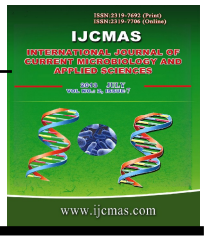
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 7 (2013) pp. 217-232**
http://www.ijcmas.com



Original Research Article

Isolation and identification of Vibrios from marine food resources

K. Arunagiri[1,2*], K. Jayashree[2] and T. Sivakumar[2]

[1]Research and Development Centre, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India
[2]Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kancheepuram, Tamil Nadu, India
***Corresponding author e-mail:** arunagirikumar01@gmail.com*

Keywords

Vibrio spp; sea food samples; Antibiotic resistant pattern; hemolytic reaction.

A B S T R A C T

In the present study encompasses the incidence of *Vibrio* spp. in sea foods as a human pathogen, in contribute to the onset of sporadic and epidemic outbreaks of diarrhoeal disease in humans. The following aspects were covered during the present investigation. Isolation of *Vibrio* spp. in sea food samples collected from Kanchipuram and Walajapet local fish market. Antibiotic resistant pattern of isolated *Vibrio* spp. Hemolytic activity of randomly selected in *Vibrio* spp. High lights of the pattern investigations are, *Vibrio* spp. was readily recoverable in all the 21 samples tested *viz*., Finfishes and crustaceans. The entire biochemical test was carried out to identify the *Vibrio* spp. Antibiotic resistant pattern was observed in co-trimoxzole, amikacin, Gentamycin, Oxytetracycline were performed to find out antibiotic sensitivity pattern. A total of 21 strains were tested for hemolytic reaction 15 strains were positive for hemolytic production in blood agar plates and the remaining 6 strains were failed to lyses blood erythrocytes.

Introduction

Members of the *Vibrio* genus are gram-negative, halophilic bacteria indigenous to coastal marine systems (Thompson *et al.*, 2003). While these common bacteria persist as a natural component of the marine microbial flora, a small percentage of environmental isolates carry the genetic determinants for human pathogenesis (Nishibuchi and Kaper, 1995; Chakraborty *et al.*, 2000; Rivera *et al.*, 2001). Currently, *Vibrio* infections are the leading cause of seafood - borne bacterial gastroenteritis in the United States and together, *Vibrio cholera*, *V. parahaemolyticus* and *V. vulnificus* account for the majority of those infections (Mead *et al*., 1999). Among the 4,754 *Vibrio* infections reported to the Centers for Disease Control and Prevention (CDC) from 1997-2006, 3,544 (75%) of those infections were foodborne in origin and 1,210 (25%) of those infections were non-foodborne in origin (Dechet *et al*., 2008).

The association of Vibrios with planktonic organisms, especially copepods, has been suggested as an important component of *Vibrio* ecology, especially for *V. cholerae* (Sochard *et al.*, 1979; Huq *et al.*, 1983; Huq *et al.*, 2005). Plankton represent organic-rich microenvironments (Grossart *et al.*, 2005), and the high nutrient concentrations of the plankton microhabitat can selectively enrich heterotrophic bacteria, including vibrios (Huq *et al.*, 1983; Tamplin *et al.*, 1990; Lipp *et al.*, 2003; Long *et al.*, 2005).

The characterization of *Vibrio spp.* isolated from cultured fish or marine water (Zorrilla *et al.*, 2000). It is a ubiquitous organism of the saprophytic micro biota. *Vibrio* spp. is usually isolated in the spring and summer from marine sources. i.e.: depend on water temperature greater than 10˚C. *V. alginolyticus* as well as *V. parahaemolyticus* should be considered in tissues infections exposure to ocean water. The present study has therefore been under taken in the following objectives; to isolate and identify potentially pathogenic *Vibrio* species from sea foods, to know the resistant pattern of isolated Vibrios against various antibiotics, to find out the hemolytic activity of randomly selected isolates and to study ecological aspects of *Vibrios*.

Materials and methods

Collection of seafood samples

Totally, 20 different sea food samples such as Finfishes and crustaceans (shrimp and crab) were collected from different sites in Kanchipuram, and Walaja fish market. All sea food samples were transported in individually labeled and sealed new plastics bags to avoid contamination. The samples were placed in sealed containers with dry ice and transported frozen to the laboratory for bacterial analysis. The time between sample collection and analysis was approximately 24 hours.

Sample processing for bacteriological examination

Isolation of *Vibrio* spp. from sea foods

Finfish and crustaceans were washed thoroughly with sterile distilled water prior to bacteriological examination. The heads and tails of the fishes were cut into small pieces using sterile scissors and the guts were removed. The Crustaceans and finfish samples were then homogenized in blenders, and 25g of each homogenate was placed in 225ml of alkaline peptone water (APW) pH 8.6, and incubated at 37˚C for 24 hours. At the end of incubation period, two loopful of culture from pellicle of each flask (Enrichment broth APW) were then streaked on to Thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubated at 37˚C for 24 hours.

Identification of *Vibrio* spp.

Additional characterization tests included Gram staining, motility test, Biochemical test, catalase, cytochrome oxidase activity test, Triple sugar iron test, ornithine, Arginine, lysine, valine, leucine, dehydrolase test, Nitrate reduction test, Gelatin hydrolysis test, starch hydrolysis test, and glucose, lactose, mannitol, maltose and sucrose fermentation test were carried out.

Antibiotic resistant pattern

Vibrio spp. all strains were isolated from seafood samples were grown in nutrient broth containing 2% NaCl. Muller-Hinton (MHA) agar medium (Hi media) was used for antibiotic resistances pattern. Gentamycin, Co-Trimoxazole, Bacitracin,

Amikacin, and Oxytetracycline were usder and the 24 hours broth culture of *Vibrio* spp. were swabbed on MHA agar plates and the disc were placed by using alcohol dipped and flamed forceps on the surface of MHA agar medium. All the plates were lifted for 20 minutes and then incubated in an inverted position at 37˚C for 18-24 hours. The results were recorded by measuring the zone of inhibition. Then Kirby – Bauer method (agar diffusion method) was followed to determine the susceptibility of MRSA to antibiotics.

Haemolytic activity

Vibrio spp. strain to produce hemolysin was tested on blood agar supplemented with 5% sheep anticoagulant blood. All the selected cultures were seeded in blood agar plates, and the plates were incubated at 37˚C for 24hours. Hemolytic activity was determined (Kishishita *et al.*, 1998).

Ecological aspects of *Vibrio* spp.

Effect of pH on bacterial growth

All the bacteria were inoculated into Nutrient broth containing different pH ranges (6, 7, 8, 9, 10) and incubated at 37°C. After incubation, the optical density was measured at 600 nm.

Effect of Temperature on bacterial growth

All the bacterial were inoculated into Nutrient broth (NB) broth and the tubes were incubated at different temperature range (20, 30, 40, 50 and 60°C) and incubated at 37°C. After incubation, the optical density was measured at 600 nm.

Effect of Salinity on bacterial growth

All the bacterial were inoculated into Nutrient broth containing different salinity ranges such as 5, 10, 15, 20, 25% and incubated at 37°C. After incubation, the optical density was measured at 600 nm.

Effect of Carbon and Nitrogen Sources on bacterial growth

All the bacterial strains were inoculated into Nutrient broth (NB) broth containing different carbon sources (Glucose, Sucrose, Lactose) and incubated at 37°C.. After incubation, the Optical density was measured at 600 nm.

Effect of Heavy Mineral sources on Bacterial growth

All the bacterial strains were inoculated into Nutrient broth (NB) broth containing different mineral sources (Magnesium sulphate, Calcium chloride and Zinc sulphate) and incubated at 37°C. After incubation, the Optical density was measured at 600 nm.

Result and Discussion

Isolation of *Vibrio* spp. from the samples

A total number of 20 sea food samples (Finfishes and crustaceans) were collected from Kanchipuram and Walajapet fish market. Result revealed that, totally 21 species of *Vibrio* spp. were isolated and identified from finfish and other marine samples based on the staining and biochemical test (Table.1). In this, 19 species of *Vibrio* spp. were isolated from fish samples and each one from crab and prawn samples (Table.2).

Table.1 Identification of Marine isolates of *Vibrio* spp.

S.No	Morphology and Biochemical test	Results
1.	Gram staining	Gram Negative rods
2.	Motility	Motile
3.	Indole	+Ve
4.	Methyl Red	+Ve
5.	Voges proskauer	+Ve
6.	Citrate Utilization test	+Ve
7.	Triple sugar iron agar	Acid/Acid, No gas, and H2S
8.	Catalase	+Ve
9.	Oxidase	+Ve
10.	Nitrate reduction test	+Ve
11.	Gelatin hydrolysis test	+Ve
12.	Starch hydrolysis test	+Ve
13.	Carbohydrate Fermentation test	
	Glucose	+Ve
	Maltose	+Ve
	Mannitol	+Ve
	Sucrose	+Ve
	Lactose	-Ve
14.	Amino acid decarboxylase test	
	Arginine	-Ve
	Lysine	+Ve
	Ornithine	+Ve
	Leucine	-Ve
	Valine	-Ve
15.	Trypticase soy agar	+Ve
16.	Halophilisms test	
	0%NaCl	-Ve
	1%NaCl	+Ve
	3%NaCl	+Ve
	6%NaCl	+Ve
	8%NaCl	+Ve
	10%NaCl	+Ve

+ = Positive, - = Negative

Table.2 Isolation and Identification of Marie isolates of *Vibrio* spp.

S.No	Name of the *Vibrio* spp.	Sources isolated
1	V. *alginolyticus*	Fish
2	*Vibrio* sp.1	Fish
3	V. *campbellii*	Fish
4	V. *cincinnatiensis*	Fish
5	V. *ctioteree*	Fish
6	V. *costicote*	Fish
7	V. *fumissii*	Fish
8	V. *harveyi*	Fish
9	V.*logei*	Fish, Crab
10	V. *mediterranei*	Fish, Prawn
11	V. *metschnikovii*	Fish
12	V. *mimicus*	Fish
13	V. *netreiqens*	Fish
14	V. *orientalis*	Fish
15	V.*parahaemolyticus*	Fish
16	V. *pelagius*	Fish
17	V. *proteolyticus*	Fish
18	*Vibrio* sp.2.	Fish
19	V. *splendidus*	Fish
20	*Vibrio* sp.3	Fish
21	V. *vulnificus*	Fish

Antibiotic resistant pattern

The pattern of resistance to various antimicrobial substances in *Vibrio* spp. isolated from sea foods samples is given in (Table 3).Among the strains tested against 5 antibiotics resistant pattern was observed. In co-trimoxazole antibiotic, the maximum zone of clearance was observed in *V. campbellii* with represented by 31mm and minimum inhibitory activity was observed in *V. logei* with 13 mm.
In Gentamycin antibiotic, the maximum zone of clearance was observed in *V. harveyi* with represented by 23mm and minimum inhibitory activity was observed in *Vibrio* sp.1 with 14 mm. In Amikacin antibiotic, the maximum zone of clearance was observed in *V. campbellii* with represented by 23mm and minimum inhibitory activity was observed in *V. splendidus* with 13 mm. In oxy tetracycline antibiotic, the maximum zone of clearance was observed in *V. metaschinikovii* with represented by 19 and minimum inhibitory activity was observed in *V. parahaemolyticus* with 5 mm.

Hemolytic activity

Among the 21 strains tested, 15strains (80%) showed positive hemolytic activity on blood agar plates and remaining 6 strains were non-haemolytic activity were observed. (Table. 4)

Ecological aspects of *Vibrio* spp.

Effect of temperature on the growth

In this study, the maximum growth was observed in temperature range of 37ºC after incubation. In this temperature study, *V.campbllii* showed maximum growth with 0.65 (optical density). Minimum growth rate was observed *in V. splendidus* with 0.8. (Table 5)**.**

Effect of pH on the growth

In this study, the maximum growth was observed in pH 8 after incubation. In this pH, showed maximum growth in *Vibrio* sp.2 with 0.96 (optical density). Minimum growth rate was observed in *V.mimicus* (0.01) (Table 6)**.**

Effect of salinity on the growth

In this study, the maximum growth (optical density) was observed in salinity after incubation. In this salinity study, showed maximum growth in *V.vulnificus* (1.02). Minimum growth rate was observed in *V.parahemolyticus* (0.02) (Table 7)**.**

Effect of carbon sources on the growth

In this study, the maximum growth was observed after incubation. In this study, showed maximum growth in *V.metschnikov*i with 1.48 (OD). Minimum growth rate was observed in *V.alginolyticus* with 0.08 (OD) (Table 8)**.**

Effect of mineral sources on the growth

In this study, showed maximum growth in *V.metschnikov*i with 1.23 (OD). Minimum growth rate was observed in *V.pelagius* with 0.07 (OD) (Table 9)**.**

Discussion

In the present study, the several species of finfishes and crustaceans were collected from the market found to harbour Vibrio. From available reports, it is well known

that sea foods were found to be they carrier of Vibrios and the ubiquitous

Table.3 Antibiotic sensitivity pattern of bacterial pathogens

Name of the *Vibrio* spp.	**Co-Trimaxazole**	**Gentamycin**	**Amikacin**	**Oxy Tetrcycline**
V. *alginolyticus*	24mm	14mm	14mm	13mm
Vibrio sp.1	2mm	1.4mm	2.1mm	16mm
V. *campbellii*	31mm	18mm	25mm	-
V. *cincinnatiensis*	20mm	16mm	16mm	-
V. *ctioteree*	-	20mm	20mm	-
V. *costicote*	20mm	15mm	19mm	-
V. *fumissii*	16mm	19mm	22mm	14mm
V. *harveyi*	20mm	23mm	25mm	10mm
V.*logei*	1.3mm	2mm	23mm	10mm
V. *mediterranei*	10mm	12mm	18mm	16mm
V. *metschnikovii*	14mm	9mm	15mm	19mm
V. *mimicus*	13mm	13mm	20mm	15mm
V. *netreiqens*	22mm	19mm	25mm	12mm
V. *orientalis*	5mm	2mm	22mm	14mm
V.*parahaemolyticus*	12mm	14mm	19mm	5mm
V. *pelagius*	19mm	22mm	23mm	14mm
V. *proteolyticus*	15mm	15mm	16mm	-
Vibrio sp.2	16mm	14mm	13mm	11mm
V. *splendidus*	12mm	16mm	19mm	-
Vibrio sp.3	12mm	12mm	1.3mm	11mm
V. *vulnificus*	2mm	13mm	15mm	14mm

Table.4 Haemolytic activity of Marine *Vibrio* spp

S.No	Name of the *Vibrio* spp.	Heamolytic activity
1	V. *alginolyticus*	Negative
2	*Vibrio* sp.1	Alpha haemolysis
3	V. *campbellii*	Negative
4	V. *cincinnatiensis*	Gamma haemolysis
5	V. *ctioteree*	Negative
6	V. *costicote*	Beta haemolysis
7	V. *fumis.ii*	Gamma haemolysis
8	V. *harveyi*	Gamma haemolysis
9	V.*logei*	Gamma haemolysis
10	V. *mediterranei*	Negative
11	V. *metschnikovii*	Negative
12	V. *mimicus*	Beta haemolysis
13	V. *netreiqens*	Gamma haemolysis
14	V. *orientalis*	Gamma haemolysis
15	V.*parahaemolyticus*	Alpha haemolysis
16	V. *pelagius*	Positive
17	V. *proteolyticus*	Gamma haemolysis
18	*Vibrio* sp.2.	Negative
19	V. *splendidus*	Gamma haemolysis
20	*Vibrio* sp.3	Gamma haemolysis
21	V. *vulnificus*	Beta haemolysis

Table.5 Effect of different temperature on *Vibrio* spp. growth
(The Values are measured in Optical Density)

S.No	Name of the *Vibrio* spp.	20°C	30°C	40°C	50°C	60°C
1	V. *alginolyticus*	0.24	0.18	0.22	0.19	0.24
2	*Vibrio* sp.1	0.23	0.27	0.15	0.25	0.39
3	V. *campbellii*	0.22	0.32	0.65	0.36	0.8
4	V. *cincinnatiensis*	0.19	0.36	0.42	0.19	0.12
5	V. *ctioteree*	0.25	0.33	0.10	0.25	0.22
6	V. *costicote*	0.16	0.30	0.41	0.35	0.34
7	V. *fumissii*	0.35	0.26	0.42	0.29	1.8
8	V. *harveyi*	0.43	0.24	0.63	0.48	0.38
9	V.*logei*	0.58	0.30	0.27	0.38	0.39
10	V. *mediterranei*	0.25	0.35	0.45	0.27	0.64
11	V. *metschnikovii*	0.25	0.35	0.38	0.42	0.22
12	V. *mimicus*	0.24	0.29	0.26	0.10	1.32
13	V. *netreiqens*	0.26	0.33	0.0	0.40	1.38
14	V. *orientalis*	0.18	0.27	0.20	0.40	0.26
15	V.*parahaemolyticus*	0.18	0.25	0.24	0.32	0.58
16	V. *pelagius*	0.19	0.48	0.36	1.19	0.16
17	V. *proteolyticus*	0.17	1.47	0.35	1.26	1.19
18	*Vibrio* sp.2.	0.19	1.35	0.47	0.9	0.33
19	V. *splendidus*	0.20	0.32	0.14	0.32	0.41
20	*Vibrio* sp.3	0.20	0.31	0.8	0.16	0.10
21	V. *vulnificus*	0.19	0.23	0.45	0.24	0.20

Table.6 Effect of different pH on Vibrio spp. growth
(The Values are measured in Optical Density)

S.No	Name of the *Vibrio* spp.	6	7	8	9	10
1	V. *alginolyticus*	0.68	0.53	0.61	0.61	0.10
2	*Vibrio* sp.1	0.84	0.56	0.55	0.50	0.15
3	V. *campbellii*	0.74	0.73	0.90	0.79	0.27
4	V. *cincinnatiensis*	0.24	0.45	0.84	0.70	0.27
5	V. *ctioteree*	0.84	0.75	0.87	0.80	0.07
6	V. *costicote*	0.60	0.70	0.84	0.77	0.23
7	V. *fumissii*	0.78	0.65	0.76	0.58	0.05
8	V. *harveyi*	0.58	0.60	0.85	0.94	0.17
9	V.*logei*	0.79	0.69	0.83	0.76	0.07
10	V. *mediterranei*	0.97	0.75	0.91	0.75	0.10
11	V. *metschnikovii*	0.54	0.61	0.90	0.65	0.21
12	V. *mimicus*	0.01	0.43	0.58	0.56	0.06
13	V. *netreiqens*	0.79	0.76	0.85	0.82	0.08
14	V. *orientalis*	0.56	0.73	0.77	0.80	0.07
15	V.*parahaemolyticus*	0.00	0.29	0.38	0.32	0.03
16	V. *pelagius*	0.93	0.70	0.91	0.81	0.17
17	V. *proteolyticus*	0.94	0.67	0.91	0.67	0.10
18	*Vibrio* sp.2.	1.02	0.77	0.96	0.89	0.10
19	V. *splendidus*	0.69	0.67	0.95	0.77	0.13
20	*Vibrio* sp.3	0.73	0.76	0.84	0.80	0.12
21	V. *vulnificus*	0.56	0.78	0.94	0.86	0.23

Table.7 Effect of different Salinity on Vibrio spp. growth
(The Values are measured in Optical Density)

S.No	Name of the *Vibrio* spp.	5%	10%	15%	20%	25%
1	V. *alginolyticus*	0.53	0.75	0.19	0.32	0.46
2	*Vibrio* sp.1	0.73	0.70	0.60	0.29	0.57
3	V. *campbellii*	0.90	0.69	0.82	0.64	0.13
4	V. *cincinnatiensis*	0.76	0.61	0.23	0.73	0.18
5	V. *ctioteree*	0.85	0.73	0.05	0.16	0.23
6	V. *costicote*	0.34	0.70	0.96	0.52	0.08
7	V. *fumissii*	0.52	0.66	0.11	0.33	0.76
8	V. *harveyi*	0.43	0.64	0.26	0.46	0.56
9	V.*logei*	0.47	0.75	0.82	0.64	0.47
10	V. *mediterranei*	0.78	0.84	0.81	0.17	0.78
11	V. *metschnikovii*	0.63	0.93	0.67	1.23	0.46
12	V. *mimicus*	0.57	0.53	0.02	0.34	0.66
13	V. *netreiqens*	0.48	0.56	0.17	0.47	0.47
14	V. *orientalis*	0.33	0.32	0.21	0.63	0.51
15	V.*parahaemolyticus*	0.46	0.03	0.8	0.10	0.02
16	V. *pelagius*	0.58	0.17	0.10	0.21	0.16
17	V. *proteolyticus*	0.51	0.29	0.16	0.83	0.21
18	*Vibrio* sp.2.	0.39	0.77	0.86	0.02	0.25
19	V. *splendidus*	0.45	0.78	0.49	1.04	0.17
20	*Vibrio* sp.3	0.35	0.56	0.25	0.56	0.07
21	V. *vulnificus*	1.02	0.21	0.36	0.77	0.10

Table.8 Effect of different Carbon sources on Vibrio spp. growth
(The Values are measured in Optical Density)

S.No	Name of the Vibrio sp.	Glucose	Sucrose	Lactose
1	V. *alginolyticus*	0.19	0.36	0.8
2	*Vibrio* sp.1	0.10	0.8	0.24
3	V. *campbellii*	0.36	0.38	0.12
4	V. *cincinnatiensis*	0.23	0.64	0.32
5	V. *ctioteree*	0.29	0.48	0.30
6	V. *costicote*	0.19	0.42	0.24
7	V. *fumissii*	0.9	0.36	0.29
8	V. *harveyi*	0.16	0.27	0.27
9	V.*logei*	0.33	0.28	0.25
10	V. *mediterranei*	0.41	0.24	0.48
11	V. *metschnikovii*	0.8	0.16	1.48
12	V. *mimicus*	0.24	0.18	1.32
13	V. *netreiqens*	0.36	0.30	0.31
14	V. *orientalis*	0.43	0.15	0.23
15	V.*parahaemolyticus*	0.24	0.42	0.24
16	V. *pelagius*	0.17	0.27	0.32
17	V. *proteolyticus*	0.43	0.63	0.47
18	*Vibrio* sp.2.	0.22	0.45	0.32
19	V. *splendidus*	0.23	0.27	0.16
20	*Vibrio* sp.3	0.27	0.4	0.24
21	V. *vulnificus*	0.19	0.10	0.20

Table. 9 Effect of different Mineral sources on Vibrio spp. growth
(The Values are measured in Optical Density)

S.No	Name of the *Vibrio* sp.	CaCl2	MgSO4	ZnSO4
1	V. *alginolyticus*	0.32	0.53	0.75
2	*Vibrio* sp.1	0.57	0.57	0.70
3	V. *campbellii*	0.13	0.76	0.27
4	V. *cincinnatiensis*	0.28	0.60	0.15
5	V. *ctioteree*	0.56	0.69	0.17
6	V. *costicote*	0.47	0.61	0.23
7	V. *fumissii*	0.78	0.73	0.16
8	V. *harveyi*	0.46	0.70	0.52
9	V.*logei*	0.66	0.76	0.46
10	V. *mediterranei*	0.47	0.66	0.64
11	V. *metschnikovii*	0.02	0.64	1.23
12	V. *mimicus*	0.16	0.75	0.47
13	V. *netreiqens*	0.21	0.84	0.49
14	V. *orientalis*	0.25	0.56	0.16
15	V.*parahaemolyticus*	0.17	0.32	0.21
16	V. *pelagius*	0.07	0.29	0.25
17	V. *proteolyticus*	0.16	0.77	0.56
18	*Vibrio sp.2.*	0.86	0.78	0.86
19	V. *splendidus*	0.56	0.56	0.49
20	*Vibrio* sp.3	0.32	0.21	0.17
21	V. *vulnificus*	0.63	0.77	0.19

distribution of this pathogenic Bacteria in sea foods. Bacterial adhesion to host surface has been described as one of the initial steps in microbial pathogenesis. It has been suggested that hydrophobicity is a determining factor in the adhesive process and in the survival of pathogen in cells. The strains of *V. alginolyticus* assayed did not agglutinate sea bream erythrovytes were agglutinated. The abilities of different *Vibrio* species to adhere to skin mucus were studied previously and observed that only the most pathogenic strains of *V.alginolyticus* and *V.anguillarum* possessed this ability.

The results obtained in this study, the isolated gram negative halophilic bacterium *Vibrio* spp. requires at least 3% NaCl and can tolerate up to 10% NaCl for growth. Sea water and sea food is an important vehicle of transmission of this species and other Vibrios and the possible spread of Vibrios to marine invertebrates.

Antibiotic susceptibility of *Vibrio* spp. were determined by disc diffusion on Muller Hinton agar (Merck) supplemented with 3% NaCl. The diamerer of the national committee for clinical laboratory standards. Antibiotic resistant pattern was observed in Ampicillin, Tetracycline, and Chloramphenical. The prevalence of antibiotics resistant microorganisms is ecologically very important and this character is plasmid borne. R plasmid is responsible for antibiotics resistant characters. These R plasmids have been found in *V.alginolyticus* and other Vibrios and carry transferable drug resistance. The data suggest that antibiotic resistant *Vibrio alginolyticus* may survive better than sensitive organisms in surface water. In general, resistance of bacteria to antibiotics may be due to enzymatic destruction of antibiotics impermeability of the cell wall to the antibiotics destruction of antibiotics impermeability of the cell wall to the antibiotics additional of chemical group to antibiotics of the 15 strains Vibrio tested 12 strains 80% were positive for hemolytic activity.

Resistance to bile and low pH is another factor favouring the colonisation of vibrios in the intestine. In the present study, all the dominant *Vibrio* species in the intestine except *V. alginolyticus,* were found to tolerate 0.3% bile. However growth as well as bile tolerance was not observed at pH below 6. Contradictory to this observation, Sera and lshida (1972) reported growth of Vibrio at 2% ox bile and at pH 5.5. Heterogeneity among the strains could be attributed for the disparity in the observation. Since high levels of these organisms were present in the intestine, possibly other tolerance mechanisms might also be acting in *in vivo* conditions.

Studies on the survival pattern and tolerance properties depicting the fate of the *Vibrio* species entering the intestinal tract of edible fish are scarce. Such studies could bring out the probability of the occurrence of pathogenic *Vibrio* species in the seafoods. Muroga (1995) in his review of literature on the bacterial diseases stated that the high mortalities in aquacultured animals had its source from live food contaminated with the opportunistic pathogens.

In general, these species and especially the genes associated with their virulence exhibited distinct seasonal variation attributed to changes in temperature, salinity and DO. Furthermore, increases in the prevalence of *V. parahaemolyticus* and *V. cholerae* coincided with shifts in the composition of the plankton community, cited as a primary reservoir for *Vibrio*

species (Huq *et al*., 1983; Lipp *et al*., 2003; Baffone *et al*., 2006).

While this study confirms the role of temperature, salinity and DO in Vibrio ecology, these results show how warmer temperatures can select for potentially pathogenic Vibrio strains. Furthermore, this study suggests that some plankton taxa, such as diatoms and copepods, may be especially important Vibrio reservoirs. Together, these results highlight the complexity of Vibrio seasonality and stress both the role of seasonal changes in environmental parameters and seasonal shifts in the composition of the plankton community.

Public health professionals at the levels of government should lead a multi disciplinary approach to prevent the recreational water illness that includes surveillance, health education, epidemiologic studies, laboratory support and environmental health research. The sea foods are safe from contamination. Fish handlers and the public should be educated on the possible microbial hazards of fishes and methods for their prevention. The importance of sanitation is during handling, storage, transportation and marketing. The sea foods are thoroughly cooked. Avoid cross contamination of cooked sea food and other raw sea foods. Avoid exposure of open wound or broken skin to warm salt or brackish water.

References

Baffone, W., R.Tarsi, L. Pane, R. Campana, B. Repetto, G.L. Mariottini *et al.,* 2006. Detection of free-living and plankton-bound vibrios in coastal waters of the Adriatic Sea (Italy) and study of their pathogenicity-associated properties. Environ. Microbiol.8: 1299-1305.

Chakraborty, S., A.K. Mukhopadhyay, R.K. Bhadra, A.N. Ghosh, R. Mitra, T. Shimada *et al.*,(2000. Virulence Genes in Environmental Strains of *Vibrio cholerae*. App. Environ. Microbiol. 66: 4022-4028.

Colorni, A., I. Paperna and Gordin, H. 1981 Bacterial infections in gilt-head seabream sparus aurata cultured at Elat. Aquacult. 23:257-267.

Dechet, A.M., P.A. Yu, N. Koram and Painter, J. 2008. Non-foodborne *Vibrio* infections: an important cause of morbidity and mortality in the United States, 1997-2006. Clin. Infect. Dis. 46: 970-976.

Grossart, H.P., F. Levold, M. Allgaier, M. Simon and Brinkhoff, T. 2005. Marine diatom species harbour distinct bacterial communities. Environ. Microbiol. 7: 860-873.

Heidelberg, J.F., K.B. Heidelberg and Colwell, R.R. 2002. Bacteria of the γ-Subclass Proteobacteria Associated with Zooplankton in Chesapeake Bay. App. Environ. Microbiol. 68: 5498-5507.

Huq, A., R.B. Sack, A. Nizam, I.M. Longini, G.B. Nair, A. Ali A *et al.*, (2005. Critical Factors Influencing the Occurrence of *Vibrio cholerae* in the Environment of Bangladesh. App. Environ. Microbiol. 71: 4645-4654.

Huq, A., E.B. Small, P.A. West, M.I. Huq, R. Rahman and Colwell, R.R. 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. App.Environ. Microbiol. 45: 275-283.

Lipp, E.K., I.N.G. Rivera, A.I. Gil, E.M. Espeland, N. Choopun, V.R. Louis *et al.,* 2003. Direct Detection of *Vibrio cholerae* and *ctx*A in Peruvian Coastal

Water and Plankton by PCR. App. Environ. Microbiol. 69: 3676-3680.

Long, R.A., D.C. Rowley, E. Zamora, J. Liu, D.H. Bartlett and Azam, F. 2005. Antagonistic Interactions among Marine Bacteria Impede the Proliferation of *Vibrio cholerae*. App. Environ. *Microbiol.* 71: 8531-8536.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee and Shapiro, C. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5: 607-625.

Nishibuchi, M,, and Kaper, J.B. 1995. Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. Infect. Immunity. 63: 2093-2099.

Rivera, I.N.G., J. Chun, A. Huq, R.B. Sack and Colwell, R.R. 2001. Genotypes Associated with Virulence in Environmental Isolates of *Vibrio cholerae*. App. Environ. Microbiol. 67: 2421-2429.

Shewan, J.M., and Veron, M. 1974. Genus I. *Vibrio pacini*1854,411. In Bergey"s Manual of determinative Bacteriology 8th edn. ed. Buchanan, R.E. and Gibbons, N.E., Baltimore: Williams and Wilkins, pp.340-345.

Sochard, M.R., D.F. Wilson, B. Austin and Colwell, R.R. 1979. Bacteria Associated with the Surface and Gut of Marine Copepods. App. Environ. Microbiol. 37: 750-759.

Tamplin, M.L., A.L. Gauzens, A. Huq, D.A. Sack and Colwell, R.R. 1990. Attachment of *Vibrio cholerae* serogroup O1 to zooplankton and phytoplankton of Bangladesh waters. App. Environ. Microbiol. 56: 1977-1980.

Thompson, F.L., T. Iida and Swings, J. 2003. Biodiversity of Vibrios. Microbiol. Mol. Biol. Rev. 68: 403-431.

Thompson, J.R., M.A. Randa, L.A. Marcelino, A. Tomita-Mitchell, E. Lim and Polz, M.F. 2003. Diversity and Dynamics of a North Atlantic Coastal *Vibrio* Community. App. Environ. Microbiol. 70: 4103-4110.

Zorrilla, I., 2000. Characterization intraespecifica de aislados de *Vibrio alginolyticus* procedentes de peaces *Marinos cultivados,* Ph.D.Thesis, Malaga, Spain.