

SOME CHARACTERISTICS OF *AEROMONAS HYDROPHILA* AND *VIBRIO* SPECIES ISOLATED FROM BACTERIAL DISEASE OUTBREAKS IN ORNAMENTAL FISH CULTURE IN SRI LANKA

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Abstract: Bacterial fish disease outbreaks of freshwater ornamental fish hatcheries and farms, reported during the period from January, 1993 to February, 1994 in Sri Lanka were used in the study. Twelve fish species belonging to 11 genera were involved. Gross external and internal lesions were recorded. Samples were plated on selective (*Aeromonas* agar, *Pseudomonas* agar and thiosulfate citrate bile sucrose agar -TCBS) media and non-selective (blood agar and nutrient agar) media. Both API 20E and API GP test strips and conventional identification procedures were followed for characterization of the isolates. Following identification, 11 selected antibiotics were tested for their effectiveness against all the isolates using disk diffusion method. *Aeromonas hydrophila* was involved in 78.26% of the total (23) disease outbreaks while *Vibrio* sp. was involved in the rest. Eroding fins, hemorrhagic lesions on the skin and at the base of tail fin, sloughing scales, hemorrhagic intestinal wall and other visceral organs were among the lesions observed due to *A. hydrophila* infection. Lesions observed due to vibriosis were, erratic swimming, hemorrhagic patches on skin and on areas around anus, base of pelvic and tail fins, hemorrhagic peritoneum and visceral organs, fluid filled erythemic intestine and blindness among some survivors. All isolates of *A. hydrophila* were resistant to the tested antibiotics of the families tetracycline and penicillin, and trimethoprim, trimethoprim+sulfamethoxazol and streptomycin. Antibiotics of the family quinolones (norfloxacin and flumequine), nitrofurantoin and gentamicin proved to be the most efficacious on *A. hydrophila* isolates. All *Vibrio* sp. isolates were sensitive to 11 antibiotics tested except colistin and streptomycin.

Key words: *Aeromonas*, antibiotics, bacterial diseases, fish, *Vibrio*

INTRODUCTION

As the global ornamental fish trade has been growing steadily over the years, the number of hatcheries, farms and trade agencies has increased markedly over the last decade. In 1988, the global value of the aquarium industry was estimated at US \$ 4 billion.¹ Singapore, the world's largest exporter of tropical fish, earned US \$ 38 million from this trade in 1988 while Sri Lanka which earned slightly over US \$ 1 million in 1985 doubled its export in 1988 and has been projected to earn US \$ 4 million in 1994 from this industry.^{1,2} Although the industry has been growing, almost all farmers experience setbacks in production during larval rearing, growout and transportation especially due to high mortality associated with diseases and related disorders which estimates roughly to about 40% of the total production.¹

It is well established in ornamental fish industry that bacterial infections are responsible for heavy losses from the farm level to the hobbyist tank. Gratzek *et al.*³ and Shotts *et al.*⁴ established that 15% of the 73 bags of pet fish originating from Southeast Asia had *Aeromonas hydrophila*. However, literature on bacterial diseases of ornamental fish culture in Sri Lanka is scarce.⁵

This work was therefore undertaken to investigate the bacterial disease outbreaks in ornamental fish culture in Sri Lanka, characterize the pathogens and to determine the sensitivity of the bacteria involved to commonly used antibiotics with a view to provide the farmers with the information on effective treatment.

METHODS AND MATERIALS

Bacterial diseases of freshwater ornamental fish that were reported to our laboratory from hatcheries and farms during the period January, 1993 to February, 1994 were investigated.

Selection of specimens: The fish species involved were: Zebrafish (*Brachydanio rerio*), Penguinfish (*Thayeria boehllei*), Black Widow (*Gymnocorymbus ternetzi*), Super Serpae (*Hyphessobrycon callistus callistus*), Cuming's Barb (*Puntius cumingi*), Cherry Barb (*Puntius titteya*), Angelfish (*Pterophyllum scalare*), Guppies (*Poecillia reticulata*), Red Swordtail (*Xiphophorus helleri*), Kissing Gourami (*Helostoma temmincki*), Goldfish (*Carassius auratus*) and Giant Gourami (*Osphromenus gorami*). Only affected live or moribund fish were selected for the study.

Physical examination: Fish were examined for the presence of external and internal parasites and macroscopic lesions were recorded. Samples for bacteriological culture were taken directly from lesions as well as from liver, kidney, peritoneal cavity and intestinal contents aseptically. When fry and juveniles were involved, sampling was done according to Tanasomwang and Muroga⁶ and direct plating was carried out with the homogenate.

Media and culture techniques: Primary isolation of bacteria was carried out on *Pseudomonas* agar, *Aeromonas* agar, thiosulfate citrate bile sucrose agar, blood agar and nutrient agar (Oxoid, England). Plates were incubated at room temperature (26°C) for 24 to 48 h and predominant colonies, both from selective and non-selective media were subcultured and purified on nutrient agar. One such pure culture was taken from each outbreak of disease.

Identification of bacteria: The cultures were identified using miniaturized biochemical tests of API 20E and API GP (Analytab Product, Plainview, England) with species identification of *Aeromonas* and confirmed by biochemical tests according to Frerichs.⁷ Characteristics of *Vibrio* sp. were compared with those of the isolate of Pena *et al.*⁸

ABST: The isolates were subjected to antibiotic sensitivity tests using susceptibility disks with the standard disk diffusion method on PDM antibiotic sensitivity medium (AB BIODISK, Solna, Sweden). Following incubation at room temperature (26°C) for 24 h, inhibition zones were measured and the bacteria were classified as resistant and sensitive. Susceptibility disks used were: norfloxacin (NX, 10µg), flumequine (AR, 30µg), ampicillin (AM, 10µg), tetracycline (TC, 30µg), chloramphenicol (CL, 30µg), nitrofurantoin (NI, 100µg), streptomycin (SM, 30µg), gentamicin (GM, 30µg), trimethoprim (TR, 5µg), trimethoprim + sulfamethoxazol (TS, 1.2µg+23.8µg) and colistin (CO, 30µg).

RESULTS

Aeromonas hydrophila was the most dominant isolate in 18 outbreaks out of the 23 cases investigated. The fish involved in these outbreaks were: Goldfish juveniles and adults, Kissing gourami fry, Angelfish juveniles, Giant gourami, Zebrafish; Guppies, Super serpae, Swordtail, Cuming's barb, and Cherry barb. Eroding fins, sloughing scales, hemorrhagic skin, intestine and other visceral organs, ulcerative necrosis at the base of tail fin were among the lesions observed due to *A. hydrophila* infections.

The dominant isolate in the other five disease outbreaks was *Vibrio* sp. in which Swordtail, Black widow fish, Super serpae, Penguinfish, Angelfish, and Cherry barb were involved. Lesions observed due to vibriosis were, erratic swimming, hemorrhagic peritoneum and visceral organs, fluid filled erythemic intestine and blindness among some survivors.

Table 1: Inconsistent cultural and biochemical characteristics of *Aeromonas hydrophila* isolated from freshwater ornamental fish during disease outbreaks from Jan., 1993 to Feb., 1994. (n = 18).

Test	Reaction*
Citrate utilization	- (66.7%)
Voges Proskauer	+ (77.8%)
Lysine decarboxylase	+ (50.0%)
Acid from L+arabinose	- (88.9%)
Acid from inositol	- (94.4%)
Acid from salicin	+ (50.0%)
Acid from rhamnose	- (94.4%)
Acid from sorbitol	+ (89.0%)
Acid from melibiose	+ (61.1%)
Acid from amygdalin	+ (72.2%)

* Percentage of isolates showing given reactions.

Many of the cultural and biochemical characteristics of *A. hydrophila* isolated from the disease outbreaks were consistent while some varied among the isolates. Those inconsistent characters are shown in Table 1. *Vibrio* sp. isolated

showed growth characteristics of 1-2 mm, low convex, circular colonies on blood agar (after 24 h at 26 °C), less than 1 mm light green colonies on TCBS agar after 24 h which turned white (2mm) with a large yellow surrounding after 48 h. The 5 *Vibrio* sp. isolated showed almost the same characteristics except for reactions in Voges-Proskauer and salt requirement as shown in Tables 2 and 3. For comparison, the characteristics of the *Vibrio* sp. reported⁸ as the causative agent of vibriosis in the Kuruma prawn are presented in these tables. All *A. hydrophila* isolates were sensitive to NX,AR,GM,and NI and resistant to AM and TC. Twenty seven percent were resistant to CL, 11.70 % were resistant to TM and TS. The *Vibrio* sp. were sensitive to the tested antibiotics except CO,NI and SM. The percentage sensitivity of *A. hydrophila* and *Vibrio* sp. to the antibiotics are shown in Tables 4 and 5.

Table 2: Cultural and biochemical characteristics of *Vibrio* sp. isolated from freshwater ornamental fish during disease outbreaks from Jan., 1993 to Feb., 1994 (n = 5).

Test/characteristic	<i>Vibrio</i> sp. Present isolate	<i>Vibrio</i> sp. Pena <i>et al.</i> ⁸
Mortality	+	+
Shape	CR	
Swarming	-	+
Oxidase	+	+
Catalase	+	+
Hugh-Leifson's (OF)	F	F
Gas from glucose	+	+
Nitrate reduction	+	+
Voges Proskauer	-(80.0%)*	-
Indole	+	+(72.0%)*
Hydrogen sulfide	-	-
Citrate (Simmon's)	-	+(52.0%)*
Beta-galactosidase	+	+(99.0%)*
Arginine decarboxylase	-	-
Lysine decarboxylase	-	-
Ornithine decarboxylase	-	-
Gelatine liquefaction	+	+
Sensitivity to 0/129	+	+
Growth at 4°C	-	-
37°C	+	
40°C	+	-
Growth in peptone water		
0% NaCl	-(80.0%)*	-
0.5% NaCl	+	-
3% NaCl	+	+
6% NaCl	-	-

* Percentage of isolates showing given reactions, CR = curved rod.

Table 3: Carbohydrate utilization of *Vibrio* sp. isolated from freshwater ornamental fish during disease outbreaks from Jan.,1993 to Feb.,1994 (n = 5).

Characteristic	<i>Vibrio</i> sp. Present isolate	<i>Vibrio</i> sp. Pena <i>et al.</i> ⁸
Acid from:		
Arabinose	-	-
Esculin	-	-
Glucose	+	+
Glycerol	-	-(93.0%)*
Inositol	-	-
Mannitol	+	+
Mannose	-	+
Melibiose	-	-
Raffinose	-	-
Rhamnose	-	-
Salicin	-	-(54.0%)*
Sorbitol	-	-
Sucrose	+	-
Starch	+	+

* Percentage of isolates showing given reactions.

Table 4: Antibiotic sensitivity of *Aeromonas hydrophila* isolated from freshwater ornamental fish during disease outbreaks from Jan.,1993 to Feb.,1994 (n = 18).

Antibiotic	Percentage sensitivity (%)	
	Sensitive	Resistant
Norfloxacin	100.0	-
Flumequine	100.0	-
Ampicillin	-	100.0
Tetracycline	-	100.0
Chloramphenicol	72.2	27.8
Nitrofurantoin	100.0	-
Streptomycin	-	100.0
Gentamicin	100.0	-
Trimethoprim	11.7	88.3
Trimethoprim+Sulfamethoxazol	11.7	88.3

Table 5 : Antibiotic sensitivity of *Vibrio* sp. isolated from freshwater ornamental fish during disease outbreaks from Jan., 1993 to Feb., 1994 (n = 5).

Antibiotic	Reaction	
	Sensitive	Resistant
Norfloxacin	5/5*	-
Flumequine	5/5	-
Ampicillin	5/5	-
Tetracycline	5/5	-
Chloramphenicol	5/5	-
Colistin	-	5/5*
Nitrofurantoin	3/5	2/5
Streptomycin	2/5	3/5
Gentamicin	5/5	-
Trimethoprim	5/5	-
Trimethoprim+Sulfamethoxazol	5/5	-

* Number of isolates showing given reactions/total isolates tested.

DISCUSSION

Aeromonas hydrophila was involved in 78.26% of the total (23) bacterial disease outbreaks investigated during the study period. This, an opportunistic pathogen was reported to be the predominant pathogen found in lesions of Epizootic Ulcerative Syndrome (EUS) affected fish^{9,10,11}. Costa and Wijeyaratne,¹² Subasinghe *et al.*⁹, Pathiratne *et al.*¹³ reported that over 21 species of brackish and freshwater fish in Sri Lanka were affected by EUS. EUS lesions (hemorrhages and ulcerative necrosis in the skin on various parts of the body) were observed in the present study on *A. hydrophila* affected fish except larvae of Kissing Gourami and juveniles of Angelfish. Dixon *et al.*¹⁴ found that about 43 % of the *Aeromonas* isolates from a variety of tropical pet fish imported from Singapore were *A. hydrophila* which were causing monomicrobial infections in these fish. Shotts *et al.*⁴ also established *A. hydrophila* as a major disease problem in ornamental fish. These and the results of the present study demonstrate the potential of *A. hydrophila* as a serious pathogen in ornamental fish industry.

A. hydrophila isolated in the present study were resistant to TC. This finding is in agreement with the observation of Dixon *et al.*¹⁴, Supriyadi and Rukyani.¹⁵ In the present study, 27.77% of the *Aeromonas* isolates showed multiple resistant to TC and CL. This is comparable with the observations made by Aoki and Watanabe¹⁶ who isolated multiple drug resistant (to TC and CL) *A. hydrophila* from freshwater fish in Thailand. Of the antibiotics tested, GM, AR, and NX were the most efficacious on *A. hydrophila* isolates. Dixon *et al.*¹⁴ also found the quinolone family antibiotics as the most effective antimicrobial drug on freshwater *A. hydrophila* from ornamental fish. Though the development of plasmid mediated resistant to quinolones is rare,¹⁷ Supriyadi and

Rukyani¹⁵ noted that 70% of the thirty *A. hydrophila* isolates from affected freshwater fish were resistant to AR in Indonesia.

The *Vibrio* sp. isolated from the five disease outbreaks were categorized as belonging to one species. Most of the characteristics of these isolates coincide with those of the *Vibrio* sp. reported by Pena *et al.*⁸ from diseased Kuruma prawn, *Penaeus japonicus*. However, the true freshwater character of the present isolates was well supported by their ability to grow in salt level as low as 0.5%.

Internal and external lesions of the disease caused by this bacteria were similar to those of vibriosis described by Post.¹⁸ Vibriosis of freshwater fish has not been recorded earlier in Sri Lanka. The list of susceptible freshwater fish species to vibriosis by Post¹⁸ did not include any of the species involved in the present outbreaks of vibriosis.

All five *Vibrio* sp. isolated were sensitive to the eleven antibiotics tested except CO, NI and SM. In contrast, many authors reported resistant *Vibrio* sp. to commonly used antibiotics from marine environments.^{19,20,21}

In conclusion, this investigation provides valuable information to the farmers on the two major bacterial killers of freshwater ornamental fish in Sri Lanka and a choice of the most effective antimicrobials against them. This also provides information for the scientists on the emergence of vibriosis of freshwater fish which has not been recorded earlier in this country.

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