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Distribution of *Vibrio parahaemolyticus* in Marine Water, Sediments and Marine Invertebrates Collected from Rameswaram Island, Tamil Nadu, India

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Abstract: Samples of seawater, sediments and invertebrates like bivalve (*Dona faba* and *Scapharca inegerivalis*) and gastropod (*Littorina scabra* and *Cerithium trailli*) were collected from the bathing place, Agnitheertham (AT) and Sewage Mixing Place (SMP) of Rameswaram island from August 2006 to July 2007. A total of twelve seawater samples and twelve sediments samples and 312 marine invertebrates were analyzed to detect the occurrence of *Vibrio parahaemolyticus*. *V. parahaemolyticus* was isolated from all the samples throughout the year. No seasonal variation in the counts of *V. parahaemolyticus* in water, sediments and marine invertebrates was observed. Water samples of SMP showed low density of *V. parahaemolyticus* compared to seawater of AT. Sediments of both AT and SMP contained high counts of *V. parahaemolyticus* compared to water samples. Invertebrates collected from both AT and SMP showed high counts of *V. parahaemolyticus*, compared to other samples. Invertebrates collected from SMP showed high density of *V. parahaemolyticus*. Among the marine invertebrates, bivalves at both AT and SMP showed high counts of *V. parahaemolyticus*. Bivalves collected from SMP showed high counts of *V. parahaemolyticus* compared to bivalves collected from AT. Occurrences of high counts of *V. parahaemolyticus* in marine organisms are dependent on the nature of feeding habits and the habitats from where the animals have been collected. Proper monitoring and attention should be given to the pilgrim town, Rameswaram, otherwise marine organisms may be infected by these pathogens, which may cause health hazards to the consumers.

Key words: *Vibrio parahaemolyticus*, bivalves, *Scapharca ineguivalli*, *Donax faba*, gastropod, *Littorina scabra*, *Cerithium trailli*

INTRODUCTION

Vibrio parahaemolyticus is considered as a potential enteropathogen, distributed throughout the world, from estuaries, through coastal waters (Joseph *et al*., 1982). Even though these organisms are isolated from marine environment, they are also distributed and isolated from fresh water environment (De *et al*., 1977). *V. parahaemolyticus* is a moderately halophilic, gram negative, motile, rod, fermentative, anaerobic, oxidase positive organisms. *Vibrio parahaemolyticus* causes disease in association with the ingestion of raw or insufficiently cooked seafoods, improper post harvests storage conditions or poor handling of seafood during preparation (Cavallo and Stabili, 2002). This organism is collected from the case of gastroenteritis in Japan, in 1950. Many researchers reported that *V. parahaemolyticus* is also isolated from human diarrheal stool, seafood and marine environment, from different parts of the world (Binta and Nyaga, 1982; Fujino *et al*., 1974).

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Sewage contains high counts of microbes. Microbes present in contaminated water may easily come in intimate contact with surface of the organisms, inhabiting in contaminated water. Various studies showed that Vibrios are not normal flora in the intestinal tract of fish and other organisms. Presence of vibrios in the tissues and organ of organisms indicated that organism's intake the bacteria from the surrounding environment (Geldrich and Clarke, 1966). The genus *Vibrio* is more frequently isolated from the surface and gut of fish and other marine invertebrates (Colwell, 1981). *V.* parahaemolyticus is also associated with wound infection in humans (Ronald, 1970), disease of crab (Krantz *et al*., 1969) and shrimp (Lightner and Lewis, 1975). A number of studies showed that there are outbreaks and cases of illness associated with consumption of shellfish grown in contaminated water, insufficiently cooked seafood, eaten as raw (Janda *et al*., 1988). Sen *et al*. (1977) reported that 8-10% of the suspected cases of gastroenteritis annually admitted to the infectious disease Hospital in Calcutta, are due to *V. parahaemolyticus* infection. Studies on isolation of *V. parahaemolyticus* from different environment sources indicated, not only the importance of the etiology of the organisms, but also about their potential impact on human health.

Rameswaram is an island and a historically important pilgrim town. People from different parts of India come and take holy at Agnitheertham. Local people also use the shore for excreting their waste materials. Through wave and tidal action the waste materials get in to the sea and the water is heavily polluted. All along the coast the sewage collected from different parts of the town is disposed in to the sea directly without any treatment. The sewage pollutes the seawater and enhances the growth of the pathogenic bacteria and fungi. The pathogenic organisms cause infectious diseases to marine organisms and make them unfit for consumption. When such infected organisms are consumed, they may cause disease outbreaks. Based on the above-mentioned information the present work was under taken to find out the distribution of *V. parahaemolyticus* in different sources of the pilgrim town Rameswaram.

MATERIALS AND METHODS

Samples were collected from the bathing place called as Agnitheetham (AT) and Sewage Mixing Place (SMP) at Ramewaram, from August 2006 to July 2007, including seawater, sediments, bivalves (*Scapharca ineguivalli* and *Donax faba*) and gastropods (*Littorina scabra* and *Cerithium trailli*). Samples were collected once in a month and were kept in refrigerator box and analyzed within three hours of sample collection.

Physico-chemical characteristics of water such as water temperature (°C), pH, Dissolved oxygen and salinity were observed simultaneously with sampling (APHA, 1982).

Bivalves and gastropods were washed with sterile saline and aseptically opened (Hunt *et al*., 1984). One gram of meat was homogenized in Phosphate Buffer Saline (PBS, pH 7), with Stomacher machine (Twedt, 1984) and the final volume was brought up to 10 mL. One gram of sediment and one gram tissues of the invertebrate were homogenized with Phosphate Buffer saline and brought up to 10 mL. One milliliter of water sample was diluted with 9 mL of Phosphate Buffer saline. Then the samples were serially diluted up to 10^6 dilutions from the initial 1: 10 diluted samples.

Finally diluted samples were filtered through the membrane filter (Millipore membrane filter 0.44 mm diameter and 0.45 μ pore size). Membrane filter was placed on the surface of the TCBS agar medium containing petri plate and incubated at 37°C for 24 h. Blue or green and yellow colour colonies were developed on the membrane filter and were used for further biochemical tests to confirm the isolates were *V. parahaemolyticus*. The biochemical tests were performed for the identification of isolates by following FAD, Bergey's Manual (Twedt, 1984; Baumann and Schubert, 1984).

RESULTS AND DISCUSSION

A total of 12 water samples, 12 sediments samples and 312 samples of marine invertebrates including two species of molluscan bivalves and two species of gastropods were collected from both sampling sites, once in a month. They were used to isolate *V. parahaemolyticus*.

No marked variation in water temperature was observed on both sampling sites, throughout the study period (Table 1). The reason is that India being a tropical country, where the temperature variation is very narrow throughout the year.

Variation in pH, salinity and dissolved oxygen concentration was observed between the sampling sites AT and SMP. Low alkaline pH, Low concentration of dissolved oxygen and low salinity were observed at the sampling site SMP, compared to the samples of AT (Table 1). The water quality of samples from the SMP reflected the high influence of the sewage on water throughout the year of study. This sampling site is highly influenced by the sewage throughout the year. This place is meant for disposing the sewage collected from the southern part of the pilgrim town Rameswaram, which was influenced by human activities like bathing.

The occurrence of enteropathogen *Vibrio parahaemolyticus*, in water, sediments and marine invertebrates collected from both the sampling sites has been studied in the present work. Results of the previous studies indicated the occurrence of *V. parahaemolyticus* in different samples like marine water (Chan *et al*., 1986), estuarine (Simidu *et al*., 1982) sediments and fresh water (Sarkar *et al*., 1985), finfish, shellfish and muscle of fish, from different parts of the world (Joseph *et al*., 1982; Ortigosa *et al*., 1989).

High counts of *V. parahaemolyticus* were observed in water, sediments and marine invertebrates (Table 2) in the present study. Difference in the counts of *V. parahaemolytiocus* between environmental samples and marine organisms was also observed in the present study. Wong *et al*. (1992) also reported the difference in the counts of *V. parahaemolyticus* in marine foods and ecological samples. The present study is well corroborated with the studies of Baross and Liston (1968). They have reported that the count of *Vibrio parahaemolyticus* in seawater ranged between 35 and 350 cfu mL^{-1}; in sediments 100 and 2000 cfu g^{-1} and in oyster 65 and 4000 cfu g^{-1}. Thompson and

Table 1: Physico-chemical characteristics of water of Agnitheertham (AT) and Sewage Mixing Place (SMP)

	Agnitheertham (AT)		Sewage Mixing Place (SMP)	
Parameters	Minimum	Maximum	Minimum	Maximum
Temperature (°C)	25.10	35.00	26.00	35.00
pH	7.50	8.40	8.20	9.20
Dissolved oxygen (mg L^{-1})	3.10	4.50	0.06	3.17
Salinity (‰)	26.69	36.85	8.32	28.00

Table 2: *Vibrio parahaemolyticus* counts in water, sediments and marine invertebrates (bivalves and gastropods) collected from Agnitheertham (AT) and Sewage Mixing Place (SMP)

			Counts of *Vibrio parahaemolyticus* (cfu/100 mL or 1 g)			
	No. of samples		AT		SMP	
Types of sample	AT	SMP	Minimum	Maximum	Minimum	Maximum
Water[a]	12	12	1.2×10^{4}	1.9×10^{4}	8.3×10^{3}	6.4×10^{4}
Sediments[b]	12	12	2.7×10^{4}	5.3×10^{4}	5.8×10^{3}	9.5×10^{4}
Littornia scabra[b]	30	40	15	1.7×10^{3}	30	44
Cerithium trailli	40	45	61	152	14	102
Donax faba	50	50	97	2.1×10^{2}	1.5×10^{3}	4.1×10^{3}
Scapharca ineguivalivis	25	27	51	2.2×10^{3}	91	2.2×10^{3}

[a]: Counts per 100 mL of water, [b]: Counts per gram of sediments or gram of flesh of invertebrates

Vanderzant (1976b) also reported high counts of *V. parahaemolyticus* in seawater, sediments and marine invertebrates. In contrast to the present study El-Sahn *et al*. (1982) reported low counts of *V. parahaemolyticus* in above-mentioned samples.

The high counts of *V. parahaemolyticus* in the water of AT compared to that of SMP (Table 2) reflects the influence of human activities and sewage on the two sites, respectively. Presence of high density of enterobacteriaceae members' and low salinity may inhibit the growth of *V. parahemolyticus*. Temperature, organic content and salinity form a complex interrelationship and aid in the development and survival of *Vibrio* species. Temperature and salinity produce certain influence on the growth and recoverability of Vibrios. Vibrios species grow well at the salinity ranged between 10 and 30‰ and temperature between 20 and 30°C.

Compared to water and sediments of AT, SMP contained high counts of *V. parahaemolyticus* (Table 1). The reason may be the availability of more nutrients in the sediments. The bacteria utilize the nutrients available in the sediments and multiply and increase in number. Sediments of SMP showed high counts of *V. parahaemolyticus* compared to that of AT. The sewage contains high organic matter and nutrients and they are settled in the sediments and increase in their concentration. Moreover the sediments at SMP are muddy, containing high concentration of organic matter and nutrients. It provides better habitat for the growth and multiplication of *V. parahaemolyticus*. But the sediments of AT, are sandy in nature. Generally the sandy nature of the sediments possesses very low concentration of nutrients and low organic matter. Hence low density of *V. parahaemolyticus* was observed in the sediments of AT (Table 2).

Vibrio parahaemolyticus was isolated from all the samples throughout the study period. In the present study no significant seasonal variation in the counts of *Vibrio. parahaemolyticus* was observed in water and sediments. In contrast to the present study, different studies conducted in temperate regions showed definite seasonal variation and the organism did not isolate in winter season. During winter season, the water temperature was between 10 and 20°C and the organism were unable to survive at this temperature and hence they adhered to the surface of the marine organisms (Sizemore *et al*., 1975; Kaneko and Colwell, 1973). But in tropical countries like India, no such high variation in water temperature is observed. Generally in tropical countries the temperature ranged between 26 and 36°C, throughout the year, which is favorable for the development and growth of *V. parahaemolyticus* and hence no seasonal variation in the counts of this organism was observed. Sarkar *et al*. (1985) also made a similar observation in water and sediments samples collected from fresh water environ.

V. parahaemolyticus was isolated from all the marine invertebrates collected from both the sampling sites throughout the year. Similarly Quintoil *et al*. (2007) also isolated *Vibrio parahaemolyticus* from finfishes and shellfishes, collected from different ecosystem of West Bengal. Oxley *et al*. (2002) also reported the occurrence of high density of *V. parahaemolyticus* in the gut of *Penaeus merguiensis*.

In the present study no seasonal variation was observed in the counts of *V. parahaemolyticus* in marine invertebrate. Similarly Thompson and Vanderzant (1976a) also did not observe the seasonal variation in the marine invertebrates collected from Galveston Bay. But most of the investigators (Baross and Liston, 1968, 1970; Bartely and Slanetz, 1971; Murakami *et al*., 1975; El-Sahn *et al*., 1982; Montilla *et al*., 1994) reported, seasonal variation in the counts of *V. parahaemolyticus* in marine animals. They have observed high counts of *V. parahaemolyticus* during summer season in all marine invertebrates. Their counts declined during winter season in all marine invertebrates, when the temperature fell to 10-20°C. Some investigators were unable to isolate the *V. parahaemolyticus* from the invertebrates, when the temperature fell into 10-20°C. Because, at this temperature this organism is viable but is unable to grow. They grow at the temperature between 15 and 42°C and maximum growth is seen between the temperatures of 30 and 35°C.

In the present study, the counts of *V. parahaemolyticus* were higher in marine invertebrates than water (8 times) and sediments (4 times). The results of the present study concur with that of Bartely and Slanetz (1971) in oyster and Baffone *et al.* (2000), in mussels. They have reported a higher counts of *V. parahaemolyticus* in fresh oysters than seawater, sediments collected from estuarine water in New Hampshire. In contrast to the results of the present study. Thompson and Vandezant (1976b) reported high counts of V. *parahaemolyticus* in water and sediments than marine invertebrates collected from Galveston Bay. In contrast to the present study, Duan and Su (2005) reported the occurrence of high density of *V. parahaemolyticus* in sediments compared to water and invertebrates.

Among the marine invertebrates examined, bivalves collected from both sampling sites showed high counts of *V. parahaemolyticus* (Table 2). Bivalves collected from SMP showed high counts of *V. parahaemolyticus.* It may be due to this site being highly influenced by sewage, shallow and turbid. In addition to the availability of nutrients, alkaline pH and moderate salinity may enhance the development and growth of *V. parahaemolyticus* .

Moreover, bivalve nutrition is based on filtration. Plankton forms the main base of the nutrition cycle. The planktonic bacteria in sewage and seawater may concentrate in bivalves. The concentrated *V. parahaemolyticus* may utilize the nutrients available in the sewage and may multiply and increase in their counts.

Even though, bivalves collected from both the sampling sites show the same feeding habit, the counts of *V. parahaemolyticus* was low in bivalves collected from AT compared to the bivalves collected from SMP. The reason is, in addition to the feeding habit and other inherent biological differences between the organisms, the degree of infestation could be related to the habitat. The counts of *V. parahaemolyticus* in marine organisms are also dependent on the habitat; from where they have been collected. Seawater of AT was transparent and contained low concentration of nutrients. The sediments of AT were also somewhat sandy, containing low concentration of nutrients and organic matter. It might have influenced the growth and development of *V. parahaemolyticus* in marine invertebrates collected from the sampling site of AT.

In conclusion it can be stated that great attention has to be paid to the pilgrim town Rameswaram, since both sampling sites were contaminated by human activities. Before disposal into the sea, the sewage should be treated. Otherwise it may increase the organic load. As a result of high organic load through sewage and human excreta the marine water is polluted, may enhance and influence the development and growth of opportunistic pathogens like *V. parahaemolyticus* and other human and animal pathogens like *Salmonella*, *E. coli*, etc. They may infect the marine animal, which are used as seafood. The occurrence of high counts of pathogens in marine food may cause food poisoning; especially in individuals who consume this seafood raw, or lightly or insufficiently cooked.

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