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Recovery and Antibiogram Studies of *Aeromonas hydrophila* and *Pseudomonas fluorescens* from Naturally and Experimentally Infected Tilapia Fishes

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Abstract: Seventy five Tilapia fishes (*Oreochromis niloticus*) showing signs of septicemia and skin lesions were collected during mass mortality in fish farms during the summer season to investigate the etiology of the disease. Bacteriological isolates from these fishes were mainly *Pseudomonas fluorescens* and *Aeromonas hydrophila.* Experimental infection of fishes with the naturally isolated pseudomonas and aeromonas produced the same disease and aeromonas and pseudomonas were isolated. Biochemical analysis of the naturally infected fish aquarium water showed a significant increase in its physicochemical properties compared to the controlled aquarium water. An antibiogram investigation of the recovered isolates showed that oxytetracycline and furaltadone were the most effective antibacterial agents. It is suggested that improving water quality may improve fish health condition.

Key words: *A. hydrophila, P. fluorescens,* tilapia fish

Introduction

Disease outbreaks due to *Aeromonas* spp., especially in the case of motile Aeromonas septicaemia, where the *Aeromonas hydrophila* is the etiological agent, cause the most economic losses in both intensively and extensively cultured fish as well as in wild fish (Austin and Austin, 1987; Post 1987; Noga, 1996). Together with motile aeromonad bacteria, there are *Pseudomonas* species such as *Pseudomonas fluorescens, P. anguilliseptica* which are associated with disease outbreaks in aquiculture, (Austin and Austin, 1987; El-Attar and Moustafa, 1996). Moreover, certain strains of Aeromonas species have been associated with a large spectrum of human infections, such as gastroenteritis, pneumonia, peritonitis, endocarditis, meningitis, septicaemia, urinary tract infections and wound infections (Janda and Duffey, 1988; Sacho *et al.,* 1990; Krovacek *et al.,* 1993).

The objective of this investigation was to characterise Aeromonas and Pseudomonas recovered during mass mortality among intensively cultured Nile Tilapia (*Dreochromis niloticus*) a newly developed industry in Saudi Arabia, as well as possibility of treatment for infected fish with antimicrobial agents.

Materials and Methods

Natural infection: Seventy five male and female Nile Tilapia fish (*Dreochromis niloticus*) with average body weight of 100 ± 20 g showing septicemic and skin lesions were collected during mass mortality in fish farm located in Al-Ahsa province, Saudi Arabia in summer season. The fish were transported to the laboratory in large double plastic with contained water from the same source in the farm and were supplied with oxygen through battery aerators. Then the fishes were finally transferred into glass aquaria ($10 \times 45 \times 45$ cm each) containing dechlorinated tap water. They were provided with electric aerators and the water temperature was adjusted at 25°C. These fishes were subjected to clinical and postmortem examinations using the methods described elsewhere (Austin and Austin, 1993).

Isolation and identification of bacterial strains: Colonies growth criteria, and motility characteristics of bacteria were carried out (Cruickshank *et al.,* 1982). Briefly, samples were taken aseptically from kidneys, liver, spleen of diseased fish and cultivated on different selective media (Aeromonas, agar base, M 833) with rehydrated ampicillin supplement (SR 136, oxoid, MacConkey agar, brain heart infusion agar, nutrient agar). The plates were incubated at 30°C for 24 hours. Samples were also inoculated into sheep blood agar and incubated at 30°C for 24 hours. Suspected pure colonies were subjected to Gram's stain and oxidase test. For biochemical identification, a single colonies from the resultant growth were picked up and resuspended into peptone water and biochemical identification was carried out using specific AP120E (Bio-Merieux, 1984).

Experimental infection: A total number of 30 male and female Nile Tilapia fishes with a mean body weight of 65 ± 10 g were obtained from fish farm at Al-Ahsa province. The fishes were apparently healthy, free from any visible systemic or skin lesions and with normal behavioural reflexes. The fishes were transported to the laboratory and assigned randomly to 3 groups and placed in circular fibreglass tanks each containing 2200 L of water. The fishes were maintained under flow-through condition of natural daylight. Water temperature was maintained at 26 ± 1°C and pH of 7.3 ± 0.04. The fishes were allowed to acclimatise for 2 weeks prior experimental infection with isolated fish pathogens of *A. hydrophila* and *P. fluorescens.* Experimental fish were fed commercial non-medicated fish pellet (Grow Big Floater, SF Services In. USA) at a rate of 20% body weight once daily. Group 1 and 2 fishes were inoculated intramuscularly (i.m) under the dorsal fin, using 21 gauge needle, with 0.1 ml of 24 hour broth Culture containing approximately 2.4×10^7 CFU/ml of isolated fish pathogens of *A. hydrophila* and *P. fluorescens,* respectively. Control fishes (group 3) were inoculated i.rn with 0.1 ml of sterile broth. Fishes were observed for 7 days. Reisolation of bacteria was attempted. In addition, clinical signs, postmortem lesions and mortality rate were recorded.

Table 1: Results of the biochemical identification of *A. hydrophila* and *P. fluorescence*

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A. hydrophila	v	v	+	+	+	v	-	v	-	-	-	+	v	+	+	-	-	-	-	+	-
P. fluorescence	-	+	+	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-

V = Variable, + = Positive, - = Negative

1-Arnygdlin 2-Arabinose 3-Oxidase Production 4-β-glactosidase 5-Argininedihydrolase 6-Lysine decarboxylase 7.0mithine decarbox viase 8-Citrate utilization 9-H2S production 10-Urease prduction 11-Tryptophane deaminase 12-Indole production 13-Vogesproskauser 14-Gelatinehydrolysis 15-Gulcose 16-Mannitol 17-Inositol 18-Sorbitol 19-Rhamnose 20-Sucrose 21-Melibiose

Table 2: Expermental infection of A. Hydrophila (Group 1) and *P. fluorescens* (Group 2) into healthy *O. nilaticus* fish

Group (n)	Dose Per fish	Morbidity rate %	Mortality rate %
A. hydrophila (10) infected fish	0.1 ml (2 4×10^7 CFU/ml)	60	60
(*P. flouroescens*)	0.1 m1(2.4×10^7 CFU/ml)	30	50
Control fish (10)	0.1 ml (Sterile broth)	0	

Table 3: Physicochemical properties (Mean ± SD) of water collected either from naturally infected fish aquarium (A) or experimentally controlled aquarium (B)

Parameters	A	B
Temperature (°C)	28.0 ± 1.0	26.0 ± 1.00
pH	9.2 ± 0.05	7.3 ± 0.04
Total alkalinity (mg/L)	201.0 ± 11.0	130.0 ± 6.00
Chlorosity (mg/L)	37.0 ± 3.0	20.0 ± 2.00
Total hardness (mg/L)	40.0 ± 3.0	20.2 ± 3.00
Nitrate (mg/L)	1.3 ± 0.2	0.23 ± 0.041
Phosphate (mg/L)	1.2 ± 0.2	0.11 ± 0.02

Sensitivity test: The antibiograms of the recovered pathogens were done using the disc diffusion method (Bauer *et al.,* 1966). The interpretation of zones of inhibition were estimated and recorded (Bio-Merieux, 1984).

Water quality parameters: Water samples were taken from the centre of the aquarium (20 cm in depth). Water temperature was recorded using a type T flat thermistor probe attached to a digital themocouple thermometer (Cole-Palmer Instrument, Chicago, IL, USA), pH of water was measured with a Corning pH meter 125 and an Orion Ross Electrode (American Scientific products, McGraw Park, IL, USA). Total alkalinity, chlorosity, total hardness, nitrate and phosphates were measured using commercially available kits. (Cobas Miras Roche Daignostic Systems Inc. Monclair, USA).

Treatment: Medicated fish feed containing oxytetracycline (Tetraplex, Hand/pH, Middx, UK) at a daily dose of 55 mg/kg body weight was fed for ten days. Control fish were fed the non-medicated feed throughout the experiment (Post, 1987).

Results and Discussion

The examination of the naturally infected *O. niloticus* revealed skin ulceration with narrow hyperemic zones and darkness in colour of the skin.

Others showed progressive erosions in tail and fins, exophthalmia and haemorrhagic patches all over the body. Postmortem lesions showed congestion and enlargement of internal organs. These signs were suggestive of aeromonad and pseudomonad disease (Shotts and Bullock, 1975; Post, 1987; Noga, 1996; El-Attar and Moustafa, 1996).

Microscopic examination of Gram's stained smear, morphological characteristic of colonies and biochemical test identified the pathogenic bacterial strains which were isolated from the Septicemic fish as *A. hydrophila* (Table 1) and *P. fluorescens* (Ahne *et al.,* 1982; Austin and Austin, 1987; Post, 1987; Noga, 1996).

The experimental infection of fish showed the same clinical signs observed in natural infection, indicating the septicemic bacterial disease. The infected bacteria were reisolated from freshly dead and clinically diseased fish (Table 2) and proved to be *A. hydrophila* and *P. fluorescens.* Although both bacteria produced disease of similar lesions when inoculated separately into experimental fish, but the morbidity and mortality of aeromonad was greater than the pseudomonad disease. Also the aeromonas group has the ability to prod uce different potential virulent determinants such as haentolysin cytotoxin, enterotoxin and protease. Such substances were the causative agent of fever gastroentritis, colic and diarrhoea in humans (Goodwin *et al.,* 1983; Rosner, 1964; Beuchat, 1991; Hudson and De Lacy, 1991; Kuhn *et al*., 1997).

Chemical analysis of water samples collected from intensive fish farm revealed significant ($p<0.01$) increase in its physiochemcial properties compared to water collected from experimental tanks (Table 3). Most epizooties of aeromonad and pseudomonad disease are stress related. Indeed the increase in the physicochemical characteristics of water from intensive fish farm was conducive to invasion of opportunistic bacteria, Diseases produced by *Aeromonas* and *Pseudomonas* species have been detected in association with environmental factors Such as water temperature, season of year and water quality parameters (Snieszko, 1974; Whipple and Rohovec, 1994; Noga, 1996).

The drug sensitivity test revealed that *A. hydrophila* was highly sensitive to oxytetracycline and furaltadone. *P. fluorescens* was highly sensitive to oxytetracycline and neomycin (Table 1) as shown by the size of the zone of inhibition. Treatment of experimentally infected fish with oxytetracycline resulted in complete recovery of the fishes from the disease, which is similar to previous reports (Fernandez *et al*., 1990; Emad, 1992). The difference in drug susceptibility might be due to prevalence of different strains of organism, indiscriminate usage of antimicrobial agents in feed or drinking water, or their usage at low therapeutic level. Furthermore, resistance of bacteria to other antibiotics could be a reflection of misuse of antibiotics, since easy access to various antibiotics is the case. It was suggested that the effectiveness of any antibiotic decreases in the absence of a rational utilisation program (Bulger *et al.,* 1966).

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