OPEN ACCESS
DOI: 10.5455/javar.2015.b120

J. Adv. Vet. Anim. Res., 2(4): 469-474.
Available at- *http://bdvets.org/JAVAR*

JAVAR Journal of Advanced Veterinary and Animal Research

ORIGINAL ARTICLE

Volume 2 Issue 4 (December 2015)

Detection of multidrug resistance *Aeromonas hydrophila* in farm raised fresh water prawns

Md. Bakhtiar Lijon, Mst. Mousumi Khatun, Ariful Islam, Mst. Minara Khatun and Md. Ariful Islam*

Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
**Corresponding author's e-mail: islamma@bau.edu.bd*

A B S T R A C T

This study was undertaken for isolation, identification and determination of antibiogram profile of *Aeromonas hydrophila* in farm raised fresh water prawn (*Macrobrachium rosenbergii*) on five commercial ghers. Fresh water prawns (n=25) were collected from five ghers located at Satkhira, Bagerhat and Khulna districts of Bangladesh. Brain (n=25), muscle (n=25) and intestine (n=25) samples were collected aseptically from fresh water prawn and inoculated into alkaline peptone (APW) water for enrichment at 37℃ for 8 h. Enriched cultured was streaked into Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar to isolate bacteria. Bacteria were identified by cultural, Gram staining, biochemical properties and polymerase chain reaction (PCR) assay. The antibiogram profiles of bacteria were investigated against 5 commonly used antibiotics (Gentamicin, Cefalexin, Ampicillin, Azithromycin and Ciprofloxacin) by disc diffusion method. Thirteen *A. hydrophila* isolates were identified and the prevalence of the *A. hydrophila* in fresh water prawn was 17.33%. All 13 (100%) isolates were sensitive to Ciprofloxacin, Gentamicin, Azithromycin and resistant to Ampicillin and Cefalexin. The results of this study indicate that farm raised fresh water prawn harbor multidrug resistant *A. hydrophila* which might causes public health problem if enter into human food chain.

Keywords

Aeromonas hydrophila, Antibiogram profile, Fresh water prawn, Prevalence, PCR assay

ARTICLE HISTORY

Received : 1 November 2015, **Revised**: 24 November 2015,
Accepted : 25 November 2015, **Published online**: 1 December '15.

INTRODUCTION

Fresh water prawn (*Macrobrachium rosenbergii*) is a popular food item and the second largest exportable commodity in Bangladesh. Fresh water prawn is cultured in the ghers (modified rice field) of southern region of Bangladesh like Satkhira, Bagerhat, Khulna, and Cox's Bazar. Prawn is one kind of shellfish. Bacteria, virus and parasites are common to cause infection in shellfish (FDA, 2011). Ten bacterial genera are known to cause diseases in shellfish. Feldhusen (2000) reported three groups of pathogenic bacteria in shellfish namely indigenous bacteria (bacteria commonly found in fresh and marine water including *Vibrio* spp., *Aeromonas hydrophila, Listeria monocytogenes* and *Clostridium botulinum*), non indigenous bacteria (enteric bacteria of fecal origin including *Salmonella* spp.), and bacterial contamination during processing. Shellfish infected with *A. hydrophila* are unsuitable for export because these bacteria can cause food borne infection in humans (Li and Saghaian, 2011). These bacterial infections cause tail rot, fin rot, and hemorrahagic septicemia both in fresh water and marine fishes (Aoki, 1999).

Gram-negative, facultative anaerobic A. *hydrophila* is normal bacterial flora of fish and responsible for diseases in fish at the time of stress (Peters et al., 1988). In humans, it has been associated with gastroenteritis and localized wound infection (Nemetz and Shotts, 1993). Waters get contaminated with antimicrobial agents from human and animal wastes which leads to the emergence of multidrug resistance (MDR) bacterial flora in the aquatic environment (Morita et al., 1994).

The multidrug resistance was reported in the genus of *Aeromonas* (Albert et al., 2000; Palu et al., 2006). Few reports are available on the isolation of *A. hydrophila* from fresh water fish and prawn sold at market in

Bangladesh (Rahim et al., 1984; Rahim and Aziz, 1994). However, no study has been conducted so far on the detection of *A. hydrophila* in farm raised fresh water prawn at ghers in the Southern districts of Bangladesh. The objectives of this study were (i) Isolation and identification of *A. hydrophila* from farm raised fresh water prawn, and (ii) determination of antibiogram profile of *A. hydrophila* against five commonly used antibiotics.

MATERIAL AND METHODS

Collection of samples: A total of 25 fresh water prawns were collected from five ghers which were located at Satkhira (n=2, Satkhira Sadar and Assasuni Upazillas), Bagerhat (n=2, Bagerhat Sadar and Rampal Upazillas) and Khulna (n=1, Dumuria Upazilla) districts of Bangladesh **(Table 1)** during the period from January to June, 2014. The samples were packed into sterile polyethene bags in an ice box and transported to the Department of Microbiology and Hygiene at the Bangladesh Agricultural University (BAU), Mymensingh for bacteriological study.

Table 1. Number of samples collected from each study area

Study area (District)	Location of gher (Upazila)	No. of samples collected
Satkhira	Satkhira Sadar	5
	Assasuni	5
Bagerhat	Bagerhat Sadar	5
	Rampal	5
Khulna	Dumuria	5
	Total	**25**

Processing of samples: Brain (n=25), muscle (n=25) and intestinal (n=25) specimens of prawns were aseptically collected. Muscle samples (0.5 g) were mixed with 4.5 mL alkaline peptone water (APW) and grinded by pastle and morter to prepare a 10% tissue suspension. Intestinal samples were cut by scissors into several small pieces and homogenized in PBS to prepare a 10% tissue suspension.

Enrichment of samples: A loopful of brain fluid was inoculated into 4.5 mL of APW and incubated at 37°C for 8 h. Suspension of muscle (0.5 mL) and intestine (0.5 mL) were separately inoculated into test tubes containing 4.5 mL APW and incubated at 37°C for 8 h.

Isolation of bacteria: One loopful of enrichment culture of brain, muscle and intestine was separately streaked duplicate onto Thiosulfate citrate bile salts sucrose (TCBS) agar (Himedia, India) and incubated aerobically at 37°C for 24 h. Single colony grown onto the TCBS agar was further sub cultured onto TCBS agar until pure cultures were obtained.

Identification of bacteria: Identification of bacteria was conducted by observing cultural characteristics and colony morphology on the TCBS agar and growth of bacteria into nutrient broth containing 0% and 6% sodium chloride (NaCl). Gram's staining method, motility test, sugar fermentation and biochemical tests (oxidase test, catalase test, citrate test, indole test and MR-VP test) were performed to identify bacteria.

Molecular detection of bacteria by PCR: A genus specific PCR assay was performed to identify *Aeromonas* spp. by amplifying 276-bp fragment of lipase gene (Delamare et al., 2012).

Antibiotic sensitivity test: Antibiogram profile of 13 *A. hydrophila* isolates was done against five different antibiotic such as: Gentamicin, Azithromycin, Ciprofloxacin, Ampicillin and cefalexin (Himedia, India). The antibiotics sensitivity testing was carried out according to instructions of the Clinical and Laboratory Standard Institute (CLSI, 2011).

RESULTS AND DISCUSSION

Multidrug-resistant *Aeromonas* strains have been emerged as serious public health pathogens which can cause gastroenteritis, septicemia and skin infections in humans (Igbinosa et al., 2012). *A. hydrophila* is naturally found in the aquatic environment (Austin and Adam, 1996). It produces various diseases in fishes. These bacteria enter into human body through ingestion of contaminated food and water and open wounds. *A. hydrophila* have been found in various fish species (Daskalov, 2006; Deng et al., 2009).

In the present study, isolation of *Aeromonas* spp was done on TCBS agar since it is used for the growth of both *Vibrio* spp. and *Aeromonas* spp. (Kaysner and Depaola, 2004). The most frequently isolated species of *Vibrio* in fresh water are *V. cholera* and *V. mimicus* (Fouz et al., 2002). In TCBS agar *V. cholera* produce yellow colour colony and *V. mimicus* produce green colour colony (Kaysner and Depaola, 2004). *Aeromonas* spp. on the other hand also produce yellow colour colony on TCBS agar. In this study, DNA extracted from yellow colour colony grown on TCBS agar successfully amplified 276-bp fragment of lipase gene confirmed

prawn bacterial isolates belonged to *Aeromonas* spp. (Delamare et al., 2012) (**Figure 1**). In PCR assay positive control was not available for use. In this study, differential diagnosis between *Vibrio* spp. and *Aeromonas* spp. were also performed by Gram's staining technique, lactose fermentation test and VP test. *Vibrio* spp. are Gram negative, comma shaped, non lactose fermenter and VP negative (Brooks et al., 2007; Islam et al., 2013; Kaysner and Depaola, 2004). In this study, bacteria isolated from farm raised fresh water prawn were Gram negative, rod shaped, lactose fermenter and VP test positive which are characteristics for *A. hydrophila*. Another differential diagnosis carried out to differentiate *Vibrio* spp. from *Aeromonas* spp. by their growth characteristics in nutrient broth containing 0% and 6% NaCl (Kaysner and Depaola, 2004). In the present study, prawn bacterial isolates grew in nutrient broth containing both 0% and 6% NaCl which were important growth characteristics of *A. hydrophila*.

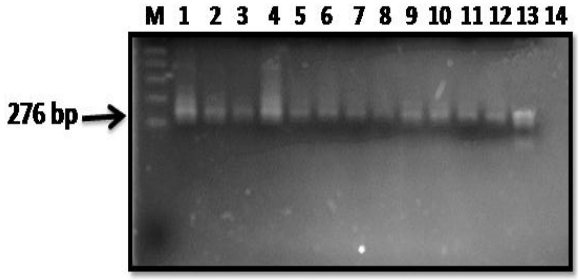

Figure 1. PCR assay to amplify lipase gene of *Aeromonas* spp. of fresh water prawn. Lane M: 1 kb DNA marker (Promega, USA); lanes 1, 2, 3 and 4: DNA of *Aeromonas* spp. isolated from prawn of Satkhira Sadar gher; lanes 5 and 6: DNA of *Aeromonas* spp. isolated from prawn of Assasuni gher, lanes 7,8 and 9: DNA of *Aeromonas* spp. isolated from prawn of Dumuria gher; lanes 10 and 11: DNA of *Aeromonas* spp. isolated from prawn of Rampal gher, lanes 12 and 13: DNA of *Aeromonas* spp. isolated from prawn of Bagerhat Sadar gher and lane 14: Negative control without DNA.

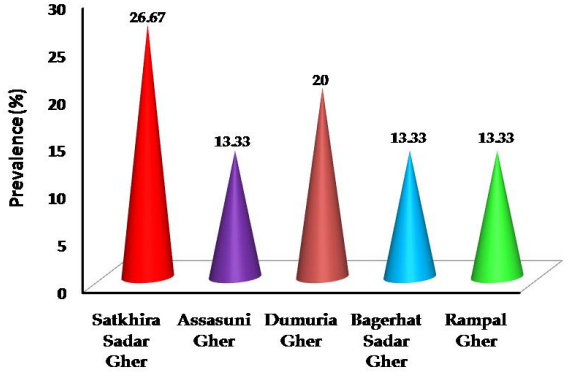

Figure 2. Prevalence of *A. hydrophila* in fresh water prawn in five commercial ghers located at Satkhira, Khulna and Bagerhat districts of Bangladesh

Several *Aeromonas* spp. were reported in fresh water fish and shellfish. Ashiru et al. (2011) reported *A. hydrophila*, *A. caviae* and *A. sobria* both in Tilapia and Catfish. These three species of *Areromonas* can be differentiated by lactose and sucrose fermentation tests. *A. hydrophila* can ferment lactose and sucrose. On the contrary, *A. caviae* and *A. sobria* cannot ferment lactose and sucrose (Ashiru et al., 2011). In this study, bacterial isolates of prawns fermented lactose and sucrose confirming their identity as *A. hydrophila*. Results of sugar fermentation and biochemical tests of *A. hydrophila* isolates from fresh water prawn are summarized in the **Table 2**.

In present study, 13 *A. hydrophila* were isolated and identified from the farm raised fresh water prawns. The prevalence of *A. hydrophila* in fresh water farm raised prawns in this study was 17.33%. Rahim and Aziz (1994) recorded 31% prevalence of *A. hydrophila* in fresh water prawn which were collected from fish market of Dhaka, Bangladesh. In shrimp 13.89% prevalence of *A. hydrophila* was reported in Iran by Khamesipour et al. (2014). Vivekanandhan et al. (2005) reported 17.62% prevalence of *A. hydrophila* in prawns in India. In the current study, brain, muscle and intestine samples were screened for *Aeromonas* spp. since these samples were also analyzed by other investigators (Ashiru et al., 2011; Jayasinghe et al., 2008). This study detected the presence of *A. hydrophila* in brain, muscle and intestine samples of prawns. The prevalence of *A. hydrophila* was the highest in intestine (28%) followed by muscle (16%) and brain (8%). The overall prevalence of *A. hydrophila* in fresh water prawn was 17.33% (**Table 3**). Vivekanandhan et al. (2005) also recorded the highest prevalence of *A. hydrophila* in the intestine (38.43%) as compared to body surface (32.46%) and gill (29.10%).

Prevalence of *A. hydrophila* was 26.67% in Satkhira gher, 20% in Dumuria gher, 13.33% in Assasuni gher, and 13.33% in Bagerhat gher and 13.33% in Rampal gher (**Figure 2**).

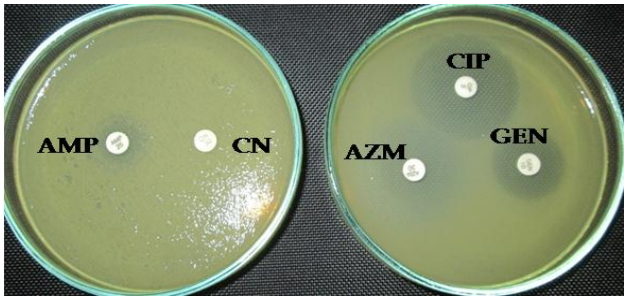

Figure 3. Antibiogram profiles of *A. hydrophila* against ampicillin (AMP), cefalexin (CN), azithromycin (AZM), ciprofloxacin (CIP) and gentamicin (GEN). The isolate was found resistant to ampicillin and cefalexin and sensitive to azithromycin ciprofloxacin and gentamicin.

Table 2. Summary of sugar fermentation and biochemical test results for *Aeromonas hydrophila*.

Name of tests	Results of this study	Results of Bergey's Manual*	Results of other investigators		Interpretation
			Results	References	
1. Sugar fermentation tests profiles using					*Aeromonas hydrophila*
Dextrose (DX)	A	A	A	Kannan et al. (2011)	
Sucrose (S)	A	A	A	Popovic et al. (2000)	
Lactose (L)	A	NS	A	Chandrakanthi et al. (2000)	
Maltose (ML)	A	A	A	Popovic et al. (2000)	
Mannitol (MN)	A	A	A	Chandrakanthi et al. (2000)	
2. Motility test using MIU media	Motile	Motile	Motile	Chandrakanthi et al. (2000)	
3. Biochemical test					
Oxidase	+	+	+	Kaysner and Depaola (2004)	
Catalase	+	+	+	Kannan et al. (2011)	
Citrate	+	NS	+	Kannan et al. (2011)	
Indole	+	NS	+	Al-Fatlawy et al. (2013)	
MR	+	NS	+	Chandrakanthi et al. (2000)	
VP	+	+	+	Kaysner and Depaola (2004)	

Legend: DX= Dextrose, ML= Maltose, L= Lactose, S= Sucrose, MN= Mannitol; A=Acid, + = positive, NS= Not stated, *= Bergey's Manual of Systematic Bacteriology (Krieg, 1984).

Table 3. Prevalence of *Aeromonas hydrophila* in brain, muscle and intestine samples of fresh water prawn.

Serial No.	Name of samples	No. of samples tested	No. of culture positive samples	Prevalence (%)	Overall prevalence (%)
1.	Brain	25	2	8	
2.	Muscle tissue	25	4	16	17.33
3.	Intestine	25	7	28	

Table 4. Summary of Antibiogram profile of *Aeromonas hydrophila* against five commonly used antibiotics.

No. of isolates tested	Antibiogram profiles of *A. hydrophila*														
	GEN			AZM			CIP			AMP			CN		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
13	0	0	13	0	0	13	0	0	13	10	0	3	10	0	3

Legend: GEN: Gentamicin; AZM= Azithromycin; CIP=Ciprofloxacin; AMP=Ampicillin; CN=Cefalexin

A. hydrophila are sensitive to ciprofloxacin and gentamicin (Ko et al., 2003; Truong et al., 2008; Overman, 1980) and resistant to ampicillin (Geiss and Freij, 1989; Overman, 1980). In this study, all *A. hydrophila* were found sensitive to ciprofloxacin, gentamicin and azithromycin and resistant to ampicillin and cefalexin **(Figure 3).** Antibiotic resistance pattern among bacterial strains may be varied which is linked to place of origin of the strains (Ko et al., 1996). In the present study, *A. hydrophila* isolated from prawns in the Dumuria Gher of Khulna were sensitive to all five antibiotics (**Table 4**).

CONCLUSION

Data of this study suggest that *A. hydrophila* are prevalent in fresh water farm raised prawn in the ghers of the southern part of the Bangladesh. Data of antibiogram study suggest that farm raised fresh water prawn harbors multidrug resistant *A. hydrophila* which may cause public health hazard if enter into the food chain.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

The research was partially funded by a grant provided by the Bangladesh Agricultural University Research System (BAURES) (Project No. 2014/77/UGC).

REFERENCES

Albert MJ, Ansanizzaman M, Talukeler KA, Chopra AK, Kuhn I, Rahman M (2000). Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhoea, healthy controls and the environment. Journal of Clinical Microbiology, 3: 3785-3790.

Al-Fatlawy HNK, Al-Ammar MH (2013). Molecular study of *Aeromonas hydrophila* isolated from stool samples in Najaf (Iraq). International Journal of Microbiology Research, 5: 363-366.

Aoki T (1999). Motile *Aeromonads* (*Aeromonas hydrophila*). Fish Diseases and Disorders. Vol 3: Viral, Bacterial and Fungal Infections. Eds P. Woo, D. Bruno. Wallingford, CABI Publishing; pp 427-453.

Ashiru AW, Uaboi-Egbeni PO, Oguntowo JE, Idika CN (2011). Isolation and antibiotic profile of *Aeromonas* species from Tilapia Fish (*Tilapia nilotica*) and Catfish (*Clarias batrachus*). Pakistan Journal of Nutrition, 10: 982-986.

Austin B, Adams C (1996). Fish pathogens, in The Genus *Aeromonas*, B Austin, M Altwegg, PJ Gosling and S Joseph Edn., Wiley, Chichester, UK; pp 198-243.

Brooks GF, Carroll KC, Butel JS, Morse SA (2007).*Vibrio*, *Campylobacters*, *Helicobacter* and associated bacteria. Jawetz, Melnock and Adelberg's Medical Microbiology. 24th Edn; pp 270-279.

Chandrakanthi WHS, Pathiratne A, Widanapathirana GS (2000). Characteristics and virulence of *Aeromonas hydrophila* isolates from freshwater fish with epizootic ulcerative syndrome (EUS). Journal of National Science Foundation of Sri Lanka, 28: 29-42.

Clinical and Laboratory Standards Institute (CLSI) (2011). Performance standards for antimicrobial susceptibility testing; twenty-second informational supplements. CLSI document M100-S22. Wayne, Pennsyslvania; 32: 3.

Daskalov H (2006). The importance of *Aeromonas hydrophila* in food safety. Food Control, 17: 474-483.

Delamare APL, Lucena RF, Thomazi G, Ferrerini S, Zacaria J, Echeverrigaray S (2012). *Aeromonas* detection and characterization using genus-specific PCR and single–strand conformation polymorphism (SSCP). World Journal of Microbial Biotechnology, 28: 3007-3013.

Deng GC, Jiang XY, Ye X, Liu MZ, Xu SY, Liu LH, Bai YQ, Luo X (2009). Isolation, identification and characterization of *Aeromonas hydrophila* from hemorrhagic Grass carp. Microbiology (Chinese), 36: 1170-1177.

FDA (Food and Drug Administration) (2011). Fish and fishery products hazards and controls guidance. Fourth Edition. Department of Health and Human Service Public Health Service.

Feldhusen F (2000). The role of seafood in bacterial foodborne diseases. Microbes and Infection, 2: 1651-1660.

Fouz B, Alcaide E, Barrera R, Amaro C (2002). Susceptibility of Nile Tilapia (*Oreochromis niloticus*) to Vibriosis due to *Vibrio vulnificus* biotype 2 (Serovar E), Aquaculture, 212: 21-30.

Geiss H, Freij B (1989). *Aeromonas* as a human pathogen. Critical Reviews in Microbiology, 16: 253-386.

Igbinosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI (2012) . Emerging *Aeromonas* species infections and their significance in public health. The Scientific World JournalVolume 2012, Article ID 625023. doi: 10.1100/2012/625023

Islam KMI, Kabir SML, Saha S, Khan MSR (2013). Prevalence and antimicrobial resistance patterns of *Vibrio Cholerae* from Bangladesh Agricultural University dairy farm. International Journal of Medical Sciences and Biotechnology, 1:13-25.

Jayasinghe CVL, Ahmed SBN, Kariyawasam MGIU (2008). The isolation and identification of *Vibrio* species in marine shrimps of Sri Lanka. Journal of Food and Agriculture, 1: 36-44.

Kannan KS, Jayavignesh V, Bhat AD (2011). Biochemical characterization and cytotoxicity of the *Aeromonas hydrophila* isolated from Catfish. Archives of Applied Science and Research, 3:85-93.

Kaysner CA, Depaola A (2004). Bacteriological Analytical Manual: Vibrio, Chapter 9. US Department of Health and Human Services.

Khamesipour F, Moradi M, Noshadi E, Shahraki MM (2014). Detection of the prevalence of *Aeromonas hyrophila* in shrimp samples by polymerase chain reaction (PCR) and cultural method in the Iran. Journal of Biology and Environmental Science, 4: 47-52.

Ko WC, Chiang SR, Lee HC, Tang HJ, Wang YY, Chuang YC (2003). In vitro and in vivo activities of fluoroquinolones against *Aeromonas hydrophila*. Antimicrobial Agents and Chemotherapy, 47: 2217-2222.

Ko WC, Yu KW, Liu CY, Huang CT, Leu HS, Chuang YC (1996). Increasing antibiotic resistance in clinical isolates from clinical and environmental sources. Antimicrobial Agents and Chemotherapy, 40: 1260-1262.

Krieg NE (Edn.) (1984). *Bergey's Manual of Systematic Bacteriology*, Vol 1, (Williams and Wilkins, Baltimore).

Li X, Saghaian SH (2011). Impact of HACCP on US Seafood Exports. Paper presented at IFAMA meeting, Frankfurt, Germany, June 20-23.

Morita K, Watanabe N, Kurata S, Kanamori M (1994). Beta-lactam resistance of motile *Aeromonas* isolates from clinical and environmental sources. Antimicrobial Agents and Chemotherapy, 38: 353-355.

Nemetz TG, Shotts EB (1993). Zoonotic diseases. In: M.K. Stoskopf (Edn.), Fish Medicine, WB Saunders, Philadelphia; pp 214-220.

Overman TL (1980). Antimicrobial susceptibility of Aeromonas hydrophila**.** Antimicrobial Agents and Chemotherapy, 17**:** 612-614.

Palu AP, Gomes LM, Miguel MA, Balassiano IT, Queiroz ML, Freilas-Almeida AC (2006). Antimicrobial resistance in food and clinical *Aeromonas* isolates. Food Microbiology**,** 23: 504-509.

Peters G, Faisal M, Lang T, Ahmed I (1988). Stress caused by social interaction and its effect on susceptibility to *Aeromonas hydrophila* infection in rainbow trout, Salmo gairdneri. Disease of Aquaculture**,** 4: 11-22.

Popovic TN, Teskeredzi ICE, Strunjak-Perovic I, Ciozi-Rakovac R (2000). *Aeromonas hydrophila* isolated from wild freshwater fish in Croatia. Veterinary Research Communications, 24: 371-377.

Rahim Z, Aziz KMS (1994). Enterotoxigenicity, hemolytic activity and antibiotic resistance of *Aeromonas* spp. isolated from freshwater prawn marketed in Dhaka, Bangladesh. Microbiology and Immunology**,** 38**:** 773-778.

Rahim Z, Sanyal SC, Aziz KMS, Huq MI, Chowdhury AA (1984). Isolation of enterotoxigenic, hemolytic and antibiotic resistant *Aeromonas hydrophila* strains from infected fish in Bangladesh. Applied and Environmental Microbiology, 48**:** 865-867.

Truong TH, Areechon NS, Wasde MS (2008). Identification and antibiotic sensitivity test of the bacteria isolated from Tra Catfish (*Pangasianodon hypophthalmua* [Sauvage, 1878]) cultured in pond in Vietnam. Nature and Science, 4**:** 54-60.

Vivekanandhan G, Hatha AAM, Lakshmanaperumalsamy P (2005). Prevalence of *Aeromonas hydrophila* in fish and prawns from the seafood market of Coimbatore, South India. Food Microbiology**,** 22: 133-137.

****