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16S rRNA GENE SEQUENCE BASED IDENTIFICATION OF V*IBRIO* SPP. IN SHRIMP AND TILAPIA HATCHERIES OF BANGLADESH

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Abstract

Presence of *Vibrio* spp., one of the deadliest fish and shrimp pathogens in aquaculture facilities worldwide for which hatchery owners often suffer hectic economic losses, were identified in shrimp and tilapia hatcheries of Cox's Bazar and Mymensingh, Bangladesh. Thirty seven *Vibrio* isolates, selected on the basis of their morphological dissimilarities in thiosulphate citrate bile salt sucrose agar (TCBS) plate, were subjected to amplified 16S ribosomal DNA restriction analysis (ARDRA) by using *Alu*I restriction enzyme following their DNA extraction and amplification of 16S rRNA (1450 bp). From this analysis, representative isolates of 8 ARDRA groups, named as ARH 1 to ARH 8, of different band patterns were sequenced and identified as *Vibrio alginolyticus*, *Aeromonas veronii, A. hydrophila, Vibrio vulnificus, V. cholerae, Edwardsiella hoshinae, Bacillus methylotrophicus* and *Aeromonas veronii*, respectively. *Vibrio* species identified in this study are pathogenic for human and aquatic organisms, and were found only in shrimp hatchery with the dominance of *V. alginolyticus*. Findings of this study indicate the poor quality of water treatment and management of the hatchery. It was also observed that all the three *Vibrio* species were present in the *Artemia* rearing tank which indicates the possible source of pathogens.

Introduction

To date, 130 species of *Vibrio* have been described and 12 were classified as human pathogens implicated mostly in food- or water-borne diseases, including *V. cholerae* as the main cause of diarrhoea, *V. parahaemolyticus* as the cause of food-borne gastroenteritis and *V. vulnificus* which is known to cause 95% of all deaths associated with seafood consumption(1). Other pathogenic species include *V. alginolyticus, V. damsela, V. fluvialis, V. furnissii, V. hollisae, V. metschnikovii* and *V. mimicus*(2). Some vibrios may cause disease in both aquatic animals and humans. Austin (2010) categorizes *V. cholerae, V. parahaemolyticus* and *V. vulnificus* as high risk organisms for zoonoses, and *Grimontia (=Vibrio) hollisae, Photobacterium (=Vibrio) damselae* subsp. *damselae*, *V. alginolyticus*, *V. harveyi, V. fluvialis, V. furnissii, V. metschnikovii* and *V. mimicus* as low risk organisms(3).

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Vibrios occur in a wide range of aquatic environments, including estuaries, marine coastal waters, and aquaculture settings worldwide[4]. Several cultivation-dependent and independent studies have shown that Vibrios appear particularly in high densities in and/or on marine organisms, e.g., fish[5], shellfish[6], shrimps[7]. Vibriosis caused by *Vibrio* spp., is one of the most prevalent diseases in fishes and other aquaculture-reared organisms and is widely responsible for mortality in aquaculture systems worldwide[8]. Due to intensification and feeding loads modern intensive shrimp systems provide almost ideal conditions for the propagation of diseases like vibriosis[9]. During outbreaks in larval and postlarval shrimp rearing, luminescent *V. harveyi, V. campbellii*, and probably *V. splendidus* have been isolated. Vibrios are considered opportunistic pathogens, but evidence suggests that some strains can be regarded as primary pathogens, especially in the case of *V. penaeicida*. *Artemia* spp. can also be susceptible to infection by Vibrios, as is the case of *V. proteolyticus* and strains of *V. parahaemolyticus* and *V. campbellii*[10].

Disease prevalence has increased with intensification of aquaculture and with increased fisheries production in Bangladesh. However, virulence of pathogens, especially bacterial diseases caused by *Vibrio* spp. has been found to be a crisis in the shrimp industry over the last few years[11]. Aquaculture industries of both saltwater and freshwater are becoming increasingly vulnerable to bacterial infection due to easy transmission of pathogens within farm area. Diseases caused by *Vibrio* spp. and *Aeromonas* spp. are commonly found to cause mortality[12]. These pathogens cause serious infections, decreased production both in the hatchery and grow-out ponds, reduced feed conversion and growth rates in surviving individuals, thus having a negative impact on the overall financial efficiency of the business. That is why efficient methods for detection, differentiation and characterization of *Vibrio* spp. are required to be included in screening programs in order to prevent infections and diseases associated with the pathogenic strains. However, little is known on the prevalence of *Vibrio* spp. in Bangladesh, except for *V. cholerae*[13]. Therefore, it is of utmost importance to detect the prevalence of vibrios in shrimp and fish hatcheries as a basis for preventive protection policy as well as public health concern.

Identification of the causative organisms up to the species level is very useful as it helps in determining the exact source of any outbreak and in devising strategies to reduce the severity of the disease. However, the conventional identification techniques involving a series of biochemical tests and agglutination with specific antisera are time-consuming and ambiguous[14]. Therefore, there is a need to characterize the sample isolates by 16S rRNA gene sequencing for species-specific identification of Vibrios. The objective of the study was to understand the prevalence of *Vibrio* community in the sampled shrimp and tilapia hatcheries of Bangladesh using culture and molecular techniques.

Materials and Methods

Sample collection and preparation: A total of 30 samples, 16 from coastal shrimp hatchery of Cox's Bazar and the rest 14 from tilapia hatchery of Mymensingh, have been collected and examined. The sampling was done during June, 2015 to August, 2015. The samples include shrimp post larvae (PL), artemia (live feed) nauplii, fry of cultured tilapia and the cultured water. Collected samples were kept in icebox maintaining temperature at –4ºC and kept at –20ºC after transferring in the laboratory. All samples were collected following the method of American Public Health Association[15]. Fry and PL samples were aseptically grinded in a mortar and blended with physiological saline (0.85% NaCl). The water samples were kept just as it was. All blended samples were kept at a distance to reduce cross contamination.

Isolation of bacteria: Alkaline peptone water (APW) was used for the enrichment of the samples in order to provide a suitable environment for *Vibrio* spp. to grow and reach a detectable level for the presumptive identification. One ml blended solution from each sample was taken in 9 ml alkaline peptone water in a test tube. These tubes were kept in the incubator at 37^0C for 6 to 24 hrs. After the incubation period, 2/3 loopful of culture were transferred to TCBS agar media aseptically and then streaked. The streaked TCBS plates were kept in the incubator at 37^0C over a period of 24 hrs. After observing the morphology of bacterial colonies which grew in the TCBS, only single colonies with dissimilar traits were picked and then subcultured in new TCBS plates. The subcultured single colonies were further screened on their morphological appearance and only variant isolates were stored in Luria-Bertani (LB) broth supplemented with 30% glycerol at –80ºC for future use.

Molecular analysis of the isolates for identification: Chromosomal DNA of the selected 37 isolates was extracted followed by amplification of the 16S rRNA gene of the isolates by polymerase chain reaction (PCR) and amplified ribosomal DNA restriction analysis (ARDRA) to group the isolates into different genotypes. 16S rRNA gene amplicons of selected representative isolates of each genotype were sequenced followed by phylogenetic analysis to unveil the phylogenetic relatedness.

The conserved region of 16S rRNA of the isolates was amplified by PCR for further analysis. The sequences of oligonucleotide primers used for amplification of the ribosomal subunit 16S rRNA were as follows: 27F 5′- AGA GTT TGA TCM TGG CTC AG -3′[16] and 1492R 5′- CGG TTA CCT TGT TAC GAC TT-3′[17], with an approximate length of 1500 bp. Reactions of the amplification were conducted in a reaction volume of 15 µl containing 7.5 µl of GoTaq® (2X) Master Mix, 1.5 µl of DNA template, 4.5 µl of nuclease-free water, 0.75 µl of each forward and reverse primer. PCR amplification was done in a thermal cycler (Veriti 96-Well Thermal Cycler, Applied Biosciences, USA). The program initially consisted of the following steps: 94°C for 5 min for denaturation, then 40 cycles at 94°C for 1 min, 57°C for 1 min and 72°C for 1 min 30 s, followed by an extension step

at 72°C for 7 min. Successful amplification of the desired genes was visualized by resolving the PCR products in 1% agarose gel (w/v). One kb DNA ladder (Promega, USA) was also electrophoresed along the side of the amplified sample DNA, which served as a reference for the determination of the molecular weights of the fragments obtained in the PCRs. The gel was viewed using Alpha Imager HP Gel-documentation system (Cell Bioscience, USA).

Table 1. List of samples collected from Cox's Bazar (shrimp hatchery) and Mymensingh (tilapia hatchery).

Shrimp hatchery of Cox's Bazar		Tilapia hatchery of Mymensingh	
Sample ID	Sample	Sample ID	Sample
C1	Artemia nauplii from Tank 1	M1	Tilapia fry, 40 days
C2	Artemia nauplii from Tank 2	M2	"
C3	Shrimp PL of 10 days, Tank 1	M3	"
C4	Water from PL (10) Tank 1	M4	Tilapia fry, 25 days
C5	Shrimp PL of 10 days, Tank 2	M5	"
C6	Water from PL (10) Tank 2	M6	"
C7	Shrimp PL of 12 days	M7	Tilapia fry, 28 days
C8	Water from PL (12) Tank	M8	"
C9	Artemia nauplii from Tank 1	M9	"
C10	Artemia nauplii from Tank 2	M10	Tilapia fry, 33 days
C11	Shrimp PL of 8 days	M11	"
C12	Water from PL (8) Tank	M12	"
C13	Shrimp PL of 12 days	M13	Water from hapa- Tilapia of 25 days
C14	Water from PL (12) Tank	M14	"
C15	Shrimp PL of 10 days		
C16	Water from PL(10) Tank		

Amplified ribosomal DNA restriction analysis (ARDRA): Enzymatic digestion of 16S rRNA gene amplicons of the 37 presumptive *Vibrio* isolates was performed using the *Alu*I (Promega, USA) restriction enzyme. The restriction digestion (20 µl of final volume) was carried out for 4 hrs at 37°C. The preparation for the reaction was done in a reaction volume of 20 µl containing 0.5 µl of *Alu*I restriction enzyme, 2 µl of reaction buffer, 3 µl of template and 14.5 µl of nuclease free water. The resulting digestion products were resolved by agarose gel electrophoresis using 2% agarose (w/v).

16S rRNA gene sequencing and phylogenetic analyses: The PCR products of specific genes were purified with the Wizard PCR SV Gel and PCR CleanUp System kit (Promega, USA) according to the manufacturer's instruction prior to sequencing. The PCR products were sent to First BASE Laboratories Sdn Bhd (Malaysia) where the cycle

sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems®, USA) according to manufacturer's instruction and extension product was purified followed by capillary electrophoresis using ABI genetic analyzer (Applied Biosystems®, USA). Bidirectional (5′ to 3′ and 3′ to 5′) sequences were done for all 8 representative isolates.

Geospiza's Finch TV version 1.4 was used to view DNA sequences. Homology searching using BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov) was executed for comparing primary sequence identity. MEGA v 6.0[18] was used for the comparative analysis of molecular sequence data for reconstructing the phylogeny. Distance matrices were calculated using Kimura's 2-parameter distances. Robustness of topologies were assessed by the bootstrap method with 1,000 replicates.

Nucleotide sequence accession numbers: The reported sequences in this study have been submitted to NCBI GenBank database under accession numbers KY084544-KY084551.

Results and Discussion

Isolation of presumptive Vibrio spp.: Green, yellow and other colored colonies were selected from TCBS cultures and were then streaked on Luria Bertani Agar (LBA; with 3% NaCl supplementation). On TCBS, yellow colonies were presumptively identified as *V. alginolyticus*, *V. cholerae*, *V. fluvialis*, *V. metschnikovii* and *V. furnissii.* Green or blue-green colonies were assumed to be *V. parahaemolyticus*, *V. vulnificus* and *V. mimicus*. A total of 37 pure colonies were selected by TCBS, from the 30 samples collected from Cox's Bazar and Mymensingh. Presumptive *Vibrio* colonies were screened from the TCBS media based on their morphological dissimilarities (Table 2) so that as many variations as possible could be taken for further confirmation through molecular approaches.

Identification of the presumptive *Vibrio* isolates

Amplified Ribosomal DNA Restriction Analysis (ARDRA): The genomic DNAs purified from the 37 suspected *Vibrio* colonies were subjected to a polymerase chain reaction in order to amplify their respective 16S rDNA. Four isolates (5, 6, 14 and 21) did not amplify with the primers. These produced amplicons of about 1450 bp, which were used as substrate for cleavage by a restriction enzyme, *Alu*I to address their ARDRA pattern. The pattern produced eight different groups for all of the 37 isolates (Fig. 1). The corresponding bacterial isolates respective to each ARDRA group and the best representative colony from each group is summarized in Table 3. The ARDRA approach was practiced in this study for the purpose of accurate grouping among the morphologically dissimilar isolates. Grouping of isolates reduces the time and cost of molecular identification[19]. *Alu*I, the restriction enzyme used in ARDRA, was best recommended by Szczerba *et al.* 2009[20].

Table 2. Thirty seven presumptive *Vibrio* isolates and their colony morphology in TCBS plate that were selected for molecular analysis.

Colony ID	Sample ID	Color	Size	Shape	Elevation	Surface
1	C1	Yellow	Medium	Round	Convex	Smooth
2	C3	Blue	Medium	Round	Flat	Smooth
3	C6	Yellow	Small	Round	Convex	Smooth
4	C7	Yellow	Medium	Round	Convex	Smooth
5	C9	Greyish-green	Small	Irregular	Raised	Smooth
6	C10	Green	Small	Round	Flat	Smooth
7	C11	Yellow	Large	Round	Convex	Smooth
8	C12	Yellow	Medium	Round	Convex	Smooth
9	C13	Greenish black	Large	Round	Convex	Smooth
10	C14	Yellow	Large	Round	Convex	Smooth
11	C15	Yellow	Small	Round	Convex	Smooth
12	C16	Yellow	Small	Round	Convex	Smooth
13	C7	Yellow	Small	Round	Raised	Smooth
14	C9	Dark green	Small	Round	Convex	Smooth
15	C10	Green	Medium	Round	Convex	Smooth
16	C2	Yellow	Large	Round	Raised	Smooth
17	C3	Blue	Small	Round	Convex	Smooth
18	C5	Blue	Medium	Round	Convex	Smooth
19	M1	Dark green	Medium	Round	Convex	Smooth
20	M3	Blue green	Medium	Round	Convex	Smooth
21	M4	Greenish	Small	Irregular	Convex	Smooth
22	M5	Blue green	Medium	Round	Convex	Smooth
23	M7	Blue green	Small	Round	Convex	Smooth
24	M7	Greenish	Small	Round	Convex	Smooth
25	M9	Blue green	Medium	Round	Convex	Smooth
26	M12	Blue green	Medium	Round	Flat	Smooth
27	M14	Blue green	Small	Round	Convex	Smooth
28	M1	Blue	Medium	Round	Convex	Smooth
29	M3	Blue green	Small	Round	Convex	Smooth
30	M5	Blue green	Large	Irregular	Flat	Smooth
31	M5	Blue	Small	Round	Convex	Smooth
32	M8	Green	Small	Round	Convex	Smooth
33	M8	Blue green	Medium	Round	Convex	Smooth
34	M11	Blue green	Medium	Round	Convex	Smooth
35	M12	Dark green	Small	Round	Convex	Smooth
36	M13	Blue green	Medium	Round	Convex	Smooth
37	M13	Blue green	Medium	Round	Convex	Smooth

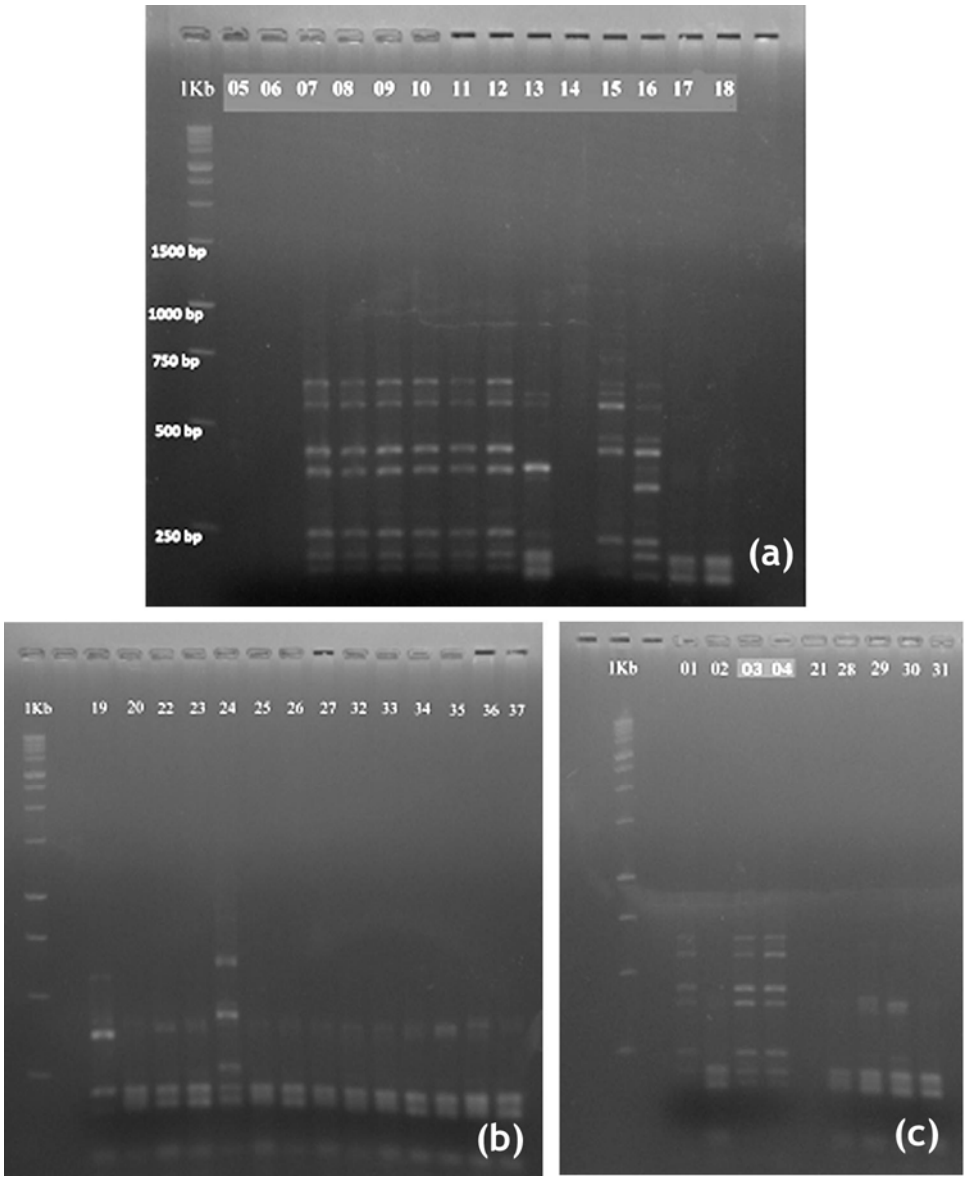

Fig. 1a-c: Denotes ARDRA pattern analysis of 37 isolates (four isolates did not amplify with 16S rDNA primers). Restriction digestion of bacterial isolates using *Alu*I enzyme, the left most lane in all 3 figures indicates 1 kb DNA marker. The number above each lane represents the respective bacterial isolate.

16S rRNA sequence based identification: The identification of the 16S rDNA gene sequences of eight representative isolates of the eight ARDRA groups (ARH 1, ARH 2, ARH 3, ARH 4, ARH 5, ARH 6, ARH 7 and ARH 8) through nucleotide BLAST of NCBI is summarised in Table 4.

Table 3. The ARDRA group of 33 isolates of bacteria from shrimp and tilapia hatchery samples.

ARDRA group	Representative colony ID	Colony ID
ARH 1	7	1 ,3, 4, 7, 8, 9, 10, 11, 12
ARH 2	18	2, 17, 18, 28, 31
ARH 3	13	13
ARH 4	15	15
ARH 5	16	16
ARH 6	19	19
ARH 7	24	24
ARH 8	26	20, 22, 23, 26, 25, 27, 29, 30, 32, 33, 34, 35, 36, 37

Table 4. 16S rRNA sequence (1320 bp) based identification of representative eight isolates from hatchery environment.

Group	Closest relative (obtained from BLAST search)	% homology with the GenBank sequence	GenBank accession number of corresponding sequence	GenBank accession number of isolates under present study
ARH 1	*Vibrio alginolyticus* strain PE2 16S rRNA	100%	KT036618.1	KY084544
ARH 2	*Aeromonas veronii* strain WX153415 16S rRNA	100%	KT964297.1	KY084545
ARH 3	*Aeromonas hydrophila* strain A-X4 16S rRNA	99%	KJ806490.1	KY084546
ARH 4	*Vibrio vulnificus* strain FORC_009 chromosome 2	99%	CP009985.1	KY084547
ARH 5	*Vibrio cholerae* strain BB31	100%	KF446244.1	KY084548
ARH 6	*Edwardsiella hoshinae* strain ATCC 35051 16S rRNA	99%	KM676416.1	KY084549
ARH 7	*Bacillus methylotrophicus* strain LD34 16S rRNA	100%	KR855694.1	KY084550
ARH 8	*Aeromonas veronii* strain K11 16S rRNA	100%	KU041801.1	KY084551

As group ARH 1 is identified as *Vibrio alginolyticus,* all the isolates of this group can also be identified as *Vibrio alginolyticus.* Similar conclusions can also be drawn with the other groups about their corresponding isolates. In Table 5, a total summary of identification of all the isolates is shown with their corresponding sample name that were

Table 5. Summary of molecular identification of the bacteria (33 isolates) isolated from shrimp and tilapia hatchery environments of Bangladesh.

Group	Identified species	Colony ID	Sample ID	Sample
ARH 1	*Vibrio alginolyticus*	1	C1	Artemia from Tank 1
		3	C6	Water from PL(10) tank
		4	C7	Shrimp PL of 12 days
		7	C11	Shrimp PL of 8 days
		8	C12	Water from PL(8) tank
		9	C13	Shrimp PL of 12 days
		10	C14	Water from PL(12) tank
		11	C15	Shrimp PL of 10 days
		12	C16	Water from PL(10) Tank
ARH 2	*Aeromonas veronii*	2	C3	Shrimp PL of 10 days
		17	C11	Shrimp PL of 8 days
		18	C5	Shrimp PL of 10 days
		28	M1	Tilapia fry, 40 days
		31	M5	Tilapia fry, 25 days
ARH 3	*Aeromonas hydrophila*	13	C7	Shrimp PL of 12 days
ARH 4	*Vibrio vulnificus*	15	C10	Artemia from Tank 2
ARH 5	*Vibrio cholerae*	16	C2	Artemia from Tank 1
ARH 6	*Edwardsiella hoshinae*	19	M1	Tilapia fry, 40 days
ARH 7	*Bacillus methylotrophicus*	24	M7	Tilapia fry, 28 days
ARH 8	*Aeromonas veronii*	20	M3	Tilapia fry, 40 days
		22	M5	Tilapia fry, 25 days
		23	M7	Tilapia fry, 28 days
		25	M9	Tilapia fry, 28 days
		26	M12	Tilapia fry, 33 days
		27	M14	Water of 25 day old tilapia fry pond
		29	M3	Tilapia fry, 40 days
		30	M5	Tilapia fry, 25 days
		32	M8	Tilapia fry, 28 days
		33	M8	Tilapia fry, 28 days
		34	M11	Tilapia fry, 33 days
		35	M12	Tilapia fry, 33 days
		36	M13	Water of 25 day old tilapia fry pond
		37	M13	Water of 25 day old tilapia fry pond

collected from hatchery environments of Bangladesh. Three of eight representative isolates ARH 1, ARH 4 and ARH 5 were identified as *Vibrio alginolyticus, Vibrio vulnificus* and *Vibrio cholerae*, respectively. The table shows that the samples in which these *Vibrio* species were found were exclusively collected from the coastal environment of Cox's Bazar. Among these three groups, ARH 1, which was identified as *Vibrio alginolyticus,* represents the highest number of isolates including samples. Of the eight representative groups, three were identified as genus *Vibrio (V. alginolyticus, V. vulnificus* and *V. cholerae)*, three *Aeromonas* (two *A. veronii* and one *A. hydrophila*), one *Edwardsiella hoshinae* and one *Bacillus methylotrophicus.* The dominance of *Vibrio* species in hatchery environments is well established[21,22]. Interestingly, good number of isolates showed similarity with *Aeromonas* species even though TCBS (selective for *Vibrio* spp.) media was used for isolation. This might be due to the fact that *Aeromonas* spp. has partial inhibitory growth in TCBS agar media[23].

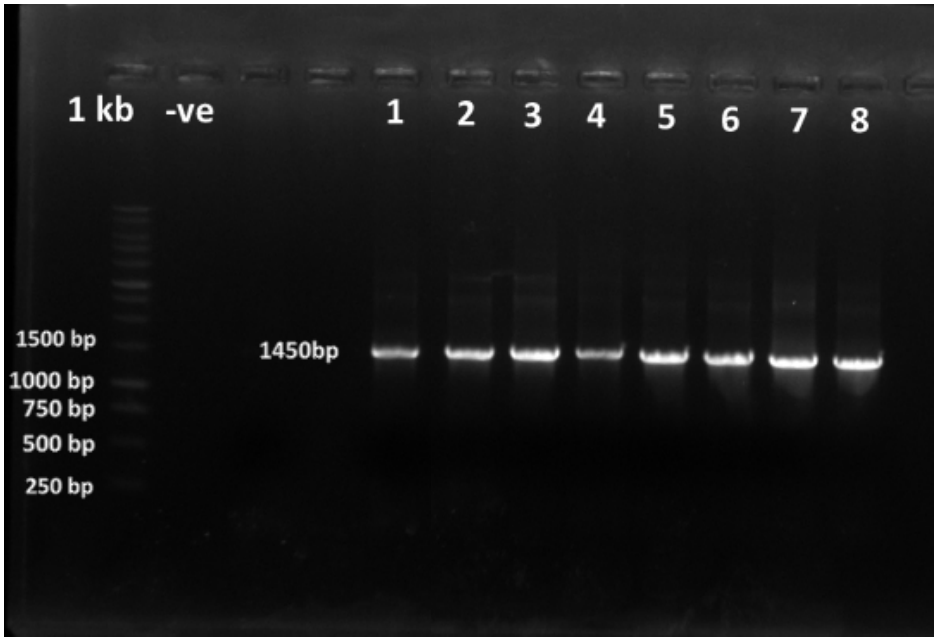

Fig. 2. Agarose gel (1%) electrophoresis of 16S rDNA gene of representative strains of eight ARDRA groups. The far left lane is 1 kb DNA ladder, while the next lane is used as negative control.

Phylogenetic analysis: The constructed phylogenetic tree involved a total of 18 (seven of our isolates and eleven downloaded from NCBI GenBank) nucleotide sequences to robust the positioning of isolates and to ascertain about their taxonomic position. The optimal tree with the sum of branch length = 0.24803246 is shown. The percentage of

replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. There are a total of 1320 positions in the final dataset, which is in compliance with the recommended ideal guidelines (for less than 1% ambiguities) for use of 16S rRNA gene sequencing for microbial identification.

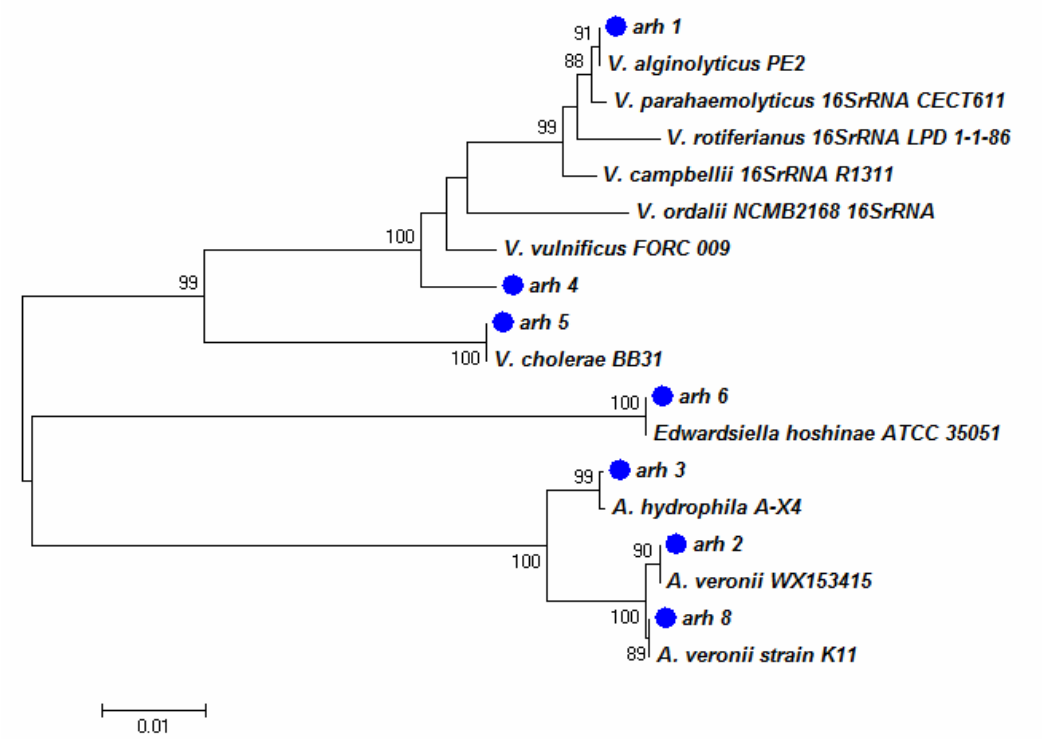

Fig. 3. The neighbour-joining (NJ) phylogenetic tree based on partial 16S rRNA gene sequences. The evolutionary distances were computed using the Maximum Composite Likelihood method. Numbers in tree are bootstrap values. Blue circle indicates position of the representative strains of seven ARDRA groups.

Phylogenetic analysis (Fig. 3) of the representative eight isolates confirmed the taxonomic position of the isolate ARH 1, ARH 4 and ARH 5 of the genus *Vibrio*, and allocated ARH 1 to the strain *Vibrio alginolyticus* PE2, ARH 4 to the strain *Vibrio vulnificus* FORC_009 and ARH 5 to the strain *Vibrio cholerae* BB31. From the tree it is also clear that ARH 1 is closely related to the strain of *Vibrio parahaemolyticus* CECT 611, *V. rotiferianus* LPD 1-1-86, *V. campbellii* R 1311, *V. ordalii* NCMB 2168 and *V. vulnificus* FORC 009. The phylogenetic tree confirms the taxonomic position of ARH 3, ARH 2 and ARH 8 in the genus *Aeromonas* supporting their similarity with the strain *A. hydrophilla* A-X4, *A. veronii* WX153415 and *A. veronii* K11, respectively. The taxonomic relation of ARH 6, which is allocated to *Edwardsiella hoshinae* ATCC 35051, is closer with the genus *Aeromonas* than with *Vibrio* spp.

In the sequenced 8 representative isolates, 3 *Vibrio* species have been found among which the maximum number of isolates has been identified as *Vibrio alginolyticus* (ARH 1). The samples, in which these 3 *Vibrio* species are found, were exclusively collected from coastal environment. This finding is in agreement with the observation that occurrence of Vibrios is plentiful in marine and coastal environment than in freshwater[10]. However, in support of the current study, it might be stated that the dominance of *V. alginolyticus* in the shrimp hatchery samples was also found by another study[24]. Felix (2000) also described *V. alginolyticus* as the most common *Vibrio* species in the shrimp hatchery environments[25]. This species has been implicated as the causal agent of vibriosis or gas gut disease of many marine aquaria fishes[26]. *V. alginolyticus* is also described as a pathogen for shrimp farming[27]. The presence of this bacterium also indicates its resistance against the treatments applied on the hatchery water.

The representative strain ARH 4 is identified as *Vibrio vulnificus* which is an established human pathogen[10] and responsible for many food borne diseases[28]. Similar to the findings of this observation Rao *et al*. detected the bacteria only in shrimp hatchery samples[24]. The source of this strain was the water of artemia tank that was also accused as a source of *V. harveyi* in the works of Vaseeharan and Ramasamy[29]. *Vibrio cholerae* was found as the isolate ARH 5 which was also from the same source as *V. vulnificus.* The presence of this bacterium denotes fecal contamination in the water that might have happened due to poor sanitation or uncleanliness of the hatchery laborer or operators. This bacterium is a well-recognized human pathogen associated with cholera disease[10].

Conclusion

This study describes the presence of *Vibrio* species in the sampled hatcheries and helps to comment on the quality of the hatchery environment. The presence of pathogenic *V. alginolyticus, V. vulnificus* and *V. cholerae* in the shrimp hatchery indicates a possibility of future outbreak of vibriosis and other diseases. The findings of this study also questions about the way of using live food (artemia) in the respective shrimp hatchery as all of the 3 pathogenic *Vibrio* species were found in the samples collected from the artemia tank. However, the absence of *Vibrio* species in the freshwater fish hatchery is not unquestionable and demands further research on this aspect.

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