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Pseudomonas elongata Infection in Scattered Mirror Carp (*Cyprinus carpio*): Bacteriology, Gross Pathology and Treatment

[1]Nejdet Gültepe and [2]Seyit Aydin
[1]Department of Aquaculture, Faculty of Fisheries,
Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
[2]Faculty of Kastamonu Education, University of Kastamonu, 37100 Kastamonu, Turkey

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, irrigation in kidney, anemia, swollen intestine, fins rot and hyperaemia in operculum and skin. Lethal $Dose_{50}$ (LD_{50}) of *Pseudomonas elongata* was calculated 2.24×10^{5}. No significant difference was obtained among enumered 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 interdial 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 $Dose_{50}$ (LD_{50}) 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 (GSP) 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 GSP 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 (Küfrevioglu *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 GSP agar was employed to determine their sensitivity to chemotherapeutic agents. Plates were read both at 24 and

Corresponding Author: Nejdet Gültepe, Department of Aquaculture, Faculty of Fisheries, Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey

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 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. Totaly, 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 10^5 live cells *P. elongata* and 3rd group was injected with 5×10^5 live cells *P. elongata* for the patogenicity test. Also, 3 groups were used for the chemoteraphy. 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 (LD_{50}), was calculated by the method of Reed and Müench (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 5×10^5 live cells *P. elongata*. The 1st chemotherapy group treated with orally 100 mg kg^{-1} fish dosage of enrofloxacin (baytril) 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, enumered 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 hypotesis of the difference of 2 rates using Minitab-User Quide 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, ofloxacin, 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 10^5 live cells of *P. elongata* (10% mortality) and 9 of 10 scattered mirror carp infected with 5×10^5 live cells of *P. elongata* (90% mortality) died between 3-15 days following the inoculation. LD_{50} of the *P. elongata* isolate was calculated 2.24×10^5.

Table 1: Biological and biochemical characteristics of bacteria (*Pseudomonas elongata*) isolated from diseased scattered mirror carp

Characteristic	Response
Gram stain	-
Growth at RT* (30°C)	+
Morphology of colonies on GSP agar	Small, pink, round, convex
Morphology of colonies on TS agar	Small, cream, round, convex
Morphology of cell	Rod
Motility (RT), Oxidase, Catalase	-
Voges-Proskauer, Metil red, Gelatin hydrolysis, Starch hydrolysis, Aesculin hydrolysis, Growth at KCN, Arginin dihydrolase	+
Lysin decarboxilase, Simmon's citrate, Indol, NO_3 reduced NO_2, Urease, H_2S production, Gas production from glucose	-
O/F	O
Production of acid from carbonhydrates	
Glucose, trehalose, arabinose, xylose, galactose, inulin, sucrose, fructose, dextrin, mannose, sorbitol	+
Maltose, valin, raffinose, dulcitol, mannitol, lactose, erythritol, inositol, adonitol, glycogen	-

*= Room temperature

Table 2: Results of susceptibility test of *Pseudomonas elongata* isolate to chemothrerapeutants

Chemotherapeutics (μg disc^{-1}) against, which *P. elongata* was sensitive			
Azithromycin	15	Azlocillin	75
Cefadroxil	30	Cefoperazone	75
Cefoperazone+Sulbactam	75+30	Cefotaxime	30
Ceftriaxone	30	Cephazolin	30
Chloramphenicol	30	Ciprofloxacin	5
Clarithromycin	15	Doxycycline	30
Enoxacin	10	Enrofloxacin	5
Imipenem	10	Netilmicin	30
Norfloxacin	10	Novobiocin	30
Ofloxacin	5	Pefloxacin	5
Rifamycin SV	30	Sulbactam+Ampicillin	10+10
Tetracycline	30		
Chemotherapeutics (μg disc^{-1}) against which *P. elongata* was intermediate			
Cefuroxime	30	Cephalexin	30
Erythromycin	15	Gentamicin	10
Chemotherapeutics (μg disc^{-1}) against which *P. elongata* was resistant			
Amikacin	30	Ampicillin	10
Amoxycillin	25	Amoxicillin+ Clavulanic acid	20+10
Aztreonam	30	Carbenicillin	100
Cefaclor	30	Cefixime	5
Cefoxitin	30	Ceftizoxime	30
Cephalothin	30	Kanamycin	30
Lincomycin	2	Methicillin	5
Mezlocillin	75	Nalidixic acid	30
Oxacillin	5	Penicillin	G (10U)
Piperacillin	100	Thiamphenicol	30
Tobramycin	10	Trimethoprim+ Sulphamethoxazole	1.25+23.75
Vancomycin	30		

The clinical signs of experimental infection such as dark colored of a location on body surface, cataract in eyes, hemorrhagic damage of liver, irrigation 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 3.65×10^{11}-2.45×10^{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 coccoid and longer cells had aerobic, having a strictly respiratory type of metabolism with oxygen. The results were almost identical with those of isolates from interdial sand, sea water or bottom sediments (Palleroni, 1984; Holt *et al*., 1994). In this study, *P. elongata* produced acid from arabinose and lactose but not from inulin and sorbitol, unlike it was reported in Bergey's Manual of Systemic Bacteriology (Palleroni, 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 2.15×10^{7}-1.08×10^{8} 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 2.24×10^{5}. The chemotherapy with orally enrofloxacin and chloramine T bathes provided complete recovery of the experimentally infected fish in treatment. According to these results, of orally enrofloxacin and chloramine T bath treats 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.

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