Study of gross and histopathological changes in *Cyprinus carpio* infected by non- hemolytic *Streptococcus* Part 1: Isolation and Identification of non-hemolytic *Streptococcus*

Asaad M. R. Al-Taee[1], Ali A. S. Al-Mayah[2], Faiha S. Khadban[2]

1- Marine Science Center, 2- Veterinary Medicine College, Basrah University

ABSTRACT

Twenty four of non- hemolytic *Streptococcus* was isolated from the pond of healthy carp of Marine Science Center- Basrah University. The bacteria were identified depending on colonies morphology of growing isolates on Brain Heart Infusion (BHI) agar. Colonies were small, round and convex shape. The cells of non- hemolytic *Streptococcus* were Gram positive, and may occur in pairs or chains. The cells are spherical or ovoid shape and small.

Sixty fishes of common carp (*Cyprinus carpio*) were obtained from the farm of Marine Science Center, University of Basrah. These fishes were divided into six groups (10 fishes/20 liters aquaria). One of those groups was injected intraperitoneally (I.P.) with an isolate of non-hemolytic *Streptococcus* at a concentration of 1 X 10^4 CFU/ ml. The other two groups were inoculated with 2.5 X 10^7 CFU/ ml of the same isolate via water pollution, one with and the other without skin abrasion. The other three groups were left as control and the experimental period was 35 days. The mortality rate was 100% in the I.P. injected group during the first week of the experiment, while nil in the other groups.

This article is a part of the thesis of the third author

INTRODUCTION

Streptococcus species infections occur in a variety of species of fresh and marine water fishes (27).

Streptococcal infections of fish which were rarely reported before 1970 (15, 20) which became a major problem worldwide with the intensification of aquaculture (4, 3, 13, 7, 16). These diseases may be

either chronic or acute and usually were associated with some types of environmental stress, such as high stocking density, high organic load in the water, or sub-lethal external protozoan infections or trauma to the fishes (25,24).

Acute outbreaks often occur during the warmer months of the year or when fish were subjected to stress, with cumulative mortality reaching 80% within 10 days. After an acute outbreak, a low level chronic mortality can carry on for weeks or months with a small number of fish dying every day. Any size of fish can be affected by *Streptococcus* sp. but most outbreak place on juvenile fish of at least 10 g.

There were about twelve species of *Streptococcus* that have been associated with some type of disease condition in fish (2). *Streptococcus* infections have been reported in Central America, Japan and United State of America. The disease has been reported in over twenty-four species of fishes from fresh and marine water and from both culture and wild fish population. This disease can be transmitted from fish to human, especially when fish were purchased to the market as live fish and the consumers were spine or cut while dressing the fish (25, 26).

The aim of this study is the isolation and identification of non-hemolytic *Streptococcus* from fish water, observing the rout of infection on the mortality rate and the effect of the antibiotics, salt concentrations and temperature on the growth of bacteria.

MATERIALS AND METHODS

Sampling

Water Samples: Twenty-four water samples were obtained from the fish pond of Marine Science Center-Basrah University during the period of March to October 2005. The samples were collected in sterile 500 ml glass bottle. The samples were analyzed in the Marine Bacteria Laboratory within 1-2 hours following the collection.

Fish Samples: Sixty fishes of common carp (*C. carpio*) in average weight 20-25 gm. had been obtained from the fish farm of Marine Science Center. They were brought a live to the pathology laboratory by 4 liters plastic jar.

Isolation of Bacteria: The *Streptococcus* spp. were isolated from water by the spread method on Brain Heart Infusion agar (BHI) and incubated at 37 C˚ for 48h. Figure (1) describes the procedures used for the isolation and identification of bacteria in this study (14, 22, 6).

Bacterial Count: In order to estimate the number of non-hemolytic *Streptococcus* spp., eight serial dilutions of normal saline (9 ml in each tube) had been done under aseptic condition. One ml of the stock culture was added to the first tube, and then serial dilutions have been performed. After that 1 ml of each dilution was transferred aseptically to sterile duplicate Petri-dishes and mixed with about 17 ml of nutrient agar. The Petri- dishes were incubated at 37 C° for 24 h. The colonies were counted in the duplicate plates; the results were averaged and reported as a number of bacteria per ml. (9).

Experimental Design: 60 fish were divided into six groups at a density of 10 fish/20 liters aquaria in order to treat with bacteria. The average weight of fish was 20-25 gm. The fishes were reared for about 35 days.

The fishes were acclimated to the experimental conditions for 7 days from arrival to the laboratory and starting with the treatment. Each aquarium was continuously aerated by air pump and maintained at 37 C°. These groups were treated as follows (21, 8)

Group 1: It was intraperitoneally(I.P.) injected with non- hemolytic *Streptococcus* at a concentration of 1 X 10^4 CFU/ ml.

Group 2: It was intraperitoneally injected with normal saline and served as control for group 1.

Group 3: 2.5 X 10^7 CFU/ ml. of non-hemolytic *Streptococcus* was added to the aquarium.

Group 4: It was used as a control for group 3 without addition of bacteria.

Group 5: Fish were subjected for abrasion through removal of scales at both sides of fish body by a sterile scalpel. The aquarium of these fishes was contaminated by 2.5 X 10^7 CFU/ ml. of non-hemolytic *Streptococcus*.

Group 6: The fish were subjected for abrasions of the body and left in the aquarium without any treatment and act as a control for group 5.

Daily, one-third of the water was changed, dead fish were removed and feces were siphoned off, and fish behavior was observed. Mortalities were recorded daily over a four weeks.

Isolation of non-hemolytic *Streptococcus* from dead fish:

Bacterial swabs were taken from kidney, liver and brain of dead and moribund fish and cultured on BHI agar for 48 h. at 37 C°, then Gram stain were made. The Gram positive short cocci chains of bacteria were sub cultured on BHI agar for 24 h. at 37 C°, and subjected to the biochemical tests (22, 6).

RESULTS

24 isolates of non-hemolytic *Streptococcus* were isolated from the fish pond of Marine Science Center. The bacteria were identified depending on the colonies morphology of grown isolates on BHI agar. Colonies were small, round and convex shape. The cells of non-hemolytic *Streptococcus* were Gram positive, and may occur in pairs or chains. Cells spherical or ovoid shape and small. Table (1) showed the biochemical tests for identification of non-hemolytic *Streptococcus*.

Bacteriological examination of liver and kidney of ten dead fish with gross lesion repeatedly resulted in the isolation of non-hemolytic *Streptococcus*. In BHI agar the *Streptococcus* colonies were grey colored, translucent, circular slightly convex pin head. Gram stain showed large numbers of non-hemolytic *Streptococcus* which occur in chains and isolated in pure culture.

The biochemical tests of all isolates were typical of member of the genus *Streptococcus*. All isolates were subjected to antibiotics test (Table 2) growing on temperature (15, 20, 25, 30, 35, and 40 C˚) and salt tolerance concentration 0.5, 1, 3, 6.5 and 10% (Table 3).

DISCUSSION

24 isolates of *Streptococcus* were isolated from the water of fish pond. The bacteria were identified as non-hemolytic *Streptococcus* spp.

according to their morphological and biochemical characteristics (Table 1, 2, and 3) which agree with (14). This indicating that streptococci can survive in the water and mud of fish farm because it can be from the fish itself, and it can survive under high temperature degree (17) which mentioned also that, *Streptococcus* may be present in salt water, fresh water and mud, with higher incidence during summer months. This implies that, in aquaculture system some *Streptococcus* occurs naturally in the environment may become endemic and cause disease on a periodic basis. Non-hemolytic *Streptococcus* were opportunistic on stress to assert pathogenicity, so that (6) suggested that, *Cyprinus carpio* can be experimentally infected with non-hemolytic *Streptococcus* by using some stress like high temperature, low dissolve oxygen level and high nitrite concentration.

The dual effect of high summer water temperature on the growth of the *Streptococcus* D organism appear to have been a predisposing circumstance for the pathogenicity of this organism (5).

The present study revealed that intraperitoneally injection of *C. carpio* with non-hemolytic *Streptococcus* resulted in cloudy eyes with erratic swimming as the most important clinical signs (1). This result is in agreement with many researchers, who found that, the I.P. injection of experimental fish's cause some clinical signs and lead at end to death (3, 16, 18. 23, 12, 10, 11).

The present study indicated that *C. carpio* is highly susceptible to I.P. injection whereas contamination of water has no effect in spite that bacteria will enter the digestive system because it may be died due to the effect of enzymes secreted by the digestive system and the affect of secondary immune defense. While I.P. injection makes it easy to enter the macrophage and live inside it, so that bacteria circulating with the blood and cause septicemia infection. Rasheed and Plumb (19) failed to reproduce the disease in Gulf killifish dipped in bacterial suspensions unless they injured the fish prior to the dipping. While in the present study *C. carpio* were failed to show the disease by dipping in bacterial suspension neither with nor without skin abrasion, so normal skin is not a usual portal of entry of non-hemolytic *Streptococcus* into *C. carpio* because it has a highly defense mechanism represented by skin which in addition to the cuticle layer it contain specific immunoglobulin, lysozyme and free fatty acids. All of these have anti pathogenic activity.

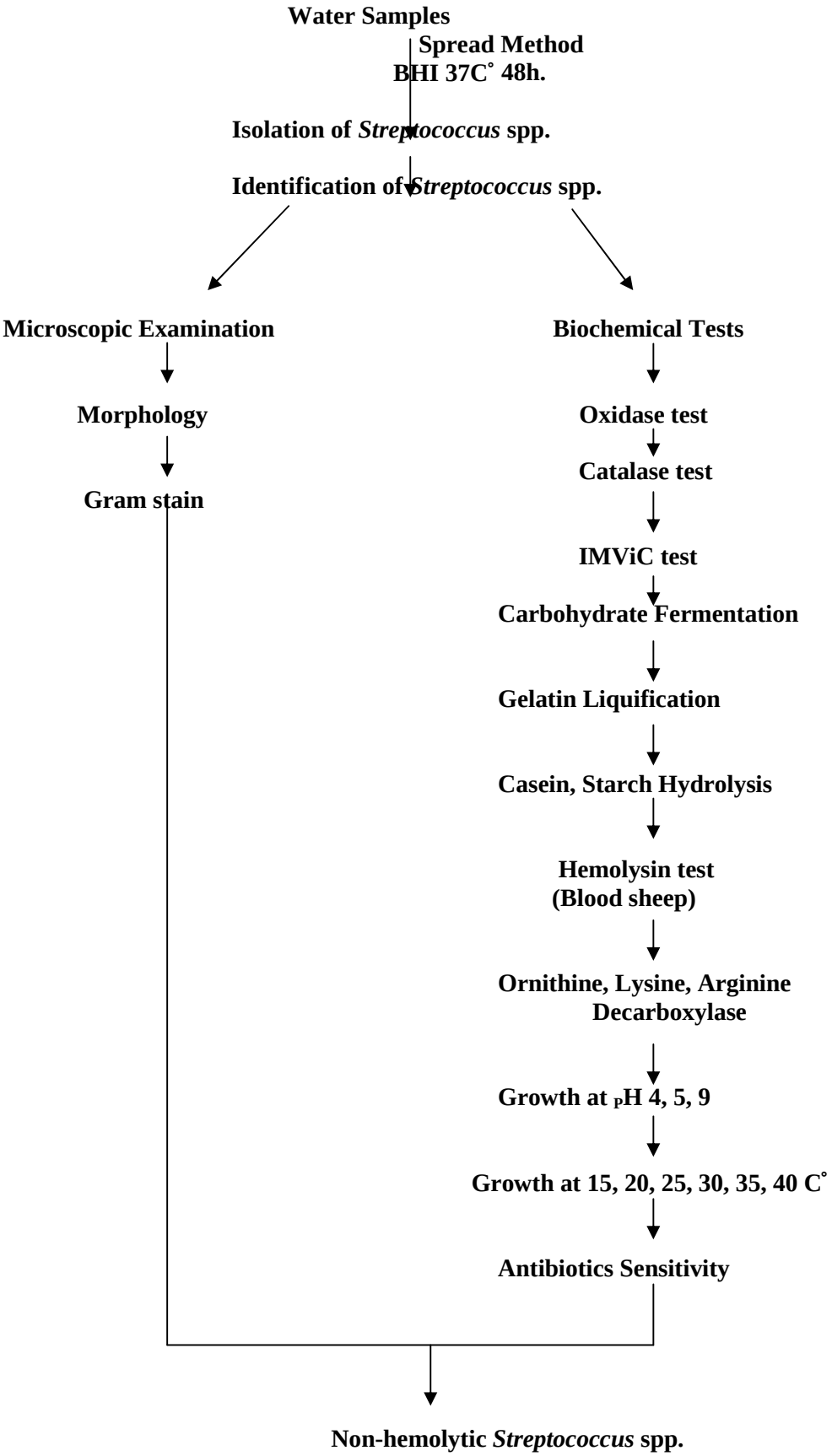

Fig (1): Diagram for isolation and identification of non- hemolytic *Streptococcus* spp.

Table (1): Biochemical characteristics of non-hemolytic *Streptococcus* Spp.

Test	Result
Gram stain	+
Shape	Cocci
Motility	–
Oxidase	–
Catalase	–
IMViC tests	– + + –
O/ F reaction	–/–
Acid from: Sucrose	+
Arabinose	–
Maltose	+
Galactose	–
Rhamnose	–
Glucose	+
Sorbitole	–
Mannitol	–
Mannose	+
Gelatin Liquification	–
Hydrolysis of: Casein	–
Starch	–
Hemolysin test	–
Decarboxylase of :Arginine	+
Ornithine	–
Lysine	–
Growth at pH: 4	–
5	–
9	+
Growth at Temp.:15	–
20	+
25	+
30	+
35	+
40	–

الوفيات 100% في الأسماك المحقونة عن طريق الخلب بينما لم تتأثر الأسماك في المجاميع الأخرى.

البحث مستل من أطروحة الباحث الثالث