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Evaluation of some antibiotic residues in some cultured fishes

Y.A. Abdel-Hakeim* and R.H. Zaki **

Food Hygiene Unit., Animal Health Research Institute, Alex., Egypt*
Food Hygiene Unit., Animal Health Research Institute Zagazig., Egypt**
Agriculture Research Central (ARC)

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Abstract

Owing to excessive use of antibiotics in the field of fish industry which may results in a serious effect to human health as a result of frequent consumption of fish, this study aimed to monitor the occurrence of four antibiotics from different classes including Sulphonamides (sulphadimethoxine sodium), Quinolone (enrofloxacin), Macrolides (erythromycin) and Tetracyclines (Oxytetracycline), in two types of farmed fish (tilapia and carp).

A total of 80 fish samples tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio),* (40 of each) were collected randomly from farmed fish in Alexandria governorate and examined for quantitative determination of antibiotic residues using HPLC. The detected antibiotics were Sulphadimethoxine sodium (40 and 60%), Enrofloxacin (50 and 60%), Erythromycin (10% and 0%) and Oxytetracycline (70 and 90%), with a mean value of (88.71±2.88 and 203.33 ±2.30µg/kg), (160.31±1.84 and 230.35 ±0.46 µg/kg), (110.75 ±1.20 µg/kg and ND) and (310.15 ±2.40 and 270.61±1.12 µg/kg) in tilapia and carp muscular tissue respectively. A total of (20 and 40%) Sulphadimethoxine sodium, (30 and 50%) Enrofloxacin, and (50 and 70%) Oxytetracycline,in tilapia and carp tissue samples respectively were found exceeding the maximum residue limits (MRLs) as recommended by EOS 1735/2010. The results were discussed together with the public health significance of such antibiotic residues.

Keywords: *Antibiotics residues, Sulphonamide, Oxytetracycline, Enrofloxacin, Erythromycin, Tilapia, carp.*

Introduction

Fish is one of the healthiest food sources which is low in fat, high in protein and omega-3 fatty acids (**Fayet-Moore *et al.*, 2015**). As awareness for the substantial health benefits of eating fish get improved, the global demand for seafood products is continuing to increase dramatically. Due to such increases in demands, aquaculture became one of the fastest growing food-production sectors which contribute significantly to regional communities and economies (**Yarsan and Yipel, 2013** and **Yarsan *et al.,* 2014**).

Industrial aquaculture is a rapidly growing industry in many developed and developing countries. It is expected that this growth will increase at an even faster rate in the future stimulated by the depletion of fisheries and the market forces that globalize the sources of food supply (**Glodburg *et al.,* 2001** and **Glodburg and Naylor, 2005**). The last 20 years have seen a fourfold growth in industrial aquaculture worldwide (**Naylor *et al.,* 2000; 2003** and **Naylor and Burke, 2005**). This impressive industrial development has been accompanied by some practice potentially damaging to human and animal health (**Glodburg and Naylor, 2005** and **Naylor and Burke, 2005**), that include passing large number of veterinary drugs into the environment (**Haya *et al.,* 2000** and **Boxall *et al.,* 2004**).

Most of the aquaculture systems in the world continue to intensify cultivation methods. These methods are characterized by high stock density and volume, heavy use of formulated feeds containing antibiotics, antifungals and other pharmaceuticals (**Sapkota *et al.,* 2008**). Antibiotics are a group of natural and synthetic compounds that destroy bacteria (bactericidal) or inhibit their growth (bacteriostatic). Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants. These uses of antibiotics can create antibiotic resistance, the resistance genes of which can be transferred to human, resulting in antibiotic-resistant infections (**Sergio *et al*.,2016**). It is recognized that another route of transmission of resistant microorganisms from animals to humans is through the food chain, in fish farming (aquaculture), the widespread use of antibiotics for treating bacterial diseases has been associated with the development of antibiotic resistance in *Aeromonas hydrophila, A. salmonicida, Edwardsiella trada, E. icttaluri, Vibrio anguillarum, V. salmonicida, Pasteurella piscida, and Yarsinia ruckeri* (**Hernandez, 2005**) and controlled studies are needed to determine the effect of antimicrobial therapy on the ecology of aquaculture ponds, particularly at the microorganism level.

Another problem created by the excessive use of antibiotics in industrial aquaculture is the presence of residual antibiotics in commercialized fish and shellfish products (**Goldburg *et al*., 2001; Cabello, 2003; 2004; Angulo *et al*., 2004** and **Sorum, 2006**). This problem has led to undetected consumption of antibiotics by consumers of fish with the added potential alteration of their normal flora that increases their susceptibility to bacterial infections and also selects for antibiotic-resistant bacteria (**Mc -Dermot *et al.,* 2002; Greenlees, 2003; Cabello, 2004** and **Salyers *et al*., 2004**).

Moreover, undetected consumption of antibiotics in food can generate problems of allergy and toxicity, which are difficult to diagnose because of a lack of previous information on antibiotic ingestion (**Cabello, 2004**). Allergy to antibiotics and problems of toxicity can also be created for the unprotected workers in the aquaculture industry through the use of large amounts of antibiotics that come in contact with the skin, and intestinal and bronchial tracts as workers medicate the food in food mills, distributing it, and administer it to fish (**Lillehaug *et al*., 2003**).

Low level doses of antibiotics in food stuffs consumed for long periods may lead to an increase in resistant strains of bacteria. To protect human health, the European Union has established safe maximum residue limits (MRLs) for these drugs and other veterinary substances, for use as veterinary drugs in animal products entering in the human food chain. The use of veterinary drugs is regulated through **EOS 1735/2010**, which describes the procedure for establishing MRLs for veterinary medicinal products in foodstuffs of animal origin. MRLs values for antibiotics in fish, as set by Egyptian Organization Standard, **Council Directive 96/23/EC**, contains guidelines for controlling veterinary drug residues in animals and their products with detailed procedures. Prohibition of the use of growth-promoting agents such as hormones and B-agonists is laid down in **Council Directive 96/22/EC**.

Maximum residue levels (MRL) for antibiotics in fish musculature according to EOS 1735 /2010.

Pharmacologically active substance	MRLs µ /kg
-**Sulfonamides**	
(Sulfadimethoxine Sodium) SDM	100
-**Quinolones**	
(Enrofloxacin) ENF	100
-Macrolides	
(Erythromycin) EM	200
-Tetracyclines	
(Oxytetracycline) OTC	100

MRLs were determined for fish muscle and skin, bovine and chicken muscle (EOS) 1735 /201and (EC)No 37/2010.

To improve food safety for consumer, routine monitoring fore the presence of antibiotic residue is necessary in different food products. There are numerous techniques developed for the determination of the drug residues in various food of animal origin, including gas chromatography, liquid chromatography -tan- dem mass spectrometry (LC-MS/MS) and four plate tests (**Kilinc and Cakli, 2008** and **Chafer-Pericas *et al.*, 2010**). Among these chromatographic techniques, the capability of LC-MS/MS for the analysis of antibiotic residues has been investigated by many researchers from all around the world and LC-MS/MS has been reported as rapid, efficient and sensitive tool (**Horie *et al.*, 2003; Chafer-Pericas *et al.*, 2010** and **Eduardo *et al.,* 2013**).

The aim of the present study was to monitor the incidence of some antibiotics residues in fish tissue samples collected from different fish farms in Alexandria, by using High-Performance Liquid chromatography to evaluate their levels in relation to the international recommended permissible limits to ensure consumers safety.

Materials and Methods

Sampling: A total number of 80 cultured fresh water fishes ,40 samples from each, Nile tilapia *(Oreochromis niloticus),* and Common Carp (*Cyprinus carpio),* were collected randomly from different private fish farms at Alexandria governorate.

These fish farms have suffered from previous bacterial infections and the fishes were treated with a course of different antimicrobials. The fishes were randomly collected at the day of catching(3days) post-treatment. The average body weight of the collected fishes was (150±5g.) and an average length of (10 ±2cm.) for Tilapia, and (250 ±10g,) with an average length of (20 ±4cm,) for Carp.

Sample preparing: The collected fishes were filleted, the skin and bones were removed, and the musculature were cut and frozen at -20ºC before being analyzed. Prior to analysis, frozen fish tissue samples were thawed overnight in a refrigerator. The musculature samples (100-150g) were diced into small pieces.

Standard solutions:

Sulphadimethoxine sodium (Sulphonamides), and Oxytetracycline were kindly supplied by Pharma Swede, Cairo, Egypt. Sulphadimethoxine sodium and Oxytetracycline purity were found to be 99.87±0.854% and 100.63±0.777% according to the United States Pharmacopeia 34NF29-2011. Standard Enrofloxacin (Quinolones) and Erythromycin (Macrolides) were kindly supplied by European Egyptian Pharmaceuticals, and their purity were found to be 99.70±1.235% and 99.41±0.958% according to the British Pharmacopeia, Her Majesty's Stationary Office, London. 2007.

Stock standard solution of individual compounds (each 100µg/ml) were prepared by accurately weighting and dissolving the powder in 25ml methanol: water (50: 50 v/v) into four separate 100-ml volumetric flasks and the volume was completed to the mark with same solvent mixture. The prepared solutions were then stored at -20ºC and protected from light (**Chico *et al.*, 2008)**.

Sample preparation and extraction procedure: according to the procedure described by **Granelli *et al.*, (2009)**.

Instrumental analysis: Chromatographic analysis was performed using high performance liquid chromatography system (HPLC) Agilent 1260 series. Chromatographic separation of analytes was carried out on a reversed-phase C18 column (50mm×3mm, 2.7µm, Agilent, Poroshell 120EC) using an isocratic mobile phase composed of 0.1% formic acid in water :0.1%formic acid in methanol (20:80, by volume) at a flow of 0.4mL/min. The column temperature was maintained at 25ºC.

Statistical analysis: Maximum, Minimum, mean and standard error were calculated according to **Petrie and Watson, (1999)**.

Results

Table (1). Antibiotic residue levels detected in cultured Tilapia musculature samples (N.=40)

Drug residues	Mean ±SE (µ /kg)	Range (µ /kg)	No. of positive samples	No. of samples (percent %) Above MRL	MRLs µ /kg
-Sulfonamides (Sulfadimethoxine Sodium) SDM	88.71±2.88	45 - 160.5	16 /40 (40%)	8 (20%)	100
-Quinolones (Enrofloxacin) ENR	160.31±1.84	62 - 285	20 /40 (50%)	12(30%)	100
-Macrolides (Erythromycin) EM	110.75 ±1.20	ND - 119.75	4/40 (10%)	------	200
-Tetracyclines (Oxytetracycline) OTC	310.15 ±2.40	79 – 543	28/40 (70%)	20 (50%)	100

Positive sample =sample which containing antibiotic residues by any amount
Sample Above MRL= samples which are exceeding the MRLs

Table (2). Antibiotic residue levels detected in cultured Carp musculature sample (N.=40)

Drug residues	Mean ±SE (µ /kg)	Range (µ /kg)	No. of positive samples	No. of samples (percent %) Above MRL	MRLs µ / kg
-Sulfonamides (Sulfadimethoxine Sodium) SDM	203.33 ±2.30	39 - 537	24 /40 (60%)	16(40%)	100
-Quinolones (Enrofloxacin) ENR	230.35 ±0.46	43 - 354	24 /40 (60%)	20(50%)	100
-Macrolides (Erythromycin) EM	ND	ND	-------	------	200
-Tetracyclines (Oxytetracycline) OTC	270.61±1.12	85 – 412	36/40 (90%)	28(70%)	100

Positive sample = sample which containing antibiotic residues by any amount
Sample Above MRL= samples which are exceeding the MRLs

Discussion

Approximately over hundred different antibiotic agents are at present being used in human and veterinary practice. Drugs used in animal husbandry considered one of the main sources of chemical contaminants present in food that arise from agricultural practices. Misuse and overuse of antibiotics in farm animals is potentially harmful to humans due to the growing problem of antibiotic resistance and the presence of veterinary drug residues in the food of animal origin, which produce worldwide public health concern (**Dasenaki and Thomaidis, 2015**).

Sulfonamides, the oldest and largest group of synthetic antimicrobial agents, have an antibacterial activity and are commonly used for prophylactic or therapeutic purpose in veterinary medicine, including sulfamethizole, sulfadiazine, sulfamethoxazole, sulfasalazine, sulfisoxazole. At present, sulfonamides and other drugs (chlortetracycline, penicillin, and several ionophores) are the most common contaminating antimicrobials in animal feed, development of drug-resistant strain. Therefore, maximum residue limits were established according to the European (EU) regulations or JECFA of WHO/FAO for the protection of consumers of animal products. The MRL for sum of sulfonamides or single compound in milk and meat has been set at 100µg/kg (**Commission Regulation, 1992** and **EOS 1735 /2010)**

From Tables (1 and 2), the present data revealed that sulfonamides ((Sulfadimethoxine

Sodium) were detected in 16 out of 40 examined cultured tilapia tissue samples and in 24 out of 40 cultured carp tissue samples, with total mean values were 88.71±2.88 and 203.33 ±2.30 (µg/kg), respectively. Only 8 tilapia tissue samples (20%) and 16 carp tissue samples (40%) exceed the MRL 100 (µ/kg).

Limi *et al.*, (2018) reported that the level of sulfonamides residue in edible tissues of carp was found to be higher than MRL (472.97µ/kg) in China. Sulfonamides residues were detected in tilapia fillet with a mean value of 140.1 (µ/kg) reported by **Katia *et al.*, (2018).**

From 107 catfish samples in Spain, only three contained residues of sulfonamides without exceeding MRLs (**De-Mendoza *et al.*, 2012**). 36% of the sampled fish muscles from five rainbow trout farms had detectable levels 69± 0.21µ/kg (**Gokmen *et al.*, 2014)**, **Yiepel *et al.*, 2017** reported that the level of residue in edible tissues of rainbow trout was found to be higher than MRL in 17.3% farmed fish samples suggesting incorrect use of antibiotics in aquaculture in Turkey.

Quinolones and fluoroquinolones are an important family of synthetic antibacterial used in both human and veterinary medicine. In the veterinary field, they are used for the prophylaxis and for treatment of veterinary diseases in most types of farmed animals, they are also used in aquaculture (**Carlucci, 1998**). The introduction of the fluorinated quinolones had important therapeutic advantages, because this antibiotic group has higher antibacterial activity than the parent compounds (**Jackson *et al.*, 1998**). Their extensive administration to fish destined for human consumption has become a serious problem because their residues can persist in edible animal tissues. Quinolones may be directly toxic or be the source of resistant human pathogens representing a possible risk to human health (**Juan-Garcia *et al.*, 2006**). Recently, **Samuelsen.,** (**2006)**, reported the pharmacokinetics of several quinolones in freshwater species and in sea water species, as oxalinic acid, flumequine, enrofloxacin, and ciprofloxacin in plasma and tissue of channel catfish, rainbow trout, Atlantic halibut, Atlantic cod, Atlantic salmon and gilthead sea bream, among others were determined by HPLC to calculate maximum concentration and time to maximum concentration in plasma and tissue were established in each type of fish.

The obtained results in tables (1 and 2) revealed that, ENR residues were detected in 20 out of 40 cultured tilapia tissue samples and 24 out of 40 cultured carp tissue samples with a main value of 160.31±1.84 and 230.35 ± 0.46 (µ/kg), respectively. Twelve tilapia tissue samples (30%) and Twenty (50%) carp tissue samples exceeded the MRL 100 (µ/kg).

The use of Quinolones has been totally restricted in aqua culture in industrialized countries, not only because they are a highly effective group of antibiotics for human infections but also because of their ability to generate cross-resistance among all the members of this group (**Gorbach, 2001; Cabello, 2004; Moellering, 2005** and **Sorum, 2006**). Quinolones also remain active in sediments for prolonged periods of time as they are not readily biodegradable (**Jacoby, 2006**).

Macrolides belong to the family of macrocyclic antibiotics. These drugs are often used as feed additives for growth promotion, but they can also be used for therapeutic purposes because they have a wide range antibacterial spectrum. Their use leave residues in edible products that not only can have direct toxic effects but also may lead to allergic reactions in consumers and to the development of resistant bacteria. Macrolides are the most effective medicine against diseases produced by mycoplasmas and have been widely used in the rearing of food-producing animals, including fish, to prevent and treat diseases. The antimicrobial spectrum of macrolides is slightly wider than that of penicillin and therefor, macrolides are common substitutes for patients with a penicillin allergy **(Baydan *et al.*,2012).**

EM was only detected in four tilapia tissue sample with a mean value of 110.75±1.20 (µ/kg), that was lower than the acceptable level of MRL 200 (µ /kg), while EM was not detected in carp tissue tables (1 and 2).

Tetracyclines antibiotics (TCAs), which represent oxytetracycline OTC, tetracycline TC, chlortetracycline CTC, and doxycycline DC, are commonly used all over the world as veterinary medicines and feed additives (**Oka *et al.*, 2000**). Recently, OTC, CTC, and TC products are approved by the US Food and Drug Administration (FDA) for use in food-producing animals.

The obtained results revealed that Oxytetracycline OTC residues were detected in 28 out of 40 tilapia tissue samples and 36 out of 40 carp tissue samples with a mean value of 310.15 ±2.40 and 270.61±1.12 µ /kg respectively, which indicate that contamination of fish tissue samples with Tetracyclines was very high.

20 (50%) and 28(70%) tilapia and carp tissue samples exceeded the MRL (100 µ /kg) for tetracycline residues (Tables 1 and 2).

OTC was found in 39.4% of examined catfish fish samples in Nigeria by **Otatoye and Basiru, (2013)**. In the other study, 107 catfish samples examined in Spain, 10(9.3%) were found to contain TCs with concentration between 3.9 and 80.8 µ /kg (**De Mendosa *et al*.,2012)**. also, **Yiepel *et al*., (2017)** found tetracycline 12 %of examined rainbow trout tissue samples.

Important differences in the frequency and concentration of residues were observed among the fish species tested. This could be attributed to some factors related to changes in pharmacodynamics and pharmacokinetics of drugs in different fish species and also changes in environmental factors such as salt and temperature level of waters (**He *et al*.,2012 and Di Salvo *et al*.,2013**). In addition to species - dependent factors. **He *et a*l.,2012** noted that a variety of other factors such as administration routes, period and dosage of antibiotics as well as quantity and harvesting time of fish may be involved.

It is possible that low doses of antimicrobial agents, such as those found as residues in food could alter intestinal enzyme activity and have an effect on certain hormones and drugs, since in most cases the lowest doses at which the perturbation in the intestinal microflora occur have not been determined. In order to ensure human food safety, FDA, considered data gathered from a large number of compounds and determined that the maximum safe concentration of antimicrobial products is 1 ppm in total diet of 1.5 kg. This equals a maximum antibiotic dose of 1.5 mg/day from consuming residues in food (**Paige *et al.,* 1997**).

It is clear that the use of antibiotics for prophylaxis and treatment of some infectious diseases of fish shortly before harvesting (3dayes) resulted in the presence of their residues in the tissue. To safe consumer against the hazards which resulted from consumption of fish containing antibiotic residues, the following instruction should be recommended.

1-Prohibit administration of antibiotics before harvesting is necessary for withdrawal of any antibiotic residues.

2-An administration of antibiotics should be done under the supervision of veterinarians.

3-Regular examination of farm fish for detection of antibiotic residues.

4- Sufficient heat treatment or cold storage for fish may cause degeneration of antibiotics in the food.

5-Wastewater treatment plants to reduce residues entering the environment to a minimum

6-Nile tilapia is more safer than common carp regarding antibiotic residues.

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