_Pseudomonas elongata_ Infection in Scattered Mirror Carp
_(Cyprinus carpio): Bacteriology, Gross Pathology and Treatment_

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**Abstract:** _Pseudomonas elongata_ was isolated from naturally infected scattered mirror carp (Cyprinus carpio) and its pathogenicity was tested by intramuscular injection. The infection caused mortality in scattered mirror carp with gross clinical abnormalities such as dark coloured of a location on body surface, cataract in eyes, haemorrhagic damage of liver, irritation in kidney, anemia, swollen intestine, fins rot and hyperaemia in operculo and skin. Lethal Dose$_{10}$ (LD$_{10}$) of _Pseudomonas elongata_ was calculated 2.24×10$^7$. No significant difference was obtained among enumerated of pathogenic bacteria isolated from gill, liver, kidney tissues and total pathogenic bacteria. Sensitivities of _Pseudomonas elongata_ against 50 chemotherapeutants were tested. Minimum inhibitory concentrations of enrofloxacin and chloramine T to the isolate were calculated 5 mL L$^{-1}$ and 10 mg L$^{-1}$, respectively. Best treatment method was no mortality performed with orally enrofloxacin application and chloramine T bath.

**Key words:** _Pseudomonas elongata_, scattered mirror carp, chemotherapy, chloramine T, enrofloxacin

**INTRODUCTION**

The species of _Pseudomonas genus_ can be widely distributed in nature. Some species are pathogenic for humans, animals and plants (Holt _et al._, 1994). _Pseudomonas elongata_ was isolated from intertidal sand, sea water and bottom sediments (Palleroni, 1984). No information is currently available regarding its isolation and pathogenicity in aquatic animals. _P. elongata_ was isolated from naturally infected scattered mirror carp in Turkey.

The present study was designed to test the pathogenicity of _P. elongata_ in scattered mirror carp, bacteriology of this infection Lethal Dose$_{10}$ (LD$_{10}$) and sensitivity of some chemotherapeutics.

**MATERIALS AND METHODS**

Nine different 1000 L capacity fiberglass tanks supplied with freshwater circulation daily (once/day) and continuous aeration (9 ppm dissolved O$_2$ content) were used for infection of scattered mirror carp used in the present study. The water temperature was 22±0.5°C with pH 7.8 in basins.

Bacteria were isolated from naturally infected scattered mirror carp. For the isolation of bacteria, inocula were aseptically obtained from kidney, liver and gills of naturally infected fish and immediately streaked on enriched Tyriptic Soy (TS) agar and _Pseudomonas-Aeromonas Selective_ (GPS) agar. Incubations were generally carried out at 25°C for 48 h (Halkman, 2005; Aydin _et al._, 2000).

After initial incubation, individual colonies grown on GPS agar at 25°C to 48 h. Individual colonies grown on these re-streaked plates were used in the identification tests (Leloglu and Erdogan, 1979; Plumb and Bowser, 1983; Austin and Austin, 1993; Halkman, 2005). All the bacteriological media used in this research were purchased from Merck (Merck, Germany). Also, MIS (Microbial Identification System) was examined in the identification of bacteria. Individual colonies grown on re-streaked plates were identified with fatty acid profiles (Küfevicioglu _et al._, 1999).

Aliquots (0.1 mL) were used to test the sensitivity of the bacterium to several chemotherapeutics. The agar disc diffusion method (Bauer _et al._, 1966) with enriched GPS agar was employed to determine their sensitivity to chemotherapeutic agents. Plates were read both at 24 and
48 h incubation, at the end of incubation, the diameter of the zone of inhibition was measured to the nearest millimeter with calipers. According to the standards set forth by national committee for clinical laboratory standards or by the antibiotic manufacturer's recommendations (Plumb et al., 1995).

For the MIC's (Minimum Inhibitory Concentration) of formalin, enrofloxacin and chloramine T were prepared dilutions 20, 10, 5, 1, 0.1 and 0.01 μL mL⁻¹ in test tubes containing 5 mL sterile phosphate buffer solution. Standardized bacterial isolates (0.1 mL) were added to each tube and left to stand at room temperature for 1 h, after which a loopful of material from each tube was inoculated onto plates containing GSP agar medium. These were incubated for 3 days at 25°C and then examined for growth of P. elongata (Cipriano et al., 1996).

Trial was performed triPLICATE. Totally, 180 scattered mirror carp, average 21.06±2.49 g body weight, were used for the experiment. Stock density carryout 10 fish/tanks. First group was control group, 2nd group was injected with 10⁴ live cells P. elongata and 3rd group was injected with 5×10⁵ live cells P. elongata for the patogenicity test. Also, 3 groups were used for the chemotheraphy. Following 20 days of preliminary adaptation period fish were injected on muscle around dorsal fin.

The degree of virulence, expressed as the 50% mean Lethal Dose (LD₅₀), was calculated by the method of Reed and Muench (1938).

P. elongata was re-isolated from kidney, liver and gills of dead fish quantitatively by using GSP agar and identified as P. elongata by characterization tests. During the experiments of the experimental infections, behaviours of the diseased fish as well as their gross external and internal symptoms were recorded.

For the chemotherapy, the experimentally infected fish with 5×10⁵ live cells P. elongata. The 1st chemotherapy group treated with orally 100 mg kg⁻¹ fish dosage of enrofloxacin (Baytrol) per day for 7 days. The 2nd chemotherapy group treated with orally 100 mg kg⁻¹ fish dosage of enrofloxacin and chloramine T bath (20 mg L⁻¹ water for 1 h) per day for 7 days. The 3rd chemotherapy group was bathed for 7 days with 20 mg L⁻¹ dosage of chloramine T for 1 h day⁻¹.

Number of microorganisms was used dilution plate method. At the end of the incubation that is at 25°C for 48 h, enumerated results were given as cfu (colony forming units).

The data obtained from the organs of moribund fish was tested variance analysis. The mortality rates of fish groups were compared by test the hypothesis of the difference of 2 rates using Minitab-User Guide package programme. A value of p<0.05 was considered to be significant.

**RESULTS**

The isolate was identified using standard biochemical profiles as *Pseudomonas elongata* (Table 1). The bacterial isolate could not be determined with MIS. Standard biochemical tube and plate tests were more successful than MIS for identification of *P. elongata*.

Isolate was observed susceptible to all antibiotics except ampicillin after 24 h, incubation period. The 48 h incubation period was chosen because in a preliminary study, it was shown that zones of inhibition did change between 24 and 48 h and they were more clearly measurable at 48 h. Antimicrobial sensitivity of the isolate the end of 48 h incubation period was summarized in Table 2. According to the results of study, enrofloxacin, ciprofloxacin, norfloxacin, oxefloxacin, pefloxacin, imipenem, enoxacin, netilmicin antibiotics could be recommended to treat fish infected with P. elongata.

In vitro assays indicated that MICs were 5 mL L⁻¹ of enrofloxacin, 10 mg L⁻¹ of chloramine T against *P. elongata*, but formalin was not effective to isolate.

One of 10 scattered mirror carp infected with 10⁵ live cells of *P. elongata* (10% mortality) and 9 of 10 scattered mirror carp infected with 5×10⁵ live cells of *P. elongata* (90% mortality) died between 3-15 days following the inoculation. LD₅₀ of the *P. elongata* isolate was calculated 2.24×10⁴.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Response</th>
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<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 37°C (30°C)</td>
<td>Small, pink, round, convex</td>
</tr>
<tr>
<td>Morphology of colonies on GSP agar</td>
<td>Small, cream, round, convex</td>
</tr>
<tr>
<td>Morphology of colonies on TS agar</td>
<td>Rod</td>
</tr>
<tr>
<td>Morphology of cell</td>
<td></td>
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<tr>
<td>Motility (OC), Oxidase, Catalase</td>
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</tr>
<tr>
<td>Voges-Proskauer, Metil red, Gelatin hydrolysis, Starch hydrolysis, Aesculin hydrolysis, Growth at KCN, Arginin dehidrolase</td>
<td>+</td>
</tr>
<tr>
<td>Lysin decarboxilase, Simonson's citrate, Indol, NO₂ reduced NO₃, Urease, H₂S production, Gas production from glucose</td>
<td>-</td>
</tr>
<tr>
<td>O/F</td>
<td>0</td>
</tr>
<tr>
<td>Production of acid from carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Glucose, trehalose, arabinose, xylose, galactose, inulin, succrose, fructose, dextrin, mannose, sorbitol, maltose, salicin, raffinose, dulcitol, mannitol, lactose, erythritol, inositol, adonitol, glycerol</td>
<td></td>
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<td>*= Room temperature</td>
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Table 1: Biological and biochemical characteristics of bacteria (*Pseudomonas elongata*) isolated from diseased scattered mirror carp.
The clinical signs of experimental infection such as dark colored of a location on body surface, cataract in eyes, hemorrhagic damage of liver, irritation in kidney, anemia in gills, swollen intestine and degeneration of fins and hyperemia in operculum and body surface in scattered mirror carp were observed. The counts of *P. elongata* in organs (gill, liver and kidney) from death fish ranged from \(3.65 \times 10^{12} - 2.45 \times 10^{14}\) cfu g\(^{-1}\).

Four of 10 scattered mirror carp treated with orally enrofloxacin died (40% mortality) and one of 10 scattered mirror carp bathed with chloramine T died (10% mortality) in 7 days following the inoculation. But 10 scattered mirror carp treated with orally enrofloxacin and chloramine T bath no died.

**DISCUSSION**

The biological and biochemical characteristics of the isolate both coccoïd and longer cells had aerobic, having a strictly respiratory type of metabolism with oxygen. The results were almost identical with those of isolates from intertidal sand, sea water or bottom sediments (Pulleroni, 1984; Holt *et al.*, 1994). In this study, *P. elongata* produced acid from arabinose and lactose but not from insulin and sorbitol, unlike it was reported in Bergey’s Manual of Systemic Bacteriology (Pulleroni, 1984).

Microbial Identification System (MIS) uses gas chromatography analysis of whole-cell Fatty Acid Esters (FAMEs) between 9 and 20 carbons in length to characterize a wide range of bacterial genera and species. Cellular fatty acid compositions are widely used as a basis for the characterization of bacteria. The identification is done by one chromatographic run, which usually requires less than half an hour. The MIS uses quantitative analyses of fatty acid profiles for reliable identification of many bacteria to the subspecies level. But MIS can not identify some bacterial genera and species due to similarities of fatty acid profiles of bacterial cells and insufficient database of this system.

Koch’s postulates were satisfied with the counts of *P. elongata* in organs (gill, liver and kidney) from death fish. No significant difference was obtained among numbered of pathogenic bacteria isolated from gill, liver, kidney tissues and total pathogenic bacteria (*P. elongata*) of death fish in groups \((p>0.05)\). These results support that injection of the increasing number of *P. elongata* did not seem to significant effect on the number of bacteria, which would be isolated from the organs of moribund fish following the bacterial challenge. The number of *P. elongata* in organs (gill, liver and kidney) from death fish ranged from \(2.15 \times 10^2 - 1.08 \times 10^5\) cfu g\(^{-1}\).

The treatment of orally enrofloxacin application with chloramine T bath was significantly successful than orally enrofloxacin application group \((p<0.01)\).

**CONCLUSION**

The present results demonstrated that *P. elongata* could be a pathogen for scattered mirror carp. LD\(_{50}\) for this bacterium was calculated \(2.24 \times 10^3\). The chemotherapy with orally enrofloxacin and chloramine T baths provided complete recovery of the experimentally infected fish in treatment. According to these results, oral enrofloxacin and chloramine T bath treatments were best treatment for *P. elongata* infection.

In addition, pathogenicity of *P. elongata* needs to be examined for different fish species and under different conditions.

**REFERENCES**


