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IDENTIFICATION OF *VIBRIO* SPECIES OCCURRING IN FOOD FISHES AND STUDIES ON THEIR ANTIBIOTIC RESISTANCES

S. Saravanan and R. Sivakami*

PG & Research Department of Zoology, Arignar Anna Govt. Arts College, Musiri -621211, Tamil Nadu, India. e-mail id:

***Corresponding Author Email: drsiva17@gmail.com**

ABSTRACT

Of the Crustaceans suitable for aquaculture, shrimp plays an important role. To increase production, aquaculture have resorted to high stocking densities leading to diseases resulting in heavy losses to aquaculture industry. Among the various pathogens, Vibrio has been found to be one of the most studied and diverse genus of microorganisms found in the aquatic systems. Hence, the present study was attempted to identify the different species of Vibrio that occurs in fishes and shrimps grown in this part of the country during the different seasons. A total of 11 species of Vibrio could be isolated of which four species dominated during the rainy season and two species each during the pre-summer and summer season. The differences in the distribution and occurrence of these species is attributed to the changes in the physicochemical and climatic conditions during each season. Studies on antibiotic resistance reveals that among the various substances tested, herbal extract appeared to be the most effective.

KEY WORDS

Fish disease, Vibrio, Fish, Shrimp, Antibiotic resistance

INTRODUCTION

The brackish water area available in India has been estimated to be around 1.2 million ha (Heran *et al.*, 1992) of which about 65000 ha are under aquaculture. Of the crustaceans suitable for aquaculture, shrimp plays an important role as it is extensively cultivated throughout the world. However, to increase production, aqua culturists have resorted to high stocking densities and supplementary feeding. This has led to deterioration in water quality leading to diseases thereby resulting in heavy losses to the aquaculture industry. The diseases are more commonly found in extensive and modified extensive systems where water quality management practices have been poor (Ramaiah, 2006).

Among the water and food borne pathogens, especially in coastal and marine ecosystems, *Vibrio* has been found to be the major species (Lekshmy *et al.*, 2014) with members of the family *Vibrio*naceae contributing as much as 60 percent of the total bacterial population (Simidu and Tsukamoto, 1985).

After the discovery of the first *Vibrio* species (*V. cholerae*) in 1854 by the Halian physician Filippo Pacini in Florence in 1854 (Thompson *et al.*, 2004) many species belonging to this genus have been identified. According to the Association of *Vibrio* biologists, 99 species of *Vibrio* including two sub-species have been identified (January 2013). Today, *Vibrio* is one of the most studied and diverse genera of microorganisms found in the aquatic systems of both marine and estuarine environment.

Literature reveals that *Vibrio* sp. have been frequently isolated from edible shell fish and other fishes (Thompson *et al.*, 2004). In addition, several species

have been characterized as probionts (Gomez-Gill *et al.*, 2000) and as pathogens (Lee *et al.,* 1996). Recently, Austin (2010) classified Zoonotic *Vibrio* with higher risk *Vibrio* and lower risk *Vibrios*. Hence the present study was attempted to identify the different species of *Vibrio* that occurred in fishes and shrimps grown in this system.

MATERIAL AND METHODS

Collection of seafood samples: a total of 20 different sea food samples such as Finfishes and crustaceans (shrimps) were collected from different sites in Pudukkottai estuary, Tamil Nadu. 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 less than 10 hours.

Isolation of *Vibrio* spp. from sea foods: Finfish and crustaceans (shrimps) 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 (shrimps) and finfish samples were then homogenized in blenders, and 25 g of each homogenate was placed in 225 ml 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* species: 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; glucose, lactose, mannitol, maltose and sucrose fermentation tests.

Antibiotic resistant pattern: *Vibrio* sp. isolated from seafood samples (shrimps and fin fishes) were grown in nutrient broth containing 2% NaCl. Muller-Hinton (MHA) agar medium (Hi media) was used for antibiotic resistance pattern. Gentamycin, Co-Trimoxazole, Bacitracin, Amikacin, and Oxytetracycline were used and the 24 hours broth culture of *Vibrio* sp. 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 incubated in an inverted position at 37°C for 18-24 hours. The results were recorded by measuring the zone of inhibition. Kirby-Bauer method (agar diffusion method) was followed to determine the susceptibility of MRSA to antibiotics.

Haemolytic activity: *Vibrio* sp. produced hemolysin was tested on blood agar supplemented with 5% sheep anticoagulant blood. All the selected cultures were seeded in blood agar plate, and the plates were incubated at 37°C for 24 hours. Hemolytic activity was determined as per Kishishita *et al.* (1992).

RESULTS AND DISCUSSION

The various *Vibrio* species that could be isolated from the fishes and shrimps that occurred in this system during the three seasons of the year are presented in Table 1. As evident from the table, a total of 11 species could be isolated based on the various biochemical tests (Table 2). However, each species recorded different counts during the four seasons. During the rainy season, four species dominated in terms of abundance (*V. alginolyticus, V. campbellii, V. mediterranei* and *V. mimicus*) while during pre-summer season, two species dominated (*V. fumissi* and *V. harveyi*) and during summer season, three species dominated (*V. cincinnatiensis, V. ctioteree* and *V. logei*). However, two species (*V. costicote* and *V. metschnikovii*) were found to occur at almost the same abundance for all the three seasons. The differences in the distribution and occurrence can possibly be related to changes in factors like temperature, salinity, dissolved oxygen and pH.

The resistance of *Vibrio* species to various antimicrobial substances are presented in Table-1. Each species showed different resistance pattern and responded differently in different treatments. Among the five substances tested (four antimicrobial and one herbal extract), three species (*V. cholerae, V. harveyi* and *V. metschnikovii*) recorded maximum zone of clearance to Oxytetracycline. However, among the three species, the maximum zone of clearance was observed in *V. cholerae* followed closely by *V. harveyi*. With regard to Gentomycin, only *V. mediterranei* recorded maximum zone of clearance. However, with the Streptomycin extract, three species namely *V. costicolus, V. fumissii* and *V. logei* recorded maximum zone of clearance with the maximum zone of clearance being recorded by *V.*

costicolus followed by *V. fumissii.* With regard to ampicillin, none of the *Vibrio* species recorded maximum zone of clearance when compared to the remaining four tested materials. In the herbal extract, four species (*V. algniolyticus, V. campbellii, V. cincinnatiensis* and *V. mimicus*) recorded highest zone of clearance. Nevertheless, among the four species, the maximum zone of clearance was observed in *V. alginolyticus* followed by *V. campbellii*. Thus among the various substances that were tested, the herbal extract appeared to be the most effective followed by *Oxytetracycline* while the least inhibitory effect was recorded with Ampicillin.

Literature reveals that *Vibrio* species have frequently been isolated from edible fin fish and shell fish. Some species like *V. parahaemolyticus* and *V. vulnificus* cause serious food borne gastroenteritis in humans (Thomson *et al.*, 2004). In addition, some species such as *V. harveyi and V. campbelli* have been found to be pathogenic for shrimps as well as fishes (Le Roux *et al.*, 2002; Austin and Austin, 2007; Austin, 2010). According to Horii *et al.* (2005), *V. alginolyticus* has been associated with ear, soft tissue and infections and antibiotic resistance has been cited as a major issue while *V. harveyi* has been reported to be major pathogens for aquatic animals causing serious losses to aquaculture industry (Cano-Gomez *et al.*, 2011). *V. mimicus* so named for its resemblance to *V. cholerae* have also been well known as pathogens of vertebrates and other aquatic invertebrates (Austin, 2010). Thus, the results of the present study clearly reveal that the system contains potential pathogens that can cause losses not only to aquaculture industry but endanger man as well. The study also clearly indicates the need to study the health-related aspects, necessitating the need of health professionals to work in hand with the industry. In addition, the study also highlights the need to educate the fish handlers and the public on possible hazards and ways for prevention.

Table-1: Isolation and identification of marine isolates of *Vibrio* sp. from Kodikulam Estuary

S. No.	Species	Sources isolated	Rainy season	Pre-summer season	Summer season	Heamolytic activity
1.	*Vibrio alginolyticus*	Fish	+++	++	++	Negative
2.	*V. campbellii*	Fish	+++	++	+++	Negative
3.	*V. cincinnatiensis*	Fish	+++	++	+++	Gamma haemolysis
4.	*V. ctioteree*	Fish	++	+++	+++	Negative
5.	*V. costicote*	Fish	+++	+++	+++	Beta haemolysis
6.	*V. fumissii*	Fish	++	+++	+++	Gamma haemolysis
7.	*V. harveyi*	Fish, Crab	++	+++	+	Gamma haemolysis
8.	*V. logei*	Fish, Prawn	+++	++	+++	Gamma haemolysis
9.	*V. mediterranei*	Fish, Prawn	+++	++	++	Negative
10.	*V. metschnikovii*	Fish, Prawn	+++	+++	+++	Negative
11.	*V. mimicus*	Fish, Crab	+++	++	+	Beta haemolysis

'+++' ??; '++'; '+'

Table-2: Antibacterial activity of various antibiotics and herbal extracts on *Vibrio* species

S. No.	Species	Oxytetracycline (mm)	Gentamicin (mm)	Streptomycin (mm)	Ampicillin (mm)	Neem + Sunberry Herbal extracts (mm)
1.	*Vibrio alginolyticus*	24	7	10	24	36
2.	*V. campbellii*	31	3	12	10	34
3.	*V. cincinnatiensis*	20	4	-	14	28
4.	*V. ctioteree*	30	-	12	13	28
5.	*V. costicote*	16	12	20	14	18
6.	*V. fumissii*	16	7	18	-	16
7.	*V. harveyi*	29	-	10	8	26
8.	*V. logei*	1.3	-	8	7	4
9.	*V. mediterranei*	10	16	4	8	10
10.	*V. metschnikovii*	14	11	8	10	9
11.	*V. mimicus*	13	12	7	16	18

'–' No infection

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***Corresponding Author:**
R. Sivakami*
Email: drsiva17@gmail.com