International Journal of Natural Sciences (2011), 1(4):82-88

www.ijns.net

pISSN : 2221-1012
eISSN : 2221-1020

Antibiotic sensitivity and *in vitro* antimicrobial activity of plant extracts to *pseudomonas fluorescens* isolates collected from diseased fish

Foysal MJ, Rahman MM * and Alam M

Department of Genetic Engineering and Biotechnology,
Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh

[Received: November 23, Accepted: December 26, 2011]

ABSTRACT

Studies were conducted to identify *Pseudomonas fluorescens* isolates from a collection of bacteria isolated from bacterial haemorrhagic septicaemia infected carp and catfish, evaluate their antibiotic sensitivity pattern and screen the antibacterial activity of some medicinal plant extracts against the isolates.. A total of 10 isolates were identified as *P. fluorescens* by morphological, physiological and biochemical tests. *In vitro* antibiotic sensitivity test of the *P. fluorescens* isolates were conducted by disc diffusion method for seven antibiotics where, all of the isolates were found to be sensitive only against streptomycin and gentamycin but, most of the isolates (80%) were found resistant to chloramphenicol (C). Moreover, eighty percent of the isolates showed resistance to multiple antibiotics. A total of 118 plant extracts were screened for their antibacterial activity against the *P. fluorescens* isolates where the isolates exhibited sensitivity to 30 samples. Leaf extracts of *Tamarindus indicus, Terminalia chebula, Citrus aurantifolia, Eugenia caryophyllata* and *Spondias pinnata* were found to inhibit the growth of all of the *P. fluorescens* isolates.

Keyword: Fish disease, *Pseudomonas fluorescens*, Antibiotic sensitivity, Herbal sensitivity

INTRODUCTION

Bacterial fish diseases constitute one of the major challenges facing sustainable aquaculture production in Bangladesh and elsewhere. Approximately 15% of the total of freshwater aquaculture production has been estimated to be lost every year in Bangladesh due to disease infestation [1]. In Bangladesh, scientific information about bacterial diseases in fish are in preliminary stage while it is scare in the field of *Pseudomonas fluorescens* which has been denoted as one of important disease causing agents of both farmed and wild fish. *P. fluorescens* was originally described as the causative agent of Bacterial Hemorrhagic Septicemia disease of pond-cultured fish [2, 3]. It is considered as a primary pathogen of freshwater fish and opportunistic pathogen for different fish species cultured in marine and brackish waters worldwide [4, 5]. In Bangladesh, *P. fluorescens* has been isolated from eye surface and mouth lesions of diseased Rajpunti (*Barbodes gonionotus*) along with some other fish pathogenic bacteria [6] from. Isolation and characterization of *P. fluorescens* from gills of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), African magur (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) has also been reported elsewhere [7, 8].

In vitro sensitivity pattern of *P. fluorescens* to commercial antibiotics have been done earlier by some scientists [9, 10]. They reported that *P. fluorescens* was generally resistant to β-lactum antibiotics but sensitive to gentamycin and torbamycin. Antibiotics are long been used for the treatment of bacterial infections of fish but habitual use of antibiotics can lead to problems with bacterial resistance and with hazardous residues released in aquaculture environment [11, 12]. Thus, applications of antibiotics as a therapeutic agent for fish health management are now strongly discouraged in different countries [13]. However, a large number of herbs are known to contain strong antibacterial activity that can be used to control different fish diseases. But, only a few scientific studies have yet been conducted in this field. Considering the above facts, the present study was carried out to isolate and identify *Pseudomonas fluorescens* from bacterial haemorrhagic septicaemia infected carp and catfish, to find out their antibiotic sensitivity pattern and to screen the inhibitory effect of available medicinal plant extracts for the isolates.

MATERIALS AND METHODS

Collection of Diseased Fish Samples:

*Corresponding author**:** mahbub_biotech@yahoo.com

Table 1. Pseudomonas fluorescens isolates with their origin

Isolates	Host	Organ of isolation	Symptoms of the diseased fish
$PuKL_2$	Rajpunti (*Barbodes gonionotus*)	Lesion in eye	Haemorrhagic lesion in the eye
$PuKL_{22}$	Common Carp (*Cyprinus carpio*)	Lesion in skin	Small haemorrhagic lesions in the skin, Loss of scale
PFN_3	Magur (*Clarias batrachus*)	Kidney	Haemorrhagic lesions in the body surface
PFK_{13}	Tengra (*Mystus tengara*)	Lesion in tail	Haemorrhages in the tail
Cla_1B_8	Magur (*Clarias batrachus*)	Kidney	Haemorrhagic lesions in the body surface
Cla_1B_{10}	Magur (*Clarias batrachus*)	Lesion in skin	Moderate to large haemorrhagic lesions in the body surface
Cla_1B_{18}	Magur (*Clarias batrachus*)	Lesion in skin	Moderate to large haemorrhagic lesions in the body surface
Cla_2B_7	Magur (*Clarias batrachus*)	Kidney	Haemorrhagic lesions in the body surface
P_2F_2	Tengra (*Mystus tengara*)	Lesion in eye	Haemorrhagic lesion in the eye and mouth region
P_2F_4	Tengra (*Mystus tengara*)	Lesion in eye	Haemorrhagic lesion in the eye and mouth region

Carp (Rajpunti, *Barbodes gonionotus* and Common Carp, *Cyprinus carpio*), and catfish (Magur, *Clarias batrachus* and Tengra *Mystus tengara*) suspected to be suffering from bacterial hemorrhagic septicemia with severe mortality was collected from different fish farms of Bangladesh during October 2010 to January 2011. Most of the fish was collected in alive condition with water of the affected water body. When the fish was dead, it was collected aseptically in plastic box with ice. The fish samples were transported to the USDA-Project Laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh for further studies.

Table 4. Antibiotic sensitivity of *Pseudomonas fluorescens* isolates

Strain name	Streptomycin (S)	Sulphamethoxazole (RL)	Erythromycin (E)	Chloramphenicol (C)	Cephradine (CE)	Gentamycin (GM)
$PuKL_2$	1.4mm	R	R	R	3.4mm	2.2mm
$PuKL_{22}$	1.6mm	2.6mm	2.8mm	3.8mm	3.6mm	2.4mm
PFN_3	2.2mm	R	3mm	R	3.2mm	2.4mm
PFK13	2.8mm	R	R	R	R	2.2mm
Cla_1B_8	3.4mm	R	1.6mm	R	3.8mm	2.8mm
Cla_1B_{10}	1.8mm	R	R	R	2.8mm	3.2mm
Cla_1B_{18}	1.8mm	R	R	R	R	3.0mm
Cla_2B_7	3.0mm	3.4mm	1.8mm	2.4mm	R	3.2mm
P_2F_2	2.6mm	R	R	R	R	3.4mm
P_2F_4	2.8mm	1.8mm	R	R	3.2mm	2.6mm

C: Chloramphenicol (30 µg disc^{-1}), E: Erythromycin (10 µg disc^{-1}), OT: Oxytencycline (30 µg disc^{-1}), S: Streptomycin (10 µg disc-^{1}), SXT: Sulphamethaxazole (15 µg disc^{-1}), CE: Cephradine (30 µg disc^{-1}), GM: Gentamycin (10 µg disc^{-1}), R: Resistant, ±: Confusing zone.

External signs of the Diseased Fish:

The external signs of the diseased fish were recorded. Hemorrhagic lesions in the skin were observed in some diseased fish while in some other

fish hemorrhages was found in fin, tail, and eye. Loss of scale was also observed in some fish. The external signs of the diseased fish have been summarized in Table 1.

Table 3. Phenotypic properties of the *Pseudomonas fluorescens* isolates.

Traits	Results
Colony shape	Round
Colony size	Medium
Colony color	Yellowish
Gram stain	-
Shape	Rod
Motility	+
Oxidase	+
Polar flagella	+
Catalase	+
O-F test	Oxidative
Acid production in Glucose	+
H2S production	-
Methyl-Red test	-
Growth at Casein	+
Growth at Gelatin	+
Growth at Tween20	+
Growth at 4^{o}C	-
Growth at 25^{o}C	+
Growth at 37^{o}C	+
Growth at 42^{o} NaCl	+
Growth in 0% NaCl	+

Isolation of Bacteria:
Isolation of bacteria was carried out from eye, tail and skin lesions as well as from kidneys of the diseased fish. Smears from haemorrhages or lesions and a portion from kidneys were aseptically inoculated on nutrient agar (NA) plate by streak plate method as described by Ferdowsy *et al*., 2011 [14]. The plates were then incubated at 25ºC for 24-36 hours. Individual colonies grown on the agar medium were separated from the plates to obtain pure culture of bacteria.

Identification of bacterial genera *Pseudomonas*:
A series of morphological, physiological and biochemical tests were performed to identify the assumed *P. fluorescens* isolates from a large collection of bacteria obtained from the diseased fish samples. A presumptive identification was performed by Gram staining, motility test, oxidase test, catalase activity, acid production from glucose, oxidation-fermentation (OF) test, methyl red test, casein hydrolysis, gelatin hydrolysis, growth in tween 20 medium and H_2S production. Growth temperature of the isolates was studied by incubation of the isolates on NA medium at 4^{o} C, 25^{o} C and 37^{o} C for 24-48 hours. The Bacterial isolates were identified up to species level following the tests described in the Cown and Stell's Manual for the Identification of Medical Bacteria (Barrow and Feltham, 1993 [15]).

Antibiotic sensitivity test:
In vitro sensitivity of the *P. fluorescens* isolates to different commercial antibiotics was determined by disc diffusion method as described by Rahman *et al*. (2010) [16]. Briefly, individual isolates were cultured into nutrient broth and incubated at 25°C for 12 hours. Fifty micro liter of individual broth culture was dropped on the NA plate with micropipette. The broth on the plate was spread aseptically by a sterile 'L' shaped glass rod. Seven commercially prepared antibiotic discs viz., streptomycin (10 µg/disc), chloramphenicol (30 µg/disc), sulphamethoxazole (25 µg/disc), erythromycin (15 µg/disc), oxytetracycline (30 µg/disc) and cephradine (30 µg/disc) manufactured by Oxoid Ltd. and gentamycin (10 µg/disc) manufactured by Becton Disc kinson & company were placed on the surface of the medium with sterile forceps and pressed gently to ensure good contact with the surface of the medium. The plates were then incubated at 25°C for 24 h. After incubation the organism was considered sensitive if there was zone of complete inhibition around the disc and resistant if there was no zone of inhibition. The diameter of the zone of inhibitions was measured in millimeter (mm) by measuring scale.

Herbal sensitivity of the *Pseudomonas fluorescens* isolates:
A total of 118 randomly selected herb extracts was used in this study to screen their sensitivity to the *P. fluorescens* isolates. Most of the herbs were collected from a nursery of Adamdighi, Bogra and different parts of Sylhet district. A list of the plants has been given in Table 2. The fresh parts of plants such as young leaves, bark, bulb, root, flower, rhizome or petiole were collected and washed several times with distilled water. The plant parts were cut into small pieces and paste was made by using mortar-pestle. Approximately 10µl of a herb extract was inoculated on the individual spread plate culture. The plate was then allowed to incubate at 25^{0}C for overnight. After incubation, the herb extract was noted for zone of inhibition for each *Pseudomonas fluorescens* isolates. The diameter of the herb extracts and the diameter of the zone of inhibitions were measured by measuring scale. The ratio between the diameters was calculated.

RESULTS

Identification of the Isolates:
A total of 154 bacterial isolates was isolated from the disease infected carp and catfish. The isolates were

assessed for their morphological, physiological and biochemical characteristics. Among these, 10 isolates were Gram negative, rod shaped, motile with polar flagella, catalase positive, oxidative bacteria, produced acid from glucose in paraffin free media but unable to ferment glucose with paraffin, H_2S production negative, methyl red test negative, hydrolyzed casein and gelatin, showed positive growth in tween 20 media and able to grow at 25°C and 37°C but unable to grow at 4°C and 42° (Table 3). Based on the characteristics these isolates were identified as *P. fluorescens*.

Antibiotic sensitivity of the *Pseudomonas fluorescens* isolates:

The *P. fluorescens* isolates were found to vary in their antibiotic sensitivity pattern to the seven antimicrobial agents tested. All of the isolates showed sensitivity to two antibiotics such as streptomycin and gentamycin. But, most of the isolates (80%) were found resistant to chloramphenicol (C). Moreover, 70%, 60%, and 30% isolates exhibited resistance to sulphamethoxazole (RL), erythromycin (E) and cephradine (CE), respectively (Table 4). Only one isolate ($PuKL_{22}$) was sensitive to all of the antibiotics tested and another isolate (Cla_2B_7) was resistant to Cephradine. However, 80% of the isolates were resistant to multiple antibiotics.

Herbal sensitivity of the *Aeromonas* sp. isolates:

The *P. fluorescens* isolates were found to be sensitive to 30 herbs out of 118 herbs samples tested. The leaf extract of *Terminalia arjuna, Eugenia caryophyllata, Tamarindus indicus, Ipomoea fistulosa, Spondias pinnata, Terminalia chebula, Lawsonia inermis, Citrus aurantifolia, and Pterygota alata* inhibited the growth of all of the *P. fluorescens* isolates (Table 5). The *P. fluorescens* isolates exhibited 90% sensitivity to the leaf extracts of *Eucalyptus globulus, Piper betle, Artabotrys uncinatus, and Emblica officinalis* respectively. The crude herb extract of *Mikania cordata, Shorea robusta, Crocus sativus, Hydnocarpus kurzii, Annona squamosa, Cerodendrum indicum* also inhibited the growth of 60-80 % of the bacterial isolates. The extracts of *Allium cepa, Mikania cordata, Vitex negundo, Datura suaveolens, Dipterocarpus turbinatus, Allamanda schottii, Campsis radicans, Duranta repens, Lannea coromandelica* and *Cassia alata* also showed sensitivity against some of the isolates.

DISCUSSION

Bacterial haemorrhagic septicaemia is a disease that causes mass mortality in both freshwater and marine fish. The primary symptom is a red ulcer or lesion

Table 5. Inhibitory effects of plant extracts against Pseudomonas fluorescens isolates

Name of the Plants	Sensitivity of herb extracts to *Pseudomonas fluorescens* isolates.									
	$PuKL_2$	$PuKL_{22}$	PFN_3	PFK_{13}	Cla_1B_{10}	Cla_2B_7	Cla_1B_8	Cla_1B_{18}	P_2F_2	P_2F_4
Terminalia arjuna	0.8	1.7	1.75	1.57	2.0	2.14	2.0	2.2	2.2	2.17
Eucalyptus globulus	d	2.0	2.0	1.63	1.75	1.78	1.86	1.63	1.67	1.5
Allium cepa	-	-	-	1.5	-	-	-	-	-	-
Piper betle	1.5	1.87	1.92	d	1.9	1.97	2.02	d	2.12	1.85
Ipomoea fistulosa	1.8	3.0	3.22	2.3	2.91	2.7	2.53	2.58	3.11	2.32
Spondias pinnata	2.14	2.86	2.6	2.76	2.0	2.5	2.4	2.5	3.0	2.9
Lawsonia inermis	1.89	2.13	2.43	2.54	2.6	2.25	2.17	2.14	2.87	2.67
Mikania cordata	1.6	1.5	-	-	1.6	1.8	-	-	1.6	1.8
Citrus aurantifolia	1.9	2.34	2.7	2.5	2.8	2.1	2.15	2.78	2.92	2.1
Vitex negundo	1.87	-	-	-	-	-	-	-	d	-
Terminalia chebula	2.03	2.36	2.0	1.8	1.82	2.13	1.9	1.88	2.42	2.23
Eugenia caryophyllata	d	1.46	1.8	1.9	1.8	2.0	2.08	2.25	1.82	2.13
Terminalia bellirica	d	1.7	2.22	1.73	1.64	1.57	1.38	1.33	d	1.23
Withania somnifera	1.6	1.43	1.29	d	1.32	1.4	-	d	1.31	1.17
Tamarindus indicus	2.33	2.6	2.0	2.06	2.5	2.48	2.0	2.35	2.03	2.04
Shorea robusta	-	d	1.44	1.34	1.23	1.7	1.22	1.38	1.3	1.28
Crocus sativus	-	1.17	1.2	1.29	1.25	1.4	-	d	1.2	1.33
Datura suaveolens	-	1.4	-	-	-	-	-	-	1.3	-
Dipterocarpus turbinatus	1.44	d	-	-	-	-	-	-	1.28	-
Allamanda schottii	-	1.6	-	-	-	1.38	-	-	1.44	-
Hydnocarpus kurzii	d	d	1.28	1.2	1.25	1.3	d	1.28	1.51	1.2
Campsis radicans	d	d	-	-	-	-	-	-	1.15	-
Emblica officinalis	1.57	1.5	1.23	1.2	1.2	1.54	d	1.27	1.38	1.25
Artabotrys uncinatus	1.38	d	1.56	1.4	1.75	1.45	1.6	1.8	1.27	1.27
Lannea coromandelica	1.2	1.5	1.28	1.44	1.5	1.4	1.3	d	1.5	1.38
Clerodendrum indicum	1.2	d	1.23	1.2	1.2	1.33	1.38	-	1.2	1.3
Annona squamosa	d	1.88	1.63	1.67	1.6	1.67	d	d	1.89	1.33
Adhatoda vasica	-	-	1.2	1.3	-	-	1.4	-	1.56	1.44
Pterygota alata	1.25	1.27	1.44	1.51	1.2	1.23	1.36	1.5	1.4	1.38
Duranta repens	-	-	1.2	1.34	-	-	-	-	-	-

-: negative result; d: zone of inhibition not clear.Number indicates zone ratios.

which can quickly deteriorate through layers of skin, tissue, and muscle. *Pseudomonas fluorescens* is known to be the causative agent of the disease. In the present study, several fish was collected with expression of haemorrhagic lesions in the eye and skin of different carp and catfish from different districts of Bangladesh. A total of 154 bacterial isolates collected from these fish were initially characterized for their morphological, physiological and biochemical properties. Among these, 10 isolates were identified as *P. fluorescens* since they exhibited the morphological and phyo-biochemical properties resembled to *P. fluorescens*. Chowdhury (1998) [6] isolated and identified some *Pseudomonas* isolates from diseased farmed fish of Bangladesh. Paul *et al*. (1998) [17] reported isolation of Pseudomonads from diseased Rajpunti (*Barbodes gonionotus*). Hossain *et al*. (2011) [18] also identified several Pseudomonad isolates recovered from diseased fish from different types of water bodies.

In vitro antibiotic sensitivity of the *P. fluorescens* isolates to seven commercial antibiotic discs *viz*., streptomycin, chloramphenicol, sulphamethoxazole, erythromycin, oxytetracycline, cephradine and gentamycin was performed by disc diffusion method. In the present study, all of the isolates were found sensitive to only streptomycin and gentamycin. Eighty percent of the isolates exhibited resistance to chloramphenicol followed by sulphamethoxazole (70%) and erythromycin (60%). El-Atta and Tantawy (2008) [19] conducted antibiotic sensitivity test of *P. fluorescens* isolates collected from skin ulcers of *Tilapia nilotica* (= *Orechromis niloticus*) and found the isolates to be sensitive to Nalidixic acid, chloramphenicol, Ciprofloxacin, Streptomycin and resistant to Amoxicillin and Ampicillin. Ghosh *et al.* (2011) [9] conducted *in vitro* screenings of antibiotics against the two most commonly found opportunistic bacteria related to epizootic ulcerative syndrome of fish—*Aeromonas hydrophila* (MTCC 646) and *Pseudomonas fluorescens* (MTCC 103) and reported that gentamycin, ofloxacin, kanamycin, and tobramycin were effective. These reports correlate with our findings. However, in the present study, 80% of the isolates were found resistant to multiple antibiotics. Olasumbo *et al*. (2007) [12] evaluated the sensitivity of 15 antibiotics to 129 *Pseudomonas* spp. isolates collected from rainbow trout (*Oncorhynchus mykiss*) farms of Australia and reported multiple antibiotic resistance patterns of the isolates.

Antibiotics are traditionally used for fish health management. But careless use of antibiotics has potential risk associated with the transmission of resistant bacteria from aquaculture environments to humans (Denev *et al*., 2009 [13]). There is also risk associated with the introduction of nonpathogenic bacteria, containing antimicrobial resistance genes in the human and subsequent transfer of such genes to human pathogens (FAO 2005 [20]). In addition, antibiotics inhibit or kill beneficial micro flora in the gastrointestinal tract of fish and also made accumulation of antibiotic residue in fish products to be harmful for human consumption (WHO 2006 [21]).

Now a days, uses of antibiotics for fish and animal health management are discouraged and efforts are given to develop alternative therapeutics like antimicrobial agents from plant sources for the treatment of fish, animals as well as humans.

In the present study, a total of 118 herb extracts were screened for their inhibitory activity to the *P. fluorescens* isolates. The isolates were found sensitive to 30 herb samples. Among these, the leaf extracts of *Terminalia arjuna, Eugenia caryophyllata, Tamarindus indicus, Ipomoea fistulosa, Spondias pinnata, Terminalia chebula, Lawsonia inermis, Citrus aurantifolia,* and *Pterygota alata* exhibited antibacterial activity against all of the *P. fluorescens* isolates. The leaf extracts of *Eucalyptus globulus, Piper betle, Artabotrys uncinatus, and Emblica officinalis* were capable to inhibit the growth of 90% of the isolates. The crude extracts of *Mikania cordata*, *Shorea robusta*, *Crocus sativus*, *Hydnocarpus kurzii*, *Annona squamosa*, *Clerodendrum indicum* suppressed the growth of 60-80 % of the isolates. The extracts of *Allium cepa, Mikania cordata, Vitex negundo, Datura suaveolens, Dipterocarpus turbinatus, Allamanda schottii, Campsis radicans, Duranta repens, Withania somnifera* and *Cassia alata* also showed evidence of antimicrobial activity for some of the *P. fluorescens* isolates. Muniruzzaman and Chowdhury (2004) [22] evaluated sensitivity of certain local medicinal herbs against fish pathogenic *P. fluorescens* isolates and argued that the extract of bulb of *Allium sativum* had significant inhibitory effects. In the present study, no inhibitory effect was observed for the bulb extract of *Allium sativum* to the bacterial isolates tested. The reason might be due to the difference of isolates. However, Rahman and Hossain (2010) [11] reported that leaf extracts of. *Eugenia caryophyllata, Spondias pinnata, Terminalia chebula, Eucalyptus globulus, Terminalia arjuna,* and *Tamarindus indicus* possess potential antimicrobial activity for fish pathogenic *Aeromonas* sp. isolates. Ferdowsy *et al*. (2011) [14] also claimed that the juice of Labu (*Citrus aurantifolia*), extract of bulb of garlic (*Allium sativum*) and leaf extracts of Mehdi (*Lawsonia inermis*), Horitoki (*Terminalia chebula*), Amra (*Spondias pinnata*), Guava (*Psidium guajava*), Labanga (*Eugenia caryophyllata*), Tentul (*Tamarindus indicus*) and Amloki (*Emblica officinalis*) had inhibitory effects for *Edwardsiella* sp. isolates collected from diseases infected catfish. Ghosh *et al*. (2011) [9] conducted screening of antibacterial activity of plant extracts and reported that both aqueous and methanolic extract of *Terminalia chebula, Polyalthia longifolia, Terminalia bellirica*, and *Emblica officinalis* were sensitive against the bacterial strain of *P. fluorescens* (MTCC 103) isolated from Epizootic Ulcerative Syndrome affected fish. These reports support our present findings.

It was the first known report where more that 100 herbs were screened for their antimicrobial activity against fish pathogenic and multiple antibiotic

resistant *P. fluorescens* isolates collected from Bangladesh. The herbs that inhibited the growth of all of the *P. fluorescens* isolates could be used for the treatment of fish disease caused by *P. fluorescens*. In the present study, leaf extracts of *Ipomoea fistulosa* and *Eucalyptus globulus* were found to possess high antibacterial activity against the isolates but, whether the extracts hold any toxic effects on fish is need to be evaluated. Further research works are needed for purification of particular compounds responsible for antibacterial activity of the herb extracts.

ACKNOWLEDGEMENTS

The present study was conducted under a research project titled "Molecular Detection of Bacterial and Fungal Diseases of Carp and Fish and Herbal Treatment for Remedy of the Diseases" funded by United States Department of Agriculture.

REFERENCES

1. M A R Faruk, M M R Sarker, M J Alam, M B Kabir (2004). Economic loss from fish diseases on rural freshwater aquaculture of Bangladesh. *Pakistan J. Biol. Sci*. 7 (12): 2086-2091
2. Wakabayashi H. and Egusa S (1972). Characteristics of a *Pseudomonas* sp. from an epizootic of pond cultured eels (*Anguilla japonica*). *Jpn. Soc. Sci. Fish.* 38: 577–587.
3. Shiose J, Wakabayashi H, Tominaga M and Egusa S (1974). A report on a disease of cultured carp due to a capsulated Pseudomonas. *Fish Pathol*. 9: 79–83.
4. Alicia E, Toranzo T, Magarinos B and Romalde SL (2004). A review of the main bacterial fish diseases in mariculture systems, *Aquaculture* 246: 37-61.
5. Hadi TK, Morshino JL and Das PG (2002). Infectious disease caused by *Pseudomonas fluorescens* in silver carp. *J. Fish dis.* 36(3): 45-53.
6. Chowdhury MBR (1998). Involvement of Aeromonads and Pseudomonads in diseases of farmed fish in Bangladesh. *Fish. Pathol*. 33: 247-254.
7. Miyashita T (1984). Isolation of *Pseudomonas fluorescens* and *Edwardsiella tarda* from diseased tilapia. *J. Fish Pathol*. 19: 45–50.
8. Uddin MM and Ahmed AH (2004). Infectious disease of fish, *J. Appl. Aquacult.* 2(1), 53-61.
9. Ghosh A, Das BK, Roy A and Chandra G (2011). Antibiotic resistance and herbal treatment of bacterial fish pathogens causing Epizootic Ulcerative Syndrome. *J. Herbs spic. Med. Plants*. 17 (1): 47-51.
10. Nusbaum KE and Shotts EB (2006). Action of selected antibiotics on four common bacteria associated with diseases in fish. *J. Fish dis.* 5(4): 394-404.
11. Rahman MM and Hossain, MN (2010). Antibiotic and herbal sensitivity of some *Aeromonas* sp. collected from diseased carp fish. *Progress. Agric.* 21(1&2):117-129.
12. Olasumbo L. Akinbowale, Peng H, Grant P and Barton D (2007). Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *Int. J. Antimicrobial Age*. 30 (2) 177-182.
13. Deven S, Staykov Y, Moutafchieva R and Beev G (2009). Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. *Int. Aquat. Res*. 1: 1-29.
14. Ferdowsy H, Foysal MJ, Hossain, MN and Rahman MM (2011). Isolation of *Edwardsiella* sp. from diseased catfish and their sensitivity to some antibiotics and medicinal plant extracts. *Int. J. BioRes*. 11 (1): 48-53.
15. Barrow GI and Feltham RKM (1993). Cown and Stell's Manual for the Identification of Medical Bacteria. Third edition. *Cambridge University Press*: 331.
16. Rahman MM, Ferdowsy H, Kashem MA and Foysal MJ (2010). Tail and fin rot disease of carp and climbing perch in Bangladesh. *J. Biol. Sci*. 10 (8): 800- 8004
17. Paul HK, Chowdhury MBR, Uddin MN and Rahman MM (1998). Experimental infection of silver barb (*Puntius gonionotus*) with som recovered Pseudomonad isolates. *Bangladesh J. Fish.* 20 (1-2): 77-80.
18. Hossain MMM, Rahman MA, Mondal S, Sahadat Mondal ASM and Chowdhury MBR (2011). Isolation of some emergent bacterial pathogens recovered from capture and culture fisheries of Bangladesh. *Bangladesh Res. Publ. J*. 6 (1): 77-90.
19. El-Atta MEA and Tantawy MME (2008). Bacterial causes of skin ulcers in *Tilapia nilotica* (*Oreochromis niloticus*) with special references to its control. In: *Proceedings of 8 th International symposium on Tilapia in Aquaculture*. 1419-1436.
20. FAO. 2005. Responsible Use of Antibiotics in Aquaculture (Ed. Serrano PH), FAO Fisheries Technical Paper 469, FAO, Rome, Iraly, pp 98.
21. WHO. 2006. Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and

antimicrobial resistance: Seoul, Republic of Korea, 13-16 June 2006.

22. Muniruzzaman M and Chowdhury MBR (2004). Sensitivity of Fish Pathogenic Bacteria to Various Medicinal Herbs. *Bangladesh J. Vet. Med*. *2* (1) : 75-82