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Prevalence of Microbial Load in Shrimp, *Penaeus monodon* and Prawn, *Macrobrachium rosenbergii* from Bangladesh

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Abstract: A comparative studies regarding prevalence of microbial flora in the muscle of locally available tiger shrimp (*Penaeus monodon*) and giant water prawn (*Macrobrachium rosenbergii*) have been analyzed in terms of aerobic plate count (APC), enterobacteriaceae and Salmonella-Shigella (SS) counts. The total counts ranged from $2.04x10^2$ to $4.5x10^5$ CFU/ml for shrimp and $1.08x10^2$ to $1.2x10^5$ CFU/ml for prawn. The total coliforms count ranged between $5.4x10^2$ and $8.5x10^5$ cells for the shrimp and for the prawn $5x10^2$ and $4.4x10^4$ cells. Furthermore, the Salmonella-Shigella (SS) count ranged from $0.2x10^2$ to $1.1x10^2$ cells for the shrimp and between $0.26x10^2$ and $0.96x10^4$ cells for the prawn. Sixteen isolates were characterized from all the samples on plate count agar with percentage of different microbes characterized as follows: *Staphylococcus auerus* (6.25%), *Salmonella* sp. (25%), *Shigella* sp. (12.5%), *Flavobacterium* sp. (12.5%) and *Vibrio* sp. (43.75%). Results revealed that the microbial load found to be higher in samples taken from the departmental chain shops, without having proper treatment.

Key words: Food safety · HACCP · Fish borne pathogens · Microbiological quality · *Penaeus monodon* · *Macrobrachium rosenbergii*.

INTRODUCTION

Food security is a complex issue, where fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants. In addition fish, shellfish and fish products have occupied second position (next to ready made garment and knitwear) in the list of exportable commodities of Bnagladesh, whereas frozen shrimp occupied 72.4% i.e. about US$ 428 million [1]. So, to increase fish quality assurance in accordance with microbial load assessment is deemed necessary.

The Food and Agricultural Organization of the United Nations and the World Health Organization [2] state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity [3]. Biological contaminants such as bacteria, viruses, protozoa, fungi and helminthes constitute the major cause of food borne diseases such as cholera, *E. coli* gastroenteritis, salmonellosis, shigellosis, campylobacteriosis, brucellosis, amoebiasis, typhoid fever and poilomyletis with varying degrees of severity, ranging from mild indisposition to chronic or life threatening illness [4]. Therefore, for the assurance of consumers European Commission Health and Consumer Protection Directorate General (EC/DG), United States Food and Drug Administration (USFDA) and Canadian Food Inspection Agency (CFIA) legally mandated in 1999 that the hazard analysis critical control point (HACCP) system and complementary standard sanitation operation procedures (SSOPs) be implemented for processing all purchased seafood products [2]. Actually they consider the detectable presence of pathogens like *Salmonella* sp. as an indicator of adulteration [5]. Moreover, the faecal coliforms as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals [6]. Moreover, estimation of bacterial numbers in fish is frequently used to retrospectively assess microbiological quality or to assess the presumptive safety of the product. When total count

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reaches 10^6 CFU (Colony Forming Units) per gram or milliliter of product [7], the product is assumed to be at, or nearing, spoilage. In raw shrimp, acceptable component limit of fecal coliforms less than 20 CFU/g, *Staphylococcus aureus* i.e. coccal enterotoxin level equal to or greater than 10^4 CFU/g, *Salmonella* sp. presence of organism [8]. According to Higgins [9], standards of sanitation, method of handling and the time/temperature of holding fish are all crucial element to assure quality. There have been several reports on the health risks associated with the consumption of seafood, ranging from allergic reactions, stomach and intestinal cancerous growths, a general degeneration of peripheral cellular tissues, to gradual breakdown of the digestive and excretive systems in a statistically high percentage of people [3].

This investigation aimed to evaluate the incidence of bacterial load and pathogens in locally available shrimp and prawn species, with a view to provide potential approaches for improving the quality assurance and create awareness among the consumers.

MATERIALS AND METHODS

Sampling Area and Period of Study: Samples were obtained from five representative sources of Bangladesh such as tiger shrimp (*Penaeus monodon*) from District of Cox's Bazar (A) and Satkhira (B) and giant water prawn (*Macrobrachium rosenbergii*) from Noakahli Dist. (E) To assess the microbial load in the present study; both were obtained from local markets (C) and departmental chain shops (D) in Dhaka city. Samples have been collected from December, 2005 to February, 2006. To avoid further contamination, during transportation from the source to laboratory, samples were carried by special sterile bags packed in insulated box with ice to maintain the temperature around 4 to 6°C.

Isolation and Identification of Indicators and Pathogens: Samples were rinsed thoroughly with sterile distilled water. Alongside the microbial evaluation of the sterile distilled water washing, the washed samples were also assessed for bacterial growth by mutilating, macerating and smashing into the distilled water. Dilution and plating by spread plate method were carried out soon after sampling. Approximate 15 ml of plate count Agar (PCA), MacConkey Agar, Membrane Fecal Coliforms (mFC) Agar, Eosin Methylene Blue (EMB) Agar, Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar, Xylose Lysine Deoxycholate (XLD) Agar and Salmonella-Shigella Agar (SSA) has been melted and brought to 45° C was poured into the sterile Petri plates and different initiatives were taken for equal distribution of the media. After solidifying and inoculation, plates were inverted and placed in incubator at 37°C for 48 hours for evidence of growth. Total sixteen samples have been analyzed in terms of aerobic plate count (APC), enterobacteriaceae counts and Salmonella-Shigella (SS) counts. For the isolation of *Vibrio* spp. a pre-enrichment step was done [10]. Pure isolates of resulting growth were identified using different biochemical tests for example Kligler's Iron Agar (KIA) test, Indole production test, Methyl Red (MR) test, Voges-Proscauer (VP) test, Citrate utilization test, Motility Indole Urea (MIU) test, Carbohydrate fermentation test and salt tolerance test as described by Jolt *et al.* [11].

RESULTS AND DISCUSSION

Table 1 showed the total counts, coliform and Salmonella-Shigella counts of different shrimp and prawn samples. The total counts ranged from $2.04x10^2$ to $4.5x10^5$ and $1.08x10^2$ to$1.2x10^5$ cells for the shrimp and prawn samples respectively where the highest count were found in the sample D_1 ($4.5x10^5$) and D_2 ($1.2x10^5$); followed by sample B ($5.9x10^3$), C_1 ($4.5x10^3$) and C_2 ($1.2x10^3$) while sample A and E had a low count of $2.04x10^2$ and $1.08x10^2$ respectively (Table 1). Total counts for all the samples were generally high exceeding the limit of $1.0 x10^2$ CFU/ml.

The coliform counts ranged between $5.4x10^2$ and $8.5x10^5$ cells for the shrimp and from $5x10^2$ to $4.4x10^4$ cells for prawn also exceed the limit of zero CFU/ml (Table 1). This indicates that sample D_1 ($8.5x10^5$) and D_2 ($4.4x10^4$) also have the highest count, followed by Sample C_1 ($4.5x 10^3$) and C_2 ($5.1x10^3$) while sample E had the lowest coliform count ($5x10^2$), followed by sample A ($5.4x10^2$) and B (5.3x102) as shown in Table 1. The Salmonella-Shigella (SS) count ranged between $0.26x10^2$ and $0.96x10^4$ cells for the prawn and from $0.15x10^2$ to $1.1x10^4$ cells for the shrimp, also exceed the limit of $1.0x10^2$ CFU/ml (Table 1). This indicates that Sample D_1 and D_2 had the highest SS count ($1.1x10^4$) and ($0.96x10^4$), respectively, followed by sample B ($0.45x10^3$) and C_2 ($1.2x10^3$) while sample A, C_1 and E had a low SS count of $0.15x10^2$, $0.2x10^2$ and $0.26x10^2$ respectively (Table 1). Similar trend of bacterial growth was also reported by Harrison [12] where the total psychrophilic-mesophilic count was $3.0x10^4$ and $1.3x10^4$ CFU/g of shrimp, where the initial microbial flora, in order of predominance, was *Acinetobacter-Moraxella, Flavobacterium,* gram-positive *cocci* and *Bacillus* spp. Abrahamson [13] found bacterial counts of $1.6x10^3$ to $1.6x10^5$ CFU/g of fresh shrimp caught in the Gulf of Mexico.

Table 1: Total counts, coliform counts and Salmonella-Shigella (SS) counts isolated from shrimp and prawn samples

Sample		Total count (CFU/ml)	Coliform count (CFU/ml)	Salmonella-Shigella count (CFU/ml)
P. monodon	A	$2.04x10^2$	$5.4x10^2$	$0.15x10^2$
	B	$5.9x10^3$	$5.3x10^2$	$0.45x10^3$
	C_1	$4.5x10^3$	$4.5x10^3$	$0.2x10^2$
	D_1	$4.5x10^5$	$8.5x10^5$	$1.1x10^4$
M. rosenbergii	C_2	$1.2x10^3$	$5.1x10^3$	$1.2x10^3$
	D_2	$1.2x10^5$	$4.4x10^4$	$0.96x10^4$
	E	$1.08x10^2$	$5.0x10^2$	$0.26x10^2$

Samples A from District of Cox's Bazar; B from Satkhira Dist.; C_1, C_2 and D_1, D_2 from local markets and departmental chain shops in Dhaka city, respectively; E from Noakhali Dist. of Bangladesh

Table 2: The different types of bacteria isolated from the surface of the different processed frozen seafood products

	Frequency	*P. monodon*				*M. rosenbergii*		
Bacterial Isolates	No (%)	A	B	C_1	D_1	C_2	D_2	E
Salmonella sp	4(25)	-	-	+	+	+	+	-
Shigella sp.	2(12.5)	-	-	-	+	-	+	-
Staphylococcus auerus	1(6.25)	-	-	-	+	-	-	-
Vibrio sp.	7(43.75)	+	+	+	+	+	+	+
Flavobacterium sp.	2(12.5)	-	-	-	+	-	+	-
Total	16(100.0)	1	1	2	5	2	4	1

Samples A from District of Cox's Bazar; B from Satkhira Dist.; C_1, C_2 and D_1, D_2 from local markets and departmental chain shops in Dhaka city, respectively; E from Noakhali Dist. of Bangladesh

Table 2 depicted the following status of the bacterial pathogens from the surface of different shrimp and prawn samples i.e. *Vibrio* sp. [7 (43.75%)] and *Salmonella* sp. [4(25%)] were most frequently isolated being present in almost all samples, followed by *Flavobacterium* sp. [2 (12.5 %)] and *Shigella* sp. [2 (12.5%)] were isolated from sample D_1 and D_2. *Staphylococcus auerus* [1 (6.25%)] was only isolated from sample D_1. Similarly, Williams [14] for Gulf shrimp and Shaikhmahmud *et al.* [15] for Bombay prawns both reported *Achromobacter, Bacillus* and *Pseudomonas* as the main groups present on fresh shrimp. Okonko *et al.* [16]. The isolation and identification of bacteria from the surface of frozen seafood products were *Enterobacter aerogenes, Salmonella* sp., *Flavobacterium* sp. and *S. auerus*. Fagade *et al.* [17] reported the isolation of a total of 2 genera of bacteri and 3 genera of fungi in a similar study on non-carbonated orange drink. Ikenebomeh *et al.* [18] isolate *S. auerus, B. cereus* and other organisms in a similar study on a fresh and roasted edible worms (*Rhynchophorus phoenics*) larvae from 5 locations in Delta state of Nigeria.

Since, according to ICMSF [7], total coliform limits, $1.0x10^2$ CFU/g can be present in the food, but all of the shrimp and prawn samples were exceeding the limit. The presence of total and fecal coliform indicated that other harmful and pathogenic microorganisms such as *Salmonella sp., Shigella sp.* and *Vibrio sp.* might be present in the samples. For this reason, further identification was carried out. It was found that 2 samples contained *Flavobacterium* and *Shigella* spp., 4 samples had *Salmonella* spp. samples had *Vibrio sp.* among the sixteen collected samples. The most frequent isolated index of water quality and indicators of faecal contamination; *E. coli* and *Streptococcus faecalis* reported in this study, might be the result of possible contamination during sales or unhygienic handling of seafood that could be contaminated water used for processing [16] or ice used to freeze [19].

During this study it was observed that collected samples from departmental chain shops were more contaminated. The fact is that, all frozen products such as meat, fish and shrimps are stored together in departmental store; as a result cross contamination may occur. Not only that but also the product is stored for relatively long period until sold and sometimes storage condition cannot be ensured properly due to technological disruption [20]. Sanchita [21] also found contamination carried over

from the source of water, poor hygiene and sanitation condition of the processing premises. Therefore suggested that food processors should be educated on the adverse effect of contamination. Government should also come forth to implement the quality and precaution that are adopted in case of export products.

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