

## Surveillance of Bacteria Species in Diseased Freshwater Ornamental Fish from Aquarium Shop

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### SHORT REPORT

A survey of bacteria disease infected in freshwater ornamental fish in retail pet shop in Kuala Terengganu, Terengganu, Malaysia was conducted from July to September, 2007. The collected diseased fish were Dwarf Gourami (*Colisa lalia*), Discus (*Symphysodon aequifasciatus*), Discus Cichlids (*Symphysodon* spp.), Black Tetra (*Gymnocorymbus ternetzi*), Swordtail (*Xiphophorus helleri*), Platy (*Xiphophorus maculatus*), Variegated platy (*Xiphophorus variatus*), Black Ruby Barb (*Barbus nigrofasciatus*), Tiger Barb (*Barbus pentazona hexazona*), Sumatra Barb (*Barbus tetrazona*), Fighting Fish (*Betta splendens*), Guppy (*Poecilia reticulata*), Mollies (*Poecillia* spp.) and Silver Catfish (*Pangasuis sutchi*). The bacteria were isolated using blood agar plate, cytophaga agar plate, GSP agar plate, XLD agar plate and MacConkey agar without crystal violet plate. The isolated bacteria were identified using commercial identification kit. Antibigram of the isolated bacteria of the present study were also determined. Nowadays, bacterial disease is a common problem faced by ornamental fish industry. Bacterial Gram negative is recognized as causative agent of many bacterial diseases attacking ornamental fish. *Aeromonas*, *Citrobacter*, *Flavobacterium*, *Edwardsiella*, *Mycobacterium*, *Pseudomonas* and *Vibrio* are Gram negative bacteria usually isolated from the diseased ornamental fish. These bacteria were opportunistic and ubiquitous in the aquatic environment. Many factors could contribute to bacterial infection in ornamental fish namely poor water quality, crowding, transportation and inadequate nutrition. Many cases of bacterial infections in ornamental fish have been reported worldwide. For

instance, the study of Dixon and Contreras [1] showed the presence of multi drug resistant *Edwardsiella tarda* in the imported ornamental fish. Furthermore, the imported gourami from Asia was found infected by *Yersinia ruckeri*, a causative agent of enteric red mouth disease. Therefore, this study was conducted to survey bacterial disease infected in ornamental fish in Kuala Terengganu, Malaysia and antibiogram of isolated bacteria. Approximately fifty diseased freshwater ornamental fish were collected from an aquarium shop in Kuala Terengganu, Terengganu, Malaysia. They were brought back to laboratory using plastic bag filled with provided aquarium water from pet aquarium shop. Externally, cotton bud was used to swab onto the lesion while internally intraperitoneal fluid of the diseased fish was swabbed aseptically and spread on blood agar plate, cytophaga agar plate, glutamate starch phenol red (GSP) agar (Merck, Germany) plate, Xylose Lysine Deoxycholate (XLD) agar (Merck, Germany) plate and MacConkey agar without crystal violet (Difco, USA) plate, separately. After incubation for 24 h, the inoculated plates were examined for the suspected single and pure bacterial colony. The bacteria were then kept in Trypticase Soy Agar (TSA) (Merck, Germany) deep tube for identification purpose. The identification of the suspected bacteria was done using commercial identification kit (BBL Crystal, USA). The identified bacterial were cultured in Trypticase Soy Broth (TSB) (Merck, Germany) for 24 h at room temperature. The bacterial suspensions were adjusted into 10<sup>6</sup> CFU/ml and spread on Mueller Hinton agar (Oxoid, England). Antibiotic disks were then placed on the MH agar plate and incubated for 24 h at room temperature. The diameter of inhibition zones of the each tested antibiotic disk was measured and interpreted as sensitive

Table 1: Antibiotic sensitivity test of the bacterial isolates from diseased freshwater ornamental fish collected from aquarium shop in Kuala Terengganu, Terengganu, Malaysia

Isolate	Source	E	FR	RL	C	K	NA	OTC
<i>Acinetobacter iwoffii</i>	<i>Symphysodon aequifasciatus</i>	I	R	I	S	S	S	S
<i>Acinetobacter baumannii</i>	<i>Barbus pentazona hexazona</i>	I	R	R	R	S	S	R
<i>Aeromonas hydrophila</i>	<i>Xiphophorus maculatus</i>	I	S	R	S	S	S	R
<i>Aeromonas hydrophila</i>	<i>Barbus pentazona hexazona</i>	I	R	R	S	S	R	S
<i>Aeromonas hydrophila</i>	<i>Symphysodon</i> spp.	I	S	R	S	S	S	R
<i>Aeromonas hydrophila</i>	<i>Colisa lalia</i>	I	S	R	S	S	S	R
<i>Aeromonas hydrophila</i>	<i>Gymnocorymbus ternetzi</i>	I	S	R	S	S	S	I
<i>Aeromonas hydrophila</i>	<i>Poecilia reticulata</i>	I	R	R	S	S	R	R
<i>Aeromonas hydrophila</i>	<i>Pangasuis sutchi</i>	R	S	R	S	S	R	R
<i>Aeromonas hydrophila</i>	<i>Colisa lalia</i>	S	R	R	R	S	S	R
<i>Aeromonas hydrophila</i>	<i>Barbus pentazona hexazona</i>	S	R	R	I	S	R	R
<i>Aeromonas hydrophila</i>	<i>Poecilia reticulata</i>	I	R	R	S	S	S	R
<i>Aeromonas hydrophila</i>	<i>Osphronemus goramy</i>	I	S	R	S	S	R	R
<i>Aeromonas hydrophila</i>	<i>Pangasuis sutchi</i>	I	I	R	S	S	R	R
<i>Aeromonas hydrophila</i>	<i>Poecilia reticulata</i>	I	R	R	R	S	R	R
<i>Aeromonas hydrophila</i>	<i>Pangasuis sutchi</i>	I	S	R	S	S	R	R
<i>Aeromonas hydrophila</i>	<i>Poecilia reticulata</i>	I	S	R	S	S	S	R
<i>Chromobacterium violaceum</i>	<i>Pangasuis sutchi</i>	I	S	R	S	S	R	R
<i>Chromobacterium violaceum</i>	<i>Gymnocorymbus ternetzi</i>	S	S	R	S	S	R	R
<i>Chromobacterium violaceum</i>	<i>Symphysodon</i> spp.	I	S	R	S	S	R	R
<i>Edwardsiella tarda</i>	<i>Colisa lalia</i>	I	S	R	S	S	R	R
<i>Enterobacter</i> sp.	<i>Symphysodon aequifasciatus</i>	I	R	I	S	S	S	S
<i>Flavobacterium</i> sp.	<i>Pangasuis sutchi</i>	R	R	R	I	R	S	R
<i>Serratia marcescens</i>	<i>Xiphophorus maculatus</i>	I	S	R	S	S	S	R
<i>Stenotrophomonas maltophilia</i>	<i>Osphronemus goramy</i>	R	R	R	S	I	I	R
<i>Yersinia</i> sp.	<i>Symphysodon aequifasciatus</i>	I	S	R	S	S	S	R

Keys:

R = Resistance, I = Intermediately sensitive, S = Sensitive

E = Erythromycin 15µg/disk, FR = Furazolidone 15 µg/disk, RL = Sulphamethoxazole 25 µg/disk, C = Chloramphenicol 30 µg/disk,

K = Kanamycin 30 µg/disk, NA = Nalidixic acid 30 µg/disk, OTC = Oxytetracycline 30 µg/disk

or intermediary sensitive or resistant based on National Committee for Clinical Laboratory Standards (NCCLS) provided by manufacturer. Antibiotic test was run in triplicates. In the present study, 25 isolates were successfully isolated from diseased ornamental fish. One isolate of each *Edwardsiella tarda*, *Flavobacterium* sp., *Stenotrophomonas maltophilia*, *Serratia marcescens*, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Yersinia* sp. and *Enterobacter* sp., 3 isolates of *Chromobacterium violaceum* and 15 isolates of *Aeromonas hydrophila*. The result of this study showed that majority of the isolated bacteria was *Aeromonas hydrophila*. *A. hydrophila*, *E. tarda*, *Yersinia* sp. and *Flavobacterium* sp. were bacteria species commonly isolated from diseased fish. Although *S. maltophilia* and *S. marcescens* were rarely reported in cultured fish but several studies claimed these types of bacteria have been isolated from the diseased

fish. For instance, 6 isolates of multidrug resistant of *S. maltophilia* was successfully isolated from cultured yellowtail (*Seriola quinqueradiata*) from a marine fish farm in Japan [2]. Whereas, the virulent (LD<sub>50</sub>) of *S. marcescens* was reported ranging from 5 x 10<sup>3</sup> to 1 x 10<sup>5</sup> CFU/ml for White Perch (*Morone americanus*) [3]. Table 1 showed antibiogram of the present isolates against 7 types of antibiotics. In the present study, 41.8% cases of antibiotic resistance were reported. On the contrary, 23.7 and 34.5% cases of intermediary sensitive and sensitive, respectively against the tested antibiotics were noted. Most of the present isolates were resistant to sulphamethoxazole except for *Acinetobacter iwoffii* which was found to be intermediary sensitive. In the present study, kanamycin was found to be effective in controlling the present isolates because only one isolate showed resistance to it.

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