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INVESTIGATION OF THE RESISTANCE OF *PSEUDOMONAS* SP. IN THE CARP *CATLA CATLA* TO SOME ANTIBACTERIAL AGENTS

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Abstract

Investigation was carried out to know the presence of *Pseudomonas* sp. in kidney and slime of the catla (*Catla catla*), in respective pond water and their sensitivity patterns against various antibacterial agents. A medium; Pseudomonas Agar Base supplemented with C. F. C. SR 103 (Oxoid) was used as a selective medium for the isolation of *Pseudomonas* sp. A number of antibacterial agents including some common antibiotics were tested against the isolated *Pseudomonas* sp. in the pond water; kidney and slime of fish catla (*Catla catla*). The number of *Pseudomonas* sp. varied from $2.5x10^2$ to $7.4x10^4$ CFU/ml, $2.9x10^2$ to $3.5x10^3$ CFU/g and $3.1x10^4$ to $5.9x10^2$ CFU/g in pond water, kidney and slime respectively, where as total load of bacteria varied $1.7x10^4$ to $2.9x10^6$ CFU/ml, $2.7x10^3$ to $3.8x10^4$ CFU/g and $3.1x10^6$ to $6.1x10^7$ CFU/g in pond water, kidney and slime. The antibacterial agents, used to know the resistance pattern, were Oxolinic acid (2µg/disc), potentiated Sulphonamides (25µg/disc), Chloramphenical (10µg/disc), Streptomycin (10µg/disc), Oxytetracycline (30µg/disc) and Erythromycin (10 µg/disc). The highest percentage of resistance was found in Oxytetracycline (30µg/disc) and Erythromycin (10 µg/disc).

Keywords: Resistane, Pseudomonas, Carp

Introduction

After China and India, Bangladesh is the third inland fish producing country in the world (Nuruzzaman, 1990). Recently fish disease, especially microbial fish disease outbreak has been one of the major barriers for the production of fish. Chowdhury (1993) in an investigation of fish farm by spot observation suspected that many farmed fishes were suffering from diseases like Septicemia, Columnaris etc. caused by bacterial pathogens. But research work on bacterial diseases in Bangladesh is in elementary stage. As a result, the farmers do not have the knowledge to take any action against diseases. Some times they use different chemicals and antibiotics to control these diseases without any recognized doses and thus making bacteria / pathogens resistant.

In aquaculture, species selection is an important factor influencing sustainable yield. There are many indigenous edable species in our country and among them catla (*Catla catla*) is very popular cultivable fish due to its faster growth and taste. This species is known to be more susceptible to various bacterial diseases. So effort has been made for the investigation of the resistance pattern of this fish.

Materials and Methods

Samples for study

Five carp ploy culture ponds were selected under Dhaka Fisheries Ltd. in Gazipur district for the sampling of pond water and the fish, catla (*Catla catla*). The sampled fishes were 50g to 100g in weight. The ponds under investigation were designated as P-1, P-2, P-3, P-4 and P-5. Samples were brought to the Fish Disease Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Culture media

A selective medium, Pseudomonas Agar Base supplemented with C – F - C SR 103

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(Oxoid) found suitable for the isolation of *Pseudomonas* sp. both from fish kidney as well as slime and pond water in preliminary test was used for the present study. However, for the other purpose such as total bacterial content, transfer of bacteria, Tryptone Soya Agar (TSA, Oxoid) was used. For antibiotic sensitivity test, Isosensitest Agar (Oxoid) was used.

Antibacterial agents

In the present study six antibacterial agents were used. They were oxolinic acid (2µ g/disc), potentiated sulphonamides (25µg/disc), chloramphenical (10µg/disc), streptomycin (10µg/disc), oxytetracycline (30µg/disc) and erythromycin (10 µg/disc). All the antibacterial agents were prepared by Oxoid.

Determination of bacterial content

The number of bacteria in pond water, kidney and slime of the fish were determined by standard plate count (spreading) method. Total number of bacteria was determined on TSA. *Pseudomonas* sp. were determined using Pseudomonas Agar Base.

Sensitivity test of Pseudomonas sp. *against the various antibacterial agents.*

From the Pseudomonas Agar Base medium a number of well-developed colonies were randomly selected to observe the sensitivity pattern of *Pseudomonas* sp. to various antibacterial agents, using separate vials. Suspensions were prepared for each of the isolates in physiological saline and 0.1 ml of the solution was transferred to the ISO-Sensi-Test agar plates and spread with the sterile "L" shaped glass rod. Six antibacterial discs were dispensed on the inoculated plates by placing the oxoid Unipath Disc Dispenser Mark-II and incubated at a temperature of 25^0 C for 24 hours. Sensitivity was recorded according to normal growth response. Resistance (R) was recognized when the growth was normal and when the clear zone was observed surrounding the disc, was recognized as a sensitive one by measuring the zone of inhibition in mm. However zone of confusing growth around the discs were noted.

Results

Total bacterial content in the sampled pond water, fish kidney, and fish slime varied in number from $1.7x10^4$ to $2.9x10^6$ CFU/ml, $2.7x10^3$ to $3.8x10^4$ CFU/g and $3.1x10^6$ to $6.1x10^7$ CFU/g respectively (Table-1). *Pseudomonas* sp. were present in water and fishes (slime and kidney) of all ponds with some variation in numbers (Table-1). The numbers of *Pseudomonas* sp. in the water of different ponds varied from $2.5x10^2$ to $7.4x10^4$ CFU/ml, and those in kidney and slime from to $2.9x10^2$ to $3.5x10^3$ CFU/g and to $3.1x10^4$ to $5.9x10^2$ CFU/g, respectively.

Sensitivity patterns of *Pseudomonas* sp. against various antibacterial agents were found highly diversified, either resistant or sensitive with different magnitude (Table. 2).

Table 1: Total bacterial content and total number of *Pseudomonas* sp. in the pond water, kidney and slime of sampled fish.

Fish Ponds	Water (CFU/ml)		Kidney (CFU/g)		Slime (CFU/g)	
	Total number of bacteria	Number of *Pseudomonas* sp.	Total number of bacteria	Number of *Pseudomonas* sp.	Total number of bacteria	Number of *Pseudomonas* sp.
P-1	$3.5x10^5$	$2.5x10^2$	$1.6x10^4$	$3.7x10^2$	$9.5x10^6$	$3.1x10^4$
P-2	$2.6x10^6$	$3.4x10^3$	$2.7x10^3$	$6.1x10^2$	$6.1x10^7$	$3.2x10^3$
P-3	$3.1x10^5$	$4.1x10^4$	$2.1x10^4$	$3.5x10^3$	$3.1x10^6$	$5.9x10^2$
P-4	$1.7x10^4$	$3.1x10^3$	$3.8x10^4$	$1.1x10^2$	$5.1x10^7$	$3.1x10^3$
P-5	$2.9x10^6$	$7.4x10^4$	$5.1x10^3$	$2.9x10^2$	$3.8x10^6$	$4.2x10^3$

P-1 Pond No. 1

P-2: Pond No. 2

P-3: Pond No. 3

P-4: Pond No. 4

P-5: Pond No. 5

Table 2: Sensitivity patterns of *Pseudomonas* sp. against various antibacterial agents.

Isolates from ponds	Response to various antibacterial agents (drug disc) with their zone of inhibition (mm).					
Water	OA	OT	SXT	S	C	E
P-1	R	R	+16.5	R	+12.4	R
P-2	+15	R	±17	±08	R	R
P-3	± 09	R	R	±11	±13	R
P-4	R	R	R	R	R	R
FP-5	R	R	+12	+14.3	R	±10
Kidney						
P-1	+16	R	±10.5	±08	R	+09
P-2	±18	R	+12	R	R	R
P-3	R	R	R	±17.2	+15	R
P-4	R	+10	R	R	R	R
P-5	+10	R	R	R	R	R
Slime						
P-1	R	R	R	R	+16	+14
P-2	R	+13	R	R	R	R
P-3	+11	R	+15.1	+15.1	R	R
P-4	+15.5	R	R	R	R	R
P-5	R	R	+19	+17.2	06	R

P-1 Pond No. 1	OA: Oxolonic acid	E :Erythromycin
P-2: Pond No. 2	OT: Oxytetracycline	R : Resistant
P-3: Pond No. 3	SXT: Potentiated sulphonamides	± :Confusing zone
P-4: Pond No. 4	S: Streptomycin	
P-5: Pond No. 5	C: Chloramphenical	

Discussion

The result of the present study revealed that the pond water, fish kidney and slime are carrying a large number of *Pseudomonas* sp. Though, their numbers are different in different ponds and different parts of same fish and same part of different fish, which is similar to the findings of Chowdhury *et al* (1994). It was observed that the numbers of *Pseudomonas* sp. varies in different pond water and different seasons. The result showed a distinct multiple drug resistant characters. In this regard, the result of the present investigation corresponds to the report of Chowdhury *et al* (1994). A high percentage of resistant *Pseudomonas* sp. isolates were detected against erythromycin and oxytetracycline. The present results are correlated with those of Dixon and Issvoran (1993), who carried out an experiment on the antibacterial drug resistance in *Aeromonas* sp. isolated from domestic gold fish and koi and found that more than 60% of *Aeromonas* sp. were resistant to tetracycline. They also observed multiple resistance pattern of *Aeromonas* sp. in their study.

Though, the reason behind the occurrence of multiple drug resistant patterns in the collected bacteria was not investigated, but it was suspected that as semi intensive culture system was practiced in the fish farms and various antibacterial agents were used to protect bacterial diseases of fish without any recognized doses, so the bacteria showed drug resistance in multiple patterns (Watanabe *et al.* 1971, Toranzo *et al.* 1984, Aoki *et al.*1985).

Although, the specific pathogen were not clearly identified but the isolates were preserved in the laboratory. The detection of the resistant *Pseudomonas* sp. isolates will help to identify *Pseudomonas* sp. pathogen in the near future. It has been known that pathogenic bacteria have usually strong ability to resist drug. So, it is suspected that the isolates which had multi resistant properties and which were recovered from disease affected fish might have every possibility to be the pathogen of the diseased fish. All the resistant isolates were preserved in the laboratory properly. Further studies are recommended to detect the pathogen specificity.

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