EFFECTS OF ANTIBIOTIC ON BACTERIAL FLORA IN MRIGAL FISH (*CIRHINUS CIRHOSUS*, BLOCH, 1795) UNDER LABORATORY CONDITION

S.A.Haque[1*], M.S.Reza[2], M.A.Islam[3], V.C.Roy[4], M.A.Alam[5]

[1]Department of Fisheries Technology, Sheikh Fajilatunnesa Mujib Fisheries College, Melandah, Jamalpur, Bangladesh.
[2]Department of Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202.
[3]Scientific Officer, Bangladesh Fisheries Research Institute,Shrimp Research Station, Bagerhat, Bangladesh.
[4]Department of Fisheries Technology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.
[5]Department of Fisheries Biology and Genetics, Sheikh Fajilatunnesa Mujib Fisheries College, Melandah, Jamalpur, Bangladesh.

ABSTRACT: *The Study was conducted on the effects of antibiotic on bacterial flora under laboratory condition. Oxytetracycline (OTC), the most widely used antibiotic, was fed to the Mrigal (average body weight 25g) at the rate of 2g/kg through fish diet and bacterial content was estimated for a period of 20days.Total 8 aquariums were used, where 3 aquariums for control and 5 aquariums for replication of the treatment. Physico-chemical parameters of aquarium water were determined where temperature,of culture aquariums were more or less similar.Before antibiotic treatment dissolve oxygen (DO), pH and total hardness was 7.80±0.10mg/l, 4.10±0.10, and890.00±10.00ppm, respectively which reached a value 9.90±0.10mg/l, 5.90±0.10and 710.00±10.00ppm, respectively indicating the changes after 20 days. Prior to antibiotic treatment,bacterial load was $2.90 \pm 0.06 \times 10^3$cfu/ml in aquarium water, $6.90 \pm 0.20 \times 10^5$cfu/g in fish gills, $4.70 \pm 0.10 \times 10^7$cfu/g in fish intestine,and $85.25 \pm 3.38 \times 10^5$in fish skin respectively which was Significantly reduced to $1.25 \pm 0.03 \times 10^3$cfu/ml in water, $5.42 \pm 0.20 \times 10^5$cfu/g in gills, $3.33 \pm 0.05 \times 10^7$ cfu/g in intestine, and $11.24 \pm 0.01 \times 10^5$in fish skin respectively after 20 daystreatment period. Water and fish samples were also analyzed for bacteria were completely absent before and after antibiotic treatments.*

KEYWORDS: Antibiotic, Physico-chemical parameters, Bacterial load, *Cirhinus cirhosus*

INTRODUCTION

In aquaculture, important inputs are required for successful fish production is chemicals and drugs. Among the chemicals used in aquaculture,disinfectants (e.g.hydrogenperoxide and malachite green), antibiotics (e.g. sulfonamides, oxytetracycline and tetracycline's). Among the chemicals of Bangladesh oxytetracycline is widely employed to treat bacterial infections in aquaculture

farms.Oxytetracycline is one of the most widely used antibacterial agents in aquaculture worldwide (Smith *et al.*, 1994). The vast majority of oxytetracycline supplied in mediated feed can be found in hatchery effluent at concentrations that account for nearly the entire drug supplied.These chemical has effect on the water quality parameters, bacterial population and also the storage condition of fishes. So indiscriminately use of these chemical have long term effect on fish and also consumers.The microorganisms influence the water quality and are closely associated with the fish physiology and diseases. Nutrients increase is readily incorporated with the microbial community and ultimately into the fish biomass. The success of practical aquaculture depends on water quality which is greatly influenced by aquatic microorganism. Usually, aquatic animal including fish takes a large number of bacteria through their food and drinking water which accumulate in their intestine.

Antibiotics are a group of compounds used annually in large quantities to treatbacterial diseases in humans as well as to promote growth and prevent disease in animalssuch as cattle, swine, poultry, and fish during animal food production. Often, thesecompounds are only partially metabolized by the user and can subsequently be excretedinto wastewater, manure, or directly into water systems during fish farming in suchcases, antibiotics may further be exposed to soils and sediments as well as to surfacewater and groundwater due to agricultural runoff and wastewater effluent contamination.The presence of antibiotics in such water systems is of concern primarily due tothe potential to trigger antibiotic-resistant bacteria in the environment.

Following the discovery of the growth promoting and disease fighting capabilities of antibiotics, fish farmers began using such drugs in feeds. Antibiotics routinely used for treatment of human infections are also used for animals, for therapy, prophylactic reasons or growth promotion. For the last named purpose, sub therapeutic doses of antibiotics usually have been used, and this has contributed to promoting resistance. These uses of antibiotics can also create antibiotic resistance in non-pathogenic bacteria, the resistance genes of which can be transferred to disease-causing bacteria, resulting in antibiotic-resistant infections for humans.The report from the invitational European Union conference on *The Microbial Threat* (EU, 1998) recognized that the major route of transmission of resistant microorganismsfrom animals to humans is through the food chain.

The Mrigal is the common fish in Bangladesh. Major portion of fish protein comes from these fish. These are highly nutritious fish food. Its taste, high protein content and rapid growth make it one of the most important fish under of the Indian major carp groups. These fish are now cultured throughout the Bangladesh. In the fish pond mrigal is the major species for culture. Thoseculture ponds antibiotics are used indiscriminately.Although very little work in this field has been done in the past. The study carried out the changes of Physico-chemical parameters and the bacterial loads of different parts are easily understood**.**Considering the importance of mrigalfish in Bangladesh aquaculture, the study was carried out to determine the changes in its physico-chemical parameters and bacterial population.

MATERIALS AND METHODS

Experimental site

Eight glass aquariums (size 37cm×30cm×60cm) were set at the Laboratory of Fish Harvesting, Dept. of Fisheries Technology, BAU , where 3 aquariums for control treatment, and 5 aquariums for antibiotic treatment.These aquaria were filled to a depth of 15 cm with tape water collected from the Dept. of Fisheries Technology laboratory, BAU. The experimental aquariums were designed as control aquarium-1, control aquarium-2, control aquarium-3, and aquarium-1, aquarium-2, aquarium-3, aquarium-4, aquarium-5, for the convenience of study. The mrigal fry was collected from Field Laboratory Complex of Faculty of Fisheries, BAU and released the aquarium. In the aquarium-1,aquarium-2,aquarium-3, aquarium-4 and aquarium-5 feed contained 2g/kg oxytetracycline and was used for 3 weeks. The aerators were set with each aquarium. The aquarium water was changed daily. Physico-chemical parameters were determined for each aquarium 4 days interval.

Physicochemical analysis

The water quality parameters were recorded 4 days interval throughout the experimental period. Water quality measurements and sample collection were made between 9.00am to 11.00 am on each sampling Day. Water samples were collected from each aquarium to a depth of 35 cm. On each sampling Day, 500 ml water was collected in a clean black plastic bottle with cap from each a very carefully without any agitation. Each bottle was then marked with respective aquarium number with three replicates. Water quality parameters such as temperature, dissolved oxygen, hardness, and pH were measured. Water temperature was recorded in the laboratory with the help of a centigrade thermometer (div=0.1°C). Other Parameters such as dissolved oxygen, hardness and pH were measured using HANNA Test kit, Hanna Instruments Ltd., Germany.

Microbiological analysis

Sampling was done every alternative day for microbial analysis of water, fish gills,intestine, and skin for antibiotic treated fish.Aquarium water samples were collected in sterile glass bottles (250 ml) 15-20 cm below the water surface from three different locations in each aquarium in every sampling. Three samples were combined to make a composite sample for bacteriological analysis in the laboratory. Appropriate sample dilutions were made (10^{-1} to 10^{-5}) with sterile physiological saline (0.85% w/v NaCl) in deionized water. Aliquots of 0.1 ml of the serial dilutions were inoculated onto nutrient media in duplicate using the spread plate method (APHA, 1998) as this medium recovered most of the bacteria.

All plates in duplicate on sterile petridish were done on sterile nutrient agar media. From sample solution of different dilutions 0.1 ml samples were taken by a micropipette and transferred aseptically into the pre-prepared agar plates by raising the upper lid sufficient enough to enter the tips of the pipette. The samples were then spread homogenously and carefully by sterile flamed L-shaped glass rod throughout the surface of the media until the sample were dried out. For total heterotrophic aerobic bacterial counts of pond water, sediment, gills and intestine, all the inoculated plates were incubated at 28^{0}C for 24-48 hrs. The colony-forming units (cfu) were counted under a Quebec darkfield colony counter (Leica, Buffalo. NY. USA) equipped with a

guide plate ruled in square centimeters. Plates containing 30-300 colonies were used to calculate bacterial population results, recorded as cfu per unit of sample by using following formula:

$$\text{cfu/g} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{wt. of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Determination of *Escherichia coli* and *Salmonella* spp. by selective media
Escherichia coli and *Salmonella* spp. were determined by using selective media EMB-agar and SS-agar, respectively. Bacterial inoculums from the spread plate were transferred into the selective media. Growth of bacterial colony in EMB-agar and SS-agar media indicate the presence of *E. coli*.and*Salmonella* spp., concurrently.

Statistical Analysis
The data obtained in the experiment were recorded and preserved in computer. The data obtained in the experiment were analyzed by using CRD of MSTAT programmed. Significant differences were determined among treatments at the 5 % level ($p< 0.05$).

RESULTS AND DISCUSSION

Physicochemical parameters of water
The water quality parameters of antibiotic treated aquaria were recorded using commercial test kits (HANNA Test kit, Hanna Instrument Ltd. Germany). Water temperature (°C), pH, dissolved oxygen (mg/l), and hardness (ppm) were measured every 3 days interval. Water quality parameters of oxytetracycline treatment had been presented in Table 1.

Table 1. Water quality parameters with oxytetracycline treatment in mrigal fish (*Labeorohita*) aquariums

Days after treatment	Temperature (ºC)	pH	Dissolve Oxygen (mg/l)	Hardness (ppm)
0	28ºC	7.80±0.10	4.10±0.10	890.00±10.00
4	28ºC	8.90±0.20	4.40±0.20	860.00±15.00
8	27ºC	9.10±0.10	4.60±0.20	840.00±20.00
12	27ºC	9.30±0.10	4.70±0.10	790.00±10.00
16	29ºC	9.70±0.10	5.80±0.10	730.00±6.00
20	28ºC	9.90±0.10	5.90±0.10	710.00±10.00

Results (Mean ± S.D)

Changes in water quality parameters of aquarium water
The temperatures of the aquariums water were found similar. The mean values of water temperature were 28.33°C. Prior to the antibiotic treatment there was the lowest pH value was 7.80. After antibiotic treatment the pH values were increased. In the day 0-4, 4-8, 8-12,12-16,16-20,the pH values were increased at 9.90. The pH value before antibiotic treated in the pond water ranging

from 7.58-7.92, and after two weeks in the antibiotic treated pond water ranging from 7.57-7.69 (Kashem, 2012).The values of pH ranged from 7.9 to 8.2 in aquarium condition (Ferdous, 2012).The pH greater than 7.0 the ideal values of various physico-chemical parameters (Ferdous, 2012).The values of pH ranged from 7.9 to 8.2 in aquarium condition (Boyd, 1998).

Before treatment there was the lowest dissolve oxygen (D.O) value 4.10mg/l.After the antibiotic treatment, the dissolve oxygen (DO) values were increased. The highest dissolve oxygen (DO) value found5.70mg/l into theday 4-8, 8-12,12-16,and16-20.Without antibiotic treated pond water DO ranged from 5.52 to 5.8 mg/l, after two weeks in the antibiotic treated pond water dissolve oxygen ranged from 5.44 to 5.86 mg/l (Kashem 2012). The DO from 4 to 8 mg/l were suitable for fish culture (DoF,2001).The DO ranging from 2.2 to 8.8 mg/l, in ponds of BAU campus, Mymensingh (Dewan*et al.*, 1991).In the pond water the DO values ranged from 5.98 to 6.53 mg/l (Kunda*et al*.,2008).

The highest total hardness value 890.00ppm of mrigal fish aquarium was in the day-0(before treatment).After the antibiotic treatment, the total hardness values were decreased largely. The lowest hardness value found 730.00 ppm into the day 16-20.In the aquarium water the total hardness was above 960 ppm in aquarium water (Ferdous ,2012).
The present study result was closely related to the results of (Kashem, 2012), (Ferdous ,2012), (Dewan*et al.*, 1991), (Kunda*et al*.,2008), and (Boyd, 1998).

Quantitative analysis of bacteria
The results of the quantitative estimation of aerobic heterotrophic bacteria in the experimentalaquarium water, the gill filaments and intestine of mrigal fish are given in Table 2.
Table 2. Bacterial load in aquarium water, gills and intestinal content of mrigal fish

Table 2. Bacterial load in aquarium water, gills and intestinal content of mrigal fish

Days after treatment	Water (cfu/ml)	Gill (cfu/g)	Intestine (cfu/g)	Skin (cfu/cm^2)
0	$2.90\pm0.06\times10^3$	$6.90\pm0.20\times10^5$	$4.70\pm0.10\times10^7$	$85.25\pm3.38\times10^5$
4	$2.72\pm0.05\times10^3$	$6.75\pm0.10\times10^5$	$4.45\pm0.05\times10^7$	$76.17\pm3.38\times10^5$
8	$2.10\pm0.01\times10^3$	$6.34\pm0.20\times10^5$	$4.23\pm0.10\times10^7$	$64.87\pm1.53\times10^5$
12	$1.84\pm0.02\times10^3$	$6.12\pm0.10\times10^5$	$4.10\pm0.10\times10^7$	$42.53\pm1.06\times10^5$
16	$1.70\pm0.03\times10^3$	$5.79\pm0.20\times10^5$	$3.71\pm0.05\times10^7$	$21.73\pm0.05\times10^5$
20	$1.25\pm0.03\times10^3$	$5.42\pm0.20\times10^5$	$3.33\pm0.05\times10^7$	$11.24\pm0.01\times10^5$

Results (Mean ± S.D)

The Changes in bacterial load
Before treatment there was the highest bacterial load in aquarium water sample $2.90\pm0.06\times10^3$cfu/ml. After antibiotic treatment the bacterial load of water sample were gradually decreased. The lowest bacterial load of water sample found $1.25\pm0.03\times10^3$cfu/ml in the last day 16-20.In the starting day of the experiment the highest bacterial load in gill sample was $6.90\pm0.20\times10^5$cfu/ml. After antibiotic treatment the bacterial load of gill samples were decreased

in slow process. The lowest bacterial load of gill sample found $5.42\pm0.20\times10^5$cfu/ml into the day 16-20.

The highest bacterial load in intestine of fish sample was $4.70\pm0.10\times10^7$cfu/g in the first day of the experiment. After antibiotic treatment the bacterial load of intestine sample were decreased very slowly. The lowest bacterial load of intestine sample was found $3.33\pm0.05\times10^7$cfu/g into the day16-20. The highest bacterial load in skin of fish sample was $85.25\pm3.38\times10^5$cfu/g in the day-0(before treatment). After antibiotic treatment the bacterial load of skin samples were decreased very slowly. The lowest bacterial load of skin sample was found $11.24\pm0.01\times10^5$cfu/g into the day 16-20.

The bacterial load in culture ponds were in the ranged of $4.9\pm1.03\times10^3$-$5.75\pm1.0\times10^3$cfu/ml in water, $5.62\pm1.0\times10^7$-$6.60\pm1.02\times10^7$cfu/g in sediments, $6.77\pm1.0\times10^6$-$7.57\pm1.0\times10^6$cfu/g in mrigal gill filaments, $7.94\pm1.01\times10^6$-$9.12\pm1.0\times10^6$cfu/g in silver carp gill filaments, $6.02\pm1.02\times10^7$-$8.32\pm1.0\times10^7$cfu/g in mrigal fish intestinal content and $5.12\pm1.05\times10^7$-$6.60\pm1.0\times10^7$cfu/g in silver carp fish intestinal content. After antibiotic treatment total viable counts in culture ponds were $3.1\pm1.19\times10^3$-$3.1\pm1.20\times10^3$cfu/ml in water; $4.27\pm1.10\times10^7$ - $3.1\pm1.13\times10^7$cfu/g sediment; $5.37\pm1.01\times10^6$-$3.09\pm1.19\times10^6$cfu/g in mrigal gill; $3.16\pm1.29\times10^6$ - $4.07\pm1.20\times10^6$cfu/g in silver carp gill; $2.69\pm1.12\times10^7$ - $4.68\pm1.12\times10^7$cfu/g in mrigal intestinal content and $2.95\pm1.1\times10^7$ - $2.63\pm1.17\times10^7$cfu/g in silver carp intestinal content.Theviable counts were significantly varied between the control ponds and treatment ponds(Kashem,2012).The total viable counts were $6.7\pm2.1\times10^3$-$2.7\pm1.1\times10^3$cfu/ml in pond water (Al-Harbi and Uddin,2004).Total viable counts of bacteria (measured as colony-forming units. cfu) were in the range of $5.6\pm0.8\times10^3$ to $2.4\pm1.2\times10^4$ cfu/ml in pond water; $9.3\pm1.1\times10^6$ to $1.9\pm1.5\times10^5$ cfu/g in sediment; $7.1\pm0.7\times10^5$ to $8.7\pm1.1\times10^6$cfu/g in the gills; and $3.4\pm1.8\times10^6$ to $5.8\pm0.4\times10^7$cfu/g in the intestine of tilapia (Al-Harbi and Uddin, 2003).The organic matter influences the load and composition of microbial population (Rheinheimer, 1985). On the other hand bacterial flora in fish is the reflection of aquatic environments (MacFarlane *et al*., 1996).The bacterial load were $9.8\pm0.9\times10^5$ to $4.2\pm3.1\times10^6$cfu/g in fish gills.The total viable counts of common carp pond waters, sediment, gills and intestine and were$1.2\pm2.9\times10^4$ to $2.5\pm3.5\times10^5$ cfu/ml; $9.3\pm2.1\times10^7$ to $2.7\pm3.5\times10^9$ cfu/g; $4.3\pm2.9\times10^6$ to $1.6\pm3.9\times10^7$ cfu/g; and $8.7\pm4.1\times10^9$ to $5.4\pm3.2\times10^5$ cfu/g, respectively (Al-Harbi and Uddin,2007).
The experimented result was closely associated with (Al-Harbi and Uddin, 2007), (Kashem,2012), (Rheinheimer, 1985).

Analysis of pathogenic bacteria in aquariums water
Escherichia coli and *Salmonella spp*. were analyzed. They are called sanitary index organism. They have great influence in quality of end products. There were not present *E. coli* and *Salmonella* spp. in the aquariums before treatment and after treatment.

CONCLUSIONS

Thephysicochemical and bacteriological analysis of the aquarium fishes before treatment and after treatment were done carefully. The Oxytetracycline was used for aquarium treatment. Oxytetracycline is widely used as a antibacterial agent. Initial load of bacteria has great

contribution to the quality of end product. Before antibiotic treatment whole physicochemical and bacteriological study of experimental fishes were done. Antibiotic treatment was done at 2g/kg through pelleted feed. After antibiotic treatment both physicochemical and bacteriological analysis were done. In the physicochemical syudy after antibiotic treatment the pH and dissolve oxygen increased gradually,but the total hardness reduced destrically. Bacterial loads were reduced significantly in aquariums when antibiotic medicated diets were used. *Escherichia coli* and *Salmonella* spp. were not detected throughout the experiment period.

REFERENCES

Al-Harbi,and M.N. Uddin. (2004) Seasonal variation of bacterial ponds in Saudi Arabia used for tilapia culture. *J. Appl. Aqua*., 16:53-57.

Al-Harbi, and M.N. Uddin.(2003). Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromisniloticus× O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aqua. Res.*, 34: 43-48.

Al-Harbi, and M.N. Uddin. (2007) Seasonal trends in gill bacterial flora of hybrid tilapia, *Oreochromisniloticus× Oreochromisaureus.J. Appl. Aqua.,*19:61-69.

Boyd, C. E. and L. Massaut, (1998) Risks associated with the use of chemicals in pond aquaculture. *Aquac.Eng.*, 20.113-132 pp.

Dewan, S., M.A. Wahab, M.C.M. Beveridge, M.H. Rahman and B.K. Sarker. (1991) Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carps fry and fingerlings grown in extensively managed, rain-fed ponds in Bangladesh. *World Mari. Soc*., 10:571-574.

DoF (2001) Saronica, MatshaSaptahSankalan 2001. Department of Fisheries (DoF), the government of people's republic of Bangladesh, Dhaka, 79p.

FAO. (2010) Fisheries statistics: Aquaculture production, 88/2. FAO, Rome, Italy, pp 12.

Ferdous. J, (2012) Effects of some water treatment chemicals on water quality .M.Sc. Thesis., p.18-35.Department of Fisheries Technology.BAUMymensingh, Bangladesh.

Kashem, M.A. (2012) Effects of antibiotic on bacterial flora in fish culture ponds. M.Sc. Thesis., p.22-31. Department of Fisheries Technology.BAU,Mymensingh, Bangladesh.

Kunda, M., M.E. Azim, M.A. Wahab, S. Dewan, N. Roos, and S.H. Thilsted (2008) Potential of mixed culture of freshwater prawn, *Macrobrachiumrosenbergii* and self-recruiting small species mola, *Amblypharyngodonmola* in rottional rice-fish/prawn culture systems in Bangladesh. *Aqua. Res.,* 39:506-517.

Macfatlan RD.JJ. Maclaghim, and GI. Bollok. 1996. Quantitative and qualitative analysis of gut flora of sea bass from estuary and coastal marine environments. *J Wildlife Dis.*, 22:344-348.

Raihan, A. (2001) To assess the effects of addingpunti, *Puntiussophore* and Mola, *Amblypharyngodonmola* in carp polyculture. M.S. dissertation, Dept. of Fisheries Management, BAU,Mymensingh, Bangladesh.

Rheinheimer, G. (1985) Aquatic Microbiology. 3rdedn. John Willy and Sons.Chichster.257 p.

Smith, R. P., Hiney, M. P. and Samuelson, O. B.(1994) Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annu. Rev. Fish Dis.*, 4. 273-313.