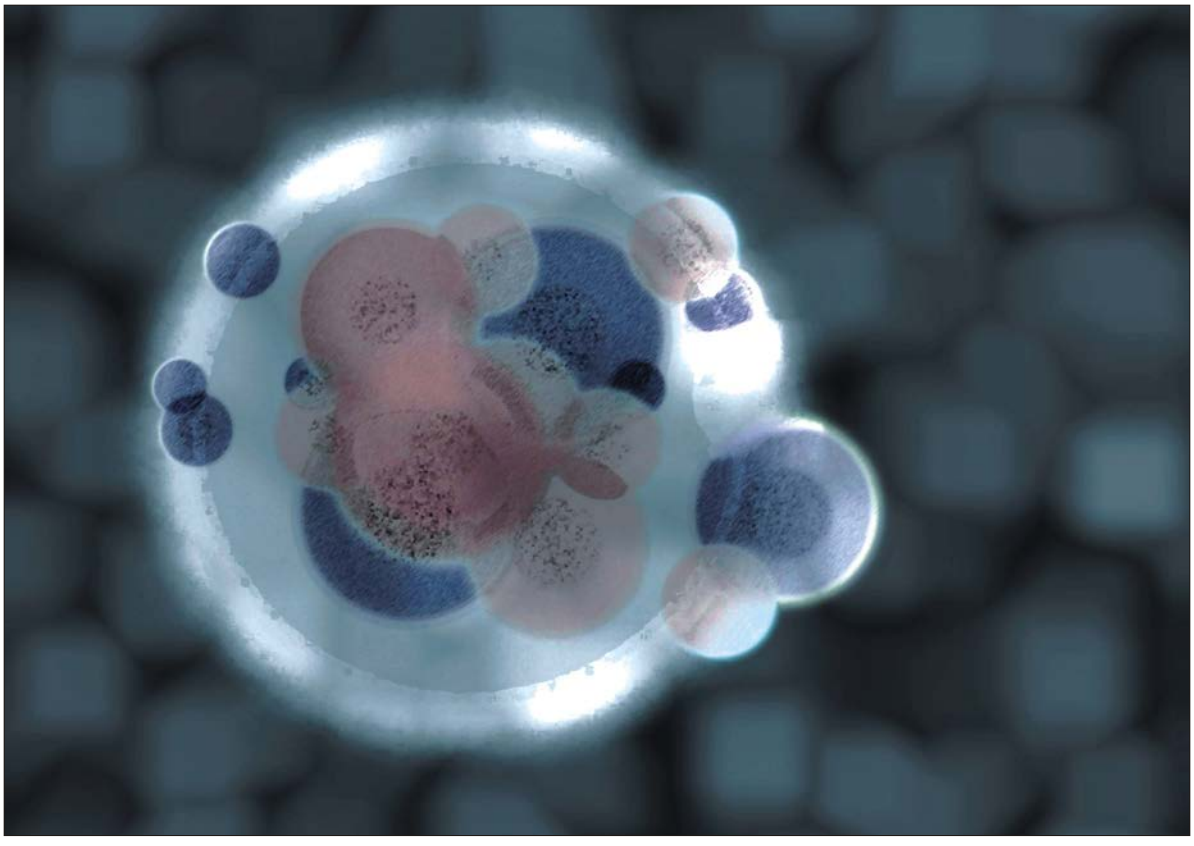

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Tail and Fin Rot Disease of Indian Major Carp and Climbing Perch in Bangladesh

[1]M.M. Rahman, [2]H. Ferdowsy, [3]M.A. Kashem and [1]M.J. Foysal
[1]Department of Genetic Engineering and Biotechnology,
Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh
[2]Department of Zoology, Dhanmondi College, Dhaka, Bangladesh
[3]Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Abstract: Tail and fin rot disease occurred in Indian major carp, catla (*Catla catla*) and climbing perch, koi (*Anabas testudineus*) in fish farms located at two districts of Bangladesh. The affected fish showed lesion and erosion on the tail and fins. Approximately, 40% mortality was recorded in those farms. The present study was conducted to isolate and identify the bacterial pathogen causing the disease, to conduct artificial infection challenge for confirmation of the pathogen and to know the antibiotic sensitivity pattern of the isolates. Bacteria were isolated from the lesions of diseased fish on Cytophaga agar medium where they developed characteristic yellowish pigmented colonies. They were identified as *Flavobacterium columnare* based on biochemical characterization tests. All of the isolates were found to be highly virulent for carp fish (*Puntius gonionotus*) in artificial infection challenge experiment but, virulence for koi fish (*A. testudineus*) were found to be varied. These isolates exhibited sensitivity to antibiotics chloramphenicol, oxytetracycline, erythromycin, streptomycin, but some of them were resistant to sulphamethoxazole and all were resistant to gentamicin and cefradine.

Key words: *Flavobacterium columnare*, fish disease, artificial infection, antibiotic sensitivity

INTRODUCTION

Bangladesh is an agrobased country where fisheries contribute as a substantial sector in its economy. Both fisheries and aquaculture play a major role in alleviating protein deficiency and malnutrition, in generating employment and foreign exchange earnings in Bangladesh (DOF, 2003). Aquaculture accounted for about 43.5% of the total fish production during 2003-04, with inland open water fisheries contributed 34.8% (DOF, 2005). The country is one of the world's leading inland fisheries producers with a production of 1646819 tonnes during 2003-04 (DOF, 2005). FAO ranked Bangladesh as sixth largest aquaculture producing country of the world (DOF, 2005). The major fish species cultured in inland water bodies are Indian major carps (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*), Chinese carps (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), Thai catfish (*Pangasius sutchi*), silver barb (*Puntius gonionotus*) and Genetically Improved Farmed Tilapia (GIFT). Recently, culture of koi (*Anabas testudineus*) also has got popularity in Bangladesh. Due to the rapid expansion of aquaculture, fish disease has become one of the crucial factors in fish production (Rahman and Chowdhury, 1996).

The major fish diseases occurred in Bangladesh are Epizootic Ulcerative Syndrome (EUS), Aeromonad septicaemia, different types of fungal and parasitic diseases etc. (Chowdhury *et al*., 2003). Moreover, tail and fin rot disease is also found in different fish farms and the rate of incidence of this type of disease is assumed to be increased in the recent years (Faruk *et al*., 2004). But a scientific study on the disease including isolation and confirmation of specific pathogen is yet to be done. Considering the importance and need of the country, the present study has been conducted to isolate and identify the bacterial pathogen causing tail and fin rot disease of Indian major carp (*Catla catla*) and climbing perch (*Anabas testudineus*), to conduct artificial infection challenge for confirmation of the pathogen and to know the antibiotic sensitivity pattern of the isolates.

MATERIALS AND METHODS

Collection of diseased fish: Tail and fin rot type disease occurred in Indian major carp, catla (*Catla catla*) and climbing perch, koi (*Anabas testudineus*) in fish farms located at Sylhet and Munshigonj districts respectively during January to April 2010. The fish was cultured in private farms. Lesion and erosion on the tail and fins were

Corresponding Author: M.M. Rahman, Department of Genetic Engineering and Biotechnology,
Shahjlal University of Science and Technology, Sylhet 3114, Bangladesh

observed in the affected fish. Approximately 40% mortality was recorded in those farms. The disease affected fish was collected from the pond and transported in insulated container in alive or moribund condition with the pond water to the Laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet for further studies.

Isolation of bacteria: Cytophaga agar medium (Anacker and Ordal, 1959) was used for isolation of suspected bacterial pathogen from the affected fish. The pH of the medium was adjusted to 7.4. Bacteria were isolated from 05 randomly chosen fish sample from each species. Swabs from lesion and eroded tail and fins were separated and spread aseptically on the agar plates following the plate dilution method. After incubation at 25°C for 48 h, subculture was done from the flat, thin and spread greenish-yellow colony to obtain pure culture.

Identification of bacterial isolates: In order to identify the suspected bacterial pathogen, various physio-biochemical tests were performed. For confirmation, a Japanese reference strain of *Flavobacterium columnare* EK-28 was used. Gram's staining, oxidase, catalase, oxidation-fermentation (O-F), indole production, gelatin liquefaction, caseinase, aesculin dihydrolase, H_2S production, production of acid from carbohydrates and other tests mentioned in Table 1 were performed following the Manual for Isolation and Identification of Fish Bacterial Pathogens (Frerichs and Millar, 1993) and the Cowan and Steel's Manual for Identification of Medical Bacteria (Barrow and Feltham, 1999). After characterization the bacterial isolates were preserved in Cytophaga agar slant.

Artificial infection challenge: Two bacterial isolates isolated from the eroded tail and fins of catla and four isolates collected from koi were randomly chosen for artificial challenge test. The isolates were grown in 200 mL Cytophaga broth (Anacker and Ordal, 1959) at 25°C for 24 h in shaking incubator. The cells were harvested from the culture broth by centrifugation at 10000 rpm for 15 min and then resuspended in sterilized distilled water. The cell number was adjusted to 3-5×10^8 CFU mL^{-1} for artificial infection challenge test.

Young climbing perch, koi (*A. testudineus*) and a carp fish species, silver barb (*Puntius gonionotus*) (average weight 25 g) were used as experimental fish for the artificial challenge experiment. The fish were acclimatized in laboratory condition for 3 days before use in the experiment. Ten koi and 10 carp fish were exposed to each bacterial suspension at 25°C for 30 min. Then the fish were transferred to aquarium where 10 fish from each species was kept in each aquarium containing 10 L sterile water. The fish was kept there for 7 days with aeration but no food was offered to the experimental fish. The infection in fish was detected by the appearance of lesion, erosion in tail and fins and mortality. The rate of infection and mortality of fish was recorded. Bacteria were isolated from the lesion and eroded tail and fins of the affected fish on Cytophaga agar medium and confirmed by physio-biochemical characterization tests as mentioned earlier.

Antibiotic sensitivity test of the selected isolates: Seven commercially prepared discs containing antibacterial agents were tested in *in vitro* condition to observe the sensitivity of the selected isolates. The antibiotics were chloramphenicol (30 μg $disc^{-1}$), oxytetracycline (30 μg $disc^{-1}$), sulphamethoxazole (25 μg $disc^{-1}$), erythromycin (10 μg $disc^{-1}$), streptomycin (10 μg $disc^{-1}$), gentamicin (10 μg $disc^{-1}$) and cefradine (30 μg $disc^{-1}$). At first suspension of individual bacterial isolates were spread on different agar plates, then the antibiotic discs were placed aseptically on the plates by the help of sterile forceps. The plates were incubated at 25°C for 48 h. After incubation of the isolates, clean zone surrounding a disc was recorded as sensitive and the zone of inhibition was measured in mm. When there was no zone of inhibition surrounding a disc, the isolate was considered as Resistant (R) to the antibiotic.

RESULTS

Identification of bacterial isolates: Bacterial colonies apparently similar to the Cytophaga group were recovered from the lesion and eroded tail and fins of the diseased fish. A few other bacteria were also grown in the agar medium which were thought to be normal environmental micro-flora and were not considered for further identification. Two isolates isolated from catla and four isolates collected from koi were randomly chosen for physio-biochemical identification. The isolates F1 and F2 were isolated from the lesion of tail and F3 and F4 were isolated from infected fins of koi. The isolates F5 and F6 were isolated from the lesion of tail of catla. The isolates developed characteristic yellowish pigmented colonies on Cytophaga agar. The biochemical and physiological characteristics of the bacteria are given in Table 1. They were Gram-negative, filamentous bacteria exhibiting flexing movements. They exhibited positive results for oxidase, catalase, H_2S production, congo red absorption and degradation of gelatine, casine and chondroitin sulfate tests, but negative result for aesculin hydrolysis. The isolated strains grew on agar supplemented with polymyxin and neomycin as yellow rhizoid colonies. The isolates were unable to metabolise carbohydrates. According to these morphological, physiological and biochemical characteristics, the isolates were identified as *Flavobacterium columnare*.

Table 1: Characteristics of the bacterial isolates

Characteristics	Isolates F 1-4	Isolates F 5-6
Colour of colony	Yellowish	Yellowish
Binding of colony to congo red dye	+	+
Ability to grow in presence of :		
Neomycin	+	+
Polymyxin B	+	+
Shape	Filamentous rod	Filamentous rod
Gram's stain	-	-
Gliding motility	+	+
Oxidase	+	+
Catalase	+	+
Oxidative-fermentative (O-F)	Oxidative	Oxidative
Arginine dihydrolase	-	-
Lysine decarboxilase	-	-
Ornithine decarboxylase	-	-
Voges-Proscure Test	-	-
Tryptophane deaminase	-	-
Indole test	-	-
Urease Production	-	-
Production of H_2S	+	+
Degradation of:		
Gelatine	+	+
Chondroitin sulfate	+	+
Aesculin	-	-
Casein	+	+
Starch	-	-
Acid production from:		
Glucose	-	-
Lactose	-	-
Sucrose	-	-
Mannose	-	-
Arabinose	-	-
Inositol	-	-
Sorbitol	-	-

Fig. 1: Artificially infected fish (*A. testudineus*)

Pathogenicity test of selected *Flavobacterium* isolates: Experimental fish were found to be affected becoming moribund with appearance of typical lesion on the body surface as well as tail and fins (Fig. 1), exposed to the isolates F1, F2, F3 and F4. In the present study, experimental carp fish (*Puntius gonionotus*) exposed to any one of the isolates (F1, F2 F3, F4, F5 and F6) exhibited 100% mortality (Table 2). Thirty percent mortality was observed in experimental koi fish (*A. testudineus*) for the isolate F1, F2 and F4 whereas, 40% mortality was observed in experimental koi fish (*A. testudineus*) when exposed to the isolate F3. On the other hand, 100% mortality was observed in experimental koi fish (*A. testudineus*) when challenged with isolates F5 and F6. Similar bacteria were reisolated from all of the infected fish.

Table 2: Experimental infection with selected isolates

		Rate of infection (%)		
Isolates	Experimental fish	Lesion	Mortality	Re-isolation
F1	*A. testudineus*	40	30	+
	P. gonionotus	100	100	+
F2	*A. testudineus*	40	30	+
	P. gonionotus	100	100	+
F3	*A. testudineus*	50	40	+
	P. gonionotus	100	100	+
F4	*A. testudineus*	40	30	+
	P. gonionotus	100	100	+
F5	*A. testudineus*	100	100	+
	P. gonionotus	100	100	+
F6	*A. testudineus*	100	100	+
	P. gonionotus	100	100	+
Control	*A. testudineus*	-	-	-
	P. gonionotus	-	-	-

Table 3: *In vitro* antibiotic sensitivity pattern of the selected isolates to different antibiotics

	Response to different antibiotics with zone of inhibition (mm)						
Isolates	C	E	OT	S	SXT	CE	GM
F1	35	30	25	20	±10	R	R
F2	30	32	27	18	26	R	R
F3	31	28	24	20	21	R	R
F4	32	28	28	18	22	R	R
F5	30	27	28	17	R	R	R
F6	33	29	27	19	R	R	R

C : Chloramphenicol (30 μg $disc^{-1}$), E: Erythromycin (10 μg $disc^{-1}$), OT: Oxytetracycline (30 μg $disc^{-1}$), S: Streptomycin (10 μg $disc^{-1}$), SXT:Sulphamethaxazole (15 μg $disc^{-1}$), CE: Cefradine (30 μg $disc^{-1}$), GM: Gentamicin (10 μg $disc^{-1}$), R: Resistant, ±: Confusing zone

Drug sensitivity of the isolates: In drug sensitivity experiment, all of the bacterial isolates exhibited sensitivity to chloramphenicol (C), oxytetracycline (OT), erythromycin (E) and streptomycin (S) (Table 3). All isolate showed resistance to gentamicin (GM) and cefradine (CE). One isolate exhibited partial sensitivity while another two isolates showed resistance to sulphamethoxazole (SXT).

DISCUSSION

Tail and fin rot disease is a bacterial disease of freshwater fish. It is widely distributed in tropical as well as temperate countries. The disease is characterized by grayish white spots on the body, skin erosion and fin rot. Most species of fish are susceptible to this disease and it may cause large mortalities (Frerichs and Roberts, 1989; Noga, 2000). In Bangladesh, the infestation of tail and fin

rot type disease is assumed to be increased in different fish species in the recent years, but until this report, no systematic studies have been conducted to confirm the specific pathogen of the disease.

Flavobacterium columnare is recognized as the etiological agent of tail and fin rot disease. It was formerly called *Flexibacter columnaris*, but in 1996 it was transferred to the genus *Flavobacterium* (Decostere *et al*., 1997). The bacterium is Gram negative, long, thin rod that exhibits flexing movement and are able to form columns. In the present study, the bacterial isolates collected from eroded tail and fins of affected Indian major carp, catla (*Catla catla*) and climbing perch, koi (*A. testudineus*) were characterized following several biochemical tests. The results of the tests were compared with a reference Japanese strain EK-28. Characteristics of all of the isolates were similar to each other and did not differ with the reference strain EK-28. Based on the colony characteristics and biochemical properties the bacterial isolates were identified as *F. columnare*. Shamsudin and Plumb (1996) isolated *F. columnare* from channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), large mouth bass (*Micropterus salmonides*) and flathead minnows (*Pimephales promelos*) exhibiting clinical signs of disease. Nusbaum and Shotts (1981) isolated *Aeromonas hydrophila* complex, *A. salmonicida*, *F. columnaris* and *Pseudomonas* sp. which were commonly associated with the disease of fish. Chowdhury and Wakabayashi (1990) also isolated *F. columnare* from gold fish (*Carassius auratus*). Decostere *et al.* (1998) isolated four Gram negative 'flexing' filamentous bacterial strains from tropical fish which were identified as *F. columnare*. The above mentioned studies support our findings which was the first isolation from climbing perch (*A. testudineus*) of Bangladesh.

The experimental infection of six *F. columnare* isolates was performed by bath exposure of experimental fish at 3-5×10^8 CFU mL^{-1} bacterial suspension. These isolates successfully produced similar type of disease in the experimental fishes and confirmed as pathogen of the disease occurred in farmed fishes. However, the virulence of these isolates differed for the two fish species used as experimental fish. All of the isolates caused high rate of infection and mortality in carp fish (*Puntius gonionotus*). High mortality rate was also observed in experimental koi fish (*A. testudineus*) for isolates collected from carp fish, but moderate infection and mortality was observed in koi fish (*A. testudineus*) for isolates collected from koi fish. The reason behind the variation in virulence was not investigated. However, it was assumed that the pathogens isolated from carp fish were highly virulent for both fish species than that of the pathogens isolated from koi fish. Soltani *et al*. (1996) studied the susceptibility of freshwater and marine fish to infection by *F. columnaris* and *F. martimus* in laboratory experiments where *F. columnaris* produced a more severe disease in barramundi (*Lates calcarifer*) compared to goldfish (*Carassius auratus*). Decostere *et al.* (1999) conducted virulence test with four strains of *F. columnare* by intramuscular injection, where the strains exhibited variations in virulence pattern. These reports support our present findings.

Antibiotic sensitivity test of the isolates indicated that, they are sensitive to antibiotics chloramphenicol, oxytetracycline, erythromycin and streptomycin. All isolate showed resistance to gentamicin (GM) and cefradine (CE). One isolate exhibited partial sensitivity while another two isolates showed resistance to sulphamethoxazole (SXT). Bernardet (1989) reported the sensitivity pattern of nine *F. columnaris* isolates collected from diseased fish of France, which were sensitive to ampicillin, cefalotin, streptomycin, tetracycline, chloramphenicol, novobiocin, nalidixic acid and furans but resistant to gentamycin, neomycin, kanamycin, erythromycin, trimethoprim and actinomycin D and variable result was obtained for sulphonamides. The results correlate with the findings of the present study except for erythromycin. The results of the present study were also very much similar with the findings of Kubilay *et al*. (2008).

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