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Fluoroquinolone Residues in Fish Collected from Farms and Retail Stores in Stara Zagora Region, Bulgaria

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HIGHLIGHTS

- Fluoroquinolones were detected in 44.3% of freshwater and 42.9% of marine fish samples in Bulgaria.
- The highest measured concentrations were established in three common carp samples from the retail stores.
- Regular monitoring of antibiotic residues is too necessary in fish distributed in Stara Zagora Region, Bulgaria.

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Acronyms and abbreviations
ELISA=Enzyme-Linked Immunosorbent Assay

ABSTRACT

Background: Fluoroquinolones are among various antibiotic groups used in livestock husbandry. The aim of this screening study was to evaluate the presence of fluoroquinolone residues in fish samples of Stara Zagora region, Bulgaria.
Methods: A total of 69 samples from freshwater, marine, and anadromous fish were collected randomly from fish farms and retail stores in Stara Zagora region, Bulgaria. Fluoroquinolone residues were determined using Enzyme-Linked Immunosorbent Assay method. Data were statistically processed using STATISTICA 10.0 software.
Results: Fluoroquinolones were detected in 44.3% of freshwater and 42.9% of marine fish samples, with no significant difference (p>0.05). Their concentrations varied from 1.03 to 271.73 µg/kg for common carp, 1.43 to 12.63 µg/kg for rainbow trout, 1.17 to 1.94 µg/kg for silver carp, 1.00 to 1.82 µg/kg for rudd and 1.27 to 2.15 µg/kg for striped catfish. Among marine fish, fluoroquinolone positive samples were found in European sprat (1.68 µg/kg), Longtail southern cod (1.30 µg/kg), and European hake (1.83 µg/kg).
Conclusion: High levels of fluoroquinolones were found in some fish samples of Stara Zagora region, Bulgaria. Regular monitoring of antibiotic residues is too necessary in fish distributed in this region.

Introduction

The continuously increasing fish consumption is responsible for the exceptional expansion of aquaculture when compared to any other livestock production sector (Santos and Ramos, 2018). Intensive fish farming is associated with emergence of bacterial diseases, often entailed by stressors like big stocking density, hypoxia, increased levels of nitrites, and ammonia. This necessitates extensive use of antibiotics for disease prevention and therapy (Conti et al., 2015; Rhodes et al., 2000), which result in antibiotic resistant bacteria and residues in fish intended for human consumption. Thus, the accidental intake of antibiotics leads to damage of normal intestinal microflora and increased sensitivity to bacterial infections in fish consumers. Other consequences of antibiotic intake with food are allergic states and intoxications, which are difficult to diagnose due to the lack of

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antibiotic intake in history of disorders (Cabello, 2006). That is why the control on antibiotic residues is essential to guarantee food safety and to protect consumers (Mahmoudi et al., 2014).

Several groups of antibiotics are used in livestock husbandry, among which fluoroquinolones are one of the most commonly applied. Fluoroquinolones are approved for treatment of infections in cattle, pigs, fish, and poultry (Barreto et al., 2017; Wagil et al., 2014). European Commission has set the following maximum residue limits for fluoroquinolones in fish (Commission Regulation, 2010), including enrofloxacin (100 μg/kg), danofloxacin (100 μg/kg), difloxacin (300 μg/kg), flumequine (600 μg/kg), oxolinic acid (100 μg/kg), and sarafloxacin (30 μg/kg in salmonids). The aim of this study was to perform a screening for presence of fluoroquinolone residues and their levels in fish from farms and retail stores in Stara Zagora region, Bulgaria.

Materials and methods

Collection of samples

A total of 69 samples from freshwater, marine and anadromous fish were collected randomly from fish farms and retail stores in Stara Zagora region, Bulgaria (Figure 1), from May to September in 2018. Freshwater fish samples included common carp (n=26), rainbow trout (n=10), silver carp (n=5), Northern pike (n=5), rudd (n=4), Prussian carp (n=4), European perch (n=3), Striped catfish (n=2), Wels catfish (n=1), and Albanian barbel (n=1). One sample was obtained from each seven type of marine fishes, including Atlantic mackerel, garfish, European sprat, Alaska Pollock, Longtail southern cod, European hake, and Atlantic herring. Also, a sample of Pontic shad was gathered as anadromous fish.

Preparation of samples

Determination of fluoroquinolone residues was performed using MaxSignal® Fluoroquinolone Enzyme-Linked Immunosorbent Assay (ELISA) Test Kit (Bioo Scientific Corporation, Austin, TX, USA). All the samples were prepared according to the manufacturer's instructions. A piece of back muscle was collected. Fat was removed and the samples were homogenized in a mortar. Four ml of 70% methanol was added to 1 g of homogenized sample weighed in a centrifuge tube. The tubes were vortexed for 10 min at maximum speed and centrifuged for 5 min at 4000 xg at room temperature. From the supernatant, 0.5 ml were transferred in an Eppendorf microtube 1.5 ml and mixed with 0.5 ml of 1X Sample Extraction Buffer.

ELISA test procedure

A volume of 50 μl of each standard and sample extract was added in each microplate well. Enrofloxacin standard solutions were used at concentrations of 0.1, 0.25, 0.5, 1, and 5 ng/ml as well as negative control. The procedure was run as per manufacturer's recommendations. Absorbance was read at λ=450 nm on ELISA plate reader (Rayto RT-2100C, China). The detection limit was 0.4 μg/kg. The data were calculated through MaxSignal® ELISA Analysis Program in Excel.

Statistical analysis

The data (means and standard deviations) were statistically processed using STATISTICA 10.0 software (StatSoft Inc., USA). Significant level was determined at $p<0.05$.

Results and discussion

From a total of 69 fish samples, 30 (43.5%) contained fluoroquinolone residues as presented in Table 1. The target group of antibiotics was found out in 44.3% of freshwater and 42.9% of marine fish. There was no significant difference between the fluoroquinolone levels in freshwater and marine fish ($p>0.05$). The concentrations detected in positive freshwater fish samples varied from 1.03 to 271.73 μg/kg. A single positive sample was demonstrated among Prussian carp and European perch samples, while from Northern pike, Wels catfish and Albanian barbel specimens contained no detectable fluoroquinolone levels. The highest measured concentrations were established in three common carp samples from the retail stores.

Our results on the presence of fluoroquinolone residues in freshwater fish were in line with other investigations. Barani and Fallah (2015) demonstrated fluoroquinolone residues in 16-37.8% of rainbow trout samples in different fish farms in Iran, showing range of 6.75-99.8 μg/kg fluoroquinolones. Pham et al. (2015) performed ELISA monitoring for antibiotic residues in 51 freshwater fish samples from retail markets in Vietnam and reported 15 (29.4%) positive samples. Six samples (40%) contained fluoroquinolones in the range of 12-66 μg/kg. Based on another study carried out on freshwater fish collected in China, He et al. (2016) detected fluoroquinolones in range of 7.0-185.7 μg/kg whereas 18% of the samples exceeded 100 μg/kg. We found 3 common carp samples (11%) out of all freshwater fish samples that contained fluoroquinolones over 100 μg/kg. Guidi et al. (2018) established that 14% out of 29 tilapia and rainbow trout samples collected from two farms in Brazil contained fluoroquinolone residues at levels from 12.54 to 19.01 µg/kg. In addition, according to Tittlemier et al. (2007), antibiotic exposure of farmed fish was probably greater compared to that of wild fish. Intensive aquaculture systems are currently used to increase fish productiveness. Hence, this environment may act as a stressor, which result in greater fish susceptibility and emerge of bacterial diseases. This necessitates extensive use of antimicrobial agents to treat suffering fish (Barani and Fallah, 2015).

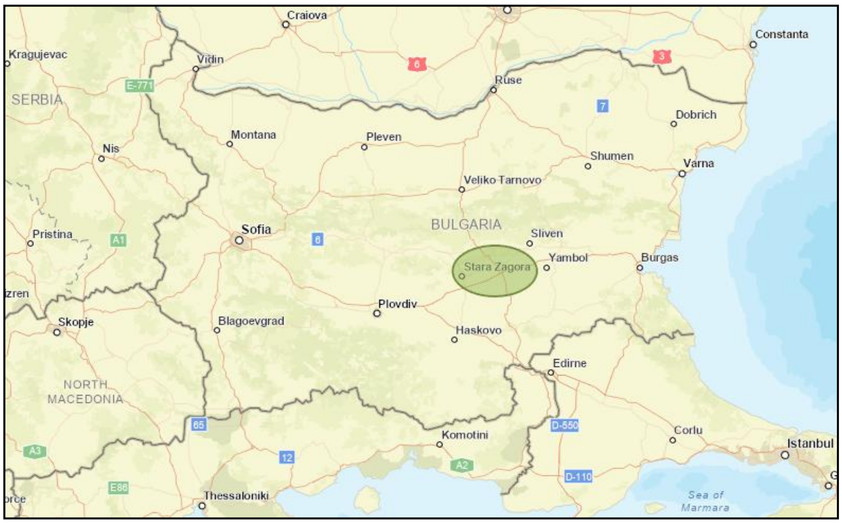

Figure 1: Map of study area (Stara Zagora Region) in Bulgaria

Table 1: Occurrence of fluoroquinolones in fish sampled from Stara Zagora Region, Bulgaria

Fish species	Positive samples n (%)	Concentration (µg/kg)	
		Range	Mean±SD
Freshwater fish			
Common carp (*Cyprinus carpio*)	13 (50)	1.03-271.73	44.26±86.71
Rainbow trout (*Oncorhynchus mykiss*)	5 (50)	1.43-12.63	6.34±5.26
Silver carp (*Hypophthalmichthys molitrix*)	3 (60)	1.17-1.94	1.67±0.44
Northern pike (*Esox lucius*)	0 (0)	-	-
Rudd (*Scardinius erythrophthalmus*)	2 (50)	1.00-1.82	1.41±0.58
Prussian carp (*Carassius gibelio*)	1 (25)	-	1.64
European perch (*Perca fluviatilis*)	1 (33.3)	-	1.83
Striped catfish (*Pangasianodon hypophthalmus*)	2 (100)	1.27-2.15	1.71±0.62
Wels catfish (*Silurus glanis*)	0 (0)	-	-
Albanian barbel (*Luciobarbus albanicus*)	0 (0)	-	-
Marine fish			
Atlantic mackerel (*Scomber scombrus*)	0	-	-
Garfish (*Belone belone*)	0	-	-
European sprat (*Sprattus sprattus*)	1*	-	1.68
Alaska Pollock (*Gadus chalcogrammus*)	0	-	-
Longtail southern cod (*Patagonotothen ramsayi*)	1*	-	1.30
European hake (*Merluccius merluccius*)	1*	-	1.83
Atlantic herring (*Clupea harengus*)	0	-	-
Anadromous fish			
Pontic shad (*Alosa immaculate*)	0	-	-

* Only a single sample was tested

Among marine fish, fluoroquinolone positive samples were found out in European sprat (1.68 μg/kg), Longtail southern cod (1.30 μg/kg), and European hake (1.83 μg/kg) whereas samples from Atlantic mackerel, garfish, Alaska Pollock, and European hake did not demonstrate detectable levels of the antibiotics. In general, it was stated that antibiotic residues could be found in marine fish (Canada-Canada et al., 2009). In the vicinity of marine fish farms, there is a large circle of ecosystems where wild fish species are caught for human consumption. Some of these wild fish species could consume antibiotic-containing feed remaining not eaten by farmed fish. In Chile, Fortt et al. (2007) proved the presence of quinolone residues in both wild fish róbalo (*Scorpaena hystrio*) and cabrilla (*Elginops maclovinus*) captured close to a farm, where salmons were fed with a diet containing quinolones. Tittlemier et al. (2007) also confirmed the presence of antibiotic in marine fish collected in Canada by detecting 0.4 μg/kg chloramphenicol in a cod and haddock sample but this antibiotic was not target of our survey. Data of other investigation on the presence of antibiotic residues in marine fish showed lower fluoroquinolone levels. Using ELISA, Conti et al. (2015) gave evidence for enrofloxacin levels from 0.1 to 0.25 μg/kg in seabass and gilthead seabream from Italy. Contrary to our findings, He et al. (2016) established higher fluoroquinolone residues in range of 2.5-47.1 μg/kg in marine fish comprising of Golden pompano, Mangrove jack, Yellow fin porgy, Crimson snapper, black porgy, ginkgo fish, little spinefoot, red drum, and white star snapper purchased in China.

Although there was no significant difference between the fluoroquinolone levels in freshwater and marine fish, we measured the highest concentrations in common carp samples. According to He et al. (2016), fish accumulate antibiotics from water through the skin and gills or ingest them by food. Different antibiotic concentrations among different fish species are due to the trophic level, specific habitat, and feeding manner. Probably, that is why quinolone levels are the highest in carnivorous fish and decrease in herbivorous and omnivorous fish. Higher quinolone concentrations may appear in freshwater fish compare to marine fish because marine cage aquaculture is open area and it can be contaminated by sewage water and wastewater.

We found out fluoroquinolone residues in 43.5% of fish samples obtained from fish farms and retail stores in Stara Zagora region, Bulgaria. The presence of antibiotics in fish marketed for consumption means that respective withdrawal periods were not observed. According to Pham et al. (2015), antibiotics are applied for maintaining the fish health in order to decrease losses and reimbursement of investments. The use of antibiotics is rather an economic than health-preserving or safety decision. Farmers often used repeatedly an antibiotic that has previously shown a good efficacy or applied multiple antibiotics consequently until diseased fish are recovered. That is why it is affirmed that both veterinarians and farmers should be well acquainted with the risks of antibiotic residues in animals used for human consumption (Mahmoudi et al., 2014).

Conclusion

High levels of fluoroquinolones were found in some fish samples of Stara Zagora region, Bulgaria. The fluoroquinolone residues are potentially dangerous for consumers, and therefore, regular monitoring of antibiotic residues is too necessary in fish distributed in this region.

Author contributions

All authors contributed equally to study designing, experimental work, data analysis, and also manuscript writing. Authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest.

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