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Isolation of human pathogenic bacteria in two edible fishes, *Priacanthus hamrur* and *Megalaspis cordyla* at Royapuram waters of Chennai, India

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Abstract

Two edible fishes, Bulls eye, *Priacanthus hamrur* (Percidae) and Hard tail scad, *Megalaspis cordyla* (Carangidae) from the waters of Royapuram coast, Chennai, Tamil Nadu were chosen for isolation of bacterial human pathogens in their gills, intestine, muscle and skin. Based on their growth characteristics on specific culture media, the following human bacterial pathogens, *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae* and *Shigella dysenteriae* were isolated in both the fishes. The medium of the fishes and the sediments of their habitat were furthermore collected and examined for pathogens. Different bacterial species were found in the medium that was analyzed, including the human pathogens isolated from the fishes. Surprisingly the five human bacterial pathogens harbored in the fishes were found in the medium but not in the sediment. This research is primarily to highlight the quality of these two edible fishes in the coastal waters of Royapuram, Chennai and to create awareness amid fish eating population.

Keywords: Fish, water pollution, *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Shigella dysenteriae*, *Priacanthus hamrur*, *Megalaspis cordyla*

Introduction

Fishery has become an important economic activity in countries having a coastal line. The Fishery Survey of India (FSI) surveys and assesses marine fishery resources in India's Exclusive Economic Zone (EEZ) to ensure optimum utilization. One of the long term visions of FSI is monitoring surveys for fish stock health in the Indian EEZ and fishery strategies. It moreover stresses the need for sustainable fishery and enhanced food security (FSI, 2007). United Nations Food and Agriculture Organization released a report on the state of the World's Fisheries. The red flag it raised is related to the declining stocks, which is not encouraging for an increasing world population, many of whom rely on eating fish to survive. In India, 80% of the infectious diseases are contaminated -water- borne such as typhoid, cholera and dysentery (WHO 2001a). Fishery products, which are of great importance for human nutrition worldwide and provide clear health benefits can act as a source of food borne pathogens and may be a potential source of infection (Kromhout *et al*., 1985). The bacterial flora of marine fish, sediments and sea water has been studied all over the world. Work on fish pathogens has been carried out in marine fishes (Ward *et al*., 2009). The bacterial diseases are caused mainly due to contaminated water and sea foods (Najiah Musa *et al*., 2008). The majority of reported sea foods - associated disease outbreaks are caused by toxins; biotoxins and histamine (Chen *et al*., 2010) and viruses (Duane *et al*., 2009). Bacterial flora of marine fish, sediments and sea water has been studied world over with a view to explain the spoilage of fish (Yagoub, 2009). Immediately after the procurement, there is a significant amount of data on the microbiology of sea food produced or imported in different countries (Ristori *et al*., 2007). Environmental degradation of coastal area of Chennai (Ennore creek) affecting fish and fisher folk has already been reported (Shanthi & Gajendran, 2009). The present intention is to investigate the occurrence of any human bacterial pathogens in the two marine fishes taken for examination and also to explore the possible route of entry into food chain through the fish.

Materials and methods

Marine water samples were collected from the Royapuram coastal region in Chennai. The surface sea water and sediments were collected one and half nautical miles away from the shore, in sterile glass stoppered bottles. A sizable number of freshly caught, *Priacanthus hamrur* and *Megalaspis cordyla* of the same area were collected from the fishermen. To examine the bacterial organisms in the fishes taken for study and in the water samples, the methods of culture and plating as described by Venkataraman and Sreenivasan (1952) were followed.

The tissues of *Priacanthus hamrur and Megalaspis cordyla* such as gills, intestine, muscle and skin were dissected and processed as described by Hossain (2008). The respective portions were cut, homogenized and after serial dilution, they were subjected in separate culture medium.

Culture technique procedures recommended by Bergey (1948) were followed. The media employed for the isolation of different types of bacteria were nutrient agar, ZoBell agar, Mac Conkey agar and Blood agar and these were time tested ones in bacterial cultures.

To identify and characterize the isolated pathogens, biochemical parameters such as test for oxidase, catalase, indole, methyl red, Vp citrate, TSI, urease, nitrate reduction, H_2S production were carried out.

Preliminary identification was done by gram staining, acid fast staining and mortality test for bacterial organisms. Initially the cultured bacteria were identified and classified as human pathogenic and human non-pathogenic.

Results and discussion

Different species of bacteria were isolated and identified and among them, the human pathogens that were found in the culture include *E. coli, Salmonella typhi,*

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Table 1.Microbial contamination of edible fishes

Bacterial species	*Priacanthus hamrur*				*Megalaspis cordyla*			
	Gills	Intestine	Muscle	Skin	Gills	Intestine	Muscle	Skin
Escherichia coli	+	-	+	+	+	+	+	+
Salmonella typhi	-	-	+	+	+	+	+	+
Pseudomonas aeruginosa	+	-	+	+	+	+	+	+
Vibrio cholerae	-	+	+	-	+	+	+	+
Shigella dysentriae	+	-	+	-	+	+	+	+

Pseudomonas aeruginosa, Vibrio cholerae and Shigella dysenteriae. The mentioned pathogens were found in all the tissues of *Megalaspis cordyla* and in the muscle of *Priacanthus hamrur* and the presence of bacterial pathogens in different tissues are shown in Table1.

Fishery products, which are of great importance for human nutrition worldwide and provide clear health benefits (Kromhout *et al.,* 1985), can act as a source of food borne pathogens. Bacterial flora of marine fish, sediments and sea water has been studied world over with a view to explain the spoilage of fish (Yagoub, 2009). One of the essential things in food hygiene is the examination of food, especially for the presence of microorganisms. This is very much needed for the protection and maintenance of community health. The main source of contamination is from water (WHO, 1996).

Evidence from this study and according to published microbiological guidelines (Gilbert *et al*., 1996), suggests that the microbiological quality of the two fishes examined are unacceptable and pose a potential risk to public health. The study fishes harbored human disease causing bacterial organisms responsible for Typhoid fever, Traveler's diarrhea, Keratitis, Swimmer's ear and Skin diseases, Cholera and Shigellosis.

Immediately after the procurement, there is a significant amount of data on the microbiology of sea food produced or imported in different countries (Ristori *et al*., 2007). Human infections caused by pathogens transmitted from fish are quite common. The isolation of enteric pathogenic bacteria from fish that might be transmitted to humans after the handling or consumption of fish was studied in Nile tilapia and 39.5% were Shigella sp.; 11.1% were *Salmonella typhi*; 25.4% were *Escherichia coli*. Ten fishes collected from open-air markets revealed *E. coli* (50%) and *S. typhi* (20%) (Onyango *et al*., 2009).

Among the different fishes analysed, the highest incidence of *S.typhi* was seen in Scopelidae (28%) followed by Trachnidae (26.9%). A well marked seasonal variation in the incidence pattern was observed in fishes with a higher incidence during monsoon season followed by post-monsoon and pre-monsoon. The region of the body that showed frequent isolation was in the alimentary canal of fishes (41.33%) (Mohamed Hatha and Lakshmanaperumalsamy, 2002).

Escherichia coli and fecal coliform bacteria were isolated from five benthic and pelagic fish species to determine their role in the fecal contamination of recreational waters by Hansen *et al*., (2008). The study showed 42% of the fecal coliforms from benthic fish were *E. coli*, only 4% of these coliform bacteria from pelagic fish were *E. coli.* Cluster analysis showed different fish species harbored identical strains of *E. coli* and some fish contained multiple *E. coli* strains. Although the result demonstrated that benthic fish contain *E. coli*, it may be more appropriate to consider these fish as a vector of *E. coli* from other sources, rather than a new source of *E. coli* contamination in aquatic environments. Ishii *et al*., (2007) observed that the beach sand and sediments of temporal sinks of *E. coli*.

Vibrio cholerae, the etiologic agent of cholera, is autochthonous to various aquatic environments, but despite intensive efforts its ecology remains an enigma. Recently it was suggested that copepods and chironomids, both considered as natural reservoirs of *V. cholerae*, are dispersed by migratory water birds, thus possibly distributing the bacteria between water bodies within and between continents. Senderovich *et al.,* (2010) have confirmed the occurrence of *V. cholerae* in one marine species of fish and suggested that fishes are reservoirs of *V. cholerae*. As fish carrying bacteria swim from one location to another (some fish species move from rivers to lakes or sea and vice versa), they serve as vectors on a small scale. Nevertheless, fish are consumed by waterbirds, which disseminate the bacteria on a global scale. Moreover, *V. cholerae* isolates had the ability to degrade chitin, indicating a commensal relationship between *V. cholerae* and fish. Better understanding of *V. cholerae* ecology can help reduce the times that human beings come into contact with this pathogen and thus minimize the health risk this poses.

Thampuran *et al*., (2005) observed the prevalence and characterization of typical and atypical Escherichia coli from fish sold at retail in Cochin, India. El Hadi *et al*., (2004) studied the prevalence of potentially pathogenic Vibrio species in the sea food marketed in Malaysia. Establishment and persistence of E. coli in the intestine of rainbow trout, *Oncorhynchus mykiss* was investigated by Rio-Rodriguez *et al*., (1997). The distribution of *P. aeruginosa* in swamps and it's infection to *O. niloticus* was investigated by Hossain *et al.,* (2006).

Members of the genus Pseudomonas are important phytopathogens and agents of human infections, while other strains and species exhibit bioremediation and biocontrol activities. Species-specific detection of Pseudomonas species in the environment may help to gain a more complete understanding of the ecological significance of these microorganisms. Comparative analysis of biochemically and PCR-ribotyping and PAGE revealed that there was extensive heterogeneity at the genetic and protein levels. SDS-PAGE clearly demonstrated the differences between fish and sediment isolates as evident from the higher range of protein

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profiling (Tripathy *et al*., 2007). The present study also confirms the occurrence of human pathogenic bacteria in fish population irrespective of inland and sea waters; pelagic, benthic and demersal waters; in the habitat and in the open-market.

Conclusion

Five human bacterial pathogens were isolated from the two species of edible fishes, *Priacanthus hamrur* (demersal) and *Megalaspis cordyla* (pelagic) which is largely consumed by coastal population of Chennai. The presence of these human pathogens in the marine waters indicates the pathogenic microbial contamination level of the habitat. This may be owing to the heavy load of sewage disposal into the seas in Chennai area which could act as a suitable culture medium for these pathogens to survive and grow. This untreated and the improper way of sewage disposal system is one of the main sources for microbial water contamination which results in the accumulation of these bacterial pathogenic species in the commercial edible fishes. Moreover these fishes act as reservoirs of human pathogens which are a serious threat to the fish consuming community. Unhygienic fish handling practices of these infected fishes and inadequate cooking may further contribute to the spread of these pathogens. Appropriate surveillance methods are needed to keep the levels of food and water borne infections to a minimum. This investigation is to mainly bring out the quality of these two edible fishes caught in the shore waters of Royapuram, Chennai and to create awareness among fish eating community.

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