



Original Research Article

Studies on antibiotic susceptibility of *Aeromonas hydrophila* Isolated from gold fish (*Carassius auratus*)

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A B S T R A C T

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The aim of this study was to describe a case of skin lesions with haemorrhage in gold fish (*carassius auratus*) hatchery farm and to determine effective antibiotic treatments. In the macroscopic examinations of 15 gold fish with haemorrhagic skin lesions, brown or red spotted skin of a varying degree were found, along their bodies. No lesions were observed in the internal organs. Pure cultures of *Aeromonas hydrophila* were isolated from the skin, kidney, heart, and liver of the gold fish. All of the isolates were found to be susceptible to neomycin, streptomycin and resistant to Ampicillin, Amphotericin, Bacitracin, cephalixin and moderately sensitive to Erythromycin, Kanamycin. Treatment of *A. hydrophila* infection can be carried out by applying neomycin or streptomycin for 3days at 10 g/ton of pool water.

Introduction

Fish diseases are among the most important problems and challenges confronting fish culturing. Fish diseases do not occur as a single caused event, but are the end result of interactions of the disease, fish and environment.

Aeromonas hydrophila, a Gram negative motile rod, a member of the family vibrionaceae, has been widely studied and regarded as the most important bacteria for

causing “aeromonosis, or harmorrhagic septicaemia or motile aeromonas septicemia” in fish (Arlgka *et al.*, 1995; Rhaman *et al.*, 2001) and other aquatic animals such as frog (Hyys *et al.*, 2003) Prawn (Vivekanadhan *et al.*, 2002), carp (Nielsen *et al.*, 2001) and mussel (Maki *et al.*, 1998).

Aeromonas hydrophila is an opportunistic pathogen of a wide variety of hosts

(Huisinga *et al.*, 2003). *Aeromonas hydrophila* infection causes a serious damage in pond and aquarium culture. The pathogenesis and histopathology of red-sore disease has been extensively studied in common carp (*Cyprinus carpio*) (Vivas *et al.*, 2004). *A. hydrophila* has been considered to be responsible for red-fin diseases in cultured eel (*Angilla japonica*) (Hoshina, 1962) red disease in common carp (Gaines, 1978) red sore disease in largemouth bass (*Micropterus Salmonides*) (Huisinga *et al.*, 2003). Many bacteria isolated from fish with hemorrhagic septicemias in fish were often misidentified, it is now recognized that certain isolations of bacteria ascribed to the genera *Pseudomonas*, *Proteus*, *Bacillus*, *Aerobacter* and *Achromobacter* actually belonged to the genus *Aeromonas*.

It is believed that *Aeromonas hydrophila* contributes to the pathogenesis of the lesions. Histopathological changes in diseased animals provide a methodological platform to determine the causes of their mortality (Iwalokun *et al.*, 2001) For example, histopathological changes in *Aeromonas hydrophila* infected cat fish, *Clarias batrachus* and *Salmo gairdneri* have been documented (Anglea 1990, Candan 1990). The liver as the centre of metabolism is also involved in breaking down toxic substances. As a result, hepatic cells are subjected to more damage than cells of other organs.

Aeromonas hydrophila out breaks have a major impact in aquaculture (Austin and Austin, 1999) and such out breaks can be controlled either by prophylactic measures, like vaccination or by antibiotic treatment. Most of the antibiotics used to control fish diseases today are of microbial origin. In medicine, problem with pathogenic microbes becoming

increasingly resistant against most commonly used antibiotics and chemicals is being experienced (Leugn and Foster, 1996). Then some derivatives of the antibiotics circumvent or even prevent rapid mutation of the pathogens into resistant forms (Hughes, 2003).

The aim of this care report is to describe a care of skin lesions with haemorrhaging in gold fish (*Carrasius auratus*) hatchery farm in Nagercoil of Kanyakumari District, Tamil Nadu to determine an effective antibiotic treatment.

Materials and Methods

Environmental conditions at the fish hatchery farm

Because of haemorrhages with dermal ulcers along their bodies, 15 gold fish were selected for the investigations. The disease history was obtained from their owner. At the farm, there are 5% mortality and 25% morbidity was observed.

Sampling and microbiological examination

The samples of the liver, gill, kidney, heart, and skin of each of fish were collected. The samples were inoculated in 5% sheep blood agar plates (Hi media) MacConkey agar (Hi media) and *Aeromonas* isolation agar (Hi media) plates and then incubated at 25°C and 37°C for 3-4 days under aerobic conditions. After incubation, the pure haemolytic yellow colonies on blood agar, green nucleated colonies on *Aeromonas* isolation agar were isolated from skin and internal organs of all the gold fish. The bacteria were identified as *A. hydrophila* on the basis of colony morphology, Gram-staining, and biochemical characteristics

(Brener *et al.*, 2005, Koneman *et al.*, 1992). Wet mounts of skin, fin, and gill smears were also examined microscopically as well as a macroscopic examination for parasites.

Antibiotic susceptibility test

The susceptibility of the isolates to the following antibiotics: ampicillin (10 µg, Hi media), streptomycin (10 µg, Hi media), amphotericin (10 µg, Hi media) Bacitracin (10 µg, Hi media) cephalixin (10 µg, Hi media) Neomycin (10 µg, Hi media), Erythromycin (10 µg, Hi media) and Kanamycin (10 µg, Hi media) were tested using disc diffusion method.

Result and Discussion

In the macroscopic examinations of the gold fish, brown or red spotted skin lesions of a varying degree were found along their bodies (fig 1). These lesions were mostly scattered over the abdomen, operculum, head, fins, and gills. No lesions were observed in the visceral organs. Gram-negative bacilli were seen in gram-stained smears, taken from the skin, liver, and kidney. No parasites in the skin, fin, gills, and internal organs were found. *Aeromonas hydrophila* was isolated from the liver, gill, kidney, heart, and skin of each fish. A biochemical characteristic of *A. hydrophila* was given in table 1 and the growth of *A. hydrophila* on specific media was shown in figure 2. *A. hydrophila* isolate were found to be susceptible to neomycin, streptomycin and resistant to Ampicilin, Amphotericin, Bacitracin, cephalixin and moderately sensitive to Erythromycin and Kanamycin (Table 2 and figure 3). In the treatment of *A. hydrophila* infection, neomycin and streptomycin can be used for treatment

based on results from an antibiotic susceptibility test. After the fish had been kept in saltwater for 30 minutes, they were removed and placed in water either neomycin or streptomycin. The antibiotic can be used for 3 day in pool water at 10 g/ton for complete curing.

As it was mentioned earlier, *A. hydrophila* causes a disease known as haemorrhagic septicaemia or ulcer disease in fish, and belongs to the most common bacteria present in aquatic environments throughout the world. The bacterium is naturally found in the intestinal tract of the fish, and does not cause the disease under natural conditions (Swann and White 1989). The disease caused by *A. hydrophila* is one of the major disease problems for farmed fishes (Guz and Kozinska, 2004). Outbreaks of the disease are usually caused by stress and changes in environmental conditions. Overcrowding, handling, transportation, poor water quality, a sudden change of temperature, low dissolved oxygen, and high CO₂, nitrite, and ammonia levels are the most common predisposing factors associated with this disease (Cipriano, 2001; Yildiz *et al.*, 2005). The parasites, such as *Lernaea*, *argulus*, and *A. salmonicida* may also be a cause of ulcerative lesions on the skins of gold fish (Laster and Roubal, 1999). But, in this study, at the level of macroscopic and microscopic examinations, no parasites were observed. Also, it is considered that the disease of the fish may be related to *A. hydrophila*, as the same bacteria in a pure culture were isolated from skin lesions and internal organs. It has been reported by several researchers that *A. hydrophila* causes haemorrhagic skin lesions in cultured and fresh water fish (Radu *et al.*, 1999). Similarly, some researchers reported that most cultured and

Table.1 Biochemical identification of *Aeromonas* sp.

| Sl. No | Biochemical character | Result |
|--------|---|---------------|
| 1. | Gram stain | Gram negative |
| 2. | Motility | Motile |
| 3. | Indole production | + |
| 4. | Methyl red | + |
| 5. | Voges proskauer | + |
| 6. | Simon's citrate | + |
| 7. | H ₂ S Production | + |
| 8. | Gelatin hydrolysis | + |
| 9. | CHO fermentation (Glucose, lactose, xylose) | + |
| 10. | Oxidase | + |
| 11. | Catalase | + |
| 12. | Arginine dihydrolase | + |
| 13. | Ornithine decarboxylase | - |
| 14. | Crease | - |
| 15. | Phenyl alanine deaminase | - |
| 16. | DNAase | + |

+ - positive, -- - negative

Table.2 Antibiotic test for *Aeromonas hydrophila*

| Serial No | Antibiotics | Nature |
|-----------|--------------|----------------------|
| 1. | Ampicilin | Resistant |
| 2. | Amphotericin | Resistant |
| 3. | Bacitracin | Resistant |
| 4. | Cephalexin | Resistant |
| 5. | Neomycin | Sensitive |
| 6. | Erythromycin | Moderately sensitive |
| 7. | Kanamycin | Moderately sensitive |
| 8. | Streptomycin | Sensitive |

Fig.1 Showing symptoms of haemorrhagic ulcerative diseases



Fig.2 Isolation of *A. hydrophila* in agar base medium



Fig.3 Antibiotic susceptibility test for *A. hydrophila*



fresh water fish, such as eel, carp, catfish, rainbow trout, ayu, tilapia, were susceptible to *A. hydrophila* infection (Aoki 1999 and Kozinska *et al.*, 2002). *A. hydrophila* isolated from fish, displays a high degree of resistance to antibiotics (Aoki 1999; Guz and Kozinska 2004; Radu *et al.*,1997). Consequently, we performed the antibiotic susceptibility test, in order to determine the susceptibility of *A. hydrophila* to different antibiotics that are used in veterinary medicine. At present, the most widely used method of controlling *A. hydrophila* infection in cultured fish is the use of antimicrobial drugs (Guz and Kozinska 2004; Son *et al.*, 1997). The widespread antibiotic use is associated with an increased antibiotic resistance in aquatic bacteria. Thus, antimicrobial susceptibility tests are important for an effective treatment. From this study we conclude that, this case report has exhibited *A. hydrophila* may cause haemorrhages with dermal ulcers in goldfish. Uncontrolled and extensive use of antimicrobial agents may cause the frequent occurrence of multiple antimicrobial resistances. Therefore, an antimicrobial susceptibility test in *A. hydrophila* infection, as well as in other bacterial infections, has to be certainly made in cultured fish hatcheries. To prevent the infection water should be regularly changed and overcrowding and stress in the fish population should be avoided.

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