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Significance of Cross-contamination on Bacteriological Quality of Black Tiger Shrimp (*Penaeus monodon* Fabricius 1798) for Export Trade Produced in Fish Processing Plant

Basudev Mandal[1], Sourabh Kumar Dubey[2]*, Dona Bhattacharya[1] and Bimal Kinkar Chand[3]

[1]*Department of Aquaculture Management and Technology, Vidyasagar University, Midnapore, West Bengal-721102, India*
[2]*Department of Aquatic Environment Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata-700094, India*
[3]*Directorate of Research, Extension and Farms, West Bengal University of Animal and Fishery Sciences, Kolkata-700037, India Sciences, Kolkata-700037, India*

Abstract

In this study, bacteriological quality of raw and processed *Penaeus monodon* produced for export trade from pre-processing, processing area and cross-contaminated sample was analysed in terms of total plate count (TPC), *Vibrio cholerae*, *Escherichia coli, Staphylococcus aureus, Salmonella* spp and *Listeria monocytogenes* from a HACCP (Hazard Analysis Critical Control Points) approved processing plant. In all cases bacterial loads were highest in cross-contaminated sample than the pre-processing and processing area. *E. coli* was present in cross-contaminated raw and processed sample; however *V. cholerae, S. aureus, Salmonella* spp *and L. monocytogenes* were not detected in any case. In pre-processing, processing and cross-contaminated samples, the TPC value was not exceeding the standard limits as per ICMSF (International Commission on Microbiological Specifications for Foods Standards) and EIC (Export Inspection Council) standard. Another side of the present work indicated that, microbial analysis of all the samples obtained from processing area were within the permissible limits. This study clearly indicated that maximum microbial load in cross-contaminated condition due to transfer of place, shifting of equipments, products etc. The present microbiological survey revealed that there is an urgent need for considerable improvement in the bacteriological quality of products and it should be free from cross-contamination. Strict observance to CGMPs (Current Good Manufacturing Practice) and HACCP plan can greatly improve the product quality and reduce the risk of cross-contamination.

Keywords: Cross-contamination; Total Plate Count (TPC); *Penaeus monodon*; *Escherichia coli*; *Salmonella; Staphylococcus; Listeria monocytogenes*

Introduction

Shrimp is the major value added exported fishery product of India [1]. Southeast Asia continued to be the largest buyer of Indian marine products with a share of 25.75% in US$ realization and 39.58% in quantity. Exports to Southeast Asia have shown a growth of 43.19% in quantity and 95.99% in US$ realization. European Union ranks second in terms of frozen shrimp export with a share of 22.02%, followed by USA 19.17%, Japan 14.09%, China 7.06%, Middle East 4.39% and other countries 7.51% [2]. In Indian shrimp culture scenario, West Bengal is the second dominant shrimp producing state across its 158 km coastline [1]. The dominant species of shrimp, *Penaeus monodon* Fabricius 1798 (black tiger) are extensively cultured in the coastal area of West Bengal due to its unique taste, high unit value and ever-expanding export demand in the global market.

Most of the farm-raised products of shrimps are being processed as either block frozen or individually quick frozen (IQF) for export to various countries in Middle East Asia, Europe, Japan, and USA [3]. Frozen shrimp in India continued to be the major export item accounting for 51.35% of the total foreign exchange earnings. Shrimp exports from India during 2010-2011 increased by 17.67%, 34.77% and 32.98% in quantity, Indian rupee value and US$ value respectively than the previous year [2]. In past decades, the frozen shrimp exported from India had faced many problems such as high bacteriological count in the cooked and peeled frozen shrimps, as well as the presence of other pathogenic bacteria such as *Salmonella* [3]. Food security is a complex issue, where animal proteins such as fish and fishery products are generally regarded as high risk commodity with respect to pathogen contamination, presence of natural toxins and adulterants [4]. Moreover coliforms, especially *Escherichia coli* are the most alarming concern in all types of food products as their presence reflects the unhygienic handling during production process, improper storage conditions and post-process contamination [5-7]. It is important to note that cross-contamination of pathogenic bacteria in the finished shrimp products is generally introduced by human contact in the various steps of the process flow. The problem of cross-contamination in relation to potential transmission of pathogens to food is a serious issue in food processing and allied industries. The possible route of pathogenic bacterial contamination in shrimp products takes place either by direct contact with contaminated containers or through airborne particles. Some potential pathogenic bacteria are attached to the surfaces of the products as their predominant form of survival in nature and man-made ecosystems [8]. Several studies indicated that various bacteria including *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp., survive on hands, cloths, utensils etc. after initial contact with the microorganisms [9-11].

Augmented import of seafood from developing countries resulted in the acceptance of international guidelines for food processing

***Corresponding author:** S. K. Dubey, Faculty of Fishery Sciences, Department of Aquatic Environment Management, West Bengal University of Animal and Fishery Sciences, Kolkata-700094, India, Tel: +91 9830094171; E-mail: sourabhkumardb@gmail.com

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such as Hazard Analysis Critical Control Points (HACCP) and European Union (EU) guidelines. In an attempt to control the microbial contamination of foods, various international agencies have recommended microbiological criteria for assessing the effectiveness of HACCP program [12]. If coliforms were effectively destroyed at the pasteurizing step, still there was a possibility to detect their presence in the cooked products. These cases were also noticed when Good Management Practice (GMP) and HACCP programs are implemented to access best hygienic conditions for human consumption. As the bacterial contamination come from various sources in the processing work flow, it is extremely important to identify the possible route of cross-contamination to establish effective control process of the bacterial contamination and strengthens the GMP and HACCP programs [13]. There are some reports on the bacteriological assessment of frozen raw, processed IQF and ready to eat shrimps produced from processing plant in India [3,14-16]. Rao et al. [17] studied the seasonal variations in the supply of raw materials at processing plants located in Cochin, Veraval and Kakinada in India. Iyer et al. [18] observed that bacterial content and handling of raw materials influence the bacteriological quality of frozen shrimps. Reilly et al. [19] reported microbiological changes of insufficiently iced and improperly stored shrimps at higher temperatures. Zuberi and Qadri [20] showed the important role of microorganisms in the deterioration of shrimp quality. Jeyasekaran et al. [21] reported the incidence of *Listeria monocytogenes* in seafood landed at the Mangalore coast of India. However, there are no such studies on differences of bacteriological load in the shrimp products before and after the food processing work flow. In continuation to the fact, the main objective of this study is designed to access the bacteriological quality of various processing steps, raw and processed black tiger shrimp in a processing plant following the HACCP guidelines.

Materials and Methods

Study area and sampling design

The experimental work was carried out in a processing plant located at East Kolkata Township (22° 30' 48.07" N and 88° 24' 06.43" E), West Bengal, India with an approved HACCP plan. The processing plant has been regularly monitored and verified by respective competent authority.

Samples (N=24; three samples from eight treatments) were taken for microbiological evaluation such as total plate count (TPC), presence of *V. cholerae*, *E. coli, S. aureus, Salmonella* spp and *L. monocytogenes* in order to access the bacteriological quality of cross-contaminated condition and also the effectiveness of HACCP program. All samples were collected aseptically from pre-processing and processing areas according to the recommendation of USFDA [22]. Cross-contaminations of equipments were established by transferring them from processing to pre-processing areas at considerable distance and in direct contact with contaminated surfaces. Water and ice were also moved from processing to pre-processing areas and were placed in contaminated surface for long duration. The distance from pre-processing and processing area of the study design was about 115 meter. Bacteriological analysis was undertaken from three important steps of the process flow viz., i) receiving table, panning crate (a metal container or tray) and workers' hands ii) water and ice sample; and iii) head on black tiger shrimp (HOBT; shrimp with whole body parts), headless black tiger shrimp (HLBT; shrimp without head) and processed shrimp product from pre-processing and processing areas after cross-contamination.

Bacteriological procedures

The frozen samples were thawed before analysis by overnight storage at 5°C in a refrigerator. After that total plate count (TPC), detection of food borne pathogenic bacteria such as *V. cholerae, E. coli*, *S. aureus*, *Salmonella* spp and *L. monocytogenes* were undertaken as part of this study as per the instruction mentioned in Bacteriological Analytical Manual of Food and Drug Administration [22]. For swab test, solutions of peptone (1 g L^{-1}) and sodium chloride (8.5 g L^{-1}) were prepared. The solutions were stored in bottles and were sterilized for 15 minutes at 121°C. Tryptone glucose beef extract (TGBE) agar (HiMedia, Mumbai) was used for the estimation of TPC after incubation at 35°C for 48 h. For estimation of *E. coli*, Tergitol-7 agar (HiMedia, Mumbai) and Macconkey agar (HiMedia, Mumbai) were used as selective media and typical *E. coli*-like colonies were confirmed by IMViC test (Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test). Thiosulfate citrate bile salts sucrose (TCBS) agar (HiMedia, Mumbai) was used to evaluate the presence of *V. cholerae* after incubation of at 37°C for 24 h in alkaline peptone water (APW). For *S. aureus*, Baird parker agar (BP) (HiMedia, Mumbai) was used at 35°C for 48 h. Typical colonies were confirmed using tube coagulase test [22]. For *Salmonella* detection, Rappaport–Vassilliadis (RV) broth (HiMedia, Mumbai) and tetrathionate broth (TTB) (HiMedia, Mumbai) were used for selective enrichment after pre-enrichment in lactose broth. Selective media used were xylose lysine deoxycholate (XLD) agar, bismuth sulphite agar (BSA), and hektoen enteric agar (HEA) (HiMedia, Mumbai). For detection of *L. monocytogens, Listeria* enrichment broth (BLEB) (HiMedia, Mumbai) was used after pre-enriched at 30°C for 4 h [22].

Statistical analysis

The data obtained in the present investigations were subjected to one-way Analysis of Variance (ANOVA) using statistical software Medcalc® version 12.7.0. (MedCalc Software bvba, Ostend, Belgium). Tukey's (HSD) test was used to determine the differences among the means. Significant differences are stated at P<0.01 level.

Results

Microbial analysis to determine the bacterial load in receiving table, panning crate and workers' hands before and after cross-contamination

Total plate count values of all cases were found to be significantly much higher (P<0.01) in cross-contaminated samples than in pre-processing and processing areas. After cross-contamination, the TPC value was found to be highest (P<0.01) in panning crates (394.66 ± 90.38 cfu sq cm^{-1}) followed by worker's hands (273.33 ± 154.38 cfu sq cm^{-1}) and receiving tables (80.56 ± 5.98 cfu sq cm^{-1}) respectively. In pre-processing areas, the TPC was highest in panning crates (61.33 ± 11.23 cfu sq cm^{-1}) followed by receiving tables (60.66 ± 8.50 cfu sq cm^{-1}) and workers' hands (48.33 ± 9.29 cfu sq cm^{-1}) respectively. In processing areas the lowest TPC was observed in receiving tables (4.33 ± 2.51 cfu sq cm^{-1}) followed by workers' hands (9.66 ± 2.88 cfu sq cm^{-1}) and panning crates (11.00 ± 3.60 cfu sq cm^{-1}) respectively.

Microbial analysis of water and ice samples before and after cross contamination

*V. cholera*e was not detected in all the samples and *E. coli* were positive in cross-contaminated samples. High TPC values were observed (P<0.01) in cross-contaminated water and ice samples than

samples from pre-processing and processing areas, such as 122.50 ± 25.35 cfu ml^{-1} and 271.66 ± 53.79 cfu ml^{-1} respectively. Bacterial load in water samples from pre-processing and processing areas was 15.33 ± 6.02 cfu ml^{-1} and 7.66 ± 5.50 cfu ml^{-1} respectively. In ice sample, TPC was lowest in processing area (12.33 ± 6.02 cfu ml^{-1}) followed by pre-processing area (16.33 ± 3.78 cfu ml^{-1}) but did not differ significantly (P>0.01). *E. coli* in cross contaminated water and ice sample was 17.0 cfu ml^{-1} and 19.0 cfu ml^{-1} respectively. However, *E. coli* were not detected in samples collected from pre-processing and processing area.

Microbial analysis of HOBT, HLBT and processed products before and after cross-contamination

The TPC was significantly maximum (P<0.01) in all cross-contaminated samples when compared to pre-processing and processing areas. After cross contamination, the TPC value was significantly higher (P<0.01) in processed products (5.1×10^4 cfu g^{-1}) followed by HLBT (4.8×10^4 cfu g^{-1}) and HBOT (4.5×10^4 cfu g^{-1}) respectively. In the pre-processing area, the TPC of HOBT, HLBT and processed samples were 3.5×10^4 cfu g^{-1}, 3.8×10^4 cfu g^{-1} and 4.9×10^4 cfu g^{-1} respectively. In the processing area, the TPC of HOBT, HLBT and processed sample was 1.6×10^4 cfu g^{-1}, 2.9×10^4 cfu g^{-1} and 3.1×10^4 cfu g^{-1} respectively. *E. coli* was present in all cross- contaminated samples, however *V. cholerae, S. aureus*, *Salmonella* spp and *L. monocytogenes* were not detected in HOBT, HLBT and processed samples from pre-processing, processing areas and cross contaminated samples. *E. coli* in cross contaminated HOBT, HLBT and processed samples were 3.0 cfu g^{-1} , 7.0 cfu g^{-1} and 11 cfu g^{-1} respectively.

Discussion

Pérez-Rodríguez et al. [23] defined cross-contamination as "a general term which refers to the transfer, direct or indirect, of bacteria or virus from a contaminated product to a non-contaminated product". According to the World Health Organization [24], 25% of foodborne outbreaks are closely associated with cross-contamination events involving scarce hygiene practices, contaminated equipment, and contamination via food handlers, processing, or inadequate storage. Chen et al. [25] demonstrated that bacterial transfer rates varied by more than five orders of magnitude depending on the nature of the surfaces involved in the cross-contamination. According to him a transfer rate of 100% was identified as the maximum value, although in some cases it was found that the recipients demonstrated higher numbers of microbial load than the contributor of contamination. Our study clearly indicated that maximum microbial load in cross-contaminated condition due to transfer or shifting of the place, equipments and products from the concerned place to another places.

The total bacterial load in all the evaluated samples was high in cross-contaminated condition. In pre-processing, processing and cross-contaminated samples, the value was not exceeding 10^4 cfu g^{-1}, which is lower than the standard limits of 5×10^5 cfu g^{-1} as per International Commission on Microbiological Specifications for Foods Standards [26,27] and Export Inspection Council, India [28,29]. Most of the previous studies indicated that bacterial load in fresh tropical shrimps ranged from 10^3 to 10^5 cfu g^{-1} [20,30-34].

The presence of *E. coli* in ready-to-eat foods is undesirable because it indicates poor hygienic conditions during processing. In the present study, *E. coli* were positive in cross- contaminated samples. In water and ice samples *E. coli* levels exceed ICMSF standards but within marginally acceptable range, however in HOBT, HLBT and processed shrimp products *E. coli* was within ICMSF acceptable limits i.e., 11 cfu g^{-1}. Ideally *E. coli* should not be detected and as such a level of <3 g^{-1} has been given as the satisfactory criteria for this organism. Levels exceeding 100 g^{-1} are unacceptable and indicate a level of contamination [27]. This finding substantiates with the study of Iyer and Shrivastava [14] and Antony et al. [35]. Jeyasekaran et al. [36] reported that *E. coli* in shrimp ranged from 0.6 to 240 cfu g^{-1}. Iyer et al. [18] also stated that season plays a role in controlling the bacterial quality of raw shrimps and observed that the bacterial counts were higher in certain specific seasons.

V. cholerae, S. aureus, Salmonella spp and *L. monocytogenes* were below detection limit in all the samples. Varma et al. [37] documented that out of the 7,238 tested shrimps, *V. cholerae* was present in only one raw shrimp. Antony et al. [35] also found similar results. ICMSF acceptable range of *S. aureus* was $<1.0 \times 10^3$ cfu g^{-1}, however, levels of $\geq 10^4$ cfu g^{-1} are considered hazardous as foods with this level of contamination may result in food borne illness if consumed. Hatha et al. [3] detected *Staphylococci* less than 1% of the IQF shrimp samples. Ready-to-eat foods should be free of *Salmonella* as consumption of food containing this pathogen indicates poor food preparation and handling practices or cross-contamination which may result in food borne illness. Their presence in high numbers (>10^4 g^{-1}) in ready-to-eat foods indicates that an unacceptable level of contamination has occurred. Consistent with the present study, Chen et al. [38] while analyzing bacteriological quality of shrimp, did not find *Salmonella* and Antony et al. [35] also observed similar findings. In Thailand, Dalsgaard et al. [39] also reported that *Salmonella* was not detected from shrimps. However, there were very few reports on the incidence of *Salmonella* in fish and fishery products in India [40]. Bhaskar et al. [41] reported the incidence of *Salmonella* from aquacultured shrimps. Food under refrigeration should be free from *L. monocytogenes*. However levels of 10^2 cfu g^{-1} indicate a failure with food handling controls and are considered a public health risk [42]. The present study is well supported by earlier studies of Manoj et al. [43], Kamat and Nair [44], Antony et al. [35] and Prabakaran et al. [16]. However, Jeyasekaran et al. [21] reported the incidence of *L. monocytogenes* in raw shrimps. As humans are the major source of contamination in the processing environment [45], good private hygiene and adherence to GMPs are a must to control contamination. Wearing of nose-mouth cover and periodical dipping of workers' hands in chlorinated water must be strictly followed in the processing unit [3].

The present study indicated that microbial analyses of all the samples obtained from the processing areas were within the safe limits suggested in the guidelines by competent authority [26-28] and these values were far lower than pre-processing areas. This finding reveals that the adherence to Current Good Manufacturing Practice (CGMPs) and HACCP plan greatly enhances the quality of food products in the processing plants. The sanitary conditions of seafood processing plant are also associated with the microbial quality of finished products [46,47].

Though raw shrimps were procured exclusively from farms, the chances of natural contamination were expected in case of samples from polluted coastal waters [48]. However, the raw material could pick up microbial load during transportation and storage. Excellent temperature control of the raw material (less than 5°C) during pre-processing might be able to control the multiplication of mesophilic microflora present in the raw material [3].

Conclusion

The risk of food-borne infection associated with cross-contamination depends on two factors: the level of contamination on the surfaces and the probability of its transfer to the foods being consumed [49]. The present microbiological survey revealed that there

is an urgent need for considerable improvement in the bacteriological quality of products and it should be free from cross-contamination. Strict adherence to CGMPs and HACCP plan can greatly augment the product quality and minimize the risk of cross-contamination. The processing plants should take paramount care while collecting raw specimens to produce uniformly high quality product. Prior to food preparation, all workers' hands should be free of all test microorganisms and changing of equipments from low risk zone to high risk zone should be avoided. Regular training programs should be organized for processing workers for effective implementation of HACCP programme and also to meet the requirements of European Commission and the other international food regulating bodies. Further studies on quantifying the cross-contamination risk associated with the various steps in the food preparation process can provide a scientific basis for risk management efforts in shrimp processing industries.

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