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Microbes of public health significance in shrimps at Kakinada port area

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ABSTRACT

A study was undertaken to detect the incidence of pathogens of Public Health significance in three varieties of shrimps (flower, White and Tiger) at Kakinada port area in market as well as processing centres samples. Five stages of processing i.e. head removal, sizing and grading, final rinse, arrangement and water filling and packing were selected for analysis. The incidence of Salmonella in samples from market and processing centres is high in flower (36% and 22%), low in tiger (24% and 14%) and moderate in white (28% and 18%). The incidence during processing reduced after first step, remained same after second step and reduced afterwards reaching 8% in all the three varieties. The Staphylococcus aureus counts were ($3.9x10^5$ and $4.1x10^3$), ($5.8x10^5$ and $5.4x10^3$) and ($4.6x10^5$ and $4.8x10^3$) respectively for market and processing centres and after final step of processing the counts were 2.0,2.2 and $2.1x10^3$ in tiger, flower and white. The incidence of Vibrio cholerae and Vibrio parahaemolyticus in market samples are high in flower (6% and 32%), low in Tiger (4% and 26%) and moderate in White (4% and 28%). The incidence is zero for Vibrio cholerae in processing centres and Vibrio parahaemolyticus is 24%, 16% and 20% for flower. The incidence of Vibrio parahaemolyticus remained the same after first and second steps, reduced during further steps reaching 12%, 8% and 6% in Flower, White and Tiger shrimps respectively.

Key words: shrimps, incidence, pathogens, local market, processing stages.

INTRODUCTION

Shrimps are widely distributed in temperate and tropical salt and fresh waters. The common commercial shrimp belongs to the genus pevers, which turns pink and white when cooked. Fresh shrimp is packed in ice for shipping or frozen and packaged. Microbial safety is one of the Public Health issues associated with seafood consumption. Contamination due to unhygienic handling entails the risk of spreading pathogenic agents of communicable diseases. [1] reported that 17.39% of shrimp samples were found to be contaminated with Salmonella out of 276 samples collected from various fish markets of Coimbatore, South India. [2] assessed the quality of shrimps destined from European market shrimps, found to be contaminated with *Staphylococcus aureus* and *Vibrio paraheamolyticus.* [3] assessed the microbiological quality of shrimps at two fish markets located in south India and isolated *Vibrio cholerae* from 2 out of 5 shrimp samples. Anand et. al., (2002) reported only one of the shrimp sample positive for salmonella in Tuticorin fishing harbour. [4] reported that 17.39% out of 276 crustaceans tested positive for Salmonella from various fish markets of Coimbatore, South India. Shrimps pick up Vibrios from the environment, in which they occur as well as during subsequent handling [5] Vibrios in general may cause variety of diseases including gastroenteritis, wound infection, ear infection and primary/secondary septicaemia [6]. [7]

reported that isolation of *Vibrio parahaemolyticus* from seafood is not unusual because *Vibrio parahaemolyticus* is a normal saprophytic inhabitant of coastal marine environment. [8] reported that 14.3% of shrimp collected from processing plants at Kakinada were positive for *Staphylococcus aureus* and the level of incidence being above the prescribed upper limit of 100 cfu/g as per specifications of EIA. The present study was conducted for the presence of pathogens of Public Health significance in three varieties of shrimps viz. White Shrimp, Tiger shrimp and Flower Shrimp collected from local market places and processing plant situated at Kakinada port area.

MATERIALS AND METHODS

Sampling of shrimps

The three varieties of Shrimp samples were collected from different local markets, immediately after reaching the markets. The samples were also collected at different processing plants of Kakinada port area immediately after receiving the lots. Samples were also collected during processing in the processing plants and the following five key stages of processing were selected for Sampling and testing a) After head removal, b) After sizing and grading c) After final rinse d) After arrangement and water filling e) After packing. Samples collected both from local markets and processing plants were packed in insulated ice box and transported to the laboratory immediately. The samples were analyzed for different microbiological parameters i.e. within 3 hours after collection. A total no. of 50 samples under each variety were collected and analyzed.

Preparation of the sample

The Shrimp samples are washed thoroughly with clean water in order to remove micro filth attached to the Shrimps. Contamination during sample preparation was controlled by wearing sterile surgical gloves and using sterilized scissors and forceps. Hand and instruments are periodically dipped in chlorinate water maintained at 50 ppm. The Shrimps are deheaded and the shell was removed using scissor. Shrimps are deveined by holding with a sterile forceps and scissor. A portion of muscle is removed and taken into a sterile sachet.

50grms of sample is blended in 450ml of sterile diluent and made into slurry using a blender. The resulting solution represents a dilution stage of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared by transferring 1ml of the previous dilution into 9ml of sterile distilled water and so on. Mixing of the sample at each dilution was done by rotating and tilting so as to resuspend the material uniformly.

Isolation and identification of pathogens of public health significance

All the media used in the present study were prepared according to standard procedure. Media were sterilized by autoclaving at 121^{o}C at 15 Psi pressure for 15 min, unless otherwise specified. Sterility of the media was checked by incubating at 37^{o}C for 24 h.

Salmonella

25g of sample is aseptically weighed and transferred into sterile blending container. 225ml of sterile lactose broth is added and blended for 2 min at high speed. The homogenized mixture is aseptically transferred into a sterile wide mouthed screw capped jar of 500 ml capacity.P^{H} is adjusted to 6.8. Jar cap is loosened by ¼ turn and incubated the sample for 24 hrs at 37^{o}C. The incubated sample mixture is gently shaked. 1ml of mixture is transferred into 10 ml of SCB. Both tubes are incubated for 24 hrs at 37^{o}C. A loop full of incubated SCB is streaked separately onto plates containing BSA. All the plates are incubated for 24 hrs at 37^{o}c. Plates are examined for suspicious Salmonella colonies.

Vibrio species

Test sample is inoculated in Alkaline Peptone Water Solution and incubated at 37^{o}C for 6-8 h. A secondary enrichment broth is inoculated by transferring 1 ml from the first enrichment broth and incubated at 37^{0}C for 18h. A loopful of culture from enrichment broth is streaked on TCBS and incubated for 24h at 37^{0}C. Characteristic colonies are picked up and cultured onto Nutrient Agar for further characterization. The culture is tested for oxidase reaction and other biochemical reactions. The *Vibrio cholerae* produces flat, yellow colonies of 2-3 diameter, where as colonies of *Vibrio parahaemolyticus* are small with bluish green center.

Staphylococcus aureus
1ml of inoculum is spread onto Mannitol Salt Agar medium plates. Plates are incubated at 37^0C for 24 to 48 hrs. Plates are examined for suspected *Staphylococcus aureus* colonies. On MSA typical *Staphylococcus aureus* appears as round , smooth, yellow colonies.

RESULTS AND DISCUSSION

Incidence of Salmonella
Fifty samples each of market and processing plants of Flower shrimp, White shrimp and Tiger shrimp were tested for the presence of pathogenic *Salmonella spp* and the results were tabulated in the Table 1. The incidence of Salmonella for the collected samples from local markets was high in Flower shrimps 36% (18/50), low in Tiger shrimp 24% (7/50) and moderate in White shrimp 28% (14/50). Incidence of Salmonella in market shrimp samples 5 out of 5 was reported by [9] and 50% incidence by [10]. Almost similar levels of incidence was reported by [11] i.e. 30.4%, [12] i.e. 38%, [13] i.e. 20% in various types of shrimps.

The incidence of salmonella in the present study was higher than the incidence reported by [14] i.e. 16%, [15] i.e. 4%, [16] i.e. 7.5% to 12.5% and 0.5% by [17] and [18]. The incidence of Salmonella in samples immediately after collection from processing plants was also high in Flower shrimp i.e.22% (11/50), moderate in White shrimp i.e. 22% (11/50) and minimum in Tiger shrimp 14% (7/50).

During the processing of shrimps after the first step (after head removal), the Salmonella incidence reduced in all the three varieties of shrimps to 20% (10/50), 16% (8/50) and 14% (7/50) in Flower shrimps, White shrimps and Tiger shrimps respectively. This might be due to removal of highly contaminated head portion. The incidence remained same after second step (sizing and grading) in all the three varieties.

After third step of processing (final rinse) the incidence was slightly reduced to 14% (7/50), 12% (6/50), and 10% (5/50) respectively for Flower shrimp, White shrimp and Tiger shrimp. This might be due to disinfection action of chlorine solution. The incidence further reduced in Flower shrimp after fourth step to 12%, but remained same in White shrimp and Tiger shrimp i.e. 12% (6/50) and 10% (5/50). These counts were further reduced after fifth step of processing (packing) to 8% (4/50) in all the three varieties of shrimps. The proper packing and cooling has reduced the counts. Similar trends of decrease in incidence of Salmonella in shrimps during various stages of processing reported by [9]. Lower incidence of Salmonella than the present study was recorded by [11] i.e. 0.2%.

Incidence of Vibrio spp.
The samples of three varieties of shrimp collected from market and processing plants were tested for the presence of two species of Vibrio i.e *Vibrio cholerae* and *Vibrio Parahaemolyticus* and the results are presented in Table 2.

Vibrio spp. are pathogenic to human and have been implicated in food borne disease. *Vibrio cholerae* is water borne pathogen that causes gastrointestinal disorders with a wide range of clinical manifestations including vomiting and rice like diarrhoea [19]. Sea food importing countries generally do not accept the presence of *Vibrio cholerae* in any sea food or sea food products. *Vibrio parahaemolyticus* was the first among the non-cholera Vibrios to be widely recognized as a human pathogen and remains as one of the important causative agent of gastrointestinal infections associated with consumption of raw sea food [20] and [21]. The incidence of *Vibrio cholera* and *Vibrio parahaemolyticus* in market samples of shrimp was high in Flower shrimp (6% and 32%), moderate in White shrimp (4% and 28%) and low in Tiger shrimp (4% and 26%). The incidence of Vibrio spp. in present study is higher with *Vibrio parahaemolyticus* than *Vibrio cholerae* compared to the findings of [22] i.e. incidence of 5% for *Vibrio parahaemolyticus*, which is lower than *Vibrio cholerae* i.e 15%.

The incidence of *Vibrio Spp* in the present study are almost similar with the findings of [23], who reported 1% of *Vibrio cholerae* in shrimps. [12] reported an incidence of 16% for *Vibrio cholerae* and 28% for *Vibrio parahaemolyticus* in South India. [10] reported 28% *Vibrio parahaemolyticus* and *Vibrio cholera* totally absent. The incidence of *Vibrio cholera* is nil in all the three varieties of shrimp samples received at processing plants, where as the incidence of *Vibrio parahaemolyticus* is 24%, 20% and 16% respectively in Flower, White and Tiger shrimps. The incidence remained the same after the first step (head removal) and second step (sizing and grading) of processing, in all the three varieties of shrimps. The chances of contamination of *Vibrio spp* through manual handling is negligible, might have been the cause for no change in the counts. The incidence of *Vibrio*

parahaemolyticus slightly reduced after the third step (final rinse) to 14% in Flower shrimps, 12% in White shrimps and 8% in Tiger shrimps and remained same in after the fourth step (arrangement and water filling). The reduction in the incidence after final rinse might be due to effect of Chlorine solution. The incidence slightly reduced after fifth step (packing) to 12% in Flower shrimps, 8% in White shrimps and 6% in Tiger shrimps.

This decrease in incidence might be due to cooling effect as well as hygienic packing of shrimps. Similar trends of decrease in incidence after packing was reported by [9]. The percentage of incidence of Vibrio species is higher in the present study compared to the findings of [11] i.e. 0.2% after packing of shrimps.

Incidence of staphylococcus aureus

Fifty samples each of the three varieties of shrimps from market and processing plants were tested for the presence of pathogenic *Staphylococcus aureus* and the results were tabulated in the Table 3.

The *Staphylococcus aureus* count (cfu/g) in market samples of Tiger shrimp is low $3.9x10^5$ ($3.6\ x10^4$ to $4.5x10^5$), high in flower shrimp $5.8x10^5$ ($2.2x10^4$ to $7.4x10^5$) and moderate in between, in White shrimp $4.6x10^4$ ($3.0x10^4$ to $5.8x10^5$). All most similar counts of Staphylococcus were reported by [24] i.e. 10^3 to 10^4 cfu/g and [17] i.e. 10^4 cfu/g. [25] reported higher counts ($2.12x10^3$ to $1.36x10^6$ cfu/g) than the counts in present study in raw shrimps marketed at Tuticorin.

The *Staphylococcus aureus* count (cfu/g) of the samples immediately after collection from processing plants was also low in Tiger shrimps $4.1x10^3$ ($3.9x10^2$ to $5.7x10^3$), moderate in White shrimps $4.8x10^3$ ($4.0x10^2$ to $5.9x10^3$) and highest in Flower shrimps $5.4x10^3$ ($5.0x10^2$ to $6.5x10^3$). The Staphylococcus counts of market samples are high compared to samples from processing plants. This might be due to unhygienic handling practices by the people in markets. The counts of present study are lower than the counts reported by Garret (10^4 cfu/g) in raw shrimps.

During processing of shrimps after first step (head removal) the *Staphylococcus aureus* counts reduced to $3.8x10^3$ ($4.3x10^2$ to $6.1x10^3$), $4.4x10^3$($3.6x10^2$ to $5.3x10^3$) and $3.8x10^3$ ($3.4x10^2$ to $5.0x10^3$) in Flower shrimp, White shrimp and Tiger shrimp respectively. This decrease might be due to removal of contaminated head portion of the shrimps. These counts slightly increased after second step (sizing and grading) to $4.2x10^3$ ($4.8x10^2$ to $6.4x10^3$) in flower shrimps, $4.6x10^3$ ($3.7x10^2$ to $5.6x10^3$) in White shrimps, $4.1x10^3$ ($3.5x10^2$ to $5.2x10^3$) in Tiger shrimps. The increase might be due to contamination from manual handling during sizing and grading.

After third step of processing (final rinse) the counts slightly reduced to $2.8x10^3$ ($2.6x10^2$ to $4.8x10^3$), $2.4x10^3$ ($2.6x10^2$ to $3.8x10^3$) and $2.2x10^3$ ($2.0x10^2$ to $3.6x10^3$) in flower, white and tiger shrimps respectively. The effect of chlorine might have reduced the counts after final rinse. Further reduction of counts observed after fourth step (arrangement and water filling) to $2.4x10^3$($2.0x10^2$ to $3.7x10^3$) in flower shrimps, $2.3x10^3$($2.1x10^2$ to $3.2x10^3$) in white shrimps and $2.1x10^3$($1.8x10^2$ to $3.0x10^3$) in tiger shrimps. Same counts were maintained after fifth step (packing) $2.2x10^3$ ($1.4x10^2$ to $2.8x10^3$) in Flower shrimps, $2.1x10^3$ ($1.3x10^2$ to $2.6x10^3$) in White shrimps and $2.0x10^3$ ($1.5x10^2$ to $2.2x10^3$) in Tiger shrimps. Similar trends of increase and decrease in staphylococcal counts during processing was reported by [9] in Penaeid shrimps.

Table 1: Salmonella

S. No.	Sample	Flower Shrimp		White shrimp		Tiger shrimp	
		Market	Plant	Market	Plant	Market	Plant
1.	Fresh	36%(18/50)	22%(11/50)	28%(14/50)	18%(9/50)	24%(12/50)	14(7/50)
2.	Processing	--	--	--	--	--	--
a.	After head removal	--	20%(10/50)	--	16%(8/50)	--	14%(7/50)
b.	After sizing and grading	--	20%(10/50)	--	16%(8/50)	--	14%(7/50)
c.	After final rinse	--	14%(7/50)	--	12%(6/50)	--	10%(5/50)
d.	After arrangement & water filling	--	12%(6/50)	--	12%(6/50)	--	10%(5/50)
e.	After packaging	--	8%(4/50)	--	8%(4/50)	--	8%(4/50)

The figure in parenthesis indicates the Number of Salmonella positive samples out of 50.

Table 2: Vibrio

S. No.	Sample	Flower shrimp				White shrimp				Tiger Shrimp			
		Market		Processing Plant		Market		Processing Plant		Market		Processing Plant	
		VC	VP	VC	VP	VC	VP	VC	VP	VC	VP	VC	VP
1.	Fresh	6% (3/50)	32% (16/50)	0	24% (12/50)	4% (2/50)	28% (14/50)	0	20% (10/50)	4% (2/50)	26% (13/50)	0	16% (8/50)
2.	Processing	--	--	--		--	--	--		--	--	--	
a.	After head removal	--	--	--	24% (12/50)	--	--	--	20% (10/50)	--	--	--	16% (8/50)
b.	After sizing and grading	--	--	--	24% (12/50)	--	--	--	20% (10/50)	--	--	--	16% (8/50)
c.	After final rinse	--	--	--	14% (7/50)	--	--	--	12% (6/50)	--	--	--	8% (4/50)
d.	After arrangement & water filling	--	--	--	14% (7/50)	--	--	--	12% (6/50)	--	--	--	8% (4/50)
e.	After packaging	--	--	--	12% (6/50)	--	--	--	8% (4/50)	--	--	--	6% (3/50)

**VC – Vibrio Cholerae*
**VP – Vibrio Parahaemolyticus*
The figure in parenthesis indicates the number of Vibrio positive samples out of 50.

Table 3: Staphylococcus

S. No.	Sample	Flower Shrimp		White shrimp		Tiger shrimp	
		Market	Processing Plant	Market	Processing Plant	Market	Processing Plant
1.	Fresh	5.8×10^5 (2.2x104 to 7.4x105)	5.4×10^3 (5.0x102 to 6.5x103)	4.6×10^5 (5.0x104 to 5.8x103)	4.8×10^3 (4.0x102 to 5.9x103)	3.9×10^5 (3.6x104 to 5.9x103)	4.1×10^3 (3.9x102 to 5.7x103)
2.	Processing	--	--	--	--	--	--
a.	After head removal	--	3.8×10^3 (4.3x102 to 6.1x103)	--	4.4×10^3 (3.6x102 to 5.3x103)	--	3.8×10^3 (3.4x102 to 5.0x103)
b.	After sizing and grading	--	4.2×10^3 (4.8x102 to 6.4x103)	--	4.6×10^3 (3.7x102 to 5.6x103)	--	4.1×10^3 (3.5x102 to 5.2x103)
c.	After final rinse	--	2.8×10^3 (2.6x102 to 4.8x103)	--	2.4×10^3 (2.6x102 to 3.8x103)	--	2.2×10^3 (2.0x102 to 3.6x103)
d.	After arrangement & water filling	--	2.4×10^3 (2.0x102 to 3.7x103)	--	2.3×10^3 (2.1x102 to 3.2x103)	--	2.1×10^3 (1.8x102 to 3.0x103)
e.	After packaging	--	2.2×10^3 (1.4x102 to 2.8x103)	--	2.1×10^3 (1.3x102 to 2.6x103)	--	2.0×10^3 (1.5x102 to 2.2x103)

The figure in parenthesis indicates the range of Staphylococcus aureus count.

CONCLUSION

In general it was observed that the shrimp samples from local market had higher levels of Salmonella, Vibrio and Staphylococcus organisms compared to the samples collected from processing centres. This might be due to good hygienic conditions in processing centres. Among the varieties of shrimps Tiger variety has lower microbiological parameters, high in Flower variety and moderate in White variety of shrimps. In all the three varieties the microbiological parameters decreased after every step of processing, which might be due to the effect of individual processing steps.

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