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Assessment of antibiotic sensitivity and pathogenicity of *Vibrio* spp. and *Aeromonas* spp. from aquaculture environment

Abstract

Bacterial and viral diseases constitute a major factor limiting the successes of shrimp farming industry. In this context, Identification of drug resistant pathogens and determination of the pathogenic processes of shrimp are fundamental for further progress in the disease management. They could be valuable in the evaluation of their epidemiology and control measures.

Keywords: aquaculture environment, pathogenic bacteria, antibiotic sensitivity, *vibrio* spp, *Aeromonas* spp.

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Abbreviations: PL, post larval; TCBS, Thiosulfate–citrate–bile salt–sucrose agar; MIC; minimum inhibitory concentrations; NaCl, Sodium chloride; µg, microgram; hrs, hours; LD_{50}, Lethal dose; ml, milliliter

Introduction

Losses due to bacterial and viral diseases constitute a major factor limiting the successes in aquaculture practices and more particularly in shrimp farming industry. Outbreaks of diseases normally occur in the early developmental stages of shrimp in the hatcheries and thus the larval (nauplius, zoea, mysis) and post larval shrimp are more susceptible for microbial infections. Viral, bacterial, fungal and protozoan infections occur either simultaneously of successively in the larval/post larval shrimp. *Vibro alginolyticus V. parahaemolyticus*, *V. anguillarim* and other strains of the genus *Vibrio* are the ubiquitous bacterial flora of the shrimp.[1–8] *Pseudomonas* spp., *Aeromonas* spp., *Flavobacterium* spp. and *Moraxella* spp. have also been shown to occur in the shrimp.[4,9] The occurrence of bacteria along the Coramandal Coast, Bay of Bengal, abundance of potentially pathogenic micro–organisms in the larval rearing hatcheries, effect of water exchange, chlorination, antibiotics, and UV radiation in the elimination of *Vibrio* population, effect of probiotics, antibiotics sensitivity, pathogenenicity, *in vitro* antibiotics susceptibility of *Vibrio* spp. and *Aeromonas* spp. isolated from black tiger shrimp *Penaeus monodon* Fabricius hatcheries and ponds were described.[9–15] Mortalities if any in the hatcheries/in the natural populations are usually related to primary or secondary invasions of bacteria.[16] Recently mass mortalities of post larval *P. monodon* has been implicated to be due to antibiotic–resistant *V. harveyi* Baumann et al. infection.[9,17] However, experimental investigations on the *in vivo* effect of bacteria (isolated from diseased shrimp) on the mortality of larval shrimp are limited.[9,18–21] Moreover identification and classification of the causative bacteria was difficult and reinfection from the cultured organisms is often unsuccessful.[16] A possible probiotic treatment for the control of pathogenic *Vibrio* spp. in *P. monodon* by Bacillus subtilis Cohn, 1872 BT23, was reported.[22,23] In view of paucity of information on the identification of pathogens from the aquatic environment and the pathogenic processes leading to death of shrimp, the present study was carried out. Such studies will be fundamental for further progress in the disease management as water health in the environmental, microbiological assessments, detection of resistant pathogenic organisms and diseases management studies could be valuable and support in the evaluation of their epidemiology and control measures following intervention. These could help improve the health status of shrimp in culture conditions and can have a positive impact in all areas of life–economic, social and ensure sustainable environment and development.

The objectives of the study are:

1. To isolate and identify the kinds of bacteria from the aquaculture environment and to resolve their pathogenecity of the isolated bacteria on the healthy post larval *P. monodon*.
2. To establish the minimum inhibitory concentration of antibiotics of the bacterial isolates.
3. In addition, *in vivo* antibiotic therapy experiments were carried out for effective management of the disease of the post larval *P. monodon*.

Materials and methods

Sample collection

Outbreaks of diseases frequently occurred in a hatchery at Chennai, India. Water samples were taken from the aquatic environments as well as from the hatchery at Chennai, India. Healthy post larval (=PL) *P. monodon* (PL 10 to 20) were taken and transported to the laboratory in an air filled polythene bags containing sea water to the laboratory where they were maintained in plastic troughs containing filtered sea water with continuous aeration and used for further experimental infection studies. Water samples from the aquaculture environment and diseased, moribund/dead post larval *P. monodon* were used for isolation and identification of bacteria and for further experimental infection studies.

In *vitro* culture of bacterial Isolates

The water samples taken from the aquatic environment were serially diluted and plated onto Zobell's marine agar or TCBS agar. Moreover diseased/moribund larval samples were examined under a carlzeiss binocular microscope for external signs of bacterial infection. Further the infected tissues of the post larval *P.monodon* were homogenized by using a sterile homogenizer and autoclaved sea water. After homogenization, the tissue samples were serially diluted and plated onto Zobell's marine agar or Thiosulfate–citrate–bile salt–sucrose (TCBS) agar. The bacteria were stained with Gram's stain. Moreover the growth characteristics of bacteria in modified nutrient broth enriched with 0% to 10% NaCl or blood agar medium with selective supplement 10µg/ml of ampicillin or DNase agar medium was determined by following the procedures of Robert Bailey et al.[24] The plates were incubated at 28°C for 24 hours. Pure cultures were maintained for further characterization and identification.

Biochemical characteristics and Identification of bacteria

Bacterial isolates were identified based on the following biochemical characteristics.[25] viz. arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, oxidase, catalase, indole production, methyl red and vogesproskauer test, esculin hydrolysis, H_2S production, growth and acid production in the presence of glucose, mannitol, sucrose and lactose, gas production in the presence of glucose were determined by following the methods of Norris et al.[26] Sensitivity of the bacterial isolates (*Vibrio* spp., *Aeromonas* spp.) to *vibrio*static agent (0/129) (2,4, diamino–6,7–diisopropylpteridine phosphate) (150µg) were determined by the following method of Seidler et al.[27]

Minimum inhibitory concentration of antibiotics for the control of the growth of the bacterial isolates

The minimum inhibitory concentrations (MIC) of the following antibiotics ciprofloxacin, chloramphenicol, erythromycin phosphate, nifurpirinol (prefuran), oxytetracycline and streptomycin required in controlling the growth of *Vibrio* spp. and *Aeromonas* spp. isolated from the water samples and also the diseased post larvae were assessed by the tube dilution method using nutrient broth.[28,29]

Mortality of experimentally infected P. monodon

To determine the pathogenicity of the isolated bacteria on the healthy post larval *P. monodon*, the following experiments were designed. 300 ml of filtered, sterilized (autoclaved) sea water was taken in each of the 10 different 500 ml conical flasks. Ten healthy posts larval *P .monodon* were introduced into each flasks containing filtered, sterilized (autoclaved) sea water. Known concentration of the bacteria was inoculated in the water in the flask with bacterial isolates of *V. anguillarum* Sakai et al. */ V.damsela* Smith et al. Kimura et al. */V. furnissii* Brenner et al.1983*./A. hydrophila* Stanier*/A. sobria* Popoff et al. Also a set of controls (bacteria uninoculated) were also maintained. The post larvae were observed every 24 hrs for 6 days (144 hours) for signs of pathogenicity and mortality. The LD_{50}) for each bacterial species was determined by the method of Reed et al.[30] LD is the number of cells of a bacterial inoculums, given all at once, which causes the death of 50% (one half) of a group of test animals.

In vivo antibiotic therapy experiments

In vivo antibiotic therapy assay experiments were carried out as described below., The methodology of experimental infection of post larval *P.monodon* is similar to that of the procedure of pathogenicity tests detailed above except that they were treated with different concentrations of various antibiotics viz. nifurpirinol (prefuran) 1µg/ml, 4µg/ml, 7µg/ml; oxytetracycline 2µg/ml, streptomycin 1µg/ml, 2µg/ml, 3µg/ml and ciprofloxacin 0.5µg/ml, 1µg/ml. For a comparison, a set of antibiotics untreated control groups of larval shrimp were maintained. Percentage mortality of post larval *P. monodon* was determined. The most effective concentration of antibiotics required to reduce / prevent the mortality of the infected post larval *P. monodon* were determined.

Results and discussion

Water samples from the aquaculture environment in and around Chennai, India were contaminated with pathogenic bacteria viz. *Vibrio anguillarum*, *V. furnissii*, *Photobacterium damselae, Aeromonas hydrophila* and *A. sobria*. They were shown to be pathogenic and cause mortality to the infected post larval *P. monodon*. These studies indicate that a large scale continuous surveillance programme for detection and monitoring the resistant/ sensitive pathogenic bacteria along the coastal surface waters is needed to ensure a consistently acceptable level of water quality for aquaculture and other purposes viz.

a. To establish and quantify the occurrence and spread of antibiotic sensitive/resistant bacteria in marine surface waters.

b. To determine epidemiology of sensitive/ resistant water borne pathogens so as to evolve better – cost effective water treatment methods.

We also report that the pathogenic bacteria can be controlled with selective use of certain effective antibiotics to improve the health status of shrimp in culture conditions and these can promote a positive impact in the areas of sustainable aquaculture environment and development.

Biochemical characteristics and identification of the bacterial isolates

Five species of bacteria representing two genera viz. *Vibrio* and *Aeromonas* were isolated and identified from the water samples of the aquaculture environment at Chennai, India. The genus *Vibrio* was represented by V. anguillarum. *V. furnissii* while the genus *Aeromonas* includes two species viz. *A. hydrophila* and *A. sobria*. All the bacterial species were found to be motile and Gram negative rods. Details of biochemical tests carried out in the study to identify the bacterial isolates from the water samples are listed in Table lA. V.anguillarum, V.damsela, V.furnissii, A.hydrophila and A.sobria were found to grow in Zobell's marine agar 2216 E and TCBS agar and they did not grow in blood agar with selective supplement ampicillin (l0µg/ml) nor exhibited a zone of clearance in DNase agar. *Aeromonas* spp. was found to grow in TCBS agar, Zobell's marine agar 2216 E and in blood agar with 10 µg/ml. In DNase agar, the species of *Aeromonas* exhibited growth and a zone of clearance. Moreover, *Vibrio* spp. were found to be sensitive to the *vibrio*static agent (0/129) (2,4diamino –6,7 diisopropylpteridine phosphate) (150 µg). The present investigation revealed the occurrence of two genera of bacteria viz. *Vibrio* and

Aeromonas represented by a total of five species viz. *V. anguillarum V. damsela, V. furnissii, A. hydrophila and A. sobria* in the diseased post larval *P. monodon*. *Vibrio* furnissii is reported for the first time from post larval *P. monodon* in India whereas *Vibrio*, *Aeromonas*, *Pseudomonas* have been recorded from eggs, larval, post larval and juveniles of P. indicus[8,29,32] *Vibrio* spp. have been reported in all life stages of *P. monodon, P. vannamei, P. setiferus, P. orientalis and* several other penaeid species and thus vibriosis represent a greatest challenges causing significant economic loss to the industry.[2,4,5,23,28,31] All the bacterial isolates (*Vibrio* spp. and *Aeromonas* spp.) from post larval *P. monodon* exhibited growth in TCBS agar, zobell's marine agar and modified nutrient broth agar enriched with NaCl. *V .furnissii* exhibited growth in 2% – 8% NaCl while *V. anguillarum* and V. damsel exhibited growth in 2%–6% NaCl. In contrast, *Aeromonas* spp. required lower salt concentrations (0%–3% NaCl) for their growth. These observations are in agreement with that of Takahashi et al.[5,32] and Jayakumar et al.[33] who have reported that their isolates of *Vibrio* exhibited growth only in 0% to 6% NaCl and 0%–7.5% NaCl respectively. 6.5% NaCl was shown to inhibit the *Vibrio* isolates of *P. japonicus*.[5,32]

Table 1a Biochemical characteristics of bacterial isolates (*Vibrio* spp. and *Aeromonas* spp.) From water samples and diseased post larval *Penaeu monodon*

S. No.	Tests	*V. Anguillarm*	*V. damselae*	*V. Furnissii*	*A. Hydrophila*	*A. Sobria*
1	Motility	+	+	+	+	+
2	Production of Indole	+	–	+	+	+
3	Methyl red	+	+	+	+	–
4	Vogesproskauer	+	+	–	+	±
5	Oxidase	+	+	+	+	+
6	Catalase	+	+	+	+	+
7	Arginine dihydrolase	+	+	+	+	+
8	Lysine decarboxylase	–	+	–	+	±
9	Ornithine decarboxylase	–	–	–	–	–
10	H_2S production	–	–	–	–	–
11	Gas from glucose	–	–	+	+	+
12	Growth on / acid production from					
	a. Glucose	+	+	+	+	+
	b. Sucrose	+	–	+	+	+
	c. Lactose	–	–	–	+	–
	d. Mannitol	+	+	+	+	+
13	Growth in 0% NaCl	–	–	–	+	+
14	Growth in 2% NaCl	+	+	+	+	+
15	Growth in 3% NaCl	+	+	+	+	+
16	Growth in 4% NaCl	+	+	+	–	–
17	Growth in 0% NaCl	+	+	+	–	–
18	Growth in 8% NaCl	–	±	+	–	–
19	Growth in 10% NaCl	–	–	–	–	–
20	Esculin hydrolysis	–	–	–	+	–
21	0/1 29 vibriostatic agent (150µg)	S	S	S	R	R
22	Growth in Zobell's marine agar	+	+	+	+	+
23	Growth in TCBS agar	+	+	+	+	+
24	Growth in Blood agar (with 10 µg/ml of ampicillin)	–	–	–	+	+
25	DNase agar	–	–	–	+	+

+: Positive
– : Negative
+/–: Weak Positive
S: Sensitive
R: Resistant

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Minimum inhibitory concentration (MIC) of antibiotics for the control of bacterial isolates

The results of the MIC of antibiotics viz. chloramphenicol, ciprofloxacin, oxytetracycline, erythromycin phosphate, streptomycin, nifurpirinol (prefuran) required to control the growth of bacterial isolates of *V.anguillarum*, *V .furnissii,damsela,A.hydrophila* and *A.sobria* to control the bacterial growth *in vivo* and *in vitro* are set out in Table lB. It is seen that ciprofloxacin and streptomycin are the most effective antibiotics in inhibiting the growth of all the species of *Vibrios*, *Photobacterium* and *Aeromonas* tested in this study while nifurpirinol (prefuran) was found to be the least effective antibiotics. MIC of the antibiotic erythromycin phosphate is significantly different for the species of *Vibrio* whereas the MIC for other antibiotics was similar for all the bacterial isolates tested.

Table lb Minimum inhibitory concentration of antibiotics to control the growth of bacterial isolates *Vibrio* spp. and *Aeromonas* spp. from water samples/ infected tissues of *Penaeus monodon*

S. No.	Antibiotics	*V. anguillarum*	*V. damsela*	*V. furnissii*	*A. hydrophila*	*A. sobria*
1	Nifurpirinol (Prefuran)	25µg/ml	25µg/ml	25µg/ml	24µg/ml	24µg/ml
2	Oxytetracycline	14µg/ml	13µg/ml	13µg/ml	14µg/ml	14µg/ml
3	Erythryomycin Phosphate	15µg/ml	9µg/ml	4µg/ml	5µg/ml	6µg/ml
4	Chloramphenicol	13µg/ml	12µg/ml	15µg/ml	12µg/ml	12µg/ml
5	Streptomycin	2µg/ml	2µg/ml	2µg/ml	3µg/ml	2µg/ml
6	Ciprofloxacin	1µg/ml	1µg/ml	1µg/ml	1µg/ml	µg/ml

Minimum inhibitory concentration of oxytetracycline (14µg/ml); chloramphenicol (12–15µg/ml) and streptomycin (2µg/ml) required in controlling the growth of *Vibrio* spp. and *Aeromonas* spp. was shown to be lower than the recommended values. A higher MIC of antibiotics has been reported to control the growth of bacterial isolates (*Vibrio* spp.) of *P. indicus* obtained from a polluted Ennore Estuary.[32] Lightner[4] showed a higher MIC of oxytetracycline (250µg/ml) to control the growth of *Vibrio* spp. whereas Sahul Hameedet al.[7] reported a lower MIC 5µg/ml of chloramphenicol but a higher MIC of oxytetracycline (150 µg/ml) was required to inhibit the growth of *V. campbelli* like bacterium from *P. indicus*. Takahashi et al.[32] reported <0.1µg to 12.5µg of oxytetracycline/ml to inhibit the growth of *Vibrio* spp. According to Baticados et al.[20] prefuran is the most effective antibiotics that inhibited the growth of their isolates of *V. harveyi* and *V. splendidus* obtained from *P. monodon*. However our findings indicate that a very high MIC of nifurpirinol (Prefuran) (25µg/ml) is required to inhibit the growth of *Vibrio* spp. The probable reason for this higher MIC concentration of prefuran required for the control of *Vibrio* spp. and *Aeromonas* spp. may be related to the antibiotics applied in the hatcheries which would have lead the bacteria to develop antibiotic resistance.

Mortality of infected P. *monodon*

Inoculation of *V. anguillarum*, *V. furnissii, P. damselae, A. hydrophila* and *A. sorbia* into the culture water of the post larval *P.monodon* have shown to be infective, pathogenic and caused mortality. When the inoculum of bacterial concentration was higher, the mortality of the post larval *P.monodon* was 100% and if the bacterial concentration was lower, the death rate was found to be proportionately lower and hence taken longer duration of time for mortality to occur. The LD_{50} values for post larval *P. monodon* were higher for *Vibrio* species than *Aeromonas* species (Table 2). In contrast, none of the control post larval shrimp exhibited mortality.

Experimentally infected post larval *P.monodon* showed reddish coloration and sluggishness in swimming and feeding and they were generally found to rest in the bottom of the tank as a sign of bacterial infection and stress. Black spots and erosion of the tail portion of the infected postlarvae were commonly noticed.

The current study has shown that the bacterial isolates of *Vibrio* spp. and *Aeromonas* spp. are shown to be pathogenic. They caused 100% mortality of the experimentally infected post larval *P. monodon* indicating that these bacteria (*Vibrio* spp. and *Aeromona* spp.) were pathogenic, and collectively/individually would have caused the death of postlarvae in the hatcheries. These results confirm .the findings of Karunasagar et al.[17] who have reported that 10^6 cells of V. harveyii caused 100% mortality of post larval *P. monodon* while 103 cells/ml caused only 30% mortality. Similarly *V. anguillarum* and *V. damsela* was shown to be pathogenic and caused mortality in penaeid shrimp.[9,34,35] The current study has shown that experimental inoculation of 3.2 x 10^5 cells/ml *V. anguillarum* and an addition of 2µg/ml of oxytetracycline reduced the mortality of the post larval shrimp *P. monodon*. However a higher concentration of bacterial inoculation (1.3 x 10^6 cells/ml) caused 40% mortality of postlarvae whereas in coulation of 5.0 x 10^s cells/ml caused only 10% mortality. Chanratchakul et al.[36] studied oxytetracycline pretreatment and subsequent injection of V. parahaemolyticus into *P. monodon*. They noticed that 5g oxytetracycline/kg of feed treated shrimp were found to have lower mortalities than that of untreated bacteria infected shrimp. Sahul Hameed[7] reated protozoea, mysis and post larval *P. indicus* with chloramphenicol to control the infection caused by the *V. campbelli* like bacterium. They showed that 3h/6h treatment of antibiotics reduced the larval mortalities significantly. The present study has shown that treatment of 0.5µg/ml of ciprofloxacin caused mortality of post larval *P. monodon* at higher bacterial inoculum concentration. In contrast, 1 µg/ml of ciprofloxacin treatment for a period of 72h prevented the mortality of post larval *P. monodon*. This study illustrates that a suitable exposure time and concentration of antibiotics are required to be determined for each bacteria for effective control of the mortality of infected post larval *P. monodon*.

Table 2 Mortality of experimentally (Vibrio spp. and Aeromonas spp.) infected post larval Penaeus monodon (without antibiotics treatment)

Bacteria	Concentration of bacteria inoculated (no. of cells/ml) into the culture water	No. of death/total postlarvae challenged	% Mortality	LD_{50} (Cells/Ml)
	1.3×10^6	10/10	100%	
	6.3×10^5	10/10	100%	
V.anguillarum	5.0×10^5	10/10	100%	1.1×10^5 (5.04*)
	3.2×10^5	8/10	80%	
	1.6×10^5	7/10	70%	
	3.6×10^6	10/10	100%	
	1.8×10^6	10/10	100%	
V.damsela	1.5×10^6	10/10	100%	2.7×10^5 (5.43*)
	1.0×10^6	10/10	100%	
	5.0×10^5	9/10	90%	
	5.0×10^6	10/10	100%	
	2.5×10^6	9/10	90%	
V.furnissii	1.8×10^6	7/10	70%	6.0×10^5 (5.71*)
	1.2×10^6	6/10	60%	
	6.0×10^5	5/10	50%	
	1.0×10^8	10/10	100%	
	5.0×10^5	10/10	100%	
A.hydrophila	3.6×10^5	9/10	90%	8.5×10^4 (4.92*)
	2.5×10^5	8/10	80%	
	1.2×10^5	7/10	70%	
	1.0×10^8	10/10	100%	
	5.0×10^5	10/10	100%	
A.sobria	3.6×10^5	9/10	90%	7.5×10^4 (4.87*)
	2.5×10^5	8/10	80%	
	1.2×10^5	8/10	80%	

In vivo effect of antibiotics against infected *P. monodon*

Nifurpirinol (prefuran) 1 µg/ml, 4µg/ml, was found to be ineffective in reducing the mortality of the experimentally infected post larval shrimp. When the concentration of antibiotic nifurpirinol (prefuran) was increased to 7µg/ml, the mortality of the post larval shrimp was only slightly reduced (Tables 3–7). In contrast, when the experimentally infected post larval *P. monodon* were treated with oxytetracycline(2µg/ml)/ciprofloxacin(0.5µg/ml)/streptomycin (1µg/ml), an appreciable reduction in the mortality was noticed. Ciprofloxacin(1µg/ml)/streptomycin(2µg/ml) proved to be the most effective antibiotics against *vibrio* spp. and A.sobria. In the case of A.hydrophila, streptomycin 3µg/ml of was found to be most effective in reducing the mortality of post larval *P. monodon*. Application of ciprofloxacin (1µg/ml) and streptomycin (2µg/ml) every 24 hrs for a period of 72 hrs was found to be sufficient in preventing the mortality caused by the bacterial isolates of the post larval *P. monodon*. The present study has shown that ciprofloxacin and streptomycin are the best antibiotics to control the growth of the isolates of *Vibrio* spp. and *Aeromonas* spp. *in vitro* and *in vivo*. In contrast, the nifurpirinol (Prefuran) was found to be the least effective antibiotics. Oxytetracycline, chloramphenicol, and erythromycin phosphate exhibited moderate effect in inhibiting the growth of the bacterial isolates tested in this study. Ciprofloxacin (1µg/ml) inhibited the growth of the *vibrio* spp. and *Aeromonas* spp. and thereby the mortality of experimentally infected post larval *P. monodon* significantly. Ruangpan et al.[37] have shown 0.3µg–0.6µg ciprofloxacin/ml to be sufficient to control the growth of *Vibrio* spp.

Table 3 Mortality (percentage) of *Vibrio anguillarum* infected post larval *Penaeus monodon* treated with antibiotics

Antibiotics	Concentration of bacteria inoculated (no. of antibiotics)					Level of significance
	1.3 x 10^6	6.3 x10^5	5.0x10^5	3.2 x 10^5	1.6 x 10^5	
	Percentage mortality					
Control (without antibiotics)	1	1	1	0.8	0.7	
Nifurpirinol (Prefuran)						
1µg/ml	1	0.9	0.8	0.8	0.7	p>0.05
"4µg/ml	1	0.9	0.8	0.6	0.6	p>0.05
"7µg/ml	0.7	0.6	0.6	0.5	0.4	p<0.01
Oxytetracycline						
2µg/ml	0.4	0.2	0.1	0	0	p<0.001
Ciprofloxacin						
0.5µg/ml	0.2	20%	0.1	0	0	p<0.001
"1µpg/mi	0	0	0	0	0	p<0.001
Streptomycin						
1µg/ml	0.2	0.1	0	0	0	p<0.001
"2µg/ml	0	0	0	0	0	p<0.001

Table 4 Mortality (percentage) of *Photobacterium damselae damselae ,Vibrio damsel* infected post larval Penaeus monodon treated with antibiotic

Antibiotics	Concentration of bacteria inoculated (no. of cells/ml) into the culture water					Level of significance
	3.6 x 10^6	1.8 x 10^6	1.5 x 10^6	1.0 x 10^5	5.0 x 10^5	
	Percentage mortality					
Control (without antibiotics)	100%	100%	100%	100%	70%	
Nifurpirinol (Prefuran)						
1µg/ml	100%	100%	100%	100%	70%	p>0.05
"4µg/ml	100%	90%	90%	90%	60%	p>0.05
"7µg/ml	80%	70%	70%	50%	40%	p<0.01
Oxytetracycline						
2µg/ml	40%	40%	20%	20%	0%	p<0.001
Ciprofloxacin						
0.5µg/ml	30%	30%	10%	0%	0%	p<0.001
"1µg/ml	0%	0%	0%	0%	0%	p<0.001
Streptomycin						
1µg/ml	20%	10%	0%	0%	0%	p<0.001
"2µg/ml	0%	0%	0%	0%	0%	p<0.001

Table 5 Mortality (percentage) of *Vibrio*. furnissii infected post larval *Penaeus monodon* treated with antibiotics

Antibiotics	Concentration of bacteria inoculated (no. of cells/ml) into the culture water					Level of significance
	5.0×10^6	2.5×10^6	1.8×10^6	1.2×10^5	6.0×10^5	
	Percentage mortality					
Control (without antibiotics)	100%	90%	70%	60%	50%	
Nifurpirinol (Prefuran)						
1µg/ml	100%	90%	70%	60%	50%	p>0.05
"4µg/ml	100%	90%	70%	50%	50%	p>0.05
"7µg/ml	70%	60%	60%	50%	50%	p<0.05
Oxytetracycline						
2µg/ml	50%	30%	20%	10%	0%	p<0.001
Ciprofloxacin						
0.5µg/ml 50%		30%	10%	0%	0%	p<0.001
"1µg/ml	0%	0%	0%	0%	0%	p<0.001
Streptomycin						
1µg/ml	20%	10%	0%	0%	0%	p<0.001
"2µg/ml	0%	0%	0%	0%	0%	p<0.001

Table 6 Mortality (percentage) of *Aeromonas hydrophila* infected post larval *P.monodon* treated with antibiotics

Antibiotics	Concentration of bacteria inoculated (no. of cells/ml) into the culture water					Level of significance
	1.0×10^8	5.0×10^5	3.6×10^5	2.5×10^5	1.2×10^5	
	Percentage Mortality					
Control (without antibiotics)	100%	100%	90%	80%	70%	
Nifurpirinol (Prefuran)						
1 µg/ml	100%	100%	90%	70%	60%	p>0.05
" 4 µg/ml	90%	80%	80%	70%	60%	p>0.05
" 7 µg/ml	80%	80%	60%	60%	50%	p<0.05
Oxytetracycline						
2 µg/ml	30%	10%	10%	0%	0%	p<0.001
Ciprofloxacin						
0.5 µg/ml 30%		20%	0%	0%	0%	p<0.001
" 1 µg/ml	0%	0%	0%	0%	0%	p<0.001
Streptomycin						
1 µg/ml	20%	10%	0% '	0%	0%	p<0.001
" 2 µg/ml	20%	0%	0%	0%	0%	p<0.001
" 3 µg/ml	0%	0%	0%	0%	0%	P<0.001

Table 7 Mortality (percentage) of *Aeromonas sobria* infected post larval *Penaeus monodon* treated with antibiotics

Antibiotics	Concentration of bacteria inoculated (no. of cells/ml) into the culture water					Level of Significance
	1.0 X 108	5.0 X 105	3.6 X 105	2.5 X 105	1.2 X 105	
	Percentage Mortality					
Control (without antibiotics)	100%	100%	90%	80%	80%	
Nifurpirinol (Prefuran)						
1 µg/ml	100%	100%	90%	80%	80%	p>0.05
" 4 µg/ml	90%	90%	80%	60%	60%	p>0.05
" 7 µg/ml	70%	60%	60%	40%	40%	p<0.01
Oxytetracycline						
2 µg/ml	30%	10%	0%	0%	0%	p<0.001
Ciprofloxacin						
0.5 µg/ml 20%		10%	0%	0%	0%	p<0.001
" 1 µg/ml	0%	0%	0%	0%	0%	p<0.001
Streptomycin						
1 µg/ml	20%	10%	0%	0%	0%	p<0.001
" 2 µg/ml	0%	0%	0%	0%	0%	p<0.001

Conclusion

In this study, we have shown that water samples from the aquaculture environment in and around Chennai, India were contaminated with *Vibrio anguillarum, V. furnissii, V.damsela, Aeromonas hydrophila* and *A. sobria*. They were shown to be pathogenic and caused mortality of the infected post larval *P. monodon* under experimental conditions. The present investigation has thus demonstrated that the clean surface water is a unique fundamental commodity and hence a large scale continuous surveillance programme of drug resistant/sensitive pathogenic bacteria along the coastal surface waters is needed to ensure a consistently acceptable level of water quality for aquaculture and other purposes. Further, there is a possibility of development/ adoption of diagnostic tools for rapid detection of the drug resistant pathogens in water and a cost effective treatment methods could possibly be evolved. We have shown that Ciprofloxacin and streptomycin were the best antibiotics for the control of the bacteria thereby to reduce the mortality of bacteria infected shrimp. Prefuran is the least effective antibiotics in reducing the mortality of the infected post larvae. Our studies establish that the pathogenic bacteria can be controlled with selective use of certain effective antibiotics and improve the health status of shrimp in culture conditions and can have a positive impact in the areas of sustainable aquaculture environment and development.[38–42]

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Conflict of Interest

We declare that there is no financial or conflict of interests.

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