



Screening of Pathogenic Microorganisms from Commercially Important Marine Fishes off Visakhapatnam Fishing Harbour, East coast of India

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Abstract

The present study was carried out for microbial investigation to screening of pathogenic microorganism from selected commercially important edible fish samples from the fishing harbour off Visakhapatnam. The study was performed during the period of April 2012 to March 2013. Selectively, three commercially important fishes were used to evaluate the quality of fish samples in terms of microbial content, using Agar plate method. Total Bacterial Count (TBC), Total coliform counts (TCC) Total faecal coli form counts (TFCC) was performed in the fishes of Areolate grouper (*Epinephelus areolatus*), Silver pomfret (*Pampus argenteus*) and Indo-Pacific king mackerel (*Scomberomorus guttatus*). 10gms of fresh muscle was swabbed from the dorsal fin region and was chosen as the target sample for the estimation of the microbial load. The TBC ranged from 1.6×10^5 cfu/g to $2.75.96 \times 10^5$ cfu/g, while the total coliform count and faecal coliform not exceeded the acceptable limit recommended by FAO. The highly pathogenic bacteria *Salmonella* sp., *Vibrio* sp., were mostly present in the collected fish samples. The main findings of this study inferred that the fish obtained from these sources passers potentially pathogenic microorganisms.

Keywords: Microbiology, TPC, TCC, TFCC.

Introduction

The importance of sea food as a source of animal protein foodstuff gained more and more attention in recent years. In India, the natural resources are highly rich where annual harvestable fishery potential to the country is estimated to increase in millions of tones day by day¹. Even though, sea foods are nutritious value and easy a rich sources of many countries. The pathogenic bacteria naturally occurring in the marine environment undesirable changes during contamination started from the postharvesting period². It is considered that the quality of seafood depends on the quality of water where the fishes are caught and the sanitary conditions of the landing centre.

Microbial contamination from environment may be transferred to the food products directly through surface contact by personnel, pests, air movements and cleaning regimes³. The study was aimed to isolate and determine the microbial flora of pathogenic bacteria present in the types of important fishes like Areolate grouper (*Epinephelus areolatus*), Silver pomfret (*Pampus argenteus*) and Indo-Pacific king mackerel (*Scomberomorus guttatus*) of Visakhapatnam area.

Study Area: Figure-1 showing the location of Visakhapatnam situated in Middle East Coast of India, the fishes Areolate grouper, Silver pomfret and Indo-Pacific king mackerel are fished throughout the year and used of consumption as fresh

forms. These fishes are characterized as long lived, fast growing fishes. The Fishing harbour of Visakhapatnam has special yard with modern facilities like ice manufacturing plants, display trays, trained personnel, electric balances and attractive packaging system available in harbour for selling and transportation of fish to different areas. Improper hygienic conditions and disposal of waste material favours the adaptability of microbial population to alter.

Material and Methods

Sample collections: The commercially important marine edible fishes were collected in fish landing centre off Visakhapatnam and transported to the laboratory for further microbiological analysis. Three common edible fishes Areolate grouper *Epinephelus areolatus*⁴, Silver pomfret *Pampus argenteus*⁵ and Indo-Pacific king mackerel *Scomberomorus guttatus*⁶ were selected for the screening of microbial population. The selective fishes were identified according to Fish Base 2010⁷.

Microbiological analysis: Five microbiological parameters were examined on fishes which include Total Bacterial count (TPC), Total Coliform Count (TCC) and Total Fecal Coliform Count (TFCC), Total Fungal Count (TFC), and qualitative analysis of *Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp., *Shigella* sp., and *Staphylococcus* sp.,

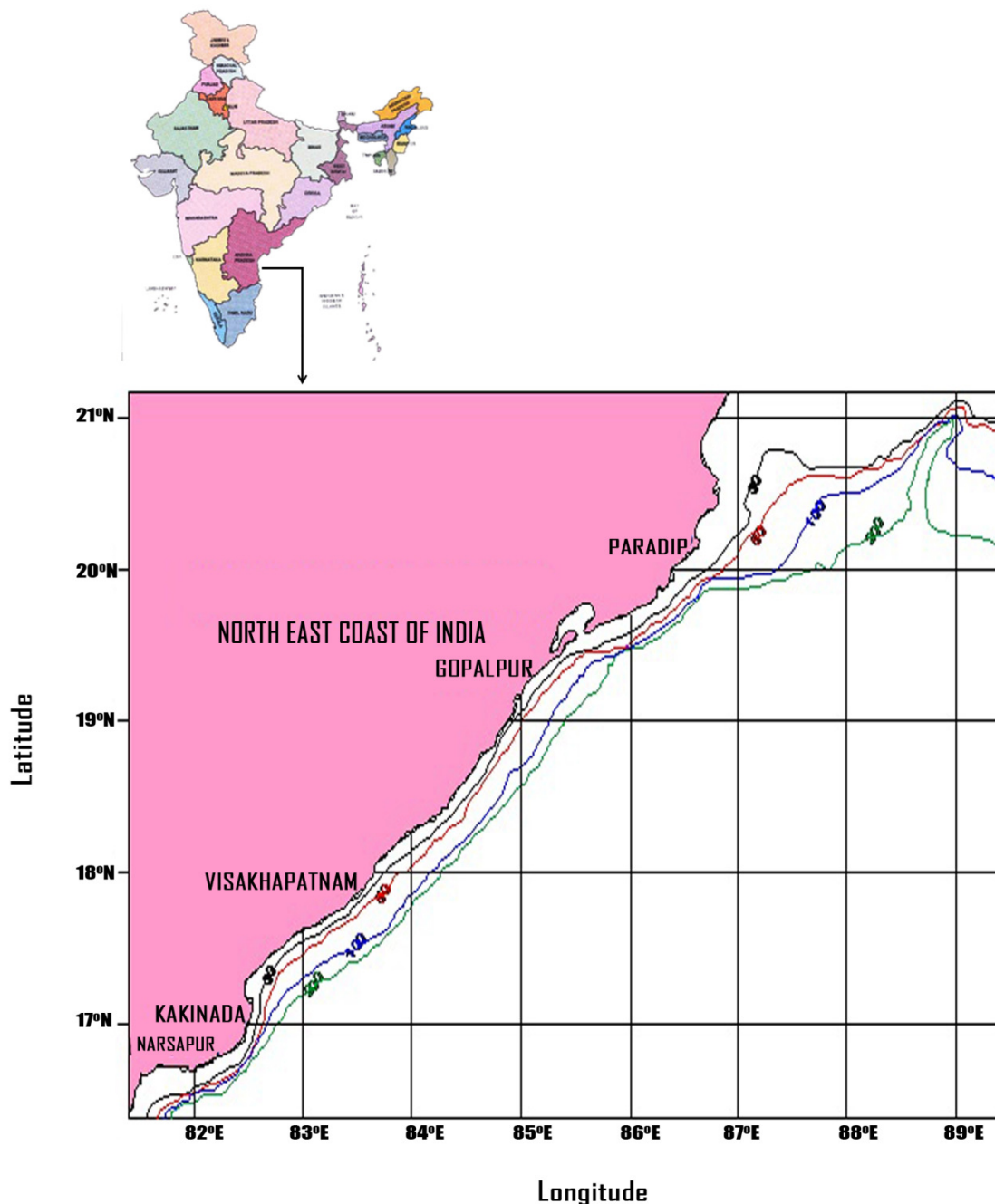


Figure-1
Map showing Visakhapatnam coastal area

Preparation of sample: About 1gm was taken from each fish homogenate was made in 10ml distilled water. The solution was serially distilled ten folds. 0.1ml of (10^{-10}) dilution was spread on to Zobell Marine Agar in duplicate and incubated for 18-24 hrs at 37°C.

Enumeration, Isolation and Identification of isolates: The pour plates were made using Nutrient Agar (NA), MacConkey Agar (MCA), Salmonella / Shigella Agar (SSA), Thiosulfate

Citrate Bile Salt Sucrose Sugar Agar (TCBS), Manital Salt Agar (MSA) and Sabouraud Dextrose Agar (SDA) for total heterophilic bacterial counts, total coliform counts, total *salmonella/shigella* counts, total vibrio count and total fungal counts respectively. The plates were incubated at 37°C for 24-28 hrs except for fungal plates and which were incubated at room temperature (28°C) for 3-7 days.

Microbial count: An automatic colony counter is determining

used for counting the microorganism number of colonies from cultured Petri plates and the count was expressed as Colony Forming Unit (cfu/g). The total count and faecal coliform bacteria were examined by using standard technique Most Probable Number (MPN) procedure.

Results and Discussion

The microbial analysis clearly showed the individual results conducted on selected 3 samples of fishes in table-1.

Table-1
Microbiological Assessment of Fish Samples

Name of fish	TPC (cfu/g)	TCC MPN/g	TFCC MPN/g	TFC cfu/g
<i>Epinephelus areolatus</i>	2.6X10 ⁵	28	20	3.1X10 ²
<i>Pampus argenteus</i>	2.75X10 ⁵	46	21	1.25X10 ²
<i>Scomberomorus guttatus</i>	1.6X10 ⁵	21	2.8	Nil

The highest count of TPC was found in Silver pomfret 2.75X10⁵ CFU/g and the lowest count 1.6X10⁵ were found in Indo-Pacific king mackerel. The environmental conditions depending upon the bacterial flora which it was catch rather than on the fish samples^{8,9}. The fungal counts ranged from 3.1X 10²-1.25X10²cfu/g. The international commission suggested the bacterial load of the fish samples were within the range of the maximum limit (10⁵cfu/g)^{10,11}.

Indicator organisms as the Total Coliform Count (TCC) and Total Fecal Coliform Count (TFCC) were found in almost all the samples of three fishes, collected from Visakhapatnam fishing harbour. The highest count of TCC (46MPN/g) found in Silver pomfret and lowest count was found in Indo-Pacific king mackerel in samples. TFCC count was always lower than TCC. Almost all values exceed the IAMS¹² limits (100/g) for total coliform and 11/gm for faecal coliform that infers on the supply of low quality fish in most of the fish. The presence of coliform group *E. coli* was in higher range, suggests contamination of the samples before or during handling, processing¹³. But, lower load of TFCC in sample Indo-Pacific king mackerel indicates lower range of contamination.

The present study showed the highest Total Fungal Count (TFC)

in Areolate grouper and lowest count found in Silver pomfret samples. TFC was absent in Indo-Pacific king mackerel. The occurrence of bacterial isolates obtained from the fish samples represented in table-2.

The presence of *Vibrio* sp., in the fish can cause pathogenic infection to the consumer. In the present investigation, *Vibrio* sp., was studied qualitatively and found in all the samples. According to recommendation of International Association of Microbiology Societies, fresh and frozen fish should be free of *Vibrio* (0/gm)¹⁴. The present study revealed that microbial quality was not good due to presence of *Vibrio* sp., in all the samples.

Salmonella sp., is highly pathogenic and found in all the three fish samples. The results indicate the consequence of contamination of the processing, improper handling, hygienic and sanitary conditions of Visakhapatnam harbour. Drinking faecal contaminated water can also lead to an outbreak of the same. Fish harvested from such water can carry *Salmonella* sp¹⁵. The isolation of *Pseudomonas* sp was also considered as potential for human and a good indicator of food quality as spoilage organism¹⁶.

Conclusion

Fish of good quality should have counts of total bacteria of less than 10⁵ per gram and faecal coliforms and total coliforms should not exceed 10/gm and 100/gm respectively FAO¹⁷. Total Plate Count and Coliform Count have been used extensively as basis of regulating the microbial quality of food. In this study, both regulatory parameters were excessively above the ICMSF guidelines¹⁸. To overcome this situation, it is mandatory to follow the code of practice concerning handling of the catch, icing, post-harvesting procedures and storage including deputation and hygienic measures. Hence, the present study provided baseline information about these pathogens causing food-borne diseases.

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Table-2
Frequency of occurrence of bacterial isolates

Organisms	<i>Epinephelus Areolatus</i> (cfu/ml)	<i>Pampus argenteus</i> (cfu/ml)	<i>Scomberomorus guttatus</i> (cfu/ml)
<i>Vibrio cholera</i>	80	102	62
<i>Salmonella species</i>	50	75	32
<i>Shigella species</i>	20	22	10
<i>Staphylococcus aureus</i>	56	52	40
<i>Pseudomonas aerogenes</i>	30	26	22
Total	236	287	166

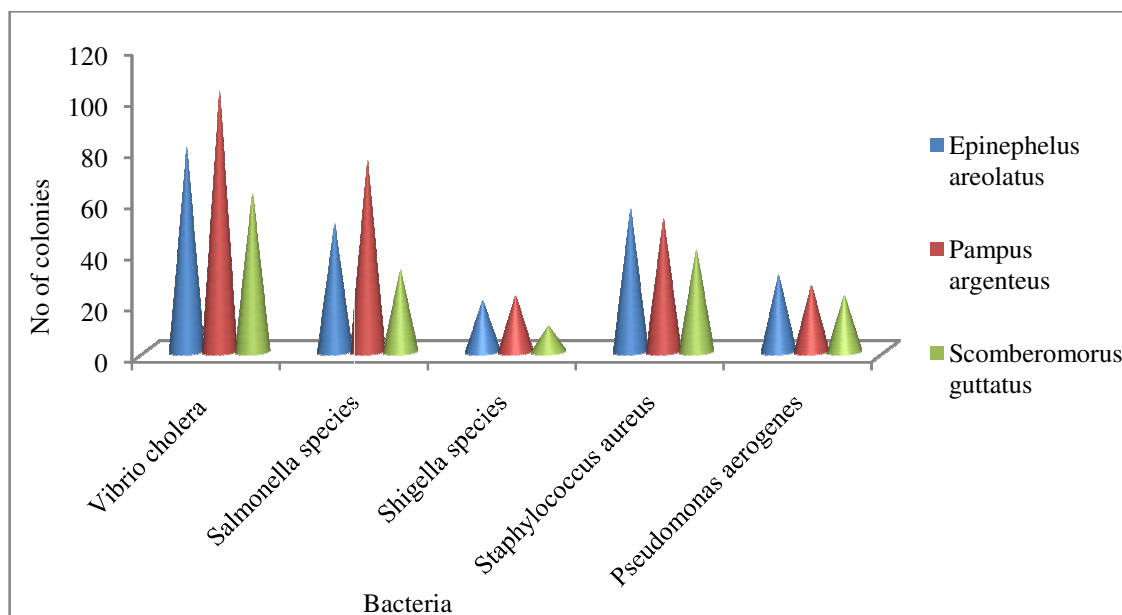


Figure-2
Incidences of the various isolates in cultures

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