



Antibacterial Activity, Biochemical Effect and Tissue Residue of Fourth Generation Cephalosporin Used in Treatment of Nile Tilapia Fish Against Bacterial Infection

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ABSTRACT

Antimicrobial drugs are used to control bacterial infections among fish in aquaculture and misuse could be associated with the spread of antimicrobial resistance and tissue residues with a negative impact on humans and animals including fish. This study was conducted to evaluate the effect of cefquinome on *Aeromonas hydrophila* and some biochemical parameters in tilapia fish, together with estimating its residue in serum, muscles, and liver using high-performance chromatography. One hundred and thirty-five Nile tilapia fish (*Oreochromis niloticus*) were used and divided into 3 equal groups. Fish of group 1 was a negative control, group 2 was a positive (infected with *Aeromonas hydrophila*) and group 3 was infected with the same bacteria and treated with Cefquinome sulphate (Cobactan® 2.5%) as a single intramuscular dose of 10 mg/kg body weight. Cefquinome showed an in-vitro inhibition zone of 29.3 ± 0.26 mm against *A. hydrophila*, and the minimum inhibitory concentration was 2 µg/ml. While, in-vivo; the mortality rate among infected fish was decreased after treatment by cefquinome from 66.6 % to 17.7 %, a slight recovery has been recorded in biochemical parameters at 7th to 14th day post-medication. Serum and tissue residue of cefquinome reached under the limit of detection at 120 hrs. post-medication while still detected in the liver even after 120 hrs post medication. It could be concluded that cefquinome had a good effect against *Aeromonas hydrophila* infection in tilapia. Fish flesh could be suitable for human consumption 5 days of post-cefquinome medication.

INTRODUCTION

Fish consider as an important source of protein, micronutrient, minerals and essential fatty acids to people all over the world (Allison, 2001). Aquaculture is a fast-

growing food production sector and its intensification may associated with a huge use of various drugs (Rahman, 2014).

Antibiotics have an important uses in human and veterinary medicine. In aquaculture, antibiotics are used mainly for therapeutic purposes and prophylactic measures which might induce antimicrobial resistance (Serrano, 2004). The use of large amounts of antibiotics might cause residues in fish tissue and fish products, which might create resistance against both Gram-positive and Gram-negative bacteria (Samanidou *et al.*, 2007).

FDA's Center for Veterinary Medicine (CVM) regulates the manufacture, distribution, and use of animal's drugs. FDA also has established safe maximum residue limits (MRLs) for these drugs and other veterinary medications. Such strict regulation were taken to ensure that the treated animals are free from potentially harmful residues (Canada *et al.*, 2012).

The current study aimed to evaluate the in-vitro and in-vivo antibiotic activity of cephalosporin against pathogenic *Aeromonas hydrophila* and their residues in tilapia fish together with recording the alterations in some biochemical parameters after treatment.

MATERIALS AND METHODS

1. In-Vitro study:

1.1.Sensitivity test (disc diffusion method):

The *in-vitro* antibacterial activity of cefquinome, ciprofloxacin, amikacin and tobramycin against *Aeromonas hydrophila* (as a common bacterial pathogen among Nile tilapia in Egypt) was carried out using disc diffusion test (Bauer *et al.*, 1966). At the end of incubation period, antibiotic inhibition zones were measured in mm using a measuring caliber. Susceptibility testing was conducted according to Quinn *et al.*, (1994).

1.2.Minimum inhibitory concentration test (MIC):

The minimum inhibitory concentration assay is a technique used to determine the lowest concentration of a particular antibiotic needed to inhibit the bacterial growth. This assay is typically performed on planktonic (free floating) bacterial cells as described by Lin *et al.*, (2014). The media, an antimicrobial agent and the microbe were prepared to be tested. The most commonly used media is cation-adjusted Mueller Hinton Broth, due to its ability to support the growth of most pathogens and its lack of inhibitors towards common antibiotics. The antimicrobial concentration is adjusted into the correct concentration by mixing stock antimicrobial with media. The adjusted antimicrobial was serially diluted into multiple tubes. The microbe, must come from the same colony-forming unit, and must be at the correct concentration.

2. *In-Vivo* study:

A total of 135 Nile tilapia (*Oreochromis niloticus*) were used in this study. Fish were divided into 3 equal groups (each group contained 45 fish subdivided in three glass aquaria as replicates) as shown in **Table (1)**. Experimental infection was done by intraperitoneal injection of 24 hrs aged broth culture of *Aeromonas hydrophila* (with 0.5 ml of 1×10^7 CFU per fish) (**Alyahya et al., 2018**). *A. hydrophila* strain was obtained from Animal Health Research Institute, Port-Said branch and used to induce the pathogenicity test.

Cefquinome sulfate (Cobactan[®] 2.5%) injectable suspension (50 ml) was used in the experiment as most efficient cephalosporin in the *in-vitro* studies. Every 1 ml of suspension contains 29.64 mg cefquinome sulphate (equivalent 25 mg cefquinome). (cefquinome was purchased from Intervet International GmbH-Germany Company[®]). Cefquinome had been injecte in a single intramuscular therapeutic dose (10 mg/kg.B.Wt.) soon after appearance of disease symptoms (at 3rd day of experimental infection with *A. hydrophila*) (**Shan et al., 2015**).

The experiment was extended 2 weeks and serum samples were collected at 1st, 7th and 14th days post cefquinome administration for biochemical analyses while serum, muscles and liver samples were collected at 2, 24, 48, 72, 96 and 120 hrs post cefquinome administration to detect drug tissues residues.

Table 1. The Experimental designe among Nile tilapia groups.

Group*	Treatment
1	(non-infected non treated group). (Negative control)
2	Infected non-treated group (inoculated intraperitoneally with 0.5 ml of 1×10^7 CFU broth culture of <i>Aeromonas hydrophila</i> (Positive control).
3	Infected as group 2 and treated with single intramuscular dose of cefquinome 10 mg /Kg B.Wt. (Shan et al., 2015).

*Each group subdivided into 3 equal replicates that reared in 3 aquaria.

2.1. Clinical examination:

The experimented fish were examined clinically for signs of diseases and the mortality rate was recorded.

2.2. Biochemical analysis:

Five fish were used from each group for blood collection at 1st, 7th and 14th days post cefquinome administration. About 1-1.5 ml of blood was collected from caudal vein in blank tube (without any coagulant) and left to clot at room temperature, then was

centrifuged at 3000 rpm for 15 minutes for serum separation and then the sera were collected in 1.5 ml eppendorff tubes and kept frozen at -20°C until analyzed (**Stoffregen, 1997**).

The serum levels of Alanine aminotransferase (ALT) commonly known as glutamic pyruvic transaminase (GPT) and serum aspartate aminotransferase (AST) commonly known as glutamic oxaloacetic transaminase (GOT), were determined colorimetrically according to the method of **Reitman and Frankel (1957)**. Determination of serum ammonia and Serum creatinine level were done using calorimeter according to method of **Meyerhoff and Rechnitz, (1976)**.

2.3.Determination of antimicrobial residues:

Sample Preparation:

The muscle, liver and blood samples of treated fish were taken from the caudal vein of 18 fish at 2, 24, 48, 72, 96 and 120 hrs (3 fish for each sampling) after drug administration. Blood samples were collected in clean Wassermann tubes to permit it to clot at room temperature for 45 minute, centrifuged at 3000 rpm for 15 minute to obtain serum sample which preserved in eppendorff tube at -20 ° C till analyzed (**Abd El-latif *et al.*, 2013**).

The muscle and liver samples were wrapped in aluminum foil and kept in deep freezer at -20°C until transported in icebox to assess the level cefquinome residues in Central Lab. of Faculty of Science, Suez Canal University.

Principle of HPLC:

HPLC follows the same basic principle as chromatography. Different components in the sample have varying affinities to the adsorbent material. This causes a difference in the flow rate for each component which leads to their separation as they come out of the column. The only difference is that the speed and sensitivity of HPLC is much higher than that of LC due to the application of a high pressure (**Susha and Afsaneh, 2018**).

Technique of HPLC:

To estimate cefquinome in muscle and liver tissues of tilapia, acetone was added to the tissue samples and they were whirl mixed, homogenized and centrifuged. Dichloromethane was added to the supernatant. The samples were shaken and centrifuged. The mobile phase was prepared fresh daily and filtered and degassed by passage through a 0.45 µm nylon filter under a vacuum for 30 min just before use. The flow rate was 1 mL/ min. The retention time was 8.4 min. The injection volume was 75µL. H₂O-based phase was filtered and injected onto the HPLC system after calibration of standard which were prepared fresh daily 0, 0.02, 0.04, 0.10, 0.40, 1, 2, 4, 10 and 12 µg/ml by spiking 190 µg blank fish plasma with 10 µl of water for zero standard sample.

All steps in the HPLC separation technique were done as described by **Uney *et al.*, (2011)** as following:

- Injection of the liquid sample into the column containing the stationary phase.
- Individual sample components were forced down the tube by high pressure from the pump.
- Components are separated under the influence of various chemical/physical interactions with the particles in the stationary phase.
- The separated analysts were identified by the detector present at the end of the column.
- The detector measured the concentration of the components.
- The UV detection wavelength was 268 nm, and the limit of detection of cefquinome was 0.01 mg/mL.
- Data from the detector was processed and a chromatogram was produced.

2.4. Statistical analysis:

The recorded data were analyzed statistically for variance (ANOVA), with the least significant difference (LSD) as described by **Snedecor and Cochran (1981)** using computerized SPSS program (1999) version 17.0.

RESULTS

1. In-vitro:

1.1. Sensitivity test:

In-vitro sensitivity test of cefquinome against *A. hydrophila* strain using agar disc diffusion method showed variable zones of inhibition (**Table 2**). It was found that, cefquinome showed higher inhibition zone than amikacin and ciprofloxacin. However, *A. hydrophila* was also sensitive to amikacin and ciprofloxacin. On the other hand, tobramycin showed no inhibition zone, that mean *A. hydrophila* was resistant to tobramycin.

Table 2. Interpretation chart for the size of inhibition zones.

Tested drugs	Diameter of inhibition zone(ml)	Interpretation
Cefquinome (CFQ)	29.3 ± 0.26^a	Susceptible
Amikacin(AK)	19.7 ± 0.26^b	Susceptible
Ciprofloxacin(CIP)	21.0 ± 0.45^b	Susceptible
Tobramycin(TOB)	---	Non-sensitive

1.2. MIC test:

Tube A& B (with 0.5 and 1 μg cefquinome / ml) did not inhibit bacterial growth. Tubes C, D, E and F on the other hand, inhibited bacterial growth. As tube C was the lowest concentration of the antibiotic that inhibited cell growth, it has been considered to have the minimum inhibitory concentration. Therefore, the *in-Vitro* MIC for this bacterium was 2 $\mu\text{g}/\text{ml}$ (**Fig. 1**).



Fig. 1. Showing MIC tubes results.

2. *In-Vivo* studies:

2.1. Clinical examination:

The effect of cefquinome administered at 10 mg/kg B.Wt as a single intramuscular dose in *Oreochromis niloticus* experimentally infected with *A. hydrophila* at third days of inoculation revealed lack of appetite, bloated appearance and falling of some scales. At 7th day post-medication, the infected non-treated fish showed excessive falling of scales with subcutaneous hemorrhage, while infected-treated group exhibited less loss of scales. The mortality started at 48 hrs and extended till 7th day post-infection (**Table 3**).

Table 3. The effect of cefquinome on cumulative mortality in Nile tilapia infected with *A. hydrophila* at the 7th day post infection.

Fish grouping	Total number	Number of dead fish	Mortality %
1. Control (non-infected non-treated)	45	3	4.4 %
2. Infected non-treated	45	30	66.6 %
3. Infected treated with Cefquinome	45	8	17.7 %

2.2. Biochemical tests:

2.2.1 Testing of liver functions:

Asparate aminotransferase (AST), Alanine aminotransferase (ALT) (μL) enzymes and total bilirubin (mg/dl) of normal and experimentally infected tilapia with *A. hydrophilla* and those infected and treatment with cefquinome were shown in **Table (4)**.

At 1st day post medication:

A significant increase ($P < 0.05$) in serum transferases enzymes AST, ALT and total bilirubin levels of infected group (gp 2) was seen in comparison with negative control group. The infected-treated group (gp 3) also reflected a significant increase ($P < 0.05$) in AST, ALT and total bilirubin in comparison with negative control group (gp 1).

At 7th and 14th day post medication :

The infected group (gp 2) recorded a significant increase ($P < 0.05$) in AST, ALT and total bilirubin in comparison with negative control group. While group 3 (infected treated group) showed a significant decrease ($P < 0.05$) in AST, ALT and total bilirubin in comparison with infected group.

Table 4. Serum enzymes and bilirubin level in Nile tilapia (with and without infection by *A. hydrophilla* and treatment using cefquinome), (M \pm S.E).

Groups	Liver biomarkers								
	1 st day post-medication			7 th day post-medication			14 day post-medication		
	AST (μ /ml)	ALT (μ /ml)	T.bilirubin (mg/dl)	AST (μ /ml)	ALT (μ /ml)	T.bilirubin (mg/dl)	AST (μ /ml)	ALT (μ /ml)	T.bilirubin (mg/dl)
1 (Control)	28.04 $\pm 3.120^b$	38.99 $\pm 4.240^b$	0.239 $\pm 0.038^c$	28.29 $\pm 4.128^b$	38.49 $\pm 3.021^b$	0.240 $\pm 0.038^c$	28.019 $\pm 3.730^b$	38.38 $\pm 2.923^b$	0.238 $\pm 0.040^b$
2 (infected)	50.18 $\pm 2.97^a$	55.18 $\pm 6.281^a$	0.483 $\pm 0.0197^a$	55.09 $\pm 4.220^a$	60.59 $\pm 3.982^a$	0.455 $\pm 0.0291^a$	63.00 $\pm 3.901^a$	65.50 $\pm 3.122^a$	0.466 $\pm 0.037^a$
3 (infected- treated)	52.38 $\pm 3.002^a$	54.09 $\pm 5.650^a$	0.402 $\pm 0.010^b$	30.16 $\pm 3.187^b$	39.02 $\pm 3.006^b$	0.272 $\pm 0.072^b$	27.88 $\pm 2.989^b$	38.42 $\pm 2.899^b$	0.238 $\pm 0.013^b$

The different letters in the same column means a significant changes at $p < 0.05$.

*Medication was done 3rd day post infection.

2.2.2 Results of kidney function test parameters:

Serum creatinine (mg/dl) and serum blood ammonia (μ g/dl) of negative and experimentally infected tilapia fish with *A. hydrophilla* and those infected and treated with cefquinome were shown in **Table (5)**.

At 1st day post medication :

The infected group (gp 2) had a significant increase ($p < 0.05$) in serum creatinine and serum blood ammonia in comparison with negative control group (gp 1). On the other hand, group 3 (infected-treated group) recorded a significant increase ($p < 0.05$) in serum creatinine and serum blood ammonia in comparison to negative control group.

At 7th and 14th day post medication:

The infected group (gp 2) recorded a significant increase ($P < 0.05$) in serum creatinine and serum blood ammonia in comparison to negative control group (gp 1). While group3 (infected-treated group) revealed a significant decrease ($p < 0.05$) in serum creatinine and serum blood ammonia in comparison with the infected group.

Table 5. Kidney function in the Nile tilapia (with and without infection by *A. hydrophilla* and treatment using cefquinome), ($M \pm S.E$).

Group	Kidney function parameters					
	1 st day		7 th day		14 th day	
	Post medication		Post medication		Post medication	
	Serum creatinine (mg/dl)	Blood ammonia (μ g/dl)	Serum creatinine (mg/dl)	Blood ammonia (μ g/dl)	Serum creatinine (mg/dl)	Blood ammonia (μ g/dl)
1 (Control group)	0.234 $\pm 0.010^b$	239.51 $\pm 9.136^b$	0.234 $\pm 0.020^c$	240.26 $\pm 8.020^c$	0.232 $\pm 0.020^b$	237.43 $\pm 10.113^b$
Group 2 (Infected)	0.423 $\pm 0.022^a$	380.29 $\pm 13.20^a$	0.503 $\pm 0.0132^a$	412.32 $\pm 9.016^a$	0.473 $\pm 0.0229^a$	500.77 $\pm 8.130^a$
Group 3 (Infected-treated)	0.404 $\pm 0.012^a$	366.78 $\pm 11.01^a$	0.351 $\pm 0.013^b$	304.21 $\pm 7.902^b$	0.242 $\pm 0.010^b$	235.28 $\pm 12.219^b$

The different letter in the same column means a significant changes at $p < 0.05$. *Medication was done 3rd day post infection.

2.2. Cefquinome residues:

The residues of cefquinome as a single I/M dose of 10 mg/kg.B.Wt. in Nile tilapia fish infected with *A. hydrophilla* were recorded in **Table (6)**:

3.2.1 Serum concentration of cefquinome (μ g/ml):

At 2 hour post-medication:

There was a significant increase ($p < 0.05$) in serum cefquinome level of infected-treated group in comparison to the same group after 24 hr. post medication.

At 24 and 48 hours post-medication:

There was a significant decrease ($p < 0.05$) in serum cefquinome level of infected-treated group at 24 and 48 hr post medication compared with the same group after 2 hrs and 24 hr post-medication; respectively.

At 72 and 96 hours post-medication:

There was a non-significant decrease ($p < 0.05$) in serum cefquinome level of infected-treated group at 72 and 96 hrs post-medication compared with infected treated group at 48 and 72 hr post-medication; respectively.

At 120 hours post medication:

The serum cefquinome level of infected-treated group at 120 hrs post medication was lower than to be detected (under limit of detection $0.01\mu\text{g}$).

2.2.2. Muscle concentration of cefquinome ($\mu\text{g}/\text{mg}$):

At 2nd hour post-medication:

There was a significant increase ($p < 0.05$) in muscle cefquinome level of infected-treated group compared with the same group at 24 hrs post medication.

At 24 and 48 hours post-medication:

There was a significant decrease ($p < 0.05$) in muscle cefquinome level of infected-treated group at 24, 48 hr post medication compared with the same group after 2, 24 hrs post-medication; respectively.

At 72 and 96 hours post medication:

There was a non-significant decrease ($p < 0.05$) in muscle cefquinome level of infected-treated group at 72 and 96 hr post-medication compared with infected treated group at 48 and 72 hrs post-medication; respectively.

At 120 hours post-medication:

The level of cefquinome residue in muscle of infected-treated group was lower to be detected (under limit of detection $0.01\mu\text{g}$).

2.2.3. Liver concentration of cefquinome ($\mu\text{g}/\text{mg}$):

At 2nd hour post-medication:

There was a non-significant increase ($p < 0.05$) in liver level of cefquinome in infected treated group compared with the same group after 24 hrs post-medication.

At 24 and 48 hours post-medication:

A non-significant decrease ($p < 0.05$) in liver level of cefquinome in infected treated group at 24 and 48 hrs post-medication was seen compared with the same group after 2 and 24 hrs post-medication; respectively.

At 72 hours post-medication:

There was a significant decrease ($p < 0.05$) in liver cefquinome level in infected treated group at 72 hrs post-medication compared with same group at 48 hr post-medication.

At 96 and 120 hours post-medication:

A non-significant decrease ($p < 0.05$) in liver level of cefquinome in infected treated group at 96 and 120 hrs post-medication was observed compared with infected treated group at 72 and 96 hrs post medication; respectively.

Table 6. Concentration of cefquinome $\mu\text{g/ml}$ in serum, muscle and liver in the Nile tilapia after a single intramuscular dose of 10 mg/ kg, ($M \pm S.E$) (n= 3).

Time post medication (hrs)	Serum $\mu\text{g/ml}$ $\pm\text{SD}$	Muscle $\mu\text{g/g}$ $\pm\text{SD}$	Liver $\mu\text{g/g}$ $\pm\text{SD}$
2nd	14.99 $\pm 1.33^a$	1.05 $\pm 0.21^a$	10.02 $\pm 2.91^a$
24	1.81 $\pm 0.613^b$	1.31 $\pm 0.01289^b$	10.29 $\pm 0.03189^{ab}$
48	0.68 $\pm 0.063^c$	0.19 $\pm 0.05189^c$	6.71 $\pm 2.39189^b$
72	0.22 $\pm 0.013^{cd}$	0.03 $\pm 0.00189^{cd}$	1.94 $\pm 0.4689^c$
96	0.05 $\pm 0.003^d$	0.02 $\pm 0.00289^d$	1.87 $\pm 0.19289^{cd}$
120	14.99 $\pm 1.33^a$	Lower to be detected	0.70 ± 0.01289

The different letter in the same column means that there were significant changes at $p < 0.05$.

DISCUSSION

The emergence of antibiotic-resistant bacteria strains led to the necessity of continuous approving of new antibiotics to be used for treatment of fish diseases, especially against Gram-negative bacteria. One of the best available alternatives was the fourth generation cephalosporins, especially cefquinome, which has a wide therapeutic range covering many Gram-negative bacteria that cause serious diseases in fish such as *Aeromonas* and *Pseudomonas* species (Olatoye and Basiru, 2013).

Cefquinome is approved for treatment of bacterial infections in several animal species in many countries (Aarestrup and Skov, 2010). Cefquinome has been extensively used for treatment of cattle, pig and pets against bacterial infections (Zonca *et al.*, 2011).

The current study had been carried to explore the efficacy of cefquinome against *A. hydrophilla* in tilapia fish. In addition to estimating the safety and residue of therapeutic cefquinome dose.

The *in-vitro* study showed the sensitivity of *A. hydrophilla* isolates to some antibiotics and indicated a high sensitivity reaction to cefquinome, this results were similar to that obtained by **Roman et al., (2011)** who mentioned that, *A. hydrophilla* infection in fish had been found to be sensitive to some of the fourth generation cephalosporins including the cefipime.

As the cefquinome is bactericidal via a time-dependent mechanism; the determination of the MIC is very important as its efficacy depends on the time that the serum cefquinome concentration exceeds the MIC for the pathogen (*A. hydrophilla* in the current study).

Toutain et al., (2002) recorded that, the antibiotic serum concentration should persist greater than the MIC for 40-60% of the treatment time course. In the present study, the cefquinome MIC against *A. hydrophilla* was found to be 2µg/ml. This value was very close to that recoded against *A. hydrophilla* by other researchers who found the cefquinome MIC against *Aeromonas* ≤ 2µg/ml (**Orozova et al., 2008**).

This study elucidated that one dose of 10 mg cefquinome/kg body weight succeeded to control the *A. hydrophilla* infection in tilapia and decreases the mortality from 66.6% to only 16.7%. The clinical symptoms associated with *A. hydrophilla* infection slightly recovered after 7days post medication, and completely recovered after 14 day post medication.

Although there are no previous studies on the efficacy of cefquinome in *A. hydrophilla* infected fish, this finding can be supported and explained by 3 previous studies. The first work was done by **Shan et al., (2015)** who determined the pharmacokinetic parameters of cefquinome in tilapia after a single intramuscular or intraperitoneal administration. Shan observed a maximum plasma concentration (C_{max}) of 49.40 µg/mL after a single intramuscular dose of 10 mg cefquinome/kg of body weight in tilapia. The second study, **Orozova et al., (2008)** investigated the multiple antibiotic resistance of 26 *Aeromonas* strains isolated from drinking water, meat and fish in Bulgaria. He stated that, most of isolates were potentially susceptible to cefquinome and only 11.5% of isolates showed resistance under the concentration of 2µg/ml. The third recent study was conducted in Northern Italy to investigate the antimicrobial activity and multidrug resistance of the motile *Aeromonas* spp. isolated from farmed and wild freshwater fish. Interestingly, all the isolates were susceptible to cefquinome and there were no single resistant isolate recorded in this study (**Borella et al., 2020**). This explains the high efficacy of the cefquinome in our study against *A. hydrophilla*.

Our result reported a significant increase in AST, ALT and total bilirubin in infected non-treated group at the 1st, 7th and 14th days post medication in comparison with negative control group. This result was similar to those of **Racicot et al., (2006)** who

mentioned that, there was a significant increase in serum enzymatic levels of infected fish suffering from bacterial infection which might be as a result to hepatic injury leading to hepatic dysfunction and disturbance in enzymatic activities.

While at 7th day post medication, the infected-treated group with cefquinome recorded a significant decrease in AST, ALT and total bilirubin in comparison to the infected non-treated group. Gradually, at 14 day post-medication the infected-treated group returned to normal values again in comparison to normal control group. Similar findings were recorded by **Yu *et al.*, (2018)** who investigated changes in mud loach fish (*Misgurnus mizolepis*) experimentally infected with *Aeromonas sobria*. Yu and his colleagues recorded a significant increase ALT, AST, and lactate dehydrogenase (LDH) activities in *Aeromonas*-infected fish compared to the control fish.

The infected non-treated group, in the current study, recorded a significant increase in serum ammonia and creatinine at 1, 7 and 14 days post-medication compared with negative control group. On the other hand, the infected-treated group with cefquinome, also recorded a significant increase in serum ammonia and creatinine only at 1st day post medication compared with normal control group, while at 7th day post-medication showed a significant decrease in serum ammonia and creatinine blood level in comparison to infected non-treated group and reached to normal level at 14 day post medication in comparison to normal control group. This result was in accordance to that had been mentioned by **Robert *et al.*, (2018)** who studied the damage of kidney cells in case of bacterial infection where elevation in ammonia and creatinine levels was seen in the blood. Also, **Abd Allah *et al.*, (2019)** recorded similar elevation in blood creatinine after 2 days of *A. hydrophila* infection in African catfish. In another study, **Yu *et al.*, (2018)** investigated the hemato-biochemical changes in mud loach fish infected with *Aeromonas sobria* and found 3 times increase in blood urea at 5 days post *Aeromonas*-infection. These elevations in blood ammonia and creatinine suggest some degrees of renal toxicity. Such toxicity is common in *Aeromonas* infection and may be resulted from *Aeromonas* cytotoxins that harm some vital internal organs including the kidney as cited by **Yardimci and Aydin (2011)**.

Cefquinome blood level decreased gradually at 48, 72 and 96 hrs post medication and reached under limit of detection after 120 hours post medication. These results were slightly longer than that was obtained by **Shan *et al.*, (2015)** who studied that cefquinome pharmacokinetics in non-infected tilapia after a single intramuscular and intraperitoneal administration of 10 mg cefquinome/kg body weight. The authors stated that, the cefquinome became under limit of detection in plasma after 96 hr from intramuscular and intraperitoneal injections of cefquinome. The reason of longer cefquinome persistence in blood in our study could be attributed to the *Aeromonas* infection which affected both liver and kidney (the main 2 drug elimination organs) as indicated by elevated serum transaminases, ammonia and creatinine. Such phenomena was typically common and

recorded several times in fish and other animals (**Wang et al., 2015 and Shiry et al., 2019**).

The current study also, showed a relatively high cefquinome concentration in muscles of the infected-treated fish at 2 hrs post medication, this concentration decreased significantly by time where at 120 hrs post medication, the level of cefquinome residue in muscle was below the detection limit. This time was noticeably shorter than that was obtained by **Martin et al., (1998)** who concluded that cefquinome became under limit of detection after 7 days of intramuscular injection of cefquinome in salmon fish. Obviously, this difference can be assigned to the species and water temperature differences, as Martin and his co-authors calculated the cefquinome residues in salmon fish at 10 °C while we calculated the cefquinome residues in tilapia at 30 °C.

Moreover, there was a high cefquinome concentration in the liver of infected treated tilapia which decreased gradually but still could be detected even after 120 hrs post medication. At that time, cefquinome concentrations were under the detection limit in serum and muscles. That result was parallel to that was obtained by **Martin et al., (1998)** who recorded that the cefquinome concentrations persisted detectable in liver up to 14 days post intramuscular injection of cefquinome in salmon fish. The persistence of drugs and antibiotics residues in liver more than in serum is typical and was recorded by most of researchers who studied the antibiotic residues in fishes such as **Wang et al., (2015), Martin et al., (1998) and Shiry et al., (2019)**.

CONCLUSION

It could be concluded that, cefquinome was efficient in treatment of *Aeromonas hydrophila* infection in Nile tilapia. Fish flesh is suitable for human consumption 5 days post-medication using the therapeutic dose of cefquinome, while liver of fish shouldn't be eaten even after 5 days post-medication.

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