Pharmacological studies On Some Antibacterial Agents In Fish

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ABSTRACT

The antibacterial activities of amoxicillin and florfenicol were investigated both *in-vitro* and *in-vivo* against *Aeromonas hydrophila* in *Clarias Lazera* . The obtained results indicated that the two drugs were effective *in vitro* against microorganism. The antibacterial activity of these drugs in their therapeutic doses decreased the mortality rate (%) and pathogenic clinical signs compared to that of infected non medicated group. The estimated hematological parameters showed that both amoxicillin and florfenicol caused a slight decrease in RBCs, WBCs counts, Hb, and PCV % and all these changes were reversible and returned back to the normal range within 7 days post treatment . The effects of these drugs on the estimated biochemical parameters showed that, amoxicillin and florfenicol induced non significant changes in the most of biochemical parameters (AST, urea and creatinine) compared to the control group. While significant changes were recorded between amoxicillin and florfenicol treated group and that infected non treated one .

INTRODUCTION

Fish is often the cheapest source of animal protein and is, therefore, important in the diets of the lowest income groups *(1)*. Outbreaks of disease have become a critical factor which has hampered the development of aquaculture in many countries. Bacterial diseases are a major problem in aquaculture and account for significant losses of fish among these various disease agents. The Gram-negative bacteria, *Aeromonas* species are commonly found in a wide range of aquatic environments including fish ponds and it is the causative agent of motile aeromonas infection (MAI), which occur in a wide variety of freshwater fish species. *Aeromonas hydrophila* and other motile aeromonads are among the most common bacteria in freshwater habitats throughout the world, and these bacteria frequently cause disease among fish *(2)*.

Amoxicillin is a semi-synthetic penicillin of a broad spectrum antibacterial activity. It has been licensed in the UK for use in fish therapy *(3)*.

In late 2005, florfenicol was made available to producers as a veterinary feed directive (VFD) drug , a relatively new category established by the FDA to more closely control new therapeutic products, primarily antimicrobials, and their use in food animals *(4)*.

Recently, attention has been focused on residues in food fish, the risk of developing resistant pathogens, and environmental bioaccumulation .Morover, other deleterious effects, such as immunosuppression, nephrotoxicity, and growth retardation have been associated with the use of drugs in aquatic organisms *(5)*.

The present study was conducted to throw the light on the efficacy of amoxicillin and florfenicol against *Aeromonas hydrophila* organism in *Clarias lazera*. In addition to the effect of the drugs on some haematological parameters and liver and kidney functions .

MATERIALS AND METHODS

Materials

Antibacterial agents

(1) Amoxicillin: E-mox® is available in vials containing 500 mg or 1 gm amoxicillin as the sodium salt obtaind from Eipico Pharmaceutical Co., Egypt.

(2) Florfenicol : Floricol® each ml contains 100 mg of florfenicol obtained from Pharma Swede –EGYPT

Tested organisms

1.*Aeromonas hydrophila*: It was obtained from Animal Health Research Institute .Dokky, Cairo

Fish

A total number of 150 apparently healthy catfish (*Clarias lazera*) were employed for this study. They were obtained from a private fish farm in Sharkia governorate with average body weight of 200 gm. Fish were acclimatized for 2 weeks before beginning of experiment.

Media

Nutrient broth; Nutrient agar for bacterial growth; Muller –Henton agar for sensitivity test .

Chemicals used for hematological studies

Heparin,Methanol, Giemsa stain, Natt & Herrik's solution and Hydrochloric acid (1\10 N)

Kits for serum biochemical analysis: kits used for determination of serum transaminases (AST&ALT) , urea and creatinine levels were supplied by biomerieux, France.

Methods

I. Efficacy of amoxicillin and florfenicol against *Aeromonas hydrophila* infection in (catfish)

***a. In-vitro:* -Sensitivity test (disc diffusion method)**

The *in-vitro* antibacterial effect of amoxicillin and florfenicol against *Aeromonas hydrophila* was carried out using disc diffusion method *(6)*. The technique was standardized by the National Committee for Clinical Laboratory Standards *(7)*

b. In Vivo

To determine the *in-vivo* efficacy of amoxicillin and florfenicol against *Aeromonas hydrophila* infection, One hundred and fifty Nile catfish *(Clarias lazera)* were divided into six equal groups each of 25. They were kept in a well aerated glass aquaria measuring 100 x 50 x 50 cm to be acclimatized on dechlorinated tap water for 15 days.

- Group 1: fish were served as non infected non treated (negative control).
- Group 2: normal fish treated with therapeutic dose of amoxicillin (80 mg/kg b.wt) in feed for 10 successive days*(3)*.
- Group 3: normal fish treated with therapeutic dose of florfenicol (10 mg/kg b.wt) in feed for 10 successive days *(8)*.
- Group 4: fish inoculated intraperitoneally (I/P) with 0.2 ml of 24 hrs broth cultures of *Aeromonas hydrophila* (2.5×10^8 / ml) and kept without medication (positive control).
- Group 5: fish experimentally infected similarly, and treated with therapeutic dose of amoxicillin
- Group 6: fish experimentally infected similarly, and treated with therapeutic dose of florfenicol.

11-Haematological studies

Blood samples were collected with EDTA as anticoagulant, from caudal veins of five fish from each group on 1st, 7th, and 14th days post administration of the drug.

Total erythrocytic and leucocytic counts were performed *(9)*, Heamoglobin concentration was *(10)*, while packed cell volume % was determined *(11)* .

111 . Biochemical analysis

Serum samples were collected from each group on 1st , 7th and 14th days post drug dosing and were used for determination of serum AST& ALT *(12)*,urea level *(13)* and creatinine *(14)*.

Statistical analysis

Data were analyzed using computerized SPSS. Results of the biochemical estimations were reported as mean ± S.E. The total variation was analyzed using one–way analysis of variance (ANOVA).Duncan test was used for determining significance, probability levels of less than 0.05 were considered significant *(15)*.

RESULTS AND DISCUSSION

***In-vitro* activity**

1. ***In-vitro*** sensitivity test of *A.hydophila* strain against amoxicillin and florfenicol using agar disc diffusion method showed that *A.hydophila* was susceptible to the tested drugs with clear zone of inhibition (Table

1). Sensitivity studies of *A. hydrophila* isolates against florfenicol indicated its high sensitivity to florfenicol. This result is similar to which showed that *A. hydrophila* was sensitive to florfenicol *in vitro* ***(16,17)***..

Sensitivity of *A. hydrophila* isolate against amoxicillin indicated that the microorganism was sensitive to amoxicillin, these results were parallel to that previously reported that *A. hydrophila* isolated from diseased fish was sensitive to amoxicillin ***(18,19)***.

In vivo activity

1. Mortality (%)

Our results revealed that experimentally infected catfish with *A. hydrophila* organism responded to the treatment with amoxicillin and florfenicol, as the recorded clinical signs were declined after 5 days post treatment .

The mortality started at the third day and reached 80% in the infected non treated group while the medicated groups with therapeutic dose of amoxicillin and florfenicol showed reduction in mortality rates (16 and 12% respectively), compared to non medicated group. (Table 2).

Table 1. Sensitivity test for amoxicillin and florfenicol against *A. hydrophila*.

Drug	Diameter of inhibition zones (mm)	Interpretation
Amoxicillin (30mg)	18	Susceptible
Florfenicol (30mg)	22	Susceptible

Table 2. The effect of amoxicillin and florfenicol on mortality (%) of experimentally infected *Clarias lazera* inoculated with *A. hydrophila* .

Fish grouping	Total number	Number of dead fish	Mortality (%)
Control (non infected non treated)	25	0	0%
Infected non treated	25	20	80 %
Infected treated with Amoxicillin	25	4	16%
Infected treated with Florfenicol	25	3	12%

2. Pathogensity

The effect of amoxicillin and florfenicol on pathogencity of *A. hydrophila* experimentally infected *Clarias Lazera*.

Two days after inoculation of *A. hydrophila* the infected fish showed some clinical signs manifested by sluggish movement, swimming near the water surface, progressive erosion allover the body, erythema at the base of the fins and some fish showed ulcer formation .

Treatment with amoxicillin (80 mg/kg in feed) daily for 10 successive days showed decline in clinical signs and mortality rate. Amoxicillin was effective for controlling *Streptococcus iniae* infection in blue tilapia ***(20,21)***. Amoxicillin at 10, 30 and 80 mg significantly increased the survival of *S. iniae* infected tilapia to 45, 75 and 93.8 % respectively, compared to 3.8 % in the positive control and the survival rate was significantly higher in the 80 mg treatment (93.8 %) than the 10 mg treatment (45 %).

Treatment with florfenicol at dose 10 mg / kg feed for 10 days was effective and increased survival of fish that have been challenged with *Aeromonas hydrophila* and in turn decrease mortalities. Florfenicol succeeded in controlling *Aeromonas Salmonicida* infections in Atlantic salmon***(22)***, and effective for the control of mortality in catfish due to enteric septicemia ***(23)***.

Hematological results

In the current work, administration of amoxicillin in therapeutic dose to non infected fish induce non significant decrease in total erythrocytic count and packed cell volume while it showed significant decrease in haemoglobin and total leucocytic count on 1st day post treatment compared with non infected group. The haematological parameters were decreased following administration of amoxicillin for five consecutive days in recommended therapeutic dose in broiler chicks ***(24,25)***.

Our results showed that florfenicol caused slight decrease in all hematological parameters manifested by decrease in RBCs count, Hb and PCV volume. This effect may be attributed to

depression of florfenicol on mitochondrial synthesis of protein in bone marrow. Inhibition of mitochondrial protein synthesis ultimately disrupts mitochondrial function, cellular function and cellular proliferation *(26)*.

Biochemical results

In the present study it has been observed that administration of therapeutic dose of amoxicillin and florfenicol to non infected fish induced significant increase in ALT on 1st day and non significant increase in AST. These findings were supported by the histopathological alteration of hepatic tissue.

Some alterations in laboratory determination may be observed specially in ALT and AST which may be elevated following amoxicillin and penicillin therapy *(27)*. Also it has been recorded that β-Lactamines cephalosporin cefoperazone in therapeutic dose in rabbits evoked a significant elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and that the drug did not affect the level of alkaline phosphatase *(28)*.

Florfenicol had no adverse effects on liver functions indicating that the drug is safe at the therapeutic dose *(29-31)*.

The effect of amoxicillin and florfenicol on urea and creatinin was studied in cat fish. The results indicated that there were non significant increase in urea and creatinine levels in both amoxicillin and florfenicol treated groups when compared to control value. These results are supported the results reported before and showed that florfenicol produced slight elevation in both urea and creatinine levels in fish *(32)*.

On the other hand, fish infected with *A.hydrophila* and treated with amoxicillin and florfenicol showed a moderate significant improvement in serum urea and creatinine levels when compared with infected non treated group.

It could be concluded that florfenicol was more effective in both *in vitro* and *in vivo* than amoxicillin .

Table 3. The effect of amoxicillin and florfenicol on erythrocytic count (10^6 / mm) of clinically healthy and experimentally infected *Clarias Lazera* with *Aeromonas hydrophila* microorganism. (M±S.E) (n=5).

Group	Erythrocytic count (10^6 / mm)		
	1st day	7th day	14th day
G1 Non infected non treated (control)	2.60±0.09a	2.69±0.06a	2.70±0.07a
G2 Non infected treated with amoxicillin	2.56±0.09a	2.62±0.05ab	2.58±0.04a
G3Non infected treated with florfenicol	2.14±0.10 bc	2.47±0.07ab	2.53±0.05a
G4 Infected non treated	1.58±0.18 d	1.62±0.12c	1.64±0.12b
G5 Infected treated with amoxicillin	2.42±0.08ab	2.45±0.04b	2.57±0.03a
G6 Infected treated with florfenicol	1.98±0.13 c	2.45±0.05b	2.53±0.07a

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 4. The effect of amoxicillin and florfenicol on total leucocytic count (10^3 / mm^3) of clinically healthy and experimentally infected *Clarias lazera* with *Aeromonas hydrophila*. (M±S.E) (n=5).

Group	Days post treatment		
	1st day	7th day	14th day
G1(control) Non infected non treated	15.30±0.27c	15.10±0.34b	15.40±0.22b
G2 Non infected treated with amoxicillin	11.75±0.51d	15.03±0.36b	15.36±0. 40b
G3Non infected treated with florfenicol	10.08±0.33e	13.66±0.15c	16.00±0.38ab
G4 Infected non treated	19.77±0.39 a	20.04±0.38a	16.76±0.38a
G5 Infected treated with amoxicillin	17.23±0.94b	15.70±0.35b	16.09±0.33ab
G6 Infected treated with florfenicol	20.03±0.13 a	15.75±0.22b	13.98±0.07c

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 5. The effect of amoxicillin and florfenicol on total Hb (gm / dl) of clinically healthy and experimentally infected Clarias *lazera* with *Aeromonas hydrophila* microorganism. (M±S.E) (n=5).

Group	Days post treatment		
	1st day	7th day	14th day
G1Non infected non treated (control)	10.80±0.25a	10.58±0.30a	11.14±0.16a
G2Non infected treated with amoxicillin	9.60±0.29b	9.60±0.30ab	10.54±0.24ab
G3Non infected treated with florfenicol	8.72±0.25 bc	9.11±0.36b	10.12±0.19b
G4 Infected non treated	7.80±0.46c	7.40±0.43c	8.00±0.35c
G5 Infected treated with amoxicillin	9.20±0.46b	9.60±0.35ab	10.20±0.25b
G6 Infected treated with florfenicol	9.50±0.35b	9.50±0.36b	9.70±0.33b

Different letters at the same column means that there was a significant change at $p<0.05$

Table 6. The effect of amoxicillin and florfenicol on total PCV % of clinically healthy and experimentally infected Clarias *lazera* with *Aeromonas hydrophila* microorganism. (M±S.E) (n=5).

Group	Days post treatment		
	1st day	7th day	14th day
G1 Non infected non treated (control)	30.00±1.37a	30.04±1.88a	28.90±1.88a
G2Non infected treated with amoxicillin	29.20±.66a	29.40±0.87a	28.40±1. 56a
G3Non infected treated with florfenicol	29.20±1.22a	28.80±0.74a	28.70±1.20a
G4 Infected non treated	21.00±1.87b	21.20±0.58b	23.00±2.26b
G5 Infected treated with amoxicillin	28.00±1.46a	28.40±2.71a	28.90±1.28a
G6 Infected treated with florfenicol	24.40±1.32b	28.60±0.67a	28.91±1.15a

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 7. The effect of amoxicillin and florfenicol on serum AST (U/ml) and ALT (U/ml) of clinically healthy and experimentally infected Clarias lazera with A. hydrophila . (M ± S.E) (n = 5).

Group	Day post treatment					
	1st day		7th day		14th day	
	AST	ALT	AST	ALT	AST	ALT
G1(control) Noninfected non treated	219.60±30.7b	17.36±0.46c	222.60±28.5b	19.60±1.28c	221.20±29.2b	21.20±1.39b
G2 Non infected treated with amoxicillin	216.8±32.5b	29.20±4.02ab	219.6±30.8b	27.41±1.75b	218.60±28.9b	21.94±3.05b
G3 Non infected treated with florfenicol	300.4±25.3ab	30.66±1.36a	227.8±30.5b	23.62±1.73bc	220.6±33b	20.87±0.99b
G4 Infected non treated	359.6±40.5a	31.14±1.77ab	411±35.4a	36.40±0.92a	447±26.5a	42.60±3.73a
G5 Infected treated with amoxicillin	268.4±33.4ab	28.00±3.48ab	231.2±19.4b	22.03±2.61bc	240±19.5b	20.05±1.67b
G6 Infected treated with florfenicol	300±25.3ab	29.27±1.82ab	231.2±19.4b	22.26±2.36bc	230.60±23.2b	20.80±1.52b

Different letters at the same column means that there was a significant change at $P \leq 0.05$

Table 8. The effect of amoxicillin and florfenicol on serum urea and creatinine of clinically healthy and experimentally infected Clarias lazera with A. hydrophila . (M ± S.E) (n = 5)

Group	Day post treatment					
	1st day		7th day		14th day	
	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
G1 (control) Non infected non treated	1.60±0.17c	0.43±0.02a	1.60±0.17b	0.42±0.02a	1.64±0.19b	0.44±0.03a
G2 Non infected amoxicillinl treated	2.13±0.14 bc	0.40±0.03a	1.72±0.24b	0.41±0.01a	1.88±0.05b	0.43±0.02a
G3 Non infected florfenicol treated	2.18±0.17bc	0.51±0.04a	1.86±0.19b	0.44±0.05a	1.58±0.14b	0.42±0.03a
G4 Infected non treated	3.22±0.31a	0.59±0.03b	3.48±0.23a	0.54±0. 02a	3.56±0.39a	0.51±0.03a
G5 Infected amoxicillin Treated	2.26±0.25bc	0.48±0.05a	2.00±0.15b	0.42±0.04a	1.72±0.11b	0.42±0.05a
G6 Infected florfenicol treated	2.35±0.35b	0.48±0.04a	1.83±0.09b	0.43±0.02a	1.68±0.11b	0.42±0.03a

Different letters at the same column means that there was a significant change at $P \leq 0.05$

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الملخص العربي

دراسات فارماكولوجية على بعض مضادات الميكروبات فى الأسماك

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استهدفت هذه الدراسة معرفه كفاءة كلا من دواء الأموكسيسيللين و الفلورفينيكول في علاج اسماك القراميط المصابة بميكروب الايروموناس هيدروفيلا معمليا . وقد أعطي الأموكسيسيللين عن طريق الأكل بجرعة علاجيه قدرها ٨٠مجم/ كيلو جرام وزن حي يوميا لمده عشرة أيام متتالية . كما أعطي الفلورفينيكول عن طريق الأكل بجرعة علاجيه قدرها ١٠مجم/ كيلو جرام وزن حي يوميا لمده عشرة أيام متتالية .

أجريت هذه الدراسة علي عدد ١٥٠ سمكة تزن الواحدة ٢٠٠ جرام تقريبا و قسمت إلي ست مجموعات متساوية كلا منها خمسة وعشرون سمكه و ذلك لمقارنه كفاءة هذه الأدوية معمليا وعلي الأسماك المصابة وعلي نسب النفوق وكذلك دراسة تأثير هذه الأدوية علي صوره الدم و أيضا علي وظائف الكبد و الكلي .

وقد أوضحت الدراسة ما يلي :-

١- عقار الفلورفينيكول أعطي أعلي تأثير مثبط على ميكروب الايروموناس هيدروفيلا معمليا و كذلك في الأسماك الحية و كان هناك تناقص ملحوظ في عدد الأسماك النافقة في المجموعة التي تلقت العلاج بهذا العقار عند مقارنتها بالمجموعة الضابطة و كذلك عقار الأموكسيسيللين حيث كانت نسبه النفوق في الأسماك الغير معالجه(٨٠%) و انخفضت إلي(١٢ & ١٦%) في الأسماك المعالجة بكلا الفلورفينيكول و الأموكسيسيللين علي التوالي .

٢- بدراسة تأثير هذه العقاقير علي صورة الدم لوحظ أن كل هذه العقاقير تحدث تغيرات في صوره الدم بدرجات متباينة ولكن تلك التغيرات رجعت إلي معدلها الطبيعي , وأن هناك تناقص معنوي في هذه القياسات بالنسبة للمجموعة المعداه بالميكروب بالمقارنة بالمجموعة الضابطة .

كما وجد أيضا زيادة معنوية في تلك القياسات في الأسماك المعداه و التي تم علاجها بالمقارنة بالأسماك الغير معداه و هذه الزيادة عادت مستوي هذه القياسات إلي معدلها الطبيعي .

٣- بدراسة تأثير هذه العقاقير علي مستوي أنزيمي الأسبرتات أمينو ترا نسفيريز و الألانين أمينو ترانسفيريز و علي مستوى البولينا و الكرياتينين في مصل الأسماك المستخدمة وجد أن عقار الأموكسيسيللين و الفلورفينيكول أحدث تغيرات غير معنوية بالمقارنة بالمجموعة الضابطة .

كما وجد أن هناك تغيرات معنوية في هذه القياسات بالنسبة للمجموعة المعداه بالميكروب بالمقارنة بالمجموعة الضابطة .و أن هذه التغيرات في تلك القياسات عادت إلي معدلها الطبيعي في الأسماك المعداه والتي تم علاجها.