

# A Bacteriological Assessment for *Salmonella* and *Escherichia coli* in Some Selected Fresh Water Prawn (*Macrobrachium rosenbergii*) Farms and Depots

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**Abstract** Golda farms and depots of selected areas of the different districts of Bangladesh viz. Khulna, Bagerhat, Jessore and Norail area were sampled for the detection of *Salmonella* sp. and *Escherichia coli*. Incidence of *Salmonella* positive samples was 39%, 25%, 50% and 42% in the farms and 30%, 20%, 20% and 30% in the depots of Dumuria under Khulna, Bagerhat Sadar under Bagerhat, Avoyanagar under Jessore and Kalia under Norail district respectively. On the other hand, *E. coli* positive samples was 23%, 42%, 25% and 17% in the farms and 70%, 30%, 50% and 30% in the depots of Dumuria (Khulna), Bagerhat Sadar (Bagerhat), Avoyanagar (Jessore) and Kalia (Norail) region respectively. The overall results indicate that the trend of *Samonella* and *E. coli* contamination in farms and depots of all the regions is more or less similar although some variations were observed among the farms and depots of different location and region.

**Key words :** Golda, *Macrobrachium rosenbergii*, depots, *Escherichia coli*, *Salmonella*

## Introduction

The initial microflora of live crustaceans depends upon the environment from which they are harvested. Crustaceans are generally cooked prior to consumption which significantly reduces the microbial flora. But major problems occur as a result of cross contamination during different activities from harvesting to consumption. Most of the published papers in the literature concerning the microbial spoilage are related with shrimps of marine origin (particularly *Penaeus* spp.).

Salmonellae are bacteria which reside in the intestinal tract of infected animals and humans and which may be shed in the faeces. Their presence in raw shellfish is indicative of sewage pollution of the coastal environment or the pond water (e.g. effluent run off from agricultural land). 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 [1]. *E. coli* is an enteric organism which is often used as an indicator organism

in the determination of the microbiological status of products and/or processes. This bacterium is used in the categorization of shellfish harvesting areas under Council Directive 91/492/EEC [2]. Its presence is indicative of faecal contamination and suggests that pathogens of an enteric nature may also be present. Based on these considerations the present study was conducted to monitor the incidence of *Salmonella*, and *E. coli* in freshwater prawn (golda) during different stages of handling and transportation at various temperatures.

## Materials and Methods

### Sample collection and sampling

Eight Golda (*Macrobrachium rosenbergii*) farms and 8 depots from four Southern districts of Bangladesh (Khulna, Bagerhat, Jessore and Norail) were sampled for the detection of *Salmonella* sp. and *Escherichia coli*. Samples were taken from two farms and two depots

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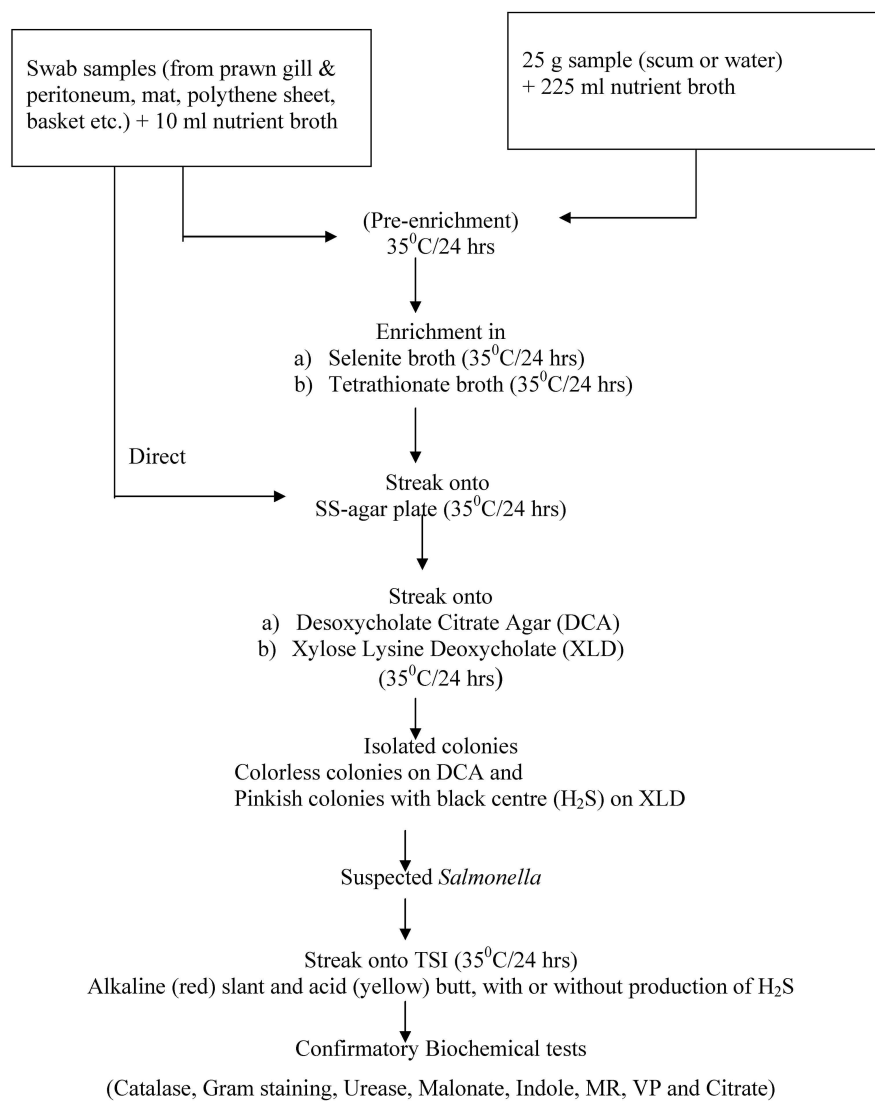
from each sampling site. Sources used for the bacterial samples in the farms were water, pond bottom scum, shrimp (body surface, gills and peritoneal cavity), basket (in most cases plastic drums) and mat/polythene sheet. Sources of bacterial samples in the depots were shrimp, basket and mat/polythene sheet. 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.

Two sampling methods were used such as (i) direct inoculation of pre-enrichment medium with water and scum 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. As soon as the sampling was completed all the samples were transported to the laboratory and subjected to bacteriological analysis for the detection of *Salmonella* sp. and *E. coli* according to the method of American Public Health Association [4]. In order to increase the number of bacteria in the sample so that they can be easily detected pre-enrichment and selective enrichment was done and then plated to different media.

### Screening and biochemical tests

Suspected colonies of *Salmonella* was inoculated in the Triple Sugar Iron (TSI) agar slant by streaking the slant and stabbing the butt and incubated at 35°C for 24 hrs. *Salmonella* cultures typically produce an alkaline (red) slant and acid (yellow) butt, with or without pro-



**Fig. 1.** Flow Diagram of Examination Procedures for *Salmonella*.

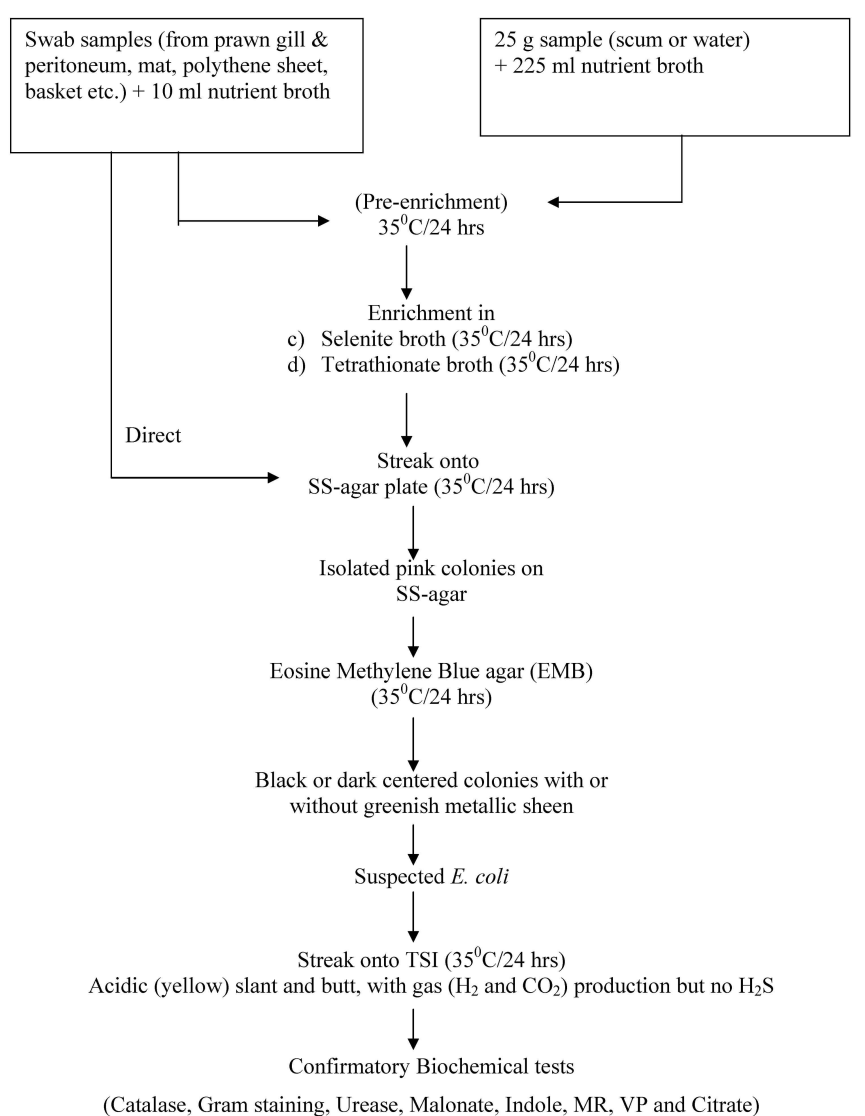
duction of  $H_2S$  (blackening of butt) in TSI agar (Fig. 1). For *E. coli* pink colonies on SS agar were streaked onto Eosine Methylene Blue agar (EMB) and incubated at  $35^\circ C$  for 18-24 hrs developed black or dark centered colonies with or without the greenish metallic sheen. In the Triple Sugar Iron (TSI) agar slant, *E. coli* cultures typically produce an acidic (yellow) slant and acidic (yellow) butt, with production of gas ( $H_2$  and  $CO_2$ ) which cause splitting of the medium but produce no black ( $H_2S$ ) color in TSI agar. The TSI cultures were purified by streaking onto McConkey agar (MCA) and incubated for 24 hrs at  $35^\circ C$  (Fig. 2). Typical colonies appear transparent and colorless, sometimes with a dark center.

*Salmonella* and *E. coli* colonies were sub-cultured in nutrient broth or in nutrient agar at  $35^\circ C$  for 24 hrs.

Using the nutrient broth culture or nutrient agar culture as inoculum the biochemical tests were performed (Table 1).

**Table 1.** The list of biochemical tests. The tests were incubated at  $35^\circ C$  for 18-24 hrs (where applicable)

Tests ( <i>Salmonella</i> )	Results	Tests ( <i>E. coli</i> )	Results
Catalase	+	Catalase	+
Gram	-	Gram	-
Urease	-	Indole	+
Malonate	-	MR	+
Indole	-	VP	-
MR	+	Citrate	-
VP	-		
Citrate	+/-		



**Fig. 2.** Flow Diagram of Examination Procedures for Coliforms and *E. coli*.

## Results and Discussion

### Detection of *Salmonella* and *E. coli* in golda farms

*Dumuria, Khulna:* From two investigated golda farms for the detection of *Salmonella* and *E. coli* 13 samples were collected from the probable sources of bacteria like pond water, bottom scum, prawn, basket and mat. Three prawn samples, one water sample and one basket samples contained *Salmonella* and one sample each of water, scum and shrimp contained *E. coli*. In rest of the samples no *Salmonella* and *E. coli* could be detected. Total numbers of suspected isolates were 34 and among them 8 isolates have been identified as *Salmonella* and 4 isolates as *E. coli*. In farm-2 from none of the samples *E. coli* could be detected (Table 3).

*Bagerhat Sadar, Bagerhat:* Twelve samples were collected from the golda farms under bacteriological investigation. No mats were available at the farm site

during sampling, so sample could not be taken from mat. Only three samples were found *Salmonella* positive but all of them were from prawn samples. No *Salmonella* could be detected in pond water, scum or basket used in the farm. Five samples were *E. coli* positive which included pond water, gills, peritoneal cavity and basket. Total number of suspected isolates was 32, of which 3 have been identified as *Salmonella* and 5 as *E. coli*. Prawns from both the farms were *Salmonella* positive (Table 2).

*Avoyanagar, Jessore:* Two golda farms of Avoyanagar were investigated for *Salmonella* and *E. coli* contamination. Twelve samples were collected from different sources of bacteria including prawn. Due to unavailability samples could not be taken from mat. *Salmonella* was detected in six samples and *E. coli* in three samples. In farm -1 water and basket samples have been found to be *Salmonella* positive and in farm-2 water, scum, prawn and basket samples have shown positive result for *Salmonella*. Only 3 samples have been found

**Table 2.** Incidence of *Salmonella* and *E. coli* in selected golda farms of Bangladesh

Bacteria	Sample	Dumuria, Khulna	Sadar, Bagerhat	Avoyanagar, Jessore	Kalia, Norail
<i>Salmonella</i>	Water	+	—	+	+
	Scum	—	—	+	—
	Prawn	+	+	+	+
	Basket	+	—	+	—
	Mat	—	*	*	*
<i>E. coli</i>	Water	+	+	+	+
	Scum	+	—	—	—
	Prawn	+	+	+	+
	Basket	—	+	+	—
	Mat	—	*	*	*

\* Sample could not be taken

\*\* One positive among the two farms sampled

**Table 3.** Total number of samples collected and number of samples found *Salmonella* and *E. coli* positive in golda farms

Bacteria	Types of sample	No. of samples	No. of samples positive	Samples positive (%)	No. of isolates	No. of isolates positive	Isolates positive (%)
<i>Salmonella</i>	Water	8	4	50	25	6	24
	Scum	8	1	12.5	17	1	5.9
	Prawn	24	11	45.8	67	18	26.8
	Basket	8	3	37.5	22	3	13.6
	Mat	1	Nil	0	2	Nil	0
<i>E. coli</i>	Water	8	5	62.5	25	6	24
	Scum	8	1	12.5	17	1	5.9
	Prawn	24	5	20.8	67	7	10.4
	Basket	8	2	25	22	3	23.6
	Mat	1	Nil	0	2	Nil	0

to be *E. coli* positive in the farms. Number of suspected isolates was 35, of which 7 have been identified as *Salmonella* and 5 of them as *E. coli*. None of the prawn samples from farm-1 contained *Salmonella* (Fig. 3).

*Kalia, Norail*: Twelve samples were collected from the selected farms of Kalia of Norail district. Two prawn samples from farm-1 and two prawn samples and one water sample from farm-2 were found to be *Salmonella* positive, on the other hand one prawn sample from farm-1 and one water sample from farm-2 were *E. coli* positive. Prawns from both the farms were *Salmonella* positive. Number of suspected strains isolated from the samples of these farms was 32 and out of which 10 isolates have been identified as *Salmonella* and 3 identified as *E. coli*. Due to unavailability sample could not be taken from mat.

A comparison of incidence of *Salmonella* and *E. coli* in golda farms of selected areas of Bangladesh is given to the Table 2 and Table 3.

Detection of *Salmonella* and *E. coli* in depots

*Dumuria, Khulna*: Two selected golda depots of the Dumuria area were also investigated for the detection of *Salmonella* and *E. coli* in prawn, basket and mat. Ten samples were collected from the two depots which included prawn body surface, gills, peritoneal cavity, basket and mat. In depot-1 two prawn samples and one basket sample was found to be *Salmonella* positive but in depot-2 all the samples were *Salmonella* negative. In depot-1 all the samples were *E. coli* positive and in depot-2 *E. coli* was detected in basket and mat samples. Total number of suspected isolates from the samples was 46, of which 4 of them identified as *Salmonella* and 21 of them were *E. coli* (Fig. 4).

*Bagerhat Sadar, Bagerhat*: Ten samples were collected from the two golda depots investigated from this area. Two prawn samples one each from the two depots were found to be *Salmonella* positive and two prawn samples and a basket sample were found to be *E. coli*

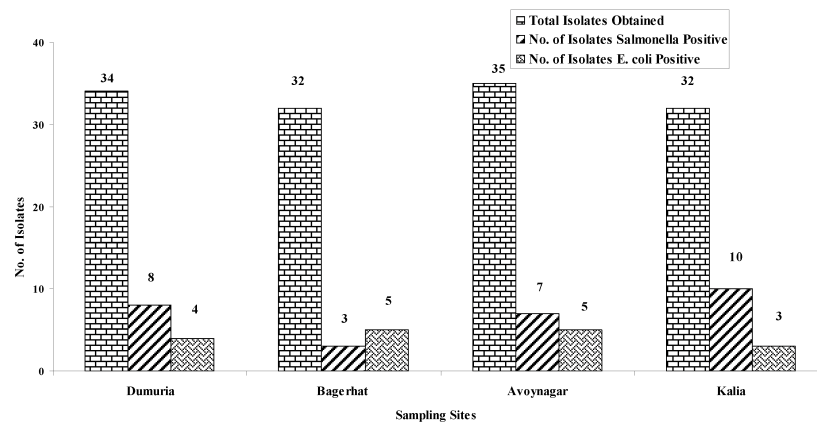


Fig. 3. Contamination of isolates in golda farms of different sampling areas.

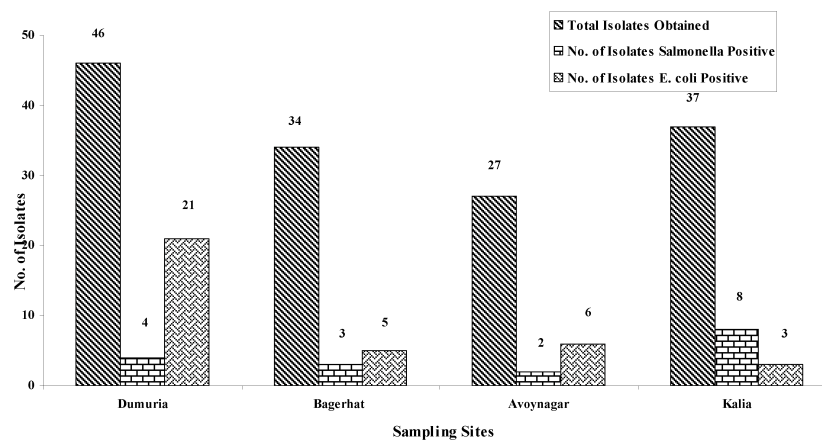


Fig. 4. Contamination of *Salmonella* and *E. coli* in golda depots of different sampling areas.

positive. Number of suspected strains isolated was 34 and among them 3 were confirmed as *Salmonella* and 5 of them as *E. coli*. Prawn samples collected from both the depots were found to be *Salmonella* positive. General sanitary and hygienic conditions of these depots were not satisfactory.

*Avoyagar, Jessore*: Ten samples were collected from the golda depots of this location. One mat and one basket samples were *Salmonella* positive and 5 samples including 3 prawn samples were *E. coli* positive. No *Salmonella* could be detected in any of the prawn samples of the depots. Number of suspected strains isolated was 27. One isolate each from mat and basket were identified as *Salmonella* and two isolates from peritoneal cavity and one each from prawn body surface, gill, basket and mat were identified as *E. coli*.

*Kalia, Norail*: Ten samples were collected from two depots of Kalia. One prawn sample each from two farms were *Salmonella* positive and one basket sample from farm-2 was also positive in *Salmonella* detection. Two prawn samples and one basket sample were *E. coli* positive. Number of suspected isolates was 37. Among the 37 isolates 8 were identified as *Salmonella* and 3 as *E. coli*. Infrastructure facilities of the depots of Kalia are not good; many of them are bamboo made huts with earthen floors.

A comparative description of incidence of *Salmonella* and *E. coli* in selected golda depots of Bangladesh is

given in Table 4 and Table 5.

### Comparison in the incidence of *Salmonella* and *E. coli* in the selected farms and depots of Bangladesh

*Prawn*: Total numbers of golda samples collected from the farms were 24 and numbers of *Salmonella* and *E. coli* positive samples were 11 and 5 respectively while total numbers of isolates were 67 among which 18 and 7 isolates were found *Salmonella* and *E. coli* positive respectively. On the other hand in the depots total numbers of samples and isolates from the prawns were 24 and 87 respectively, among which 6 and 10 samples and 13 and 14 isolates exhibited positive results for *Salmonella* and *E. coli* respectively. About 48.5% samples and 26.8% isolates found to *Salmonella* positive in farms while about 25.0% samples and 14.9% isolates provided positive results for *Salmonella* to the depots. The results exhibited that *Salmonella* contamination was relatively higher in the farms than the depots. On the other hand, in case of *E. coli* the results were found slightly inverse as about 20.8% samples and 10.4% isolates were found *E. coli* positive in the farms and about 41.6% samples and 16.1% isolates in the depots.

*Basket*: Numbers of basket samples collected from the farms were 8, among those numbers of *Salmonella* and *E. coli* positive samples were 3 and 2 respectively,

**Table 4.** Incidence of *Salmonella* and *E. coli* in selected golda depots of Bangladesh

Bacteria	Sample	Dumuria, Khulna	Sadar, Bagerhat	Avoyagar, Jessore	Kalia, Norail
<i>Salmonella</i>	Prawn	+	+	—	+
	Basket	+	—	+	+
	Mat	—	—	+	—
<i>E. coli</i>	Prawn	+	+	+	+
	Basket	+	+	+	+
	Mat	+	—	+	—

\* One positive among the two farms sampled

**Table 5.** Total number of samples collected and number of samples found *Salmonella* and *E. coli* positive in golda depots

Bacteria	Type of sample	No. of sample	No. of sample positive	No. of sample positive (%)	No. of isolates	No. of isolates positive	No. of isolates Positive (%)
<i>Salmonella</i>	Prawn	24	6	25	87	13	14.9
	Basket	8	3	37.5	32	3	9.4
	Mat	8	1	12.5	25	1	4
<i>E. coli</i>	Prawn	24	10	41.6	87	14	16.1
	Basket	8	5	62.5	32	11	34.3
	Mat	8	3	37.5	25	10	40

while total numbers of isolates were 22 with 3 positive for both *Salmonella* and *E. coli*. Total numbers basket samples collected from the depots, on the other hand, were 8, among them *Salmonella* and *E. coli* positive samples were 3 and 5 respectively. Total numbers of isolates from those samples were 32 among them *Salmonella* and *E. coli* positive were 3 and 11 respectively. About 37.5% samples and 13.6% isolates provided positive results for *Salmonella* and about 25.0% samples and 13.6% isolates provided positive results for *E. coli* in case of farms. On the other hand, about 37.5% samples and 09.4% isolates for *Salmonella* and about 62.5% and 34.3% for *E. coli* provided positive results in the depots. *Salmonella* incidence in baskets in the farms and depots were more or less similar but incidence of *E. coli* was higher in depots than the farms.

*Mat*: Mat sample was collected from only one farm and 2 isolates were obtained. No *Salmonella* or *E. coli* could be identified from the mat sample. On the other hand total numbers of mat samples collected from the depots were 8, number of *Salmonella* and *E. coli* positive samples were 1 and 3 respectively. Total number of isolates from those samples was 25, of which 1 isolate provided positive result for *Salmonella* and 10 isolates for *E. coli*. In the depots about 12.5% mat samples and 4.0% isolates from those samples were found *Salmonella* positive, while about 37.5% mat samples and 40.0% isolates provided positive result for *E. coli*.

Numerous studies indicated that crustacean shellfish from warm waters carry higher bacterial loads of mesophilic, gram positive bacteria. Present study conducted on the incidence of *Salmonella* and *E. coli* in prawns 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 prawn. The coliform contents of farmed penacids varied between 460-1100 whereas the surrounding water and sediments were between  $11 \times 10^3$  and 123 or 1100 respectively. Faecal coliforms were found in the range of 10-200 on the sediment samples [7].

Coliform bacteria, *Escherichia coli*, coagulase-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 head-

less 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 [3]. 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 [1]. 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 [6].

In the present study it was found that about 70% farms were received and drained 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 prawn samples of farms may due to the above causes. 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 [6]. 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 [5]. 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, delayed icing, improper washing, careless beheading and peeling of the prawns.

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