

Isolation and identification of *Vibrio* sp. in the Hepatopancreas of cultured white pacific shrimp (*Litopenaeus vannamei*)

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ABSTRACT. The *Vibrio* is the most common genera with crustaceans often causing various diseases in Aquaculture and significant economic losses. Many *Vibrio* species are pathogenic to human and have been implicated in food borne diseases. The present study was carried out, the isolation and identification of pathogenic bacterial flora were isolated from infected in hepatopancreas of *vannamei*. The SPDS Oceanic farm, RS Aqua farm of and Valli vilas Aqua farm Vellar estuary, Cuddalore District, Tamil Nadu, during the period of (September 2013 to November 2013). The collected samples were plated on TCBS- (Thiosulfate-Citrate-Bile salt-Sucrose) agar medium. The present study, totally 253 green colonies were isolated from TCBS agar plates and among these, 175 colonies were identified by using the biochemical tests showed the *V. parahaemolyticus*, *V. mimicus*, *V. vulnificus*, *V. damsela* and *P. shigelloides*. The maximum species was recorded in *V. parahaemolyticus* (83.4 %) and minimum was observed in *V. mimicus* (1.7 %).

1. INTRODUCTION

Aquaculture is the world's fastest growing food production sector, regarding cultured shrimp and prawn the annual rate reach up to 16.8% between 1984 and 1995 [1]. Shrimp cultivation is one of these most economically important agricultural activities in Asia and South America, and also practiced on worldwide [2]. In international trade, the most prominent product from aquaculture is marine shrimp. Which approximately 26 % of the total product comes from pond-reared *Penaeid* species [3]. *P. vannamei* and *P. monodon* (taxonomy according to [4] are the predominant species of farm-raised shrimp cultivated in both the Eastern and the Western hemispheres. As with any monoculture, raising shrimp in ponds in close proximity to each other increases the spread of diseases [5]. However, shrimp cultivation have been faced many serious problems such as shrimp diseases, unsatisfactory practices, i.e. inadequate control of water quality, etc. The most severe diseases of shrimp, causing the greatest economic losses to growers, are caused by Viruses and Bacteria [6]. This kind of diseases attacked due to bacterial infections, particularly caused by luminous *Vibrio*. [7]. *Vibrio* is well recognized as significance of disease and mortality. Two species, *V. cholerae* and *V. parahaemolyticus* are well documented as human pathogens. It also causes several fish diseases with serious problems for a wide range of wild and farmed species [8].

Pathogenic bacteria have also been involved in this crisis *vibrio* species are among the most important bacterial pathogens of cultured shrimp, responsible for up to 100% stricken, species such as *V. harveyi*, *V. anguillarum*, *V. parahaemolyticus* and *V. vulnificus* have been frequently associated with mortalities both in hatcheries and grow out ponds [9, 10]. The trend of above, the present investigation has been made on isolation and identification of *Vibrio* species from infected hepatopancreas of *P. vannamei*.

2. MATERIALS AND METHODS

Collection of samples

In the present study the infected shrimp samples were collected at three shrimp aquaculture farms (SPDS, RS and Valli vilas) which are located at southern and northern banks of Vellar estuary, Parangipettai, India. During the period of March to May 2014. The collected samples were transferred to laboratory and stored at refrigerator for further analysis.

Isolation of shrimp pathogenic Bacterial colonies:

The hepatopancreas of infected shrimp was dissected and removed for pathogenic bacteria isolation. The samples are serially diluted 10^{-1} to 10^{-9} factor. 0.1 ml of samples from 10^{-5} , 10^{-6} and 10^{-7} were sucked and spread over the sterilized TCBS- (Thiosulfate –Citrate- Bile salt-Sucrose) medium of Peptone-10.00g, Yeast extract- 5.00g, Sodium citrate- 10.0g, Sodium thiasulphate-10.0g, Sodium cholate- 3.0g, Oxgall- 5.00g, Sucrose- 20.0g, Sodium chloride- 10.0g, Ferric citrate-1.00g, Bromo thymolblue-0.04g, Thymol blue- 0.04g, Agar- 14.00g, Final pH(at 20°C) 8.6 ± 0.1 and 1000 ml distilled water and the petri plated are placed overnight at 37- 45 °C at incubator. The incubation period was maintained for 24 to 48 hours at 37°C for the pathogenic culture.

Purification and conservation of isolates

For the *Vibrio* species identification of each sample, 115 yellow colonies and 253 green colonies were selected from TCBS plates. A total of 175 green colonies were isolated then purified and stored in TCBS agar slant for further studies.

Biochemical identification of shrimp bacteria

The isolated bacterial species were identified by the following the morphological and biochemical characteristics of the individual colony was recorded. The individual colony was transferred to nutrient agar. The isolates were subjected to following different biochemical test such as Gram staining, Motility, Morphology, Indole test, Methyl red test, Pigmentation test, Voges proskauer test, Citrate test, Urease test, Lactose test, H₂S production and starch hydrolysis as described by [11].

3. RESULTS AND DISCUSSION

In the present investigation, totally 368 pathogenic bacterial colonies were isolated from infected hepatopancreas of *P. vannamei*, of which 253 green colonies and 175 yellow colonies (Table 1). In this study was showed *V. parahaemolyticus*, *P. shigelloides*, *V. damsela*, *V. mimicus* and *V. vulnificus* were record. The maximum was recorded in *V. parahaemolyticus* (83.4%) and minimum was recorded of *V. mimicus* (1.7%) (Table 2).

Similar works were done by many researchers on other aquaculture farms [12]. Have surveyed *P. monodon* culture ponds of coastal Andhra Pradesh and isolated six species of *Vibrios* viz., *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and *V. splendidus*. Farm-made feeds showed a high incidence of *V. parahaemolyticus*, *V. cholerae*, *E. coli* and *Staphylococcus aureus* [13]. Five species of *Vibrio* viz., *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis* and *V. mimicus* were detected in the pond water and the prawn body with *V. alginolyticus* and *V. parahaemolyticus* as the dominant species for all ponds [14]. [15] Isolated 143 *V. cholerae* non O1 strains from shrimp farms in Thailand. Mass mortalities due to red disease from *V. parahaemolyticus* along with other *Vibrio* spp. were isolated by from ponds in the Philippines [16]. The number of *Vibrio* in farm sediment was reported to be 10 to 20 times higher than those in water column [17]. The dominant species (47.5%) belonged to the genus *Vibrio* in water samples from *P. monodon* pond in Taiwan [18]. Severe stress and injury to shrimp under poor environmental conditions lower their resistance, rendering them susceptible to viral as well as bacterial infection [19]. *Vibriosis* is known to affect a wide range of fish and shellfish organisms [20, 21]. In the present study bacterial species were isolated from infected shrimp muscle tissues.

Epizootiology and pathogenicity of bacterial infections caused by many problems in cultured giant tiger prawn *Penaeus monodon* [22]. *Vibrio* species are considered as part of the normal flora of seawater and can invade marine animals [23]. [24] Has found total *Vibrio* loads in healthy and infected shrimp during a complete 60-day culture cycle. However, sick shrimp presented an increase in luminescent *vibrios* suggesting that infection involves the multiplication of a specific population of pathogens

4. CONCLUSION:

The present study revealed that the pathogenic bacteria are perhaps those most important pathogens in shrimp culture ponds causing several mortalities and financial losses. There is also a need to develop the shrimp culture practice and control the pathogenic microbes is very essential.

Table: 1 SPDS, Oceana farms from yellow and green colonies were isolated.

SPDS, Oceana farms	Serial dilution 10^{-5}	Serial dilution 10^{-6}	Serial dilution 10^{-7}	Total <i>vibrio</i> colonies	Yellow Colonies	Green Colonies
Pond 1	30	12	3	35	14	21
Pond 2	16	7	2	25	11	14
Pond 3	32	8	1	41	18	23
Pond 4	10	4	0	14	5	9
Pond 5	21	13	4	38	7	31
Total				153	55	98

Table 2: RS Aqua farms from yellow and green colonies were isolated.

RS Aqua farms	Serial dilution 10^{-5}	Serial dilution 10^{-6}	Serial dilution 10^{-7}	Total <i>vibrio</i> colonies	Yellow colonies	Green colonies
Pond 1	31	10	4	45	10	35
Pond 2	28	16	2	46	17	29
Pond 3	16	8	1	25	12	13
Total				116	39	77

Table 3: Valli vilas farms from yellow and green colonies were isolated.

SPDS, Oceana farms	Serial dilution 10^{-5}	Serial dilution 10^{-6}	Serial dilution 10^{-7}	Total <i>vibrio</i> colonies	Yellow Colonies	Green Colonies
Pond 1	32	15	1	48	8	40
Pond 2	33	17	0	51	13	38
Total				99	21	78

No. of colonies	Gram staining	Mortality	Morphology,	Indole	Methyl red	Pigmentation	Voges- proskauer	Citrate	Urease	Lactose	H ₂ S production	Starch hydrolysis	Identification of bacteria
Ss1	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss2	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss3	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss4	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss5	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss6	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss7	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss8	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss9	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss10	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss11	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P. shigelloides</i>
Ss12	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss13	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss14	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss15	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss16	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss17	-	+	rod	-	+	G	+	-	+	-	-	+	<i>V. damsela</i>
Ss18	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss19	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss20	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss21	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss22	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss23	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss24	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss25	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss26	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss27	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss28	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss29	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss30	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss31	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss32	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss33	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss34	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss35	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss36	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss37	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss38	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss39	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss40	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss41	-	+	rod			G	-	-	-	-	-	+	<i>V. mimicus</i>
Ss42	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss43	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss44	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss45	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss46	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss47	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss48	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss49	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss50	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss51	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss52	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss53	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss54	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>

Ss55	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss56	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss57	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss58	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss59	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss60	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss61	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss62	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss63	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss64	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss65	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss66	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss67	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss68	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss69	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss80	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss81	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss82	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss83	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss84	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss85	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss86	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss87	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss88	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss89	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss90	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss91	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss92	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss93	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss94	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss95	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss96	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss97	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss98	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss99	-	+	rod			G	+	-	+	-	-	+	<i>V. damsela</i>
Ss100	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss101	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss102	-	+	rod			G	-	-	-	-	-	-	<i>V. mimicus</i>
Ss103	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss104	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss105	-	+	rod			G	-			-			<i>V. mimicus</i>
Ss106	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss107	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss108	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss109	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss110	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss111	-	+	rod	+	+	G	-	+	+	-	-	-	<i>V. vulnificus</i>
Ss112	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P. shigelloides</i>
Ss113	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P. shigelloides</i>
Ss114	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P. shigelloides</i>
Ss115	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss116	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss117	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss118	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss119	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss120	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss121	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss122	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss123	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss124	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>

Ss125	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss126	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss127	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss128	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss129	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss130	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss131	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss132	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss133	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss134	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss135	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss136	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss137	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss138	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss139	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss140	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss141	-	+	rod			G	+	-	+	-	-	+	<i>V.damsela</i>
Ss142	-	+	rod			G	+	-	+	-	-	+	<i>V.damsela</i>
Ss143	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss144	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss145	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss146	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss147	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss148	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss149	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss150	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss151	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss152	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss153	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss154	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss155	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss156	-	+	rod	+	+	G	-	-	+	-	-	-	<i>P.shigelloides</i>
Ss157	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss158	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss159	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss160	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss161	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss162	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss163	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss164	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss165	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss166	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss167	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss168	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss169	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss170	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss171	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss172	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss173	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss174	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>
Ss175	-	+	rod	+	+	G	-	-	-	-	-	+	<i>V. parahaemolyticus</i>

Table 4. Biochemical identification of pathogenic bacterial isolate from infected Hepatopancreas *vannamei*

Name of the pathogen	% of vibrio species
<i>V. paraheamolyticus</i>	83.4
<i>V. vulnification</i>	5.3
<i>V. mimicus</i>	1.7
<i>V. damsela</i>	2.2
<i>P.shigelloides</i>	7.1

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