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Full Length Research Paper

Prevalence and distribution of *Vibrio vulnificus* in fishes caught off Chennai, Indian ocean

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Vibrio vulnificus **is a notorious sea-food borne pathogen with a high mortality rate. This is ubiquitously present in marine environments, particularly in tropical water. In these studies the prevalence of *V. vulnificus* in fishes caught off Chennai coast of Indian Ocean are determined. Commercially important fishes were analyzed for the occurrence of vibrios of which some of them were harbored fishes. The vibrios constitute up to 19 - 39% of the total aerobic flora. The prevalence of *V. vulnificus* constitutes about 13% of total vibrios isolated from fish. Other clinically important vibrios isolated are *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The comprehensive data regarding the environmental occurrence and levels, niches, survival, seasonality and strain diversity will be helpful for developing sea food strategies which helps to eliminate the risk of *V. vulnificus* in exporting sea foods.**

Key words: Distribution, *Vibrio* species, *Vibrio vulnificus,* prevalence.

INTRODUCTION

Vibrio vulnificus is a sea food borne pathogen. This causes serious wound infection and septicemia in human. It was identified in the late 1970s (Morris and Blake, 1985). It was reported that *V. vulnificus* is the most common pathogen to cause wound infection in persons with liver chronic diseases and hemochromatosis. Centre for Disease Control reported in 1976 that the genus *Vibrio* were distributed in the tropical sea water and in the aquatic environment. This genus includes 37 species among which only 11 species are pathogenic to human causing disease. There are only limited reports available in the world regarding the pathogenicity of *V. vulnificus* (Thampuran and Surendran, 1998). The species *V. vulnificus* has been divided into three sub groups based on their biochemical properties; the biotype three causes wound infection in humans during handling of fish and it causes mortality to persons with liver diseases, debilited persons and alcoholics etc It causes high mortality rate when compared with other species. High prevalence of *V. vulnificus* has been identified in the coastal and estuarine environment during warm weather due to high temperatures in the sea. During hot weather up to 25% of death rate have been reported in USA, Europe and Asia, due to exposure of wounds to sea water and handling of seafood products (Guaglientolo et al., 2006). It is necessary to update the comprehensive information on the distribution and prevalence of *V. vulnificus* in the Indian coastal environment. In this paper the prevalence of *V. vulnificus* in fish caught in Chennai located in the coast of Indian Ocean is determined.

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MATERIALS AND METHODS

Nine types of fish samples were taken freshly from fish landing center for these studies. In these studies the whole parts of the fishes were sampled according to the procedure outlined by the FDA (Eliott et al., 1992). Fishes like Russell's scad (*Decapterus russelli*), Mojarras (*Gerres filamentoses*), Red snapper (*Lutjanus sp.*), Grey mullet (*Mugil cephalus*), Thread fin bream (*Nemipterus japonicus*), Big eye scad (*Selar crumenopthalmus*), Sardine (*Sardinella sp.*), Indian mackerel (*Rastrelligar kanagurta*), Oil sardine (*Sardinella longiceps*) these are the marine fishes used for this study. These samples are the most common varieties on the Chennai coast of Indian Ocean.

Year round sampling

These fish samples were collected for the period of sixteen months from same place of two sites; one site was the Kovalam beach, Chennai coastal area where the samples were directly gotten from the fisher man. The second site was from the Marina beach of fish landing centre in Chennai. The fish samples were kept in ice box

and immediately analyzed within an hour. In fish samples, the whole part of the skin, gills and all the part of intestine were used for *V. vulnificus* isolation (Elliot et al., 1992). To avoid the surface contamination of the body of the fish, the fish was cleaned with 70% ethanol.

Isolation and identification

The FDA (Elliot et al., 1992) approved methods were used for the isolation and enumeration of *V.vulnificus*.

10 g of 70% alcohol was used to clean whole part of the fish and was weighed and blended with 90 ml of sterile Phosphate-buffered saline (PBS) with 2% NaCl (pH 7.5). Serial dilutions were made with phosphate buffered saline up to 10^{-7}. The dilutions 10^{-4}, 10^{-5} and 10^{-6} were plated in Thiosulfate-citrate-bile salts-sucrose (TCBS) agar, Cellobiose-colistin (CC) agar and Cellobiose-polymyxin B-colistin (CPC) agar media for the isolation of *V. vulnificus* in triplicates, the plates were incubated at 37˚C for 24 h. After 24 h the green color colonies were isolated and identified up to species level by using biochemical tests (Alsina and Blanch, 1994 a, b) as listed in (Table 1).

Density of vibrios between sites

Twenty-five gram of fish sample was inoculated into 225 ml of alkaline peptone water (APW) and four selective media in this experiment. TCBS, CC, CPC and marine agar with salt (MA) were prepared following the manufacturer's instructions (Hi media) and incubated the APW for 24 h at 37℃. After 16 - 18 h of incubation, a loopful of culture was streaked onto TCBS, CPC, CC and MA (2% NaCl) incubated for 24 h at 37℃. CC agar plate was incubated at 37℃ or 40℃. A minimum of four typical colonies were selected in each enrichment plate and furtherly subjected to biochemical identification (FDA, 2000 BAM).

The highest rate of *V.vulnificus* from sample site 1 (Kovalam) was (44%) in CC agar followed by (35%) in CPC, (1%) in TCBS and (0%) in MA saline, but *Vibrio* species isolates among the two sampling sites, Site 1 had 44% *V. vulnificus* and site 2 had 52% *V. vulnificus* of the isolates. The plating efficiency of CC agar with APW enrichment was found to be better than that of the other three (Karunasagar et al., 1990). The density of *vibrios* in site 1 and site 2 was analyzed using Gene Spring GX version 7.3 software (Agilent technologies. Santa Clara, California) (Sheik, 2009).

Most probable number analysis

MPN three tubes technique with alkaline peptone water saline (APWS) were performed to enumeration of *V. vulnificus*. Serial dilution tests measure the concentration of the target microbes in the sample with an estimate called the most probable number (MPN). The MPN is particularly useful for low concentrations of organisms (Peeler et al., 1992). Only viable organisms enumerated by the MPN were used for the determination of the growth unit (GUs) and colony forming units (CFU). The whole part of skin, muscle, gill and intestine of the fish (10 g) sample were taken with 2% NaCl and three tubes MPN was performed using APW broth. Positive tubes were used for the isolation of *V. vulnificus* on to thiosulphate citrate bile salts agar and the isolates were furtherly confirmed by biochemical tests.

RESULTS AND DISCUSSION

The incidence of the *V. vulnificus* in marine fish sample collected freshly from fish landing centers and 19 to 39% of other *Vibrio* sp was observed from freshly taken fish, among which 13% of *V. vulnificus* prevalence was observed from fishes in and around Chennai coast. These results indicate that the incidence of *V. vulnificus* in marine fish was low and the post harvest contamination was negligible. Table 2 shows the presence of total *Vibrio* species in the fish samples obtained from site 1 and site 2 which showed a significant difference ($P < 0.05$) of the presence of *vibrios* in the fish samples.

The species identified were *Vibrio alginolyticus, V. vulnificus, Vibrio campbellii, Vibrio orientalis, Vibrio mediterranei, Vibrio logei, Vibrio harveyi, Vibrio parahaemolyticus* in site 1 and site 2 among which the prevalence of the *V. vulnificus* was observed during the study periods of March 2007 to June 2008.

During the study period of November 2007 to January 2008 densities of *V. vulnificus* was found very low. In summer from April 2007 to October 2007 *V. vulnificus* densities were considerably higher. The marine fishes which showed the presence of *Vibrio* sp. were Russell's

Table 1. List of various biochemical tests used for the identification of V*ibrio* species.

Cultural characters	Anon(1957)
Gram staining	Anon(1957)
Oxidase test	Elliot et al. (1995)
Hugh and Leifson test	Hugh and Leifson (1953)
Decorboxylation of amino acid	Moller (1955)
Lysine dehydrolase	Moller (1955)
Ornithine dehydrolase	Moller (1955)
Arginine dihydrolase	Thornley (1960)
Salt tolerance	West and Colwell (1984)
Growth at 0,3,6,8 and 10%(NaCl)	
Citrate reaction	Anon (1957)
Gelatinase production	Anon (1957)
Gas from Glucose	West and Colwell (1984)
Indole production	Cowan and Steel (1965)
ONPG reaction	Elliot et al. (1992)
Swarming	West and Colwell (1984)
Urease production	Elliot et al. (1995)
Voges proskauer reaction	Lee et al. (1975)
Growth on single carbon source	Lee et al. (1975)
Lactose	
D-glucose	
Fermentation of carbohydrate	Elliot et al. (1995)
Mannitol, cellobiose, sucrose	
Disc diffusion tests	West and Colwell(1984)
Ampicillin resistance 10 ug	
O/129 resistance 10 and 150 ug	
H2S production	Anon(1957)

Table 2. Presence of *Vibrio* species in the fish samples obtained from site 1 and site 2 collected from Chennai.

Fish (no. of fishes sampled)	No. of isolates	*V. alg.*	*V. vul.*	*V. cam.*	*V. ori.*	*V. med.*	*V. log.*	*V. har.*	*V .par.*
Decapterus russelli (4)	73	12	12	12	8	4	1	4	20
Gerres filamentoses (4)	41	8	8	7	4	4	1	1	8
Lutjanus sp.(4)	37	4	1	4	4	12	8	3	1
Mugil cephalus (4)	61	6	12	8	10	8	9	5	3
Nemipterus japonicus (4)	75	10	11	13	9	12	7	12	1
Selar crumenopthalmus (4)	25	4	4	3	5	4	3	1	1
Sardinella sp (4)	20	1	2	2	4	5	3	1	2
Rastrelligar kanagurta (4)	68	12	7	11	10	8	7	7	6
Sardinella longiceps (4)	60	8	6	7	8	12	4	3	12
Total	460	65	63	67	62	69	43	37	54
% of each species		14	13	16	11	17	9	7	10

Table 3. Percentage of total *Vibrios* present in fishes caught off Chennai coast.

Scientific name	Common name	Halophilic bacteria x $10^{-6}g^{-1}$	Vibrio x 10^{-5} g^{-1}	Percentage of Vibrios to total halophilic bacteria
Decapterus russelli	Russell's scad	51.0	11.0	21.57
Gerres filamentoses	Mojarras	1100	210	19.10
Lutjanus sp	Red snapper	512	193	37.70
Mugil cephalus	Grey mullet	8.4	2.6	30.95
Nemipterus japonicus	Thread fin bream	360	112	31.11
Selar crumenopthalmus	Big eye scad	374	101	27.01
Sardinella sp.	Sardine	4.7	1.8	38.30
Rastrelligar kanagurta	Indian mackerel	940	370	39.37
Sardinella longiceps	Oil sardine	600	202	33.67

scad, Mojarras, Red snapper, Grey mullet, Thread fin bream, Big eye scad, Sardine, Indian mackerel, Oil sardine. Table 3 shows the different species of *vibrios* and its percentage in the representative samples of fish. The year round study of *V. vulnificus* in the site 1 and site 2 throughout the year is given in Figure 1.

Our study shows that there is no positive relation between the type of fish species studied and the type of pathogen isolated. Among the different type of fish collected samples of *sardine* and *Selar crumenopthalmus* showed low prevalence of *Vibrio* species. This accounted for the variability of *V. vulnificus* due to factors like the location of the catch, diet, climatic conditions. Our results were comparable to the reports from the West coast of India (Thampuran and Surendran, 1998). Low level of Incidence of *V. vulnificus* in the fish body has not been reported (Oliver et al., 1982). Normally the geographical area, salinity and the temperature are responsible for cell density (Kelly, 1982). The *V. vulnificus* were localized in the fish intestine. The species of the *Vibrio* have been identified worldwide as major components of the intestinal flora of wild or cultured fish (Sera and Ishida, 1972). Most of the colonies in this study were identified as *V. mediterrenei* followed by, *V. campelli, V. alginolyticus* and *V. vulnificus*. Three key characteristics for distinguishing these species from *V. vulnificus* are the ONPG

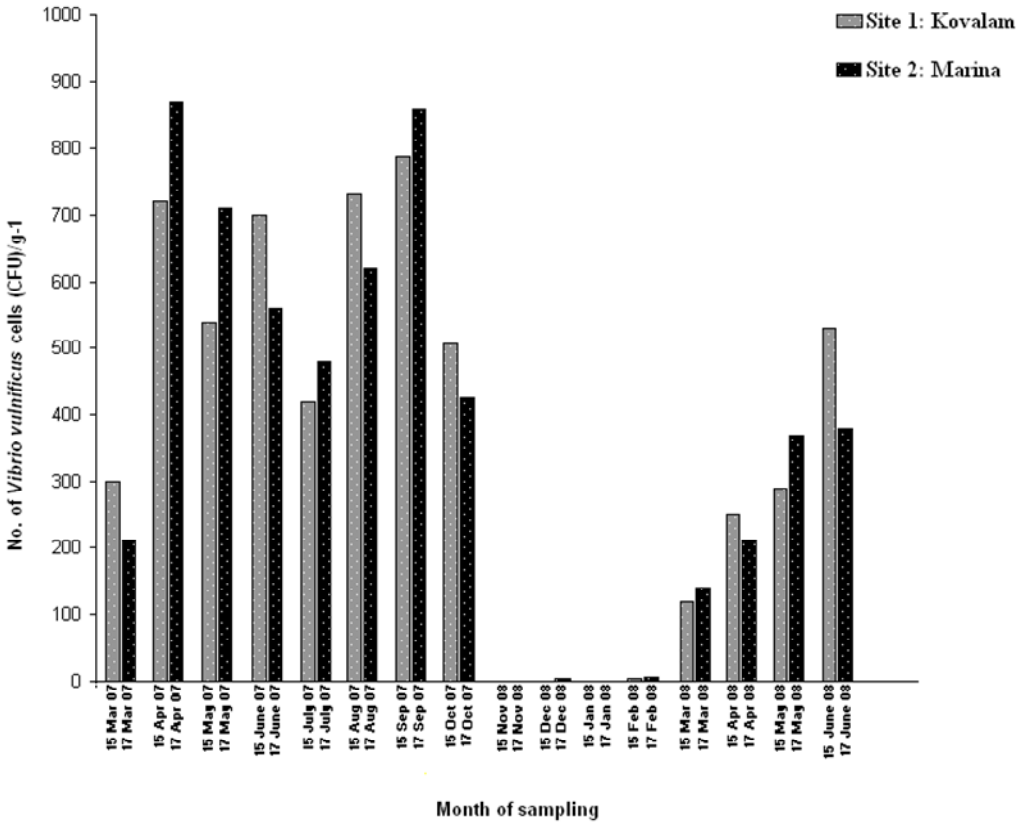

Figure 1. Year round study of *Vibrio vulnificus* in site 1 and 2 between March 2007 to June 2008 in marine fish samples by MPN technique.

reaction, lactose and sucrose fermentation.

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