

Survival of antibiotic resistant *Aeromonas* strains in different water conditions

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Abstract

Studies were conducted to find out the survival of three antibiotic resistant *Aeromonas* strains in different types of water. The selected *Aeromonas* strains were *A. hydrophila* (local), *A. sobria* (local) and *A. hydrophila* (Thai), which were only recovered from farmed fishes. Seven types of water were used. Among these experimental water, lake water, distilled water and fish farm pond water had supported the long time survival of *A. hydrophila*. In contrast, private fish farm pond water was the most favourable for *A. sobria*. Deionised water was found not to support the survival of any species but Masjid pond water and FRI pond water were found to be moderately suitable for all the species. However, the survival pattern of Thai strain of *A. hydrophila* was found to have similarity with the survival of the local strain of *A. hydrophila*.

Key words : *Aeromonas*, Antibiotic, Water

Introduction

Aeromonas spp. are very important among the major bacterial fish pathogens. Many fish diseases including ulcer disease, haemorrhagic septicaemia, etc. commonly found in freshwater fishes are caused by *A. hydrophila*. *Aeromonas* sp., *Pseudomonas* sp. and *Flexibacter columnaris* were initially suspected to have their involvement in the outbreak of these diseases (Chowdhury 1997). The *Aeromonas* spp. are widely distributed all over the world (Amos 1985). The conditions of aquatic environment is very important for the survival of these bacterial fish pathogens. Any types of environmental water which supports the long time survival may contribute to an easy outbreak of fish disease (Chowdhury and Wakabayashi 1990). The present study was undertaken to investigate the survival patterns of three *Aeromonas* strains resistant to antibiotics in seven types of waters.

Materials and methods

Aeromonas strains

Three *Aeromonas* strains were used for survival test in this study. These were as follows: 1. *Aeromonas hydrophila* (local), 2. *Aeromonas sobria* (local) and 3. *Aeromonas hydrophila* (Thai). The source of collection and the resistant level to antibiotics of the selected *Aeromonas* strains are shown in Table 1.

Table 1. Selected *Aeromonas* strains used for survival test in different types of water

Strains	Sources of collection			Resistant to the antibiotics
	Farm	Fish	Organ	
<i>A. hydrophila</i> (local)	JFF	<i>C. mrigala</i>	Kidney	E, S, OA
<i>A. sobria</i> (local)	TFF	<i>L. rohita</i>	Liver	E, SXT, C
<i>A. hydrophila</i> (Thai)	Thailand	<i>P. gonionotus</i>	Kidney	E, OA, C

JFF : Jhalak Fish Farm TFF : rishal Fish Farm E : Erythromycin (10mg/disc)
 OA : Oxolinic acid (2mg/disc) C : Chloramphenicol (30mg/disc)
 S : Streptomycin (10mg/disc) SXT : ulphamethoxazole (25mg/disc)

Experimental water

Seven different types of water were used as experimental water for the survival test of the three selected *Aeromonas* strains. These were as follows : 1) Distilled water, 2) Deionised water, 3) JFF pond water, 4) BAU Masjid pond water, 5) Ishakhan lake water, 6) FRI pond water and 7) FF pond water. The waters were used after sterilization by autoclave. Parameters like dissolved oxygen and pH of the relevant water were recorded before and after autoclaving as shown in Table 2.

Table 2. Physico-chemical parameters of the experimental water recorded during the sampling period of survival test

Types of water	Before autoclaving		After autoclaving	
	pH	DO (mg/l)	pH	DO (mg/l)
Distilled water	6.8	7.6	7.1	7.2
Deionised water	7.1	7.2	7.3	7.8
JFF pond water	7.6	8.1	7.8	7.8
Masjid pond water	7.4	8.6	7.4	7.7
Ishakhan lake water	7.4	8.3	7.4	8.1
FRI pond water	7.2	7.8	7.9	7.5
FF pond water	7.5	8.1	7.8	7.8

DO : dissolved oxygen
 JFF : Jhalak Fish Farm

FF : Faculty of Fisheries
 FRI : Fisheries Research Institute

Procedures of survival test

Individual experimental *Aeromonas* were cultured on Tryptone Soya Agar (TSA, Oxoid Ltd., UK) plate. Then a sample of freshly cultured (18 to 24 hours) inoculum weighing 20 to 30 mg was taken into the sterile test tube containing 3 to 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 dilution's were made in sterile relevant experimental water. From each dilution 0.1 ml was taken for incubation on TSA plate and was spread it by sterile L-shaped glass rod. Then the plates were placed at 25°C in the incubator for 24 to 36 hours to incubate. After incubation, the number of bacterial colonies were determined at 0 day (immediately after incubation), 1 day, 3 day, 5 day, 7 day and 10 day until completion of the experiment (Islam and Chowdhury 1997). In each circulation duplicate plates were used and bacterial load was calculated.

Results and discussion

Survival of A. hydrophila (local)

It was observed that Ishakhan lake water, FF pond water and distilled water had supported the long time survival of this strain for a longer period as no considerable variations in the total load of bacteria had been noticed in those experimental water during experimental period. Deionosed water did not support the survival of this strain. Water of JFF pond, FRI pond and Masjid pond water supported moderate survival of the strain.

Survival of A. sobria (local)

Among the seven types of experimental water, distilled water and JFF pond water were found to be more suitable to support the survival of this strain, as in both cases the total load of *A. sobria* did not show any significant variation during the experimental period. But sharp variation in total load from the initial day to the 10 day of the experimental period in case of deionised and Ishakhan lake water signifies their unsuitability for prolonged survival of this strain. FRI pond water, FF pond water and Masjid pond water were found to be moderately suitable to support the survival of this strain.

Survival of A. hydrophila (Thai)

It was observed that Ishakhan lake water, FF pond water and distilled water had supported the long time survival of this strain. Deionised water did not support the survival of this strain. JFF pond water and FRI pond water supported moderate survival of the strain as in both cases slight variations in total load had been observed.

The results of the present study showed that Masjid pond water and Ishakhan lake water supported prolonged survival of local strains of *A. hydrophila*. The reasons might be the high concentration of dissolved oxygen and favourable pH of the water in those water bodies. JFF pond water supported good survival of *A. sobria*. Deionised water did not support the survival of either of the strains and distilled water supported the survival of *A. hydrophila* in varying degrees which agree with the findings of Chowdhury and Wakabayashi (1990). Chowdhury (1997) investigated the survival of some selected isolates of *Aeromonas* and *Pseudomonas* in different water conditions. The results of the present study were similar to the results of Chowdhury's works.

Comparison of distilled water with pond water in the survival of *Aeromonas* strains, revealed that pond water was better than distilled water, though both were freshwater. The source of pond water is considered to be similar to the water of the aquaculture facilities. Pond water may contain some trace elements and rich in nutrients which probably helped *Aeromonas* strains in its long time survival. Deionised water lacking any such nutrients may have failed to produced this effect. River water, pond water and saline water were found to be the most suitable for long term survival of *Pseudomonas* strains (Islam and Chowdhury, 1997). 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 0.5-5.0% NaCl.

The present study provides information about the survival of *Aeromonas* strains recovered from the aquaculture facilities in Bangladesh and a fish farmer/scientist could utilise this knowledge in order to reduce the incidence or density of the bacteria from his farmed pond. Further studies are necessary to know the pathogenicity of these *Aeromonas* strains and to find out an appropriate control measures against these strains.

References

- Amos, K. H., 1985. Procedures for the detection and identification of certain fish pathogens. 3rd ed. Fish Health Section, *Am. Fish. Soc. Coravallis, Oregon*. 114 pp.
- Chowdhury, M. B. R., 1997. Bacterial involvement in fish disease in Bangladesh. International Symposium on Diseases in Marine Aquaculture, Japanese Society of Fish Pathology, 3-6 October, 1997, Hiroshima, Japan.
- Chowdhury, M. B. R. and H. Wakabayashi, 1990. Survival of four major bacterial fish pathogens in different types of experimental water. *Bangladesh J. Microbiol.*, **7(1)** : 47-54.
- Islam, M. S. and M. B. R. Chowdhury, 1997. Survival of antibiotic resistant *Pseudomonas* strains in different types of water. *Bangladesh J. Fish. Res.*, **1(2)** : 39-45.
- Muroga, K. and M. Tatani, 1982. Isolation of *Vibrio anguillarum* from juvenile red sea bream (*Pargus major*). *Fish Pathol.*, **16** : 211-214.
- Wakabayashi, H. and S. Egusa, 1972. Characteristics of a *Pseudomonas* sp. from an epizootic of pond cultured eels (*Anguilla japonica*). *Bull. Jap. Soc. Sci. Fish.*, **38** : 577-587.

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