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Effects of antibiotic on the bacterial microflora in two commercially important catfish species, *Clarias batrachus* and *Heteropneustes fossilis* in Bangladesh

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PEER REVIEW

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Comments

Antibiotic treatment is often unavoidable due to bacterial disease outbreaks in fish under intensive culture conditions. The authors have shown that antibiotic treatment can affect the bacterial loads in gills, skin and intestines. This reduction in bacterial loads can be beneficial in cases of disease. However, it can also have deleterious effects due to removal of beneficial bacteria present on these tissues in fish. Further studies are warranted to study the effects of antibiotic treatment in these two indigenous Indian catfish species.

Details on Page 848

ABSTRACT

Objective: To assess the effects of a widely used antibiotic, oxytetracycline (OTC) on the bacterial microflora in two catfish species under artificial culture conditions in the laboratory.

Methods: The experiment was conducted in the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh–2202. The fish were reared in six aquaria (size 37 cm×30 cm×60 cm) where three aquaria served as replicates of the antibiotic treatment groups and the remaining three aquaria served as an untreated control group. Each aquarium was stocked with 25 fish on an average body weight 15 g. OTC was administered to the fish in the treatment groups at the rate of 2 g/kg in–feed twice daily upto *ad libitum*, whereas fish in the untreated control groups were given the same feed without antibiotics for 20 d. During the experiment, bacterial loads were estimated as colony forming unit (CFU/g) by every alternate day in the aquarium water, gills, skin and intestine of fish.

Results: The administration of OTC in feed resulted in gradual decrease of bacterial loads in the gills, intestine and skin of the two catfish species tested. In contrast, the bacterial loads remain unchanged or slightly increased in the control groups not fed with OTC. Water quality parameters such as dissolved oxygen, pH and total hardness were found to be within suitable range in the test aquaria but not in control aquarium throughout the experimental period.

Conclusions: The results of this experiment showed that in–feed antibiotic OTC for a period of 20 d reduced the bacterial loads in the gills, intestines and skin of treated fish.

KEYWORDS

Antibiotic therapy, Bacterial load, Catfish

1. Introduction

Aquaculture is the fastest growing food–producing sector in the world with the greatest potential to meet the growing demand for food^[1]. In contrast to other animal production sectors, aquaculture is characterized by an

enormous diversity of species raised both in natural and artificial systems. Commercial catfish culture has been increasingly gaining importance in Bangladesh. Indigenous catfish shingi (*Heteropneustes fossilis*) and magur (*Clarius batrachus*) are widely distributed freshwater fish species in great demand. With increasing intensification in

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commercial aquaculture, disease has become a major problem.

A wide variety of pathogens including bacteria can cause disease in catfish. Most disease agents are naturally present in low numbers and normally do not cause problems. The natural defense mechanisms of fish such as undamaged skin, mucus covering the skin, and various components of the immune system, keep disease agents in check. However, when overcrowded fish in culture operations are further stressed by low dissolved oxygen or nutritionally inadequate feeds, the ability of natural disease defense system of catfish to protect against infectious diseases may be reduced.

Antibiotics are often used as chemotherapeutic agents in the treatment of bacterial diseases. Among the common antibiotics, oxytetracycline is widely employed to treat bacterial infections in aquaculture farms[2]. It belongs to the tetracycline group which exerts antimicrobial action against Gram positive and Gram negative bacteria, rickettsia and mycoplasmas. The use of antimicrobial drugs for treatment and control of the disease problem due to intensive fish farming has increased significantly. During treatment of bacterial fish diseases in aquaculture, antimicrobial agents are released into the surrounding water. The use of antibiotics in aquaculture depends on the local regulations, which vary widely between different countries. The emerging view that antibiotics should be used with more care has prompted more strict regulations on the use of antibiotics in aquaculture and on the presence of antibiotic residues in aquaculture products.

Numerous investigations have been carried out on the microbiology of freshwater and marine environment in different parts of the world[3]. Studies have shown that the bacterial flora in the gut of fish reflects their aquatic environment including water, sediment and food[4]. The bacterial compositions of the water used for growing fish affect the quality of the fish and fish products. In every microbial habitat, the nutritional competition between organisms plays an important role in influencing the composition of microflora[3]. Aquatic animals including catfish are known to take in large numbers of bacteria through their food and drinking water, which then accumulate in their intestine. Some of these ingested bacteria remain present in the intestine for a relatively long period while some are temporary residents, frequently due to incompatible physical and chemical conditions or lethal interactions between bacteria or immune responses in the gut. There is evidence that the alimentary tracts of fish are complex ecosystems, containing a large number of microorganisms. Microbial populations in the intestinal contents are much higher than those in the surrounding water. It is generally believed that bacteria can contribute to the diet of fish. The microbial populations within the digestive tract of fish are usually much denser than in the surrounding water, suggesting that the digestive tract provides a favorable ecological niche for these organisms[4,5].

There was very little published information on the bacterial load of intestinal contents in fish, and their composition, seasonal variation and biochemical characteristics. Therefore, the present study was carried out to investigate the effects of antibiotic therapy reducing bacterial load in skin, gills and intestine of experimental fish kept under laboratory condition, and any associated effects on water quality.

2. Materials and methods

2.1. Fish rearing and experimental design

The fry of shingi and magur were sourced from a nearby field hatchery complex and transported to the laboratory at Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh–2202 for experimental purpose. After an acclimation period of 2 d, the 25 fish include shingi and magur were transferred to each glass aquaria (size 37 cm×30 cm×60 cm) filled with tap water and kept under good aeration. Water temperature ranged from 28 to 29 °C maintained in heater throughout the experiments. Fish in three aquaria were designated as replicates of the treatment group, referred to as experimental aquarium 1, 2 and 3, and fish in the remaining three aquaria were designated as replicates of the control groups, and referred to as control aquarium 1, 2 and 3. Oxytetracycline was fed to the fish reared in the experimental aquarium at the rate of 2 g/kg feed twice daily upto *ad libitum* whereas fish reared under control condition was given similar amount of feed without antibiotics for 20 d. The 20% aquarium water was changed daily.

2.2. Sampling for bacteriological analysis

Sampling was done every alternate day through the 20-day experimental trial from both treatment and control aquaria. Microbiological examinations of water, skin, gills and intestine of treatment and control fish in the aquaria were carried out. For every sampling, three fish (80–140 g) from each aquarium were used for bacterial counts in fish organs (skin, gills and intestine). The fish were killed by ice slurry and the number of incidental organisms was reduced by washing the fish skin with 70% ethanol before opening the ventral surface of the abdomen with sterile scissors to expose the body cavity. 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 put in a tube containing 2 mL of sterile saline solution. One milliliter of each homogenate solution was serially diluted (10^{-3} to 10^{-7}) and treated in the same way as the aquarium samples.

2.3. Media preparation and sterilization

For preparation of media, 17.5 g of plate count agar media (Oxoid Limited) was suspended into 1 L of distilled water in a conical flask. The agar mixture was heated on a gas burner until completely dissolved, followed by sterilization in an autoclave for 20 min at 121 °C and 15 pounds per square inch pressure. The sterilized agar media was cooled down to 50 °C and poured into sterile Petri dish.

2.4. Sample preparation and culture

Standard plate count expressed as colony forming units per gram (CFU/g) were determined by performing ten-fold serial dilutions of tissue samples using plate spread method. One gram each of gills, skin and intestinal contents was homogenized in 20 mL sterile physiological saline, shaken for 1 min and 1 mL sample transferred with a micropipette to test tube containing 9 mL of physiological saline to obtain 10^{-1} dilution of original sample solution. Using the similar

process, dilutions of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} were made. A vortex mixer was used to ensure adequate mixing of each serially diluted tissue suspension.

2.5. Aerobic plate count

Each serial dilution of tissue samples was inoculated on sterile nutrient agar media, in duplicates. Using aseptic techniques, 0.1 mL of serial dilutions of each tissue sample was inoculated into sterile nutrient agar media. Inoculated tissue samples were spread on the agar media using flamed sterile L-shaped glass rod. For total heterotrophic aerobic bacterial counts of pond water, gills and intestine, all the inoculated plates were incubated at 28 °C for 24–48 h. The colony forming units were counted under a Quebec dark field colony counter (Leica, Buffalo, NY, USA) equipped with a plate-guide ruled in square centimeters. Plates containing 30–300 colonies were used to calculate bacterial loads, recorded as CFU/g of sample by using following formula:

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

3. Results

3.1. Changes in bacterial load in skin

For Day 0 (before treatment) magur, the average bacterial load in skin samples was $(5.24 \pm 0.15) \times 10^6$ CFU/cm². The bacterial load of skin samples increased then decreased gradually in the antibiotic treated fish. The highest and lowest bacterial load of skin sample was $(5.94 \pm 0.10) \times 10^6$ CFU/cm² in the Day 8 and $(3.56 \pm 0.05) \times 10^6$ CFU/cm² in the Day 20 (Figure 1). For Day 0 (before treatment) shingi, the bacterial load in skin sample was $(5.60 \pm 0.15) \times 10^6$ CFU/cm². The bacterial loads of skin sample were decreased with antibiotic treatment. The lowest bacterial load of skin sample was $(2.90 \pm 0.19) \times 10^6$ CFU/cm² in the Day 20 (Figure 1).

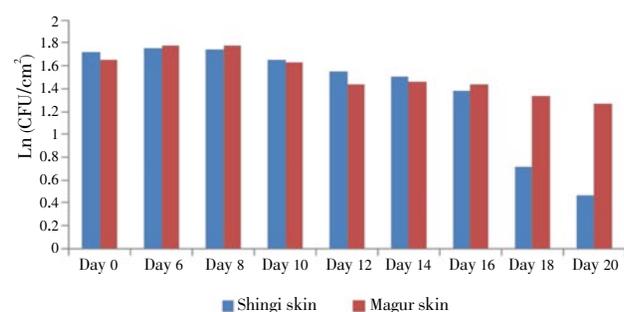


Figure 1. Changes in bacterial load of shingi and magur fish skin (CFU/cm²).

3.2. Changes in bacterial load in gill

For Day 0 (before treatment) magur, the average bacterial load in gill samples was $(7.51 \pm 0.12) \times 10^5$ CFU/g. The highest bacterial load in gill was $(8.93 \pm 0.18) \times 10^5$ CFU/g on Day 10, and which reduced to $(3.43 \pm 0.25) \times 10^5$ CFU/g on Day 20 in the antibiotic treatment groups (Figure 2). For shingi, the highest and lowest bacterial load in gill sample was $(9.60 \pm 0.15) \times 10^5$ CFU/g on Day 0 (before treatment) and $(1.40 \pm 0.05) \times 10^5$ CFU/g on Day 20 (Figure 2).

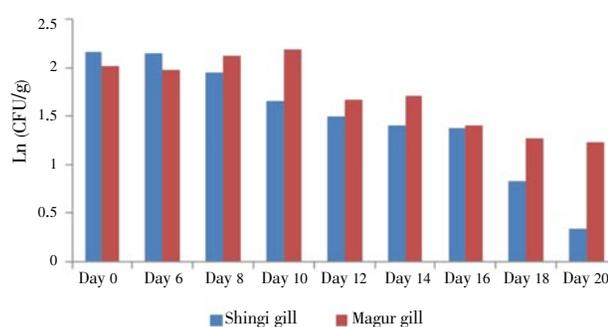


Figure 2. Changes in bacterial load of shingi and magur fish gill (CFU/g).

3.3. Changes in bacterial load in intestine

For Day 0 (before treatment) magur, the bacterial load in intestine sample was $(7.36 \pm 0.51) \times 10^7$ CFU/g. Bacterial load of intestine sample was increased $[(9.13 \pm 0.10) \times 10^7$ CFU/g] on Day 8 and decreased to $(3.86 \pm 0.15) \times 10^7$ CFU/g on Day 20 (Figure 3). For shingi, the highest and lowest bacterial load in intestine sample was $(9.5 \pm 0.2) \times 10^7$ CFU/g on Day 0 (before treatment) and $(4.63 \pm 0.15) \times 10^7$ CFU/g on Day 20 (Figure 3).

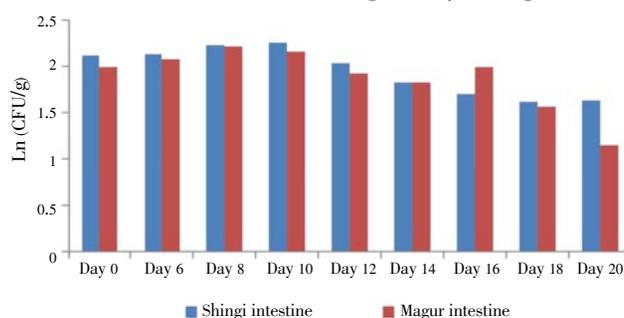


Figure 3. Changes in bacterial load of shingi and magur fish intestine (CFU/g).

4. Discussion

This is the first initiative of bacteriology study of indigenous cat fishes (shingi and magur) in Bangladesh. The result of the present study showed variations in total bacterial load of skin, gill and intestine. The total viable counts were $(6.7 \pm 2.1) \times 10^3$ to $(2.7 \pm 1.1) \times 10^3$ CFU/mL in pond water reported by Uddin and Al-Harbi[6]. Other studies revealed that 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; $(7.1 \pm 0.7) \times 10^3$ 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 intestine of tilapia[7,8]. The results were more or less similar to the present study. It was reported that the organic matter influences the load and composition of microbial population[3]. On the other hand, bacterial flora in fish is the reflection of aquatic environments[9]. Oxytetracycline is effective against a wide range of Gram-positive and Gram-negative bacteria[10]. In this study, the bacterial loads decreased more slowly in skin and intestine as compared to water and gills in response to antibiotic treatment. It was reported that intestinal load may be reduced by up to 1 log or less if the water is treated with >512 µg/mL oxytetracycline[11]. In the present study, we used oxytetracycline at a dose of 2 mg/kg feed which is approximately 4 times the dose than that reported by Kapetanaki *et al*[11]. Therefore, it may be assumed that reduction of bacterial load would be faster due to higher dose of antibiotic treatment.

Oxytetracycline is an antibiotic that is poorly absorbed in the intestinal tract and apparently undergoes degradation in seawater^[2,12]. Therefore, it can be speculated that the extent that antibiotics can affect the aquatic habitat may vary. 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. In this study, our results suggested that bacteria loads were lower in oxytetracycline-treated aquaria than control untreated aquaria.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

This study is based on laboratory experiments to determine the effects of in-feed antibiotic therapy, specifically oxytetracycline on bacterial loads on skin, gills, intestines and culture water. The study was carried out in two indigenous Indian catfish species.

Research frontiers

There has been no similar study on the effects of in-feed antibiotics in the two indigenous Indian catfish species used in this study.

Related reports

Antibiotic treatment is often unavoidable due to bacterial disease outbreaks in fish under intensive culture conditions. Bacteria forms part of the normal microflora in the environment in which fish are kept, and can cause disease when fish are stressed by husbandry procedures, poor water quality or low water temperatures.

Innovations and breakthroughs

The results obtained in this study indicated that antibiotic therapy reduced bacterial loads on skin, gills, intestines and culture water in two indigenous Indian catfish species under experimental laboratory conditions.

Applications

The normal bacterial microflora on the skin, gills or intestine of healthy fish exist in harmony. Factors that cause a change in the numbers and types of bacteria such as antibiotic treatment can have beneficial as well as harmful

effects, and warrant further future studies so that such effects can be mitigated.

Peer review

Antibiotic treatment is often unavoidable due to bacterial disease outbreaks in fish under intensive culture conditions. The authors have shown that antibiotic treatment can affect the bacterial loads in gills, skin and intestines. This reduction in bacterial loads can be beneficial in cases of disease. However, it can also have deleterious effects due to removal of beneficial bacteria present on these tissues in fish. Further studies are warranted to study the effects of antibiotic treatment in these two indigenous Indian catfish species.

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