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RESEARCH ARTICLE

PREVALENCE, DISTRIBUTION AND PHENOTYPIC IDENTIFICATION OF *VIBRIO* SP. IN FISHES CAUGHT OFF CHENNAI, INDIAN OCEAN

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ABSTRACT

Vibrio alginolyticus, Vibrioparahaemolyticus and *Vibrio harveyi* is a notorious seafood borne pathogen with a high mortality rate. This is ubiquitously present in marine environments, particularly in tropical water.in these studies the prevalence of vibrio sp 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 prevalence of vibrio Harvey constitutes about 14%, 17% and 8% of total vibrio isolated from fish. Other clinical Vibrios are also isolated and identified. Vibriosp were detected by conventional cultural and molecular methods using PCR and sequencing of 16SrDNA. This study is an initial step to provide a baseline for future molecular research targeting Vibrio spfood borne illness.

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INTRODUCTION

Seafood constitutes one of the tastiest growing sources of food. Billions of people throughout the world rely on fish as a primary source of protein, particularly in developing countries. Seafood is also the most important food commodity exported from developing countries. In the last two decades, there has been an increased awareness of the nutritional and health benefits of fish consumption. With increased fish consumption, there is also an increased in the number of food borne illness. Seafood is known to be responsible for a significant percentage of food borne disease worldwide. The genus *Vibrio* is the most diverse and abundant group of marine bacteria with 74 described species, and its taxonomy is under constant review due to the incorporation of genotypic and molecular analysis that show this genus to be highly heterogeneous (Ceccarelli *et al*., 2014; Thompson *et al*., 2004). The species of clinical importance are *V. cholera*, *V. parahaemolyticus*, *V. vulvificus*, *V. alginolyticus*, *V. fluvialis*, *V. mimicus*, *V. hollisae*, *V. damsela*, *V. furnissii*, *V. cincinnatiensis*, *V.harveyi*and *V. metschnikovii*. They are also species of ecological and probiotic importance, such as *V. fischeri*, *V. splendidus*, *V. halioticoli*, *V. mediterranei*and *V. rotiferranius* (Thompson *et al*., 2004; Asplund *et al*., 2011). This is a Gram negative, halophilic bacterium that is widely distributed in temperate and tropical coastal waters throughout the world in all varieties of fin fish and Shellfish (Deepanjali*et al*., 2005) and is recognized as important seafood borne pathogen throughout the globe. Of the seafood borne gastroenteritis cases in Japan, 70% are attributed to *V. parahaemolyticus* (Depaola*et al*., 1990) and in India, the organism accounts for about 3.5%-23.9% of gastroenteritis cases admitted to the infectious Disease Hospital in Kolkata (Pal *et al*., 1985). *V. alginolyticus*, *P.damselae* and *V.harveyi* are also opportunistic pathogens of economic significance in aquaculture, responsible for high mortality in cultured fish and Shellfish, sometimes destroying an entire aquaculture operation (Chatterjee and Halder, 2012).

Vibrio disease is described as vibriosis or bacterial disease, penaeid bacterial septicemia, penaeidvibriosis, luminescent vibriosis or red leg disease and is widely distributed. Signs of *Vibrio* disease include lethargy, tissue appendage necrosis, slow growth, slow larval metamorphism and body malformation, bolitasnigricans, bioluminescence, muscle opacity, melanization, empty midgut and anorexia (Gabriel Aguirre-Guzman *et al*., 2004). With bacterial septicemia, large numbers of bacteria have been observed in microscopic wet mount of the haemolymph. Necrosis and inflammation of organs (lymphoid organ, gills, heart, hepatopancreas, etc.,)

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granulomatous encapsulation, are also present. Malaria can be evaluated easily by normal histopathological methods (Lightner, 1996; Smith, 2000). Different extracellular products (ECP) with toxic effects on shrimp have been identified and characterized from a variety of *Vibrio* sp. and strains isolated from marine organism and the environment. These ECP have been proposed as virulence factors for shrimp and other marine organism. Extracellular products such as chitinases, hemolysins, alkaline proteases, cysteine proteases, alkaline metal chelator-sensitive proteases, serine proteases and metalloproteases have been isolated from cell free culture supernatants (CFS) of *Vibrio harveyi, Vibrio anguillarum, V. alginolyticus*and other species (Gabriel Aquirre-Guzmat*et al*., 2004).

Among pathogenic Vibrios that causes food borne illness, *V. parahaemolyticus*causes the highest number of seafood associated gastroenteritis in the United States and Asian countries (Mead *et al*., 1999), apart from few cases mediated by *V. alginolyticus* (SanthaSudha*et al*.,2012). However, not all of the environmental strains are considered to be pathogenic. The pathogenicity of *V.alginolyticus* in humans is associated with the production of thermostable direct haemolysin (TDH) and TDH related haemolysin (TRH) encoded by tdh and trh genes, respectively (Janda*et al*., 1998).Environmental gradients (temperature, salinity and nutrients) and biological factors influence the distribution and dynamics of *Vibrio* population (Takemura*et al*., 2014; Jay *et al*., 2009).As a representative of the halophilicvibrios, *V. alginolyticus* is isolated from coastal water and sediments all over the world (Chan *et al*., 1986; Eiler*et al*., 2006) and is considered to be part of the normal marine microflora. This bacterium also belongs to the most important opportunistic pathogens of aquatic animals, including fish, shellfish, crustaceans, coral and echinoids, causing serious disease and damage in cultured fish and important economic losses (Austin *et al*., 1993; Balcazar*et al*., 2010). Several virulence factors, including the iron uptake system (Litwin and Calderwood), extracellular haemolysin (Aguirre-Guzman *et al*., 2004) and proteases (Zhou *et al*., 2007) are suggested as the major contributions to pathogenicity in this species. By definition, *Vibrio harveyi*is a marine Gram negative luminous organism with a requirement for sodium chloride (Farmer *et al*., 2005). The organism was originally named as *Achromobacterharveyi*. As a result of 16S rRNA sequence analysis, *V. harveyi*is regarded as one of core species of the genus *Vibrio* (Dorsch*et al*., 1992). With the rapid development in aquaculture, particularly in Asia and South America, the organism has become recognized as a serious cause of disease, particularly of marine invertebrates, namely the economically penaeid shrimp (Austin and Zhang, 2006). It causes deep dermal lesions, gastro-enteritis, eye lesions, infectious necrotizing enteritis, vasculitis, skin ulcer and white spot on the foot in various fish species (Austin and Zhang, 2006). Despite its role as a serious pathogen of marine animals, the pathogenicity mechanisms of *V. harveyi* have yet to be fully elucidated. Extracellular products (ECP) have been considered to be important determinants of virulence in *V. harveyi* (Saeed, 1995).

Prevention and control of infections caused by *Vibrio* sp. pathogenic for humans depend on understanding their ecology, pathogenicity and modes of transmission. The contribution of climate change (in particular elevated air and surface watertemperature) and the increasing anthropogenic effects of tourism may increase the risk of emergence and spread of waterborne and food borne infections (UND programme, 2013).In light of these factors, the aim of this study was to conduct environmental surveillance to assess the abundance, diversity and phenotypic identification of *Vibrio* sp. along the Chennai coastal region.

MATERIALS AND METHODS

Seafood samples were collected from 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 sea cat fish (*Arius dussumieri*), red snapper (*Latescalcarifer*), sardine (*Sardinella* sp.), seafish (*Scomberomeruscommerson*), Barra cuda (*Sphyaena*), Malabar Trevally (*Caranzoidesmalabaricus*), crab (Brachyura), kiddi shrimp (*Parapenaeopsisstylifera*), Indian white prawn (*Penaeusindicus*), tiger prawn (*Penaeusmonodon*), white clam (*Villoritacyprinoides*), black clam (*Villoritacyproiindes*). These are the marine fishes used for the study. These samples are the most common varieties on the Chennai coast of Indian Ocean. These fish samples were collected for the period of twelve months from same place of two sites; one site was the Kovalam beach, Chennai coastal area where the samples were directly gotten from the fisherman. The second site was from the Mahabalipuram beach of fish landing centre. The fish samples were kept in icebox and immediately analyzed within an hour's. In fish samples, the whole part of the skin, gills and all the parts of intestine were used for *Vibrio* sp. 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 of *Vibrio* sp.

In FDA (Elliot *et al*., 1992) approved methods were used for the isolation and enumeration of *Vibrio* sp. 10 gm of 70% alcohol cleaned whole part of the fish samples was weighed and blended with 90 ml sterile phosphate buffered saline (PBS) with 2% NaCl (pH 7.5). Serial dilutions were made with phosphate buffered saline upto 10^{-7}. The dilutions 10^{-4}, 10^{-5} and 10^{-6} were plated in Trypton Soy Agar (TSA) and Thiosulfate Citrate-Bile salt Sucrose (TCBS) agar medium for isolation of total halophilic bacteria and *Vibrio* count respectively, the plates were incubated at 37°C for 24 hours. After 24 hours, the green and yellow color colonies were isolated and identified upto the species level by using biochemical test (Alsina and Blanch, 1994 a, b) as listed in Table 1 and 16s rDNA analysis, Phylogenic tree analysis.

Biochemical test

Presumptive *V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi* isolates were characterized by using standard methods (FDA, 1969). In addition, the following tests were performed for exoenzymes production; all strains were tested for lipase on a medium including Tween 80 and DNA hydrolysis (DNase agar, Himedia, Mumbai). The enzymes amylase, protease, caseinase and lecithinase were also detected on respective media prepared with phosphate buffer saline (PBS). After incubation upto 72 hours at 37°C, the formation of a clear zone caused by protein degradation is considered a positive test (Zanetti *et al*., 2000).

Kanagawa phenomenon

The ability to hemolyze erythrocytes was tested on Blood agar. Incubated media were incubated at 37°C for 24 hours and were examined visually for zones of hemolysis.

Antibiotic Susceptibility test

Susceptibility to several antimicrobial agents was determined using the Kirby-Bauer method and Mueller Hinton agar plates supplemented by 1% NaCl. The following antibiotics were selected for this study from previous reports (Benkahla-Nakbi *et al*., 2009; Snousi *et al*., 2006) including Amoxicillin, Ampicillin, Chloramphenicol Cephadroxil, Cefazolin, Ciprofloxacin, Erythromycin Gentamycin, Metronidazole, Lincomycin, Norfloxacin, Oxytetracyclin, Penicillin, Rifampicin, Streptomycin and Tetracycline. After 24 hours of incubation at 37°C, organism were classified as Sensitive (S), Intermediate (I) or Resistant (R) upon the diameters of inhibition zones obtained.

Identification of *Vibrio* sp. by PCR and sequencing of 16S rDNA

DNA extraction of *Vibrio* isolates

The procedure of DNA extraction of *Vibrio* isolates was done using the Hiper Bacterial Genomic DNA extraction kit (Cat# HTBM008, Himedia, Mumbai). Briefly a single colony of pure isolate was picked up from TCBS agar and inoculated into 5ml nutrient broth then incubated at 37°C. A total volume of 1-3 ml of bacterial culture was centrifuged at 10000 rpm for 2 min then supernatant was discarded. The pellet was then re-suspended by adding 100 µl of buffer. The re-suspended cells were centrifuged at 10000 rpm for 5 min then the supernatant was discarded completely. The protein pellet was denaturized by resuspension in 180 µl of lysis solution 1 and 20 µl of proteinase K, then incubated at 55°C for 30 min. Homogenization was achieved by adding 200 µl of lysis solution II and mix by inverting tube and incubation at 55°C for 10min. 200 µl of absolute ethanol was added with immediate mixing to prevent precipitation of DNA due to high ethanol centrifugation. The sample was transferred into the column and centrifuged at 10000 rpm for 1 min. The flow was discarded and the column was washed by 500 µl of wash buffer by centrifugation at 14000 rpm for 3 min. The flow was discarded the DNA was eluted in 200 µl of elution buffer, which left for 1 min at room temperature and then centrifuged at 10000 rpm for 1 min to elute the DNA (Cat# HTBM008, Himedia, Mumbai).

Amplification of 16S rDNA

Partial 16S rDNA was amplified using the universal oligonucleotide primers 27F (5'-AGAGTTTGATCCTG GCTCAG-3') and 1392R (5'–GGTTACCTTGTTACGACTT-3'). Briefly, 0.2 µg of genomic DNA was added to 25 µl Hi-Chrom PCR master mix (Cat# MBT089, Himedia, Mumbai). The mixture was then amplified in a DNA thermal cycler using the following program: one denaturation step at 94°C for 5 min; 35 cycles of denaturation, 92°C for 30 s, annealing temperature for 30 s at 55°C, extension at 68°C for 60s; and a finalextension at 72°C for 10 min.The PCR products were electrophoresed in 2% agarose gel (Himedia, Mumbai) incorporated with nucleic acid gel stain at voltage 100 volt for 1 hour. The gel was phylographed with gel documentation system with UV- trans-illuminator.

DNA sequencing and analysis

Molecular identification of the isolated strains was carried out based on 16S rDNA sequence analysis. The sequences of the 16S rDNA PCR amplicon from isolates were determined. Alignment identity of their sequence was compared with some other strains. Sequence showed more than 99% identity with the sequence of 16S ribosomal RNA gene of *V. alginolyticus, V. parahaemolyticus* (not shown) and *V. harveyi* strains. A phylogenetic tree was constructed using neighbor joining methods, and it shown in Figure 1 & 2. Therefore, the isolated strains were identified as *V. alginolyticus, V. parahaemolyticus* and *V. harveyi* based on their morphological, cultural, physiological and biochemical characteristics and finally 16S rDNA sequence analyses.

RESULTS AND DISCUSSION

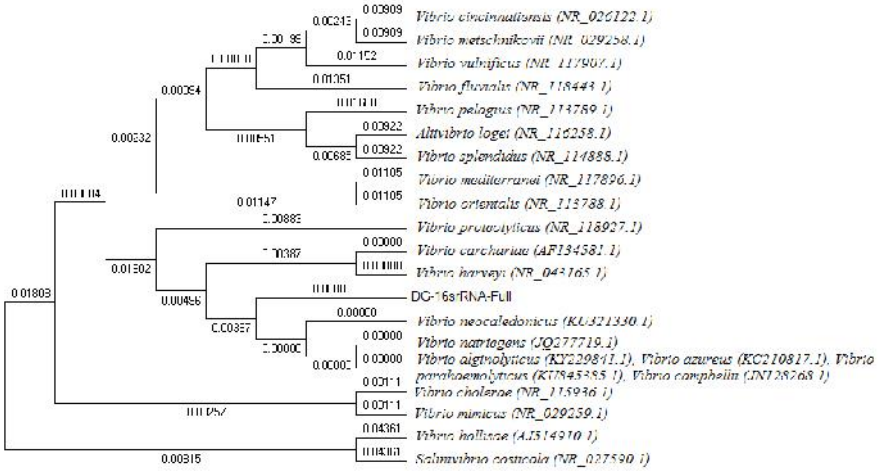
The incidence of the *V.alginolyticus, V.parahaemolyticus* and *V. harveyi* in marine fish samples collected freshly from fish landing centers and among which 14% of *V.alginolyticus*,17% of *V.parahaemolyticus* and 8% of *V.harveyi* prevalence was observed from fishes in and around Chennai coast. The results indicate that the incidence of Vibrio sp 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 of the presence of vibrio in the fish sample. From previous studies and according to literature, the prevalence of pathogenic vibrios appears to be influenced by two main physicochemical environmental factors. Firstly, temperature has a marked influence on the occurrence of vibrios. The seasonal variation and cycle are considered to correlate with water temperature that is a major factor affecting the abundance of *V. parahamolyticus* and resulting in the emergence of more virulent serotype and thereby increases opportunities for outbreak of food borne illness, which are a cause for concern for the seafood industry (Panicker *et al*., 2004). Secondly, seawater salinity exerts a strong influence on the survival of *Vibrio* sp. Low salinity may favour *V. vulvificus* growth in shellfish, while *V.parahaemolyticus* tolerates higher salinity value (Wright *et al*., 2007).

Table 1. Morphological and biochemical characteristics of *Vibrio* sp. isolated from fish samples

Biochemical test	*V. alginolyticus*	*V. parahaemolyticus*	*V. harveyi*
Gram stain	-	-	-
Growth on TCBS	Y	G	LG
Motility	+	+	+
Vogesproskauer	+	-	V
Arginine	-	-	-
Salt tolerance (1%)	+	+	+
ONPG	-	-	-
Citrate utilization	+	+	+
Ornithine decarboxylase	V	+	-
Carbohydrate fermentation			
Mannitol	+	+	V
Arabinose	-	V	-
Sucrose	+	-	V
Glucose	+	+	V
Salicin	-	-	-
Cellobiose	-	-	V
Salinity to 0/129 (100 µg)	+	+	+
0/129 (150 µg)	+	+	+
Growth at 4°C	-	-	-
Growth at 42°C	-	-	-

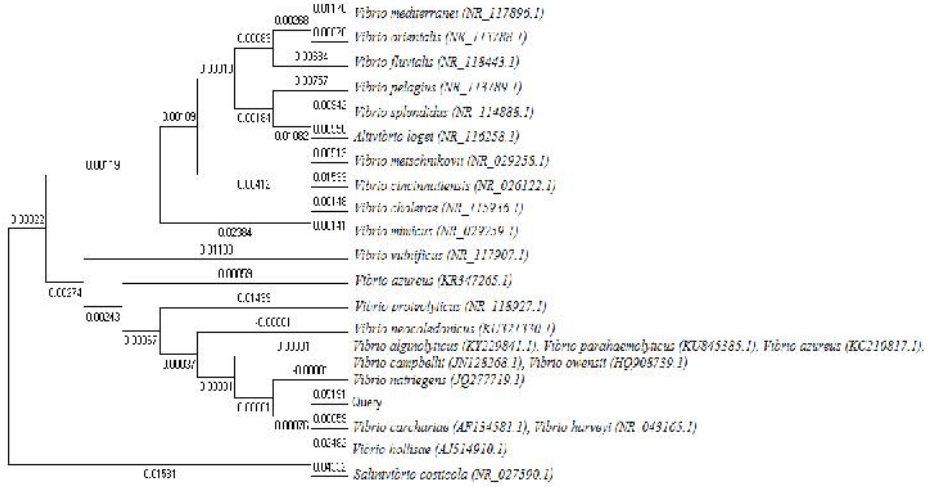
Y-yellow, V-variable, G-green, LG-light green, +-positive, -negative. The species identified were *V. alginolyticus, V.parahaemolyticus,V.harveyi,V.vulificus,V.cincinnatiensis,V.orientalis,V.mediterranei*and*V.logei* in site 1 and site 2 during the study period of March 2015 to June 2016.During the study period of November 2015 to January 2016 densities of Vibrio sp was found very low.

In summer from April 2015 to October 2015 Vibrio sp density were considerably higher. Hussein *et al.*, found only *V. parahaemolyticus* in 2.1% of the examined shrimp samples by PCR. Yang *et al.*, has previously reported that 14.9% of frozen and iced seafood samples were contaminated with *V. parahaemolyticus.* The high prevalence of *Vibrio* sp. in the examined samples could be due to temperature abuse.



DG- *V. alginolyticus*

Fig.1. Phylogenetic tree for the conformation of *V. alginolyticus*



Query- *V. harveyi*

Fig. 2. Phylogenetic tree for the conformation of *V. harveyi*

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

Fish (No. of fishes sampled)	No. of isolates	*V. alginolyticus*	*V. parahaemolyticus*	*V. harveyi*	*V. vulvificus*	*V. cincinnatiensis*	*V. orientalis*	*V. mediterranei*	*V. logei*
Aricusdussumieri	60	14	16	4	8	11	6	3	2
Latescalcrifer	71	16	18	6	11	8	7	6	2
*Sardinella*sp.	37	4	6	2	4	6	5	4	3
Scomberomeruscommerson	40	5	5	3	6	4	4	8	4
Sphyraena	60	5	8	1	3	3	3	5	2
Carangoidesmalabaricus	35	3	4	4	2	3	5	8	4
Brachura	28	2	3	3	4	4	2	4	5
Parapenaeopsisstylifera	38	2	4	7	3	2	1	4	6
Penaeusindicus	42	4	4	4	2	4	2	5	4
Penaeusmonodon	28	3	3	3	1	3	2	8	2
Villoritacyprinoides	42	8	12	2	4	2	1	6	2
Total	481	66	83	39	48	38	38	49	36
% of each sample		14	17	8	10	8	8	10	7

The short generation time of 12 minutes for *V. parahaemolyticus* permit the organisms to accumulate in millions in a few hours. Most members *Vibrio* sp. are halophilic and the addition of NaCl is often required for enzymatic activity; however, the concentration of NaCl can affect the biochemical profile and lead to erroneous identification with at least one system (Martinez-Urtaza *et al*., 2006). For these reasons, more specific, rapid and sensitive molecular methods for *Vibrio* sp. identification are needed. Adehayo-Tayo *et al*. (2011) reported the distribution and frequency of occurrence of *Vibrio* sp. isolates from seafood samples. Among the *Vibrio* sp. isolated *V. cholera* was the most predominant 25/53 (47.2%), this was followed by *Vibrio parahaemolyticus* 10/53 (18.9%), *Vibrio mimicus* 8/53 (15.1%), *Vibrio fluvicalis* 7/53 (13.2%) and *Vibrio alginolyticus*2/53 (3.8%) while *Vibrio vulvificus* was the least predominant 1/53 (1.9%). In the present study *Vibrio alginolyticus, V, parahaemolyticus* and *V. harveyi* occurred in the seafoods. This is in agreement with the studies of Gopal *et al.* (2005) and Colakogu *et al.* (2006). The study by Gopal*et al*., (2005) revealed the dominance of *V. alginolyticus*, followed by *V. parahaemolyticus* in East and West coast samples. Several reports associated *Vibrio* hemolysins (*V. vulvificus, V. parahaemolyticus* and *V. harvyi*) with shrimp disease. The hemolysins appeared to have diverged into three families, one of the thermolabilehemolysins (eg. VhhB gene of vha-Hn from *V. harveyi*), one of six thermostablehemolysins, three from *V. parahaemolyticus* (i.e., Vpa-H, Vpa-H1 and Vpa-HII) and one from *V.mimicus* (Vmi-H) and one a putative hemolysin of *V. vulvificus* (Vvu-H), recently identified as a homologus and cobalt transport protein in *V. cholera* (Gabriel Aguirre-Guzman *et al*., 2004). Vibrios are responsible for a number of clinical conditions such as cholera, gastroenteritis, and septicemia and wound infections (Jay *et al*., 2005; Oliver and Kapoor, 1997; Thompson *et al*., 2004). Twelve *Vibrio* sp. have been documented as potential food borne disease agents in humans: *V. cholerae, V. parahaemolyticus, V. vulvificus, V. alginolyticus, V. funissii, V. fluviales, V. dansela, V. mimicus, V. hollisae, V.cincinatiencis, V. harveyi*and *V. metchnikovii* (Adams and Moss, 2008; ICMSF, 1996; Thompson and Swing, 2006).

Table 3. Drug sensitivity of various bacteria isolated from site 1 and 2 off Chennai coastal region

Antibiotic	*V. alginolyticus*	*V. parahaemolyticus*	*V. harveyi*
Amoxicillin	R	R	R
Ampicillin	R	R	R
Cephadroxil	S	I	R
Cefazolin	R	R	R
Chloramphenicol	S	S	R
Ciprofloxacin	S	S	S
Clotrimazole	I	R	R
Erythromycin	I	R	R
Gentamycin	I	R	I
Metronidazole	R	R	R
Norfloxacin	S	S	S
Oxytetracycline	I	I	S
Penicillin G	R	R	R
Rifampicin	R	R	R
Streptomycin	R	R	R
Tetracycline	I	I	I

R=Resistant; I=Intermediate; S= Sensitive

The marine fishes which showed the presence of Vibrio sp were *Aricusdussumieri, Latescalcrifer, Sardinella* sp, *Scomberomeruscommerson, Sphyraena, Carangoidesmalabaricus, Brachura, Parapenaeopsi, stylifera, Penaeusindicus, Penaeusmonodon* and *Villoritacyprinoides.* 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 *Brachura*sp and *Penaeusmonodon* showed low prevalence of vibrio species, our results were comparable to the reports from the bay of Bangal coastal region, India (Rajapandian *et al*., 2009). *Vibrio* sp. are transmitted to humans mostly via sewage contaminated water or seafood – when consumed raw or partially cooked (De Paola *et al*., 2000; ICMSF, 1996; Oliver and Kapoor, 1997). Though *Vibrio* sp. has been isolated from marine environments, poor processing practices are reported as the major cause of the food contamination (Kaysner *et al*., 1992). The bacteria may persist in the food depending on storage temperatures, pH and the product water activity (ICMSF, 1996) until the food is consumed, thereby causing disease. Pathogenic *Vibrio* sp. are a health concern especially in fish harvested from poor quality waters (ICMSF, 1986). *Vibrioalginolyticus* is largely opportunistic pathogen causing systemic infections in persons with underlying diseases such as the immune-compromised individuals, those with severe burns, cancersor with a history of alcohol abuse (Oliver and Kapoor, 1997), though it has occasionally been associated with cases of gastroenteritis and diarrhea. In healthy individuals *V. alginolyticus* is associated with extra intestinal infections such as wound or ear infections (Novotny *et al*., 2004). *V. alginolyticus* is also important food spoilage organism producing histamine by the decarboxylation of histidine and is responsible for scombroid poisoning characterized by nausea, vomiting, abdominal cramps, neurological disorders and skin irritations (Novotny *et al*., 2004; Ray and Bhunia, 2008). *V. alginolyticus*is most commonly isolated *Vibrio* sp. in marine environments from all over the world. It has been isolated from both fin fish and shell fish (Oliver and Kaper, 1997). Pinto *et al.* (2006) analyzed 38 shellfish samples and detected *V. alginolyticus* from 76% of the samples while only 42% of their samples were positive for *V. parahaemolyticus*. Pathogen strains of *V. alginolyticus* carry the collagenase and TOXR genes and can be identified through detection of these genes (Cai *et al*., 2009). *V. alginolyticus* is associated with white spot in shrimp in India and Taiwan while the zoonotic hazard of this pathogen has been implicated in ear, soft tissue and wound infection in human (Merwad *et al*., 2011; Horii *et al*., 2005). Of the *Vibrio* sp. described in Bergey's manual of Systemic Bacteriology, *V. alginolyticus, V. anguilarum, V. parahaemolyticus* and *V. harveyi* has been described as pathogenic to paeneid shrimp (Lightner, 1983; Takahashi *et al*., 1985). Due to the economic importance of *V. harveyi* infection, there is considerable interest in methods to identify the type and track *V. harveyi* related populations associated with marine reared animals. Identification of *V. harveyi* strains can be a challenging task some species within the Harveyi Clade (*V. harveyi, V. campbelli, V. alginolyticus, V. rotiferianus, V. parahaemolyticus, V. mytili* and *V. natriegens*) have a high degree of both genetic and phenotypic similarity (Sanger *et al*., 1977). In the case of *V. harveyi*, it is often difficult to resolve this species from other species of the *Vibrio* core group (*V. alginolyticus, V. campbelli, V. parahaemolyticus* and *V. rotiferianus*) based solely on 16S rDNA gene heterogenicity. For instance, the species *V. harveyi, V. campbelli* and *V. rotiferianus* have more than 99% sequence identity of the 16S rDNA gene (Vandenbergh*et al*., 2003).The pathogenicity of *V. harveyi* may be attributed to extracellular products (ECPs) which were harmful to fish. Both pathogenic and non-pathogenic cultures produced ECPs

containing caseinase, gelaniase, phospholipase, lipase and hemolysins (Liu *et al*., 1997; Zhang and Austin, 2000).

Drug sensitivity studies revealed all the bacterial isolates to be sensitive to Norfloxacin and Ciprofloxacin and resistant to Ampicillin, Metronidazole, Cefazolin.*V.alginolyticus* showed maximum sensitivity to Norfloxin and Ciprofloxacin (Table 3). One of the major risks involves the consumption of raw or undercooked seafood's that may be contaminated by food borne pathogens present in the marine retail markets. such risks are further increase if the food is mishandle during handling, slaughter, transportation and processing where pathogens could multiply exponentially under favorable conditions (Oliver and Kaper, 1997). In contrast to most other food borne pathogens Vibrio sp utilize aquatichabitats as their natural niche (Oliver and Kaper, 1997; Reidle and Klose, 2002). As a results Vibrio sp are commonly associated with polluted water, seafood and other aquatic animals as the main source of contamination. Food born infection with vibrio sp are common in coastal cities where retail markets are close to the sea basin (Rebaudet et al., 2013). Finally it is empirical to mention that the identity of the retrieved *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* where presumptively identified using morphological characteristics extracted from morphological characteristics on the selective TCBS agar medium. All isolates matched the standard morphological criteria previously established (Alsina and Blanch, 1994; Perilla *et al*., 2003; Austin and Austin, 2012). Molecular conformation of the retrieved vibrio isolates was done using partial amplification of 16S r DNA using the universal oligonucleotides.

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