

# Effect of Oxytetracycline on bacterial load of *Hypophthalmichthys molitrix* (silver carp) fish in culture pond of Mymensingh, Bangladesh

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**Abstract:** Investigation was conducted to assess the influence of antibiotic on bacterial flora of *H. molitrix* where other physicochemical parameters of culture ponds were suitable for microbes and fish. The experiments were performed from April to October, 2012 in the ponds of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Oxytetracycline (OTC) was used as an antibacterial agent, which is a broad spectrum antibiotic widely use in Bangladesh aquaculture. Pelleted feed with 2g/kg OTC was applied in culture ponds. Physicochemical parameters of water like temperature, pH, dissolved oxygen, alkalinity, ammonia and nitrate were recorded weekly 28.99 to 31.09°C, 7.58-7.95 ppm, 5.36-5.86 mg/L, 86.31-111.99 mg/L, 0.20-0.30 mg/L and 0.11-0.17 mg/L, respectively. All the parameters were in suitable range for fish culture. Total viable counts of bacteria (measured as colony-forming units) were in the range of  $4.9 \pm 1.03 \times 10^3$ -  $5.75 \pm 1.0 \times 10^3$  cfu/mL in pond water,  $5.62 \pm 1.0 \times 10^7$ -  $6.60 \pm 1.02 \times 10^7$  cfu/g in sediments,  $7.94 \pm 1.01 \times 10^6$ - $9.12 \pm 1.0 \times 10^6$  cfu/g in gills,  $5.12 \pm 1.05 \times 10^7$ - $6.60 \pm 1.01 \times 10^7$  cfu/g in intestinal contents of *H. molitrix* in control ponds respectively. After OTC treatment the bacterial range were  $3.1 \pm 1.19 \times 10^3$ - $3.1 \pm 1.20 \times 10^3$  cfu/mL in water,  $3.1 \pm 1.13 \times 10^6$ - $4.27 \pm 1.10 \times 10^6$  cfu/g in sediment,  $3.16 \pm 1.29 \times 10^5$ - $4.07 \pm 1.20 \times 10^5$  cfu/g in gill,  $2.63 \pm 1.17 \times 10^6$ - $2.95 \pm 1.1 \times 10^6$  cfu/g in intestinal contents of *H. molitrix* treated ponds respectively, indicating reduction of overall bacterial load below 1 log in sediment, gills and intestinal contents of *H. molitrix* significantly. (P<0.05)

**Keywords:** Oxytetracycline (OTC) , Pelleted feed, *H. molitrix*, Bacterial load.

## Introduction

World aquaculture has cropped up tremendously during the last ten years becoming an economically important industry (Subasinghe *et al.*, 2009). In contrast to other animal production sectors, aquaculture is highly dynamic and characterized by an enormous diversity of species raised both in natural and artificial systems (Walter and Winton, 2010). The success of field aquaculture solely depends on water quality which is greatly influenced by aquatic microorganism. Numerous investigations have been carried out on the microbiology of freshwater and marine environment in different parts of the world. The bacterial contents of the water used for growing fish affects the quality of the fish as well as fish products produced. In every microbial habitat the nutritional competition between organisms plays an important role in influencing the composition of microflora (Rheinheimer, 1985). Therefore, studying aquatic microbiology is a crying need for water quality management both in aquaculture and for public health. Usually, aquatic animal including fish takes a large number of bacteria through food and drinking water which accumulate in their intestine. The microbial ecology of the gastro-intestinal (GI) tract of variety of freshwater and marine fishes has been investigated intensively by many researchers during the last decade (Kim *et al.*, 2007; Yang *et al.*, 2007; Zhou *et al.*, 2009). Intensification and commercialization of production, aquaculture industry faces major problems with bacterial diseases, and vast quantities of chemical and antibiotic products are frequently used to counteract this (Le *et al.*, 2005; Tu *et al.*, 2008). During the last decades, antibiotics were used as traditional strategy for fish diseases management but also for the amelioration of growth and efficiency of feed conversion. On the other hand antibiotics inhibit or kill beneficial micro biota in the GI ecosystem. Among antibiotics OTC is widely

employed to treat OTC belongs to the tetracycline group which exerts antimicrobial action against both Gram (-) and (+) bacteria and it is widely employed to treat bacterial infections in aquaculture farms. (Prescott *et al.*, 2000; Reed *et al.*, 2006; Wang *et al.*, 2004). Therefore, the study was conducted to determine the bacterial load in water, sediments, gills and intestine of experimental *M. molitrix* before antibiotic treatments and after antibiotic treatments in ponds.

## Materials and Methods

**Experimental site:** The experiment was conducted in three (3) earthen ponds of the Faculty of Fisheries, BAU, Mymensingh. The ponds were more or less similar in size (12×15 ft), depth, basin conformation, bottom-soil type and contour. Ponds were classified into two groups viz. (a) Control pond without antibiotic medicated diet fed (pond no.-3), (b) Oxytetracycline treated ponds (pond no. 1 and pond 2). Pond-1 and 2 were designated as treatment-I and treatment-II where pelleted feed containing 2g/kg oxytetracycline was used for two weeks (Table 1). The ponds were devoid of aquatic vegetations, well exposed to sunlight and completely free from flooding. Rain water and water from deep tube-well were the main sources of water in the experimental ponds during the study period.

**Physicochemical analysis:** Physicochemical parameters were determined for each treatment on weekly basis between 9.00-10.00 hours. Water samples were collected from each pond from surface to a depth of 15-20 cm. On each sampling day, 250 mL water was collected carefully in a clean black plastic bottle with cap from each pond. Each bottle was then marked with respective pond number with three replicates. The physicochemical parameters eg. dissolved oxygen (DO), pH, total alkalinity, ammonia and nitrite-nitrogen and temperature were observed on the spot and were determined by using univesal pocket meters.

Table 1. OTC treatment rate, feeding duration and regime of pond-1, pond-2 and pond-3

Pond no.	Treatment	Feed(Mediated with OTC)	Feeding duration	Feeding regime
1.	I	2g/kg	2 weeks	2 times/day

2.	II	2g/kg	2 weeks	2 times/day
3.	III	Control pond-no OTC	Control	2 times/day

**Sampling for bacteriological analysis:** For microbial investigations three samplings of water, sediment, gills and intestine of *H. molitrix* were done weekly in culture ponds before antibiotic treatment. After antibiotic treatment samplings were done every alternative day and means were taken.

**Pond water:** 250 ml of water were collected in sterile glass bottles (15-20 cm depth) from three different locations. The three samples were combined to make a composite sample for bacteriological analysis in the laboratory. Aliquots of 0.1 mL of the serial dilutions were inoculated onto nutrient media in duplicate using the spread plate method as this medium recovered most of the bacteria.

**Sediment:** Bottom sediment 10g were collected, with sterile glass bottles submerged to the bottom, from the same three locations in each pond. The sediment sample of 0.2 g was suspended in 1mL of sterile saline solution. 1 ml of the homogenate was serially diluted ( $10^{-1}$  to  $10^{-8}$ ) and treated as previously described for the water sample.

**Fish gills and intestine:** For every sampling, three *H. molitrix* (80-140 g) from each pond were used for bacterial counts in fish organs (e.g gills and intestine). The fish were killed by pitching. Around 0.5-1 g each of gills and intestinal content were taken aseptically and homogenized separately in a mortar. Approximately 0.2 g of each homogenate was then putted in a tube containing 2 mL of sterile saline solution. 1 ml of each homogenate solution

was serially diluted (to  $10^{-7}$ ) and treated in the same way as the pond water samples.

**Aerobic plate count (APC):** All plates in duplicate on sterile petri dish were done for every dilution. 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 spreaded 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°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 centimetres. Plates containing 30-300 colonies were used to calculate bacterial population results, recorded as cfu per unit of sample by using following formula:  $\text{cfu/g} = \{(\text{No. of colonies on petridish} \times \text{dilution factor} \times \text{wt. of total sample solution}) \div \text{wt. of fish sample in gm}\}$ .

**Data analysis:** Data were calculated by using simple MS office programme and SPSS software.

## Results and Discussion

**Physicochemical parameters:** Physicochemical parameters are shown Table 2.

Table 2. Water quality parameters of different treatments (Mean  $\pm$  SD)

Parameters	Oxytetracycline Treated Ponds		Control pond
	2 weeks Treatment (pond 1)	2 weeks Treatment (pond 2)	(Without antibiotics pond-3)
Water temperature (°C)	30.71 $\pm$ 1.263	30.25 $\pm$ 0.753	30.04 $\pm$ 1.046
DO (mg/L)	5.65 $\pm$ 0.212	5.56 $\pm$ 0.204	5.66 $\pm$ 0.142
pH	7.63 $\pm$ 0.064	7.82 $\pm$ 0.133	7.75 $\pm$ 0.165
Alkalinity	99.15 $\pm$ 12.844	97.69 $\pm$ 11.309	97.38 $\pm$ 8.996
NH <sub>3</sub> -N (mg/L)	0.25 $\pm$ 0.050	0.23 $\pm$ 0.029	0.24 $\pm$ 0.044
NO <sub>3</sub> -N (mg/L)	0.12 $\pm$ 0.012	0.14 $\pm$ 0.029	0.12 $\pm$ 0.010

### Bacterial analysis:

**Bacterial analysis of pond waters:** Before OTC treatment all control pond bacterial counts were  $4.9 \pm 1.03 \times 10^3$  cfu/mL (control pond),  $5.3 \pm 1.0 \times 10^3$  cfu/mL (before treatment pond 1(BTP1)) and  $5.75 \pm 1.0 \times 10^3$  cfu/mL (before treatment pond 2 (BTP2)). Pragmatically pond 2 (before treatment) was more bacteria than other two ponds. After 2 weeks of treatment the reduced bacterial load was  $3.1 \pm 1.19 \times 10^3$  cfu/mL for pond1(ATP1) and  $3.1 \pm 1.20 \times 10^3$  cfu/mL for pond 2 (ATP2) (Fig. 1).

**Bacterial analysis of pond sediments:** Before the treatment bacterial counts of sediments of all ponds were  $5.89 \pm 1.02 \times 10^7$ ,  $5.62 \pm 1.0 \times 10^7$  and  $6.60 \pm 1.02 \times 10^7$  cfu/g in control pond, BTP1 and BTP2, respectively. Pragmatically sediments of pond 2 (before treatment) were many bacteria than other two ponds. After 2 weeks of treatment the reduced bacterial load was  $4.27 \pm 1.10 \times 10^6$  cfu/g for treatment pond 1 and  $3.1 \pm 1.13 \times 10^6$  cfu/g for treatment pond 2 (Fig.2).

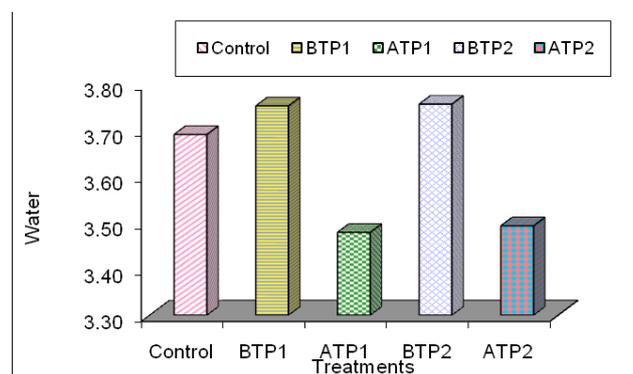
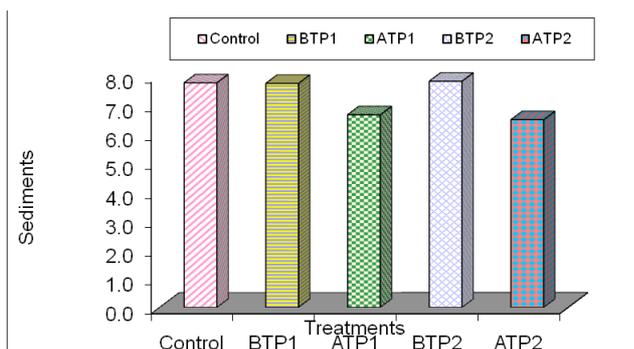
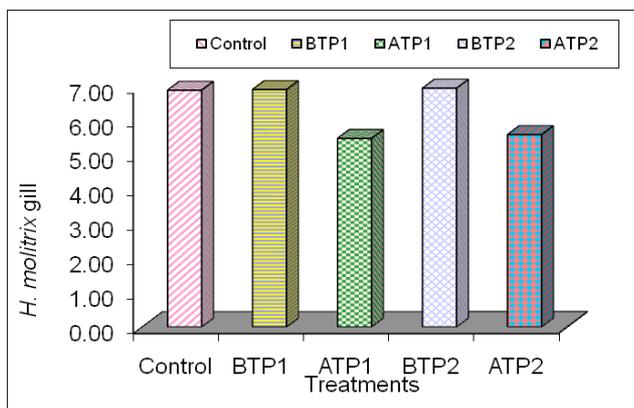


Fig. 1. Bacterial load (log value) in OTC treated and untreated pond waters



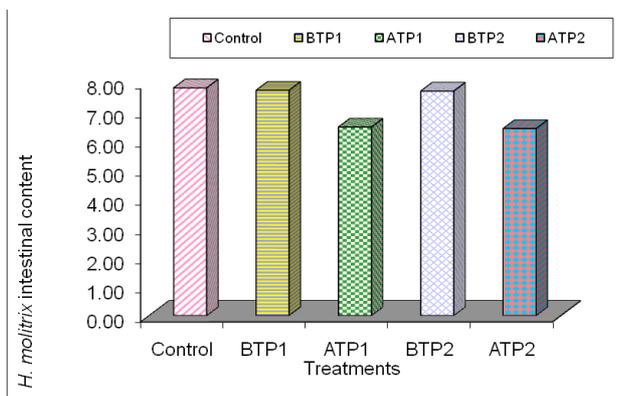
**Fig. 2.** Bacterial load (log value) in OTC treated and untreated pond sediments.

**Bacterial analysis of *H. molitrix* gills:** Before the treatment bacterial counts of *H. molitrix* gill filaments of all ponds were  $7.94 \pm 1.01 \times 10^6$ ,  $8.31 \pm 1.01 \times 10^6$  and  $9.12 \pm 1.0 \times 10^6$  cfu/g in control pond, BTP1 and BTP2. After 2 weeks of treatment the reduction of bacterial load was  $3.16 \pm 1.29 \times 10^5$  cfu/g for treatment pond 1 and  $4.07 \pm 1.20 \times 10^5$  cfu/g for treatment pond 2 (Fig. 3).



**Fig. 3.** Bacterial load (log value) in OTC treated and untreated pond of *H. molitrix* gills

**Bacterial analysis of *H. molitrix* intestinal content:** Before treatment bacterial counts of silver carp intestinal content of all ponds were  $6.60 \pm 1.01 \times 10^7$ ,  $5.37 \pm 1.01 \times 10^7$  and  $5.12 \pm 1.05 \times 10^7$  cfu/g in control pond, BTP1 and BTP2. After 2 weeks of treatment the reduced bacterial load was  $2.95 \pm 1.1 \times 10^6$  cfu/g for treatment pond 1 and  $2.63 \pm 1.17 \times 10^6$  cfu/g for treatment pond 2 (Fig. 4).



**Fig. 4.** Bacterial load (log value) in OTC treated and untreated pond of *H. molitrix* intestinal content

## Discussion

**Water quality parameters:** The experiment was conducted to investigate the effect of antibiotic on bacteriological analysis conducive aerobic plate count before and after OTC treatment where the physicochemical parameters (temperature, DO, pH, alkalinity, ammonia and nitrate) were not any effect on microbial population reduction. Mollah and Haque (1987) revealed that water temperature ranged from 26.00 to 32.44°C in the pond of BAU campus, Mymensingh. MacLean *et al.* (1994) found that water temperature ranging from 28.9 to 29.1°C were favorable for the culture of fish. Microbes can grow at wide range of temperature. The suitable temperature for the growth of bacteria is 30 to 40°C, which is more likely similar to our results. The mean values of DO of the present study were adjacent to the study of the Kohinoor *et al.* (2000). Paul (1998) recorded pH value ranging from 6.51-9.45 was suitable for pond fish culture. In the present study, pH values varied from 7.26 to 7.79, which was more or less similar to the findings of Ali *et al.* (2004). Dewan (1991) observed higher total alkalinity during winter and lower alkalinity during monsoon. (NH<sub>4</sub><sup>+</sup>) is relatively nontoxic. MacEvoy (2002) noted that the maximum safe concentration of ammonia was unknown and his inference was that the permissible level was higher than the value of 0.012 mg/L commonly accepted by fish culturists. Paul (1998) recorded NH<sub>3</sub>-N of 0.09 to 0.99 mg/L in ponds of BAU campus, Mymensingh, which were near to the present finding. The range of NO<sub>3</sub>-N values recorded by Whitton *et al.* (1988) were 0.22-0.23 and 0.0006-0.05 mg/L, respectively. The physicochemical parameters were suitable throughout the experimental period. There was not any noticeable change of physicochemical parameters that may affect the change of microflora.

**Bacteria:** The present study revealed that (before treatment) bacterial load in culture ponds were in the range between  $4.9 \pm 1.03 \times 10^3$  -  $5.75 \pm 1.0 \times 10^3$  cfu/mL in pond water,  $5.62 \pm 1.0 \times 10^7$  -  $6.60 \pm 1.02 \times 10^7$  cfu/g in sediments,  $7.94 \pm 1.01 \times 10^6$  -  $9.12 \pm 1.0 \times 10^6$  cfu/g in gills,  $5.12 \pm 1.05 \times 10^7$  -  $6.60 \pm 1.01 \times 10^7$  cfu/g in intestinal contents of *H. molitrix* in control ponds respectively. Al-Harbi and Uddin (2004) reported that total viable counts were  $6.7 \pm 2.1 \times 10^3$  -  $2.7 \pm 1.1 \times 10^3$  cfu/mL in pond water. Total viable counts of bacteria were in the range of  $5.6 \pm 0.8 \times 10^3$  to  $2.4 \pm 1.2 \times 10^4$  cfu/mL in pond water;  $9.3 \pm 1.1 \times 10^6$  to  $1.9 \pm 1.5 \times 10^5$  cfu/g in sediment;  $7.1 \pm 0.7 \times 10^5$  to  $8.7 \pm 1.1 \times 10^6$  cfu/g in the gills; and  $3.4 \pm 1.8 \times 10^6$  to  $5.8 \pm 0.4 \times 10^7$  cfu/g in the intestinal content of tilapia (Al-Harbi and Uddin, 2003) which complied with our findings. The results of Al-Harbi and Uddin, 2006, 2007, 2008, 2010; Rodrigues and prieto, 1989; Henebry *et al.*, 1990; Chowdhury *et al.*, 2003; Yoshirizumi *et al.*, 1976; was more or less agreed when compare with present findings. The organic matter influences the load and composition of microbial population (Rheinheimer, 1985). Sediment bacterial composition and load greatly influence by effluent characteristics. On the other hand bacterial flora in fish is the reflection of aquatic environments (MacFarlane *et al.*, 1996). It affects the storage life and quality of fishery products. The results showed the variation of bacterial

flora among the ponds. The variation also may influenced by nutritional composition between the ponds. After antibiotic treatment total viable counts in culture ponds were  $3.1 \pm 1.19 \times 10^3$  -  $3.1 \pm 1.20 \times 10^3$  cfu/mL in water;  $4.27 \pm 1.10 \times 10^6$  -  $3.1 \pm 1.13 \times 10^6$  cfu/g sediment;  $3.16 \pm 1.29 \times 10^5$  -  $4.07 \pm 1.20 \times 10^5$  cfu/g in gill and  $2.63 \pm 1.17 \times 10^6$  -  $2.95 \pm 1.1 \times 10^6$  cfu/g in intestinal content of *H. molitrix* fish. The viable counts were significantly varied between the control ponds and treatment ponds. Fish intestine contain 7-8 log of bacterial population (Sugita *et al.*, 1983). There is evidence that microbial population occurs within the intestine higher than the surrounding environment (Al-Harbi and Uddin, 2003). Few of them present in the intestine for a relatively long period but most of which are temporary residents, frequently because of incompatible physical and chemical conditions, lethal interactions between bacteria or immune responses in the gut (Sugita *et al.*, 1988). Antibacterial agents are used in aquaculture to prevent the bacterial disease. It may shun bacteria by killing or inhibiting growth and both process reduce the total bacterial number. OTC are effective against a wide range of gram positive and gram negative bacteria (Chopra and Levis, 2001; Ghosh *et al.*, 2011 and Huang *et al.*, 2011). Oral treatments of oxytetracycline was more effective than pelleted feed treatment (Angelo *et al.*, 1995) and water bacteria was reduced 4 log to 3 log. Pelleted feed with OTC did not have any significant change of pond water throughout the experiment. Microorganism are experimental approaches where several variable parameters can be controlled, but where the experimental set up closely mimics natural field conditions (Sengeløv *et al.*, 2003). *Acinetobacter* sp. and *Aeromonas* sp. was major dominating bacteria in freshwater body, and also they were sensitive to OTC (Guardabassi, *et al.*, 1999). Thus may reduce the bacterial load after oxytetracycline treatment in fish culture ponds. The bacterial loads were reduced in sediments, intestine and gill rather than water. There have naturally higher bacterial load in sediment and intestinal contents (Kadavy *et al.*, 2000). Intestinal load may be reduced upto 1 log or below if the water is treated with  $>512 \mu\text{g/ml}$  OTC (Kapetanaki, *et al.*, 1995). Oxytetracycline was poorly absorbed from the intestinal tract (Cravedi, *et al.*, 1987), while OTC could apparently undergo degradation in seawater (Samuelsen, 1989). The results agreed with the present study results. It is not hard to imagine the extent to which antibiotics can affect the aquatic habitat. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products (Saglam and Yonar, 2009). Clearly, these data suggested that the numbers of OTC- treated ponds bacteria were usually lower in fish ponds undergoing antimicrobial therapy because susceptible microorganisms were inhibited.

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