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Isolation and Identification of Vibrios from Diseased Tiger Shrimp (*Penaeus Monodon*)

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ABSTRACT

The study aims to isolate and identify various pathogenic vibrios associated with Tiger shrimp (Penaeus monodon). Different biochemical tests were performed which confirmed the different types of vibrios present in the diseased shrimp based on their colony morphology and response to various biochemical tests. Antibiotic resistance pattern study confirmed that all the isolates were to be sensitive to chloramphenicol, oxytetracycline, erythromycin and nalidixic acid. The isolates were also completely resistant to certain antibiotics like ciprofloxacin, oxolinic acid and cefuroxime.

KEYWORDS: *Tiger shrimp, Vibrios, biochemical tests, ABST*

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INTRODUCTION

The majority of infections are attributed to the consumption of raw and insufficiently cooked sea food products. The number of *Vibrio species* classified as pathogenic is at least 11 (Janda *et al*., 1988 and Holmberg 1992) including *V. cholerae* as the main cause of diarrhoea, *V. parahemolyticus* as the cause of food borne gastroenteritis (Ozer *et al*., 2008; Pruzzo *et al*., 2005) and *V. vulnificus* which is known to cause 95% of all deaths associated with the consumption of sea food (Rosche *et al*., 2005). Other pathogenic species includes *V. alginolyticus, V. holisae, V. mimicus* etc (Pruzzo *et al*., 2005). *The Vibrio sps isolated from marine infected shrimp should be cooled immediately into about 7°C-10°C and then should be analysed as soon as possible. Vibrios might be injured if they undergo a rapid cooling. It is better to avoid any direct contact of the samples with ice in order to maximise the survival and existence of Vibrios. TCBS have been recommended as the standard method for the isolation of various Vibrio sps. Most Vibrio sps have a considerable growth on TCBS while the growth of non Vibrios is inhibited on this medium.*

The method also includes an enrichment in Alkaline Peptone Water (APW) at 35±2° C overnight and then isolation on TCBS medium. Preliminary identification of *Vibrio sps* can be performed on the basis of colony appearance on selective media such as thiosulfate – citrate – bile salts – sucrose (TCBS) agar (Di Pinto *et al*., 2011), followed by conventional biochemical tests like oxidase, TSI, sulphur reduction, motility, Indole, Methyl Red (MR), Voges Proskauer (VP) and salt tolerance tests. Media should be prepared with 2- 3 % NaCl to allow the growth of halophilic species. The present study was aimed to isolate and identify different *Vibrio sps* and understand their quorum sensing molecules.

MATERIALS AND METHODS

Collection of pathogenic bacteria

Based on the occurrence and prevalence the bacterial samples were collected from Tiger shrimp. The collection procedure and sampling techniques are as follows: -

Isolation of pathogenic Vibrio from shrimps

Shrimps (*Penaeus monodon*) were collected from shrimp farms at Kancheepuram district of Tamil Nadu. The collected shrimp were kept in an icebox and transported to the laboratory and stored at – 20° C (Kumaran T and Citarasu T. 2016). The infected samples were washed 4 times with 100 ml of sterile sea water on sterile filters. It was then homogenised in a sterile homogeniser with sterile water and the samples were serially diluted upto about 10-fold. Approximately one hundred micro litres of these samples

were placed on TCBS agar medium. All the plates were incubated between 28°C and 30°C.

Biochemical characterisation

Selected colonies which had the characteristic morphology of *Vibrio sps* were confirmed by conventional biochemical methods. The tests were conducted as described by Feltham and Barrow (1993) and Kaysner *et al*., (2004). The various tests performed were morphology, Gram staining, motility, oxidase test, catalase test, IMViC, carbohydrate fermentation test, nitrate reduction test, hydrogen sulphide production and ONPG hydrolysis. Halophilism tests were performed on Trypticase soya agar (TSA) and supplemented with different concentrations (0%, 0.5%, 1%, 3%, 6%, 8% and 10%) of NaCl.

Antibiotic Susceptibility Test

The isolates were grown in nutrient broth containing 2% NaCl. Antibiotic sensitivity tests were then carried out on Muller Hinton Agar (MHA) medium (HIMEDIA) plates by Kirby Bauer disk diffusion methods (Bauer *et al*., 1966). The antibiotic discs used were Chloramphenicol (CK 30 mcg), Furozolidone (FZ 50 mcg), Norfloxacin (NF 10mcg), Ciprofloxacin (CI 5 mcg), Oxytetracycline (O 30 mcg), Gentamycin (GM 10 mcg), Erythromycin (ER 15 mcg), Ofloxacin (OF 5 mcg), Rifampicin (RN 5 mcg), Cefuroxime (CR 30 mcg), Ampicillin (AP 10 mcg), Nalidixic acid (NA 30 mcg). The 24-hour broth cultures of the isolates were lawn cultured and the antibiotic discs were placed by using alcohol dipped and flamed forceps on the surface of the medium. The plates were left for 20 minutes before incubation at 37°C for 24 hours. Characterisation of the isolates like susceptible or resistant was done based on the size of the inhibition zones around each disc.

RESULTS AND DISCUSSIONS

Isolation of the pathogenic bacteria

The organisms were isolated from pathogenic shrimps and the positive controls on TCBS were identified based on their color and morphology on TCBS. The isolates were named as SPB 1, SPB2, SPB 3and SPB 4.

Characterization of the pathogenic bacteria

In the initial stages, conventional biochemical tests were conducted to identify the Vibrio colonies, the results obtained were presented in the table 1.

Table1. Biochemical characteristics of the bacterial isolates

Characteristics	SPB1	SPB 2	SPB 3	SPB 4
Shape	Short rod	Short rod	Short rod	Short rod
Gram Staining	_	_	_	_
Growth on TCBS agar	Yellow colonies.	Large yellow colonies.	Flat yellow colonies.	Blue to green centered colonies.
Sensitivity to 0/129 phosphate	+	+	+	+
Luminescence	+	+	-	+
Swarming	+	+	+	_
Oxidase production	+	+	+	+
Catalase production	+	+	+	+
Oxidative-fermentive test	F	F	F	F
Acid/gas production: Glucose	_	Acid	Acid	Acid
Sucrose	Acid	Acid	Acid	_
Mannitol	Acid	Acid	Acid	Acid
Maltose	Acid	Acid	Acid	Acid
Sorbitol	_	_	_	_
Lactose	_	_	-/+	_
Galactose	Acid	Acid	+	_
Arabinose	Acid	_	_	Acid
Nitrate reduction	+	+	_	+
Indole production	+	+	+	+
Methyl red	+	+	_	_
Voges-Proskauer	_	_	-/+	_
Simmon's citrate		+	+	_
Hydrogen sulfide production	_	_	_	_
ONPG hydrolysis	+	_	+	+
Decarboxylase of: Arginine	-	-	-	-
Lysine	+	+	+	+
Ornithine	+	+	+	+
Growth in: 4ºC	_	_	+	_
40ºC	+	+	+	+
Growth in peptone with NaCl 0%	_	_	+	_
0.5%	+	_	+	_
1%	+	_	+	_
3%	+	+	+	+
6%	+	+	_	+
8%	+	_	_	+
10%	_	+	_	_

Production of exo-cellular enzymes: Amylase	+	+	+	+
Caseinase	+	_	_	_
Gelatinase	+	+	+	+
Chitinase	+	+	_	+
Urease	_	_	_	-/+

Antibiotic Sensitivity test of the strains

The antibiotic sensitivity test of the isolates was carried out and the results are compiled in table 2 and the zone of inhibition is represented in table 3.

Table2. Antibiotic resistant pattern of the isolates

S. No	Antibiotics used	Sensitivity of the isolates			
		SPB 1	SPB 2	SPB3	SPB4
1.	Chloramphenicol	S	S	S	S
2.	Furozolidone	S	R	S	S
3.	Norfloxacin	R	R	R	R
4.	Ciprofloxacin	R	R	R	R
5.	Oxytetracycline	S	S	S	S
6.	Gentamycin	R	S	S	S
7.	Erythromycin	S	S	S	S
8.	Oxolinic acid	R	R	R	R
9.	Rifampicin	R	R	R	R
10.	Cefuroxime	R	R	R	R
11.	Ampicillin	R	S	S	R
12.	Nalidixic acid	S	S	S	S

S---Sensitive R---Resistant

Table3. Zone of inhibition of the isolates

S. No	Antibiotics used	Zone of inhibition of isolates in mm (mean ± SD)			
		SPB 1	SPB 2	SPB 3	SPB 4
1.	Chloramphenicol	16 +1	13+0	11+2	12+1
2.	Furozolidone	18+2	_	12+1	10+0
3.	Norfloxacin	_	_	_	_
4.	Ciprofloxacin	_	_	_	_
5.	Oxytetracycline	16+1	17+2	10+2	13+1
6.	Gentamycin	_	15+2	9+1	11+0
7.	Erythromycin	10+1	11+1	8+0	15+0
8.	Oxolinic acid	_	_	_	_
9.	Rifampicin	_	_	_	_
10.	Cefuroxime	_	_	_	_
11.	Ampicillin	_	14+2	13+1	_
12.	Nalidixic acid	18+1	21+1	17+2	20+2

Table4. The isolates identified based on their colony morphology and biochemical characterisation

Designation of the isolate	Organism
SPB 1	*Vibrio harveyi*
SPB 2	*Vibrio alginolyticus*
SPB3	*Vibrio cholerae*
SPB4	*Vibrio parahemolyticus*

DISCUSSION

The shrimp aquaculture industry is experiencing incredible monetary misfortunes because of mass mortalities experienced because of the rate of *Vibrios*. In this manner this present part of the investigation is devoted for the portrayal of the *Vibrios* by biochemical methods. On TCBS, particular yellow isolates were recognized as that of *V. alginolyticus, V. harveyi* and *V. cholerae*. Green or blue isolates were the *V. parahemolyticus*. Di Pinto *et al.,* 2008 proposed that *Vibrio* development is more particular in TCBS agar. Sucrose fermentation was utilized to separate *V. parahemolyticus* from *V. alginolyticus*, *Vibrio harveyi* and *Vibrio cholerae*, since this disaccharide can ferment and express the outcomes in an unexpected way. The sucrose fermenters show up as yellow colonies and non-sucrose fermenters show up as green colonies. As per the Bergey's Manual of Systematic Bateriology (2005), the different Vibrio species that can absorb glucose are *V. cholera*e (100%), *V. parahemolyticus* (0%), *Vibrio alginolyticus* (100%), *Vibrio harveyi* (83%), *Vibrio vulnificus* (20%), *Vibrio proteolyticus* (0%) and *Vibrio fischeri* (0%). Difficulties were found with the recognizable proof of different *Vibrios* isolated from food stuffs utilizing biochemical tests on the grounds that the sample contain related types of *Vibrio* or yet uncharacterised microscopic organisms which may give

comparable outcomes amid biochemical distinguishing proof (Thompson *et al.,* 2006).

Antibiotic resistant pattern was seen in all the utilized twelve anti-microbials. The cultures were observed to be sensitive just to specific anti-microbials like chloramphenicol (all the isolates), furozolidone (aside from *V.alginolyticus*), oxytetracycline (all the detaches), gentamycin (with the exception of *V. harveyi),* erythromycin (all the isolates), ampicillin (just *V. alginolyticus* and *V. cholerae* were sensitive) and nalidixic acid (all the isolates). As indicated by Gomathi *et al*, (2013) the frequency of anti-infection safe microorganisms is biologically imperative and this character is plasmid borne. These plasmids additionally have the ability to convey transferable medication obstruction. The anti-toxin safe vibrios may survive preferable in the water over the delicate ones. The antibiogram profiling uncovered that all the *Vibrio* isolates additionally now and then demonstrated multi sedate protection from nalidixic acid, oxytetracycline, chloramphenicol. This resistance may be due to the enzymatic destruction of antibiotics completely, impermeability of the cell wall to the antibiotics, addition of chemical groups to the antibiotics.

REFERENCE

[1] Di Pinto A, Ciccarese G, De Corato R, Novell L and Terio V. 2008. Detection of pathogenic *V. parahemolyticus* in Southern Italian shell fish. *Food Control*. 19: 1037-1041.

[2] Feltham R K A and Barrow G I. 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge Uni. Press.

[3] Janda J M, Powers C, Byrant R G and Abbott S L.1988. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio sps*. Clinical Microbiology Reviews. 1:245-267.

[4] Kaysner C A, DePaola A. 2004. Bacteriological analytical manual chapter 9: Vibrio.

[5] Gomathi R S, Vinothkumar R, Arunagiri K. 2013. Isolation and identification Vibrios from marine sea food samples. *Int. J. Curr. Microbiol. Appl. Sci.*2: 36-43.

[6] Ozer S, Aslan G, seda Tezchn, Bulduklu P S, Mehmet sami serin and Emekdas G. 2008. Genetic Hetero genetic and Antibiotic susceptibility of V alginolyticus strains isolated from Horse-Mackerel (*Trachurus trachurus* L.). *J. Vet. Anim. Sci*. 32(1):45-53.

[7] Pruzzo, C. A., R. Huq, R. Colwell and G. Donelli. 2005. Pathogenic *Vibrio Species* in the Marine and Estuarine Environment. In Oceans and Health: Pathogens in the Marine Environment, ed. Shimshan S. Belkin and Rita R. Colwell: 217-252.

[8] Rosche, T. M., Y. Yano, and J. D. Oliver. 2005. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol. Immunol*. 49:381-389.

[9] Kumaran, T. and Citarasu, T., 2016. Isolation and characterization of Vibrio species from shrimp and Artemia culture and evaluation of the potential virulence factor. *Intel Prop Rights*, *4*(153), p.2.

[10] Holmberg, S. D., 1992. Cholera and related illnesses caused by Vibrio species and Aeromonas. *Infectious diseases. Philadelphia: Saunders*.

[11] Thompson F L, Austin B and Swings J.2006. The Biology of Vibrios. Washington D C., A S M Press.