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*Full Length Research Paper*

# Effect of Oxytetracycline on bacterial load of *LABEOROHITA* (Rohu) fish in culture pond

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**The study was aimed to outline the anti biogram profiling of oxytetracycline against usual ecological bacterial flora of culture ponds of *L. ROHITA*. After applying pelleted feeds treated with oxytetracycline (OTC) at 2gm/kg the 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. The total viable counts of bacteria were found $4.9\pm1.03\times10^3$- $5.75\pm1.0\times10^3$cfu/mL in pond 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 gills, $6.02\pm1.02\times10^7$-$8.32\pm1.0\times10^7$cfu/g in gut of *L. ROHITA* in control ponds. After OTC treatment the total viable count of bacteria ranged from $3.1\pm1.19\times10^3$-$3.1\pm1.20\times10^3$cfu/mL in water, $3.1\pm1.13\times10^6$-$4.27\pm1.10\times10^6$cfu/g in sediment, $2.82\pm1.25\times10^5$- $3.09\pm1.19\times10^5$cfu/g gill, $2.69\pm1.12\times10^6$-$4.68\pm1.12\times10^6$cfu/g in guts of *L. ROHITA* respectively, indicating reduction of overall bacterial load below 1 log in sediment, gills and guts of *L. ROHITA* significantly ($P<0.005$).**

**Keyword:** Bacterial load, culture ponds, *L. rohita,* oxytetracycline.

## INTRODUCTION

World aquaculture has coped up tremendously during the last ten years becoming an economically important industry Subasinghe et al. (2009). It is the fastest growing food-producing sector in the world with the greatest potential to meet the growing demand for aquatic food FAO (2006). 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 (Rheinheimer, 1985). The bacterial contents of the water

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**Table 2.1** 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 |

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; Al-Harbi and Uddin (2004). 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. 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. (1987).Intensification and commercialization of production, aquaculture industry faces major problems with bacterial diseases, and vast quantities of chemical and antibiotic products are frequently used (Le et al., 2005; Tuet al., 2008). Although vaccines are being developed and marketed, cannot be used as a universal disease control measure in aquaculture. 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.

Most bacteria that infect fish are Gram negative, including *Aeromonashydrophila, Aeromonassalmonicida, Flavo bacterium columnare, Vibrio* and *Pseudomonas* species. The major groups of Gram-positive that cause disease in fish are *Streptococcus*(Wang et al., 2004). Marine bacteria with antagonistic activity could be employed to combat epizoonotics in aquaculture systems as probiotics or biocontrol agents at prophylactic or curative doses. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. But, the pathogenic bacteria becoming resistant to drugs are common due to indiscriminate use of antibiotics (Reed et al., 2006). It becomes a greater problem in the treatment against resistant pathogenic bacteria. Marine organisms are a rich source of structurally novel and biologically active metabolites. Marine plants have long been recognized as producers of biologically active substances.

Therefore, study was conducted to determine the bacterial load in water, sediments, gills and intestine of experimental *L. rohita* before antibiotic treatments and after antibiotic treatments in ponds. It works as life giving medicine for aquaculture and the post-harvest technology of fish.

## MATERIALS AND METHODS

### 2.1 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 following groups.

a) Control pond without antibiotic medicated diet fed (pond no.-3)

b) Oxytetracycline treated ponds (pond no. 1and 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. 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.

### 2.2 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 universal pocket meters.

### 2.3 Sampling for bacteriological analysis

For microbial investigations three samplings of water, sediment, gills and intestine of *L. rohita* were done weekly in culture ponds before antibiotic treatment. After antibiotic treatment samplings were done every alternative day and means were taken.

**Table 3.1.1** Water quality parameters of different treatments (Mean ± 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 ± 1.263 | 30.25 ± 0.753 | 30.04 ± 1.046 | |
| DO (mg/L) | 5.65 ± 0.212 | 5.56 ± 0.204 | 5.66 ± 0.142 | |
| pH | 7.63 ± 0.064 | 7.82 ± 0.133 | 7.75 ± 0.165 | |
| Alkalinity | 99.15 ± 12.844 | 97.69 ± 11.309 | 97.38 ± 8.996 | |
| $NH_3$-N (mg/L) | 0.25 ± 0.050 | 0.23 ± 0.029 | 0.24 ± 0.044 | |
| $NO_3$-N (mg/L) | 0.12 ± 0.012 | 0.14 ± 0.029 | 0.12 ± 0.010 | |

**2.3.1 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 APHA (1998) as this medium recovered most of the bacteria.

**2.3.2 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.

**2.3.4 Fish gills and intestine**

For every sampling, three *L. rohita* (80-140 g) from each pond were used for bacterial counts in fish organs (e.g. gills and intestine). The fish were killed by pithing. 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.

**2.3.5 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 $28^0$C for 24-48 hrs. The colony-forming units (cfu) were counted under a Quebec dark field 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: cfu/g=

$$\frac{\text{No.of coloniesonpetridish} \cdot 10 \cdot \text{dilutionfactor} \cdot \text{wt.of totalsamplesolution}}{\text{Wt.of fishsample(g)}}$$

**2.4 Data analysis**

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

**RESULTS**

**3.1 Physicochemical parameters**

**3.2 Bacterial analysis**

**3.2.1 Bacterial analysis of pond waters**

Before OTC treatment all control pond bacterial counts were $4.9\pm1.03\times10^3$cfu/mL (control pond), $5.3\pm1.0\times10^3$cfu/mL ( before treatment pond 1,BTP1)and $5.75\pm1.0\times10^3$cfu/mL (before treatment pond 2,BTP2). Pragmatically pond 2 (before treatment) were more bacteria than other two ponds. After 2 weeks of treatment the reduced bacterial load was $3.1\pm1.19\times10^3$cfu/mL for pond1(ATP1) and $3.1\pm1.20\times10^3$cfu/mL for pond 2 (ATP2).

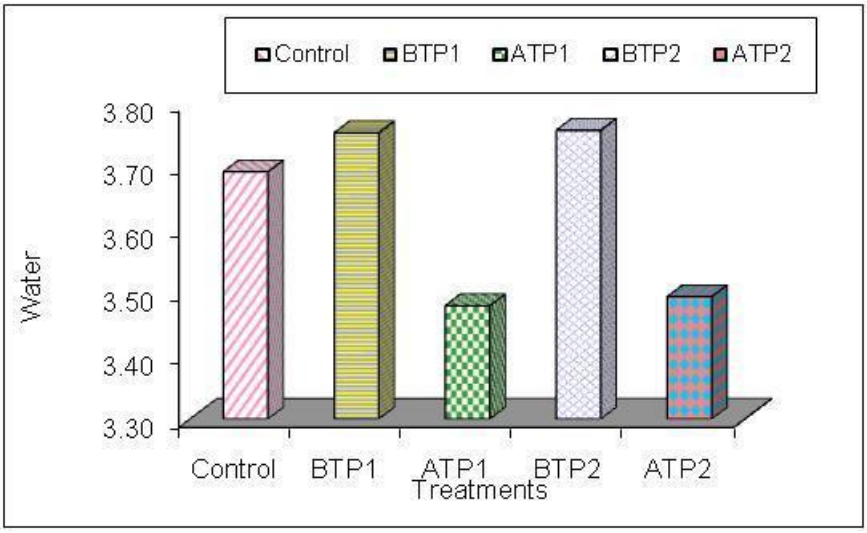


**Figure 3.2.1** Bacterial load (log value)in oxytetracycline treated and untreated pond waters.

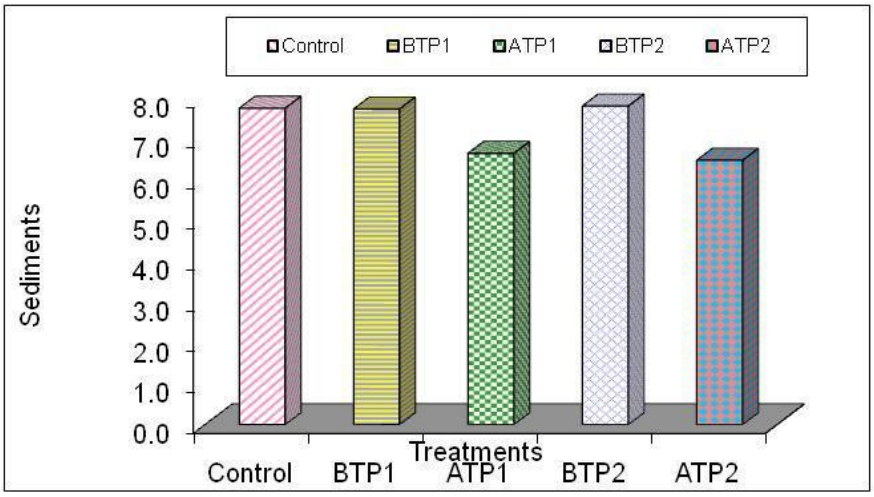


**Figure 3.2.2** Bacterial load (log value) in oxytetracycline treated and untreated pond sediments.

### 3.2.2 Bacterial analysis of pond sediments

Before the treatment bacterial counts of sediments of all ponds were 5.89±1.02×10 , 5.62±1.0×10 and 6.60±1.02×10 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±1.10×10 cfu/g for treatment pond 1 and 3.1±1.13×10 cfu/g for treatment pond 2.

### 3.2.3 Bacterial analysis of *L. ROHITA* gills

Before the treatment bacterial counts of *L. rohita* gill filaments of all ponds were $6.77\pm1.0\times10^{6}$, $5.37\pm1.01\times10^{6}$ and $7.57\pm1.0\times10^{6}$ cfu/g in control pond, BTP1 and BTP2.After 2 weeks of treatment the reduction of bacterial load was $2.82\pm1.25\times10^{5}$cfu/g for treatment pond 1 and $3.09\pm1.19\times10^{5}$cfu/g for treatment pond 2.

### 3.2.4 Bacterial analysis of *L. ROHITA* gut

Before treatment bacterial counts of rohu gut of all ponds were $6.45\pm1.01\times10^{7}$, $6.02\pm1.02\times10^{7}$ and $8.32\pm1.0\times10^{7}$cfu/g in control pond, BTP1 and BTP 2.After 2 weeks of treatment the reduced bacterial load was 2.69±1.12×10 cfu/g for treatment pond 1 and 4.68±1.12×10 cfu/g for treatment pond 2.

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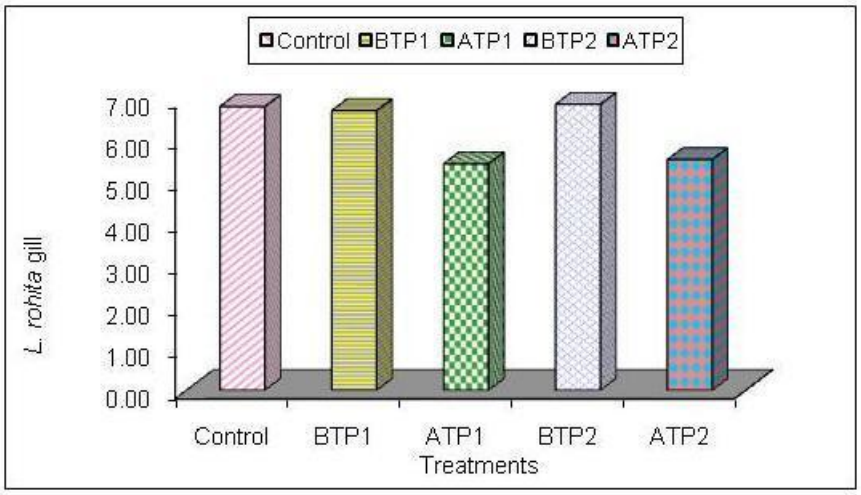


**Figure 3.2.3** Bacterial load (log value) in oxytetracycline treated and untreated pond *L. rohita* gills.

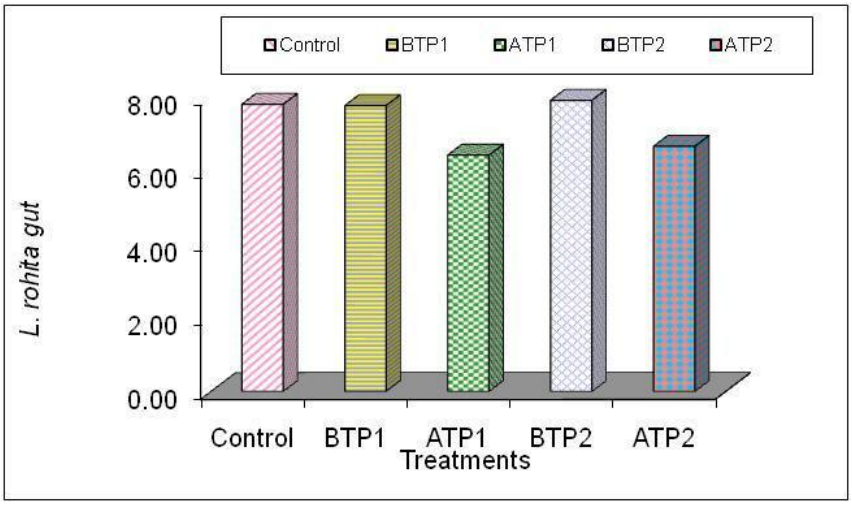


**Figure 3.2.4** Bacterial load (log value) inoxytetracycline treated and untreated pond *L. rohita* gut.

## DISCUSSION

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) reported that DO from 5 to 8 mg/L were suitable for fish culture. The results were found to lie within productive range (5.4 to 8.25 ppm) reported by Alikunhi (1957).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).Strauss et al.(1991) stated that high-unionized ammonia nitrogen ($NH_3$-N) and high pH had synergistic toxic effect. ($NH4^+$) 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. Wahabet al.(1995).Paul (1998) recorded $NH_3$-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 $NO_3$-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, Wahab et al. (1995).There was not any noticeable change of physicochemical parameters that may affects the change of microflora**.**

The present study revealed that (before treatment) bacterial load in culture ponds were in the range between$4.9\pm1.03\times10^{3}$-$5.75\pm1.0\times10^{3}$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 *L. rohita* gill filaments, $6.02\pm1.02\times10^{7}$-$8.32\pm1.0\times10^{7}$cfu/g in intestinal content. Harbi and Uddin (2004) reported that total viable counts were $6.7\pm2.1\times10^{3}$-$2.7\pm1.1\times10^{3}$cfu/mL in pond water. Total viable counts of bacteria were in the range of $5.6\pm0.8\ x10^{3}$ to $2.4\ \pm1.2\times\ 10^{4}$ cfu/mL in pond water; $9.3\pm1.1x10^{6}$ to $1.9\pm1.5x10^{5}$ cfu/g in sediment; $7.l\ \pm\ 0.7x10^{5}$ to $8.7\ \pm1.1\ x10^{6}$cfu/g in the gills; and $3.4\pm1.8x10^{6}$ to $5.8\pm0.4\ x\ 10^{7}$cfu/g in the intestinal content of tilapia Harbi and Uddin (2003) which complied with our findings. The results of Harbi and Uddin (2006,2007,2008,2010); Rudrigues and prieto (1989); Henebry et al. (1990); Trust et al. (1980); Chowdhury et al. (2003); Yoshirnizu et al. (2006); 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 $3.1\pm1.19\times10^{3}$ - $3.1\pm1.20\times10^{3}$cfu/mL in water; $4.27\pm1.10\times10^{6}$ - $3.1\pm1.13\times10^{6}$cfu/g sediment; $5.37\pm1.01\times10^{5}$ - $3.09\pm1.19\times10^{5}$cfu/g in *L. rohita* gill and $2.69\pm1.12\times10^{6}$ - $4.68\pm1.12\times10^{6}$cfu/g in intestinal content. 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 Harbi and Uddin (2003).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 Roberts (2001); Ghoshet 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 contentsKadavy et al.(2000). Intestinal load may be reduced up to 1 log or below if the water is treated with >512 µg/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.

**REFERENCES**


Ali ML, Rashid MM, Ahmed KR, Hasan SU, Alam MM (2004). Effect of high and low cost brood feeds on the hatching and survival rate of freshwater prawn, *Macrobrachiumrosenbergii* larvae, *J.* Bang. Agril. Univ., 2,135-139 (2004).

Alikunhi KH (1957). Fish culture in India, Fm. Bull. Indian Coun. Agri. Res., 20, 144 (1957).

Angelo D, Peeler JT, Rodrick G (1995). Effect of oxytetracycline-medicated feed on antibiotic resistance of gram-negative bacteria in catfish ponds, American Soc. Microbiol.*,*61,2335-2340 (1995).

Chopra I, Levin BK (2001). The bacteriological study of antibiotic resistence*,* Curr. Opin. Microbiol*.,* 2,489-193 (2001).

Chowdhury MBR, Muniruzzaman M, Uddin MN (2003). Study on the intestinal bacterial flora of Tilapia, *Oreochromisniloticus.* Bang. J. Aqua., 11, 65-70 (2003).

Cravedi JP, Choubert G, Delous G (1987). Digestibility of chloramphenicol, oxolinic acid and oxytetracycline in rainbow trout and influence of these antibiotics on lipid digestibility. Aquaculture*,* 60,133-141 (1987).

FAO (2000). Fisheries statistics: Aquaculture production, 88/2. FAO, Rome, Italy, pp 12 (2006)

Ghosh A, Das BK, Roy A, Chandra G (2011). Antibiotic resistance and herbal treatment of bacterial fish pathogens causing epizootic ulcerative syndrome, J. of Herbs, Spices and Medicinal Plants, 17,47-51 (2011).

Guardabassi L, Petersen A, Olsen JE, Dalsgaard A (1999). Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant*,* Appl. Environ. Microbiol., 64,3499–3502 (1999).

Harbi AH (2003). Faecal coliform in pond water, sediments and hybrid tilapia *Oreochromisniloticus*× *Oreochromisaureus* in Saudi Arabia, Aqua.Res., 34,517-524 (2003).

Harbi AH, Uddin MN (2004). Seasonal variation of bacterial ponds in Saudi Arabia used for tilapia culture J. Appl. Aqua., 16,53-57 (2004).

Harbi AH, Uddin MN (2005). Quqntitative and qualitative bacterial flora of giant freshwater prawn *Macrobrachiumrosenbergii* cultured in earthen ponds in Saudi Arabia, J. Aqu. Animal Health, 17,244-250 (2005).

Harbi AH, Uddin MN (2005). Quqntitative and qualitative bacterial flora hybrid tilapia (*Oreochromisniloticus*× *Oreochromisaureus)* cultured in earthen ponds in Saudi Arabia, J. Aqua. Res., 34, 43-48 (2005).

Harbi AH, Uddin MN (2006). Seasonal change of bacterial flora in fish pond sediments in Saudi Arabia, J. Appl. Aqua., 18, 35-45 (2006).

Harbi AH, Uddin MN (2007). Seasonal trends in gill bacterial flora of hybrid tilapia, *Oreochromisniloticus*× *Oreochromisaureus,* J. Appl. Aqua*.,* 19, 61-69 (2007).

Harbi AH, Uddin MN (2008). Aerobic flora of common carp (*Cyprinuscarpio* L.) cultured in earthen pond in Saudi Arabia, J. Appl. Aqua., 20, 108-118 (2008).

Harbi AH, Uddin MN (2010). Bacterial population of African catfish, *Clariasgariepinus* cultured in earthen ponds, J. Appl. Aqua., 22, 187-193 (2010).

Henebry MS, Gorden RW, Buck DH (1990). Bacterial populations in the gut of the Silver carp *(Hypophthalmicthysmolitrix)* ASFA, Part-I, 20, 270 (1990).

Hovda MB, Lunestad BT, Fontanillas R, Rosnes JT (2007). Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmosalar* L.), Aquaculture, 272, 581-588 (2007).

Huang WS, Jen LH (2011). Screening compounds of Chinese herbal medicine and antimicrobial agents for controlling bacterial diseases of eels, J. Fishery Sci. of China,18, 230-236 (2011).

Kadavy DR, Hornby JM, Haverkost T, Nickerson KW (2000). Natural antibiotic resistance of bacteria isolated from larvae of the oil fly, *Helaeomyiapetrolei*, Appl. Environ. Microbiol., 66, 4615–4619 (2000).

Kapetanaki M, Kerry J, Hiney M, Brien C, Coyne R, Smith P (1995). Emergence, in oxytetracycline-free marine mesocosms, of microorganisms capable of colony formation on oxytetracycline-containing media*, Aquaculture,* 134, 227– 236 (1995).

Kim DH, Brunt J, Austin B (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchusmykiss*), J. Appl. Microbiol., 102, 1654-1664 (2007).

Kohinoor AHM, Islam ML, Wahab MA, Thilsted SH (2000). Effect of mola (*A. mola*, Ham.) on the growth and production of carps in polyculture, Bang. J. Fish. Res., 2, 119-126 (2000).

Le XT, Munekage Y, Kato S (2005). Antibiotic resistance in bacteria from shrimp farming in mangrove areas, Sci. Total Envir.,349 95-105 (2005).

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

MacLean MH, Brown JH, Ang KJ, Jauncey K (1994). Effects of manure fertilization frequency on pond culture of the fresh water prawn, *Macrobrachiumrosenbergii* (de Man), Aquacult. Fish.Mangt., 25, 601-611 (1994).

Mc Evoy GDJ (2002). Contamination of animal feeding stuffs as a cause of residues in food: A review of regulatory aspects incidence and control, Analytica Chimica Acta*.,* 473, 3-26. (2002).

Mollah MFA, Aminul Haque AKM (1987). Studies on monthly variations of plankton in relation to the physico-chemical conditions of water and bottom soil of two ponds, Bang J. Fish.,1, 99-103 (1987).

Paul S (1998). Comparison between carp polyculture system with silver carp (*Hypophthalmichthysmolitrix*) and small indigenous fish mola (*Amblypharyngodonmola*). M.S. dissertation, Department of Fisheries management, Bangladesh Agricultural University, Mymensingh. 85 p. (1998).

Prescott JF, Baggot JD, Walter DR (2000). Tetracyclines and Glycylcyclines, *In: Antimicrobial Teraphy in Veterinary Medicine.* Giguére, S.; Prescott, J.F.; Baggot, J.D.; Walker, R.D.; Dowling, P.M. (Ed), pp.231, Blackwell Publishing, Iowa State (2000).

Reed LA, Siewicki TC, Shah AC (2006). The biopharmaceutics and oral bioavailability of two forms of oxytetracycline to the white shrimp, *Litopenaeussetiferus*, Aquaculture*,* 258, 42-54 (2006).

Rheinheimer G (1985). Aquatic Microbiology.3rd Edition. John Wiley and Son, New York, New York, 1985.

Ringø E, Sperstad S, Myklebust R, Refstie S, Krogdahl A (2006). Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadusmorhua* L.) The effect of fish meal, standard soybean meal and a bioprocessed soybean meal, Aquaculture, 261, 829-841 (2006).

Rudriguez C, Prieto A (1989). Aerophilicmicroflora of the intestine of cultured *Oreochromisaureus* and the surrounding water Quantitative analysis, ASFA., 19, 319 (1989).

Saglam NY, Enis M (2009). Effects of sulfamerazine on selected haematological and immunological parameters in rainbow trout (Onchorhynchusmy kiss ,Walbaum, 1792), In. Aqua. Res*.,* 40, 395-404 (2009).

Samuelsen OB (1989). Degradation of oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm, Aquaculture*,* 83, 7-16 (1989).

Sengeløv GY (2003). AgersøHalling-Sørensen B. Baloda SB. Andersen JS.and Jensen LB. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry, Environ. Int., 28, 587–95 (2003).

Skrodenyte-Arbaciauskiene V, Sruoga A, Butkauskas D (2006). Assessment of microbial diversity in the river trout (*Salmotruttafario*L.) intestinal tract identified by partial 16S rRNA gene sequence analysi, Fish.Sci., 72, 597-602 (2006).

Spanggaard B, Huber I, Nielsen J, Nielsen T, Appel KF, Gram L (2000). The microflora of rainbow trout intestine: a comparison of traditional and molecular identification, Aquaculture, 182, 1-15 (2000).

Spencer R (1961). The occurrence of luminous bacteria in the intestinal tract of marine fish, J. Appl. Bacteriol., 24, 34-37 (1961).

Stewart MM (1932). The bacterial flora of the slime and intestinal contents of the haddock*,* J. Mar. Biol. Ass. U.K., 18, 35-50 (1932).

Subasinghe R, Soto D, Jia J (2009). Global aquaculture and its role in sustainable development, Reviews Aquaculture, 1, 2-9 (2009).

Sugita H, Oshima K, Tamura M (1983). Bacterial flora in the gastro intestine of freshwater fishes in the river, Bull. Jap.Soc. Sci. Fish., 49, 1387-1395 (1983).

Sugita HM, Tsunohara TO, Deguchi Y (1988). The establishment of an intestinal micro flora in developing goldfish (*Carassiusauratus*) of culture ponds, Microbial. Ecol., 15, 333-344 (1988).

Sugita HR, Ueda Berger LR, Deguchi Y (1987). Microflora in the gut of Japanese Coastal Crustacea*, Bull.Jap.Soc. Sci. Fish*., 53, 1647-1655 (1987).

Trust TJ, Bull LM, Currie BR, Buckley JT (1980). Obligate anaerobic bacteria in the gastrointestinal micro flora of grass carp (*Ctenapharyngodonidella*) gold fish, Total Environment*,* 346, 87-98 (1980).

Tu TH, Silvestre F, Bernard A, Douny C, Phuong TN, Tao TC, Maghuin-Rogiste G, Kestemont P (2008). Oxidative stress response of black tiger shrimp (*Penaeusmonodon*) to enrofloxacin and to culture system, Aquaculture, 285, 244-248 (2008).

Wahab MA, Ahmed ZF, Islam MA, Rahmatullah SM (1995). Effect of introduction of common carp, *Cyprinuscarpio* (L) on the pond ecology and growth of fish in polyculture, Aqua. Res., 26, 619-628 (1995).

Walter JP, Winton RJ (2010). Emerging viral diseases of fish and shrimp, Vet.Res.,51, 51-75 (2010).

Wang Q, Liu Q, Li J (2004). Tissue distribution and elimination of oxytetracycline in perch *Lateolabraxjaponicus* and black seabream (*Sparusmacrocephalus*) following oral administration, Aquaculture*, 2*37, 31–40 (2004).

Whitton BA, Aziz A (1988). Francis P. Rother J.A. Simon T.W. and Tahmida Z.N. Ecology of deep water rice in Bangladesh, Physical and Chemical environment, 169, 3-67 (1988).

Yang G, Bao B, Peatman E, Li H, Huang L, Ren D (2007). Analysis of the composition of the bacterial community in puffer fish *Takifugu obscures*, Aquaculture, 262, 183-191 (2007).

Yoshimizu M, Kimura T, Sakai M (1976). Studies on the intestinal microflora of Salmonids-1.The intestinalmicroflora of fish reared in freshwater and marine water, Bull. Jap. Soc. Sci. Fish., 42, 91-99 (1976).

Zhou A, Liu Y, Shi Y, He S, Yao B, Ringø E (2009). Molecular characterization of the autochthonous microbiota in the gastrointestinal tract of adult yellow grouper (*Epinephelusawoara*) cultured in cages, Aquaculture*,* 286, 184-189 (2009).