



CHLORAMPHENICOL, SULFONAMIDE AND TETRACYCLINE
RESIDUES IN CULTURED RAINBOW TROUT MEAT
(*ONCORHYNCHUS MYKISS*)

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Summary

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There are many concerns about safety of food contaminated with antibacterial residues. This study was designed to investigate the occurrence of tetracyclin (TC), chloramphenicol (CAP) and sulfonamide residue in cultured rainbow trout meat. A total of 100 samples of *Oncorhynchus mykiss* were collected from various markets in northwest regions of Iran. The prepared samples were analysed for these antibiotic residues using ELISA method. Results showed that up to 56% of the samples were contaminated with three antibiotics. The antibiotic residues concentrations in the positive samples ranged within 0.09–22.12 ng/g and the TC contamination (30%) was the highest percentage of antibiotic residues in fish meat samples. Amount of mean concentration of TC residue (8.44±6.03 ng/g) in positive samples was higher in comparison with other antibiotics. In all positive samples the TC and sulfonamide levels were below the maximum residue limit (200 and 100 µg/kg respectively). Of the 100 samples analysed for residues, CAP was detectable in only seven samples. These data showed that despite the prohibition of CAP application in food animals including aquaculture, CAP residues were detectable indicating an illegal use of this antibiotic. So, the obtained results from analysis of fish meat samples were considered to be a positive sign in terms of food safety. Also, these analyses are performed as routine according to the national residue monitoring plan of the Republic of Iran.

Key words: antibiotics residue, food safety, *Oncorhynchus mykiss*

INTRODUCTION

Fish is reported by the Food and Agriculture Organization to contribute about 60% of the world's supply of protein and that 60% of the developing world derives

more than 30% of their annual protein from fish (Anonymous, 2004).

The accelerated growth of aquaculture has resulted in a series of harmful effects

to human health (Victoria & Samanidou, 2007). The widespread and unrestricted use of antibiotics in this industry to prevent bacterial infections, leads to remaining amounts in the aquatic environment. This has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments, increase in antibiotic resistance in fish pathogens as well as in the transfer of these resistance determinants to human pathogens (Victoria & Samanidou, 2007; Mastovska, 2011). Moreover, the use of large amounts of antibiotics may lead to the presence of residual antibiotics in fish tissue and fish products.

Tetracyclines are a group of broad-spectrum antibacterial compounds that are widely applied in the aquaculture industry (Wen *et al.*, 2006). In addition, fluoroquinolones, sulfonamides and penicillins exhibiting activity against both Gram-positive and Gram-negative bacteria, are widely used for treatment and prevention of diseases in fish (Samanidou & Evagelopoulou, 2007; Wang, 2009; Mastovska, 2011). In Iran, there has been an increase in the production and consumption of freshwater fish reared in aquaculture systems in recent years, mainly the rainbow trout (*Oncorhynchus mykiss*). Therefore, the drug residues in the fish tissues have reduced the quality and safety of fish as food. However, there is less attention to the residual drugs in cultured fish than in domestic animals (Ueno *et al.*, 1999). With increasing use of veterinary drugs in food production, there is global consideration about the consumption of antimicrobial residues in aquatic foods and their effects on human health. The presence of antibiotics in human food is associated with several adverse public health effects including hypersensitivity, gastrointestinal disturbance, tissue damage and neurological disorders. Additionally,

the use of antibiotics in animal husbandry and its occurrence in related food, may lead to selection of resistance in bacterial populations that do not respond to treatment commonly used for human illnesses (Wassenaar, 2005).

This study was aimed to evaluate the CAP, sulfonamide and TC residues in rainbow trout meat from markets in northwest regions of Iran.

MATERIALS AND METHODS

One hundred samples of cultured rainbow trout fish with an average body weight of 300 g were collected within a 12-month period (from April 2011 to March 2012) in northwest regional markets in Iran. Then fish muscle samples were removed and homogenised in a blender for 2 min and was used to detection of antibiotics residue.

The antibiotic residues analysis was performed by ELISA method (Martela *et al.*, 2006). In order to measure the amount of chloramphenicol, TA and sulfonamide residues, the ELISA technique was performed according to manufacturer's instruction (RIDASCREEN Chloramphenicol ELISA kit (R1505), Tetracycline ELISA kit (R3503) and Sulfonamide ELISA kit (R3004) r-biopharm, Germany). In brief, 4 g of homogenised meat sample was weighed, then the homogenised samples were mixed with 3 mL of distilled water and 6 mL of ethyl acetate was added. The suspension was vortexed for 10 min and centrifuged at 3000×g for 10 min at room temperature. A 4 mL aliquot of ethyl acetate supernatant (corresponding to 2 g of sample) was transferred into a fresh tube and dried at 60 °C under a weak stream of N₂. The residue was re-dissolved in 1 mL isooctane/chloroform (2:3) mixture. Then, 0.5 mL from the each

antibiotic buffer was added to this solution and vortexed intensively for approximately 1 min. The solution was centrifuged at 3000×g for 10 min at room temperature. Fifty µL of the aqueous (upper) layer was used per well in the assay. The absorbance of the samples was read at 450 nm and the amount of each antibiotic was calculated based on calibration curve. The level of each antibiotic was expressed as ng/g of tested tissues. The mean lower detection limit of the RIDSCREEN CAP, TC and sulfonamide was 0.05, 2 and 2 ng/g and the recovery rates were >80% for all samples.

The concentration of three antibiotics in positive samples was computed as mean ± SD. The results were also compared with the MRLs.

RESULTS

One hundred cultured rainbow trout meat samples were analysed for CAP, sulfonamide and TC residues during this study. The maximum and minimum amounts, mean and standard deviation for three antibiotic residues are shown in Table 1. Results showed that up to 56% of the samples were contaminated with three antibiotics. We found that mean concentration of TC (8.44±6.03 ng/g) in positive samples was higher in comparison with other antibiotics. The level of antibiotics contamination is depicted in Fig. 1A–C. Most of contaminated samples contained TC (30%) followed by sulphonamide (19%) and CAP with the lowest rate (7%).

In all positive samples, TC and sulfonamide levels was below the MRLs (200 and 100 µg/kg respectively) (Table 1). Of the 100 samples analysed for residues of CAP, the drug was detectable only in seven samples (Fig.1C).

DISCUSSION

The presence of antibiotic remains in meat and edible viscera of food-producing animals has attracted extensive worldwide attention from national and international public health agencies (Salehzadeh *et al.*, 2006; Asperger *et al.*, 2009). Residues of antimicrobial agents are a potential hazard for the consumer and may cause undesirable changes in bowel microflora and induce immunological reaction in susceptible persons (Mottier *et al.*, 2003; Salehzadeh *et al.*, 2006; Nafisi *et al.*, 2008). For this reason, control of antibiotic residues is necessary to ensure food safety and to prevent exposure of the consumers to drug residues.

In present study we examined the 100 muscle samples of rainbow trout for presence of TC, CAP and sulfonamide residues. Out of the 100 assayed samples using the ELISA method, 30 (30%), 7 (7%) and 19 (19%) had detectable levels for TC, CAP and sulfonamide residues respectively. In this study, TC compared to other antibiotics had the highest mean values (4.15±3.89 µg/kg) and also exhibited the highest number of infected samples (Table 1, Fig.1A).

Table 1. Concentrations of TC, CAP and sulfonamide residues in rainbow trout meat samples.

Antibiotics	Mean±SD (ng/g)	Min–Max	Positive samples (%)	MRL (µg/kg)	>MRL (%)
Tetracycline	8.44±6.03	2.09–22.12	30	200	0
Chloramphenicol	0.14±0.05	0.09–0.25	7	0	7
Sulfonamide	3.89±1.67	2.01–7.06	19	100	0

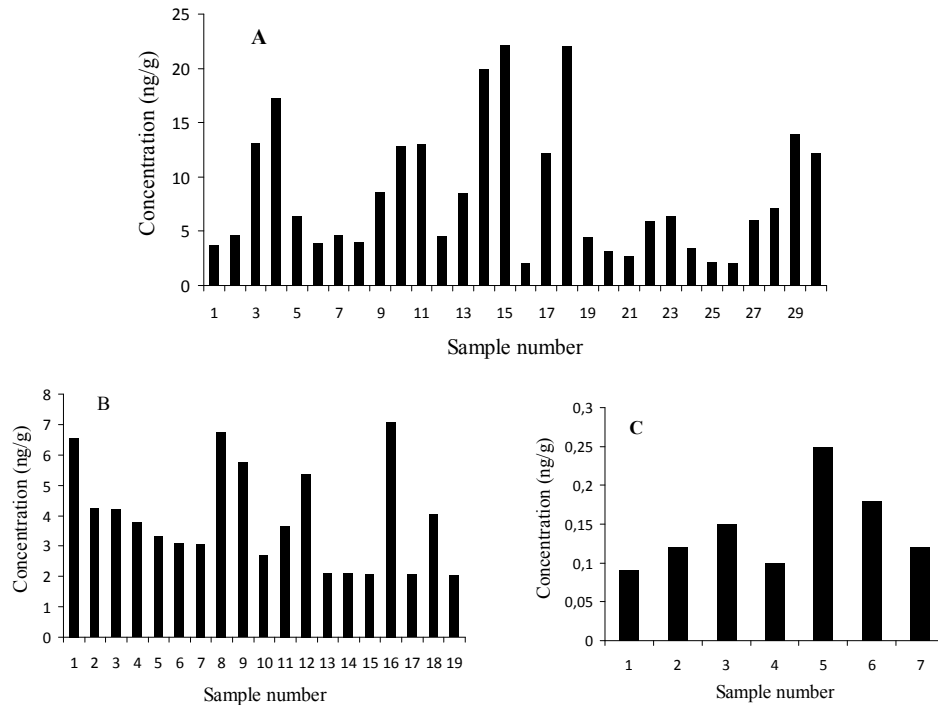


Fig. 1. Antibiotic residues in the rainbow trout meat samples. A. Tetracycline (n=30), B. Sulfonamide (n=19), C. Chloramphenicol (n=7).

Antibiotics examined in this study, as veterinary drugs, are widely used to treat and prevent infections, such as gastrointestinal and respiratory infections and to promote growth of livestock and fish (Ueno *et al.*, 1999; Botsoglou & Fletouris, 2001). Sulfonamide residues in food are an important concern, due to the possibility of risk to human health, such as resistance development and toxicity (Botsoglou & Fletouris, 2001). There are numerous reports indicating the undesirable effects of CAP in humans such as aplastic anaemia, which has resulted to its limited usage. Many countries including the USA, Canada, Australia and EU member states have banned its use in food animals (Anonymous, 1994). TC in meat may potentially stain the teeth of young

children. Allergic reactions and development of resistant strains of bacteria following the ingestion of subtherapeutic doses of antimicrobials are some of the reported hazardous effects (Wilson *et al.*, 2003).

Because of concerns they posed on human health and for regulatory purposes, the European Commission (EC) adopted a MRL of 100 and 200 µg/kg for sulfonamide and TC in foodstuffs of animal origin respectively (Salehzadeh *et al.*, 2006; Asperger *et al.*, 2009; Anonymous, 2010).

Our results indicated that the levels of contamination with TC and sulfonamide residues in the analysed samples were less than MRL (200 and 100 µg/kg). The results of Senyuva *et al.* (2000) for detection of OTC residue in cured meat using

HPLC in Turkey showed that 7 out of 10 meat samples marketed in Turkey had higher OTC residue than the levels accepted by the EU and FDA. HPLC was a rapid and specific method for the determination of OTC with recoveries from 78 to 100%. By this method, the level of OTC in 47 samples (94%) before frying and in 38 samples (76%) after frying were higher than MRLs.

Recently, the presence of CAP residues detected in imported products to EU including poultry, shrimps and honey was reported and its huge economical impact was discussed. As the administration of CAP in food animals has been legally banned, a maximum residual level (MRL) of zero should be anticipated (Okerman *et al.*, 1998; Ferguson *et al.*, 2005). In the present study, 7% of samples contained CAP at a level higher than the MRL (0 µg/kg). As CAP is a prohibited antibiotic in food animal industry, a zero tolerance must be applied for this antibacterial agent. Our data indicates that illegal uses of CAP in fish industry should be taken into account seriously. At the same time, food control centres should provide more sensitive analytical methods such as chromatography or biosensor systems to detect the low CAP concentrations.

CONCLUSION

Many antibiotics are usually used in fish farms, besides our data proved illegal use of CAP. Therefore, routine drug residues surveillance and monitoring programmes in edible animal products like fish meat should be continued to ensure food safety in the country. Nevertheless, veterinarians must be well aware of the importance of drug residues in food animals and their possible risk to the general public and should educate livestock producers on

good agricultural practices and responsible use of antibiotics in food animals.

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