

Short communication

Efficacy of Some Antibiotics used for the Treatment of Diseased Koi (*Anabas Testudineus*) Fish

Md. Mamnur Rashid^{1*}, Muhammad ForhadAli², Muhammad Mamunur Rahman³ and Ashish Kumar Saha⁴

¹Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh.

²Department of Aquaculture, Sheikh Fajilatunnesa Mujib Fisheries College, Melandah, Jamalpur, Bangladesh.

³Gazi Fish Limited, Dakup, Khulna, Bangladesh.

⁴Agroni Bank Limited, Khulna Corporate Branch, Khulna, Bangladesh.

*Corresponding author's email: mamun_aqua@yahoo.com

ABSTRACT

Naturally diseased climbing perch *Anabas testudineus* was confirmed to be caused by *Aeromonas hydrophila* bacteria by aeromonas isolation medium (AIM), 0/129 vibriostatic agent and esculin hydrolysis. Such naturally diseased koi fish were treated with four antibiotics: captor (chlortetracycline hydrochloride BP 45%), renamox 15% -Vet (amoxicillin trihydrate BP), oxy-Dox-F (oxytetracycline 20% and + doxycycline 10%), renaquine 20% -Vet (flumequine) at lower, recommended and higher doses were performed to examine the efficacy of the drugs. Captor was given at doses of 0.8, 1.0 and 1.2 g/3 litres of water separately. Doses of renamox 15% -Vet were given at 0.8, 1.0 and 1.2 g/litre of water. Doses of oxy-Dox-F were 0.8, 1.0 and 1.5 g/Kg body weight and doses of renaquine were 10, 12, and 15 mg/Kg body weight. Among the four antibiotics, effect of captor and renaquine at recommended dose showed the best result where 100% fish were recovered. However, renamox and oxy-Dox-F showed best result at higher dose.

Key Words: Climbing perch, *Anabas testudineus*, Antibiotics.

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Introduction

With the expansion of aquaculture especially intensive culture, fish folk have become susceptible to diseases. In these conditions drugs, especially antibiotics are fruitful for cure (Mamnur Rashid *et al.*, 2008). But in Bangladesh there is no ban on antibiotics. Using this opportunity many different pharmaceutical companies have been established and they are producing a huge numbers of antibiotics, whose doses and dosages are not clear. Many marginal farmers face the lack of the efficacy of them. They are not truly benefited through using the recommended dose of drugs from different pharmaceutical companies. On the other hand, if the dose of antibiotic is too low or treatment time is too short, the bacteria will not be killed and this greatly increases the risk of the bacteria developing resistance against the antibiotic. Thereafter, when bacteria become resistant to a specific antibiotic, even high concentrations of that drug will not be effective (Mamnur Rashid, 1997). Lipton (1991) studied the effect of antibiotic compounds on the growth inhibition of fish pathogen *Aeromonas hydrophila* isolated from the haemorrhagic lesions of *Labeo rohita*. He found that among the ten antibiotics, gentamycin, tetracycline, streptomycin, penicillin and neomycin inhibited the growth of the bacteria. Antibiotics such as, gentamycin, tetracycline and streptomycin were effective at 10 mg/ml. Tetracycline was effective at 20 g/ml and gentamycin, neomycin and streptomycin, at 50 g/ml for *A. hydrophila*. Chowdhury *et al.*, (2003) reported that the antibiotic, renamycin (oxytetracycline), had positive effect against bacterial infection at a dose of 50mg/Kg body wt/day applying four days and 80-90% fish were recovered under laboratory condition. Islam (2010) examined the effects of different chemotherapeutics against *A. hydrophila* infection in climbing perch. Only oxytetracycline showed the best result where 100% fish were recovered. Mahmud (2011) studied the efficacy of some antibiotics used for treatment of naturally diseased koi fish and observed that combined use of oxytetracycline 20% and doxycycline 10% showed expected result at recommended dose (100% recovery of fish) than single use of oxytetracycline and amoxicillin. The present work was undertaken to check the efficacy of four different well known antibiotics.

Materials and Methods

Experimental fish. A total of 24 naturally diseased climbing perch *Anabas testudineus* were collected from various parts of Tarakanda Upazila, Mymensingh district and observed for clinical disease

symptoms. Secondly, the internal organs of representative fish were homogenized and 100 µl were plated and incubated to observe the growth of the pathogenic bacteria, for confirmation of the infection.

Bacterial identification. Shortcut identification technique was adopted to identify the pathogenic bacteria. The colonies were sub-cultured on to *Aeromonas* isolation medium (AIM) plates. The grown colonies were tested with 0/129 vibriostatic agent and growth on esculin for the identification.

Antibiotics tested. Four different types of antibiotic preparations were used in this study. The pharmaceutical company Novartis produced captor (chlortetracycline hydrochloride BP 45%) with a recommended dose of 1.0 g/3 litres of water for 5-7 days. Renamox 15% -Vet (amoxicillin trihydrate BP) with a recommended dose of 1.0 g/litre of water for 3-5 days, and renaquine 20% -Vet (flumequine) with 12 mg/kg fish for 3-5 days. ACI had oxy-Dox-F (oxytetracycline 20% + doxycycline 10%) with a recommended dose of 1 g / 4 Kg body weight for 7-10 days.

Experimental setup. The trial was conducted in twelve separate aquaria. For each drug, three aquaria were set for three different doses with two fish in each. The used doses of four different antibiotics are shown in Table 1. Each aquarium was filled with 20 litres of ground water and then antibiotics were added to the aquarium. Total medicinal trial period was 10 days. However, the recommended dosages were followed.

Results

Gross clinical features of the diseased fish. Diseased climbing perch showed external haemorrhagic lesion, erosion of anal region and fins, loss of scales, darkening body colour and exophthalmia. From the inoculated plates of internal organs, heavy monoculture type growths of bacterial colonies were evident, confirming bacterial infection in the experimental fish.

Bacteria identified. The colonies on AIM medium grown on 0/129 vibriostatic agent and hydrolyzed esculin confirming them as *Aeromonas hydrophila* bacteria.

Antibiotic test results. Results found after treatment with antibiotics are shown in Table 2. After captor treatment by recommended dose, hemorrhagic lesions were recovered. Higher doses of renamox and oxy-Dox-F Vet could cure erosion in anal region and caudal fin ray loss. Ulcerative lesions did not exist, feeding affinity was increased and

Table 1. Lower, recommended and higher dose of four different antibiotics for their efficacy test

Name of antibiotics	Lower dose	Recommended dose	Higher dose
Captor	0.8 g/3 litres of water	1.0 g/3 litres of water	1.2 g/3 litres of water
Renamox 15% -Vet	0.8 g/litre of water	1.0 g/litre of water	1.2 g/litre of water
Oxy-Dox-F	0.8 g/Kg fish	1 g/Kg fish	1.5 g/Kg fish
Renaquine 20% -Vet	0.8 g/Kg fish	1 g/Kg fish	1.5 g/kg fish

Table 2. Effects of antibiotics on *Anabas testudineus* naturally infected with bacteria

Antibiotics	Selected dose	No. of fish treated	No. of fish cured	No. of fish not recovered	% of recovery
Captor	Lower : 0.8 g/3 L of water	2	0	2	0
	Recommended : 1.0 g/3 L of water	2	2	0	100
	Higher : 1.2 g/3 L of water	2	2	0	100
Renamox 15% -Vet	Lower : 0.8 g/L of drinking water.	2	0	2	0
	Recommended : 1.0 g/L of drinking water	2	1	1	50
	Higher : 1.2 g/L of drinking water	2	2	0	100
Oxy-Dox-F	Lower : 0.80 g/kg fish.	2	0	2	0
	Recommended : 1 g/kg fish	2	1	1	50
	Higher : 1.5 g/kg fish	2	2	0	100
Renaquine 20% -Vet	Lower : 10 mg/kg fish.	2	0	2	0
	Recommended : 12 mg/kg fish.	2	2	0	100
	Higher : 15 mg/kg fish.	2	2	0	100

there was no more dark discoloration. By renaquine at recommended dose, hemorrhagic ulcerative lesions on skin were cured.

Discussion

During the present study use of two antibiotics at their recommended doses showed good results against *Aeromonas hydrophila* bacteria, but other two did not. The doses of antibiotics reduced the level of infection. These findings correspond to the findings of Olagh and Farkasj (1978). The present doses differed from those of the other two, but the results were found to be very similar. Lio-Po and Sanvictores (1987) found positive effect of oxytetracycline in controlling *Pseudomonas* sp. in tilapia fry. Chowdhury *et al.*, (2003) found positive effect of renamycin (oxytetracycline) against bacterial infection. It was found that the best result was obtained with 100% recovery of infected fish when the antibiotic oxytetracycline, was used at a higher dose than the recommended dose in laboratory condition. Treatments using antibiotic have to be administrated at the effective dosage to ensure elimination of bacteria (De Kinkelin *et al.*, 1985). As a consequence of inappropriate use of an antibiotic, the bacteria, such as *Aeromonas hydrophila* (Aoki *et al.*, 1971) and *A. salmonicida* (Popoff and Davaine, 1971) developed resistance to this antibiotic which was transmitted to the next generations. In this aspect, the fate of unused antibiotics or their effect in the environment is very important. Obviously, the potential exists for the antibiotics to affect natural bacterial communities adversely.

Studies of freshwater salmonid farms by Austin (1985) showed that bacterial numbers decreased in effluent during chemotherapy. Moreover, it took many weeks for compounds such as oxytetracycline to be breakdown, depending upon temperature, oxygen and light levels (Samuelson, 1989). It was observed that farmers were not aware about the mode of action of particular chemical. As a consequence, during disease treatment first they were reported to try with one chemical and if it did not work, they tried for another one. They used doses of particular chemical either from their own experiences or from the instruction of the packet, if there is any and from the suggestion of chemical's sellers. Sometimes it was found that they did not complete the full course of the antibiotics. They thought it was loss of money, as the full course was quite expensive. Small farmers did not agree to use the antibiotics. At that situation medicine sellers convinced the ignorant farmers to use antibiotics once or twice in case of 7 or 10 times. This type of insincere practice of the seller was their benefit of selling the medicine merely. Darwish and Ismaiel (2003) found positive effect of amoxicillin in controlling *Streptococcus iniae* infection in hybrid striped bass *Morone chrysops* female × *Morone saxatilis* male. Amoxicillin are most effective against gram-positive bacteria such as *Streptococcus* species. In the present study, the negative effect was found by the recommended dose of amoxicillin (renamox) and oxy-Dox-F, but at higher dose, satisfactory result was found. However, captor and renaquine showed the best result at recommended doses. Mahmud (2011) treated naturally diseased koi fish and observed that combined use of oxytetracycline 20% and doxycycline 10% showed expected result at recommended dose (100% recovery of fish) than single use of oxytetracycline and amoxicillin. However, the present study was carried on limited population of fish and application of selected antibiotics. A further detail study involving larger population size and wide range of antibiotic are crucial for more accurate results.

Conclusions

Aeromonas hydrophila was confirmed as the etiology of Naturally diseased climbing perch *Anabas testudineus*. Such naturally diseased koi fish were treated with four antibiotics: captor (chlortetracycline hydrochloride BP 45%), renamox 15% -Vet (amoxicillin trihydrate BP), oxy-Dox-F (oxytetracycline 20% and + doxycycline 10%), renaquine 20% -Vet (flumequine) at lower, recommended and higher doses and among them captor and renaquine at recommended dose showed the best result. However, renamox and oxy-Dox-F showed best result at higher dose.

References

- Aoki T, S Egusa, Y Ogata and T Watanabe, 1971. Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*. J GenMicrobiol, 65: 343-349. <http://dx.doi.org/10.1099/00221287-65-3-343>
- Austin B, 1985. Antibiotic pollution from fish farms: effects on aquatic micro flora. Microbiol Sci, 2:113-117.
- Chowdhury MBR, M Muniruzzaman, UA Zahura, KZ Habib and MD Khatun, 2003. Ulcer type of disease in the fish of small-scale farmer's pond in Bangladesh. Pakistan J Biol Sci, 6(6): 544-550. <http://dx.doi.org/10.3923/pjbs.2003.544.550>
- DarwishAM and AA Ismaiel, 2003. Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in sunshine bass. J Aquat Anim Health, 15 (3):209-214. <http://dx.doi.org/10.1577/H03-016>
- De Kinkelin P, C Michel and P Ghittino, 1985. Precis de pathologie des poissons. INRA-OIE Edit.
- Islam MT, 2010. Effects of chemotherapeutics against *Aeromonas hydrophila* infection in climbing perch *Anabas testudineus*. MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.
- Jacobsen P and I Berglund, 1988. Persistence of oxytetracycline in sediments from fish farms. Aquacult. 70:365-370. [http://dx.doi.org/10.1016/0044-8486\(88\)90120-2](http://dx.doi.org/10.1016/0044-8486(88)90120-2)
- Lio-Po GD and MEG Sanvictores, 1987. Studies on the causative organism of *Oreochromis niloticus* (Linnaeus) fry mortalities I. Primary isolation and pathogenicity experiments. J Aquacult Trop, 2: 25-30.
- Lipton AP, 1991. Control of *Aeromonas* and *Pseudomonas* infections in freshwater aquaculture systems. In: *Proceedings of National Symposium on New Horizons in Freshwater Aquaculture*. Association of Aquaculturists India. pp. 171-173.
- Mahmud AM, 2011. Efficacy test of antibiotics on naturally diseased climbing perch *Anabas testudineus*. MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.
- Mammur Rashid M, 1997. Studies on *Edwardsiella tarda* infection in Japanese flounder *Paralichthys olivaceus*. PhD Thesis, Graduate school of Biosphere Sciences, Hiroshima University, Japan.
- Mammur Rashid M, MA Hasan, K Mostofa and MA Islam, 2008. Isolation of *Aeromonas hydrophila* from EUS affected shing *Heteropneustes fossilis* from a fish farm of Mymensingh. Progress Agric, 19(1): 117-124.
- Olagh R and OFarkasj, 1978. Effect of temperature, pH, antibiotics, form malachite green on the growth and survival of *Saprolegnia* and *Achlya* parasitic on fish. Aquacult, 13 :273-288. [http://dx.doi.org/10.1016/0044-8486\(78\)90009-1](http://dx.doi.org/10.1016/0044-8486(78)90009-1)
- Popoff Mand Y Davaine, 1971. Facteurs de resistance transferable chez *Aeromonas salmonicida*. Ann Inst Pasteur, 121: 337-342.
- Samuelson 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. Aquacult, 83: 7-16. [http://dx.doi.org/10.1016/0044-8486\(89\)90056-2](http://dx.doi.org/10.1016/0044-8486(89)90056-2)
- Srivastava RC, 1978. Chemotherapeutical studies on fish mycoses. An Vet Med, 35(7): 267-269.