Bacterial Diversity of Rainbow Trout (Oncorhynchus Mykiss) Affected by Aquamin-T Disinfectant

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***Abstract*—**Chloramine-T is presently available in the USA to control mortalities associated with bacterial gill disease or external columnaris only through an Investigational New Animal Drug Permit authorized by the US Food and Drug Administration (FDA).

In this study the bacterial flora occurring in gills and skin of healthy Rainbow Trout fries cultured in Coldwater Fishes Research Center, Iran were estimated both quantitatively and qualitatively after disinfection by Aquamin-T.

Aquamin-T was applied once daily for 30 min on five consecutive days to determine the effect of Aquamin-T bath exposures on skin and gill bacteria of Rainbow Trout. The study design incorporated two treatment groups that were exposed to Aquamin-T at 10 and 15mg/lit recommended daily dose and one control group. Fish were observed for five days after each exposure to monitor for latent mortality caused by exposure to Aquamin-T. Treatment groups for all tests consisted of 9 tanks (capacity 900 lit). Water chemical parameters including temperature, dissolved oxygen, pH,hardness, CO2, Nitrate, ammonium and alkalinity were determined daily in all groups. Total viable counts of bacteria in skin and gills were calculated.

The aim of this study is survey on different doses of Aquamin-T.on bacterial diversity and load in rainbow trout skin and gills.

***Keywords*—**Aquamin-T, Bacterial Diversity, Rainbow Trout.

I. Introduction

n-Sodium-n-chloro-p-toluenesulfonamide (chloramine-T) is a disinfectant that is effective in reducing fish mortalities caused by BGD (From, 1980; Bullock et al., 1991).Chloramine-T (Cl-T) is an external disinfectant whose therapeutic use to reduce mortalities associated with external bacterial infections of freshwater fish in U.S. aquaculture is presently under review by the US Food and Drug Administration(FDA) (Gaikowski,M. P. *et al.*, 2007). chloramines are the most widely used disinfectants in water treatment plants and istribution systems because they are effective, relatively inexpensive, and remain active within the systems for a considerable length of time. (Calvert, 1940; Morris, 1967).Therefore, understanding the chloramines, is important for adequate disinfection of pathogens during water treatment and prevention of microbial regrowth in the water distribution systems. With large numbers of water utilities in the U.S.(Lee, W & Westerhoff, P. 2009).

From (1980) reported that a 1 h exposure to Cl-T at 6.5 mg/L controlled bacterial gill disease (BGD) in brown trout

Salmo trutta, Atlantic salmon Salmo salar, and brook trout Salvelinus fontinalis. Studies conducted by the U.S. Fish and Wildlife Service and the State of Pennsylvania indicate that Cl-T treatment at 10 to 20 mg/L for 60 min can control mortalities associated with BGD in salmonids and prevent mortalities associated with BGD in esocids (Bullock et al., 1991; Bowker and Erdahl, 1998; D. Prater, US Food and Drug Administration, personal communication; V.K. Dawson, US Geological Survey, personal communication). Chloramine-T also effectively controlled external columnaris disease on walleye Sander vitreum when administered at 10 mg/L (J.J. Rach, US Geological Survey, personal communication) and on goldfish Carassius auratus intentionally infected with Flavobacterium columnare when administered at 15 mg/L (Altinok, 2004). The proposed treatment regimen for Cl-T in U.S. aquaculture is up to four 60-min exposures of between 10 and 20 mg/L administered once daily on nsecutive or alternate days (Meinertz et al., 2004). Our objective was to determine the effects of on different doses of Aquamin-T 10 mg/L and 15 mg/L on bacterial diversity and load in rainbow trout (*Oncorhynchus Mykiss*) skin and gills in Iran.

II. Materials & Methods

A trial was conducted to evaluate the effects of 10 and 15 ppm Aquamin-T to disinfect 360 healthy rainbow trout (oncorhynchus mykiss) fries (69±3 g, 34.2±0.37cm) cultured in the coldwater researches center, Iran.

Aquamin-T was applied once daily for 30 min on five consecutive days to determine the effect of Aquamin-T bath exposures on skin and gills bacteria of rainow trout.The study design incorporated two treatments 10 mg/L and 15 mg/L and one control group.The fishes were randomly divided in nine 900 liter round concrete ponds each containing 40 fish, with a continuous water flow of 5 liter per minute. The ponds were supplied with a well water (temp. range: 14±0.2; DO2: 6.8

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±0.2 mg/l; pH= 7±0.3).Treatment group for test consist of 4 fish picked randomly from each pond . The changes in bacterial colony forming units (CFUs) per gram of gills and per cubic cm of skin were monitored after each exposure. Live fries specimens were transfered to the laboratory to evaluate the changes in bacterial colony forming units.

A. Gills

All specimens were washed once with a sterile saline solution. Gills were removed with sterile scissors, weighed, and washed three times with sterile saline and suspended in 9 ml of saline solution.The suspension was serially diluted to 10^{-3} and 0.1 ml of the solution was spread in triplicate on TSA plates. The plates were incubated at 25 °C for 24-48 hours.Colonies were divided according to their macroscopic morphology in each plate and bacterial counts was determined.

B. Skin

A cubic centimeter of skin was removed with sterile scissors, washed three times with sterile saline and suspended in 9 ml of it.The suspension was serially diluted to 10^{-4} and 0.1 ml of the solution was spread on TSA plates. The plates were incubated at 25° C for 24-48 hours. Colonies were divided according to their macroscopic morphology in each plate and bacterial counts was determined and consequently colonies were subculture in differential medium plates for identify bacterial genera.

C. Statistics

All data were statistically analyzed by one way analysis of variance (ANOVA) to test the effect of copper sulfate on bacterial flora counts skin and gills. Duncan Multiple range testing was used to compare mean differences. The statistical tests were performed using a SPSS (ver. 10.0) program.

III. Results

No mortality was observed in any of the groups at the end of 5 days disinfection period. The results indicated that Aquamin-T could effectively reduce the number of total bacteria on skin and gills. A mild reduction of the bacterial flora was observed on skin. Aquamin-T with 15 mg/l concentration decreases bacteria flora on gills after 5 times disinfection significantly($p \leq 0.05$).

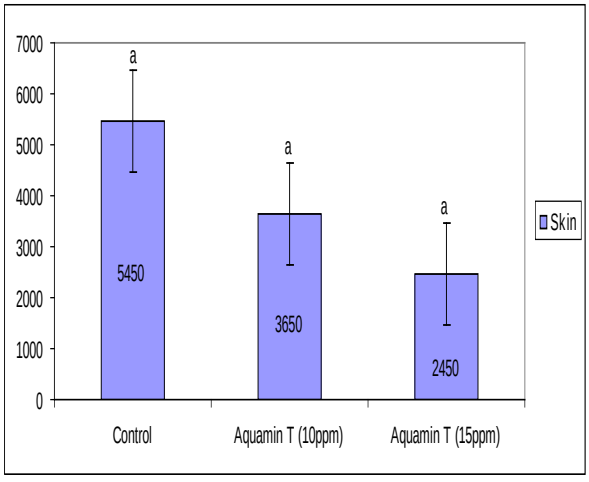

Fig. 1. Bacterial total count in skin in 3 treatment groups affected by Aquamin-T disinfectant

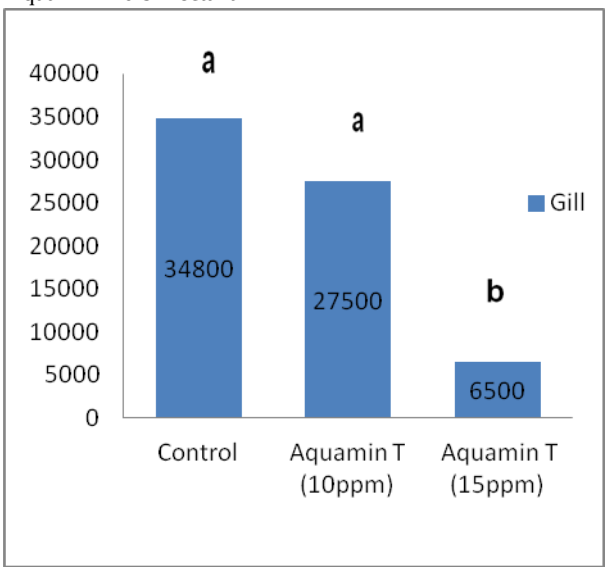

Fig. 2. Bacterial total count in gills in 3 treatment groups affected by Aquamin-T disinfectant

According to fig. 3, In totally 4 different general of bacteria Corinebacterium, Eenterobacteriacea, Staphylococcus, Streptococcus were found in skin specimen that Corinebacterium ,Eterobacteriacea, Staphylococcus, and Streptococcus isolated from control group, as well as only staphylococcus isolated from Aquamin-T 10ppm and Aquamin-T 15ppm treatment

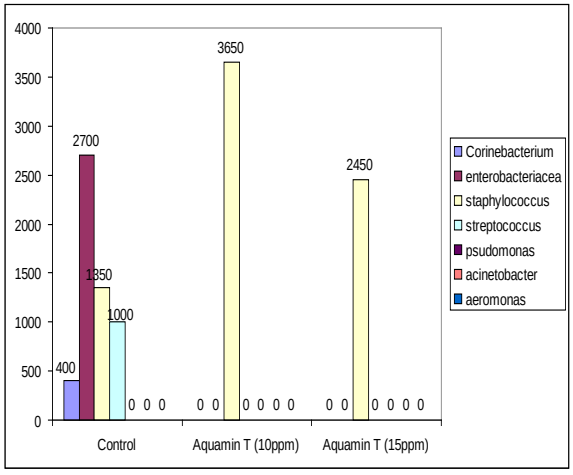

Fig. 3. Bacterial diversity in skin in 3 treatment groups affected by Aquamin-T disinfectant

In case of gill, shown as fig. 4, In totally 6 different general of bacteria were found that Enterobacteriacea, Staphylococcus and Aeromonas from control group, Corinebacterium ,Enterobacteriacea and Staphylococcus from Aquamin-T 10 ppm treatment and Enterobacteriacea, Staphylococcus, Acinetobacter and Psudomonas isolated from Aquamin-T 15ppm treatment.

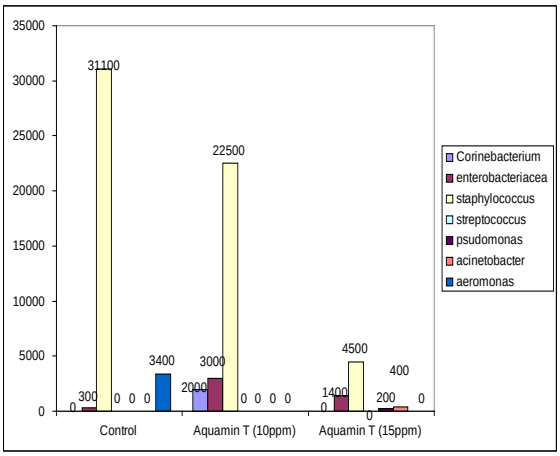

Fig. 4. Bacterial diversity in gills in 3 treatment groups affected by Aquamin-T disinfectant

In this study, 4 kinds of bacteria isolated from skin that all kinds of them including Staphylococcus, Enterobacter, Streptococcus and Corynebacterium observed in control group.

After using Aquamin-T 10 ppm only 1kind of bacteria that belong to coccus gram positive group (Staphylococcus) grown up and after using Aquamin-T 15 ppm only Staphylococcus grown up too.

IV. Discussion

According to colwell (1962) isolated 8 types of bacteria from various fish species in pugeet sound , USA that Enterobacteria, Coryneforms, Micrococcus and Aeromonas were the same as research.And similar studies observed by Horsely (1973) in Atlantic Salmon (*Salmo salar*) that Micrococcus, Staphylococcus and Aeromonas were the same as our result.Gillespi and Macrae (1975) in sea Mullet *(Mugil* cephalus), whiting (*Sillago ciliate),* bream (*Mylio australlis*) and flat head (*Platycephalus fuscus*) reported Micrococcus, Coryneforms , Staphylococcus and Aeromonas that were the same as our result.Gilmour *et al* (1976) reported isolating Anaerogenic Aeromonas and Enterobacteriacae in Plaice (*pleuronectes platessa*).Austin (198) isolated Coryneforms and Enterobacteriacea from Turbot (*Scophetalmus maximus*).In this study, six kinds of bacteria isolated from gill that four kinds of them including Staphylococcus, Enterobacter, Aeromonas and micrococcous observed in control group. After using Aquamin-T 10 ppm 3 kinds of bacteria that belong to coccus gram positive group (Staphylococcus) and gram negative bacilla group (Enterobacter and Aeromonas) grown up and after using Aquamin-T 15 ppm Staphylococcus, Corynebactrium and Enterobacter grown up.Colwell (1962) isolated Corynebacterium, Micrococcus and Enterobacteriacea from various fishes.Simidue *et al* (1962) reported Aeromonas, Corynebacterium and Micrococcus in flat fish in fish markets, Japan.Horsely (1973) reported isolating Coryneforms, Micrococcus, Enterobacteriacea and Aeromonas from Atlantic Salmon.Trust (1975) isoletad Aeromonas, Coryneforms, Micrococcus and Enterobacteriacea from Rainbow trout.

V. Conclusion

The above studies show similar ranges of bacterial agenera on fish skin and gills although proportions of the different types varied with geographical location. In addition to in present study after using disinfectant Aquamin-T obvious reduction observed in bacterial count. Especially after using Aquamin-T 15 mg/L as disinfectant in gills specimen. So Aquamin-T 15mg/L is a very useful selection to prevent mortalities associated BGD in Rainbow Trouts.

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