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Microbiological Analysis of Major Sea Food Consumed in Coastal Area of Chennai, Tamil Nadu

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A B S T R A C T

The main source of microbial contamination of coastal waters is domestic waste water and sewage discharge. In this present investigation sea foods were collected from Ennore creek Chennai Coast. The sea foods like Catla, Malabar trevally, prawn, Threadfin bream, mussels and oysters were degutted and bacteria were isolated using selective and non-selective agar medium and further confirmed by biochemical tests. The organisms were identified to generic or group level according to Bergey's manual of systematic Bacteriology. Different group of fecal coliforms and Vibrio spp. have been isolated. *Escherichia coli, Vibrio parahaemolyticus, Vibrio cholera, Staphylococcus aureus, Shigella dysentriae* were the dominant species. Shell fishes ingest bacteria along with their feed which is dangerous to humans who eat the infected seafood. Sea food has to be handled, stored and processed properly.

Introduction

Today's world is witnessing the resurgence in the consumption of fish due to the new awareness about its low cholesterol, fat content and good quality of animal protein. Fish and other sea foods are among the most important protein rich foods for human beings especially in a country like India. Since consumption of raw or uncooked sea foods leads to a variety of diseases which are harmful. Water resources and its distribution system have the chances of pollution with various microorganisms, hospitals, farms and domestic sewages. The fishes and shellfishes living in this water sources are at risk of acquiring the antibiotic resistance. This may cause serious health hazards to human and animals especially in countries where the sanitary and hygiene measures are not up to the mark. Keeping in view the above points the present study was envisaged to study the occurrence of microorganisms in fishes and shellfishes from mogathuwara kuppam, Ennore -Chennai coastal region.

Ennore creek is a backwater located in Ennore, Chennai along the Coromandel Coast of the Bay of Bengal. It is located in the zone comprising lagoons with salt marshes and backwaters, submerged under water during high tide and forming an arm of the sea with the opening to the Bay of Bengal at the creek. The creek receives wastewater from

numerous sources including untreated wastewater and treated effluents from industrial sources in the surrounding area. Studies reveal that permitted discharges account for less than 40% of the total BOD load measured in the creek. Heavy metal concentrations are found to be higher near the creek mouth compared with the near shore waters. Majority of heavy metal pollutants are likely to be present in a close proximity range of 0.5 km from the creek mouth after which there is a steady decline in their concentration up to 1.5 km.

The pollution in backwater ecosystem resulting from sewage discharges is manifested by the presence and persistence of pathogenic bacteria. Water borne pathogens like *Shigella sps, Salmonella sps* and *Vibrio sps* show persistence in polluted marine and estuarine waters. Environmental surveys are necessary for understanding and documenting the occurrence and distribution of pollution indicator and human pathogenic bacteria. In order to quantify and understand their relationship with relevant environmental factors, several investigators have examined distribution of these groups of bacteria and certain viruses in coastal waters (Colwell *et al.,* 1977; Marchand, 1986; Patti *et al.,* 1987; Piccolomini *et al.,* 1987; Ramaiah and chandramohan, 1993; Ruiz *et al.,* 2000; Ramaiah and de, 2003). Mortality and survival rates of faecal contamination indicator *Escherichia coli* in the marine regimes have also been studied (Darakas, 2001). Further, different species of bacteria including pathogenic ones (Colwell *et al.,* 1981; Xu *et al.,* 1982; Huq *et al.,* 1984; McCarthy and Khambaty, 1994; McCarthy *et al.,* 1996; Wait and Sobsey, 2000; Darakas, 2001; Ramaiah *et al.,* 2002a) survive in sea water for one to several weeks.

The impact of these indicator bacteria and pathogens on the fishery and coastal waters are significant since these organisms got ingested into the gut and alimentary canal of fishes, prawn and bivalves. In order to confirm the presence of pathogenic organisms present in the sea foods majorly consumed in Chennai coastal area such as fishes, prawns, oysters, mussels were microbiologically analysed.

Samples collected

Fish samples of Malabar trevally, catla, threadfin bream, mullet, oysters, prawns, and mussels were collected and analysed. The currently recommended method of monitoring shellfish for the presence of microbial pathogens requires culturing followed by a series of presumptive and confirmatory tests that can often take more than 5 days to complete (APHA, 1985; D'Aoust, 1989; Miescier *et al.,* 1992).

Materials and Methods

Isolation of bacteria

A small piece of flesh or tissue was extracted from the sample and it was homogenised well with sterile distilled water using a sterile mortar and pestle. 1 gm of the homogenate was suspended into a test tube containing sterile distilled water. The diluted sample was plated in Zobells marine agar and was incubated at 37^0C overnight.

Isolation and Identification of Individual colonies from Zobell Marine Agar media were randomly selected and subcultured. After purification, the organisms were tested for Gram reaction, motility and Biochemical tests (IMViC), H_2S production, sucrose, lactose and mannitol fermentation. The organisms were identified to generic or group level according to Bergey's manual of systematic Bacteriology and (Baumann and Baumann, 1981).

Results and Discussion

Out of the seven seafood sample collected from the Ennore estuary, the microorganisms present in it include *Vibrio sps.* (sample1, 3, 4, 5, 6, 7) and *Staphylococcus sps.* (sample 1,2,3,4,7) and several *Enterobactericeae* (sample 1,2,3,4,5,6,7) family (Table 1 and Fig. 1–5).

Sewage contamination of aquatic habitats is detected by enumerating the coliform groups of bacteria (Fujioka, 2002). As is universally accepted, higher sewage contamination would lead to increased number of coliforms in natural water bodies. Indiscriminate, deliberate, accidental or regular/routine disposals of sewage in most developing countries lead to higher abundance of coliform groups. Ecological surveillance for microbiological analysis is therefore necessary on a continuous basis for realizing the impacts of effluent discharges. Further, as innumerable pathogenic bacteria will constitute the microflora of effluents discharged from domestic, urban, agricultural and certain manufacturing practices, quantifying different groups of pathogenic prokaryotes ought to be a part of such surveys. For instance, information on occurrence, abundance and distribution of potent human pathogens *Vibrio cholera* (causing cholera in humans), *Vibrio parahaemolyticus* (gasteroenteritis), *Salmonella* and *Shigella* spp (typhoid fever; food poisoning), and *Staphylococcal* spps (causing skin infections) in aquatic ecosystem may prove useful in public health management (Nagvenkar and Ramaiah, 2009). Microbiologists rely on the principle that higher the incidence of sewage indicator bacteria in any environment, higher would be the chances for human pathogenic bacteria to be present (Brock *et al.,* 1994; Fujioka, 2002). Further, bacterial metabolism is such that, if a particular group, say *Vibrio cholerae* is the dominant bacterium in the sewage discharges, it can compete and rapidly outgrow the native microflora leading to increased levels of indicator bacteria in natural water bodies. Pathogenic bacteria of human health concern have mostly been studied for their survival in the marine environment (Huq *et al.,* 1984, Byrd and Colwell 1990; Smith *et al.,* 1994; Oliver *et al.,* 1985).

Pollution problems are encountered in Ennore estuary as it receives industrial effluents and domestic sewage mostly in untreated condition. These affect water quality and living organism. Major industries like Kothari chemicals, alkali chemicals, madras refineries, madras fertilizers, petrochemical industries, many other industries and government installations like Ennore Thermal power station are located around Ennore estuary. It has been estimated that about 4.49,000 litres / day of industrial effluents carrying heavy metals are let out in this estuary by the industrial establishments. Another source of pollution that poses danger of fishes and other aquatic life is the flow of domestic sewage and about 4million litres/day are discharged into this estuary, Tamilnadu water supply and sewerage board, report 1980 (Shanthi and Gajendran, 2009).

Even though the bar mouth of this estuary is kept open throughout the year by dredging operations by Ennore thermal power station for maintaining free flow of sea water into this estuary, a large number of fish, prawn and other organisms die every year in this estuary due to water contamination. The physiochemical characteristics of the water such as temperature, salinity, dissolved oxygen, pH and water transparency were the major cause for the presence of pathogenic microorganism in it which further makes entry into the sea foods (Ambrose et al., (1998).

Samples collected

Sample	Common Name	Scientific Name
1	**Malabar trevally**	*Carangoides malabaricus*
2	**Mullet**	*Mugil cephalus*
3	**Catla**	*Gibelion catla*
4	**Threadfin bream**	Nemipterus virgatus
5	**Prawn**	*Penaeus monodon*
6	**Oyster**	*Crassostrea virginica*
7	**Mussel**	*Perna viridis*

Table.1 Predominant organisms in the sea food samples

Samples	Organism Isolated	Organism Isolated	Organism Isolated
1.MALABAR TREVALLY	*Staphylococcus aureus*	*Vibrio cholera*	*Shigella dysenteriae*
2.MULLET	*Salmonella enterica*	*Staphylococcus epidermidis*	*Staphylococcus aureus*
3.CATLA	*Vibrio cholerae*	*Staphylococcus aureus*	*Salmonella enteric*
4.THREADFIN BEAM	*Staphylococcus aureus*	*Escherichia coli*	*Vibrio parahaemolyticus*
5. PRAWN	*Vibrio parahaemolyticus*	*Shigella dysentriae*	*Vibrio cholerae*
6.OYSTER	*Vibrio parahaemolyticus*	*Salmonella enteric*	*Escherichia coli*
7.MUSSEL	*Vibrio parahaemolyticus*	*Staphylococcus aureus*	*Vibrio cholera*

Fig.1 *Vibrio cholera* in TCBS agar

Fig.2 *Escherichia coli* in EMB agar

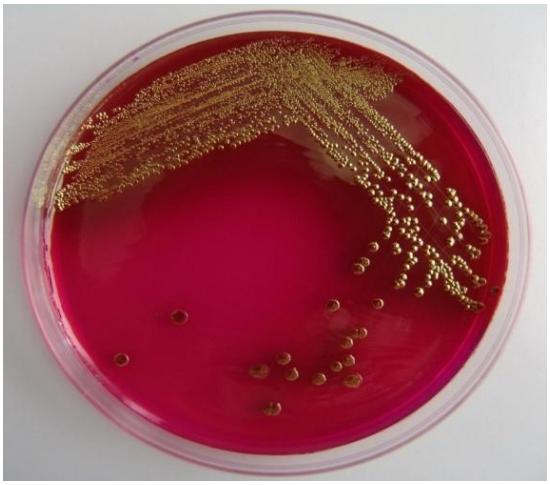

Fig.3 *Staphylococcus aureus* in Mannitol salt agar

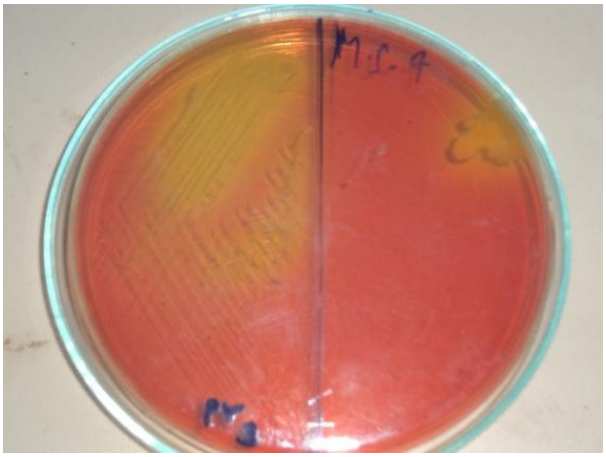

Fig.4 *Salmonella enterica* in SS agar

Fig.5 *Vibrio parahaemolyticus* in TCBS agar

When these contaminated sea foods are consumed as raw or undercooked, we humans get several diseases.

The most pathogenic organism happens to be *Vibrio sps.* The genus *Vibrio* is gram negative, oxidase reaction positive, grow on TCBS agar (Fig. 1 and 5), oxidative-fermentative test positive. Besides these characteristics, they also give reaction with arginine, lysine, ornithine, amylase, indole, citrate, Voges-Proskauer, urease, gelatin, growth on 0, 6, and 8% NaCl, growth at temperature 4, 35 and 40^0C, resistance with O/129µg, produce acid from some sugars, etc. The genus *Staphylococcus* is a gram positive, catalase positive, grow on MSA agar, hydrolyse urea, reduce nitrates to nitrites, liquefy gelatin. Besides these they are coagulase positive, greater biochemical activity; ferment mannite, produce clear haemolysis on blood agar, produce phosphatase, in a medium containing potassium tellurite, reduce tellurite to form black colonies (Alsina and Blanch, 1994a).

Vibrio parahaemolyticus is a marine bacterium that occurs naturally in shellfish, oyster, prawn, crab, raw fish, blue mussels, fresh water fish, sea fish, etc. fish is a good protein source and Chennai is one of the places in India where consumption is very high (Beuchat, 1982). In Chennai some of the fish variety is delivered from other states of India. Moreover this bacterium is commonly found in other South East Asian countries.

Vibrios constitute a major portion of the micro biota in brackish water pond ecosystem. In shrimp farms from India, (Otta *et al.*, (1999) and Vaseeharan and Ramasamy (2003) noted that *vibrio* species accounted for 38-81% of the bacterial biota. In this study, the edible sea food samples analysed were found to contain S*taphylococcus* spp, *Enterobactericeae* spp and *vibrio* species of which *Vibrio parahaemolyticus* is an organism of concern not only because some strains of this species were associated with diseases in sea foods but also because some strains of this species are human pathogens, causing gasteroenteritis (Saqkazaki *et al.*, 1968; Honda *et al.*, 1987; Farmer and Hickman-Brenner, 1992; Powell, 1999). The natural habitat of *vibrio* spp is estuarine and marine water and sediment. *Vibrios* often occur in association with invertebrate and vertebrate animal surfaces and internal organs (Huq *et al.*, 1983; Grimmes *et al.*, 1984b). Stress can compromise the animal host resulting in a commensal species switching to pathogenicity as the mode of interaction (Grimmes *et al.*, 1984c). Out of the 7 seafood samples from Chennai coastal region tested

for presence of marine pathogens, 4 samples were found to be positive for *vibrio parahaemolyticus.* This percentage is in agreement with other studies that reported about 50 - 70% of seafood showed the presence of *vibrio parahaemolyticus* (Fletcher, 1985; Linda *et al.,* 2006). Earlier works of Sakazaki *et al.,* (1968) reported that 1 - 2% of environmental samples contain virulent isolates. Recently Deepanjali *et al.,* reported that the environmental samples (oysters) contain 93.87% *Vibrio parahaemolyticus* in south west coast of India. In this study it is further more confirmed that the sea food seemed to contain *vibrio* species which are pathogenic to human due to the pollution of water resources. So in order to ensure a good and hygienic seafood, pollution of water resources should be avoided which is achieved by proper treatment of effluents from factories and sewage which treat waste water to remove major pathogenic organisms. Upon ensuring the above mentioned two strategies, pollution of sea water can be prevented and seafoods becomes safe for humans to be consumed.

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