



Original Research Article

Prevalence of *Staphylococcus aureus* in Fish Samples of Local Domestic Fish Market

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ABSTRACT

Keywords

Prevalence, *Staphylococcus aureus*, Freshwater fishes, Local fish markets, Seasonal variations, Hygiene.

A study was conducted to evaluate the prevalence of *Staphylococcus aureus* from commercially important food fishes collected from domestic fish market of Guntur, Andhra Pradesh, South India. A total of one hundred and ninety two fishes belonging to eight species were collected from the fish market over a period of one year. Of which 24.47% of fishes were found to be contaminated with *S. aureus*. The highest incidence of *S. aureus* was seen in *Cirrhinus mrigala* (50%) followed by *Cyprinus carpio* (45.83%), *Catla catla* (41.66%), *Labeo rohita* (37.5%), *Anabas testudineus* 29.16%), *Channa striatus* (16.66%), *Wallago attu* (12.5%) and *Clarias batrachus* (4.16%). A well-marked seasonal variation in the incidence pattern was observed with a higher incidence during post-monsoon followed by monsoon and pre-monsoon seasons. It was also observed that the poor sanitary conditions prevailed in the local market are crucial for the microbial contamination of fishes. The reasons for seasonal variations in the incidence of *S. aureus* in fishes of Guntur fish market have been discussed.

Introduction

Fish has high consumer preference due to its inherent nutritive value, taste and easy digestibility. It is one of the most important sources of animal protein available in the tropics and has widely been accepted as a good source of protein and other elements for maintenance of healthy body (Andrew, 2001). Fish is a highly perishable food item and the biological degradation is faster than vegetables. Therefore, it has to be handled, stored and marketed with extreme care in minimum possible time. Best hygiene has to

be maintained in the fish handling areas for prevention of contamination and loss of quality of fish. Cross contamination with harmful agents through bad handling and unhygienic practices cause illness to the consumers.

Food safety is one of the major challenges for the 21st century implying a significant redirection of food microbiologist efforts in many parts of the world toward the prevention of food borne diseases (Sperber

and Tatini, 1975). Fishery products, which are of great importance for human nutrition and provide clear health benefits, can also act as a source of various food borne diseases (Darlington and Stone, 2001). One-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone (Huis in'tVeld, 1996). Even though the safety of food has dramatically improved overall, progress is uneven and food borne outbreaks from microbial contamination, chemicals and toxins are still common in many countries (WHO, 2007).

The genus *Staphylococcus* comprises several species, of which *S. aureus* is one of the major bacterial agents causing food borne diseases in humans worldwide (EFSA, 2010; Le-Loir *et al.*, 2003). The staphylococci are Gram-positive cocci with their primary habitat in the skin, glands and mucous membranes of warm-blooded animals including humans. Infected sores and scratches are often harbourage sites for *S. aureus*. The bacteria survive well in the environment and may also be isolated from a range of sources that come into contact with man and animals. *S. aureus* can cause severe food poisoning. It has been identified as the causative agent in many food poisoning outbreaks and is probably responsible for even more cases in individuals and family groups than the records show (Bennett and Lancette, 1998). Staphylococcal food poisoning is a common food borne disease that occurs in most countries of the world, especially in India due to its warm and humid climate (Bergdoll, 1989). So far no work has been done on the microbiology of freshwater fishes of local fish markets in South India. Hence the present study was undertaken to determine the incidence of *S. aureus* to evaluate the hygienic quality of 8 different food fishes marketed in domestic fish market of Guntur city, Andhra Pradesh, India in different seasons. It was also

proposed to analyze the reasons for the contamination of fishes and the seasonal variations in the incidence of *S. aureus* 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 at Guntur fish market were purchased and analyzed 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 *S. aureus*, 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.

Preparation of fish homogenate: Twenty five grams of raw fish meat from each fish was homogenized with 225 ml of sterile phosphate buffer solution. 10 ml of this homogenate was added to a test tube containing 90 ml PB solution to get a dilution of 10^{-2} . Similarly serial dilutions up to 10^{-7} were prepared.

Isolation, Enumeration and Identification of *S. aureus*:

The isolation, enumeration and identification of *S. aureus* was carried out using standard methods of USFDA (BAM, 2001). Selective medium used for the isolation of *S. aureus* was Baird parker agar (BPA) (HiMedia Pvt. Ltd.). One ml from each dilution (10^{-1} to 10^{-7}) was spread over a dry surface of BP agar plate in duplicate. Inoculated plates were incubated at 37°C for 48hours.

Characteristic appearance of black shining convex colonies of 1–1.5 mm in diameter with narrow white margin and surrounded by a clear area extending into opaque medium were considered to be presumptive *S. aureus* (Fig. 1). The number of colonies was enumerated and the average number per gram was calculated (cfu/g). The pure cultures were also streaked on Nutrient agar (HiMedia Pvt. Ltd.) and incubated for 24 hours at 37°C and was further characterized by biochemical tests.

Morphological characteristics: The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes (Table 1).

Biochemical confirmation: Biochemical tests were performed to confirm *S. aureus* using catalase test, coagulase test, thermonuclease test, anaerobic utilization of glucose and mannitol test (Table 2).

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 4.

Results and Discussion

The present investigation on the incidence of *Staphylococcus aureus* in different fishes showed that 24.47 percent of fishes out of 192 fishes analyzed were contaminated (Table 3). Among the different varieties of fish, *Cirrhinus mrigala* showed the highest *S. aureus* contamination (50%) followed by *Cyprinus carpio* (45.83%), *Catla catla* (41.66%) *Labeo rohita* (37.5%), *Anabastes tudineus* (29.16%), *Channa striatus* (16.66%), *Walla goattu* (12.5 %) and *Clarias batrachus* (4.16%). The number of fishes analyzed, the number of positives, percentage incidence and minimum and maximum values of *S. aureus* incidence in 8 fishes during the study period are given in Table 3. The percentage incidence of *S. aureus* in different fishes during three seasons is given in Figure 2. It was observed that the prevalence of *S. aureus* was high during post-monsoon season followed by monsoon and pre-monsoon seasons. Statistical analysis of the data showed significant ($P < 0.01$) variation in the incidence levels during various seasons (Table 4).

Staphylococcus species are one of the most important food borne opportunistic bacteria in fishes and some are potential pathogens and the high population of these bacteria indicates the degree of the spoilage it might have undergone (Albuquerque *et al.*, 2007; Ayulo *et al.*, 1994; Leung *et al.*, 1992). The present study highlights the considerably high prevalence of *S. aureus* in fishes of Guntur market. The percentage incidence of *S. aureus* reported in the present study was relatively higher than those of freshwater fishes reported by Ali (2014) and El-olemy *et al.* (2014). 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 conventional practices coupled with unhygienic handling during transportation could result in high level of *S. aureus* 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. Lack of proper drainage facilities and heavy fly infestation in this market also promotes tertiary contamination to a great extent.

In the present study, lowest value of 3.6×10^1 cfu/g of *S. aureus* was observed in *Walla goattu* and highest value of 5.9×10^5 cfu/g was observed in *Labeo rohita*. These values indicate that the incidence of *S. aureus* was high in fishes which were subjected to more human handling and unhygienic status of fish handlers. A questionnaire survey revealed that *Labeo rohita* has high consumer preference with relatively more handling operations. Hence there is a possibility of microbial contamination in fishes with more routine commercial operations at different retail sale locations in the market. Clucas and Ward (1996) also reported that *S. aureus* contamination of fresh fish has been found to be from human handlers. According to Simon and Sanjev (2007) the incidence of *S. aureus* in fish samples indicates unhygienic conditions because the product contamination could be the result of a combination of improper handling, improper storage and cross contamination.

In the present study, it was also observed that the percentage incidence was highest in post-monsoon followed by monsoon and pre-monsoon seasons. This high incidence in post-monsoon season might be due to the cross contamination of *S. aureus* from marine fishes which were highly marketed in Guntur fish market during this season. It could be explained by the fact that seafoods act as vehicle for all important species of food-borne pathogens particularly *S. aureus* (Ali and Hamza, 2004; Lorca *et al.*, 2001; Reilly *et al.*, 1992 and Sanjeev *et al.*, 1996). Another reason for high prevalence of *S. aureus* during post-monsoon season could be due to low or negligible populations of other bacteria. It was also reported that Staphylococci are poor competitors and do not grow well in the presence of other organisms (Sahu *et al.*, 2012). Thus the higher incidence of *S. aureus* in post-monsoon season could be explained.

The present study demonstrated that the raw fish sold at domestic fish market in Guntur City could be a source of *S. aureus* 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).

Table.1 Morphological and culture characteristics of *S. aureus*

Isolated bacteria	Gram staining	Culture characteristics on selective media
<i>Staphylococcus aureus</i>	Gram positive cocci (in clusters) Pink colour	BPA: Typical black shining convex colonies (1-1.5 mm) surrounded by halo zone

Table.2 Biochemical characterization of *S. aureus*

Biochemical test		Reaction
Catalase activity		positive
Coagulase production		positive
Thermonuclease production		positive
Lysostaphinsensitivity		positive
Anaerobic utilization of	Glucose	positive
	Mannitol	positive

Table.3 Incidence of *Staphylococcus aureus* in fish samples

S.No.	Fish sampled	Number analyzed	Number of positives	Percentage incidence (%)	<i>S. aureus</i> (cfu/g)	
					Minimum	Maximum
1	<i>Catlacatla</i>	24	10	41.66	5.3×10^1	2.5×10^4
2	<i>Labeorohita</i>	24	9	37.5	3.5×10^1	5.9×10^5
3	<i>Cirrhinusmrigala</i>	24	12	50	3.1×10^1	4.0×10^5
4	<i>Cyprinuscarpio</i>	24	11	45.83	5.1×10^1	9.0×10^3
5	<i>Wallagoattu</i>	24	3	12.5	3.6×10^1	1.7×10^4
6	<i>Clariasbatrachus</i>	24	1	4.16	6.3×10^1	7.5×10^3
7	<i>Channastriatius</i>	24	4	16.66	$61. \times 10^1$	2.4×10^5
8	<i>Anabas testudineus</i>	24	7	29.16	7.1×10^1	2.8×10^3

Table.4 Seasonal variation in the incidence of *S. aureus* in fishes

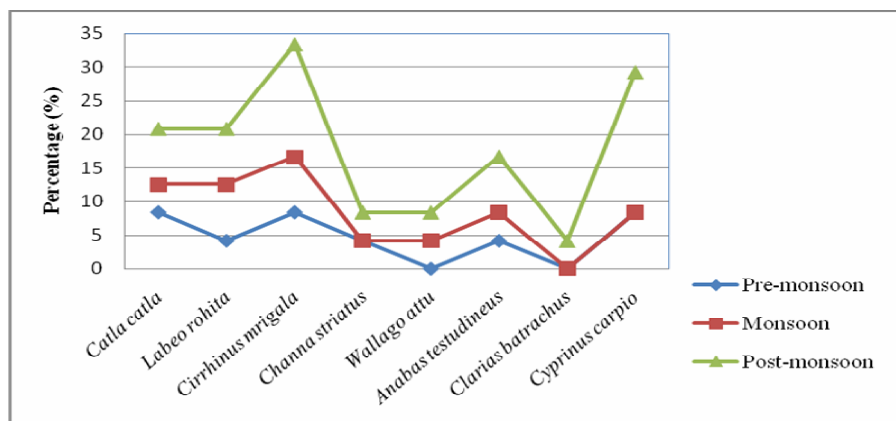
Seasons	Incidence in fish (%)
Pre-monsoon	14.06 ^a
Monsoon	25.00
Post-monsoon	53.00

^aF value 7.2318
Significant at 0.01 level

Fig.1 *Staphylococcus aureus* on BP agar medium



Fig.2 Percentage incidence of *S. aureus* in three seasons



Acknowledgements

One of the authors, P. Bujjammais thankful to the UGC for providing financial assistance under the Rajiv Gandhi National Fellowship. Authors are grateful to the authorities of Acharya Nagarjuna University for providing necessary laboratory facilities to carry out this work.

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