

Pathogenicity of *Aeromonas sobria* to Thai silver barb (*Barbodes gonionotus* Bleeker) and its sensitivity to some antibiotic agents

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Abstract

Five isolates of *Aeromonas sobria*, collected from the diseased fish were selected for detection the pathogenicity following water-born infection method on silver barbs (*Barbodes gonionotus*) at the selected exposure dose 2.5×10^8 CFU/ml which was standardized by preliminary test. In the experimental condition lesion and mortality were found in fishes. Among the isolate, Ass₁₇, Ass₁₉, Ass₃₁ and Ass₃₆ were successfully infected 20-60% fishes. Another isolate Ass₂₀ was found non-pathogenic. Drug sensitivity test was performed by six antibiotics viz. Oxytetracycline, Oxolinic acid, Chloramphenicol, Sulphamethozazole, Streptomycin, Erythromycin. All the isolates showed variable reaction patterns to antibiotics. Most of the isolates were found sensitive to Oxytetracycline (OT), Oxolinic acid (OA) and Chloramphenicol (C) but resistance to Erythromycin and Sulphamethoxazole (SXT). Isolate Ass₃₁ found resistant to Oxolinic acid.

Key words: *Aeromonas sobria*, Pathogenicity, Sensitivity, *B. gonionotus*

Introduction

Disease is one of the most constraining factor in aquaculture of Bangladesh. Both farmed and wild fishes have been found to be affected by various kinds of diseases (Rahman 1997). Common diseases of fresh water fishes are ulcers including Epizootic Ulcerative Syndrome (EUS), Septicaemic disease, tail and fin rot, bacterial gill rot, dropsy, various types of fungal, parasitic and protozoan disease (Chowdhury 1997). Bacteria, one of the major causative agents found to be associated with many diseased fish as primary causative agent and secondary invaders of ulcers and other lesions. Among all other bacteria, Aeromonads are the major pathogens with are widely distributed in farmed fish and water in Mymensingh region (Banu 1996).

Motile members of *Aeromonas* are ubiquitous in fresh water and known to cause haemorrhagic septicemia in both warm and cold water fishes (Wakabayashi *et al.* 1981). The principal motile species of *Aeromonas* are *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* (Plumb 1994). Many works on *Aeromonas hydrophila* have been done by

the scientists all over the world including Bangladesh and recognized as pathogen to fish (Banu 1996 and Rahman 1997). But *Aeromonas sobria* was not detected as pathogen until this work in Bangladesh. So, the present study was undertaken considering evaluation of pathogenicity of *Aeromonas sobria* to silver barb (*Barbodes gonionotus*).

Materials and methods

Isolation of bacterial isolates

Some Aeromonad isolates were collected from the ulcer affected different fish species of different water bodies during June to December'97. After collection the fish samples were immediately brought to the laboratory. A selective media Aeromonas Agar Base (Oxoid) supplemented with ampicillin. SR 136 E was used for isolation of Aeromonads isolates. It was found to be suitable for specific culture of Aeromonads (Choudhury and Inglis 1994b). Swabs were taken from the lesions kidney and liver of the the affected fish and stricked on the plate containing Aeromonas selective agar by sterile inoculating loop and subsequently pure culture were obtained on TSA media using conventional separation techniques.

Identification of Aeromonas sobria

Primary characterization were carried out upon Cowan and Steel's Manual for the identification of medical Bacteria edited by barrow and Felthaim (1993). For species level identification of *A. sobria* biochemical tests were performed according to barrow and Felthan (1993) and finally confirmed with the Bergesy's Manual for Systematic Bacteriology (Krieg and Holt 1984).

Pathogenicity test for A. sobria isolates

Five *Aeromonas sobria* isolates selected to investigate the pathogenic power to fish. The study was performed by water born infection method. The isolates are listed below :

<i>Aeromonas sobria</i> Isolates	Host fish	Location
ASS ₁₇	<i>Clarias gariepinus</i> (Fingerling)	Wet laboratory, FF, BAU
ASS ₁₉	<i>C. gariepinus</i>	Fisheries Bio. and Gen. Lab. FF, BAU
ASS ₂₀	<i>C. gariepinus</i>	Wet laboratory, FF, BAU
ASS ₃₁	<i>Barbodes gonionotus</i>	Faculty pond, FF, BAU
ASS ₃₆	<i>C. gariepinus</i>	Field laboratory, FF, BAU

FF= Faculty of Fisheries.

The experiment was conducted in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Healthy young silver barb collected from an experimental pond under Fisheries Faculty were selected for pathogenicity test. These were further acclimatized to aquarium conditions before use in the experiment. The fish was a exotic carp, *Barbodes gonionotus* weighing 10-15 gm.

Isolates were cultured on the TSA medium by spreading method and incubated at 25 °C for 24 h. The stock suspension of bacteria was prepared in sterile tap water with required amount of bacteria. In 30 l capacity aquarium, 15 l of bacterial suspension was prepared in the tap water with the stock suspension in such way that the bacterial density became 2.5×10^8 CFU/ml. This exposure dose of bacteria was selected by a preliminary experiment. Five fishes were exposed to bacterial suspension in aquarium under aerated condition at room temperature (water temperature ranged 24-27 °C). After 24 h of exposure 80% of the bacterial suspension was exchanged with tap water and from the following day 60% of water was exchanged at every 24 h of experimental period. The experimental period was 15 days.

Two replications were set up for the same isolate and the experiments were repeated for confirmation of infection, and thus in total 20 fish were used for single species against the individual bacterial isolate. For each set of experiment control fish were maintained in the same way. Aeration system was controlled by air pump. No feed was applied during the experimental period.

The appearance of lesion and mortality of experimental fish confirmed the infection. The pathogen was confirmed by reisolation of bacteria from the exposed fish. In the experimental condition radish zone appeared on the body surface, tail region, lower jaw and fin base of the fish. Then it turns in to dip hemorrhagic lesion and died. Some time fishes were died with out appearing any lesion on the body surface. For these case internal infection was found. Experimental fish with lesion or died fishes were collected with the sterile forcep and container. Then isolation was done by the previous way.

Antibiotic sensitivity test

Sensitivity test were performed by disc dispenser method according to Islam and Chowdhury (1997). The drug discs (Oxoid Ltd.) were Oxytetracycline (30 µg/disc), Chloramphenicol (30 µg/disc), Streptomycin (10 µg/disc) and Oxolinic acid (2 µg/disc) were used to observed the resistance pattern of *A. sobria* isolates.

Results

Among the five isolates of *Aeromonas sobria*, four were capable of causing lesion and mortality in experimental fish. But the appearance of lesion and mortality of silver barb varied from one isolate to another. Four isolates produced lesion in 20-50% fishes and mortality ranged 30-60% and reisolation was positive (Table 1).

Isolate Ass₁₇ showed haemorrhagic lesions and mortality in fishes. Lesions were observed at 6th day but the mortality was started at the 8th day of exposure. Appearance of lesion and mortality gradually increased day by day and 60% fishes were died where lesions appeared in 50% of fishes (Fig. 1).

When the fishes were exposed to the bacterial suspension with isolates Ass₁₉ the appearance of lesions were observed at 7th day and gradually increased with the increase of time. Mortality first recorded at the 10th day of exposure. Half of the fishes (50%) were died where 30% found to be affected by lesion (Fig. 2). In case of isolate Ass₃₁ 20% fishes were died where haemorrhages were observed also in 20% fishes (Fig. 3). Isolates Ass₃₆

also showed lesion and mortality in fish. Mortality began at the 7th day of exposure. Lesion appeared in 30% fishes and 50% mortality were observed (Fig. 4). However, no lesion and mortality was recorded with Ass₂₀.

Table 1. Pathogenicity test of some isolates of *Aeromonas sobria* against silver barb

<i>Aeromonas sobria</i> isolates	No. of fish challenged	With lesion		Fish Mortality		Reisolation
		No.	(%)	No.	(%)	
ASS ₁₇	20	10	50	12	60	+
ASS ₁₉	20	6	30	10	50	+
ASS ₂₀	20	0	0	0	0	Nd
ASS ₃₁	20	4	20	4	20	+
ASS ₃₆	20	6	30	10	50	+
Control	20	0	0	0	0	Nd

Nd = Not detected, + = Confirmed by reisolation

The data summarizes to repeated trials. In each 10 fishes were used for individual *Aeromonas sobria* isolates n = 20.

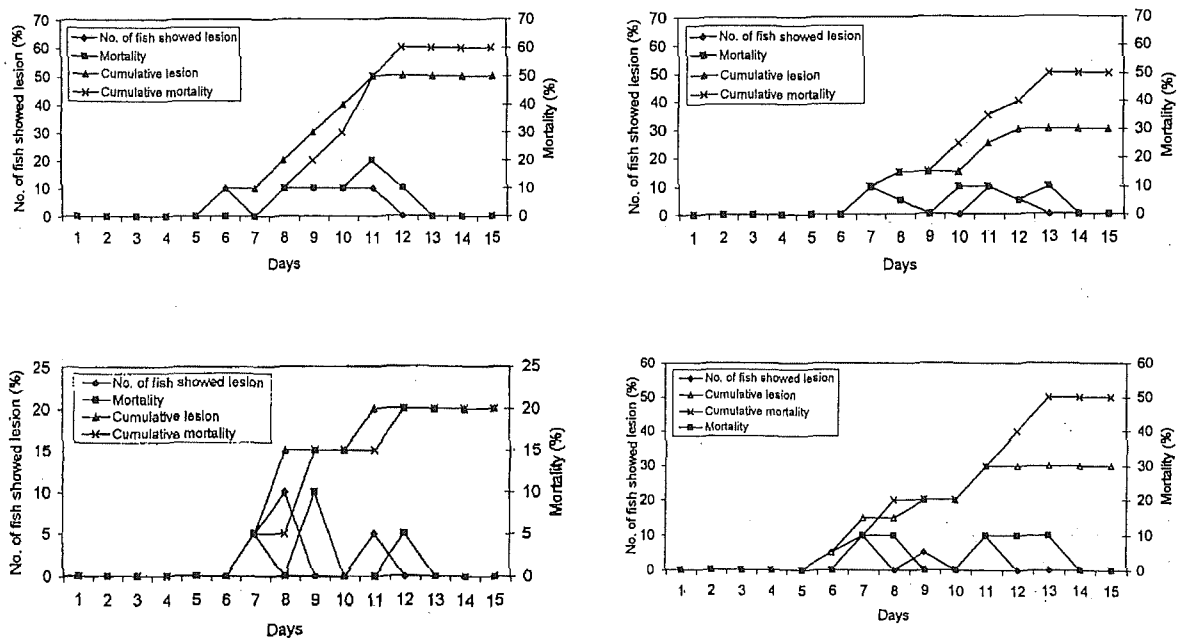


Fig. 1-4. Daily and cumulative percentage of lesion and mortality of silver barb exposed to different isolates.

The *Aeromonas sobria* isolates showed various sensitivity patterns to the six different antibiotics tested (Table 2). Among these Ass₁₇, Ass₁₉ and Ass₃₆ were highly sensitive to Oxytetracycline (OT), Oxolinic acid (OA) and Chloramphenicol (C) and resistant to Erythromycin (E) and Sulphamethoxazole (SXT).

Table 2. Reaction of *Aeromonas sobria* to some antibiotic agents

<i>Aeromonas sobria</i> Isolates	Response to different antibiotic agents with their zone of inhibition (mm)					
	OT	OA	S	C	E	SXT
ASS ₁₇	25	20	17	22	R	R
ASS ₁₉	20	15	R	15	R	R
ASS ₂₀	± 20	± 12	± 15	± 16	R	± 20
ASS ₃₁	15	R	10	17	10	11
ASS ₃₆	22	17	R	22	R	10

OT = Oxytetracycline OA = Oxolinic acid C= Chloramphenicol SXT = Sulphamethoxazole

S = Streptomycin E = Erythromycin

± = Confusing zone R = Resistant

Discussion

The experimental infection of five selected *Aeromonas sobria* isolates were performed by bath exposure of fish host to 2.5×10^8 CFU/ml bacterial suspension. Four isolates successfully caused infection and mortality in fishes. The results of the present study was correlated with a number of scientists in the world. Rogulska *et al.* (1994) performed artificial subepidermal infection with *Aeromonas sobria*, 10^7 bacterial cells in 0.2 ml of 85% PBS in 2 year old carp fish and found it as pathogen. Xu *et al.* (1985) stated that *Aeromonas sobria* is the causal bacterium of the caudal peduncle disease of grass carp. Panjagua *et al.* (1996) carried out an investigation on pathogenicity of *Aeromonas* strains and found 72.02% of *Aeromonas hydrophila*, 63% of *Aeromonas sobria* isolates were virulent for fish by intramuscular challenge. Sopinska *et al.* (1997) identified *Aeromonas sobria* as pathogen to carp. Gantam *et al.* (1992) carried out an experiment and stated the *Aeromonas sobria* was potentially pathogenic.

The results of drug sensitivity tests were variable to six antibiotics. Most of the isolates were highly sensitive to Oxytetracycline, Oxolinic acid and Chloramphenicol. On the other hand, they were resistance to Erythromycin and Sulphamethoxazole. Banu (1996) observed similar results for *Aeromonas* isolates. The results partially correlated with the observation of Chowdhury and Baqui (1997). Bornemann (1989) observed that strains of *Aeromonas sobria* are resistant to Ampicilin, 72% to Chlortetracycline, 12% to Kanamycin and 8% to Chloramphenicol.

In the present study, the selected *Aeromonas sobria* isolates under *Aeromonas* genus were capable of producing ulcerative disease.

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