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Incidence of *Salmonella* species in fish and shellfish of Guntur domestic fish market, Andhra Pradesh, India

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A B S T R A C T

A study was undertaken to detect the incidence of food-borne pathogen, *Salmonella*, of public health significance from fish and shellfish of a domestic fish market, Guntur city, Andhra Pradesh, India. A total of 192 fish (8 species) and 72 shellfish (2 species of shrimps and one species of prawn) samples were collected from the fish market for sale in retail outlets over a period of one year (2010-11). Of the samples examined, 5.72% of fishes and 9.72% of shellfish were found to be contaminated with *Salmonella*. The highest incidence of *Salmonella* was seen in giant freshwater prawn, *Macrobrachium rosenbergii* (16.66%) followed by the catfish, *Clarias batrachus* (12.5%). A marked seasonal variation in the incidence pattern was observed in both fin fishes and shellfishes with higher incidence during monsoon season followed by post-monsoon and pre-monsoon seasons respectively. The reasons for seasonal variations in the incidence of *Salmonella* in fishes of Guntur fish market have been discussed. It was also observed that the poor sanitary conditions prevailed in the local market are causative for the microbial contamination of fish and shellfish.

Introduction

Fish and shellfish are considered to be the most nutritive and highly desirable food. It provides a good quantity of animal protein in the diet with high biological value (HanyEl-Said, 2004). Fish food, in addition to being a healthy food with nutritional value can act as a source of food borne pathogens (Hudecova *et al*., 2010; Upadhyay *et al*., 2010; Kamat *et al*., 2005 and Bakr *et al*., 2011). Foodborne pathogens remain a public health threat globally and *Salmonella* is considered as one of the primary bacterial foodborne pathogens to humans (Little *et al*., 2007 and Sharp and Reilly, 1994). Aquatic environments are the major reservoirs of

Salmonella especially in tropical regions (Reilly and Twiddy, 1992; Heinitz *et al.*, 2000 and Much, *et al.*, 2009). *Salmonellae* have strong association with animals, and as such foods of animal origin must be considered potentially contaminated in a fresh, unprocessed condition (Dickson, 2000). Therefore, fish and fishery products have been recognized as a major carrier of foodborne pathogens (Saheki *et al*., 1989; Brasher *et al.*, 1998; Ali and Hamza, 2004; Kamat *et al*., 2005 and Upadhyay *et al*., 2010).

Salmonella is a facultatively anaerobic, rod shaped, Gram-negative bacterium that can cause illness in humans such as enteric fever and gastroenteritis (Anonymous, 1995 and D'Aoust, 1997). About 1.3 billion annual cases of human gastroenteritis are resulting from the ingestion of contaminated food products such as undercooked beef, pork, eggs, shell fish and fish (Esaki *et al*., 2004). Inspite of constant surveillance and intensive efforts, food-poisoning outbreaks due to salmonellosis are on the increase in western countries and fishery products account for significant portion of the outbreaks reported (Joseph *et al*., 1982 and Bean *et al*., 1990). In developing country like India, there is no such continuous monitoring system and the number of exact cases is not known.

Fish, crustaceans and molluscs were implicated as vehicles of many cases of foodborne outbreaks. Therefore, in recent years, emphasis has been placed on the importance of fish and shell fish as vehicles of *Salmonella*-induced gastroenteritis. Domestic fish markets in India are poorly maintained hygienically and are prone to several microbial pathogens leading to the spread of food safety illness such as typhoid, diarrhea, etc. Most of the low income group people access their food fishes from the local fish markets alone and are unaware of the food safety of the fishes purchased. The issues of food safety attract more attention from the government and public worldwide in recent years. For the formulation and recommendation of quality standards for fish in domestic trade, an in-depth study of microbial pathogens is necessary.

The genus *Salmonella* became a challenge to the global food system, from production through processing and consumption. Hence, the knowledge about *Salmonella* is important to ensure the safety and quality of food. So far no work has been done on the microbiology of fishes at retail outlets for sale in local fish markets of Andhra Pradesh, India. Hence the present study was undertaken to determine the seasonal incidence/distribution of *Salmonella* in 8 fin fishes and 3 shell fishes marketed in domestic fish market of Guntur city, Andhra Pradesh, India. It was also proposed to analyze the reasons for the contamination of fishes and the seasonal variations in the incidence of *Salmonella* in fishes of Guntur market.

Materials and Methods

***Study area*:** The domestic fish market chosen for the present study is located in Guntur City (16° 20' N 80°27' E) of Andhra Pradesh, India. Fish and fishery products being marketed in this Guntur market are coming from the surrounding aquaculture farms and natural water bodies of River Krishna. The Guntur fish market administrated by the Corporation of Guntur is the largest authorized wholesale and retail fish market with 42 fish stalls and 29 platforms.

***Sampling*:** A total of 192 fishes belonging to 8 species and 72 shell fishes belonging to 2 species of shrimp, *Penaeus* spp. and giant freshwater prawn, *Macrobrachium rosenbergii* (Table 2) were sampled at

Guntur fish market between February 2010 and January 2011. Fish samples were collected at fortnight intervals and the collections were made between 7 a.m. and 9 a.m. To study the seasonal variation in prevalence of *Salmonella*, the study period has been divided into pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January) seasons. The samples were collected individually in sterile polythene bags stored in thermoplastic box and transported to the laboratory. Microbial analysis of the samples was completed within 2-4 h of collection. Aseptic procedures were strictly adopted during the analysis.

Detection and isolation of Salmonella

Detection and isolation of S*almonella* was carried out using the standard methods of USFDA (BAM, 2007). All the bacteriological media were procured from Hi-media Pvt. Ltd. Mumbai.

Step 1: *Non-selective pre-enrichment*: The sample of 25 grams of fish muscle was blended with 225 ml of lactose broth in a stomacher bag and incubated at 37^{o}C for 24 hours to provide available nutrients required for the survival and repair of stressed and injured *Salmonella* cells.

Step 2: *Selective Enrichment*: About 0.1 ml of the pre-enriched sample was transferred to 10 ml of Rappaport-Vassiliadis broth (RV). Another 1ml of the pre-enriched sample was transferred to 10 ml of Tetrathionate broth (TT). Both media were incubated at 42ºC for 24 hours.

Step 3: *Isolation*: Each selective enrichment broth was shaken and then a loopful from each of them was streaked onto plates of Hektoen Enteric Agar (HEA), Bismuth Sulphite Agar (BSA), and Xylose lysine desoxycholate (XLD) agar. All plates were incubated in inverted position at 37°C for 24 hours and then examined for typical *Salmonella* colonies.

Step 4: *Confirmation of Salmonella*: Characteristic colonies on the plates were identified morphologically by microscopic examination and also submitted for biochemical testing. Suspected *Salmonella* colonies on HEA appeared blue to blue-green, entire glossy and without black centers, on BSA they are observed as brown, gray, convex, entire glossy colonies surrounded by brilliant red zones whereas on XLD they were pink colored, black centered, convex, entire glossy colonies. Suspected colonies were subjected to preliminary screening by inoculation to Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) by streaking the slant and stabbing the butt. TSI and LIA slants were incubated at 37^{0}C for 24-48 h. The development of yellow color in butt and red color on slant of TSI, and purple color in slant and butt of LIA indicates *Salmonella* positive. 2 or 3 typical (or suspected) colonies were selected from each HEA, BSA and XLD agar and subjected to gram staining and several biochemical tests such as lactose, sucrose, salicin, dulcitol, indole, urease, methyl red, Voges-Proskauer, simmons citrate and malonate were conducted for further confirmation of *Salmonella*. All cultures giving biochemical reactions were confirmed by agglutination tests with polyvalent H and somatic O antisera (Table 1).

***Statistical analysis*:** One-way analysis of variance (ANOVA) was used to study the significance and the statistical package used was SPSS version 17 software. The F Statistical value and level of significance are given in the footnotes of Table 3.

Table.1 Results of biochemical tests of *Salmonella*

Biochemical test	Reaction
Lactose	Negative
Sucrose	Negative
Salicin	Negative
Dulcitol	Positive
Indole	Negative
Urease	Negative
Methyl red	Positive
Voges-Proskauer	Negative
Simmons citrate	v
Malonate	Negative
Polyvalent (H)	Positive
Somatic (O)	Positive

*V-variable

Result and Discussion

The number of fishes and shell fishes analyzed and the number of positives for *Salmonella* are given in Table 2. The results showed that out of 192 fin fish and 72 shell fishes analyzed, 5.72% of fishes and 9.72% of shell fishes were contaminated with *Salmonella* (Table 2). Of these, the highest incidence of *Salmonella* was seen in freshwater giant prawn, *Macrobrachium rosenbergii* (16.66%) followed by the catfish, *Clarias batrachus* (12.50%). A well marked seasonal variation in the incidence pattern was observed in both fin fishes and shell fishes with higher incidence during monsoon season (Fig.1) followed by post-monsoon and pre-monsoon.

Table.2 Incidence of *Salmonella* in fin fish and shell fish

Fish	Number analyzed	Number of positives	Percentage incidence (%)
Fin fishes			
Catla catla	24	0	0.00
Labeo rohita	24	1	4.16
Cirrhinus mrigala	24	1	4.16
Cyprinus carpio	24	2	8.33
Wallago attu	24	2	8.33
Clarias batrachus	24	3	12.50
Channa striatus	24	0	0.00
Anabas testudineus	24	2	8.33
Total	**192**	**11**	**5.72**
Shell fishes			
Penaeus indicus	24	1	4.66
Penaeus monodon	24	2	8.33
Macrobrachium rosenbergii	24	4	16.66
Total	**72**	**7**	**9.72**

Incidence of *Salmonella* in fin fishes

The number of fishes analyzed, the number of positives for *Salmonella* and their percentage incidence are given in Table 2. The study revealed that 11 out of 192 samples were found to be positive for *Salmonella* (5.72%). In fin fishes, *Clarias batrachus* (12.50%) showed the higher incidence of *Salmonella* followed by *Anabas testudineus*, *Wallago attu*, *Cyprinus carpio*, *Labeo rohita* (8.33%) and *Cirrhinus mrigala* (4.16%). However, *Catla catla* and *Channa striatus* did not contaminate with *Salmonella* (Fig. 1).

Incidence of *Salmonella* in shell fishes

The number of shell fishes analyzed, the number of positives and percentage incidence are given in Table 2. The results of this study revealed that 7 out of 72 samples were found to be positive for *Salmonella* (9.72%). Of these, *Macrobrachium rosenbergii* showed the higher incidence (16.66%) of *Salmonella* followed by *Penaeus monodon* (8.33%) and *Penaeus indicus* (4.66%).

Seasonal variation in the incidence of *Salmonella* in fin fish and shellfish:

The incidence of *Salmonella* in fin fish and shellfish during various seasons was given in Table 3 & Fig. 1. The results revealed that the percentage incidence of *Salmonella* was more during the monsoon season both in fin fish (10.93%) and shell fish (17.85%) followed by post-monsoon and pre-monsoon seasons. Statistical analysis of the data showed significant ($P<0.01$) variation in the incidence levels during various seasons (Table 3) and (Fig. 1). However, there was no significant variation in the incidence level between fishes and shell fishes analyzed during various seasons.

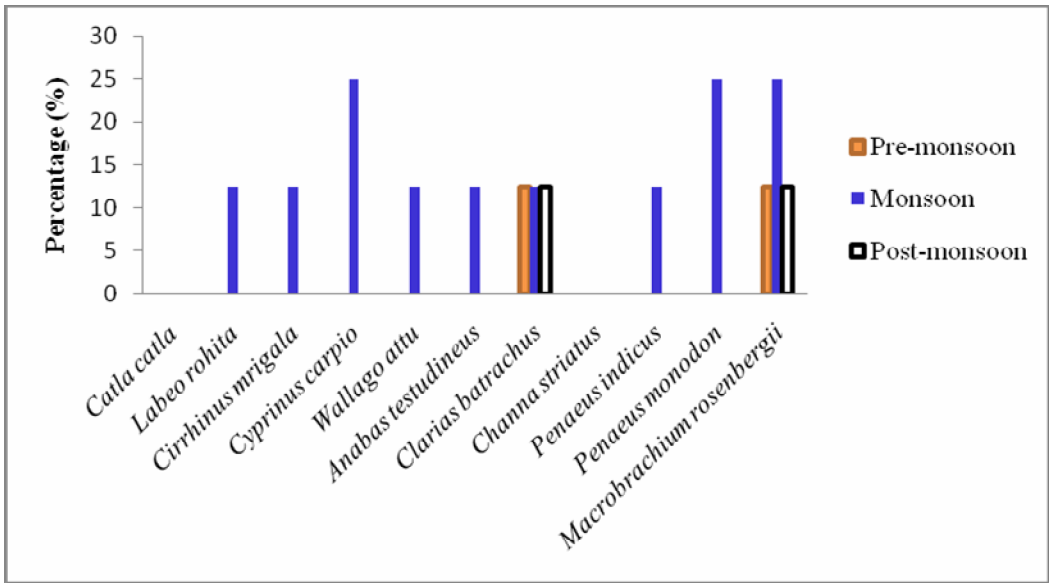
Table.3 Seasonal variation in the incidence of *Salmonella* in finfish and shellfish

Seasons	Fin fishes (%)	Shell fishes (%)
Pre-monsoon	1.56[a]	4.16
Monsoon	10.93	17.85
Post-monsoon	4.63	4.16

[a]F value 1.8571
[*] Significant at 0.01 level

Contamination of food with *Salmonella* is a major public health concern and a zero tolerance has been prescribed for *Salmonella* in fish for export trade (Liston *et al*., 1971). It is evident from the present study that the fish and shell fish of Guntur market were contaminated with *Salmonella* at 5.72% and 9.72% respectively (Table 2). According to ICMSF (1986), FDA (2001), CFIA (2011), *Salmonella* should be absent in fish and fish products. The occurrence of *Salmonella* in the marketed fish may be due to unhygienic handling, processing, improper method of storage and sanitary conditions in the market. This is in agreement with the works of Amagliani *et al*.(2011). Hatha and Lakshmanaperumalsamy (1997) also reported that the high prevalence of *Salmonella* in fish and shellfish is attributed to the poor and unhygienic handling practices and also during transportation from landing centres to fish markets. During transportation, periodical dampening of fish with contaminated water is customary to prevent over heat and drying. It was observed that the cumulative effect of such practices coupled with unhygienic handling during transportation could result in high level of *Salmonella* in marketed food fish. The use of contaminated water for cleaning and processing of fish in the fish market is presumably the cause of secondary contamination.

Fig.1 Percentage incidence of *Salmonella* in fish and shellfish in three seasons



Lack of proper drainage facilities and heavy fly infestation in this market also promotes tertiary contamination to a great extent.

In the present study, it was also observed that the percentage incidence was highest in monsoon followed by post-monsoon and pre-monsoon seasons (Table 3). The highest isolation of *Salmonella* during the monsoon season might be due to precipitation and run-off of drainage that pollutes the river and coastal waters where the presence of high organic substances promoted multiplication of these organisms. Environmental conditions prevailing during monsoon also favors a high degree of another cause to contamination, as well as an extended survival of these organisms in the aquatic systems. This is in agreement with the earlier works of Feachem (1974), Goyal *et al.* (1977), Venkateswaran *et al.* (1989), O´Shea and Field (1991), Hatha and Lakshmanaperumalsamy (1997), Baudart *et al.* (2000), Martinez-Urtaza *et al.* (2003) and Brands *et al.* (2005).

Thus the present study demonstrated that the raw fish sold at domestic fish market in Guntur City could be a source of *Salmonella* with unsatisfactory microbial quality. Fish marketing systems should be maintained clean with improvements in handling and processing to minimize the prevalence of pathogenic bacteria. In order to provide quality fish to the consumers, strict hygienic practices should be followed in fish markets (NFDB, 2011). This *Salmonella* surveillance can provide data to formulate control measures for effective treatment and prevention of foodborne illness to the consumers.

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