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Short Communication

Bacterial load in Tiger Shrimp, *Penaeus monodon* (Fabricius, 1798) from Four Different Regions of Bangladesh

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The study was conducted to assess the bacteriological hazards in both raw and processed tiger shrimp, *Penaeus monodon* from four different regions of Bangladesh. A total of 36 shrimp samples were randomly selected for this bacteriological analysis. In raw samples, total bacterial count (TBC) were $4.58x10^5$cfu/g, $3.78x10^5$cfu/g, $3.95x10^5$cfu/g and $4.38x10^5$cfu/g, whereas, total coliforms (TC) were 32.66 MPN/g, 20 MPN/g, 36.66 MPN/g, and 22.33 MPN/g from Cox's Bazar, Khulna, Satkhira and Bagerhat respectively. TBC loads in processed samples (Frozen block and HL/SO IQF) were reduced to 65.36%, 64.81%, 73.92%, 70.77% in frozen block and 71.4%, 64.02%, 68.86%, 69.63% in IQF shrimp samples from Cox's Bazar, Khulna, Satkhira and Bagerhat respectively. Total coliforms were reduced to 54.07%, 30.00%, 60.00% and 34.34% in Frozen block and 58.14%, 31.7%, 60.01% and 41.78% in IQF shrimp samples. Faecal coliforms were less than 3 cfu in all raw and processed samples. Pathogenic bacteria *Escherichia coli*, *Salmonella* sp. and *Vibrio cholerae* were absent in all raw and processed samples. Findings of the present study suggest that shrimp may be contaminated with pathogenic bacteria at any steps of post harvest procedure. The (HACCP) principles should be more strictly maintained to reduce bacterial load in processed shrimps and keep the quality of shrimp of international standard.

Keywords: Bacteriological hazard, HACCP, Ready to cook/eat

Favorable climatic condition and geographical location of the country blessed Bangladesh with vast fisheries resources. Fisheries sector is the second highest earner of foreign exchange and shrimp is the main exported products contributing about 85% of the total exports of fish products of Bangladesh[1]. Shrimp production of Bangladesh has increased steadily over the last 20 years from 72,809 metric tonnes in 1986-87 to 223,095 metric tonnes in 2007-08 in which culture based production contributes to 42.23% of total production. Shrimp exports have also grown steadily from 19,224 tonnes in 1992-93 to 49,907 tonnes in 2007-08[2].

To ensure safe and quality product major seafood importing countries have introduced hygiene regulation and legislation. Periodically, USA and EU countries with a view to improve quality of imports have laid down new requirements. On July 30, 1997, the European Commission banned imports of fishery products from Bangladesh into the EU (EEC 1997) as the result of European Community inspections of seafood processing plants in Bangladesh. Frozen shrimp export to EU market was zero in between August to December, 1997 due to this ban on seafood from Bangladesh[3]. Continuous awareness about safety, quality and for quality determination the USFDA HACCP (Hazard Analysis and Critical Control Point) regulation becomes mandatory on December 18, 1997 and seafood shipped to the USA after December 17, 1997 will have to be processed under HACCP plan in compliance with regulation[4]. Keeping the above things in view, the present study was carried out to evaluate bacteriological loads in black tiger shrimp (*Penaeus monodon*) from four major production sites of Bangladesh.

A modern shrimp processing plant was selected from each sampling site for bacteriological assessment of shrimp samples, which had modern shrimp cooking facilities with an approved HACCP plan. The processing plant had been regularly monitored and verified by respective competent authority. The sample collection sites, sample condition and number of samples considered are summarized in Table 1.

Raw shrimp samples were collected at the gate of the processing plant. The plant purchased shrimps are brought to the processing plant in boxes by insulated truck. On arrival of raw material a code was given according to the purchase date, center and arrival number. Among these received raw Shrimps, three samples were taken randomly and brought to the laboratory of the processing plant by a sterile plastic bag to

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Table 1. *Shrimp sampling sites and number of samples examined*

Shrimp Sampling Site	Condition of Sample	Number of Samples
Cox's Bazar	Raw	3
	Head Less/ Shell On (BT. HL/SO, Frozen block)	3
	Head Less/Shell On (BT. HL/SO, IQF) IQF	3
Khulna	Raw	3
	Head Less/ Shell On (BT. HL/SO, Frozen block)	3
	Head Less/Shell On (BT. HL/SO, IQF) IQF	3
Bagerhat	Raw	3
	Head Less/ Shell On (BT. HL/SO, Frozen block)	3
	Head Less/Shell On (BT. HL/SO, IQF) IQF	3
Satkhira	Raw	3
	Head Less/ Shell On (BT. HL/SO, Frozen block)	3
	Head Less/Shell On (BT. HL/SO, IQF) IQF	3
Total Samples		36

isolate bacteria. The finished products Head Less/ Shell On (HL/SO, Frozen block) Frozen block and Head Less/Shell On (HL/SO,IQF) shrimp samples were identified according to codes and three samples from both products were brought to the laboratory with sterile plastic bag for bacteriological analysis.

Bacteriological enumeration was conducted according to ICMSF[5, 6] and USFDA[9] recommended methods. Standard plate count (SPC) was performed by pour plate method using plate count agar (PCA), which was incubated at 37±1°C for 48±2h. Total coliforms and fecal coliforms were enumerated by most probable number (MPN) technique. For isolation and enumeration of *Salmonella* sp., Xylose Lysine Desoxycholate (XLD) and Brilliant Green Agar (BGA) were used and for biochemical confirmation, Urease test, Indole test and Serological test were done. Triple Sugar Iron Agar (TSI) and 1% Tryptone (Tryptophane) broth containing 30g NaCl / litre were used for isolation and enumeration of *Vibrio cholerae*. Lauryl Sulphate Tryptose (LST) broth was used for isolation of *Escherichia coli*. After incubation at 35±1°C for 48±2h, the broth tube that showed gas production was selected and a loop-full of the broth culture was transferred to EC broth that was further incubated at 45.5±0.2° C for 48±2h. Gassing tube was selected for *E. coli* enumeration using most probable number (MPN) method.

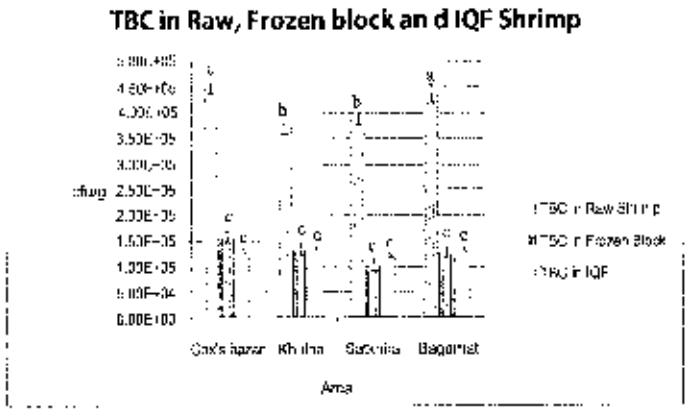

Figure 1. *TBC in Raw, Frozen block and IQF Shrimp collected from Cox's Bazar, Khulna, Satkhira and Bagerhat. Bars (mean ± SEM) with different letters are significantly different (p<0.05)*

The mean bacterial loads were compared using ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical software SPSS version 16.00 was used to analyze data with the level of significance at p<0.05.

Quantitative analysis of bacterial population was conducted to assess the bacterial load between the raw shrimp and processed shrimp. In this investigation, bacterial load of finished shrimp from two commonly used shrimp processing methods (HL/SO frozen block and HL/SO IQF) was compared with the bacteriological loads of raw shrimp samples which were collected from four main Shrimp production regions (Cox's Bazar, Khulna, Satkhira and Bagerhat) in Bangladesh.

Total bacterial count (TBC) of raw shrimp from Cox's Bazar, Khulna, Satkhira and Bagerhat was $4.58x10^5±2.40x10^5$cfu/g, $3.78x10^5±9.20x10^3$cfu/g, $3.95x10^5±4.58x10^3$cfu/g and $4.38x10^5±9.38x10^3$cfu/g respectively. In other studies, total bacterial counts from $1.4x10^5$/g to $2.6x10^7$/g, with a majority of shrimp samples in between 10^6-10^7/g (45.5%) and 10^5-10^6/g[10]. This study also found total bacterial counts ranging from 10^5-10^8/g for raw materials used in shrimp processing. *Fonseka et al.*[11] reported counts of 10^6/g for shrimp. Green *et al.*[12] performed the quantitative studies on bacteria of received shrimp in plant and reported that counts ranged from 10^6 to 10^7cfu/g.

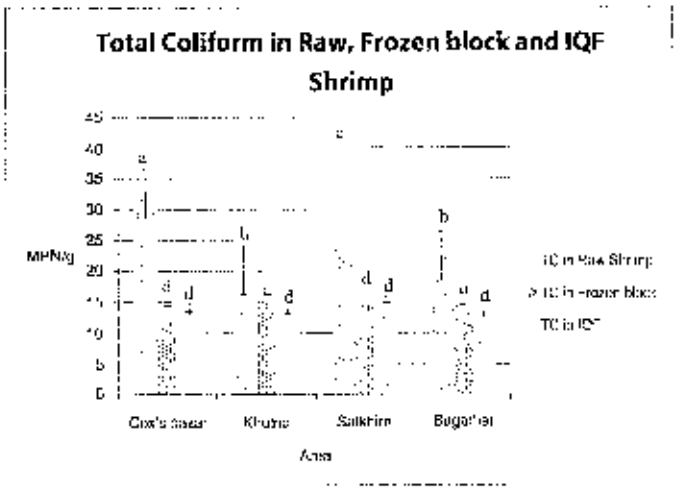

Figure 2. *Total coliforms (TC) in Raw, HL/SO Frozen block and HO/SO IQF shrimp from Cox's bazaar, Khulna, Satkhira and Bagerhat. Bars (Mean ± SEM; MPN/g) with different letters are significantly different (p<0.05)*

Total bacterial count (TBC) was reduced by 65.36%, 64.81%, 73.92% and 70.77% with HL/SO frozen block method and 71.15%, 64.02%, 68.86, and 69.63% with HL/SO IQF in shrimp samples from Cox's Bazar, Khulna, Satkhira and Bagerhat respectively. These reductions were due to washing, chilling, deheading, peeling and deveining, freezing, glazing. Shrimps washed with potable chilled water (4°C), resulted 43% reduction in bacterial counts. The bacterial count of deheaded washed shrimps was much lower than the reports of Tiger[13], who described that deheading of shrimps has lead to reduction of bacterial counts by 75%. This less reduction was probably due to delay of icing and deheading which caused

proliferation of bacteria and spreading from the head to the rest of the body of shrimp. Koseki and Isobe[14] observed that on the black tiger shrimp (*Penaeus monodon*) the combined treatment of distilled water (35°C, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) had the same bactericidal effect as treatment with ozonated water (5 ppm, 5 min) or sodium hypochlorite (NaOCl, 200 ppm, 5 min), giving a reduction in bacteria numbers of 1.2 to 1.4 log cfu/g. Layrisse and Matches[15] stated that deheading and good manufacturing practices would keep the total bacterial counts of processed shrimps to a reasonably low level. After washing the shrimp was graded for export manually according to buyer's specification. Contamination from the hand of the graders and contact surfaces might cause the excess bacteria found in export graded shrimp. It is reported that during processing steps bacterial numbers and types were increased on sorting, peeling, grading and packing and decreased on washing, cooking and freezing stages[5,6]. With proper washing (use of clean, potable, chilled water) the bacterial load of shrimps can be reduced by as many as 65%[17-19].

Total coliforms in raw shrimp from Cox's Bazar, Khulna, Satkhira and Bagerhat were 32.66±1.20 MPN/g, 20±2.64 MPN/g, 36.66±1.20 MPN/g and 22.33±.88 MPN/g respectively. Total coliforms in HL/SO Frozen block Shrimp from Cox's Bazar, Khulna, Satkhira and Bagerhat were 15±2.646 MPN/g, 15±2.30 MPN/g, 14.67±1.202 MPN/g and 14.66± 0.33 MPN/g respectively. Total coliform in HL/SO IQF Shrimp from Cox's Bazar, Khulna, Satkhira and Bagerhat was 14.00±2.0 MPN/g, 13.67±1.202 MPN/g, 15.33±0.882 MPN/g and. 13.55 ±1.2 MPN/g. A 13± incidence factor during processing reveals a large achievement in quality and good yield in peeled product[20]. Washing with chlorinated water reduced 70% bacterial load[21]. But the processing plant did not use chlorine in the washing water for shrimp in accordance. The total coliforms and fecal coliforms in HL/SO frozen block shrimp product and HL/SO IQF Shrimp product were under the standard limit.

Faecal coliform was less than 3 cfu in raw, HL/SO Frozen block and IQF Shrimp Samples. The limit is not harmful to health of human. *Salmonella* sp. which causes infection such as gastro enteritis and enteric fever in man, was absent from a sample of 25g during the present study. *Salmonella* sp. was not detected in shrimp samples from any stage of processing. Although the results of the confirmative *Salmonella* test were negative for all samples but it was positive when inoculated with *Salmonella* sp. incubated for 24 hours at 37°C. Thus the reliability of the methods was demonstrated and the negative results of *Salmonella* sp. for shrimp gut band surface were confirmed.

The result in this study indicates that *V. cholerae* was absent in the samples tested suggesting that its occurrence was not correlated with other microbial indicators of faecal pollution[22]. From Statistical analysis (Paired sample T-test, at 5% level of significance), it was found that TBC and TC (Total coliforms) were significantly varied with raw and processed samples (HL/SO. Frozen block and HL/SO. IQF) but TBC and TC were not significantly varied between HL/SO. Frozen block and HL/SO. IQF shrimp samples. The present study also conducted in a factory having traceability checklist, hazard analysis worksheet and HACCP plan form. From the present analysis, it is clear that every steps of processing have to reach the possible potential hazards, their significant justification for inclusion and exclusion as a significant hazard, preventive measures applied to prevent or reduce and eliminate the significant hazard of biological, chemical and physical in nature. The present study suggested that shrimp may be contaminated with bacteria at any steps of post harvesting procedure. So, HACCP should be maintained properly in processing industry to reduce the bacterial loads in processed shrimps.

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References

1. Hayes D. 2006. New horizons, focusing on new fisheries in Bangladesh. *World Fishing*. 54(11): 14-16.
2. Jatio Mosho Pakho Sankolon, 2009. p 108-110. Department of Fisheries (DoF), Ministry of Fisheries and Livestock.
3. Cato JC and Lima dos Santos CA.1998. European Union 1997 Seafood-Safety Ban: The Economic Impact on Bangladesh Shrimp Processing. Marine Resource Economics, 13: 215-227.
4. Choudhury SN and Islam R. 1999. HACCP implementation and quality control in the fish processing industry of Bangladesh. p 17. Dhaka International Sea Food Fair '99, Dhaka.
5. EEC. 2005. Commission regulation (EC) No. 2073/2005 on microbiological criteria for foodstaffs. Council of the European Communities (EEC). *Off J Eur Commu*. L338: 22.
6. ICMSF (International Commission on Microbial Specifications for Foods).1986. Microorganisms in Foods. Application of the Hazard analysis Critical Control Point (HACCP) system to ensure microbiological safety and quality. Blackwell Scientific Publications.
7. ICMSF. 1986. *Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications*, 2nd edn, p 190. International Commission on Microbiological Specification for Foods (ICMSF), University of Toronto Press, Toronto.
8. ICMSF. 1978. *Microorganisms in Foods 1. Their Significance and Methods of Enumeration*, 2nd edn, p 8. International Commission on Microbiological Specification for Foods (ICMSF), University of Toronto Press, Toronto.
9. USFDA. 1998. *Bacterial Analytical Manual (BAM)*, 8th edn, Revision A. United States Food and Drug Administration (US FDA), Washington DC.
10. Sumner JL, Jayaweera V and Fonseka G. 1982. A survey of process hygiene in the Srilanka prawn industry. *J Sci Food Agric*. 33: 802-808.

11. Fonseka TSG. 1985. A qualitative study of aerobic micro flora of shrimp (*Penaeus idicus*) caught in the sea of Negombo, M.Sc Thesis, University of Kelaniya.

12. Green M and Williaons. 1949. Bacteriology of Shrimp, Quantitative studies of freshly caught and iced shrimp. *Food Res.* 14: 372-383.

13. Figer EA, Bailey ME and Novak AF. 1957. Effect of delayed handling upon quality during subsequent refrigerated storage. FAO/UV. Bangkok, Thailand.

14. Koseki S and Isobe S. 2005. "Effect of ozonated water treatment on microbial control of black tiger shrimp (*Penaeus monodon*)." Food Processing Laboratory, National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki, 305-8642, Japan.

15. Layrisse ME, Matches JP. 1984. Microbiological and chemical changes of spotted shrimp (*Panaeus platyceros*) stored under modified atmosphere. *J Food Prot.* 47(6): 453-457.

16. Cann DC. 1977. Bacteriology of shellfish with reference to international trade proc. Conf. The handling processing and marketing of tropical fish, Tropical product institute, London. 377.

17. Silva N. 1985. Washed on quality and shrimp value. *J Sci Ind Res.* 124: 501-567.

18. Lisac HL. 1983. Wash me but well. INFOFISH Marketing Digest, 6: 22-23.

19. Rajadoraic PN. 1985. Bacteriology of shrimp. Quantitative studies of freshly caught and iced shrimp. Food Res.14: 372-383.

20. Ahmed MK. 1999. Sea food quality control and HACCP program in Bangladesh present status. Paper presented in the national workshop on export promotion of value-added products and their sustainable development, Hotel Sonargaon, Dhaka, 26th Feb., 1994.

21. Lyer TSG, Chanudhurr DR and Pillai VK. 1969. Bacteriological aspects of frozen prawn and their significance in quality evaluation. Technical conference on fish inspection and quality. Halifax Canada, FAO. *Fish Rep.* 81.

22. Johnsosn H and Liston J. 1973. Sensitivity of *Vibrio parahaemolitics* to cold in oysters fish fillets and crab meat. *J Food Sci.* 38: 437-441.