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Investigation of a bacterial pathogen isolated from farmed *Ompok pabda*

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Abstract. Studies were conducted to isolate and identify a pathogen collected from the hemorrhagic ulcer of farmed *Ompok pabda* and to determine the pathogenic potentiality as well as antibiotic susceptibility of the recovered isolate. Infected fish were collected from a local fish farm located at Ishwarganj under Mymensingh district, Bangladesh. The bacterial isolate was identified by using Hi Assorted™ Biochemical Test kit and further investigated to determine the antibiotic susceptibilities. To determine the virulence, a challenge model was performed using the experimental bacterium, Ar-9 by intramuscular injection of *O. pabda*. A total of 50 healthy experimental fish (2.3 ± 0.44 g) were used in another challenge test by using single intramuscular injection with a pre-fixed dose (10^7 CFU/ fish) to find out the therapeutic effects of various available antibiotics. The results demonstrated that the collected isolate was Gram-negative rod shaped bacterium exhibiting non-wrinkled, smooth and slightly yellowish colony on TSA from 8 to 42^0C. Biochemical identification revealed that causative bacterium (Ar-9) was *Aeromonas* sp. that showed variable susceptibilities against different antibiotics but marked resistance to ampicillin. Ar-9 was found virulent ($LD_{50} = 2.2 \times 10^{6.0}$) to *O. pabda*. Moreover, experiment on the therapeutic effects against the infectivity of Ar-9 revealed that CFCIN have successfully recovered 100% of challenged fish followed by O. T. C. power, Tylovet and ATIVET. Thus, the present study revealed a virulent and ampicillin resistant pathogenic bacterium which may be harmful in pabda culture in Bangladesh.
Keywords: Bacterial pathogen, *Ompok pabda*

Introduction

Recent aquaculture practices in Bangladesh have mainly been associated with the culture of carps, tilapia (*Oreochromis niloticus*), pangasius (*Pangasianodon hypophthalmus),* stinging catfish *(Heteropneustes fossilis),* walking catfish (*Clarias batrachus),* pabda (*Ompok pabda*), climbing perch (*Anabas testudineus*), shrimp (*Penaeus monodon*) and prawn (*Macrobrachium rosenbergii)*. The demand of fish for human consumption is increasing rapidly with the proliferation of population but it fails to meet the optimum production level. There are many reasons for not achieving target and disease is one of the most constraining factors in the aquaculture practices in Bangladesh. Both farmed and wild fishes have been found to be affected by various kinds of diseases every year.

Bacterial fish diseases especially bacterial hemorrhagic septicemia and motile aeromonas septicemia in freshwater fish cause great losses (Roberts *et al.* 1989, Lio Po *et al.* 1992). Motile aeromonas septicemia is probably the most common bacterial disease of freshwater fish. This disease has been associated with several members of the genus *Aeromonas,* including *A. hydrophila, A. sobria, A. schuberti* and *A. veronii* (Suthi 1991). Chowdhury *et al.* (2003) studied ulcer type of disease in the small-scale rural farmer's pond and recovered a number of susceptive bacterial pathogens from the lesions and kidney of the ulcer affected fishes during mostly January and February. Among these, *Aeromonas* is the major bacterial fish pathogens which are widely distributed in aquatic organisms in nature (Banu 1996, Islam 2006) and are

frequently observed in farmed fishes as well as in the water in Bangladesh (Iqbal *et al*. 1996, Dipu 2012). There is strong evidence that many EUS affected fish die as a result of septicemia caused by an opportunistic bacterial pathogen, *Aeromonas* sp., notable *A. hydrophila* (Khan *et al*. 2011).

Ompak pabda is a catfish under family Silurideae of the order Siluriformes. The catches of the fish have drastically declined from open waters like rivers, *beels, haors* etc. in recent years due to various ecological changes in the inland water bodies. Keeping this in mind to increase its production, breeding technology is developed. Induced breeding is recorded in hatcheries of Jessore district, Bangladesh (Galib and Samad 2011). Now-a-days, hatcheries are showing interest for artificial breeding and their culture tendency is increasing day by day but farmers are facing problems that are exclusively related to the water quality and microbial diseases. In such cases, the proliferation of pathogenic and opportunistic microorganisms and fungi lead to decreased growth and food utilization, and in many instances, massive mortalities. In the present study, a bacterial isolate recovered from pabda (*O. pabda)* during natural disease outbreaks was identified and used for experimental infections.

Materials and Methods

***Collection of diseased fish and isolation of bacteria*:** Diseased *O. pabda* (average weight 33.4±1.8g) (Fig. 1.) were collected from a commercial fish farm, located at Ishwargonj, Mymensingh in February, 2018. Infected fish were caught using a cast net, placed in strong, clean and aseptic plastic bags then packed in column, surrounded with ice bags and immediately brought to the Fish Disease Laboratory of the Department of Aquaculture, Bangladesh Agricultural University. Upon arrival, fish were surface-disinfected with 70% ethanol and dissected. Samples for bacteriological examinations were collected by inserting sterile inoculating loop into the lesions and ulcers. The inocula were sampled from the surface as well as deeper portion of lesions and internal organs particularly from skin, fin, liver and kidneys. They were streaked on Tryptone Soya Agar (Hi-media) plates and incubated at 30°C for 48 h. Interestingly, a common type of smooth, non-wrinkled and flat colonies were appeared from most of the specimens. A representative single colony from the body lesion sample was coded as Ar-9. After performing routine biochemical identification methods, pure culture was stored using Tryptone Soya Broth (Hi-media) and 20% glycerol (v/v) (Merck) in 1.8 mL cryo-tubes (Thermo) at -20°C until required for further use.

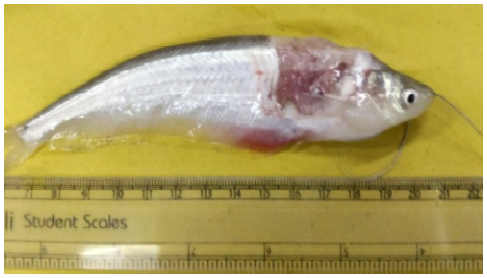

Fig. 1(a). Deep ulcerative lesion from dorsal to abdominal region.

Fig. 1(b). Hemorrhagic lesion on caudal region.

***Biochemical identification of Ar-9*:** Identification of bacteria was carried out based on the morphological and biochemical tests. Morphological test of the isolate, Ar-9 was done by Gram's staining using Grams stain kit (Hi-media) and biochemical identification was done using Hi Assorted™ Biochemical Test kit (Hi-media). The tests were based on the principle of pH change and substrate utilization. Pure culture of the isolate Ar-9 was done on the Nutrient Agar (Hi-media), freshly cultured bacterial cells were suspended in 0.85% sterile physiological saline and the suspension was adjusted to slightly more than 0.1 OD at 620 nm. The kit was opened aseptically and 50 μl of the above inoculum was inoculated in each well by surface inoculation method. The kit containing the bacterial sample was incubated at 37°C for 24 h. Identification was confirmed by comparing the biochemical reaction results of Ar-9 with the identification index (http://himedialabs.com/TD/KB002.pdf) provided with the kit.

***Experimental infection using Aeromonas sp. Ar-9*:** Experimental infections were conducted to test pathogenicity as well as to determine the virulence of isolated bacterium using *O. pabda*. Healthy *O. pabda* fingerlings (weight 2.3 ± 0.44 g) were collected from a private fish hatchery, located adjacent to BAU for experimental infection and were acclimatized in aquaria with aeration for 72 h and checked for the disease before using in the challenge test. Ar-9 was cultured on TSA plates at 37°C for 24 h, harvested and suspended in sterile 0.85% physiological saline. Tenfold serial dilutions of the suspension were prepared and five fish per dilution were used for intramuscular injection with 0.1 ml of the suspension. Challenge doses employed for intramuscular injection were ranged from 2.2×10^5 to 2.2×10^8 CFU/ fish. Control fish received only sterile 0.85% physiological saline and kept in a separate aquarium. The average water temperature was determined. After injections, the challenged fish were kept in 35 L capacity glassaquaria facilitate with continuous aeration. Around 50% of water was exchanged daily. Water temperature was maintained with room temperature. Pathogenic activity of the pathogen, Ar-9 on the experimental fish was investigated daily by a routine observation of lesions on skin, fin, fin bases, body surface and head. Gross pathological changes, moribundness and mortalities were recorded and considered as the primary diagnosis of infection. Samples from the external lesions and internal organs, *i.e.*, the skin, fins, liver and kidney of dead fish were directly streaked onto TSA plates and incubated at 37°C for 48 h to confirm that *Aeromonas* sp. Ar-9was the cause of mortality. The observation was continued up to 10 days without any feed support.

***Determination of Virulence (LD_{50})*:** LD_{50} value was calculated using the method described by Reed and Muench (1938). The formula is as follows:

$$\text{Proportionate distance} = \frac{(\%\text{ mortality at dilution next above } 50\%) - 50\%}{(\%\text{ mortality at next-Dilution above } 50\%) - (\%\text{ mortality at next dilution below } 50\%)}$$

Dilution factor (DF) = Negative Log of lower dilutions (next above 50% mortality)
The 50% endpoint dilution can be calculated thus:
Negative logarithm of LD_{50} titre = (negative logarithm of the next dilution above 50% mortality + PD) x dilution factor (DF)

Antibiogram study: Ar-9 was tested for its antibiotic-resistance by disc diffusion method (Bauer *et al*. 1966) on TSA. The antibiotic discs (Hi-media) were tested included Azithromycin (10 µg), Doxycycline (30 µg), Erythromycin (15 µg), Tetracycline (10 µg), Streptomycin (25 µg), Cefotaxime (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg), Kanamycin (30 µg) and Ampicillin (10 µg). Zones of inhibition were measured after 24 h and again after 48 h of incubation at 37°C. The isolates were classified as sensitive (S) and resistant (R) based on the size of the zone of bacterial growth inhibition.

Efficacy of antibiotics on the experimentally infected *O. pabda*

***Selection of antibiotics*:** The virulent isolate, Ar-9 was further used for another experimental infection of *O. pabda* under laboratory condition. Four veterinary grade antibiotics *viz.,* CFCIN (Ciprofloxacin 10%; FnF), Renamycin (Oxytetracycline, USP 200 mg), DT-10 (Doxycycline 10%+Trimethoprim 10%; FnF) and Sulfatrim (Sulphadiazine BP 40%+ Trimethoprim BP 8%) were selected based on the availability in the local market. Such antibiotics were used in the experiment according to a prefixed dose of 50 ppm to find out their therapeutic effects against the infectivity of examined fish pathogenic bacteria (Table I).

Table I. Selected antibiotics and their doses applied to experimentally infected fish

Sl. No.	Name of antibiotics		Used Doses
	Trade name	Types of antibiotics	
1.	Tylovet	Tylosin, Doxycycline, Colistin and Bromhexine 100g	50 ppm
2.	O.T.C Power	Oxytetracyclin Power 30%	50 ppm
3.	CFCIN	Ciprofloxacin 10%	50 ppm
4.	ATIVET	Sulphadiazine + Trimethoprim	50 ppm

***Experimental infection*:** Apparently healthy *O. pabda* fingerlings (weight 2.3±0.44 g) were collected from a private fish hatchery situated adjacent to BAU. Before starting the challenged test, fish were acclimatized for three days. The experimental challenge study was performed using seven 35 L capacity plastic bucket with continuous aeration. Total 5 buckets were set for the fish to carry out this experiment of which each contained 10 *O. pabda.*During injection, fish were smoothly and carefully handled to avoid any physical injury and stress. All fish received a single IM injection of 0.1 mL of 2.5×10^7 CFU/mL of Ar-9 suspension. Control group of 10 fish was injected with 0.1 mL of sterile 0.85% saline solution. Gross pathological changes and moribundness were checked daily. The average water temperature was 31.5±0.9°C. No feed was given during experimental infection. Rearing water of each bucket was changed up to 50% as per needed. Just after the appearance of experimental infection, selected antibiotics were applied to observe their effects on the infected fish.

Results

Collection and isolation of bacteria: Pure colonies of Gram-negative bacteria were isolated on TSA from the body lesions and internal organs *viz.,* kidney and liver of moribund fish collected from different private fish farms during mass mortalities. A total of 8 phenotypically similar isolates were collected and preserved. Isolates were coded and a representative single colony from the pure culture of the isolate, Ar-9 was selected randomly for further studies. The isolated

bacterium was Gram-negative, short rod-shaped, strictly aerobic and optimum growth temperature was 37^0C but able to grow up to 42^0C. After using the Hi Assorted™ Biochemical Test kit (Fig. 2(a) and 2(b)), following characteristics of this bacterium were revealed: positive results for citrate utilization, urease, nitrate reduction and utilization of glucose; negative results for Lysine decarboxylase, ornithine decarboxylase, H_2S production, TDA, utilization of lactose, arabinose and sorbitol. Comparison between phenotypic and biochemical characteristics of *Aeromonas* sp. (Ar-9) and the published description of Jin *et al*. 2010 and Hickman-Brenner *et al*. 1987 is given in Table II. Results were checked and verified using the identification index for Gram-negative rods supplied with the kit. According to the biochemical test results and identification index (Fig. 3), the experimental bacterium, Ar-9 was identified as *Aeromonas* sp. which ultimately showed closer biochemical similarities with *A. veronii*.

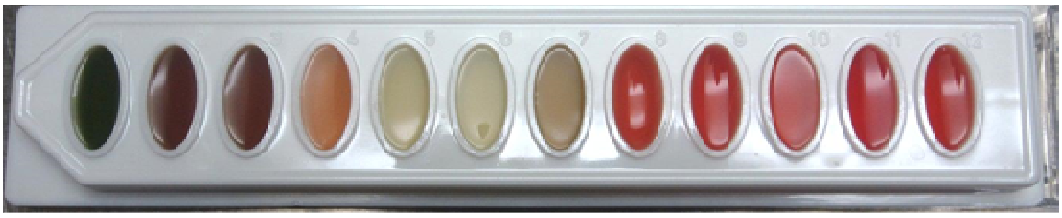

Fig. 2(a). Hi Assorted™ Biochemical Test kit (Hi-media) before inoculation of experimental bacterium.

1	2	3	4	5	6	7	8	9	10	11	12
+	+	+	+	-	+	-	-	-	-	-	-

Fig. 2 (b). Hi Assorted™ Biochemical Test kit (Hi-media) after inoculation of experimental bacterial suspension followed by incubation at 37^0C for 24 h. *Aeromonas* sp. Ar-9 was identified by comparing the visible color change (due to biochemical reaction result) and the identification index provided with kit. (+) and (-) indicating the positive and negative reactions.

Note: Well 1: Medium for Citrate utilization Test**,** Well 2: Medium for Lysine utilization Test**,** Well 3: Medium for Ornithine utilization Test, Well 4: Medium for Urease detection Test**,** Well 5: Medium for Phenylalanine deamination Test, Well 6: Medium for Nitrate reduction Test**,** Well 7: Medium for H_2S Production Test, Well 8-12: Medium for Carbohydrate Utilization Test (with five different sugars in respective wells as Glucose, Adonitol, Lactose, Arabinose, Sorbitol)

Table II. Comparison of phenotypic and biochemical characteristics of *Aeromonas* sp.(Ar-9) isolated from disease *O. pabda* following description of Jin *et al.* 2010 and Hickman-Brenner *et al.* 1987

Characteristics	*Aeromonas* sp. (Ar-9)	*A. veronii* (DJ-2)	*A. sobria* (ATCC 43979)	*A. veronii* (ATCC 35623)	*A. veronii* (ATCC 35604)
Pigment	Slightly yellow	Slightly yellow	Slightly yellow	Slightly yellow	Slightly yellow
Gram stain	-	-	-	-	-
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci
Growth on TSA	+	+	+	+	+
Growth on McConkey agar	+	+	+	+	+

Growth on SS agar	+	ND	ND	ND	ND
Growth on cetrimide agar	-	-	-	-	-
Growth on TSA media with:					
4% NaCl	+	ND	ND	ND	+
6% NaCl	-	ND	ND	ND	-
Growth on TSA at:					
4°C	-	-	-	-	-
37°C	+	+	+	+	+
42°C	+	ND	ND	ND	ND
Indole production	+	+	+	+	+
Methyl red	+	ND	ND	ND	+
Voges-Proskauer	+	+	+	+	+
Catalase production	-	+	+	+	ND
Coagulase test	-	ND	ND	ND	ND
Citrate utilization	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+
Ornithine decarboxylase	-	-	-	+	+
Urease	-	-	-	-	-
TDA	-	ND	ND	ND	ND
Nitrate reduction	+	+	+	+	+
H_2S production	-	-	-	-	-
Glucose	+	+	+	+	+
Adonitol	-	-	-	-	-
Lactose	-	-	-	-	-
Arabinose	-	-	-	+	-
Sorbitol	-	-	-	-	-

N. B. (+) = positive reaction, (-) = negative reaction, ND = Data not available

***Virulence study*:** *O. pabda* showed 100% mortality at the dose of 2.2×10^8 and 2.2×10^7CFU/ fish. Lower doses *viz.,* 2.2×10^6 CFU/ fish and 2.2×10^5CFU/ fish showed mortality rates of 80% and 60% respectively (Table III). The challenged fish exhibited signs of hemorrhages on the site of injection as well as different parts of the body surface, ulceration, especially at dorsal parts and erosions at the base of fins. The bacterium was re-isolated from the challenged fish on TSA plates and its further identification revealed the same characteristics of the injected bacteria. None of the control fish died or showed any sign of disease within the experiment.

Identification Index for Gram-negative rods

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
Aeromonas caviae	V	–	–	–	–	+	–	+	–	V	+	–
Aeromonas eucrenophila	–	–	–	–	V	+	–	+	–	–	+	–
Aeromonas hydrophila	V	V	–	–	–	+	+	+	–	V	+	–
Aeromonas media	V	–	–	–	V	+	–	+	–	V	+	–
Aeromonas veronii	+	+	+	–	V	+	–	+	–	–	–	–
Budvicia aquatica	–	–	–	V	–	+	V	+	–	V	V	–
Buttiauxella agrestis	+	–	+	–	–	+	–	+	–	+	+	–
Cedecea davisae	+	–	+	–	–	+	–	+	–	V	–	–
Cedecea lapagei	+	–	–	–	–	+	–	+	–	V	–	–
Cedecea neteri	+	–	–	–	–	+	–	+	–	V	–	+
Citrobacter amalonaticus	V	–	+	V	–	+	–	+	–	V	+	+
Citrobacter diversus	+	–	+	V	–	+	–	+	+	+	+	+
Citrobacter freundii	+	–	V	V	–	+	V	+	–	V	+	+

Note: Based on % strains showing reactions following symbols: + = Positive (more than 90%), - = Negative (More than 90%), V =11-89% positive, nd =Na dada available

Fig. 3. Identification index (http://himedialabs.com/TD/KB002.pdf) provided with the Hi Assorted™ Biochemical Test kit (Hi-media) exhibited that *Aeromonas* sp. Ar-9 is closely related to *A. veronii*.

Table III. Virulence of *Aeromonas* sp. Ar-9 studied in *O. pabda*

Bacterium	Challenge method	Challenge Dose (CFU/mL or fish)	No. of dead fish during the periods of (n=5)				Mortality (%)	LD_{50} (CFU/mL or fish)
			0-1 d	2-3 d	4-6 d	7-10 d		
*Aeromonas*sp. (Ar-9)	Intramuscular (i.m.) injection	2.2×10^8	4	1	-	-	100	$2.2 \times 10^{6.0}$
		2.2×10^7	2	2	1	-	100	
		2.2×10^6	0	1	3	1	80	
		2.2×10^5	0	0	2	1	60	
Control	Injection with 0.85% PS only	Not challenged with the bacterium	0	0	0	0	0	0

Weight (g) of challenged fish (Ave. ± S.D.), 2.4 ± 0.56 g
Water temperature (Ave. ± S.D.), 31.4 ± 1.1°C
(-): Fish do not exist in the aquarium due to mortality; PS= 0.85% Physiological saline

***Antibiogram study*:** Antibiotic susceptibility test indicated that *Aeromonas* sp.(Ar-9) was sensitive to Azithromycin, Doxycycline, Erythromycin, Tetracycline, Streptomycin, Cefotaxime, Chloramphenicol, Ciprofloxacin, Gentamycin and Kanamycin (Table IV). The results also showed that Ar-9is resistant to Ampicillin (Fig. 4.). Some of the effective antibiotics can be considered efficacious in controlling the *Aeromonas*.

***Effects of selected antibiotics on the infectivity of Aeromonas sp.*:** After experimental infection with virulent *Aeromonas* sp. (challenge dose: 2.5×10^7 CFU/ fish), the antibiotic CFCIN and OTC power were found to have distinguished effects where 90% fish were recovered (dose: 50 ppm) with the application of prolonged bath treatment (Fig. 5). Tylovet also showed good effect and found to recover 90% of challenged fish but slightly weaker recovery was reported in the case of the antibiotic ATIVET (80% recovery only). All the control fish died and showed signs of disease within the experimental period.

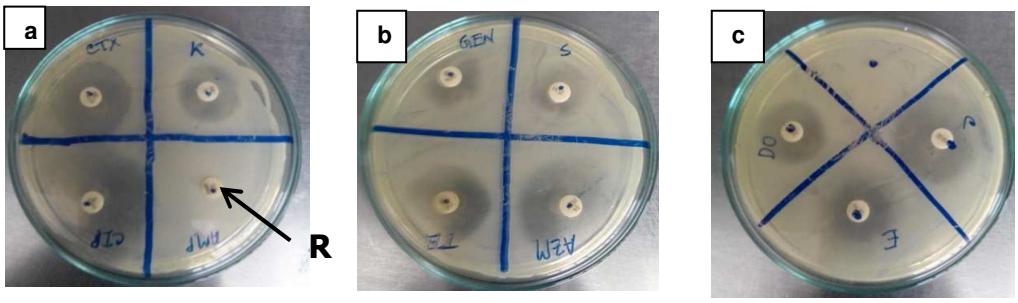

Fig. 4. Antibiogram study of *Aeromonas* sp., Ar-9 using different antibiotic discs. **Note**: AZM= azithromycin; DO= doxycycline; E= erythromycin; T= tetracycline; S= streptomycin; CTX= cefotaxime; C= chloramphenicol; CIP= ciprofloxacin; GEN= gentamycin; K= kanamycin; AMP = ampicillin and R = resistant.

Table IV. The sensitivity of *Aeromonas* sp.(Ar-9) to various antimicrobial agents

Antimicrobial agents	Disc content (µg)	Sensitivity
Azithromycin	10	S
Doxycycline	30	S
Erythromycin	15	S
Tetracycline	10	S
Streptomycin	25	S
Cefotaxime	30	S
Chloramphenicol	30	S
Ciprofloxacin	10	S
Gentamycin	10	S
Kanamycin	30	S
Ampicillin	10	**R**

S= sensitive; R = resistant

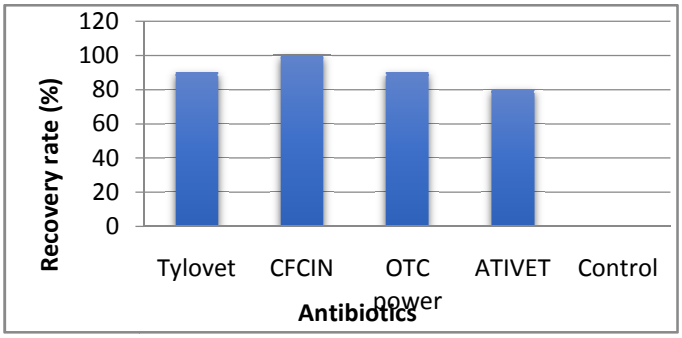

Fig. 5. Therapeutic effects of antibiotics on *O. pabda* experimentally infected with *Aeromonas* sp. Ar-9.

Discussion

During mass mortalities of *O. pabda* in the private fish farm of Ishwarganj, Mymensingh, the collected moribund fish showed irregular hemorrhages on body surface, especially at the ventral part of abdomen, abdominal ascites, hemorrhages and erosions at the bases of fins, skin lesions and shallow to deep ulcers in the advance stages of infection which were very similar to the signs found in fish suffering from motile aeromonad infections (Ausin and Austin 1987, Inglis *et al*. 1994, Park and Yu 2008). Again, both *A. hydrophila* and *A. veronii* were found associated with hemorrhagic septicemia in freshwater fish (Paniagua *et al*. 1990, Inglis *et al*. 1994, Cipriano 2001). *Aeromonas* spp. have previously been isolated from EUS diseased fish in the Southeast Asian countries by Iqbal *et al*. (1998). They found that 27% (12 of 44) *Aeromonas* isolates from fish with EUS in Malaysia, Thailand, and Bangladesh belonged to *A. veronii* biovar *sobria* and that 6 of the 11 isolates from Bangladeshi fish belonged to this species. In Bangladesh, *A. veronii* was isolated previously from various diseased fish *viz.,* African catfish (*Clarias gariepinus*), rajputi (*Puntius gonionotus*), rui (*Labeo rohita*), catla (*Catla catla*), and shol (*Channa striatus*) during the period of 1997 and 1998 from different fish farms located in Mymensingh district (Rahman *et al*. 2002). So, *Aeromonas* infection is very common in aquaculture zone of Mymensingh, Bangladesh.

According to the identification index of Hi Assorted™ Biochemical Test kit (Hi-media) the bacteria investigated in the present study is closely related to *A. veronii*. The identified bacterium is able to grow up to 42^0C on TSA which differs from many species of *Aeromonas* genus including previously reported fish pathogenic *A. hydrophila* (Chowdhury 1998). However, the descriptions of the identified *Aeromonas* sp. are too brief and incomplete to make a positive identification up to species level. It is possible that the organism discussed in this paper has not been previously described. Besides, biochemical identification tests are effective but not always conclusive (Vijai *et al*. 2012) and cannot always give reliable results because of diversities in metabolic pathways exhibited through biochemical reactions (Onuk *et al*. 2013). Further molecular investigations based on the sequence of 16S rRNA are necessary to confirm the bacterium up to species level.

The results showed that Ar-9 is resistant to ampicillin but sensitive to other examined antibiotic discs. Joseph *et al*. (1991) reported *A. veroni* bv. *veroni* was resistant to ampicillin but sensitive to enrofloxacin and kanamycin. Actually, *A. veronii* had an antibiogram typical of other *Aeromonas* strains: resistance to penicillin, ampicillin and carbenicillin but susceptibility to chloramphenicol, colistin, gentamicin and tetracycline (Hickman-Brenner *et al*. 1987). In order to avoid therapeutic failures and resistance development in *A. veronii* to antibiotics in the future, proper fish health management with exact dosage should be ensured. Fishes showed external signs and symptoms such as discoloration of the skin which could be due to the development of different patches of hemorrhagic and ulcerative skin, tail and fin rot and skin lesions were superficial. Result of the external examination of the disease was much restricted to fin base and skin which could be due to the well-documented pathogenicity mechanism induced by the *A. veronii*heat stable proteases (Austin and Austin 2007, Thune *et al*. 1993). Such potent proteases are effective proteolytic agents that directly liquefy the proteinaceous material (hyaluronic acid and collagen) in the cement substance that links cells together with an ultimate result of skin ulcers and fin rot. Although antibiotics in aquaculture are a direct threat to the health of humans and terrestrial animals as antibiotic resistance can be horizontally transferred to other pathogenic bacteria, still, antibiotics are using as quick recovery agent in the case of bacterial fish diseases. According to Shariff *et al*. (1996), Oxytetracycline (about 20 ppm) in a dip or bath solution is used against bacterial disease in Malaysia and Singapore. Chowdhury *et al*. (2003) also found a positive effect of Renamycin (Oxytetracycline) against bacterial infection. Like the present study, Rahman (2007) also used CFCIN and Renamycin (Oxytetracycline) and found a very good recovery rate in the case of the infectivity of *Aeromonas* under laboratory condition.

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