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Article

Prevalence of *Salmonella* and *Escherichia coli* contamination in shrimp (*Penaeus monodon*) farms, depots and processing plants in different areas of Bangladesh

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Abstract: A study was conducted to determine the level of contamination by indicator organisms (*Salmonella* and *Escherichia coli*) in shrimp (*Penaeus monodon*) farms, depots and processing plants of Cox's Bazar, Khulna, Bagerhat and Satkhira districts of Bangladesh using conventional technique of bacterial isolation and identification. The results of this study revealed that prevalence of *Salmonella* positive samples was 43.7%, 62.5%, 20% and 0.0% for water, pond scum, shrimp and basket samples, respectively at farm level. 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. In case of depots, shrimp, basket and mat samples were analyzed and found 20%, 56.3% and 23% positive for *Salmonella* and 53.3%, 37.5% and 92.3% positive for *E. coli*. Bacteriological assessment of the shrimp samples obtained from four seafood processing plants revealed that all the samples were found contaminated with *Salmonella* and *E. coli* except the samples of one industry. Investigation finally showed that with few exceptions all the samples from farms, depots and processing plants have different levels of contamination by *Salmonella* and *E. coli*, which is very much alarming for shrimp industry of Bangladesh.

Keywords: *Penaeus monodon*; farms; depots; processing plants; *Salmonella; Escherichia coli*

1. Introduction

Shrimp is considered as 'white gold' for Bangladesh and contributing an important role in national economy. It represents the second largest export industry for Bangladesh after garments with 97% of the shrimp produced being exported (Kruijissen *et al*., 2012), contributing about 4% to national GDP (Haque *et al*., 2012) and employing approximately 1.2 million people for production, processing and marketing activities.

From harvesting to the consumers table shrimp may be contaminated with various kinds of hazards some of which are natural and others are introduced by the handlers. There are serious safety concerns of raw fish and shell fish due to presence of biological (bacteria, viruses, parasites) and chemical hazards (Hosseini *et al.*, 2004). Salmonellae are gram-negative, non-spore forming motile aerobic rods that characteristically ferment glucose and manose but fail to ferment lactose or sucrose (Jawetz *et al.*, 1982). The natural habitat of *Salmonella* is the gastrointestinal tract of animals including birds and man (Pelzer, 1989). These organisms find its way into the river water, coastal and estuarine sediments through fecal contamination. Aquatic environment is the major reservoirs of *Salmonella* and aids its transmission between the hosts (Cherry *et al.*, 1972). The genus *Salmonella* has a wide variety of species, which are pathogenic and cause different types of food poisoning. Contamination of seafood with *Salmonella* is major public health concern. The occurrence of *Salmonella* in fish and shellfish, either in fresh or marine waters has normally been associated with fecal contamination of the area from which they were harvested (Buttaiux, 1962) On the other hand, *Escherichia coli*, the representative of coliforms, are gram-negative, rod-shaped facultatively anaerobic bacteria.

Identification criteria used are production of gas from glucose (and other sugars) and fermentation of lactose to acid and gas within 48 h at 35ºC (Hitchins *et al.*, 1998). *E. coli* are naturally found in the intestinal tracts of all warm-blooded animals, including humans. Most forms of the bacteria are not pathogenic and serve useful functions in the intestine. Pathogenic strains of *E. coli* are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. *E. coli* food infection causes abdominal cramping, water or bloody diarrhea, fever, nausea and vomiting (Ward, 1997) Shrimps Sea foods are usually contaminated by *Salmonella* and *E. coli* due to presence of human and animal wastes. Shrimp are also contaminated from the handlers who are not conscious about the hygiene and sanitation during handling of shrimp at different stages. These microorganisms are pathogenic and cause different types of food poisoning. Since importing countries are very much conscious about hazardous pathogens, the importing agencies always reject the shrimp and shrimp products if they contain any *Salmonella* and *E. coli*. Therefore, it is very much important to identify the route of *Salmonella* and *E. coli* contamination in shrimp farms, depots and processing industry. This study revealed the survey results on the prevalence of *Salmonella* and *E. coli* contamination at different shrimp farms, depots and processing plants of some selected areas of Bangladesh.

2. Materials and Methods

2.1. Sampling area and sample collection

Samples were collected from farms and depots of 8 upazilla under four prominent Shrimp (*Penaeus monodon*) producing districts of Bangladesh viz. Cox's Bazar, Khulna, Bagerhat and Satkhira. Samples were taken from two farms and two depots from each upazilla. Samples were also taken from the shrimps at the receiving point of four shrimp processing industry located at Cox's Bazar and Khulna. Sources of samples in the farms were water, pond bottom scum, shrimp (body surface, gills and peritoneal cavity), basket (in most cases plastic drums) and mat/polythene sheet. Sources of samples in the depots were shrimp, basket and mat/polythene sheet. Sources of samples in processing industries were only shrimp (body surface, gill and peritoneal cavity). In some of the sampling sites bacterial samples could not be taken from all sources due to practical reasons like unavailability of the sample sources during the time of sampling (Table 1).

Two sampling methods were used such as (i) direct inoculation of samples in pre-enrichment medium and (ii) cotton swabs taken from shrimp, basket and mat/polythene sheet were directly streaked on the surface of Salmonella-Shigella agar (SS agar) plates and the swabs were then inoculated in the pre-enrichment medium. All the samples were shipped to the laboratory within 48 hours of the sample taken. After arrival of the samples in the laboratory, bacteriological analysis was done for the detection of *Salmonella* sp. and *E. coli* according to the method of American Public Health Association (Association of Official Analytical Chemists, 1984).

2.2. Screening and biochemical tests

A suspected colony of *Salmonella* was picked up with inoculating loop and inoculated in the Triple Sugar Iron (TSI) agar slant by streaking the slant and stabbing the butt and incubated at 35^{o} C for 24 hrs. *Salmonella* cultures typically produce an alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of butt) in TSI agar. The TSI cultures were purified by streaking onto McConkey's agar (MCA) and incubated for 24 hrs at 35^{o} C. Typical colonies appear transparent and colorless, sometimes with a dark center (Figure 1). For *E. coli* pink colonies on SS agar were streaked onto Eosine Methylene Blue agar (EMB) and incubated at 35^{o} C for 18-24 hrs. Suspected *E. coli* colonies had black or dark center with the greenish metallic sheen (Figure 2).

Salmonella and *E. coli* colonies were sub-cultured in nutrient broth and are incubated at 35^{o} C for 24 hrs. Using the nutrient broth culture as inoculums the biochemical tests were performed using fresh bacterial culture (Table 2).

3. Results and Discussion

3.1. Contamination in shrimp farms

The level of *Salmonella* and *E. coli* contamination in different samples (water, scum, shrimp, basket and mat) collected from Shrimp farms of different sampling sites of Bangladesh were different.

3.1.1. Water samples

Water samples collected from Chokoria, Teknaf, Mongla, Koira and Munshigonj Shrimp (*P. monodon*) farms have shown positive result in *Salmonella* test and farms of other three locations Rampal, Paikgacha and Ashashuni have shown negative results. About 43.7% of the water samples were *Salmonella* positive. In case of *E. coli* water samples from all the locations except Paikgacha have shown the positive result and about 62.5% of the samples have shown the positive result.

Table 1. Total number of samples collected and number of samples found *Salmonella* and *E. coli*.

Bacteria	Type of Sample	Farm			Depot		
		No. of sample	No. of sample positive	Percent of positive	No. of sample	No. of sample positive	Percent of positive
Salmonella	Water	16	7	43.7	–	–	-
	Scum	16	10	62.5	–	–	-
	Shrimp	30	6	20.0	45	9	20.0
	Basket	10	Nil	0.0	16	9	56.3
	Mat	6	Nil	0.0	13	3	23.1
E. coli	Water	16	10	62.5	–	–	-
	Scum	16	7	43.7	–	–	-
	Shrimp	30	18	60.0	45	24	53.3
	Basket	10	6	60.0	16	9	56.2
	Mat	6	Nil	0.0	13	12	92.3

Table 2. Biochemical tests for phenotypic characterization of *Salmonella* and *E. coli.*

Tests (*Salmonella*)	Results	Tests (*E. coli*)	Results
Catalase	+	Catalase	+
Cytochrome oxidase	-	Cytochrome oxidase	-
MR	+	MR	+
VP	-	VP	-
Indole	-	Indole	+
Citrate	+/-	Citrate	-
KCN	-		
Malonate	-		
Urease	-		
Lactose	-		
Sucrose	-		

The tests were incubated at 35^{o} C for 18-24 hrs.

Table 3. Incidence of Salmonella and *E. coli* contamination in selected shrimp (*P. monodon*) processing plants (at receiving point) of Bangladesh.

Bacteria	Sample	Conception Seafood, Cox's Bazar	Kuliarchar Seafood, Cox's Bazar	Rupsha Seafood, Khulna	Fresh Seafood, Khulna
Salmonella	Shrimp	+	+	-	+
	Basket	*	*	*	*
	Mat	*	*	*	*
E. coli	Shrimp	+	+	+	+
	Basket	*	*	*	*
	Mat	*	*	*	*

* Sample could not be taken

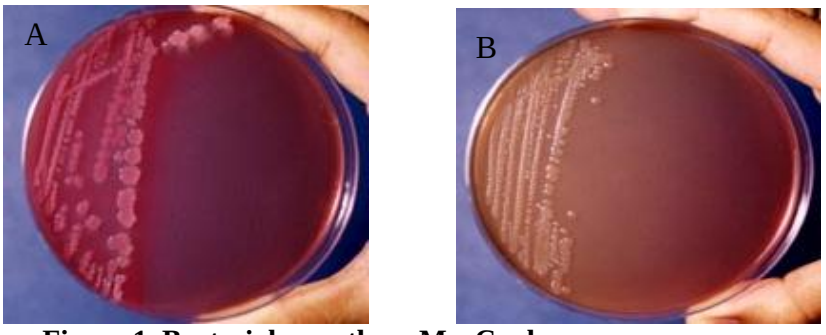

Figure 1. Bacterial growth on MacConkey agar.
A. *Escherichia coli* on MacConkey agar: growth, pink color colonies
B. *Salmonella* on MacConkey agar: growth, colorless colonies

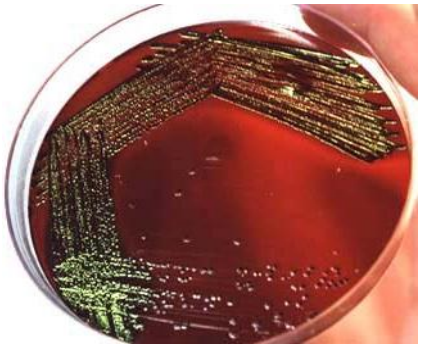

Figure 2. *Escherichia coli* on EMB agar with metallic sheen.

3.1.2. Pond bottom scum

Scum samples from all the locations except Paikgacha have been found *Salmonella* positive. About 62.5% of the scum samples have been tested positive for *Salmonella*. On the other hand scum samples from Chokoria, Rampal Mongla, Munshigonj and Ashashuni have been tested positive for *E. coli* and samples from other three locations Teknaf, Paikgacha and Koira tested negative. About 43.7% of the samples have been tested *E. coli* positive.

3.1.3. Shrimp

Shrimp samples of both the farms of Chokoria and Teknaf and one farm of Rampal have been tested *Salmonella* positive and samples from the farms of other locations tested *Salmonella* negative. 20% of total shrimp samples have been tested *Salmonella* positive. On the other hand shrimp samples collected from the farms of all the locations have been tested *E. coli* positive. 60% of all the shrimp samples have been tested *E. coli* positive.

3.1.4. Baskets and mats/polythene sheet

Samples taken from baskets and mats from Shrimp farms have been tested *Salmonella* negative. However, due to unavailability of baskets and mats samples could not be taken from some farms. Samples taken from the baskets found in the farms of Teknaf, Rampal Munshigonj and Ashashuni were *E. coli* positive but the samples taken from the mats were *E. coli* negative. 60% of basket samples tested were *E. coli* positive.

3.2. Contamination in shrimp depots

The bacteriological assessment of different samples obtained from different shrimp depots of different areas revealed different level of *Salmonella* and *E. coli* contamination.

3.2.1. Shrimp

Shrimp samples from at least one depot of each sampling location have been tested *Salmonella* positive except Paikgacha where samples collected from both the depots were *Salmonella* negative. Bacteriological samples collected from the depots of all the locations have been tested *E. coli* positive except Koira where samples from both the depots were *E. coli* negative. 20% of all the samples were *Salmonella* positive. On the other hand 53.3% of all samples were tested *E. coli* positive.

3.2.2. Basket

Bacteriological samples collected from the baskets of at least one depot of each sampling location were tested *Salmonella* positive and except one location (Teknaf) samples collected were *E. coli* positive. Among the samples 56.2% were *Salmonella* and *E. coli* positive.

3.2.3. Mat/polythene sheet

Bacteriological samples collected from the mats/polythene sheet used in the depots of Mongla, Paikgacha and Koira were *Salmonella* positive. About 23% of the samples collected have been tested *Salmonella* positive. On the other hand almost all the samples collected from the mats of all the depots have been tested *E. coli* positive.

3.3. Contamination in processing plants

Bacteriological samples were collected from shrimps at the receiving point of 4 processing plants of Cox's Bazar and Khulna region. Except Rupsha Seafood Industry of Khulna all the shrimp samples were found contaminated with *Salmonella* and *E. coli* (Table 3).

Present study conducted on the incidence of *Salmonella* and *E. coli* in Shrimp from different sources like culture ponds (water, scum), containers (mat, polythene sheet, basket/tanks etc.) transport and other sources related to post-harvest handling and transportation of shrimp. Results found are comparable to previous studies conducted elsewhere. But no information is available on Bangladesh.

A number of researchers studied the *Salmonella* and *E. coli* contamination in seafoods particularly in penaeid shrimps (*P. monodon*). The coliform contents of farmed penaeids varied between 460-1100 whereas the surrounding water and sediments were between 11 x 10^3 and 123 or 1100 respectively. Faecal coliforms were found in the range of 10-200 on the sediment samples (Putro *et al.*, 1990). Coliform bacteria, *Escherichia coli*, coagulate-positive staphylococci, *Salmonella*, and *Listeria monocytogenes* contamination was studied in individually quick frozen (IQF) shrimp products. *S. typhimurium* was isolated from one sample of raw, peeled tail-on. Coliforms were detected in all the products, though at a low level. Prevalence of coliforms was higher in headless shell-on (26%) shrimps followed by raw, peeled, and deveined tail-off (19%), raw, peeled tail-on (10%), and cooked, peeled tail-on (3.8%) shrimps. The highest prevalence of *E. coli* (4.8%) was noticed in headless shell-on shrimps. Overall results revealed that the plant under investigation had exerted good process control in order to maintain superior bacteriological quality of their products (Hatha *et al.*, 2003). Occurrence of *Salmonella* in fish and shellfish, either in fresh of marine waters has normally been associated with fecal contamination of the area from which they were harvested (Buttaiux, 1962). Thus it is generally suggested not to use animal manure as a source of fertilizer for aquaculture ponds because this practice introduces *Salmonella* which was later isolated from the harvested product (Miget, 1991).

In the present study it was found that about 70% farms received from and drain out water to another pond in all survey areas. Although the use of organic manure like cow-dung or chicken waste is limited in the ponds but there is a chance of contamination of the water with sewage through the water receiving canal from other sources. Introduction of *Salmonella* and *E. coli* to the water and scum as well as shrimp samples of farms may due to the above reasons.

Post process contamination can occur by coliforms, fecal coliforms including *E. coli, Staphylococcus* and *Salmonella* to the raw materials particularly when significant hand peeling is involved (Miget, 1991). Pathogens also introduced via product handlers-from personnel on the harvest boat, through processing plant and food service handlers, and ultimately the consumers. The hands of workers have been identified as sources of these pathogens .In the present study, the percentage of *Salmonella* and *E. coli* contamination to the samples from depots was higher than those from farms may due to improper and rough handling, delay icing, improper washing, careless beheading and peeling of the shrimps.

4. Conclusions

Detection of *Salmonella* and *E. coli* in samples collected from pond water and scum indicates various degrees of contamination and pollution. The same organisms detected in baskets, mats and shrimp indicate the poor sanitation and hygienic conditions of farms. Isolation of *Salmonella* and *E. coli* in the depots indicates the poor infrastructure facilities, poor sanitation and contamination in the depots. Most of the Shrimp farms are located nearby villages and the farms are ultimate recipient of community disposal including human and animal excreta, which may carry potential pathogens. This must be stopped. The incidence of disease in Shrimp farms is very frequent. Good farming practice including regular water quality monitoring is important to tackle the problem. Training programs are required for farmers, suppliers and depot owners for implementation of HACCP at the field level for effective quality management.

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Conflict of interest

None to declare.

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