

# Microbiological Quality of Raw Shrimps Processed in Seafood Processing Plants of Tuticorin, Tamil Nadu, India

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## Abstract

The microbiological characteristics of raw shrimps collected from three seafood processing plants (A, B and C) located in Tuticorin were tested. Total bacterial load (TPC) of the shrimps were almost uniform with  $10^4$  to  $10^5$  cfu·g. Raw shrimps from plants A and B had lower counts of total bacteria and coliforms, followed by plant C. The pathogens like *Vibrio cholerae*, *Salmonella* and *Listeria monocytogenes* were totally absent in raw shrimps. Significant difference ( $p < 0.05$ ) was observed only in *E. coli* of the shrimps collected from different seafood processing plants at different months. Results suggest that seafood processing industries should exercise utmost care in the collection of raw materials from the landing centers of different regions in various seasons to produce uniformly good quality products.

## Introduction

Shrimps are the most important items among seafoods being exported by India. Frozen shrimps, the highest foreign exchange earner among the seafood items, account for more than 70% of the total earnings of Indian marine products export. The quality of the finished product depends on the quality of raw materials, and it is difficult to preserve the freshness of raw materials when there is a long gap between the harvesting and processing periods. During this gap, improperly handled shrimps continue to deteriorate and further processing can never restore its freshness. It is obvious that spoilage of any food product is attributed to microbial growth due to improper handling and inadequate processing. Frozen shrimps are normally subjected to preshipment inspection based on physical and sensory characteristics followed by microbiological quality.

Seafood processing plants usually get their raw materials from nearby and outside fishing centers. Rajadurai (1985) reported that the time interval between the landings of shrimps and their arrival at the processing plants is

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very important. Rao et al. (1986) studied the seasonal variations in the supply of raw materials to processing plants located at Cochin, Veraval and Kakinada in India. It has been observed by Iyer et al. (1970) that bacterial content and handling of raw materials influence the bacteriological quality of frozen shrimps. Reilly et al. (1986) reported microbiological changes that occur when shrimps are insufficiently iced and improperly stored at higher temperatures. Zuberi and Qadri (1992) reported the important role of microorganisms in the deterioration of shrimp quality. Dalsgaard et al. (1995) examined the shrimp samples for prevalence of *Vibrio* and *Salmonella* while Jeyasekaran et al. (1996) reported the incidence of *Listeria* spp., particularly *L. monocytogenes* in seafoods landed in the Mangalore coast of India.

The recent introduction of HACCP system and EU hygienic regulations in seafood industries will pave the way for the production of safe and high quality seafoods. Tuticorin region on the east coast of India has about 19 seafood processing plants that export a substantial quantity of frozen shrimps. The loss due to the export of marginal quality seafoods or seafoods contaminated with pathogens by any exporter is heavy, as it affects the entire seafood industry located in that region. Since raw material quality determines the quality of final product, the present study was undertaken to assess the quality of shrimps used for processing in seafood industries located in Tuticorin, Tamil Nadu, India.

## Materials and Methods

Fresh shrimps belonging to the species *P. indicus*, *P. monodon*, *P. semi-sulcatus*, *M. dobsoni* and *P. stylifera* were obtained from three seafood processing plants (A, B and C) located in and around Tuticorin, Tamil Nadu, India. The processing plants were selected based on their facilities, capacity and status as representative plants for the region. Shrimps procured from these places were immediately placed in ice inside insulated boxes (13.5 l capacity) with a drain valve and brought to the laboratory within half an hour for analysis.

Fresh shrimps (both headless and whole) from these seafood processing plants were collected in triplicates for a period of five months (September to January) at monthly intervals to determine their microbiological quality as per the sampling proforma, which included the name and location of the processing plant, pack type, date and time of sampling, temperature and sanitary status of raw material receiving hall, place and date of catch, distance between the place of catch and plant, etc. The first batch of shrimp samples was taken in September. The raw materials for plant A were from fishing centers such as Kottaiappattinam, Thondi, Ramanathapuram and Mandapam; for plant B, Tuticorin, Ramanathapuram and Ovari and for plant C, Rameswaram, Kanyakumari and Tuticorin.

Raw shrimps were analyzed for TPC, indicator organisms like total coliforms, fecal coliforms, *E. coli* and *S. aureus* and human pathogens such as *Salmonella*, *V. cholerae*, *V. parahaemolyticus* and *L. monocytogenes*. The shrimps, brought in insulated boxes, were aseptically removed, peeled and weighed separately in sterile containers. To one part of shrimp, nine parts of diluent (physiological saline)

was added and homogenized in a sterile homogenizer. Dilutions were made from shrimp homogenate and appropriate dilutions were selected for enumeration. Isolation and identification of bacteria were done as per APHA (1976) methods. Identification of the isolates was as per the methods described by EIC (1995a) and Jeyasekaran (1996).

Appropriate dilutions of shrimp homogenate were spread plated onto plate count agar (PCA) and incubated at 37°C for 24 to 48 h and the colonies were counted and reported as total bacterial load (TPC). The MPN technique was used to determine the level of total coliforms, fecal coliforms and *E. coli* in shrimp samples. Shrimp homogenate was transferred to Lauryl sulphate tryptone broth (LSTB) tubes and incubated at 37°C for 24 h for estimation of total coliforms. Samples from positive LSTB tubes were transferred to EC broth tubes and incubated at 44.4±0.5°C for 18 to 24 h to estimate fecal coliforms. Samples from positive EC broth tubes were streaked onto eosine methylene blue (EMB) agar plates and incubated at 37°C for 24 to 48 h to estimate *E. coli*. Typical colonies were subjected to biochemical tests for final confirmation.

Shrimp homogenate was spread plated onto Baird parker agar (BPA) and incubated at 37°C for 24 to 48 h for estimation of *S. aureus*. Typical colonies were counted, purified and subjected to further biochemical tests for final confirmation. Shrimp homogenate was spread plated onto thiosulphate citrate bile salt sucrose (TCBS) agar plates and incubated at 37°C for 24 to 48 h and colonies were counted to determine *V. parahaemolyticus*. Typical colonies were subjected to biochemical tests for confirmation, 25 g of shrimp sample was aseptically taken and homogenized with 225 ml of alkaline peptone water (APW) and incubated at 37°C for 6 h for enrichment. A loopful of enriched sample was streaked onto TCBS agar plates and incubated at 37°C for 24 to 48 h to test the presence of *V. cholerae*. After incubation, the plates were checked for colony morphology. Typical colonies were confirmed by subjecting them to different biochemical tests.

Shrimp sample (25 g) was taken aseptically and homogenized with 225 ml of lactose broth and incubated at 37°C for 24 h for pre-enrichment. One ml of pre-enriched sample was transferred to 10 ml of selenite cystine broth and tetrathionate broth and incubated at 37°C for 24 h for selective enrichment. A loopful of enriched sample was streaked onto bismuth sulphite agar (BSA) and xylose lysine deoxycholate (XLD) agar plates and incubated at 37°C for 24 to 48 h for examination of *Salmonella*. After incubation, the plates were observed for typical colonies. Suspected colonies were later subjected to various biochemical tests for confirmation. The shrimp sample (25 g) was aseptically taken and homogenized in 225 ml of listeria pre-enrichment broth and incubated at 37°C for 24 h for pre-enrichment. Ten ml of pre-enriched sample was transferred to 90 ml of University of Vermont I (UVM I) broth and incubated at 37°C for 24 h for primary enrichment. For secondary enrichment, 0.1 ml of enriched sample from UVM I broth was transferred to 10 ml of UVM II broth and incubated at 37°C for 24 h. A loopful of secondary enriched sample from UVM II broth was streaked onto polymyxin aesculin lithium chloride ceftazidime acriflavin mannitol (PALCAM) agar and modified McBride's listeria agar (MMLA) plates and incubated at 37°C for 24 to 48 h to test the presence of *L. monocytogenes*. After incubation, the plates were checked for typical colonies

and suspected colonies were further subjected to biochemical tests for confirmation.

Analysis of variance (ANOVA) technique was used (Snedecor and Cochran 1962) to find out whether any significant difference exists between the samples of shrimps collected in different months and seafood processing plants in relation to overall microbiological characteristics.

## Results

The TPC of the shrimps from the processing plants were within the range of  $10^4$  and  $10^5$  cfu-g (Table 1). Shrimps collected in November had a bacterial load of  $10^5$  cfu-g, while those collected in September and October had a lower bacterial load of  $10^4$  cfu-g. Total coliforms were detectable in shrimps collected from all the plants and their MPN counts ranged from 12 to 1600-g (Table 2). Shrimps from plant A recorded a lower level while shrimps from plant C had the highest total coliforms. Faecal coliforms were not detected in shrimps collected in October from plant A and in September from plant C. Shrimps from plant A recorded a low level of fecal coliforms, followed by shrimps from plants B and C. In shrimps collected from plant A (September and October), plant B (September and November) and plant C (September) *E. coli* was not detected. Shrimps from plants A and B collected in December had lower *E. coli* counts compared to those from plant C, which recorded a higher count of 542-g in December (Table 2).

Shrimps collected from plant A had a higher *S. aureus* count of  $10^4$  cfu-g, while shrimps from plant B had lower counts ( $10^3$  cfu-g) except in September (Table 3). Shrimps collected from plant C (October) had the lowest count, but had higher counts during the other months. *V. parahaemolyticus* was not detected in September in shrimps from plants A and B. It was found to be high in shrimps collected from plant C with  $10^4$  cfu-g (November and December) while shrimps from plants A and B

Table 1. Total bacterial load (cfu-g) in raw shrimps collected from different seafood processing plants.

Sampling month	Plant "A"	Plant "B"	Plant "C"
September	$7.60 \pm 0.50 \times 10^4$	$3.35 \pm 1.05 \times 10^4$	$4.00 \pm 0.20 \times 10^4$
October	$1.40 \pm 0.10 \times 10^4$	$5.40 \pm 0.60 \times 10^4$	$1.75 \pm 0.05 \times 10^4$
November	$1.61 \pm 0.10 \times 10^5$	$1.95 \pm 0.35 \times 10^5$	$1.70 \pm 0.09 \times 10^5$
December	$4.30 \pm 0.20 \times 10^4$	$4.50 \pm 0.10 \times 10^4$	$2.05 \pm 0.10 \times 10^5$
January	$1.83 \pm 0.09 \times 10^5$	$8.95 \pm 0.25 \times 10^4$	$5.20 \pm 0.40 \times 10^4$

Table 2. Bacterial indicator organisms (MPN counts/g) in raw shrimps collected from different seafood processing plants.

Sampling month	Plant "A"			Plant "B"			Plant "C"		
	Total coliforms	Faecal coliforms	<i>E. coli</i>	Total coliforms	Faecal coliforms	<i>E. coli</i>	Total coliforms	Faecal coliforms	<i>E. coli</i>
September	33	33	ND*	13	13	ND	34	ND	ND
October	12	ND	ND	1600	1600	48	1600	1600	53
November	920	240	17	542	348	ND	1600	1600	345
December	1600	920	26	920	348	130	1600	920	542
January	1600	345	278	1600	1600	221	1600	348	138

ND\* - Not detected

recorded lower counts ( $10^3$  cfu·g), with the exception of shrimps collected from plant B (October) which had the lowest count (Table 4). All the shrimps examined from the three processing plants were found to be free from *V. cholerae*, *Salmonella* and *L. monocytogenes* (Table 4).

## Discussion

The TPC of the shrimps collected from the processing plants varied from  $10^4$  to  $10^5$  cfu·g, with about 66% having  $10^4$  cfu·g (Table 1). It was found that TPC was lower in plant B, with about 80% of the shrimps having an average count of  $10^4$  cfu·g, whereas in plants A and C, only 60% of the samples had a similar bacterial load. Most of the earlier reports indicated that bacterial load in freshly landed tropical shrimps ranged from  $10^3$  to  $10^5$  cfu·g (Vanderzant et al. 1970, Zuberi et al. 1987, Thampuran and Gopakumar 1990, Zuberi and Quadri 1992, Karunasagar et al. 1992 and Iyer and Joseph 1995). The TPC of the shrimps tested was lower than the standard limits ( $5.00 \times 10^5$  cfu·g) prescribed by EIC (1995b).

The occurrence of total and fecal coliforms was very low in the samples collected in September (Table 2). Total coliforms were relatively high in January in all the plants. However, shrimps from plant C collected from October to January recorded a higher level of total coliforms. In respect to fecal coliforms, the counts were lower in September in plants A and B, while it was not detected in plant C. On the contrary, the fecal coliforms were relatively high in plants B and C in

Table 3. Levels of *S. aureus* (cfu·g) in raw shrimps collected from different seafood processing plants.

Sampling month	Plant "A"	Plant "B"	Plant "C"
September	$1.20 \times 10^4$	$2.50 \times 10^4$	$3.00 \times 10^3$
October	$1.02 \times 10^4$	$5.85 \times 10^3$	$4.00 \times 10^2$
November	$2.70 \times 10^4$	$4.45 \times 10^3$	$1.51 \times 10^4$
December	$1.05 \times 10^4$	$3.65 \times 10^3$	$3.20 \times 10^4$
January	$7.50 \times 10^4$	$4.25 \times 10^3$	$1.05 \times 10^4$

Table 4. Bacterial pathogens in raw shrimps collected from different seafood processing plants.

Sampling month	<i>Vibrio parahaemolyticus</i> (cfu·g)			<i>V. cholerae</i>			<i>Salmonella</i>			<i>Listeria monocytogenes</i>		
	Plant			Plant			Plant			Plant		
	"A"	"B"	"C"	"A"	"B"	"C"	"A"	"B"	"C"	"A"	"B"	"C"
September	ND*	ND	$5.00 \times 10^3$	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
October	$4.50 \times 10^3$	$4.55 \times 10^2$	$1.35 \times 10^3$	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
November	$1.87 \times 10^3$	$3.00 \times 10^3$	$2.35 \times 10^4$	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
December	$1.55 \times 10^3$	$4.85 \times 10^3$	$1.25 \times 10^4$	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
January	$3.50 \times 10^3$	$3.65 \times 10^3$	$9.50 \times 10^3$	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

ND\* - Not detected

October. The total and fecal coliform counts were less than 100-g in only 40% of shrimps collected from plant A. Iyer and Joseph (1995) reported that the incidence of total coliforms in cultured *P. indicus* was 230-g, while Jeyasekaran et al. (1990) reported a MPN total coliform count of >240-g in tropical shrimps. Jeyasekaran et al. (1990) and Karunasagar et al. (1992) reported MPN fecal coliform counts of 11 to 240-g and 2 to >2400-g, respectively in freshly caught penaeid shrimps. In the present study, the MPN total and fecal coliform counts were also found in the range of 12 to 1600-g and 0 to 1600-g, respectively. Fecal coliform contents in shrimps vary depending on the sanitary and hygienic conditions of the landing centers.

Wide variation in *E. coli* counts was observed among the shrimps and it ranged from 0 to 542-g (Table 2). Significant difference ( $p < 0.05$ ) in *E. coli* counts of shrimps collected in different months was noticed. Jeyasekaran et al. (1990) reported that *E. coli* was present in shrimps in the range of 0.6 to 240-g. About 40% of the samples from plants A and B had a lower *E. coli* count than the limit (20-g) prescribed by EIC (1995b). It has been observed that the occurrence of *E. coli*, fecal and total coliforms in shrimps collected in September from the processing plants were low, and increased in subsequent months due to seasonal variation. In the Tuticorin region, the rainy season generally starts at the end of October, resulting to heavy runoff and carries away domestic sewage from the land to the sea. Iyer et al. (1970) also stated that season plays a role in controlling the bacterial quality of fresh shrimps and observed that the bacterial counts were higher in certain specific seasons. They recorded a high incidence of *E. coli* in raw shrimps during the rainy season, which is probably due to the high degree of fecal pollution of the water during that period. Among the different microbial quality characteristics tested, only *E. coli* counts were found to differ considerably among the different seafood processing plants ( $p < 0.05$ ). This shows that by estimating the *E. coli* counts in raw shrimps, the minimal quality differences among the shrimps can be determined.

*S. aureus* counts were found to be in the range of  $10^3$  to  $10^4$  cfu-g in all the shrimps collected from October to January, except in September (Table 3). It was found that 80% of the shrimps collected from plant B had a *S. aureus* count of about  $10^3$  cfu-g, whereas plant C had only 60% of shrimps with that load. On the other hand, all the shrimps from plant A had a load of more than  $10^3$  cfu-g. However, the differences were not statistically significant ( $p > 0.05$ ). Krishnamurthy and Karunasagar (1986) also reported that *Staphylococcus* was present in significant numbers in raw shrimps. Higher counts of *S. aureus* observed in raw shrimps collected from plant A show that personnel hygiene was not given much importance during handling and transportation of raw shrimps. The lower occurrence of *S. aureus* in plant B might have been due to the proper care taken by the plant workers in handling the shrimps upon arrival. However, the *S. aureus* counts in shrimps were higher than the limit (100-g) prescribed by EIC (1995b). During sensory evaluation, it was observed by the panelists that a strong chlorine odor was perceived in the shrimps collected in October from plant C, which had a *S. aureus* count of about  $10^2$  cfu-g. This could be due to excess chlorination.

*V. parahaemolyticus* was not detected in the shrimps collected in September from plants A and B (Table 4). About 40% of the shrimps collected from

plant B had *V. parahaemolyticus* counts below  $10^3$ .g, while it was only 20% for plant A. With regard to plant C, none of the shrimps had counts less than  $10^3$  cfu.g. Bandekar et al. (1982) reported that all the shrimps tested had *V. parahaemolyticus*. Karunasagar et al. (1985) have reported that freshly caught prawns from Mangalore coast were contaminated with *V. parahaemolyticus* in the range of  $10^2$  to  $10^4$  cfu.g, which is almost similar to the results of the present study. Venkateshwaran et al. (1996) reported the incidence of *V. parahaemolyticus* in headless and whole shrimps at a level of  $1.60 \times 10^2$  and  $3.60 \times 10^2$ .g, respectively. Lower levels of *V. parahaemolyticus* in shrimps collected from plant B show that the raw material was received from the fishing area where population of *V. parahaemolyticus* may be low. *V. cholerae* was absent in all the raw shrimps collected from the three processing plants (Table 4) but Varma et al. (1989) reported that out of the 7,238 shrimps tested, *V. cholerae* 01 was present in only one raw shrimp.

*Salmonella* was not reported in any of the shrimps collected from the three processing plants (Table 4). Chen et al. (1990) analyzed the bacteriological quality of *P. monodon* and observed similar results. Dalsgaard et al. (1995) also reported that *Salmonella* was not recovered from shrimps tested in Thailand. However, there were very few reports on the incidence of *Salmonella* in fish and fishery products (Varma et al. 1985). Bhaskar et al. (1995) also reported the incidence of *Salmonella* in cultured shrimps. The results of the present study is supported by the findings of Hood et al. (1983), who observed that the low coliform levels indicate the absence of *Salmonella*, but high levels of fecal coliforms are somewhat limited in predicting the presence of *Salmonella*. *L. monocytogenes* was not found in the raw shrimps collected from the three processing plants (Table 4), which is well supported by earlier studies of Manoj et al. (1991) and Kamat and Nair (1994). However, Jeyasekaran et al.(1996) reported the incidence of *L. monocytogenes* in raw shrimps.

## Conclusion

There are differences in the microbiological characteristics of the shrimps collected in different months from three seafood processing plants. The quality of the frozen products differs from the raw materials received in different months and processed in the processing plants. Hence, the present findings suggest that seafood processing plants should take utmost care while collecting raw materials from fish landing centers of different regions in various seasons to produce uniformly high quality product.

## Acknowledgments

The authors thank the Dean, Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University for providing the necessary facilities to carry out this study, the Management of the Seafood Processing Plants for allowing the regular sampling and Th.R.Lakshmanan.

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