Occurrence, Distribution and Antibiotic Resistance Patterns of *Vibrio* species associated with viral diseased Shrimp of South Indian Aquaculture Environment

Srinivasan P. and Ramasamy P.*
Department of Bioinformatics, Alagappa University, Karaikudi-630003, Sivagangai District, TamilNadu, India,
Ph: 09442135200, 09444022693, Fax: +91-4565-225202, Email:ramasamy_p@hotmail.com, ramasamy_p@sify.com

Abstract- *Vibrio* sp. has been implicated as one of the major bacterial pathogens of shrimp. A total of 121 isolates of *Vibrio* spp. were isolated from nineteen different sources of samples collected from shrimp aquaculture environment, located along the East coast of Bay of Bengal at Thulukenkulam and Chennai (Tamil Nadu) and Nellore (Andhra Pradesh), India during 2006-2007. The samples were tested for the presence of *Vibrio* spp. from different sources of aquaculture environments. All isolates were phenotypically characterized and the most frequently *Vibrio* spp. includes *Vibrio harveyi*, *V. anguillarum*, *V. damsela*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. alginolyticus*, *V. mimicus*, *V. furnissi*, *V. cholerae* and *V. ordalli*. The most predominant species was *V. harveyi* followed by *V. anguillarum*, *V. damsela*. All the isolates of *Vibrio spp*. were 100% resistant to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G and furazolidone and followed by the enduring antibiotics used.
Key words: *Vibrio* spp. Antibiotic resistance, viral diseases, shrimp aquaculture

1 Introduction

Shrimp culture has become a commercially important industry and is widespread throughout tropical countries. In India, there are 150 hatcheries and 100,000 shrimp farms occupying an estimated area of 140,000 hectares [25]. However, shrimp production has decreased in recent years due to the incidence and spread of infectious diseases [7, 25]. Disease, particularly that caused by bacterial infection, has been cited as the single largest source of economic loss in the aquaculture industry [14, 32]. The distribution of Vibriosis is worldwide, caused by various *Vibrio* spp. [4, 12-13, 34] and they are responsible for the high mortality and severe economic loss in shrimp industry in all the shrimp producing countries [7, 18-19, 38-39].

Vibrio spp., have been studied for many years and reported to cause not only serious infections and also lower shrimp production (mortality, tissue lesion or necrosis, body malformation, low growth). Although India practices both the harvest of captive-wild as well as semi-intensive culture of shrimp, problems with the bacterial disease and the estimated loss of wild stocks are unknown, except for a few reports on the occurrence of bacteria in the processed iced-storage shrimp [19]. Meagre information is available on the frequency of infection of *Vibrio* sp. and management strategies of shrimp in tropical India and such information are essential to the farmers to understand the impact of the infection and to take possible remedial measures. Although bacteria causing diseases in penaeid shrimp are considered opportunistic and manageable, the economic implication of losses due to mortality of shrimp or rejection of the infected shrimp is enormous [4, 26, 35]. Hence, the present study was initiated to assess the occurrence and distribution of *Vibrio* spp. and to determine species composition of *Vibrio* spp. in hatcheries and culture ponds of *P. monodon* along the South East coast of India. Many attempts have been made to reduce the loss due to bacterial and other diseases by chemotherapy. Antibiotics and probiotics have been widely used to control bacterial pathogens of shrimp [20, 21, 31]. In hatcheries, it is common practice to use antibiotics for prophylaxis and chemotherapy, especially when larval development is hampered. However, administration of antibiotics to infected stocks of shrimp is usually impracticable in large-scale culture enterprises, as the only routes of administration are through the culture water or in pelletized feed. Antibiotic resistant strains of *V. harveyi* have caused mass mortality in cultured *P. monodon* larvae and *Macrobrachium rosenbergii* in India [14, 24]. Throughout Asia, shrimp farmers use antibiotics in large quantities. Potential consequences of antibiotics use in culture and in animal feeds are the transfer of resistant characters to bacteria thereby leading to the development of antibiotic resistant bacterial strains, and thereby reduced efficacy of antibiotic treatment for human and animal diseases [4, 40]. Resistance of marine fish and shellfish pathogens to commonly used antibiotics has also been reported in Japan [42] and Denmark [43]. Antibiotic resistance of isolates of *Vibrio* spp. from aquaculture systems of fish, shellfish and sea foods has been found in Saudi Arabia [11], Scotland [17], USA [6, 37], Nigeria [5], India [13] and Taiwan [30]. Since, the luminescent *Vibrio* spp. infecting shrimp do not always respond to antibiotics and other chemical control methods, numerous efforts have been made to find new chemotherapeutic agents to replace those antibiotics against which the disease causing bacteria have become resistant [9, 10, 20, 31]. Currently, there are no universally acceptable pharmaceutical agents that are approved by the FDA for treating infections in shrimp aquaculture, although studies are underway to improve disease control and treatment [39]. In view of increasing concerns surrounding the evolution of antibiotic resistant bacteria in aquaculture

environments, the present study was undertaken to determine the antibiotic sensitivity patterns and percentage antibiotic resistance expressed by *Vibrio* spp. isolated from marine waters and aquaculture environments in South India.

2 Materials and Methods

2.1 Bacteriological culture media

Thio-sulphate Citrate Bile salts Sucrose agar (TCBS), Nutrient broth (NB), Luria Bertaini broth (LB), Nutrient agar (NA) and Luria Bertaini agar (LBA) were used for isolating *Vibrio* spp. The agar disc diffusion assay (Nutrient agar /Luria Bertaini agar) was used to screen and characterize the antibiotic susceptibility pattern of *Vibrio spp*. All assays in liquid and solid media were carried out at 32ºC.

2.2 Sampling sites and Sample collection

2.2.1 Farm sites

Samples were collected at shrimp farms located along the coast of the Bay of Bengal at Thulukenkulam, [30 kms from Tuticorin (08°–40' 05–08° 48'05N, 78° 10' 05–78° 14'10E)], Chennai (Tamil Nadu) and Nellore (Andhra Pradesh), India during 2006-2007. Samples of source seawater and sea sediments, shrimp culture pond water and pond sediments were collected before and after stocking the culture ponds with shrimp. Water samples from the ponds and source seawater were collected in sterile bottles of 100 ml capacity. Tissue samples were taken from *Penaeus monodon* on 30th, 45th and 60th day after seeding the ponds. From the 54th day after the start of sampling, an outbreak of *White Spot Syndrome Virus* (WSSV) occurred in all of the ponds. Shrimp were collected by cast netting and placed immediately in a sterile container with dry ice. Sediment samples from the sea and pond bottom were collected, packed in sterile polythene bags, stored and transported on ice layers in an insulated container and brought to the laboratory for bacteriological analysis. Pelletized feed samples were collected aseptically using sterile scoops and held in sterile 'whirl packs' bags until examination.

2.2.2 Hatchery sites

P. monodon eggs, post larvae, *Artemia* nauplii and source seawater were collected from various hatcheries located at Kovalam, Marina beach, Boat house (Chennai) and Tuticorin, Tamil Nadu, India and were subjected to bacteriological analysis. Various stages of post larval (PL 5 – PL 25) *P. monodon* obtained from hatcheries were maintained in well aerated, filtered sea water and fed with pelletized feed. Shrimp were sampled at intervals and the hepatopancreas was examined in squashed preparations to reveal evidence of Monodon Baculovirus (MBV) infection as described by Ramasamy *et al*. (2000). *Vibrio* spp. were isolated from MBV-infected and uninfected larvae and also from the larval rearing water, in order to clarify the pathogenic role of these bacteria. All the samples were subjected to bacteriological analysis. Similarly, the source seawater was also subjected to bacteriological examination.

2.3 *Vibrio* spp. isolation

Vibrio spp. present in the source sea water, shrimp eggs, *Artemia* nauplii, different larval stages of MBV-infected and uninfected *P. monodon*, larval rearing tank water from different hatcheries, pond water and its sediments and various tissues of WSSV-infected and uninfected shrimp (viz., gills, muscles, stomach, hepatopancreas and intestine) were isolated on Thio-sulphate Citrate Bile salts Sucrose agar (TCBS).

2.4 Bio-chemical characterization and identification of *Vibrio* isolates

Vibrio isolates were picked from TCBS agar plates according to their colony morphology, size and pigmentation variability. They were biochemically characterized and identified with the help of *Bergey's Manual of Determinative Bacteriology* [15]. Each of the *Vibrio* isolates were transferred to LB broth, cultured at 32°C for 18 hours and then stored at –20°C after the addition of 30% (v/v) glycerol for further studies. *Vibrio* isolates were subjected to the staining procedures, biochemical tests and were cultured in various media to identify the genus and species. The isolates of *Vibrio* species were characterized and identified by using the standard biochemical and morphological tests.

2.5 Antibiotic resistance of the *Vibrio* isolates

Vibrio spp. isolated from shrimp culture systems were tested for susceptibility to several commonly used antibiotics using the agar-disc diffusion method of Bauer *et al*. [8]. Commercially available antibiotic discs obtained from Hi-Media, Mumbai, India were used. The antibiotics tested include ampicillin (10 mcg), azithromycin (15 mcg), amikacin (30 mcg), nalidixic acid (30 mcg), neomycin (30 mcg), gentamycin (10 mcg), cefaclor (30 mcg), cloxacillin (1 mcg), rifampicin (5 mcg), ciprofloxacin (10 mcg), erythromycin (15 mcg), cepholaxime (30 mcg), vancomycin (30 mcg), chloramphenicol (30 mcg), oxacillin (1 mcg), streptomycin (10 units), norfloxacin (10 mcg), chlorotetracycline (30 mcg), furazolidone (50 mcg), penicillin G (10 units) and oxytetracycline (30 mcg). *Vibrio spp.* were grown in Nutrient broth (NB)/ LB liquid medium by incubating over night at 32°C. Each culture was spread on Mueller-Hinton agar (Himedia) plates and the antibiotic discs were placed on the agar surface. Inhibition zones were measured after 24 to 36 hours of incubation at 32°C.

3. Results

3.1. Bio-chemical and phenotypic characterization of the isolates of *Vibrio* spp. from the shrimp aquaculture environment

Bacteria (*Vibrio* spp.) were isolated from the shrimp egg samples of *P. monodon*, *Artemia* nauplii, *Artemia* reared water, hatchery tank water, uninfected post larval *P. monodon*, both *Monodon baculovirus* (MBV) infected and unifected post larval *P. monodon*, seawater, sea sediment, shrimp culture pond water, shrimp culture pond sediment, pond water and pond sediment from *White Spot Syndrome Virus* (WSSV) infected shrimp farm; WSSV infected tissues of *P. monodon viz*., hepatopancreas, intestine, stomach, muscles and gills obtained from shrimp farms located at Tuticorin and Chennai (Tamil Nadu) and Nellore (Andhra Pradesh), India during 2006-2007 and also from hatcheries and shrimp farms. *Vibrio* spp. were biochemically characterized and identified. The phenotypic characterization of the bacterial isolates revealed that all the isolates were rod shaped, motile, Gram negative, oxidase and catalase positive and halophilic. All *Vibrio* spp. fermented D-Glucose without gas production, reduced nitrate except a strain of *V. ordalli*. All *Vibrio* spp. were sensitive to 0/129 *Vibrio*static agent. Results of oxidase test and carbohydrate fermentation differentiation discs test showed that *V. anguillarum* were oxidase positive, lactose negative and positive to carbohydrates such as maltose, galactose, fructose, and sucrose. *V. damsela* showed oxidase positive and negative to the utilization of carbohydrates *viz*., lactose, mannitol and sorbitol. *V. parahaemolyticus* showed positive to maltose and negative to sucrose and galactose .None of the *Vibrio* isolates produced acid from inositol or rhamnose. They did not exhibit growth at 60℃ (Table-1).

3.2. *Vibrio* spp. isolation from shrimp aquaculture environment

During the course of the present study, the presence of 121 bacterial isolates belonging to genus *Vibrio* spp. The identified genus *Vibrio* were consisted of the following species, *V. harveyi* (26 isolates), *V. anguillarum* (21 isolates), *V. damsela* (15 isolates), *V. parahaemolyticus* (11 isolates), *V. vulnificus* (10 isolates), *V. fluvialis* (9 isolates), *V. alginolyticus* (8 isolates), *V. mimicus* (7 isolates), *V. furnissi* (7 isolates), *V. cholerae* (4 isolates) and *V. ordalli* (3 isolates). The species composition of the *Vibrio* spp obtained from different sources of aquaculture environment and these species were as follows; 21.48% of *V. harveyi*, 17.35% of *V. anguillarum*, 12.39% of *V. damsela*, *V. parahaemolyticus* (9.09%), 8.26% of *V. vulnificus*, 7.43% of *V. fluvialis*, 6.61% of *V. alginolyticus*, 5.78% of *V. mimicus*, 5.78% of *V. furnissi*, 3.3% of *V. cholerae* and 2.4% of *V. ordalli* respectively (Table-2). The occurrence frequencies of these species were as follows; 2.47% (*V. harveyi* and *V. vulnificus*) in the eggs of *P. monodon*, 6.61% (*V. harveyi V. furnissi V. mimicus V. damsela* and *V. anguillarum*) in the nauplii of *Artemia*, 2.47% (*V. harveyi and V. mimicus*) in the *Artemia* reared water. *P. monodon* hatchery tank water samples revealed the presence of *Vibrio* spp (3.3%) like *V. ordalli V. harveyi* and *V. vulnificus*. 9.09% of the *Vibrio* spp. (*V. harveyi V. anguillarum V. mimicus, V. fluvialis, V. damsela* and *V. furnissi*) were present in the MBV infected post larvae than in the other samples of aquatic environment. *Vibrio* spp (4.13%) like *V. harveyi*, *V. fluvialis, V. mimicus* and *V. anguillarum* from MBV uninfected post larval samples of *P. monodon*. Source sea water samples contained 4.95% of the *Vibrio* spp. like *V. parahaemolyticus V. anguillarum* and *V. harveyi*. Sea sediments. *Vibrio* spp. (4.13%) like *V. parahaemolyticus V. harveyi, V. anguillarum* and *V. damsela*. *Vibrio* spp. (8.2%) *V. damsela V. vulnificus V. alginolyticus, V. cholerae, V. anguillarum* and *V. furnissi* of the bacterial isolates from shrimp culture pond water at Tuticorin and Nellore. Shrimp culture pond sediment showed the presence of *Vibrio* spp (4.13%) like *V. damsela, V. cholerae, V. furnissi, V. anguillarum* and *V. alginolyticus*. MBV/WSSV uninfected *P. monodon* samples showed the presence of 3.3% of the *Vibrio* spp like *V. harveyi*, *V. vulnificus, V. anguillarum, V. furnissi* and *V. ordalli*. Based on the present investigation showed the occurrence of 13.22% of *Vibrio* spp. *viz*., *V. harveyi, V. alginolyticus, V. anguillarum, V. damsela, V. parahaemolyticus, V. vulnificus* and *V. fluvialis* in the WSSV infested hepatopancreas, 8.26% (*V. damsela, V. alginolyticus, V. vulnificus, V. fluvialis* and *V. harveyi*) in the WSSV infested stomach and followed by the other bacterial species, 6.61% each (*V. cholerae, V. anguillarum, V. damsela, V. vulnificus* and *V. harveyi*) in the WSSV infected pond sediment and (*V. fluvialis, V. parahaemolyticus, V. mimicus, V. anguillarum* and *V. damsela*) in the WSSV infested muscles, 4.95% (*V. alginolyticus, V. anguillarum, V. vulnificus, V. furnissi, V. parahaemolyticus* and *V. harveyi*) that were encountered in the WSSV infected pond water, 4.13% (*V. anguillarum, V. harveyi* and *V. parahaemolyticus*) in the WSSV infested intestine, and 3.3% (*V. harveyi, V. anguillarum* and *V. fluvialis*) in the WSSV infected gills of *P. monodon* (Table-2). These data revealed the prominent occurrence of *Vibrio* species in the WSSV infected shrimp culture ponds and also in the MBV infected post larval *Penaeus monodon* than in other sources of bacterial isolation *viz., Artemia* nauplii, *Artemia* reared water, egg samples, hatchery tank water, MBV and WSSV uninfected *P. monodon,* sea

sediment, sea water, shrimp culture pond sediment and shrimp culture pond water.

3.3 Antibiotic resistance pattern of *Vibrio* spp. isolated from shrimp culture ponds and hatcheries

Antibiogram patterns obtained for the *Vibrio* spp. are presented in Table-3. They show varying degrees of inhibition against the 21 different antibiotics tested. The results were compared with standard antibiotic tests conducted by Molitoris *et al*. [33]. All the isolates of *Vibrio spp*. were 100% resistant to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G and furazolidone. In tests with the remaining antibiotics, 90.90% of isolates were found to be resistant to cefaclor, 81.82% to streptomycin and rifampicin, 72.73% to oxytetracycline, 63.64% to nalidixic acid, 54.55% to cepholaxime and chlortetracycline, 45.45% to norfloxacin and azithromycin, 27.27% to neomycin, chloramphenicol and gentamycin, 18.18% to ciprofloxacin and amikacin. The results also indicated that antibiotic resistant *Vibrio spp*. were more commonly encountered in hatchery reared post larvae than in the farm reared *P. monodon.* The incidence of antibiotic resistance was higher in ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G and furazolidone than in the other antibiotics used in this study.

4 Discussion

In the present study has shown the occurrence of 121 isolates belonging to eleven different species of *Vibrio* include, 26 isolates of *V. harveyi*, 21 isolates of *V. anguillarum*, 15 isolates of *V. damsela* (12.39%), 11 isolates of *V. parahaemolyticus* (9.09%), 10 isolates of *V. vulnificus* (8.26%), 9 isolates of *V. fluvialis* (7.43%), 8 isolates of *V. alginolyticus* (6.61%), 7 isolates of *V. mimicus* (5.78%), 7 isolates of *V. furnissi* (5.78%), 4 isolates of *V. cholerae* (3.3%) and 3 isolates of *V. ordalli* (2.4%). Similar observations were made by Abraham and Palaniappan [1] who have reported the distribution of luminous bacteria in the eggs, nauplii, zoea, mysis, post larvae, adult shrimp of *P. monodon* and also in the *Artemia* nauplii and rearing tank water. Vandenberghe *et al*. [45] have isolated a total number of 1473 *Vibrio* isolates from bivalves, shrimp, fish, sea urchins, live feed (algae, Artemia, rotifers), seaweed, aquaculture market products and from the aquaculture (tank water, seawater and sediments) and reported the presence of rod shaped, motile, gram negative, oxidase positive, catalase positive and halophilic bacterial isolates. They have further shown that all the isolates of *Vibrio* spp. fermented D-Glucose without gas production, reduced nitrate except a strain of *V. ordalli*. Hosseini *et al*. [16] reported the biochemical tests used for identification of *Vibrio* spp. from 16 (2.1%) samples out of a total of 770 shrimp samples. None of them belonged to *V. cholerae* though they have identified *V. parahaemolyticus*, *V. damsela*, *V. alginolyticus* and *V. fluvialis*. In mackerel, shrimp, and squid, *V. alginolyticus* and *V. parahaemolyticus* were isolated and identified [33]. The occurrence of a high percentage of bacteria in the shrimp culture system may be due to a number of factors such as contaminated rearing tank, inadequate chlorination of sea water, the use of infected brood stocks for spawning and poor management practices.The present study has shown that the Vibrios in the source seawater were higher than those previously reported in other areas [4, 13, 26]. *Vibrio* spp. were also present in the hepatopancreas and gills and was highest (100%) recorded in male and female shrimp of *P. indicus* of all size classes sampled during all months [46]. *V. harveyi* was present in almost all the sources except in shrimp culture pond water and pond sediment. *V. harveyi* was reported to be a dominant flora (94.05%) invariably in all hatchery components [3], [10]. The current study has shown that the WSSV infected hepatopancreas to harbour the highest percentage of *Vibrio* spp. followed by MBV infected post larval tissues and WSSV infected stomach tissues. These results may further suggest that shrimp affected by WSSV/MBV may become more susceptible to invasion of *Vibrio* spp. that is present in the immediate environment. Similar observations were reported by Karunasagar *et al*. [22] who have shown that the shrimp affected by WSSV became more susceptible to the invasion of *Vibrio viz*. *V. harveyi*, *V. damsela* species. Though WSSV infection in shrimp culture systems have been reported [22, 38], this report shows the occurrence of different kinds of *Vibrio* spp. in WSSV infected shrimp tissues. The present study showed the presence of *Vibrio* species especially *V. mimicus* (7 isolates) in the tissues of *Artemia* nauplii, *Artemia* reared tank water, uninfected post larval tissues of *P. monodon*, MBV and WSSV infected tissues of shrimp. These results are comparable to the report of Vandenberghe *et al*. [45]. They have reported the presence of *V. mimicus* (15 isolates), a typical *Vibrio* profiles obtained from cluster *Vibrio* VIB 449 (2 isolates) obtained from different fish species and from diseased tissues of penaeid shrimp. The present study showed the presence of 3.3% of *Vibrio* spp. consisting of one isolate each of *V. vulnificus, V. anguillarum, V. furnissi* and *V. ordalli* (0.82%) in the MBV/WSSV uninfected *P. monodon* samples. The results also showed the presence of 4.13% of *Vibrio* spp., consisting of one isolate each of *V. harveyi, V. fluvialis, V. mimicus* and *V. anguillarum* (0.82%) in the uninfected (normal) post larval samples of *P. monodon*. The mysis and post-larval stages of *P. monodon* harboured

a higher luminous bacterial populations compared to nauplii and zoeal stages. This could be attributed to the varied types of feed viz. artificial feed, concentrated plankton, algal powder and diatom were the common food items used in the hatchery production of zoea in the hatcheries [13, 27]. In the present observation suggest that the strategies for the mode of bacterial infection in the shrimp culture industry, the digestive tracts of marine animals are reported to be the most important habitat of luminous *Vibrio* bacteria [2, 4, 27]. The proliferation of luminous *Vibrio* bacteria expelled on faecal particles from brood stock increased the luminous population of brood stock tank water and also recorded in source water [41]. Brood stock can introduce *Vibrio* spp., into the hatchery system during spawning most significantly through the faecal matter, as observed in one of the spawning tank water samples, which contained luminous Vibrios. However, the results of the present study differ from those of Yasuda & Kitao [47] who noticed an increase in the *Vibrio* isolates from eggs to mysis of *P. japonicus* and a decline thereafter. The egg and larval surfaces provide a suitable microenvironment for the bacterial growth in eutrophic rearing water and the interaction between the egg/larval surface and bacterial adhesion may be responsible for the association of a greater number of bacteria with these surfaces. These bacterial epibionts play an important role either favourably [3] or adversely [28, 29] in the well being of the shrimp. Other research findings report the occurrences of *Vibrio* spp. that were detected from shrimp eggs, healthy and diseased or dead larvae, and adult organisms were sampled from cold-water species and moderate to warm-water species [45]. All of the 121 isolates of *Vibrio* spp. were found to be 100% resistant to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin, penicillin G and furazolidone, and partially resistant to cefaclor, streptomycin, rifampicin, oxytetracycline, nalidixic acid, cepholaxime and chlorotetracycline. Adeleye, et al, [5] and Jun et al, [20] reported the antimicrobial susceptibility test showed that all the *Vibrio* isolates (100%) were resistant to amoxicillin, augmentin, chloramphenicol and nitroforantoin. They also showed multiple resistance patterns to gentamycin, nitrofurantoin, tetracycline, augmentin, chloramphenicol, amoxycilin, ofloxacin, cotrimozazole, ceftriazone, and ciprofloxacin [31]. Resistance to all ten antibiotics occurs in 8 (18%) of the isolates from in Lagos, Nigeria. Furthermore, antibiotic resistant *Vibrio* spp. were more commonly isolated from hatchery reared post larvae than from farm reared *P. monodon.* Jayakumar and Ramasamy [18] and Molitoris *et al*. [33] also reported a high degree of resistance to ampicillin and furazolidone, and incidence of resistance to chloramphenicol, neomycin and gentamycin has also been observed [18, 36, 44]. Karunasagar *et al*. [23] found that *V. harveyi* could form a biofilm on various surfaces such as plastic, cement and stainless steel even in the presence of antibiotics such as chloramphenicol and tetracycline. *V. parahaemolyticus* is reported to be resistant to the same antibiotics as *V. alginolyticus*. *V. parahaemolyticus* exhibit an intermediate resistance to tetracycline, an antibiotic important in the treatment of gastroenteritis [6, 20, 36, 46]. This is consistent with the results of the present study in which it was found that *Vibrio* isolates obtained from hatcheries showed more antibiotic resistance than those from *P. monodon* culture ponds. In the hatcheries, larger quantities and newer antibiotics are used as compared with the situation on farms. Though the use of antibacterial agents is a convenient means of controlling bacterial infection, rapid development of antibiotic resistance in bacteria and emergence of drug resistant microbial diseases in aquaculture industries poses serious environmental, economic and management problems and in addition creates human health hazards. Treatment of resistant microbial infections in fish and shrimp hatcheries involves increasing the dosage of broad-spectrum chemotherapeutic agents or using newer more potent antibiotics in the culture medium or feed. Clearly these practices exacerbate the existing problems of antibiotic resistance and residue contamination of the environment and the product. In conclusion of the current study also indicates that the source seawater in the Bay of Bengal is highly contaminated with bacteria especially Vibrios. They are mainly originated from industry and domestic sources. The shrimp hatcheries and culture ponds in India are mostly located along the coastal areas as they have easy access to sea and fresh water. The untreated aquaculture effluents from the shrimp hatcheries and farms contaminated with *Vibrio* spp. are drained off into the coastal areas and these may be one of the primary sources contributing to the increased concentration of bacteria in the coastal area of Bay of Bengal, India. The results also indicated that persistent use of antibiotics against diseases in human beings and other life forms may pollute the aquatic system and their impact on developing antibiotic resistant Vibrio sp may be a serious threat in addition to the use of antibiotics in aquaculture farms. The use of few antibiotics on shrimp farms has been banned in recent years and at present there are no licensed chemotherapeutic compounds that are effective against emerging bacterial pathogens. Control of disease depends on improvement of management practices to minimize the risk of introduction of infection into aquaculture systems,

and to reduce predisposing factors such as overcrowding and over feeding.

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Table 1: Biochemical and Phenotypical characteristics of Vibrio spp. (n – 121) isolated from the aquacultural environment

Sl. No.	Biochemical tests	*Vibrio harveyi* (n-26)	*Vibrio anguillarum* (n-21)	*Vibrio damsela* (n-15)	*Vibrio parahaemolyticus* (n-11)	*Vibrio vulnificus* (n-10)	*Vibrio fluvialis* (n-9)	*Vibrio alginolyticus* (n- 8)	*Vibrio mimicus* (n-7)	*Vibrio furnissi* (n- 7)	*Vibrio cholerae* (n-4)	*Vibrio ordalli* (n-3)
1.	Gram staining	-	-	-	-	-	-	-	-	-	-	-
2.	Citrate Utilization	+/-	+	-	-	+/-	-	-	+	+	+	-
3.	Methyl red test	+	+/-	+	+	+	+/-	NT	+	+	+	+/-
4.	Vogas Proskauer test	+/-	+	+	-	-	-	+	-	-	-	-
5.	Motility	-	+	+/-	+	+	+/-	+	+	+	+	+
6.	Starch hydrolysis	-	+	-	-	-	-	-	-	-	-	+
7.	H_2S production	-	-	-	-	-	-	+	-	-	-	-
8.	Oxidase test	+	+	+	+	+	+	+	+	+	+	+
9.	Gelatin hydrolysis	-	+	-	+	+	+	+	+/-	+	+	-
10.	Catalase test	+	+	+	+	+	+	+	+	+	+	+
11.	Arginine dehydroxylase	-	-	+	+	-	+	-	-	+	-	-
12.	Indole production test	+	+	-	+	+	-	+	+	-	+	NT
13.	Amino acid decarboxylase (lysine)	+	+	+/-	+	+	-	+	+	-	+	-
14.	Urease test	+	-	+/-	+	+	+	+	+	+	+	+
15.	Growth in DNAse agar	+	+	+	+	+	+	+	+	+	+	NT
16.	Lipid hydrolysis	+	NT	+	+	+	+	+	+	+	+	+
17.	Nitrate reduction test	+	+	+	+	+	+	+	+	+	+	-
18.	Carbohydrate fermentation test											
	a. Sucrose	+/-	+	-	-	-	+	+	-	+	+	+
	b. Lactose	-	-	-	-	+	-	-	-	-	-	-
	c. Glucose	+/-	+/-	+	+	+	+	-	+	+	+	+/-
	d. Fructose	+	-	+	-	+	+	+	+	+	+	+
	e. Maltose	+	+	+	+	+	+	+	+	+	+	-
	f. Galactose	-	+	+	-	+	+	+	+/-	+	+	-
	g. Sorbitol	-	+	-	-	-	-	-	-	-	-	-
	h. Inositol	-	-	-	-	-	-	-	-	-	-	-
	i. Arabinose	-	+	-	+	-	+	-	-	+	-	-
	j. Mannitol	+/-	+	-	+	+/-	+	+	+	+	+	-
	k. Melibiose	-	-	-	-	+/-	-	-	-	+/-	-	-
	l. Trehalose	+/-	+	+/-	+	+	+	+	+	+	+	-
	m. Rhamnose	-	-	-	-	-	-	-	-	-	-	-
19.	Growth rate											
	4°C	-	-	-	-	-	-	-	-	-	-	-
	10°C	+	+	+	+	+	+	+	+	+	+	-
	30°C	+	+	+	+	+	+	+	+	+	+	-
	40°C	+	+	+	+	+	+	+	+	+	+	-
	50°C	-	+/-	-	+	+	+	-	+/-	-	+	+
	60°C	-	-	-	-	-	-	-	-	-	-	+
20.	Growth in peptone containing different % of NaCl											
	0%	-	+/-	-	-	-	-	-	+	-	+	-
	2%	+	+	+	+	+	+	+	+	+	+	+
	4%	+	+	+	+	+	+	+/-	+	+	+	+
	6%	+	+	+	+	+/-	+	+	+/-	+	+/-	+
	8%	-	-	-	+	-	+/-	+	-	+/-	-	-
	10%	-	-	-	+	-	-	+	-	-	-	-
21.	Sensitivity to 0/129 *Vibrio* static agent	+	+	+	+/-	+	+/-	+	+	+	+	+
22.	Growth in TCBS agar	+	+	+	+	+	+	+	+	+	+	+
23.	Polymixin – B	+	-	+	+	-	+	NT	+	+	+/-	+

NT = Not tested, (+) - Positive, (-) = Negative

Table 2: Sources and total number of Vibrio spp. isolates recovered from the shrimp aquacultural environments

Sources of *Vibrio* isolation	**Total number of different species of *Vibrio***											
	V. harveyi	*V. anguillarum*	*V. damsela*	*V. parahae molyticus*	*V. vulnificus*	*V. fluvialis*	*V. algino lyticus*	*V. mimicus*	*V. furnissi*	*V. cholerae*	*V. ordalli*	**Total**
Egg samples	2	-	-	-	-	1	-	-	-	-	-	**3**
Artemia nauplii	2	1	1	-	-	-	-	2	2	-	-	**8**
Artemia reared tank water	2	-	-	-	-	-	-	1	-	-	-	**3**
Hatchery tank water	1	-	-	-	1	-	-	-	-	-	2	**4**
MBV uninfected post larvae	1	1	-	-	-	1	-	1	-	-	-	**4**
MBV infected post larvae	4	3	1	-	-	1	-	1	1	-	-	**11**
Sea water	1	1	-	4	-	-	-	-	-	-	-	**6**
Sea sediment	1	1	1	2	-	-	-	-	-	-	-	**5**
Shrimp culture pond water (Tuticorin)	-	-	1	-	1	-	1	-	-	1	-	**4**
Shrimp culture pond sediment	-	1	1	-	-	-	1	-	1	1	-	**5**
Shrimp culture pond water (Nellore)	-	1	3	-	1	-	-	-	1	-	-	**6**
MBV/WSSV uninfected *Penaeus monodon*	1	1	-	-	1	-	-	-	1	-	1	**5**
WSSV infected pond water	1	1	-	1	1	-	1	-	1	-	-	**6**
WSSV infected pond sediment	1	2	2	-	1	-	-	-	-	2	-	**8**
WSSV infected hepatopancreas	4	3	2	2	1	1	3	-	-	-	-	**16**
WSSV infected intestine	3	1	-	1	-	-	-	-	-	-	-	**5**
WSSV infected stomach	1	-	2	-	3	2	2	-	-	-	-	**10**
WSSV infected muscles	-	2	1	1	-	2	-	2	-	-	-	**8**
WSSV infected gills	1	2	-	-	-	1	-	-	-	-	-	**4**
Total No. of isolates	**26**	**21**	**15**	**11**	**10**	**9**	**8**	**7**	**7**	**4**	**3**	**121**

Table 3: Antibiotic resistance pattern of selected isolates of Vibrio spp. obtained from the aquacultural environment

Antibiotics (mcg/units)	***V. harveyi***	***V. anguillarum***	***V. damsela***	***V. parahae molyticus***	***V. vulnificus***	***V. fluvialis***	***V. alginolyticus***	***V. mimicus***	***V. furnissi***	***V. cholerae***	***V. ordalli***	**Resistant (%)**	**Sensitive (%)**
Ampicillin (10 mcg)	-	-	-	-	-	-	-	-	-	-	-	100	0
Cloxacillin (1mcg)	-	-	-	-	-	-	-	-	-	-	-	100	0
Oxacillin (1 mcg)	-	-	-	-	-	-	-	-	-	-	-	100	0
Erythromycin (15 mcg)	-	R	-	-	-	R	-	R	R	-	-	100	0
Vancomycin (30 mcg)	-	R	-	R	-	R	-	-	R	R	-	100	0
Penicillin G (10 units)	R	R	R	R	R	R	R	R	R	R	R	100	0
Furazolidone (50 mcg)	R	R	R	R	R	R	R	R	R	R	R	100	0
Cefaclor (30 mcg)	R	S	R	-	R	R	-	R	R	R	-	90.0	9.10
Streptomycin (10 mcg)	S	R	R	R	R	R	R	R	R	S	R	81.2	18.18
Rifampicin (5 mcg)	R	S	-	R	-	R	R	S	-	R	R	81.2	18.18
Oxytetracycline (30 mcg)	R	R	R	R	R	R	R	S	R	S	S	72.3	27.27
Nalidixic acid (30 mcg)	S	-	-	S	-	-	-	-	S	-	S	63.4	36.36
Cepholaxime (30 mcg)	S	R	R	R	S	R	R	S	R	S	S	54.5	45.45
Chlorotetracycline (30 mcg)	S	R	R	R	S	S	R	R	R	S	S	54.5	45.45
Norfloxacin (10 mcg)	S	S	R	R	R	S	S	R	S	S	R	45.5	54.55
Azithromycin (15mcg)	-	S	-	S	S	S	-	S	-	S	-	45.4	54.55
Neomycin (30 mcg)	R	S	R	S	S	S	S	S	S	S	R	27.2	72.73
Chloramphenicol (30 mcg)	S	S	S	S	-	R	R	S	S	S	S	27.2	72.73
Gentamycin (10 mcg)	R	S	R	S	S	S	R	S	S	S	S	27.2	72.73
Ciprofloxacin (10 mcg)	S	S	S	S	R	S	S	S	S	S	-	18.2	81.82
Amikacin (30mcg)	S	S	R	S	S	S	S	S	S	S	R	18.2	81.82

R = Resistant S = Sensitive (-) = No zone of lysis