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Article

Effects of chemotherapeutics against experimentally injured stinging catfish *Heteropneustes fossilis*

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Abstract: Effects of different chemotherapeutics were examined against experimentally infected stinging catfish *Heteropneustes fossilis*. Fish were collected from a fish market in Mymensingh, acclimatized for 7 days in laboratory condition from January to February, 2016 in aquaria at Fish Clinic of Bangladesh Agricultural University, Mymensingh, experimentally injured by using forceps and knife and waited seven days for the establishment of infection. Before starting chemotherapeutic trial, it was confirmed that the fish were infected with microorganisms by clinical diagnosis in laboratory condition. They showed hemorrhages and ulcerative lesions over the infected area. A total of 80 such experimentally infected stinging catfish having average body weight of 18 g were used for the experiment. Two chemotherapeutics: antibiotic, Eryvet (erythromycin thiocyanate INN, sulphadiazine (NaUSP) & trimithoprim BP) and antifungal, methylene blue were used in separate and combined treatment with three different doses of antibiotic. The same dose of methylene blue, 0.2 mg/l, was used for separate and combined treatment by antibiotic. Doses of antibiotic (Eryvet) were 0.8 g/10 kg body weight of fish, 1g/10 kg body weight of fish and 1.2 g/10 kg body weight of fish as lower dose, recommended dose and higher dose respectively. For combined treatment the above different doses of antibiotic and the same dose of antifungal were used. Water was exchanged regularly. The chemotherapeutic trial was conducted for 7 days and observation was continued for another 8 days to observe the effect of treatment. Combined treatment with the recommended dose of the antibiotic and methylene blue showed the best result where 90% fish were recovered. By the treatment with the higher dose of the antibiotic 70% fish were recovered. Antifungal treatment showed that 20% fish were recovered. All the fish in negative control aquarium died.

Keywords: chemotherapeutics; experimentally injured; stinging catfish

1. Introduction

Fish production through aquaculture is increasing day by day. But the total production is still behind the demand. Outbreak of various diseases is one of the crucial factors in fish production both in farming system and in wild condition. Diseases impact badly on fish production in different stages, such as severe mortality destroy the entire population, reduce growth and ultimately reduce the total production, disqualifying the product with reduced market price. A wide range of biological etiologies including virus, fungus, and especially bacteria have been associated with outbreak of diseases. Chemicals and antibiotics are important components in health management of aquatic animal, pond construction, soil and water management, improvement of natural aquatic productivity, transportation of live fish, feed formulation, manipulation of reproduction, growth promotion and processing and value addition of the final product (Subasinghe *et al.*, 1996; GESAMP, 1997). A variety of other chemicals are also being used in aquaculture for health management of fish apart from antibiotics. They include sodium chloride, formalin, malachite green, methylene blue, potassium permanganate, hydrogen per oxide, copper compounds, glutaraldehyde and trifluralin (Plumb, 1992). Methylene blue is an organic dye that has been popular as a parasiticide and fungicide on fish. It is principally used in hatcheries rather than grow-out systems. Lengthy withdrawal period was essential following application of methylene blue because of persistent residues (Alderman, 1992). Antibiotics have been widely used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish. It has been applied in aquaculture for over 50 years. Antibacterial chemotherapy is applied in aquaculture throughout the world. Although selecting the correct antibiotic and accurate dose is important factor in controlling bacterial disease, proper administration of any antibiotic for the recommended number of days is equally important. Many problems have been associated with the use of aqua-medicines. Many marginal farmers face the lack of efficiency of aqua-medicines. They are not truly benefited through using the recommended dose of aqua-medicines 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 or weakened enough and this greatly increases the risk of the bacteria to develop resistance to the antibiotic. When bacteria become resistant to a specific antibiotic, even high concentrations of that drug will not be effective. Decreased efficacy has been documented in many antimicrobial drugs regardless of their mechanism of action (Dixon, 1994). The objectives of the present study were to understand the effect of selected chemotherapeutics against experimentally infected *Heteropneustes fossilis* and to determine the dose, dosage, and methods of their application.

2. Materials and Methods

2.1. Experimental fish

Stinging catfish *Heteropneustes fossilis* of average body weight of 18 g were collected from the Mechua Bazar fish market in Mymensingh. The fish were acclimatized for 7 days in laboratory condition at Fish Clinic of Bangladesh Agricultural University, Mymensingh.

2.2. Experimental injury

The acclimatized fish were experimentally injured by using forceps and knife at a dorso-lateral place, ventro-lateral portion of the caudal region, ventral region and on the dorsal surface of the head (Figure 1). The fish were kept in the same aquaria and waited for seven days for formation of lesions. After the establishment of infection, 80 such experimentally infected fish were selected for chemotherapeutic treatment.

Figure 1. Experimental injury on the (a) dorso-lateral region (b) ventro-lateral portion in the caudal region (c) on the ventral region (d) on the dorsal surface of the head of stinging catfish, *Heteropneustes fossilis.*

2.3. Clinical diagnosis

For primary detection of the establishment of infection, following general clinical symptoms were investigated. The observation were, presence of any external hemorrhage; presence of any superficial and or ulcerative lesion in the experimentally injured place; darkening of body color; condition of any exophthalmia; loss or rottening of any fin rays; any cork screw or vertical swimming; any abnormal feeding tendency; and any sluggish movement or frequent rest.

2.4. Chemotherapeutics used

The antibiotic, Eryvet (Figure 2a) was used in treating the experimental infections. The trade name was Eryvet; composition was Erythromycin thiocyanate INN, sulphadiazine (NaUSP) and trimithoprim BP; name of the company was ACI Animal Health, ACI Limited. The recommended dose was 1 g/10 kg fish for 7 days. Methylene blue, the antifungal (Figure 2b) was also used in this experiment.

Figure 2. (a) Eryvet the antibiotic (b) Methylene blue, the antifungal, used in the experiment.

2.5. Experimental setup

The water re-circulatory system in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, was consisted of 12 rectangular glass aquaria of 40 1iter capacity, 5 drums, 1 pump and one overhead tank. Water was picked up into the overhead tank by pump. Aquaria were filled up by the water of overhead tank through downward pipe. When aquaria were filled up, water was passed through collection pipe to ultra-violet tube light complex. Then the water was reserved in the plastic drum. Drum water was picked up into the over head tank by pump (Figure 3). Freshwater from underground deep pump was added to the re-circulatory system as and when needed to fill up the loss due to evaporation. The trials were conducted in eight separate aquaria. Antifungal treatment was given in one aquaria, three aquaria were treated with antibiotic, the next three aquaria were treated with the combination of antibiotic and antifungal, and rest aquaria was selected as negative control. The aquaria with the above chemotherapeutics were designed as Section-A, Section-B, Section-C and Section-D, as follows.

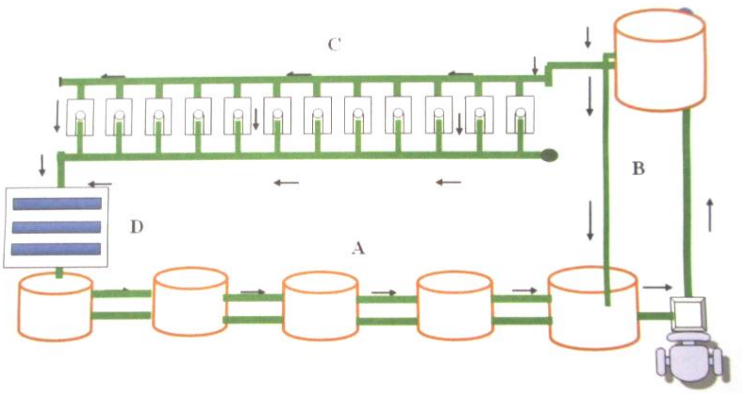

Figure 3. Layout of the re-circulatory system used for the experimentally injured catfish. A. five plastic drums; B. motor and overhead tank; C. downward pipeventilated to each aquarium and the collection pipe; D. ultra violet tube light complex.

2.5.1. Antifungal treatment (Section A)

In section-A, the aquarium number 1 was treated with the antifungal, methylene blue, at a dose of 0.2 mg/l water and the fish were intubated with 0.5 ml of sterile distilled water, once daily for 7 days.

2.5.2. Antibiotic treatment (Section B)

In Section-B, the aquarium number 2 was supplied with the antibiotic Eryvet at a dose, lower than the recommended dose, 0.8 g/10 kg fish. In Section-B, the aquarium number 3 was supplied with the antibiotic Eryvet at the recommended dose, 1 g/10 kg fish. In Section-B, the aquarium number 4 was supplied with the antibiotic Eryvet at a dose, higher than the recommended dose, 1.2 g/10 kg fish.

2.5.3. Combination of antifungal and antibiotic (Section C)

In Section-C, the aquarium number 5 was supplied with a combined treatment of the antibiotic, Eryvet, at a dose, lower than the recommended dose, 0.8 g/10 kg fish and the antifungal, methylene blue, at a dose of 0.2 mg/l of water. In Section-C, the aquarium number 6 was supplied with a combined treatment of the antibiotic Eryvet at the recommended dose, 1 g/10 kg fish and the antifungal, methylene blue at a dose of 0.2 mg/l of water. In Section-C, the aquarium number 7 was supplied with a combined treatment of the antibiotic Eryvet at a dose, higher than the recommended dose, 1.2 g/10 kg fish and the antifungal, methylene blue, at a dose of 0.2 mg/l of water.

2.5.4. Negative control (Section D)

In Section D, the aquarium number 8 was not mixed with any antifungal and the fish were intubated not with any antibiotic but with 0.5 ml of sterile distilled water, once daily for 7 days. Ten fish were released in each aquarium. Thus, in eight aquaria, 10 × 8 = 80 fish were used. Average body weight of fish was 18 g (Table 1). Medicinal trial period was 7 days and, after that period, more 8 days were for observation of the treatment result. Thus total experimental period was one month (Table 2).

Table 1. The average body weight of each of ten fish in eight different aquaria.

Aquarium number	**Section-A**	**Section-B**			**Section-C**			**Section-D**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Individual body weight (g)	15	14	16	16	14	17	18	17
	15	14	16	17	14	18	19	18
	15	14	17	17	14	19	19	18
	16	15	17	17	15	19	19	18
	17	16	18	18	15	20	19	18
	17	16	18	18	16	20	20	19
	18	17	18	18	17	21	21	20
	18	17	20	19	17	21	21	20
	19	18	20	20	18	22	22	21
	20	19	20	20	20	23	22	21
Total body weight (g)	170	160	180	180	160	200	200	190
Average body weight (g)	17	16	18	18	16	20	20	19

Table 2. Different stages of the experiment and its duration.

Experimental stage	**Duration (days)**
Acclimatization	7
Experimental injury	1
Post injured infection establishment period	7
Drug treatment period	7
Period of observation of result	8
Total	30

2.6. Calculation and application of chemotherapeutics

2.6.1. Antifungal (Methylene blue) (section A)

2.6.1.1. Aquarium number 1

Aquarium contained 30 liter of water. Dose of methylene blue = 0.2 ppm (0.2 mg/l) for prolong treatment. Total amount of methylene blue needed for aquarium = (0.2 × 30) mg = 6 mg. Every 6 mg methylene blue was weighed in an electric balance and put in an aquarium for 7 days. Everyday 50 percent water was changed and addition of methylene blue was adjusted after water change.

2.6.2. Antibiotic (Eryvet) (section B)

2.6.2.1. Preparation of antibiotic

At first 12.8 mg, 18 mg, 21.6 mg antibiotic was weighed in an electric balance and taken in three separate cleaned petridishes. Then 5 ml distilled water was added to the petridish to prepare 5 ml antibiotic solution. This 5 ml antibiotic solution was used for 10 fish. So, each fish was treated with 0.5 ml antibiotic solution. Fish was taken out from the aquarium by a scoop net and the suspension intubated orally. After 10 seconds of intubation fish was released in the aquarium. Antibiotic treatment was given once daily for 7 days.

2.6.2.2. Aquarium number 2

Number of total fish used for antibiotic treatment was 10.Selected lower than the recommended dose: 0.8 g/10 kg body weight for 7 days. Average body weight of fish was 16 g. Total body weight of 10 fish = 160 g**.** Total amount of antibiotic required for 10 fish for 1 day = 0.8 × 160 × 1000/10000 = 12.8 mg**.** So, each fish required 1.28 mg antibiotic each day.

2.6.2.3. Aquarium number 3

Number of total fish used for antibiotic treatment was 10. Selected recommended dose: 1 g/10 kg body weight for 7 days. Average body weight of fish was 18 g. Total body weight of 10 fish = 180 g. Total amount of antibiotic required for 10 fish = 1× 180 × 1000/10000 = 18 mg. So, each fish required 1.8 mg antibiotic each day.

2.6.2.4. Aquarium number 4

Number of total fish used for antibiotic treatment was 10. Selected higher than the recommended dose: 1.2 g/10 kg body weight for 7 days. Average body weight of fish was 18 g. Total body weight of 10 fish = 180 g. Total amount of antibiotic required for 10 fish = 1.2 × 180 × 1000/10000 = 21.6 mg. So, each fish required 2.16 mg antibiotic each day.

2.6.3. Combination of antifungal and antibiotic (section C)

2.6.3.1. Preparation of antifungal (Methylene blue)

Each aquarium contained 30 liter water. Dose of methylene blue = 0.2 ppm (0.2 mg/l) for prolong treatment. Total amount of methylene blue needed for aquarium = (0.2 × 30) mg = 6 mg.

6 mg methylene blue was weighed in an electric balance and put in Aquarium-5, Aquarium-6, Aquarium-7 respectively for 7 days. Everyday 50 percent water was changed and chemotherapeutics were adjusted after water change.

2.6.3.2. Preparation of antibiotic

At first 12.8 mg, 20 mg, 24 mg antibiotic were weighed in an electric balance and taken in three separate cleaned petridishes. Then 5 ml distilled water was added to the petridish to prepare 5 ml antibiotic solution. This 5 ml antibiotic solution was used for 10 fish. So, each fish was treated with 0.5 ml antibiotic solution. Antibiotic solution was used for treatment as; the amount of 0.5 ml antibiotic suspension was taken in a syringe. Fish was taken out from the aquarium by a scoop net and the suspension intubated orally. After 10 seconds of intubation fish was released in the aquarium. Antibiotic treatment was given once daily for 7 days.

2.6.3.3. Antibiotic (Eryvet)

2.6.3.3.1. Aquarium number 5

Number of total fish used for antibiotic treatment was 10. Selected lower than the recommended dose 0.8 g/10 kg body weight for 7 days.Average body weight of fish was 16 g. Total body weight of 10 fish = 160 g. Total amount of antibiotic required for 10 fish = 0.8 × 160 × 1000/10000 = 12.8 mg. So, each fish required 1.28 mg antibiotic each day.

2.6.3.3.2. Aquarium number 6

Number of total fish used for antibiotic treatment was 10. Selected recommended dose: 1 g/10 kg body weight for 7 days. Average body weight of fish was 20 g. Total body weight of 10 fish = 200 g. Total amount of antibiotic required for 10 fish = 1 × 200 × 1000/10000 = 20 mg. So, each fish required 2 mg of antibiotic each day.

2.6.3.3.3. Aquarium number 7

Number of total fish used for antibiotic treatment was 10. Selected higher than the recommended dose, 1.2 g/10 kg body weight for 7 days. Average body weight of fish was 20 g. Total body weight of 10 fish = 200 g. Total amount of antibiotic required for 10 fish = 1.2 × 200 × 1000/10000 = 24 mg. So, each fish required 2.4 mg antibiotic each day.

3. Results

3.1. Temperature of the re-circulatory system

During the study period the temperature of the re-circulatory system were ranged from 17ºC to 25ºC. The average temperature was 21.5ºC (Figure 4).

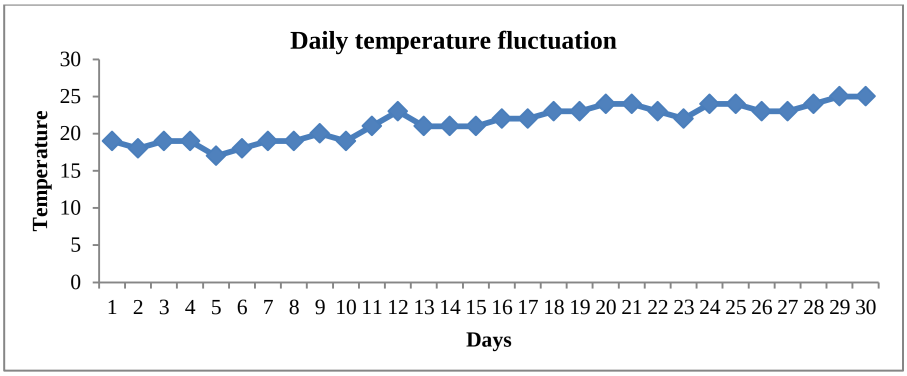

Figure 4. Daily temperature fluctuation during the experimental period.

3.2. Gross clinical features of the experimentally infected fish

Experimentally infected stinging catfish *Heteropneustes fossilis* showed the following features after 7 days of artificial injury. Reddish lesion on the dorsal surface of the head (Figure 5); cottony appearance on the dorsal region (Figure 5); lesion on the ventro-lateral portion in the caudal region (Figure 5); lesion on the ventral region (Figure 6); lesion on the fin in the caudal region (Figure 6); hemorrhagic lesion on dorsal surface in the caudal region (Figure 6); blackish spot on the ventral region(Figure 6); cottony appearance on the dorso-lateral region (Figure 6).

Figure 5. (A) Reddish lesion on the dorsal surface of the head; (B) Cottony appearance on the dorsal region; (C) Lesion on the ventro-lateral portion in the caudal and (D) Lesion on the ventral region of experimental fish.

Figure 6. (E) Lesion on the fin in the caudal region (F) Hemorrhagic lesion on dorsal surface in the caudal region (G) Blackish spot on the ventral region and (H) Cottony appearance on the dorso-lateral region of experimental fish.

3.3. Results after chemotherapeutic treatment

With the treatment of antifungal, mythelene blue two fish were cured, three fish were not recovered and five fish were died during the experiment (Figure 7). Results found after treatment with chemotherapeutics are shown in Table 3.

Figure 7. (A) No significant difference found at the seventh day of treatment; (B) Lesion present in the dorsal surface of the head at the end of the experiment; (C) Cured fish at the end of the experimental period.

With the treatment of antibiotic, Eryvet, at lower than the recommended dose three fish were not recovered but were alive and seven other fish were died during experiment (Figure 8).

Figure 8. (D) Cottony appearance present at the seventh day of treatment; (E) Improvement of infected region at the end of experimental period.

With the treatment of antibiotic, Eryvet, at recommended dose, five fish were cured, one fish was not recovered and other four fish were died during experiment (Figure 9).

Figure 9. (F) Improvement of infected region at seventh day of the treatment period; (G) Not recovery of infected region at the end of the experimental period.

With higher than the recommended dose of antibiotic, Eryvet, seven fish were cured, one fish was not recovered and two fish were died during experiment (Figure 10).

Figure 10. (H) Improvement of infected region at seventh day of the treatment period; (I) Cured fish at the end of the experimental period.

With antifungal and lower than the recommended dose of antibiotic, Eryvet, four fish were cured, four fish were not recovered and other two fish died during experiment (Figure 11).

Figure 11. (J) Improvement of infected region at seventh day of the treatment period; (K) Cured fish at the end of the experimental period.

With antifungal and recommended dose of antibiotic, Eryvet, nine fish were cured, and one fish died during experiment (Figure 12).

Figure 12. (L) Improvement of infected region at seventh day of the treatment period; (M) Cured fish at the end of the experimental period.

With antifungal and higher than the recommended dose of antibiotic, Eryvet, nine fish were cured, and one fish died during experiment (Figure 13).

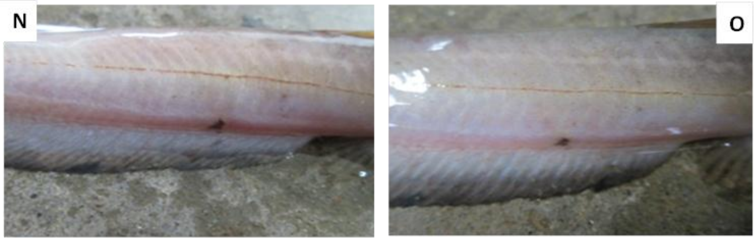

Figure 13. (N) Improvement of infected region at seventh day of the treatment period; (O) Cured fish at the end of the experimental period.

In the negative control all of the ten fish of the experiment died (Figure 14).

Figure 14. (P) Development of infected region at seventh day of the treatment period; (Q) Dead fish at the fifth day of observation.

Table 3. Effects of chemotherapeutics on experimentally infected *Heteropneustes fossilis.*

Chemotherapeutics	Selected dose	No. of fish treated	No. of fish cured	No. of fish not recovered	No. of fish dead	Percentage of recovery
Antifungal (Mythelene Blue)	0.2 mg/l	10	2	3	5	20
Antibiotic (Eryvet)	Lower	10	0	3	7	0
	Recommended	10	5	1	4	50
	Higher	10	7	0	3	70
Antifungal	Lower	10	4	4	2	40
+	Recommended	10	9	0	1	90
Antibiotic	Higher	10	9	0	1	90
Negative Control	No Treatment	10	0	0	10	0

3.4. Cumulative mortality

At the end of experiment lowest cumulative mortality, 10% was found for recommended and higher than the recommended dose of antibiotic with antifungal (CRD, CHD) treatment. For the combined treatment of antibiotic lower than the recommended dose and antifungal (CLD) showed 20% cumulative mortality. At the rate of 30% cumulative mortality was found for higher than the recommended dose of antibiotic (AHD). The treatment of recommended dose of antibiotic (ARD), 40% cumulative mortality was found. Only antifungal treatment, 50% cumulative mortality was found. For lower than the recommended dose of antibiotic (ALD) treatment, 70% cumulative mortality was found. The highest cumulative mortality was found for negative control, where all the fish were died and their cumulative mortality was 100%. Different cumulative mortality of treated fish against selected chemotherapeutics are shown in Figure 15.

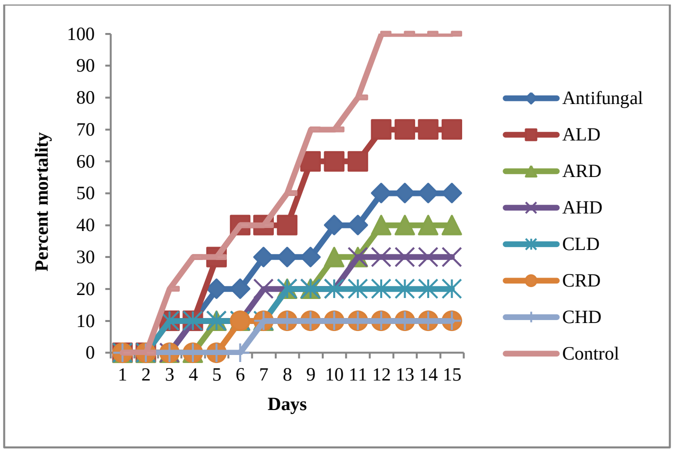

Figure 15. Cumulative mortality of treated fish against selected chemotherapeutics, by which the result, either cured or not recovered but also not died at the end of the experimental period, was understood.

4. Discussion

Commonly found traditional chemotherapeutics for disease control of fish are antibiotics, lime, salt, potassium permanganate, sumithion, melathion, formalin, bleaching powder, malachite green, methylene blue, and copper sulphate. Some previous studies also revealed the similar reports about the use of chemicals in Bangladesh aquaculture (Phillips, 1996; Brown and Brooks, 2002; Faruk *et al.*, 2005). According to, Rahman *et al.* (2016) and Ali *et al.* (2016) in the culture condition fishes were frequently affected by bacterial pathogen. Fungus attacked at the infected region as secondary invader. Different antibiotic and antifungal agents are useful to prevent bacterial and fungal disease. To establish the accurate dose, dosage and treatment method of commonly used antibiotic, Eryvet and antifungal, methylene blue, they were selected for the experiment at laboratory condition. Chemotherapeutics reduce the level of infection which either prevent multiplication of pathogen or retard their growth and the fish can overcome the disease (Olah and Farks, 1978; Srivastata, 1978; Zahura, 2001). The dose of chemotherapeutics in the present study differed from some of the previous study but the modes of action of the chemotherapeutics were found to be very similar. In any case, treatments using antibiotic have to be administrated at the effective dosage and during enough time to ensure elimination of bacteria (De Kinkelin *et al.*, 1986). The present study was carried out to justify the recommended dose and method of application of particular chemotherapeutics. Some variation was found between the information leaflet and packed indication. Neither the sellers nor the farmers or extension workers had clear idea about the ingredient of pharmaceutical companies nor were they using those without hesitation.Little is known about the fate of unused antibiotics or their effect on the environment (Rahman *et al.*, 2015a; Rahman *et al.*, 2015b; Shabuj *et al.*, 2015 and Yeasmin *et al.*, 2015). Obviously, the potential exists for the antibiotics to affect adversely natural bacterial communities. 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 broken down, depending upon temperature, oxygen and light levels (Jacobson and Berglind, 1988; Samuelson, 1989 and Neowajh *et al.*, 2015).

In the present study, methylene blue was run to prevent secondary infection caused by fungus. It was used with antibiotic combinedly at a dose of 0.2 mg/l as prolonged treatment. It showed the best result (90% recovery of fish). The relevant results were found by the study of Sharmin *et al.* (2016); Hossain *et al.* (2016); Monir *et al.* (2015); Chowdhury *et al.* (2015); Ahmed *et al.* (2015); Hasan *et al.* (2015) and Borty *et al.* (2016). Scott and Warren (1964) used 2 ppm concentration of methylene blue for complete destruction of fungal hyphae within 7 days treatment. Rahman *et al.* (2017) found the same result at the species of *L. bata*. Roberts (1993) found that 0.25 ppm of methylene blue was required to kill *Saprolegnia parasitica.* In the present study it was found that 0.2 mg/l mythelene blue was helpful to prevent fungal infection in combination with antibiotic, Eryvet. Rahman and Chowdhury (1999) conducted trials of chemotherapy to control the ulcer disease-affecting catfish as a case study. The best result was obtained by a successive bath in 1-2% NaCI suspension and subsequent oral treatment with commercial erythromycine at a dose of 75 mg per kg body weight of fish for 5 days. Haque *et al.* (2014) observed the effectiveness of antibiotic, erythromycin, reducing the bacterial load in rohu fish under artificial culture condition in the laboratory. They resulted that 2 gm/kg erythromycin in diet, twice daily is potential antibiotic to reduce bacterial load in fish and can be used commercially for maintaining the fish health in aquarium condition. The present study was conducted very carefully during uses of chemotherapeutics.

Combined use of chemotherapeutics: antibiotic and methylene blue treatment showed best results (90% recovery of fish). Single antibiotic (Eryvet) showed good results at higher dose (70% recovery of fish). Single methylene blue showed bad results (20% recovery of fish).It is suggested that combined treatment of antibiotic, Eryvet at a dose of 1 g/10 kg body weight of fish for 7 days once daily and antifungal, methyleene blue at a dose of 0.2 mg/l water for 7 days should be used for effective result. However, field level experimental trials should be conducted before its implementation.

5. Conclusions

For successful aquaculture operation selecting the correct chemotherapeutic is an important first step in controlling infection, proper administration of any chemotherapeutic for the recommended days is equally important. Generally, lesions occur in the fish body due to bacterial infection which may be cured by antibiotic treatment. But sometimes, as a secondary invader fungus may also be found on the lesion area. Thus, an anti-fungal drug use together with antibiotics should be effective measure. In treating both bacterial and fungal infection, use of mythelene blue is recommended widely. In the present study use of methylene blue together with the antibiotic showed the best result. Only single use of antibiotic did not show the best result. Obviously without antibiotic, use of methylene blue also did not show the best result. So, in the case of experimentally infection of at least stringing catfish, an effective antibiotic treatment like Eryvet together with an anti-fungal drug mythelene blue is recommended.

Conflict of interest

None to declare.

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