (*E. coli*)	Results
Gram-Stain	–	Gram-stain	–
Lysine decarboxylase	+	Indole	+
Urease	–	MR	+
Dulcitol	+	VP	–
KCN	–	Citrate	–
Malonate	–		
Indole	–		
VP	–		
MR	+		
Citrate	+/–		
Lactose	–		
Sucrose	–		

and scum samples were inoculated in the pre-enrichment medium. All the collected samples were shifted to the laboratory within 24 hours of the sample taken and incubated. Bacteriological analysis was performed for the detection of *Salmonella* sp. and *E. coli* according to the method of American Public Health Association (AOAC, 1984).

Bacteriological analysis

Pre-enrichment and Enrichment of samples

The collected samples were pre-enriched in ordinary nutrient broth and further inoculated into tetrathionate broth, selenite broth for selective enrichment and incubated at 35°C for 24 hours at the department of Fisheries Technology laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Streaking on to the Selective media

Samples were streaking into the selective media such as Salmonella-Shigella Agar (S-S), Deoxycholate Citrate Agar (DCA), and Xylose Lysine Deoxycholate (XLD) and incubated at 35^0C for 24 hrs and typical well isolated colonies were observed. Repeatedly streaking was performed where necessary.

Sample preservation

Well isolated colonies were streaking on to the surface of agar slant for samples preservation. Agar slant were prepared from ordinary nutrient agar medium. Then the preserved samples were kept into the refrigerator for further biochemical tests and phenotypic characterization of *Salmonella* and *E. coli.*

Screening and biochemical tests for phenotypic characterization of Salmonella and E. coli

Well isolated colonies of preserved samples were used to perform the screening and biochemical tests for phenotypic characterization of *Salmonella* and *E. coli*. The isolates suspected to be *Salmonella* and *E. coli* were inoculated to the TSI agar medium. Inoculation was performed with a straight needle by stabbing to the base of the butt, and streaking the slant when the needle was removed. Then it was incubated at 35^o C for 24 hrs. *Salmonella* cultures typically produced an alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of butt) in TSI agar and *E. coli* cultures typically produced an acidic slant and butt, with or without production of gases (CO_2 and H_2). The isolates suspected to be *Salmonella* and *E. coli* were inoculated to the EMB agar medium from preserved samples. Inoculation were performed with a inoculating wire by streaking into the medium and incubated at 35^oC for 24 hrs. *Salmonella* cultures typically produced translucent, amber colored or colorless colonies and *E. coli* cultures produced black or dark center with or without the greenish metallic sheen (Holt-Harris and Teague 1916). MacConkey agar is a differential media used for the isolation and differentiation of non-fastidious

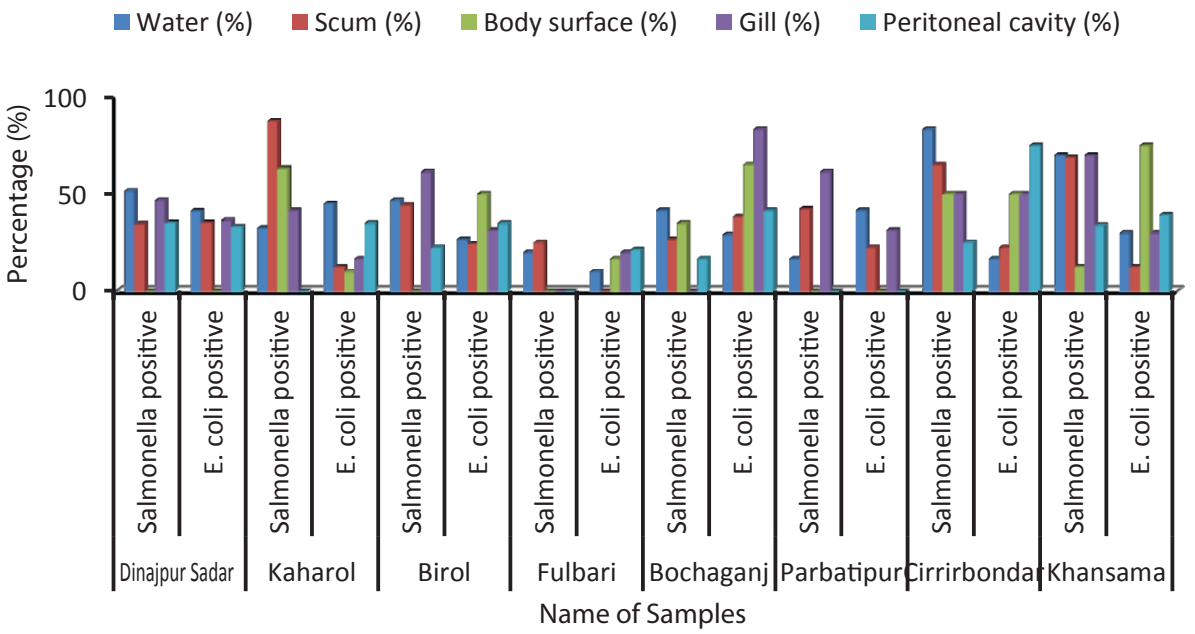

Fig. 1. Comparison of level of incidence of *Salmonella* and *E. coli* percentage at fish farms of different Upazila

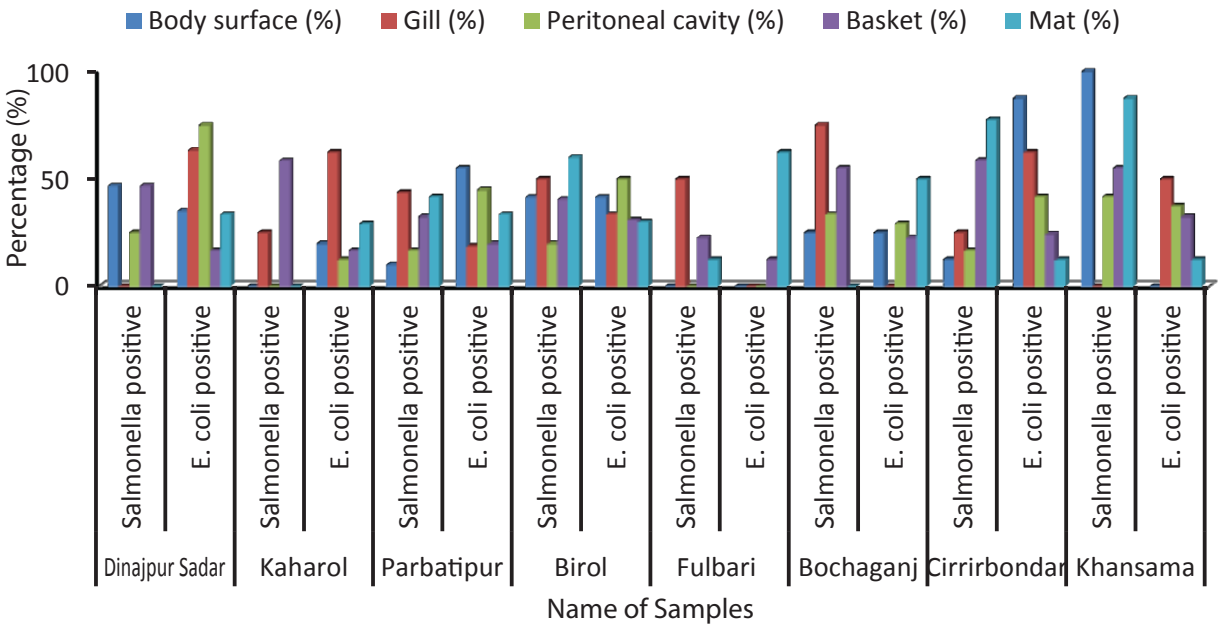

Fig. 2. Comparison of level of incidence of *Salmonella* and *E. coli* percentage at fish markets of different Upazila

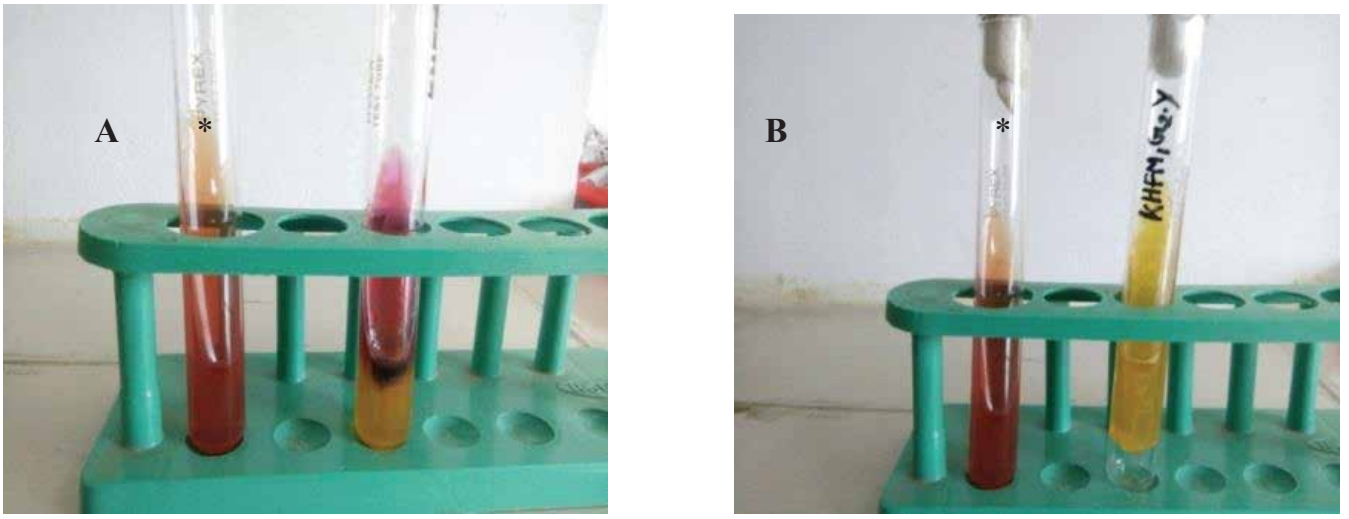

Fig. 3. Bacterial growth into TSI agar medium

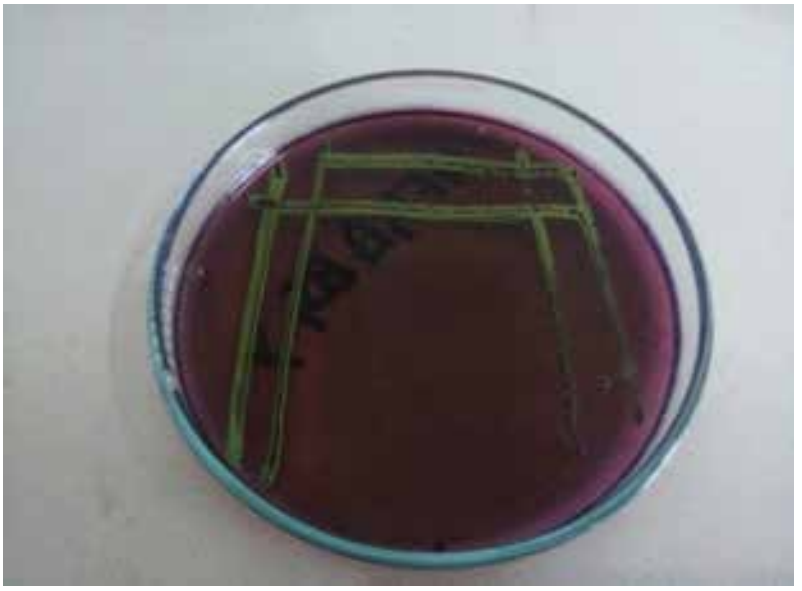

Fig. 4. *Escherichia coli* on EMB agar medium with metalic sheen

gram-negative rods, particularly members of the family enterobacteriaceae. The isolates suspected to be *Salmonella* and *E. coli* were inoculated to the medium from preserved samples and incubated at 35°C for 24hrs. *Salmonella* gave colorless colonies and *E. coli* colonies growth was showed pink colonies in these medium.

Microsoft Office Excel Worksheet 2007 was used to measure the percentage or incidence of *Salmonella* and *E. coli* in fish farms and fish markets.

Results and discussion

Contamination in fish farms

Water samples

All samples collected from different fish farms have shown positive results in respect to *Salmonella* and *E. coli* incidence. About 87.5% of the farm water samples and 46.3% isolates of water were contaminated with *Salmonella* and percentage of *E. coli* contamination for water was

81.3% of the samples and about 31.4% isolates of water showed positive result in Dinajpur district of Bangladesh. Bradd *et al*. (2008) found that, the *Salmonella* outbreaks from contaminated water to address the environment as a potential source of pathogenic *Salmonella* and investigated Salmonellae were present in 57 of the 72 water samples collected (79.2%).

Pond bottom scum

Scum samples were collected from all of the selected farms and showed *Salmonella* and *E. coli* positive. About 93.8% of the scum samples collected and 45.9% of the suspected isolates have been tested *Salmonella* positive, and about 75% of the samples and 24.8% of the suspected isolates have been tested *E. coli* positive for farm samples of Dinajpur district of Bangladesh. Anderson *et al.* (2005) studied about the fecal coliforms and enterococci indicator organisms to monitor water quality and found that freshwater and saltwater sediments were contaminated with dog feces, sewage, or other pollutant that included natural stressors such as microbial predators, radiation, and temperature fluctuations. *Salmonella* and *E. coli* both of the fecal coliform bacteria that polluted the water body and cause several impact on human body (Fish *et al.,* 1995).

Fish samples

Fish samples collected from different farms were more or less contaminated with *Salmonella* and *E coli*. About 57.8% of total fish samples and 30.9% of total suspected isolates have been tested *Salmonella* positive. On the other hand, 75.6% of all the fish samples and 41.6% of suspected isolates were *E. coli* positive. Heinitz *et al.* (2000) found that 10% of imported and 2.8% of domestic raw seafood was positive for *Salmonella. Enterococcus sp* and *Aeromonas sp*, fecal and total coliform, the presence of *Listeria sp* and *Salmonella spp* from the external surface of tilapias were shown by Morales *et al.* (2004). Maxine *et* al. (2000) found the overall incidence of *Salmonella* was 7.2% for import and 1.3% for domestic seafood whereas, around 10% of import raw seafood and 2.8% of domestic raw seafood were positive for *Salmonella.*

Contamination in fish markets

Fish samples

Fish samples collected from different fish markets of Dinajpur district were contaminated with *Salmonella* and *E. coli* except one markets of Kaharol Upazilla. About 48.9% of the samples collected and 28.6 % of all the suspected isolates were tested *Salmonella* positive. On the other hand 75.6% of all samples collected and 35.8% of all the suspected isolates were tested *E. coli* positive. Shabarinath *et al., (*2007) found *Salmonella* in 18.9% of naturally contaminated shrimp samples and 21.4% of crab, clam, mussel and oyster samples. 100 fish and shellfish samples obtained from the market and fish-landing centre in India, where *Salmonella* was detected in 70% of fish, 59% of shrimp and 30% of oyster samples. *Escherichia coli* was detected in 1.4, 1.5, and 5.9% of trout, salmon, and tilapia, respectively, no sample had _1.0 log MPN/g. However, *E. coli* was found in 13.2% of catfish, with an average of 1.7 log MPN/g (Pao *et al.,* 2008). David *et al.* (2009) studied on 120 Nile tilapia of Winam Gulf and found about 63 (52.5%) were infected with *Enterobacteriaceae*, 25 (39.7%) were *Shigella spp*, 9 (14.3%) *Salmonella typhimurium*, 7 (11.1%) *S. typhi*, 4 (6.3%) *S. enteritidis*, 16 (25.4%) *E. coli,* 1 (1.6%) *Proteus spp.* and *Enterobacter aerogenes* respectively. David *et al.* (2009) collected ten fish from open-air markets and found *E. coli* (50%), *S. typhimurium* (20%), *S. paratyphi* (10%) and *S. typhi* (20%).

Basket samples

Basket Samples were collected from baskets of different fish markets that have been found contaminated at different level with *Salmonella* and *E. coli*. Among the basket samples collected the suspected isolates were 100% and 43% *Salmonella* positive. Among the samples collected about 68.8% and 21.52% isolates were *E. coli* positive. Alam *et al.,* (2014) conducted study in two retail fish markets located in Rajbari and Barisal Sadar Upazila in the south central region of Bangladesh focusing public health and hygiene condition of retailers and indicated that due to poor infrastructural facilities, poor washing facilities, landing, handling, distribution, marketing and quality assurance, the baskets were contaminated by pathogenic bacteria.

Mat samples

About 56.3% of the mat samples collected and 38.2% of the suspected isolates have been tested *Salmonella* positive. On the other hand, 75% of the collected samples and 36.4% suspected isolates respectively were *E. coli* positive. Overall results revealed that there was no control to maintain bacteriological quality of fish markets. Hastein *et al*. (2006) outlined and discussed the hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood by health hazard bacteria.

Conclusion

Bacterial food borne illnesses are the most wide spread global public health problems of recent times, and their implication for health and economy is increasingly recognized. Bangladesh is a country with high incidence of bacterial diseases in fish farms due to the most of our water body is not hygienic. The results indicated that all of the collected samples were contaminated with *Salmonella* and *E. coli* bacteria. Fish farms condition were not satisfactory in terms of flood, sewage pollution, used of animal manure, feed and waste disposal, application of excessive fertilizer, lack of consciousness of farmers about personal hygiene and sanitation, and in fish markets, stalls, parking, spacing, sanitation, drainage and quality management systems were not improved. It is important to train up the farmers, fish handlers and fish sellers about good aquaculture practice, HACCP system, fish transportation system, cool chain management system, and overall quality management system of fishes.

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