BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

**Journal of Zoological Research**
http://ojs.bilpublishing.com/index.php/jzr

ARTICLE

# Surveillance of *Vibrio spp.* in *Penaeus monodon* Collected from Shrimp Pond of Satkhira, Bangladesh

**Abdullah-Al-Amin[1] Shahena Aktar Shipa[1*] M. Niamul Naser[2] Md. Faruque Miah[1]**

1. Zoology, Fisheries and Marine Biotechnology Research Unit, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
2. Department of Zoology, University of Dhaka, Bangladesh

ARTICLE INFO

*Article history*
Received: 1 July 2020
Accepted: 17 July 2020
Published Online: 30 July 2020

*Keywords:*
Tiger shrimp
Morphological
Physiological
Biochemical
Antibiogram

ABSTRACT

*Vibrio* is the most common genera associated with crustaceans and often causing significant economic losses. Many *Vibrio* species are pathogenic to human and have been implicated in food borne diseases. The present study was conducted to identify *Vibrio* spp. from the tiger shrimp (*Penaeus monodon*) of shrimp pond at Satkhira, Bangladesh. A total number of 33 *Vibrio* species isolates were identified from 20 shrimp samples through a series of morphological, physiological and biochemical tests. The work reports the prevalence of *Vibrio* spp. in the pond environments and the existence of three *Vibrio* species such as V. *alginolyticus*, *V. parahaemolyticus* and *V. harveyi* were identified. In the study of antibiogram, all isolates were shown 100% sensitive to streptomycin, ciprofloxacin and chloramphenicol. Maximum 41% isolates were shown resistant to co-trimethaxozole whereas 30% and 24% resistant to azithromycin and novobiocin respectively.

## 1. Introduction

Mainly tiger shrimp cultivation is almost exclusively concentrated in three districts of Bangladesh namely, Satkhira, Khulna, Bagerhat (along with Rampal) [1]. Over 70% of the total numbers of farms are located in the greater Khulna (Satkhira, Khulna and Bagerhat), which accounts for 74% of the land area under shrimp cultivation and 77% of total output. The remainder of the farm area under cultivation and their output are almost entirely accounted for by Cox's Bazar. In the major shrimp producing areas, shrimp yields range from between 150 kilogram per hectare in Cox's Bazar and just over 200 kilogram per hectare in Satkhira.

The tiger shrimp farming activities contribute significant role in the national economy of the country. Shrimp is second most important exportable commodities in Bangladesh having a high demand and price in the international market [2- 4]. Besides, its production through aquaculture and trade offers unique opportunity in providing employment and poverty alleviation. The tiger shrimp, *Penaeus monodon* an agricultural product is recognized as a considerable one for its remarkable contribution in soaring the foreign exchange earnings. Fisheries sector contributes 4.57% to the Gross Domestic Product (GDP) and shrimp alone contributes about 0.07% of total export earnings [5].

**Corresponding Author:*
*Shahena Aktar Shipa,*
*Zoology, Fisheries and Marine Biotechnology Research Unit, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh;*
*Email: shahena.shipa@gmail.com*

 DOI: https://doi.org/10.30564/jzr.v2i3.2087

Due to the increase in virulence of pathogens, especially bacterial and viral, a crisis in the shrimp industry was affected over the last few years. Low survival rates in hatchery or grow out conditions are often associated with bacterial diseases caused by *Vibrio spp*. Rather the treating the underlying cause, the diseases are treated in this cases. With the use of antimicrobial chemicals, especially antibiotics and chlorine along with the interactions of microbes, animals and their environment under the stress of commercial production of *Penaeus monodon*, have led to the emergence of more virulent pathogens [6]. For the identification of the presence of the toxic chemical, 'nitrofuran and its metabolites' that was used as broad spectrum antibiotic, several shipments of exported frozen shrimp from Bangladesh were rejected by EU, which caused a loss of about US$ 10 million in 2008 in Bangladesh [7].

The present study was conducted on the identification of *Vibrio spp*. in *Penaeus monodon* as it is the main target species for culture and high production in Bangladesh which is one of the important species for animal protein as well as great source for foreign earnings. However, the main barrier of the production of *Penaeus monodon* is pathogenic which is effect caused by the viruses and bacteria. *Vibrio* is one of the important pathogen that reducing production of this tiger shrimp. Very few researches have been done in *Penaeus monodon* with *Vibrio* in Bangladesh whereas no record was found shrimp pond of Satkhira. Therefore, this research is interested to study the observation of *Vibrio* status in *Penaeus monodon* of shrimp pond in Satkhira, Bangladesh.

## 2. Materials and Methods

### 2.1 Sample Collection

All of the experiments were carried out in the Zoology, Fisheries and Marine Biotechnology Research Unit in the Department of Genetic Engineering and Biotechnology at Shahjalal University of Science and Technology, Sylhet, Bangladesh. Samples were collected from twenty shrimps, which were selected randomly from 05 shrimp ponds in Katakhali village of Satkhira, Bangladesh. The samples of *Penaeus monodon* (5-9 cm long) were caught by harvesting with net and then transferred into the sterile polyethylene bag. All the samples were carried to the lab as soon as possible with an ice box.

### 2.2 Preparation of the Sample

All the samples were prepared and kept in different trays swabbed in 70% alcohol. For having normal temperature they were kept in the laminar airflow for 30 min. Bacterial swab from different parts of the inner and outer body were cultured into the *Vibrio* specific TCBS agar.

### 2.3 Isolation of Bacteria

Isolates with green and yellow colonies from each plate were isolated and sub-cultured into nutrient agar medium (supplemented with 2% NaCl). Bacterial specimen was collected only from single colony of each. All the sub-cultured bacteria were preserved for further biochemical tests and investigation.

### 2.4 Morphological Characterization

The studied bacterial isolates were provisionally identified on the basis of the results of morphological test such as Gram staining. Gram staining was performed using compound microscope according to the procedure described by Petersen *et al.,* 2016 [8]. The gram positive and gram negative bacteria were identified based on violet and pink color respectively.

### 2.5 Biochemical Characterization

Various biochemical tests i. e. Catalase test, Oxidase test, Oxidation fermentation (O-F) test, Motility test, Salt tolerance test, Indole test, Methyl-Red (MR) test, Voges-Proskauer (VP) test, Citrate test, Urease test, Gelatin liquefaction test, Dextrose utilization test, Hydrogen sulfide ($H_2S$) production test and Lysine decarboxylase test were performed to identify bacterial isolates.

### 2.6 Salt Tolerance Test

With varying amounts of NaCl (0%, 4%, 6%, 8%, and 10%), salt tolerance test was done on nutrient agar media supplemented. To study the salt tolerance range of the isolated species and the optimum concentration, the test was performed.

### 2.7 Determination of Antimicrobial Sensitivity Patterns

Susceptibility of bacterial isolates to different antibacterial agents was determined in vitro by disc diffusion method as described by Rahman and Hossain (2010) with slight modification. The procedure involved measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Commercially available eight antimicrobial discs such as streptomycin, erythromycin gentamycin, chloramphenicol, ciprofloxacin, co-trimethxazole, azithromycin, novobiocin were used for the test.

## 3. Resuls

### 3.1 Isolation of *Vibrio spp*.

Twenty shrimp samples were screened for observing the



DOI: https://doi.org/10.30564/jzr.v2i3.2087

presence of *Vibrio spp*. All the examined shrimps were found positive with *Vibrio spp*. A total of 33 *Vibrio* spp. isolates were collected from the experimental shrimps. The incidence of *Vibrio spp*. varied depending on the samples examined. The isolates were numbering as H1-H13, H15-H23, H25-H26 and H29-H37.

### 3.2 Morphological Characteristics of *Vibrio spp*.

Morphological studies were carried out by microscopic observations and growth characteristics in petri dishes. Fifty three yellow or green colored pin point colonies bearing colony characteristics of *Vibrio spp*. were preliminary selected from TCBS agar plates. The isolated colonies were small to medium in size and rounded. Thirty three isolates out of those fifty three were gram negative and short rod shaped. They were able to grow at $25^0$C and $37^0$C but failed to grow at $4^0$C.

### 3.3 Biochemical Characteristics of the Isolates

A series of biochemical tests were performed to identify the *Vibrio* spp. isolates up to species level (Table 1). All the isolates were positive for oxidase, catalase and O-F test. In indole test H3, H6, H10, H17, H30 and H35 showed negative result while all other isolates showed positive result. The negative results were found for all isolates in methyl-red test except the isolates H6, H10, H15, H20, H30, and H36. Again isolate no. H4, H7, H8, H13, H17, H21, H22, H31 and H33 showed positive result for Voges-Prausker test, whereas others showed negative result. In case of citrate utilization test all isolates were negative except isolates H8, H10, H15, H16, H17, H19, H25, H31 and H35. In urease test only isolates H6, H10, H16,

**Table 1.** Biochemical characterization of *Vibrio* spp. Isolates

| Isola-tes | Tests performed | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | O | M | O-F | VP | MR | I | Citrate utili-zation test | Urease test | Gelatin lique-faction test | Dextrose utili-zation test | H2S test | Lysine decarbo-xy-lation test |
| H1 | + | + | + | + | - | + | + | - | - | + | - | - | - |
| H2 | + | + | + | + | - | + | + | - | - | + | - | - | - |
| H3 | + | + | + | + | - | + | - | - | - | + | - | - | - |
| H4 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H5 | + | + | + | + | - | + | + | - | - | + | - | + | - |
| H6 | + | + | + | + | - | - | - | - | + | - | - | - | - |
| H7 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H8 | + | + | + | + | + | + | + | + | - | + | + | + | - |
| H9 | + | + | + | + | - | + | + | - | - | + | - | - | - |
| H10 | + | + | + | + | - | - | - | + | + | - | + | - | - |
| H11 | + | + | + | + | - | + | + | - | - | + | - | - | - |
| H12 | + | + | + | + | - | + | + | - | - | + | - | + | - |
| H13 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H15 | + | + | + | + | - | - | + | + | - | + | - | + | - |
| H16 | + | + | + | + | - | + | + | + | + | - | + | - | - |
| H17 | + | + | + | + | + | + | - | + | + | + | + | - | - |
| H18 | + | + | + | + | - | + | + | - | - | + | - | + | - |
| H19 | + | + | + | + | - | + | + | + | - | + | - | - | - |
| H20 | + | + | + | + | - | - | + | - | + | + | - | - | - |
| H21 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H22 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H23 | + | + | + | + | - | + | + | - | - | - | - | - | - |
| H25 | + | + | + | + | - | + | + | + | - | + | - | + | - |
| H26 | + | + | + | + | - | + | + | - | - | - | - | + | - |
| H29 | + | + | + | + | - | + | + | - | - | + | - | - | - |
| H30 | + | + | + | + | - | - | - | - | + | - | + | - | - |
| H31 | + | + | + | + | + | + | + | + | - | + | - | + | - |
| H32 | + | + | + | + | - | + | + | - | - | - | - | - | - |
| H33 | + | + | + | + | + | + | + | - | - | + | - | - | - |
| H34 | + | + | + | + | - | + | + | - | - | + | + | - | - |
| H35 | + | + | + | + | - | + | - | + | + | + | - | - | - |
| H36 | + | + | + | + | - | - | + | - | - | + | - | - | - |
| H37 | + | + | + | + | - | + | + | - | - | + | - | - | - |

***Notes:*** C= Catalase test, O= Oxidase test, M= Motility, O-F= Oxidation fermentation test, VP= Voge's-Proskauer, MR= Methyl Red test, I= Indole Test, Hydrogen sulfide ($H_2S$) production test.

 DOI: https://doi.org/10.30564/jzr.v2i3.2087

H17, H20, H30 and H35 were found positive. In gelatinase test maximum isolates were found positive, whereas the isolates H6, H10, H16, H23, H26, H30 and H32 were showed negative. Only six isolates (H8, H10, H16, H17, H30 and H34) were showed positive in dextrose utilization test, otherwise rests of the isolates were found negative. There are eight isolates (H5, H8, H12, H15, H18, H25, H26 and H31) were given positive result in $H_2S$ test. All isolates were found negative result in lysine decarboxylase test.

### 3.4 Salt Tolerance Test of *Vibrio* spp.

All the isolates were grown in nutrient agar media containing 4%, 6%, 8% and 10% NaCl. No isolates were grown in 0% NaCl (Table 2).

### 3.5 Observed Species Diversity

Three *Vibrio* species such as *Vibrio Alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* were identified. It was found that isolates H4, H7, H13, H21, H22 and H33 were motile, Gram negative, positive in catalase and oxidase test, positive in MR and indole test (Table 01). Besides these isolates were positive in VP, gelatinase and oxidase-fermentation test but were negative in urease test and were identified as *Vibrio*. *Alginolyticus* (Table 01). This study was also found that isolates H9, H11, H19, H23, H29, H32 and H36 were grown in TCBS agar medium and showed green colony. They were motile, Gram

**Table 2.** Salt tolerance test of *Vibrio* spp. isolates

| Isolates name | Tests performed | | | | |
|---|---|---|---|---|---|
| | Growth in 0% NaCl | Growth in 4% NaCl | Growth in 6% NaCl | Growth in 8% NaCl | Growth in 10% NaCl |
| H1 | - | + | + | + | + |
| H2 | - | + | + | + | + |
| H3 | - | + | + | + | + |
| H4 | - | + | + | + | + |
| H5 | - | + | + | + | + |
| H6 | - | + | + | + | + |
| H7 | - | + | + | + | + |
| H8 | - | + | + | + | + |
| H9 | - | + | + | + | + |
| H10 | - | + | + | + | + |
| H11 | - | + | + | + | + |
| H12 | - | + | + | + | + |
| H13 | - | + | + | + | + |
| H15 | - | + | + | + | + |
| H16 | - | + | + | + | + |
| H17 | - | + | + | + | + |
| H18 | - | + | + | + | + |
| H19 | - | + | + | + | + |
| H20 | - | + | + | + | + |
| H21 | - | + | + | + | + |
| H22 | - | + | + | + | + |
| H23 | - | + | + | + | + |
| H25 | - | + | + | + | + |
| H26 | - | + | + | + | + |
| H29 | - | + | + | + | + |
| H30 | - | + | + | + | + |
| H31 | - | + | + | + | + |
| H32 | - | + | + | + | + |
| H33 | - | + | + | + | + |
| H34 | - | + | + | + | + |
| H35 | - | + | + | + | + |
| H36 | - | + | + | + | + |
| H37 | - | + | + | + | + |



DOI: https://doi.org/10.30564/jzr.v2i3.2087

negative, positive in oxidase test, positive in MR (except isolate H36 showed negative) and indole test (Table 01). These isolate did not grow in trypton broth without NaCl and gave negative result in dextrose utilization test, but showed positive in O-F test and catalase test. These isolates showed negative in citrate and urese test and positive in gelatinase test (Table 01) and identified as *Vibrio parahaemolyticus*. Furthermore, the isolates H5, H12, H18, H25 and H26 were grown in TCBS agar media and showed positive in luminescence and $H_2S$ test. During the course of study it was found that, these isolates were Gram negative, motile, positive in O-F, MR and indole test and negative in VP test (Table 01). In this study it was found that these isolates were negative in dextrose fermentation test and citrate test (Table 01) and identified as *Vibrio harveyi*.

### 3.6 Antibiogram Study of *Vibrio* spp. Isolates

The bacterial isolates belong to *Vibrio spp*. was found to vary in their antibiotic sensitivity pattern to the eight antibiotic discs used. Three antibiotics were found 100% sensitive in this study. All of the isolates were susceptible to streptomycin, chloramphenicol and ciprofloxacin. Conversely majority of the isolates were resistant to co-trimethoxazole (41%), azithromycin (30%) and novobiocin (24%). The percentages of antibiotic susceptibility pattern were shown in (Figure 01). The zone of inhibition given by the antibiotics was varied with different isolates ranging from 14 mm to 40 mm in diameter (Table 3). Formation of clear zone by various antibiotic discs against *Vibrio* sp. Isolates were found.

**Table 3.** Antibiotic sensitivity pattern of *Vibrio* spp. isolates

| Isolates name | Name of antibiotics with their sensitivity pattern (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S | E | G | CH | CI | CO | AZI | NO |
| H1 | 34 | 22 | 16 | 36 | 32 | 34 | 30 | 22 |
| H 2 | 30 | 29 | 28 | 32 | 28 | 33 | 24 | 24 |
| H3 | 33 | 31 | 25 | 30 | 30 | R | 18 | R |
| H4 | 27 | 22 | 30 | 33 | 18 | 33 | 14 | 16 |
| H5 | 30 | 31 | 22 | 34 | 26 | 31 | R | 14 |
| H6 | 32 | R | 31 | 28 | 32 | R | 26 | 17 |
| H7 | 26 | 33 | 29 | 30 | 20 | 30 | 32 | R |
| H8 | 31 | R | R | 36 | 24 | R | 24 | 26 |
| H9 | 29 | 30 | 33 | 40 | 23 | R | 30 | 21 |
| H10 | 33 | 24 | 30 | 38 | 27 | 28 | R | R |
| H11 | 34 | 28 | 27 | 31 | 32 | 27 | R | 14 |
| H12 | 29 | 30 | 18 | 33 | 30 | R | 29 | 18 |
| H13 | 33 | 34 | 33 | 40 | 32 | 16 | 32 | 22 |
| H15 | 25 | R | R | 31 | 26 | 23 | R | R |
| H16 | 31 | R | 24 | 29 | 28 | R | 30 | 16 |
| H17 | 30 | 25 | 16 | 33 | 30 | 33 | R | 22 |
| H18 | 24 | R | 26 | 36 | 20 | R | 16 | 19 |
| H19 | 27 | 30 | 32 | 30 | 24 | 25 | R | R |
| H20 | 33 | 29 | R | 32 | 22 | R | R | 16 |
| H21 | 24 | 33 | 30 | 34 | 32 | R | 32 | 14 |
| H22 | 31 | R | 22 | 35 | 20 | R | 22 | 24 |
| H23 | 28 | 31 | 24 | 38 | 28 | 26 | R | 22 |
| H25 | 32 | R | R | 33 | 27 | 31 | 25 | R |
| H26 | 34 | 27 | 29 | 28 | 32 | R | 28 | R |
| H29 | 29 | 33 | R | 30 | 26 | 30 | R | 18 |
| H30 | 31 | 29 | 33 | 34 | 23 | R | 27 | 20 |
| H31 | 24 | 34 | 30 | 32 | 29 | 32 | R | R |
| H32 | 32 | R | 26 | 30 | 31 | R | 30 | 23 |
| H33 | 28 | 31 | 20 | 36 | 22 | R | 22 | 24 |
| H34 | 26 | 25 | 30 | 30 | 30 | 32 | 20 | R |
| H35 | 33 | 32 | 24 | 34 | 29 | R | 27 | 16 |
| H36 | 25 | 30 | 29 | 30 | 31 | 26 | 30 | 18 |
| H37 | 34 | R | 30 | 32 | 26 | 30 | R | 23 |

***Notes:*** E = Erythromycin; CH = Chloramphenicol; S = Streptomycin; G = Gentamycin; CI = Ciprofloxacin; CO = Co-trimethoxazole; AZI = Azithromycin; NV = Novobiocin.

 DOI: https://doi.org/10.30564/jzr.v2i3.2087

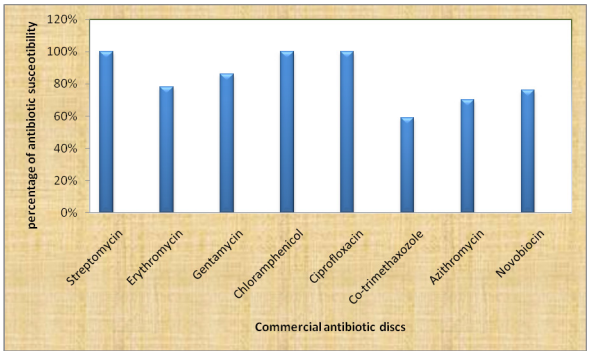


**Figure 1.** Percentage of susceptibility of *Vibrio* spp. isolates to commercial antibiotic discs

## 4. Discussion

All the isolates of the present study were found gram negative, while positive in motility test, catalase and oxidase. Most of the isolates were positive in MR and indole test but negative in VP test. These all findings are similar with the findings of Chakma *et al.* 2018 [9].

Again all isolates were positive in O-F test that means all isolates were capable of acid production but most of the isolates were unable to produce gas in dextrose fermentation test (except isolates H8, H10, H16,H17, H30 and H34). In the present study it was found that most of the isolates were negative in citrate utilization test as well as $H_2S$ test, same result was found by Teng *et al.* 2017 [10]. Maximum isolates showed negative in lysine decarboxylase test and urease test, but showed positive result in gelatin liquefaction test. Similar biochemical results are reported by other researchers [11-16].

In this study, three types of *Vibrio* species such as *Vibrio Alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* were detected. Species were identified on the basis of different biochemical test which is similar with the test results for *Vibrio Alginolyticus* [12, 17]. *Vibrio parahaemolyticus* similar results were also obtained by Jayasree *et al.,* 2006 and Alagappan *et al.,* 2010 [18, 19] and according to the findings of Jiravanichpaisal *et al.,* 1994 and Lavila-Pitago, 1996 [20, 21] the same results were for their study of *V. harveyi*.

The prevalence uses of antimicrobial agents against the *Vibrio* sp. are described by various researchers over the last 20 years [22-26]. As far in the present study, 59% isolates were resistant to co-trimethaxozole, which is mostly similar to Razvykh *et al.,* 1990 [27]. According to Abraham *et al.,* 1997 [28], it is compared with the present study that 30% and 24% resistance were shown to azithromycin and novobiocin respectively (Table 03). The present study showed that 78% sensitive to erythromycin, but Thakur *et al.,* 2003 [29] reported 100% sensitivity. According to Herwig and Gray, 1997 [30] a mostly similar comparison found with the present study that gentamycin showed 82% sensitivity pattern.

The present antibiogram study showed that 100% sensitivity to ciprofloxacin, chloramphenicl and streptomycin. Karunasagar *et al.,* 1994 [31] also found 100% sensitivity to chloramphenicol. Erythromycin are frequently used in Bangladesh in doses similar with other countries [32], and these drugs are known to yield plasmid-mediated resistance in aquatic bacteria [33].

The tiger shrimp, *Penaeus monodon* is one of the most nutritional and economic important species of Bangladesh. However, pathogenic effects reduce the production of this species and *Vibrio* is one of the vital causal agents. It was observed in the present study that *Vibrio spp*. was occurred in the *Penaeus monodon* of shrimp pond in Satkira and three species of *Vibrio* like *Vibrio Alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* were observed. The present study has been emphasized on the antibiogram of *Vibrio* spp. and study found 100% susceptibility to streptomycin, chloramphenicol and ciprofloxacin.

## 5. Conclusion

The findings of the study suggest to focus on the further molecular test with a large number of fish samples. In addition, the host-pathogen interaction also need to be analyse with the exposure of environmental factors responsible for the outbreaks of the diseases caused by *Vibrio spp*., if any.

### Acknowledgments

The authors would like to thank the Department of Genetic Engineering and Biotechnology at Shahjalal University of Science and Technology for giving necessary financial support to carry out this work.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors Contribution

All authors are equally responsible for every task of regarding the manuscript.

### References

[1] Karim, M.F., Zhang, X., Li, R. Dynamics of Shrimp Farming in the SouthwesternCoastal Districts of Bangladesh Using a Shrimp YieldDataset (SYD) and Landsat Satellite Archives. Sustainability, 2019, 11:4635.
DOI: 10.3390/su11174635



DOI: https://doi.org/10.30564/jzr.v2i3.2087

[2] BBS (Bangladesh Bureau of Statistics). Bangladesh Statistics 2017, Ministryof Planning: Dhaka, Bangladesh.

[3] Ahasan, D.A. Bangladesh's Winning Maritime Boundary, Development, Conservation and Management of Fish Resources, National Fish Week 2012 Compendum (in Bengali), Narinda, Dhaka: Department of Fisheries, Ministry of Fisheries and Livestock, GoB., 2012: 23-25.

[4] Rahman, M.M. and Hossain, M.M. (2009) Production and export of shrimp of Bangladesh: Problems and prospects. Progressive Agriculture 20(1, 2): 163-171.

[5] BER (2009 and 2010). Bangladesh Economic Review (BER) 2007. Economic Adviser's Wing. Finance Division, Ministry of Finance. Government of the People's Republic of Bangladesh.

[6] Lightner, D.V. Diseases of cultured penaeid shrimp. (Eds. McVey, J.P.) CRC Handbook of Mariculture, 2nd ed. Crustacean Aquaculture, vol. 1. CRC Press, Boca Raton FL, 1993: 393- 486.

[7] Rahman, S., Khan, S.N., Naser, M.N., Karim, M.M. Isolation of *Vibrio spp*. from Penaeid Shrimp Hatcheries and Coastal Waters of Cox's Bazar, Bangladesh. ASIAN J. EXP. BIOL. SCI. 2010, 1(2): 288 - 293

[8] Petersen, J., Mclaughlin, S. Laboratory Exercises in Microbiology: Discovering the Unseen World Through Hands-On Investigation. City University of New York (CUNY) CUNY Academic Works, 2016.

[9] Chakma, F., Sabuj, A.A.M., Haque, Z.F., Pondit, A.,Talukder, B., Kabir, S.M.L., Saha, S. Isolation and characterization of *Vibrio* sp from semi processed shrimp. Journal of Research in Biology, 2018, 8(7): 2566-2573

[10] Teng, T., Liang, L., Chen, K., Xi, B., Xie, J., Xu, P. Isolation, identification and phenotypic and molecular characterization of pathogenic *Vibrio* vulnificus isolated from Litopenaeus vannamei. PLoS ONE, 2017, 12(10): e0186135, https://doi.org/10.1371/journal.pone.0186135

[11] Pedersen, K., Tiainen, T., Larsen, J.L. Antibiotic resistance of *Vibrio* anguilarum, in relation to serovar and plasmid contents. Acta vet.scand, 1995, 36: 55-64.

[12] Austin, B., Austin, D.A. Bacterial fish pathogens, Diseases in farmed and wild fish, 2nd edn. Ellis Horwood Ltd., Chichester, 1993.

[13] Lee, K.K., Yu, S.R., Chen, F.R., Yang, T.I., Liu, P.C. Virulence of *Vibrio Alginolyticus* isolated from diseased tiger prawn, *Penaeus monodon*. Curr. Microbiol. 1996, 32: 229-231. DOI: 10.1007/s002849900041

[14] Mercedes, A., Anicet, R.B. A set of keys for biochemical identification of environmental *Vibrio* species. Journal of Applied Bacteriology, 1994, 76: 79-85.

[15] Sung, H.H., Hsu, S.F., Chen, C.K., Ting, Y.Y., Chao, W.L. Relationships between disease outbreak in cultured tiger shrimp (*Penaeus monodon*) and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. Aquaculture, 2001, 192: 101-110. DOI: 10.1016/s0044-8486(00)00458-0

[16] Choopun, N., Louis, V., Haq, A., Colwell, R.R. Simple Procedure for Rapid Identification of *Vibrio* cholerae from the Aquatic Environment. Applied and Environment Microbiology, 2002, 68(2): 995-998.

[17] Alsina, M. and Blanch, A.R. A set of keys for biochemical identification of environmental *Vibrio* species. J. Appl. Bacteriol. 1994, 76: 79-85.

[18] Jayasree, L., Janakiram, P., Madhavi, R. Characterization of *Vibrio spp*. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). J. World Aquac. Soc. 2006, 37: 523-532. https://doi.org/10.1111/j.1749-7345.2006.00066.x

[19] Alagappan, K.M., Deivasigamanim, B., Somasundaramm, S.T., Kumaran, S. Occurrence of *Vibrio parahaemolyticus* and Its Specific Phages from Shrimp Ponds in East Coast of India. Curr. Microbiol. 2010, 61: 235-240, https://doi.org/10.1007/s00284-010-9599-0

[20] Jiravanichpaisal, P., Miyazaki, T. Histopathology, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger shrimp *Penaeus monodon*. J. Aquat. An. Health, 1994, 6: 27-35. https://doi.org/10.1577/15488667(1994)006%3C0027:H-BAPOV%3E2.3. CO;2

[21] Lavilla-Pitogo, C.R., Leano, E.M., Paner, M.G. Mortalities of pond cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent bacteria, *Vibrio harveyi* in the rearing environment. SICCPPS book of abstracts, SEAFDEC, Iloilo City, Philippine, 1996, 40.

[22] Corliss, J., Lightner, D.V., Zein-Eldin, P. Some effects of oral doses of oxytetracycline on growth, survival and disease in Penaeus aztecus. Aquaculture, 1977, 11: 355-362. https://doi.org/10.1016/0044-8486(77)90085-0

[23] Mohney, L., Bell, T.A., Lightner, D.V. Shrimp antimicrobial testing-1. In vitro susceptibility of thirteen gram negative bacteria to twelve antibiotics. Journal of Aquatic Animal Health, 1992, 4: 257-261. https://doi.org/10.1577/1548-8667(1992)004%3C0257:SATIIV%3E2.3.CO;2

[24] Ruangpan, L., Kitao, T. *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. Journal of Fish diseases, 1991, 14: 383-388. https://doi.org/10.1111/j.1365-2761.1991.tb00836.x

[25] Charantchakool, P., Pearson, M., Limsuwan, C., Roberts, R.J. Oxytetracycline sensitivity of *Vibrio spp*. isolated from diseased black tiger shrimp, *Penaeus monodon* Fabricius. Journal of Fish Diseases, 1995, 18: 79-82. https://doi.org/10.1111/j.1365-2761.1995.tb01268.x

[26] Lee, S.W., Najiah, M., Wendy, W., Nadirah, M. Comparative study on antibiogram of *Vibrio spp*. isolated from diseased postlarval and marketable-sized white leg shrimp (Litopenaeus vannamei). Front. Agric. China, 2009a, 3: 446-451. DOI: 10.1007/s11703-009-0068-0

[27] Razvykh, V.M., Friauf, E.V., Givental, N.I., Bogdanova, L.F., Vedmina, E.A. Atibiotic sensitivity of *Vibrio parahaemolyticus* isolated in the turkmene SSR. Antibiot. Khimioter, 1990, 35: 23-24.

[28] Abraham, T.J., Manley, R., Papanippan, R., Dhevendran, K. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. Journal of Aquaculture in Tropics, 1997, 12: 1-8.

[29] Thakur, A.B., Vaidya, R.B., Suryawanshi, S.A. Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from moribund shrimps. India J. Mar. Sci., 2003, 32: 71-75.

[30] Herwig, R.P., Gray, J.P. Microbial response to antibacterial treatment in marine microcosms. Aquaculture, 1997, 152: 139-154. https://doi.org/10.1016/S0044-8486(96)01517-7

[31] Karunasagar, I., Pai, R., Malathi, G.R., Karunasagar, I. Mass mortalities of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture, 1994, 128: 203-209. https://doi.org/10.1016/0044-8486(94)90309-3

[32] Uddin, S.A., Kader, M.A. The use of antibiotics in shrimp hatcheries in Bangladesh. J. Fisheries and Aquatic Sciences, 2006, 1(1): 64-67. DOI: 10.3923/jfas.2006.64.67

[33] Graslund, S., Bengtsson, B.E. Chemicals and biological products used in south-east Asian shrimp farming and their potential impact on the environment- a review. The Science of the Total Environment, 2001, 280: 93-131. DOI: 10.1016/s0048-9697(01)00818-x



DOI: https://doi.org/10.30564/jzr.v2i3.2087