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In-vitro susceptibility of selectively used Antibiotics against *Vibrio spp*. isolated from Tiger Shrimp (Penaeus monodon)

Muralidharan Velappan[1] and **Deecaraman Munusamy**[2]

[1]AMET University, India
[2]Dr M G R Educational & Research Institute, India

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The sensitivity of 50 isolates of Vibrio spp. isolated from edible portion of muscle of Tiger shrimp (Penaeus monodon) was collate using selectively used antibiotics. The in vitro susceptibility of the isolates was designed by disc diffusion method employing discs contained Doxycycline, Chloramphenicol, Ofloxacin, Erythromycin, Enrofloxacin and Ciprofloxacin. Further studies explain Erythromycin and Chloramphenicol shows high degree of bacterial resistance in Tiger shrimps.

Introduction: Worldwide the bacterial disease caused by Vibrio spp. are primary responsible for mortality of cultured shrimp [1-3]. Vibriosis is induced by gram-negative bacteria belonging to the family Vibrionaceae. It is very common vibrio- related infections occur in hatcheries, the major Vibrio spp. isolated from shrimp and lobster are V. harveyi, V. parahaemolyticus, V. vulnificus, V. alginoilyticus, V. anguillarum [4]. Mortalities due to vibrios is ensued when shrimps are stressed by factors such as poor water quality, overcrowding, high water temperature, low DO and low water exchange [5]. Luminescent type of V. harveyi seems to release exotoxins and cause 80-100% mortality in P. monodon hatcheries [6]. V. anguillarum, V. campbelli, and V.cholerae (non 01) have also been reported in association with disease outbreaks in shrimps [7-9].

Currently 12 species of Vibrio genus are known to cause human infections through consumption of contaminated sea foods [10]. Where V. vulnificus is associated with severe wound and soft tissue infections such as septicemia particularly persons with compromised immune system. The presence of V. harveyi (97.30%) and V. orientalis (2.70%) in shrimp gutcontents evinced that the primary source of these bacteria in a shrimp hatcherywas the faecal matter from brood stock, perhaps at the time of spawning [11]. A highly pathogenic strains of Vibrio sp. are also continuous emerging to cause heavy mortalities among cultured shrimp [12]. Hence, the present study was investigated to determine the degree of antibacterial resistance of Vibrio spp. isolated from Tiger shrimp (Penaeus monodon) hatcheries in and around Chennai Tamilnadu.

Materials and method:

Sampling Process: Seventy five Tiger shrimps (Penaeus monodon) were caught from hatcheries, Chennai, Tamil Nadu during May 2017 to July 2018. The samples were taken into sterile bags, kept in ice bath. As the first step for the investigation, 225ml of alkaline peptone water (APW) was added to 25 g of homogenized Tiger shrimp and incubated for 370 C for 6-8hr. As per FDA, (1992) guidelines two loop full of the culture broth taken from the layer of the APW and undergone a series of 10 fold dilutions from each dilution 10ml was plated on TCBS agar by the pour plate method and incubated at 370C for 18 – 24 hr. The obtained isolates were subjected for further screening tests including Gram staining, Oxidases and catalase tests further culture in SIM (Sulphide, Indole, Motility) and TSI (Triple Sugar Iron) media, biochemical tests were performed for the species level identification as described by (Huxley et al, (2000) [13].

Antimicrobial susceptibility test: The antibiotic susceptibility testing for Vibrio isolates was determined using the following antibiotic discs: Doxycycline (10 µg), Erythromycin (30 µg), Chloramphenicol (30 µg), Ofloxacin (30 µg), Enrofloxacin (10 µg), and Ciprofloxacin (5 µg). The discs were dispensed on the surface of the medium and incubated aerobically at 250C for 24hr. The choice of antibiotics was subjected based on recommended drug for primary testing of Vibrio spp. by National Committee of Clinical Laboratory Standards NCCLS (2002).

Species	Doxycycline (10µg)		Erythromycin (30µg)		Chloramphenicol (30µg)		Ofloxacin (30µg)		Enrofloxacin (10µg)		Ciprofloxacin (5µg)	
	S	R	S	R	S	R	S	R	S	R	S	R
V.parahaemolyti cus	27	0	0	27	10	17	27	0	20	7	25	2
V.harveyi	10	0	1	0	3	7	10	0	9	1	8	2
V.vulnificus	3	0	0	3	1	2	3	0	0	3	3	0
V.cholerae	3	0	0	3	0	3	3	0	3	0	2	1
V.alginolyticus	3	0	1	2	2	1	3	0	2	1	2	1

S= Susceptibility, R= Resistance Table 1: Susceptibility Profile of Vibrio Isolates to Various Antibiotics

Results: Five Vibrio spp were identified viz., V. parahaemolyticus, V. harveyi, V. vulnificus, V. cholera, V. alginolyticus and were subjected for antibiotic susceptibility test. Antibiotic susceptibilities of 50 isolates of vibrio species are reported in Table 1. The present investigation of Vibrio isolates evinces strong resistance to erythromycin and chloramphenicol. Resistance rate of V. parahaemolyticus was 93.7% for erythromycin and 63.5% for chloramphenicol.

Conclusion: There are reports of bacterial resistance in chloramphenicol and oxytetracycline in marine fish and shrimps but higher resistance found in ampicillin in Goldlined seabream (Sparus sarba) in China [14]. According to aquaculture authority of India reports, (2002) list of 20 antibiotics was banned including pharmacologically active substance chloramphenicol and nalidixic acid for the control bacterial disease in shrimps. Previous studies also reported the occurrence of V. parahaemolyticus from various markets in southwest coast of India [15]. However, the public health significance, this pathogenic vibrio spp has not been studied extensively for the antimicrobial resistance particularly when compare to other enteric pathogens, which evinces the direct impact on the human food chain. In country like India sanitary and hygiene are not maintaining standard protocol. In this study it is further emphasized about the bacterial resistance and occurence multi- drug resistance in halophilic vibrios spp should continue to monitor to ensure the seafood safety for human consumption.

References:

1. Donald V. Lightner, Donald H. Lewi. A Septicemic Bacterial Disease Syndrome of Penaeid Shrimp. Marine Fisheries Review. 1975; 37(5).

2. Adams A. Response of penaeid shrimp to exposure to Vibrio species. Fish Shellfish Immunology. 1991; 1: pp. 59- 70.

3.Lavilla Pitogo- C.R, Leano- E.M & Paner. M.G. Mortalities of Pond cultured juvenile-shrimp, P. monodon, associated with dominance of Luminescent Vibriosis in the rearing environment. Aquaculture. 1998; 164: pp. 337-349.

4. Newman S.G. Immunization of shrimp. (Book) Aquaculture. 1992; 92: pp. 170.James Brock & Lightner. A Biopsy Procedure for the Non-destructive Determination of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) Infection in Penaeus vannamei. Journal of Animal Health. 1990; 2(2): pp. 151-153. DOI: 10.1577/1548-8667

5. Li-He., Zheng-Guoxing & Shen-Yalin. Vibrio anguillarum as a cause of disease in Penaeus orientals Kishinouye. J. Fish-China-shuichan- Xuebao. 1990; 14: pp. 1-7.

6. Chang- C.F, Su .M.S., Chen H.Y, LO. C.F, Kou G.H & Liao L.C. Effect of dietry beta 1-3, glucan on resistance to white spot syndrome virus (WSSV) in Post larval and juvenile P.monodon. Journal-Diseases of Aquatic Organisms. 1999; 36(3): pp. 163-168.

7. Evans L.H & Brock J.A. Disease of spiny lobsters in BF Phillips. J.S. Cobb. Spiny lobster management. Fishing new books Black well scientific publications London U.K. 1994: pp. 461-472.

8. Lavilla Pitogo- C.R, Leano- E.M & Paner. M.G. Morta lities of Pond cultured juvenile-shrimp, P. monodon, associated with dominance of Luminescent Vibriosis in the rearing environment. Aquaculture. 1998; 164:pp. 337-349.

9. Martin G.G. & B.L. Graves. Fine structure and classification of Shrimp haemocytes. J. Morphology. 1985; 185: pp. 339-348.

10. Johnson P.T, Stewart J.E, & Arie B. Histopathology of Halics Aerococcus Viridans, Gaffkemia infection in the Lobster Homarus americanus, and a comparison with histological reaction to a gram-negative Species, P. peroleus. J. Invertebrate Pathology. 1981; 38: pp. 127-148. Doi: 10.1128/JB.01959-06

11. Le Moullac. G. Rodriguez. J, Saulnier D & CuZon. G. Chim. L. Immunomodulation.Nutritional Aspects and Immuno stimulation. Cenaim. Aqua cop-centre, Taravao, Tahiti. 2000.

12. Huxley, A.J., Lipton, A.P. & Selvin, J. Enhancement of phagocytosis, agglutinin and bactericidins in haemolymph of the spiny lobster Panulirushomarus via immunostimulants. (In press). 2000.

13.Li-He., Zheng-Guoxing & Shen-Yalin. Vibrio anguillarum as a cause of disease in Penaeus Orientals Kishinouye. J. Fish-China-shuichan- Xuebao. 1990; 14: pp. 1-7.

14. Bo Pang, Meiying Yan, Zhigang Cui, Xiaofen Ye, Baowei Diao, Yonghong Ren, Shouyi Gao, Liang Zhang, & Biao Kan. Genetic Diversity of Toxigenic and Nontoxigenic Vibrio cholera Serogroups O1 and O139 Revealed by Array-Based Comparative Genomic Hybridization. Journal of Bacteriology. 2007: pp. 4837–4849. Doi: 10.1128/JB.01959- 06.