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European Journal of Experimental Biology, 2016, 6(4):57-61



Microbial assessment of commercially important crabs from Visakhapatnam fishing harbour, east coast of India

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ABSTRACT

The present study was conducted for microbial assessment of five selected species viz., *Scylla serrata* (Forsskal, 1755), *Portunus pelagicus* (Linnaeus, 1758) *Portunus sanguinolentus* (Herbst, 1783), *Scylla oceanica* (Forsskal, 1755), *Charybdis feriatus* (Linnaeus, 1758) from Visakhapatnam fishing harbour, east coast of India. For this, TPC (Total Plate Count) and TEC (Total Enterobacteriaceae Count) occurrence of *Staphylococcus aureus*, *Escherichia coli* and *Vibrio sp.* were determined. In this study, highest TPC was observed in *Scylla oceanica* (3.9×10^4) followed by *Portunus pelagicus* (2.75×10^3) and *Charybdis feriatus* (2.11×10^4) whereas more TEC was observed in *Charybdis feriatus* (3.6×10^6) followed by *Portunus sanguinolentus* (3.32×10^5) and *Scylla oceanica* (3.1×10^6). In overall study, more colonies were observed in *S. aureus* followed by *E. coli* and *Vibrio sp.* while overall microbial accumulation was observed high in *Charybdis feriatus* followed by *Scylla oceanica* and *Portunus pelagicus* respectively. In conclusion, five species of crab under study were more or less contaminated but comparatively microbial load in local market samples indicated that the hygienic condition of these markets were low.

Keywords: Crab, TPC, TEC.

INTRODUCTION

Marine fishery resources are living natural resources which are self renewable with dynamic habitat. In India, the natural resources are highly rich where annual harvestable fishery potential in the country is estimated to increase in millions of tonnes day by day¹. As the world's population increases inexorably at a current rate of 2% per year, the importance of seafood as a source of animal protein foodstuff gained more and more attention in recent years². Seafood is always in news as it is proclaimed to be most nutritious and healthy food as well as being linked to increasing number of food borne outbreaks across the globe. In the nutritional front, fish accounts for 16.6 percent of the global population intake of animal protein and 6.5% of all protein consumed³. Seafood are an significant fraction of a healthy diet where they have high quality protein and other indispensable nutrients can be low in saturated fatty acids and may contain omega-3 fatty acids. Infact, a well-balanced diet that includes a variety of seafood can provide a good condition of heart health, a well growth in children and development. Seafood protein is

easier to digest than other muscle, since it has small connective muscle, and rich in vitamins, minerals and other nutrients^{4,5}. In the future, it plays a vital role of animal protein for human consumption in many parts of the world^{6,7}.

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp.⁸. Seafood are prone to contamination at various stages of handling and processing Raw sea food products ,water and utensils used are highly prone to contamination⁹, Processing and packaging was done mostly by uneducated staff with deprived hygienic conditions¹⁰. TBA factor is responsible for rancid flavor, off odors, colors as well as texture deterioration¹¹. A number of reports have been proved that seafood contamination with *staphylococcus aureus*, salmonellae, coliform bacteria and others lead to health risks ranging from allergic reaction, stomach and intestinal growths, a common degeneration of peripheral cellular tissues, to gradual break down of the digestive and excretive system, abdominal cramps, vomiting, chills and fever^{12,13,14,15,16}.

Food safety has been the buzz word in recent days as there are increasing consumer awareness on hazards present in food as well the ombudsmen roles played by independent media. Although regulatory regime across the world has taken proactive steps, in most of the cases it has been a knee-jerk reaction to the impending crisis. Defining the actual goal of food safety has been an arduous task as there are umpteen interrelated factors that influence the intended goals. The pathogenic bacteria naturally occurring in marine environment cause undesirable changes during contamination starting from the post harvesting period¹⁷. The microbiological quality of sea food from any part of the world has become significant in order to avoid health hazards and also economical losses. The present investigation has been carried out to identify the microbial screening of commercially important crabs like *Scylla serrata*, *Portunus pelagicus*, *Scylla oceanica*, *Portunus sanguinolentus* and *Charybdis feriatus* as these five crabs are available extensively from the harbour of Visakhapatnam.

MATERIALS AND METHODS

Marine edible crabs were collected from landing centers of Visakhapatnam and kept in ice box in sterile bag to avoid the multiplication of microorganisms. This study was carried out during the period of December 2012 to November 2013. These crab species were identified with the help of standard identification manuals and relevant literature.

Sample Preparation:

Sample preparation was made using the method described by Lee¹⁸. About 10g of the crab sample was taken and homogenized in 10ml distilled water. The solution was serially diluted ten folds. 0.1 ml (10^{-2} to 10^{-5}) of the diluted solution was spread on to Zobell marine agar in triplicates and is incubated for 24-48 hrs at 37°C, post which the total CFU (Colony Forming Units) was counted. Serial dilutions of samples were used for microbial enumeration with the following media. Tryptone Glucose Agar was used for Total Plate Count (TPC), Violet Red Bile Glucose Agar for Total Enterobacteriaceae Count (includes all *Coliforms*, *Salmonella* and *Shigella*), Baird Parker Medium for *Staphylococcus aureus*, Eosine-Methylene Blue Agar (EMB) for *Escherichia coli* and TCBS for *Vibrio*. These methods were described by Surendran¹⁹.

RESULTS

The microbiological status of the samples is summarized in Table 1 and 2. Table 1 shows that Total Plate Count and Total Enterobacteriaceae Count of the five crab samples expressed in colony forming unit per gram (CFU g⁻¹).

Table. 1 The microbiological content of the crab samples

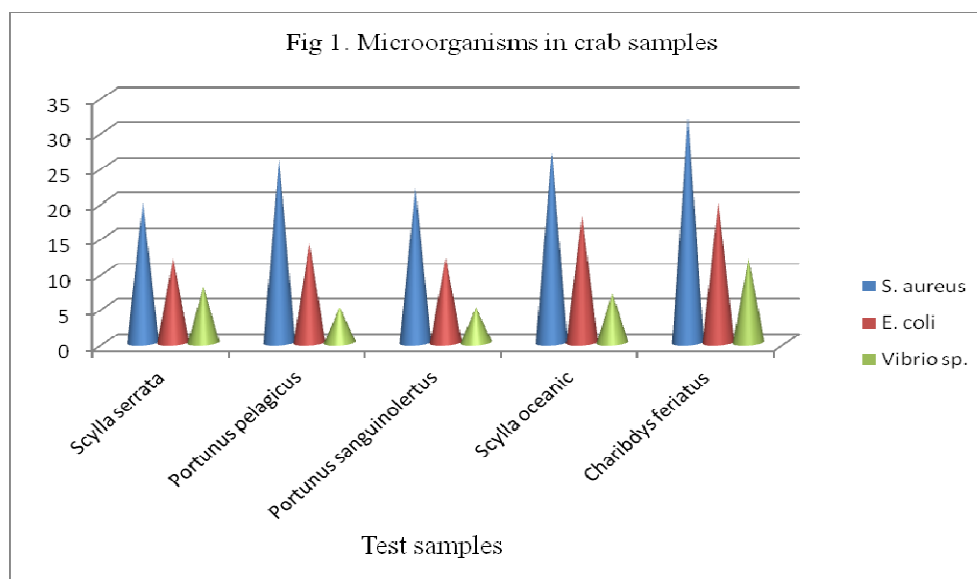
Species	TPC	TEC
<i>Scylla serrata</i> ,	1.72×10^4	2.24×10^6
<i>Portunus pelagicus</i> ,	2.75×10^3	1.6×10^5
<i>Portunus sanguinolentus</i>	1.35×10^3	3.32×10^5
<i>Scylla oceanica</i>	3.9×10^4	3.1×10^6
<i>Charybdis feriatus</i>	2.11×10^4	3.6×10^6

The Total Plate Count in *Scylla oceanica* sample showed maximum count of 3.9×10^4 cfu/g and minimum count of 1.35×10^3 cfu/g in *P. sanguinolentus*. The Enterobacteriaceae Count in crabs varied with a minimum of 1.6×10^5 to a maximum of 3.6×10^6 cfu/g respectively. The acceptable microbial level for raw crabs ranges from a minimum 10^5 cfu/g to a maximum 10^6 cfu/g as indicated by ICMSF²⁰. According to the present investigation maximum microbial level of 10^6 cfu/g was observed in TEC. This may be due to human handling and improper hygienic conditions²¹. The present results are very close to the study of Soundara pandian, and Sowmiya²¹.

From the present study, it is evident that *Staphylococcus aureus*, *Escherichia coli*, *Vibrio sp.* were the dominant microorganisms in crab samples. The microbial analysis on specific medium showed that the predominance of pathogenic microorganisms. Antai and Ibrahim²² reported the occurrence of *Staphylococcus* in crabs could have infested during improper handling and preparation of materials. In general, not all the bacteria cause disease to the host unless the necessity arises. The bacterial microorganisms that are present on the crab samples can disrupt human health. *Vibrio* sp. occurs in saline marine environments, free in the water and bound to animal and inanimate surfaces.

Table 2. Number of colonies in the culture

Species	Number of colonies per 0.1/ml sample		
	<i>S. aureus</i>	<i>E. coli</i>	<i>Vibrio sp.</i>
<i>Scylla serrata</i>	20	12	8
<i>Portunus pelagicus</i>	26	14	5
<i>Portunus sanguinolentus</i>	22	12	5
<i>Scylla oceanica</i>	27	18	7
<i>Charybdis feriatus</i>	32	20	12



DISCUSSION

Bacterial infections in the hemolymph of crabs contribute to severe mortalities, particularly within soft shell crab shedding operations. Environmental loading of fecal byproducts from humans and their associated animals is significant and can affect the quality of water and food resources in coastal ecosystems^{23,24}. In addition, infected crabs can represent a significant public health problem. The Higher microbial counts in some samples may be attributable to handling during harvest or processing. The total bacteria count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of effecting health conditions of consumers of such contamination fish²⁵. *S. aureus* strains produce a toxin that causes gastrointestinal illness. The main reservoir is humans, which carry bacterium in their nasal passages, skin, or wounds. *S. aureus* contamination of seafood is usually due to contamination by a food worker during food preparation^{26,27}. *Escherichia coli* is the most common contaminant and are often encountered in high numbers. The

isolation of these groups of organisms indicted faecal and environmental pollution and these supported the findings of Yagoub²⁸. This also confirms the findings^{29,30,31}, who isolated similar organisms from fish and fish products. It has been shown that *Escherichia coli* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment. *Vibrio* spp. is Gram-negative, facultatively anaerobic motile curved rods with a single polar flagellum. Among the *vibrio* genus, 12 species have been reported to be pathogenic to humans, where eight of these may be associated with foodborne infections of the gastrointestinal tract³². *Vibrios* are most commonly associated with seafood as natural contaminants. Foodborne infections with *Vibrio* spp. are common in Asian countries³³. The illness caused by *Vibrio* spp. food poisoning is a gastroenteritis characterised by watery diarrhea and abdominal cramps in most cases, with nausea, vomiting, fever and headache. The incubation period is usually between 12 and 24 hours³⁴ and the disease usually resolves in three days³⁵. The infection is typically acquired through consumption of contaminated seafood. 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). The present study revealed that microbial quality was not good due to presence of *Vibrio* sp., in all the samples^{36,37}.

Very few reports of illness due to the presence of these organisms in seafood have been published, but the mechanisms in these cases appear to be similar to those for other foods (Bryan, 1980). To avoid these toxin-mediated illnesses, it is important to keep foods refrigerated and to ensure proper cooling of hot foods to refrigerator temperature. Outbreaks are usually associated with foods left at inappropriate temperatures for prolonged periods, allowing multiplication of the organism and enterotoxin production³⁸.

CONCLUSION

The bacteria isolation assay evidences the presence of commensal bacterial of intestinal origin, viz: *E.coli*. The presence of *S. aureus* indicates the contamination of the fish and its natural environment by the human beings and warm blooded animals³⁹. The microbial population in the present analysis was found to be higher than the approved safety standard by FDAC. This study therefore is intended to provide basic information about the pathogenic microorganisms that are likely to cause food-borne diseases which can be prevented by following hygienic standards during the process of procuring and storage of the food material.

Acknowledgements

We would like to express our appreciation to the Department of Marine Living Resources, Andhra University, Visakhapatnam and PG Department of Microbiology, Visakha Govt. UG and PG College for Women, Visakhapatnam, for providing necessary facilities to carry out the experiments.

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