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Use of Drugs against Combating Commonly Occurring Bacterial Prawn Pathogens

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A B S T R A C T

As diseases contribute severe economic losses to aquaculturists the present study was aimed at use of drugs in combating prawn bacterial diseases. Among the five antibiotics used, results revealed that the application of neomycin sulphate and streptomycin significantly reduced the percentage of mortality in prawns, while neomycin sulphate was effective against *Pseudomonas aeruginosa* streptomycin was effective against *Vibrio harveyi* and both neomycin sulphate and streptomycin was equally effective against *Vibrio parahaemolyticus*.

Introduction

To increase production, aquaculturiests have been resorting to semi intensive and intensive culture involving high stocking densities. This in turn leads to diseases. Of the various crustaceans suitable for aquaculture, shrimp is the most important and extensively farmed all over the world (Lakshmi *et al.*, 2013).

Aquatic organisms are continuously bathed in an aqueous suspension of microorganisms and their external surfaces are always in contact with them. Existence of pathogenic microorganisms in an aquatic environment is inevitable and infections generally occur when the animal is immunologically incompetent either due to physical or biological stress (Thompson *et al.*, 2004). Of the various groups of pathogens, bacteria pose one of the most significant threats to successful fish and shell fish production throughout the world (Srivallie *et al.*, 2014). In addition, bacterial diseases are also responsible for heavy mortalities in both culture and wild fisheries throughout the world being opportunist pathogens which invade the tissue of a fish host rendered susceptible to infection (Roberts, 1989).

Among bacteria, *Aeromonas*, *Pseudomonas* and *Edwardsiella* are the major bacterial fish and shell fish pathogens which are widely distributed in nature (Banu, 1996; Islam, 1996). Among water and food borne

pathogens in coastal ecosystems, *Vibrios* constitute the major part with members of the family Vibrionaceae contributing over 60% of the total bacterial population (Simidu and Tsukamoto, 1985). In addition, contamination of hard skeleton of crustaceans and shells of bivalve mollusks with *Vibrios* and *Aeromonas* is also increasingly recognized as the cause of wound and blood infections following laceration of the skin sustained during handling of shell fish (Bonner *et al.,* 1983; Flynn and Knepp, 1987).

A number of experiments and the use of drugs have been performed in fish with antibiotics being frequently used to control bacterial diseases. However, there can be an increasing risk of developing antibiotic resistant strains of bacteria.

Though India practices both the harvest of captive-wild as well as semi-intensive culture of shrimps, bacterial disease problems and the estimated loss of wild stocks are unknown, except for a few reports on the occurrence of bacteria in the processed iced storage shrimps (Jayakumar and Ramasamy, 1999). Hence it is important to study disease conditions under natural conditions since it serves as a basis for providing information on potential risk of diseases and hence the present study in *P. indicus.*

Materials and Methods

Samples of prawn *Penaeus indicus* H. Milne Edwards of 5-30 mm carapace length were collected from the Kottaipattinam backwater of Pudukottai District, Tamil Nadu. They were transported to the laboratory in sterile seawater maintained in aerated tanks containing filtered seawater and examined within 24 h of collection; sex and carapace length (tip of the rostrum to end of carapace) were determined. The live and moribund specimens used for bacteriological studies were surface sterilized with 30 ppm iodophore to eliminate the normal microbial flora. Prawns of both sexes were sacrificed and wet mount preparation of appendages, gills, hepatopancreas, gut, muscle, gonad, hemolymph and exoskeleton were made and examined for signs of bacterial infection. Monthly prevalence (% of shrimp harbouring pathogen/parasite) of pathogenic bacteria and ciliate protozoans in relation to sex and size of the host were determined.

Bacteria were isolated on plates with Zobell's marine agar using dilutions of homogenized tissues (muscle, gills, hemolymph and hepatopancreas) or by streaking plates using exoskeleton with blisters or lesions. Bacteria that were repeatedly encountered in the tissues of *P. indicus* were further characterized and identified by standard procedures. Isolated bacteria were cultured in Zobell's marine agar at 28°C and at temperatures ranging from 10°C to 50°C. Growth characteristics of bacteria in 0% - 8% of sodium chloride in modified nutrient agar, MacConkey agar, citrate agar, Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar were determined and the authenticity of identification were confirmed with Bergey's manual of determinative bacteriology.

Minimum inhibitory concentrations of antibiotics required to control the growth of the bacteria (MIC) were determined by tube dilution method under *in vitro* conditions using 128 µg to 10 mg/ml chloramphenicol (Biochem Pharmaceuticala, India), 5 µg to 50 µg/ml of tetracycline (Sigma), penicillin 3.2 IU to 320 IV ml^{-1} (Sarabhai Chemicals, India), 4 µg to 50 µg/ml of furazolidone (Argent chemicals) by following standard procedures.

For bacterial infection experiments, prawns weighing about 5 g were acclimatized at 25-28°C in plastic troughs containing 20 liter of filtered seawater. They were divided into II experimental groups, each containing ten prawns. Various species of the bacteria *Pseudomonas* sp. and *Vibrio* sp. were grown in TCBS agar/Zobell's marine agar for 24 h. Colonies were dispersed in sterile 0.85% NaCl solution/Zobell's marine broth and the density of the suspension was adjusted with sterile saline. 100 µl of bacterial suspensions containing known colony forming units of bacteria were inoculated intramuscularly into the lateral side of second abdominal segment by adopting the method of Song *et al.* (1993). Control prawns were inoculated with a sterile Zobell's marine broth/sterile 0.85% NaCl solution. Prawns were provided with 2% biomass of body weight of pelletized feed and 100% daily water exchange. Mortality and signs of bacterial infection of prawns were recorded up to 6 days. Bacteria were isolated from the lesions/hepatopancreas of moribund/ dead prawns and identified by standard procedures.

For drug therapy experiments, *P. indicus* was intramuscularly infected with isolates of *Pseudomonas* spp. / *Vibrio* spp. and neomycin sulphate (20 µg/ml) or streptomycin (10 µg/ml) was added into culture tank water at 6, 24 and 48 h after post-infection. The mortality and. signs of disease development in the prawns were recorded by following the method of Takahashi *et al.* (1985).

Results and Discussion

The most common bacteria that were isolated from the body surface of *P. indicus* are presented in Table-1, which reveals the presence of three species, viz., *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* and *V. harveyi*. These bacteria were isolated from exoskeletal structures like carapace, uropods, pleopods, gills and appendage setae.

Five antibiotics were used experimentally to assess their minimum inhibition concentration to contain the bacterial growth *in vitro* and the results are presented in Table-2. With regard to *P. aeruginosa*, among the five types of antibiotics used, Streptomycin and Neomycin sulphate appeared to the most effective while Chloramphenicol and Furazolidine the least effective.

For *V. parahaemolyticus*, results reveal that neomycin sulphate was the most effective followed by streptomycin and chloramphenicol and Furazolidine was the least effective. The same result was also obtained for *V. harveyi* with neomycin sulphate being the most effective. However, the least effective antibiotic was Furazolidone.

The results of the experimental studies after intra muscular inoculum of the three bacterial species are presented in Table-3. As evident from the table, mortality occurred for all the three pathogens with the mortality range ranging from 66 percent (*V. harveyi*) to 86 percent (*V. parahaemolyticus*).

Results of the drug therapy experiments of *P. indicus* infected with isolates of all the three bacterial pathogens are presented in Table-3. Results reveal that the application of neomycin sulphate and streptomycin significantly reduced the percentage of mortality. While Neomycin sulphate was found to be very effective against *Pseudomonas aeruginosa*, streptomycin was effective against *V. harveyi*. However, both neomycin sulphate and streptomycin was found to be equally effective against *V. parahaemolyticus.*

Table.1 Common Bacteria Isolated from *Penaeus Indicus* of Kottaipattinam Backwaters

S. No.	Species	No. of Prawn Tested	No. of Occurrence	Percentage
1.	*Pseudomonas aeruginosa*	25	25	100
2.	*Vibrio parahaemolyticus*	25	25	100
3.	*Vibrio harveyi*	25	25	100

Table.2 Minimum Inhibitory Concentration of Antibiotics to Control The Growth (In Vitro) of Bacterial Isolation from *Penaeus indicu*

S. No.	Antibiotics	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*	*Vibrio harveyi*
1.	Chloramphenicol (mg/ml)	75	75	40
2.	Streptomycin (mg/ml)	10	10	5
3.	Tetracycline (mg/ml)	40	30	25
4.	Neomycin sulphate (mg/ml)	10	10	1
5.	Furazolidone (mg/ml)	75	75	75

Table.3 Experimental Infection of Isolates of *Pseudomonas* and *Vibrio* with Subadult *Penaeus indicus* and the Effect of Antibiotic Treatment

S. No.	Bacterial Species	Inaculum (cell/ prawn)	No. of Prawns challenged	Experimental infection (% of mortality)	Treatment with neomycin sulphate (20 µg ml^{-1})		Treatment with Streptomycin (10 µg/ml ml^{-1})		Treatment with herbal extracts (10 µg/ml ml^{-1})	
					Total to host/died	% of mortality	Total to host/died	% of mortality	Total to host/died	% of mortality
1.	*Pseudomonas aeruginosa*	3.2×10^{4}	25	70	1	10	2	20	1	10
2.	*Vibrio parahaemolyticus*	2.5×10^{4}	25	86	2	20	2	20	0	0
3.	*Vibrio harveyi*	2.6×10^{5}	25	66	3	30	1	10	1	10

The *in vitro* sensitivity of *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* and *V. harveyi* was found to differ with different bacterial species. With regard to *P. aeruginosa*, streptomycin and neomycin sulphate were found to be the most effective while for *V. parahaemolyticus* and *V. harveyi*, the most effective antibiotic was neomycin sulphate, followed by streptomycin. A perusal of literature reveals that Jayakumar and Ramasamy (1999) while studying bacterial and protozoan diseases in prawn suggested that Neomycin sulphate and streptomycin were the most effective antibiotics in inhibiting the growth of *Vibrio* sp. and *Pseudomonas* sp. respectively. Thus the results obtained in the present study are in line with those reported by Jayakumar and Ramasamy (1999).

Induced infection of the bacterial species in prawn also recorded varying degrees of mortality. While the mortality was minimum in prawns induced with *V. harveyi* recording a mortality of 66%, the highest mortality was recorded in prawns that were infected with *V. parahaemolyticus* with mortality being 86%. Literature reveals that Jayakumar and Ramasamy (1999) studying experimental infection of bacteria in prawns recorded mortality rates ranging from 30% (*V. anguillanum*) to 80% (*V. parahaemolyticus*) with *Pseudomonas* showing mortality rate of 70%. This observation is also in line with the present study, with *V. parahaemolyticus* recording highest mortality eventhough mortality rate recorded with *V. parahaemolyticus* in the present study appeared to be on the higher side.

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