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Survival of antibiotic resistant *Pseudomonas* strains in different types of water

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Abstract

A study was conducted to investigate the survival of five *Pseudomonas* strains resistant to antibiotics in different types of water. The selected *Pseudomonas* strains were designated as strain P_1(CT-29), strain P_2(CT-25), strain P_3(CT-36), strain P_4(CT-20) and strain P_5(CT-27) which were only recovered from farmed fishes. Six types of water viz., distilled water, saline water, tap water, deionised water, pond water and river water were used. Among these experimental waters, river water was found to be the most suitable for long-term survival of these strains. Deionised water did not support survival of all these *Pseudomonas* strains. Pond water, tap water and distilled water were moderately suitable for strain P_1 and strain P_4. Saline water was also found to be highly suitable for long-term survival in case of the strain P_3 and moderately suitable for normal survival of strain P_2 and strain P_5.

Key words : *Pseudomonas*, Water

Introduction

Disease is the abnormal condition of body and mind which is expressed with certain symptoms. It affects the normal health condition and causes retardation of growth, abnormal metabolic activities and death. Fish disease is the interaction among host (fish), active pathogen and aquatic bio-ecological stress. Bacterial fish disease is one of the major limiting factors to fish culture and production in Bangladesh. In an investigation of the fish farms in Bangladesh, it was found that many farmed fishes suffered from disease seemed to be caused by bacterial pathogens (Chowdhury 1993) A number of investigation about the bacteria, *Pseudomonas* spp. in various organs of fish and water have been carried in the world and a few information is available in our country about this bacteria. *Pseudomonas* sp. is an important genus among the bacterial fish pathogen, for example *Pseudomonas fluorescens* is one of the major fish

pathogens which is widely distributed throughout the world in freshwater fish. Environmental water condition is undoubtedly important for persistence of bacterial pathogen. Any water which supports long-term survival may contribute to an easy out break of disease (Chowdhury and Wakabayashi 1990). Considering the importance, survival of five selected antibiotic resistant *Pseudomonas* strains in different types of water was investigated in the present study.

Materials and methods

Bacterial strain

Five bacterial strains were used for survival test. These were as follows:

1. Strain P_1(CT-29)
2. Strain P_2(CT-25)
3. Strain P_3(CT-36)
4. Strain P_4(CT-20)
5. Strain P_5(CT-27)

The source of collection and the resistance level of the selected *Pseudomonas* strains are shown in Table 1.

Table 1. Screened *Pseudomonas* strains used for survival test in different types of water

Strains	Source of collection			Resistant to the antibiotics
	Farm	Fish	Organ	
P_1(CT-29)	BCL	*Clarias* sp.	Liver	C,SXT,E & OA
P_2(CT-25)	DFL	*Catla catla*	Kidney	C,OT,SXT,E & OA
P_3(CT-36)	BCL	*Clarias* sp.	Liver	C,E,S & OA
P_4(CT-20)	DFL	*Catla catla*	Kidney	C,SXT,E,S & OA
P_5(CT-27)	JFF	*Labeo rohita*	Slime	C,SXT,E & OA

BCL	*Bangladesh Catfish Ltd.*	*DFL*	*Dhaka Fisheries Ltd. J*
JFF	*Jhalak Fish Farm*	*P*	*Pseudomonad isolates*
C	*Chloramphenicol (30 µg/disc)*	*OT*	*Oxytetracycline (30 µg/disc)*
SXT	*Sulphamethoxazole(25 µg/disc)*	*E*	*Erythromycin (10 µg/disc)*
S	*Streptomycin (10 µg/disc)*	*OA*	*Oxolinic acid (2 µg/disc)*

Experimental water

Six different types of water were used as experimental water for survival test of the five selected *Pseudomonas* strains. These were as follows:

1. Distilled water
2. Saline water
3. Tap water
4. Deionised water
5. Pond water (Isha Khan Lake, BAU campus)
6. River water (Brahmaputra river, near BAU campus)

The waters were used for the survival test after sterilization by autoclave. Parameters like dissolved oxygen and pH of the relevant water were recorded before and after autoclaving shown in Table 2.

Table 2. Parameters of experimental water recorded during the sampling period of survival test

Experimental water	Before autoclaving		After autoclaving	
	DO (mg/l)	pH	DO (mg/l)	pH
Distilled water	7.6	7.1	7.2	7.1
Saline water	8.2	7.1	8.3	7.2
Tap water	7.8	7.2	7.7	7.1
Deionised water	7.3	7.1	7.5	7.1
Pond water	8.5	7.4	8.6	7.2
River water	8.9	7.5	8.8	7.4

Procedures of survival test

Individual experimental Pseudomonad isolates were cultured on TSA plate. Then a sample of freshly cultured (18-24 h) inoculum weighing 20-30 mg (cfu) was taken into the sterile test tube containing 3-4 ml of distilled water to make a stock suspension. Then 0.5 ml of suspension was inoculated into 150 ml of sterile individual experimental water from the stock suspension and maintained at 25°C in incubator.

At each time of sampling 0.2 ml of incubated bacterial suspension was taken separately for individual and required ten-fold dilutions were made in sterile relevant experimental water. From required each dilution 0.1 ml of fluid was taken for inoculation on TSA plate and spreaded it by L-shaped sterile glass rod. Then the plates were placed at 25°C in the incubator for 24-36 hours to incubate. After 24-36 hours incubation, the number of colonies were counted by colony counter. Viable number of bacteria were determined at 0 day (immediately after incubation), 1 day, 3 day, 7 day and 10 day until completion of the experiment (Chowdhury and Wakabayashi, 1990). In each circulation duplicate plates were used and average total load of bacteria were counted.

Results

The results of survival test of individual strain are given below :

Survival of Pseudomonas strain P_1(CT-29)

The water of Brahmaputra river supported long-term survival of this strain (Fig. 1). Distilled water and pond water did support moderate survival of this strain. The survival of this strain was declined rapidly in deionised water, where its CFU could not be determined in the end of 3 day experiment. But a gradual decrease in number occurred in saline and tap water. The highest survival was observed in river water, where the CFU number was almost similar to that of the initial value after 10 days.

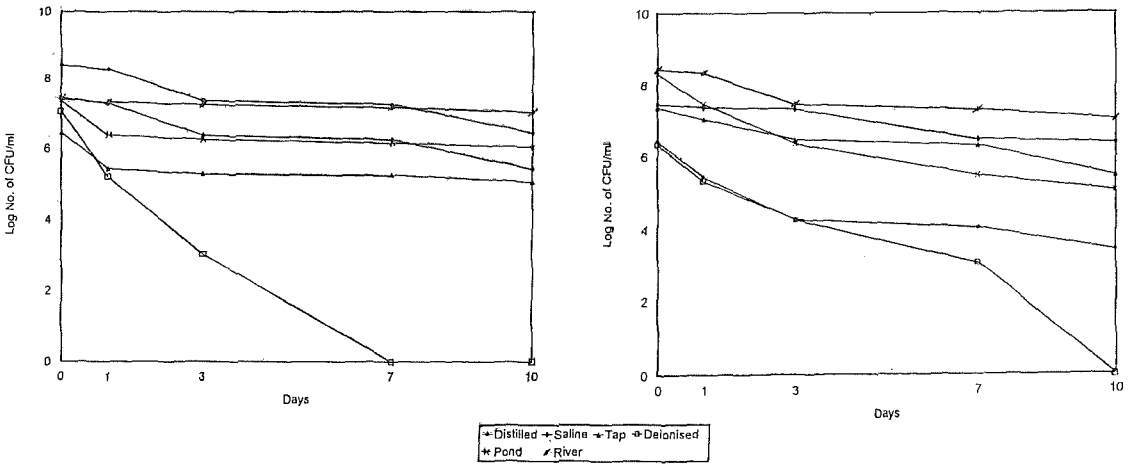

Fig. 1. Survival patterns of *Pseudomonas* strain P_1 (CT-29) in different types of water.

Fig. 2. Survival patterns of *Pseudomonas* strain P_2 (CT-25) in different types of water.

Survival of Pseudomonas strain P_2(CT-25)

The highest survival of this strain was found in river water where the CFU number after 10 days was almost similar to that of the initial value (Fig. 2). A moderate level of survival was observed in saline water with the CFU decreasing to about 10^{-1} of the initial value after 7 days. A gradual decrease of the initial number was observed in distilled water, tap water and pond water respectively. But a gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 7 days.

Survival of Pseudomonas strain P_3(CT-36)

The highest survival of this strain was found in saline water where the CFU number after 10 days was almost similar to that of the initial value and the water

supported long-term survival of this strain (Fig. 3). A moderate level of survival was observed in river water with the CFU decreasing to about 10^{-1} of the initial value after 7 days. A gradual decrease of the initial number was observed in distilled water, tap water and pond water respectively. A gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 3 days.

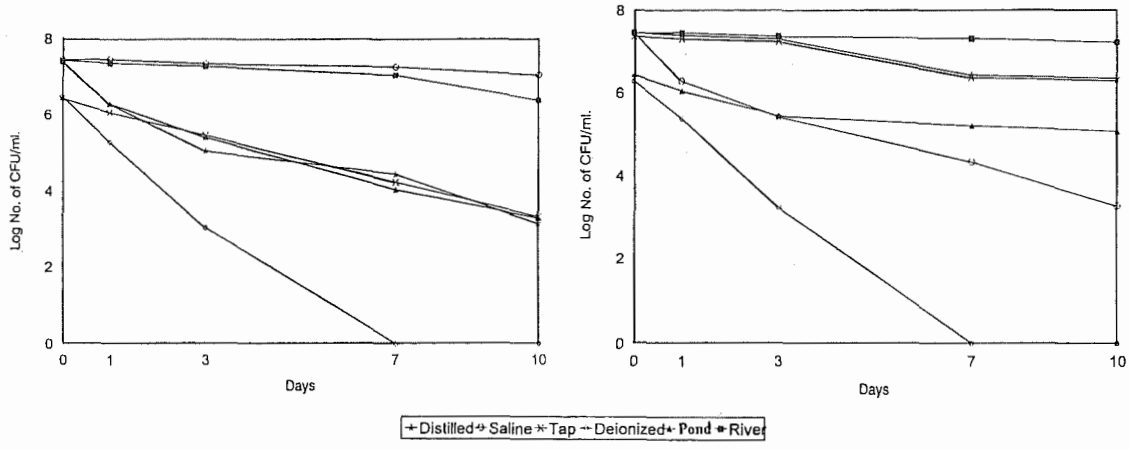

Fig. 3. Survival patterns of *Pseudomonas* strain P_3 (CT-36) in different types of water.

Fig. 4. Survival patterns of *Pseudomonas* strain P_{24} (CT-20) in different types of water.

Survival of Pseudomonas strain P_4(CT-20)

Survival of this strain was found to be the highest in river water where the bacterial number was almost the same as that of the initial value throughout the 10 days experimental period (Fig. 4). Distilled water and tap water also maintained a high survival of this strain, where the bacterial number decreased to less than 1/10 of the initial after 7 days. The bacteria gradually decreased in number in saline water. A moderate level of survival was observed in pond water with the CFU decreasing to about 1/10 of the initial value after 7 days. A gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 3 days.

Survival of Pseudomonas strain P_5(CT-27)

The highest survival of this strain was found in river water and saline water, where the bacterial number decreased to less than 1/10 of the initial after 7 days experimental period (Fig. 5). A moderate level of survival was observed in distilled water, tap water and pond water with the CFU decreasing to about 2/10

of the initial value after 7 days. A sharp decrease in the number of also occurred in deionised water where no bacteria could be recovered after 3 days.

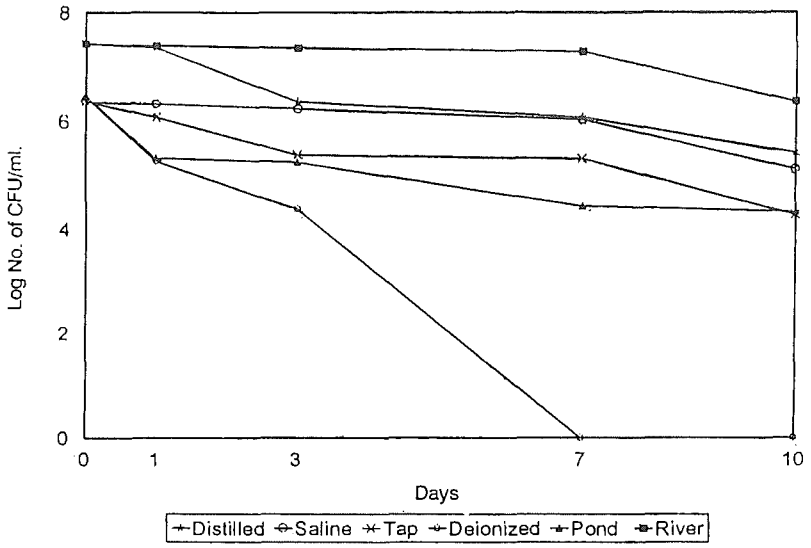

Fig. 5. Survival patterns of *Pseudomonas* strain P_5 9CT-27) in different types of water.

Discussion

Survival of five selected antibiotic resistant *Pseudomonas* strains in different types of water including distilled water, saline water, tap water, deionised water, pond water and river water was important work in the present study. Among the water tested, river water was found to be the most suitable for long-term survival of the five *Pseudomonas* strains followed by pond water. The reasons might be the high concentration of dissolved oxygen and favourable pH of these water bodies. Tap water and distilled water to be supported the survival of the strain P_1 and strain P_4 moderately. Saline water was found to be most suitable for long-term survival of the strain P_3 and moderately suitable for normal survival of strain P_2 and strain P_5. Deionised water was found not to support the survival of either of the strains.

Chowdhury and Wakabayashi (1990) reported that tap water was better than distilled water for the survival of *F. columnaris*. They mentioned that tap water might contain some trace elements which probably helped *F. columnaris* in its long-term survival. The results of the present study was related with the results of other scientists. Wakabayashi and Egusa (1972) demonstrated that high survival of *F. columnaris* in tap water. Muroga and Tatani (1982) reported that growth of *V. anguillarum* in 0.0% NaCl was negative but positive in 0.5-5% NaCl. In the present study, survival of *Pseudomonas* strain P_3 in saline (0.85% NaCl) water was found most suitable than other water. Deionised water may be lacking any such essential elements which was necessary for the survival of *Pseudomonas* strains, may have failed to produce helpful effect. Islam (1996)

found that river water, pond water and saline water were found to be the most suitable for long-term survival of *Pseudomonas* strains.

The present study provides useful information for the fish culturists on the survival of the *Pseudomonas* bacteria (fish pathogens) and with this knowledge, a fish culturist could possibly change the water in order to reduce the incidence of the bacteria. Further studies are necessary to know the pathogenicity of these *Pseudomonas* strains to various species of fish and to find out an appropriate control measures against the recovered pathogen.

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