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Antimicrobial Residues in Chicken Meat, Giblet, and Skin with Referring to Maximum Residue Limits

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Abstract

Irresponsible use of antibiotics, inability to follow label guidelines, or insufficient withdrawal periods before slaughtering poultry could result in antibiotic residues in edible poultry tissues, thereby representing hazards to public health. Therefore, this study was conducted to determine the residual levels of three commonly used antimicrobials including oxytetracycline (OXY), enrofloxacin (ENRO), and sulfadimidine (SULFA) in muscle, skin, and giblets of chicken carcasses quantitatively. Additionally, the obtained residual values were compared to the maximum residue limits (MRLs) stated by the regulatory authorities. The findings denoted that the muscles of fresh domestic broilers had significantly higher values of OXY, ENRO, and SULFA than those of fresh native breeds and imported frozen chicken ($p<0.05$). Similarly, in pooled giblets (equal weights of liver and kidneys), OXY and ENRO were significantly higher in domestic broilers than in native breeds ($p<0.05$). Likewise, ENRO and SULFA residues were higher in skin samples of domestic broilers than in native breeds. In comparison to the MRLs reported by the European Commission, the muscles from 20, 60, and 50 % of examined domestic broiler carcasses exceeded the MRLs of OXY, ENRO, and SULFA., respectively, whereas muscles from 20, 70, and 50 % of examined native breed carcasses surpassed these MRLs, respectively. Conversely, in imported frozen broilers, no muscle samples topped the MRL of OXY, while 10 % of the examined carcasses exceeded the MRLs of both ENRO and SULFA. Therefore, very extensive work is needed to monitor the antimicrobial residues in poultry tissues, as well as educational programs about the proper use of antibiotics in poultry production with emphasis on the public health risks of antibiotic residues in food should target the farmers.

KEYWORDS
Antibiotic residues, Broiler chicken, HPLC, Food safety, Maximum residue limits

INTRODUCTION

Poultry meat is a valuable source of animal protein, demand for it is globally increasing by consumers causing a progressive development in the poultry industry. Since the demand is exceeding the production, the poultry industry has a tendency to use growth promoters including antimicrobials to improve poultry production and prevent infections (Mohammadzadeh *et al*., 2022).

Antibiotics are naturally existing, semi-synthetic, and synthetic antibacterial substances that can be administered parentally, orally, or topically (Geidam *et al*., 2009). Antibiotics such as sulfonamides (SAs), tetracyclines (TCs), and fluoroquinolones (FQs) have been used in chicken farming for several years to treat bacterial infections, stimulate growth, and to prevent diseases (Nisha, 2008; Olatoye and Ehinmowo, 2010).

The Codex Alimentarius Commission created the Codex Committee on Residues of Veterinary Medications in Food, which set maximum residue limits (MRL) for about fifty-nine veterinary drugs (Codex Alimentarius Commission, 2018). MRL refers to the maximum concentration of residue arising from the use of a veterinary medicinal product (expressed in mg/kg or µg/kg on a fresh weight basis) that the community may accept as legally permissible or acceptable in or on food (McGlinchey *et al*., 2008). The maximum residue limit of sulfonamides in animal-derived foods, including chicken meat and edible giblets, is 100 µg/kg (European Commission, 2010).

Tetracyclines are important in veterinary medicine because they have a wide spectrum of antibacterial action against Gram-positive and Gram-negative bacteria in both aerobic and anaerobic bacteria. Tetracyclines are effective against *Rickettsia*, *Mycoplasma pneumoniae, Chalamydia* spp., and several atypical *Mycobacteria* and *Plasmodium* spp. that are resistant to cell-wall-inhibitor antimicrobial drugs (Kapusnik-Uner *et al*., 1996). Oxytetracycline is a natural tetracycline derivative obtained from the fungus *Streptomyces rimosus* or synthetically synthesized. Because of its broad-spectrum bacteriostatic activity, low cost, and ease of usage by oral administration through drinking

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water or feed, Oxytetracycline is one of the most commonly used antibiotics in veterinary medicine (Slana and Dolenc, 2013).

However, the insufficient withdrawal period of oxytetracycline antibiotics and misuse in chicken production resulted in the existence of residues that may affect human health (Cetinkaya *et al*., 2012), and could result in teratogenic deformity to the fetus, hypoplasia in developing teeth when supplied to infants (Senyuva *et al*., 2000). Maximum residue levels (MRLs) for the presence of oxytetracycline residues in poultry products were defined by the European Commission (2010) to be 2000 µg/Kg in muscle, 6000 µg/Kg in the liver, and 12000 µg/Kg in the kidney.

Fluoroquinolones (FQs) have broad antibacterial action against Gram-positive and Gram-negative bacteria and are used to treat respiratory, urinary, gastrointestinal, and skin diseases in animals and poultry (Barreto *et al*., 2017; Peris-Vicente *et al*., 2017; Chaowana and Bunkoed, 2019). Enrofloxacin is a second-generation bactericidal fluoroquinolone. After oral administration, it is well absorbed and distributed at the tissue level, and metabolized in the liver, producing its principal active metabolite, ciprofloxacin (Zhou *et al*., 2008). MRL for enrofloxacin has been established by the European Union. The defined MRL for muscle, and skin in broilers is 100 µg/Kg; 200 µg/Kg for the liver; and 300 µg/Kg for the kidneys. It is also noted that enrofloxacin cannot be used in poultry that produces eggs for human consumption (European Commission, 2010).

Sulfonamides (SAs) are widely utilized as therapy and for prophylactic purposes in veterinary medicine (Al-Nazawi and Homeida, 2005). For instance, infectious coryza, fowl typhoid, pullorum disease, and coccidiosis are all treated with sulfonamides in chicken (Giguere *et al*., 2006). Additionally, sulfonamides are sometimes utilized as animal feed additives because extended intake of sulfonamides in sub-therapeutic doses may promote the growth of birds and animals (Long *et al*., 1990). Because of their low cost, sulfonamides have been widely used, resulting in a surge of sulfonamide-resistant bacterial species. If insufficient withdrawal durations for the chicken are not observed or if these medications are provided indecently, SAs residues may be detected in retail chicken tissues (Kishida and Furusawa, 2001).

Sulfadimidine is one of the most commonly used SAs in human and animal medicine, with about 5400 sulfonamide derivatives. Sulfadimidine is commonly utilized by veterinarians in ruminants and poultry for infection control as well as for growth promotion. Because of its great efficacy and low cost, sulfadimidine is widely used in chicken production as a feed additive (Barceló, 2007). Sulfadimidine was found to account for nearly 95% of sulfonamide violations in animal tissues, according to many reports (SANCO, 2004; Van Boeckel *et al*., 2015; Chen *et al*., 2012). Unauthorized use of antibiotics, inability to follow label guidelines, or an insufficient withdrawal period before slaughtering poultry could result in antibiotic residue contamination of edible poultry tissues, thereby representing hazards to human health (Donoghue, 2003). Antibiotic residues in poultry meat may have a number of negative consequences on consumers. Even at relatively low dosages, they can induce direct toxicity, the development of resistant microorganisms, and allergies (Kirbis, 2007). Besides, long-term overexposure to trace levels of antibiotics through contaminated foods can cause normal microflora in the digestive system to adapt and develop antibiotic resistance (Myllyniemi *et al*., 2004; Javadi *et al*., 2011).

Therefore, antibiotic residues in chicken meat must be monitored in order to ensure food safety. As a result, numerous analytical methods for determining antibiotic residues in poultry tissue are available, the most powerful and sensitive of which is high-performance liquid chromatography (HPLC) (Abdullah *et al*., 2012).

Hence the objective of this study was to determine the residual levels of three commonly used antimicrobials including oxytetracycline (OXY) (tetracyclines), enrofloxacin (ENRO) (fluoroquinolones), and sulfadimidine (SULFA) (sulfonamides) in edible tissues and organs [muscle, skin and pooled giblets (liver and kidney)] of chicken carcasses with comparing between domestic broilers, native breeds and imported frozen broiler chicken quantitatively using HPLC.

MATERIALS AND METHODS

Chemicals and reagents

All reagents used in this study were of analytical grade. Analytical grade oxytetracycline, sulfadimidine, and enrofloxacin standards were obtained from Sigma-Aldrich (Missouri, United States). HPLC-grade acetonitrile, methanol and water were obtained from Poch SA (Poland). formic acid, citric acid, nitric acid, and potassium dihydrogen phosphate were purchased from Sigma-Aldrich (Missouri, United States).

Sampling

A total number of 30 retail chicken carcasses, 10 each of fresh domestic broilers, fresh house-reared native breed chicken (Baladi and Fayoumi), and frozen imported broiler chicken (imported from Brazil to Egypt, as identified by the package label) were collected from different retailed markets in Egypt. Each chicken carcass was represented by subsampling from muscle, skin, and pooled edible organs (equal weights from kidney and liver) for the quantitative determination of antimicrobial residues. All samples were stored at - 4°C until the time of analysis.

Determination of antimicrobial residues

HPLC Equipment

A high-performance liquid chromatography (HPLC) apparatus equipped with a liquid chromatography pump plus surveyor, an autosampler plus surveyor, variable wavelength PDA detector surveyor, and a Chromo Quest 5 software, (Thermo Fisher Scientific, USA). Besides, it was equipped with Hypersil gold C18 (10 µm, 250 x 4.6 mm) column. Additionally, syringe filters (MCE membrane, 0.45 mm, corrigtwohill company, Cork, Ireland), an ultrasonic bath (3510 Branson) and a cooling centrifuge (Centurion Scientific Ltd, UK) were used.

Quantitative determination of oxytetracyclines (OXY) by HPLC

Sample extraction

After cutting exterior fat and fascia, frozen tissue samples were thawed and coarsely diced using scissors. Using a scale, two grams of each organ, skin, or muscle sample to be analyzed were weighed, then chopped into extremely small pieces and crushed into a fine powder using a Sartorius mincer. The mixture was then homogenized in a blender for 2 minutes before adding 0.1 g citric acid. Then 1 mL of nitric acid (30%), 4 mL of methanol, and 1 mL of deionized water were added to this mixture, respectively. The solid particle suspension was mixed well in a vortex, then held in an ultrasonic bath for 15 minutes before being centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 µm nylon filter, 20 µl of the solution was injected into HPLC for analysis according to Senyuva *et al*. (2000).

Chromatographic conditions

It included a mobile phase of acetonitrile and formic acid (0.1%) with a flow rate of 1 mL/min at 25°C. The separation was done on hypersil gold C 18 (10 µm, 250 x 4.6 mm) and the detection was performed with a PDA detector set at 350 nm wavelength. The retention time was 5.8 minutes. Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the software (Chromo Quest 4.2).

Calibration curve

The calibration curve was prepared by using concentrations of 0.01, 0.03, 0.06, 1.25, 0.25, 0.5, 1, 2, 5, 10 µg/mL of oxytetracycline in eluent and spiked chicken muscle. These standards were prepared from the daily prepared stock solution and treated as 100 mg of oxytetracycline standard and were accurately weighed and put in a 100 mL volumetric flask, the powder was dissolved in 100 mL of water to make a stock solution of 1000 µg/mL. Several serial dilutions of the stock solution were carried out.

Quantitative determination of enrofloxacin (ENRO) by HPLC

Sample extraction

Frozen tissue samples were thawed and finely diced with scissors after trimming of external fat and fascia. Two grams of each sample to be analyzed were weighed using a balance and then cut into very small pieces and subsequently ground into a fine powder using Sartorius mincer. 8 mL of 5% trichloroacetic acid solution acid was added. The sample was mixed using vortex for 1 minute followed by shaking for 10 minutes in a rotary agitator, and then centrifuged for 5 minutes at 14000 rpm in a cooling centrifuge at +4 °C then filtered through a 0.45 µm nylon filter, 20 µl of the solution was injected into HPLC for analysis (Verdon *et al*., 2005).

Chromatographic conditions

It included a mobile phase of acetonitrile and phosphoric acid buffer (0.01 M, pH 3) (25:75% v/v) was utilized with a flow rate of 1.0 mL/min and the detection was carried out at 278 nm. The separation was done on hypersil gold C 18 (10 µm, 250 × 4.6 mm). The retention time was 6.9 minutes (Moghadam *et al*., 2018). Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the Chromo Quest 4.2 software.

Calibration curve

The calibration curve was prepared using concentrations 0.06, 1.25, 0.25, 0.5, 1, 2, 5, 10, 20 µg/mL of enrofloxacin in eluent and spiked chicken muscle. These standards were prepared from the daily prepared stock solution and treated as 100 mg of enrofloxacin standard and were accurately weighed and put in a 100 mL volumetric flask, the powder was dissolved in 100 mL of water to make a stock solution of 1000 µg/mL. Several serial dilutions of stock solution were carried out.

Quantitative determination of sulfadimidine (SULFA) by HPLC

Sample extraction

After trimming the external fat and fascia, frozen tissue samples were thawed and coarsely diced using scissors. Two grams of each organ to be examined were weighed with a balance before being sliced into extremely small pieces and processed into a fine powder with a Sartorius mincer. Two grams of sample were weighed and transferred to a 15 mL propylene tube. 5 mL of acetonitrile with 0.2% FA were added, vortex and mixed for 1 min, and then sonicated for 30 min, The sample was centrifuged at 8000 rpm for 5 min. After degreasing, the supernatant was filtered and transferred to a sample vial ready for Solid Phase Extraction (SPE). SPE cartridge was conditioned with 1000 µL methanol and 1000 µL water. 100 µL portion of sample extract was then loaded onto the cartridge using 1000 µL water as transfer solvent at 1000 µL/min. 20 µl of eluent was injected into HPLC for analysis (Ma *et al*., 2020).

Chromatographic conditions

It included a mobile phase of 0.01 M potassium di-hydrogen phosphate (KH2PO4) buffer and methanol (70:30 v/v) with a flow rate of 1 mL/min. The separation was done on hypersil gold C 18 (10 µm, 250 x 4.6 mm) with a PDA detector set at 264 nm. The retention time was 6.3 minutes. (Mehtabuddin *et al*., 2012). Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the Chromo Quest 4.2 software.

Calibration curve

The calibration curve was prepared by using concentrations 1.25, 0.25, 0.5, 1, 2, 5, 10, 20 µg/mL of sulfadimidine in eluent and spiked chicken muscle. These standards were prepared from the daily prepared stock solution and treated as 100 mg of sulfadimidine standard and were accurately weighed and put in a 100 mL volumetric flask. The powder was dissolved in 100 mL of water to make a stock solution of 1000 ug/mL. Several serial dilutions of the stock solution were carried out.

Statistical analysis

All the data were statistically analyzed using Minitab 19 statistical software. Significant differences among means were determined using a one-way ANOVA test. Means were considered significantly different if $P < 0.05$.

RESULTS

Antimicrobial residues in chicken muscle samples

The residual levels of three types of antimicrobials (oxytetracycline, enrofloxacin, and sulfadimidine) in muscles of examined retail-marketed fresh domestic broilers chicken carcasses were reported in Table 1. Sixty % of the chicken carcasses contained OXY, ENRO, and SULFA residues in their muscles with mean values of 2.09, 7.21, and 0.92 µg/g muscle, respectively. Regarding fresh native breed chicken carcasses, 70, 90 and 70 % of examined carcasses had OXY, ENRO, and SULFA residues in muscles with mean values of 1.62, 3.84, and 0.48 µg/g muscle, respectively (Table 1). Concerning frozen imported broiler chicken carcasses, 40, 30 and 50 % of examined carcasses were containing OXY, ENRO, and SULFA residues with mean values of 0.009, 0.046, and 0.047 µg/g muscle, respectively (Table 1). Out of the examined antimicrobial residues, obviously, ENRO showed the highest residual values in muscles of the three chicken types.

Antimicrobial residues in chicken pooled giblet samples

Shifting to the results of antimicrobial residues in pooled giblets (equal weights from liver and kidney) from various chicken carcasses, it was noticed that the pooled giblets from retail-marketed fresh domestic broilers chicken contained OXY, ENRO, and SULFA residues in 60, 60, and 10 % of the examined carcasses, with mean values accounted for 0.76, 9.34, and 0.01 µg/g, respectively (Table 2). With regard to native breed chicken, 70, 90 and 70 % of examined carcasses contained OXY, ENRO, and SULFA residues in their giblets with 0.32, 2.63, and 2.08 µg/g giblet, respectively (Table 2).

Antimicrobial residues in chicken skin samples

As regard to the antimicrobial residues in the skin of chicken carcasses, 60, 60 and 20 % of examined fresh domestic broilers carcasses contained OXY, ENRO, and SULFA residues in their skin with mean values accounted for 0.03, 1.24, 0.52 µg/g skin, respectively (Table 3). While 30, 30 and 20 % had OXY, ENRO, and

Table 1. Residual levels of antimicrobials (µg/g) in muscles of chicken carcasses

Antimicrobials	No. of positive samples	Positive samples (%)	Minimum	Maximum	Mean	Standard error
Retail-marketed fresh domestic broilers						
Oxytetracycline	6	60	0 (ND)	16.68	2.09	1.64
Enrofloxacin	6	60	0 (ND)	24.4	7.21	3.23
Sulfadimidine	6	60	0 (ND)	7.17	0.92	0.69
Retail-marketed fresh native breed						
Oxytetracycline	7	70	0 (ND)	8.24	1.62	0.82
Enrofloxacin	9	90	0 (ND)	29.92	3.84	2.93
Sulfadimidine	7	70	0 (ND)	1.84	0.48	0.21
Retail-marketed frozen imported broilers						
Oxytetracycline	4	40	0 (ND)	0.04	0.009	0.004
Enrofloxacin	3	30	0 (ND)	0.33	0.046	0.032
Sulfadimidine	5	50	0 (ND)	0.24	0.047	0.023

Table 2. Residual levels of antimicrobials (µg/g) in pooled giblets* of chicken carcasses.

Antimicrobials	No. of positive samples	Positive samples (%)	Minimum	Maximum	Mean	Standard error
Retail-marketed fresh domestic broilers						
Oxytetracycline	6	60	0 (ND)	2.68	0.76	0.29
Enrofloxacin	6	60	0 (ND)	38.85	9.34	4.17
Sulfadimidine	1	10	0 (ND)	0.16	0.01	0.01
Retail-marketed fresh native breed						
Oxytetracycline	7	70	0 (ND)	0.81	0.32	0.09
Enrofloxacin	9	90	0 (ND)	10.83	2.63	1.07
Sulfadimidine	7	70	0 (ND)	9.32	2.08	1.13

* Pooled giblets = Pooled equal quantities from liver and kidney

Table 3. Residual levels of antimicrobials (µg/g) in the skin of retail-marketed fresh domestic broilers chicken carcasses.

Antimicrobials	No. of positive samples	Positive samples (%)	Minimum	Maximum	Mean	Standard error
Retail-marketed fresh domestic broilers						
Oxytetracycline	6	60	0 (ND)	0.09	0.03	0.01
Enrofloxacin	6	60	0 (ND)	3.85	1.24	0.47
Sulfadimidine	2	20	0 (ND)	5.21	0.52	0.52
Retail-marketed fresh native breed						
Oxytetracycline	3	30	0 (ND)	3.45	0.42	0.34
Enrofloxacin	3	30	0 (ND)	4.38	0.82	0.51
Sulfadimidine	2	20	0 (ND)	2.03	0.23	0.2
Retail-marketed frozen imported broilers						
Oxytetracycline	1	10	0 (ND)	0.026	0.003	0.002
Enrofloxacin	2	20	0 (ND)	0.11	0.02	0.01
Sulfadimidine	0	0	0 (ND)	0 (ND)	0 (ND)	0

SULFA residues in the skin with mean values accounting for 0.42, 0.82, and 0.23 µg/g of skin, respectively (Table 3). Only 10 and 20 % of examined frozen imported chicken carcasses had OXY and ENRO with mean values were 0.0026 and 0.02 µg/g skin, respectively, while SULFA was undetectable in the skin of any examined chicken (Table 3). Similar to the findings in muscle and giblets, ENRO was the highest antimicrobial residues in the three chicken types in comparison with other antimicrobials

Comparison between chicken carcass based on levels of antimicrobial residues

The data illustrated in Table 4 show the comparison between chicken carcasses based on species and sources.

In the case of pooled giblets, OXY and ENRO were significantly higher in fresh domestic broilers than in fresh native breeds ($p<0.05$). Conversely, SULFA was significantly higher in native breeds than in fresh domestic broilers ($p<0.05$) (Table 4). With regard to skin samples, the significant highest value of OXY residues was recorded in native breeds followed by fresh domestic broilers and then imported frozen chicken ($p<0.05$), whereas ENRO residues in fresh broilers and fresh native breeds were significantly higher than in imported frozen chicken ($p<0.05$). Likewise, SULFA residual levels were significantly higher in fresh broilers and fresh native breeds than in imported frozen chicken ($p<0.05$) (Table 4).

Chicken samples exceeding the maximum residue limits (MRLs) of antibiotic residues

Apparently, from Table 5 the muscles of 20, 60, and 50 % of examined fresh broiler carcasses exceeded the MRLs of OXY, ENRO, and SULFA, which are 2000, 100, and 100 µg/Kg muscle, respectively. Whereas muscles from 20, 70, and 50 % of examined native breed carcasses surpassed these MRLs, respectively. In the case of imported frozen broilers, there were no muscle samples topped the MRL of OXY (2000 µg/Kg), while muscle samples from 10 % of the examined carcasses exceeded the MRLs of both ENRO and SULFA (Table 5).

There were no chicken pooled giblet samples that surpassed the MRL of OXY in the liver (6000 µg/Kg). Yet, pooled giblets from 60 and 80 % of fresh broiler chicken and fresh native breed chicken carcasses outstripped the MRL of ENRO in poultry kidney (300 µg/Kg), and the MRL in poultry liver, as well (200 µg/Kg). Additionally, pooled giblets from 10 and 60 % of fresh broiler chicken and fresh native breed chicken carcasses exceeded 100 µg/Kg, which is the MRL of SULFA in poultry liver and kidney (Table 5).
None of the examined skin samples from different chicken carcasses exceeded the MRL of OXY in poultry skin. On the contrary, skin samples from 60, 30, and 10 % of fresh broilers, fresh native breeds, and imported frozen broiler chicken carcasses topped the MRL of ENRO (100 µg/Kg skin). Furthermore, skin samples from 10 and 20 % of fresh broilers, fresh native breed chicken carcasses exceeded 100 µg/Kg, which is the MRL of SULFA in poultry skin, while there were no skin samples from imported frozen broiler carcasses surpassed such limit (Table 5).

DISCUSSION

Poultry meat is a valuable source of animal protein in the majority of the world including Egypt. The poultry meat demand is globally increasing by consumers causing a progressive development in the poultry industry. Since the demand is exceeding the production, the poultry industry has a tendency to use growth promoters including antimicrobials to improve poultry production and prevent infections (Mohammadzadeh *et al*., 2022). Unauthorized use of antibiotics, inability to follow label guidelines or an insufficient withdrawal period before slaughtering poultry could result in antibiotic residue contamination of edible poultry tissues, thereby representing hazards to human health (Donoghue, 2003). Oxytetracycline (OXY) (tetracyclines), enrofloxacin (ENRO) (fluoroquinolones), and sulfadimidine (SULFA) (sulfonamides) are three of the most commonly used antibiotics in poultry production in Egypt. Therefore, the residual levels of these three antibiotics were determined in the edible tissues of retail-marketed chicken carcasses in Egypt.

ENRO showed the highest residual values in pooled giblets in the three chicken types as compared with other antimicrobials. After administration, fluoroquinolones are rapidly absorbed, present a wide distribution volume and little binding to plasma proteins, are excreted by the urine and bile, and their residues are found in the liver and kidneys (Goetting *et al*., 2011). So, the higher MRLs allowed for the liver and the kidneys are consistent with the pharmacokinetics of fluoroquinolones, concentrating higher residue levels in these tissues (European Commission,

Table 4. Comparison between domestic broilers, native breeds and imported chicken carcasses based on antimicrobial residues in each sample type (muscle, pooled giblets, and skin).

Chicken carcass	Muscle			Pooled giblets			Skin		
	OXY	ENRO	SULFA	OXY	ENRO	SULFA	OXY	ENRO	SULFA
Fresh domestic broilers	2.09^{a}	7.21^{a}	0.92^{a}	0.76^{a}	9.34^{a}	0.01^{b}	0.03^{b}	1.24^{a}	0.52^{a}
Fresh native breeds	1.62^{b}	3.84^{b}	0.48^{b}	0.32^{b}	2.63^{b}	2.08^{a}	0.42^{a}	0.82^{a}	0.23^{a}
Imported frozen broilers	0.009^{c}	0.046^{c}	0.047^{c}	NA	NA	NA	0.002^{c}	0.02^{b}	ND^{b}

Where, pooled giblets = pooled equal quantities from liver and kidney. Oxy= Oxytetracycline, Sulfa= Sulfadimidine, Enro= Enrofloxacin. ND= not detectable, NA= not applicable. Different small letter (a, b, c, …) superscripts within the same column indicates significant differences between means at $p<0.05$.

Table 5. Percentage (%) of samples exceeding the maximum residue limits (MRLs) of antibiotic residues in poultry meat, giblets and skin.

Chicken carcass	Muscle			Pooled giblets			Skin		
	OXY	ENRO	SULFA	OXY	ENRO	SULFA	OXY	ENRO	SULFA
Fresh domestic broilers	20	60	50	0	60	10	0	60	10
Fresh native breeds	20	70	50	0	80	60	0	30	20
Imported frozen broilers	0	10	10	NA	NA	NA	0	10	0

Where, pooled giblets = pooled equal quantities from liver and kidney. NA= not applicable

2010). Moreover, the excessive rate of drug accumulation in the liver can be attributed to the metabolizing characteristic of the liver, containing a high concentration of enzymes (Karimi *et al*., 2020). The residual levels of antimicrobials in giblets of imported frozen chicken carcasses could not be investigated, as the frozen carcasses are sold without giblets.

The findings denoted that the muscles of fresh domestic broilers had significantly higher values of OXY, ENRO, and SULFA than those of fresh native breeds and imported frozen chicken ($p<0.05$), meanwhile fresh native breeds contained significantly higher values of the three antimicrobials than that of imported frozen broilers ($p<0.05$). This could be attributed to the proper use of antibiotics in birds of imported carcasses. The improper uses of veterinary drugs lead to the accumulation of drug residues in animal products (Adams, 2001). The high residual level of OXY in broilers chicken may be attributed to the production of OXY in different trade names and forms in many companies in Egypt. So, the broiler stockholders use it as a cheap effective antibiotic for control of infections and as feed additives at sub-therapeutic levels as a growth-promoting agent (Hussein and Khalil, 2013).

Moreover, the high incidence rate of SULFA in domestic broiler muscle samples may indicate the high use rate of SULFA in the intensive poultry production system, which is likely due to the high efficacy of sulfonamides in fighting diseases and promoting growth in poultry. Some of the antibiotics are quickly excreted from the animal body, however, some of them are not metabolized. Accordingly, they enter the food chain and cause pharmacological, pathological, and toxicological health hazards (Gould, 2008; Venkitanarayanan et al. 2013). Another significant reason that could lead to low antimicrobial residues in frozen carcasses in the current study is the effect of freezing on the antibiotic residues. As it was reported that the effect of freezing at -18°C/ 4 weeks was influential on the level of antibiotic residues in poultry meat samples (Al–Mashhadany *et al*., 2018).

In this regard, it was reported that these antibiotics are sometimes given by non-specialists, especially in developing countries where the guidelines for using antibiotics correctly are not strictly enforced. Excessive use of antibiotics without scientific justification may result in the development of resistant bacteria, resulting in a reduction in antibiotic efficacy in poultry treatment and, ultimately, treatment failure (Jammoul and El Darra, 2019). Furthermore, excessive use of veterinary antibiotics without regard for their withdrawal periods or side effects may result in drug residue or metabolites in chicken products, posing a health risk to consumers (Derakhshan *et al*., 2018).

In order to protect the public health from exposing to the risks of antibiotic residues in foods, maximum residues limits (MRLs), in foodstuffs of animal origin, have been established by the European Commission and reported in Commission Regulation (EU) no 37/2010 MRLs (European Commission, 2010). MRL is defined as the maximum concentration of a residue, resulting from the registered use of a veterinary chemical, that is recommended to be legally permitted or recognized as acceptable in or on a food or an animal feed. The concentration is expressed in µg / Kg of the commodity. It is important to note that a lack of monitoring programs in developing countries leads to non-compliance with animal health measures and the irrational use of veterinary medicinal products (Baazize-Ammi *et al*., 2019). Herein, we compared the obtained residual levels to the MRLs recommended by European Commission (2010) for each antibiotic in poultry meat, giblets, and skin. The obtained results showed that the majority of muscles, giblets and skin samples from domestic broilers and native breed chickens exceeded the MRLs of the examined antibiotics reported by the European Commission (2010). This could be linked to the use of antimicrobial medicines inappropriately in food-producing animals including chickens, as discussed above. As a consequence of exceeding MRLs, consumers of chicken may have toxicological consequences and allergy reactions due to chicken consumption. On the other hand, the majority of edible tissue samples from frozen imported broiler chicken were below these MRLs. This could be ascribed to the effect of freezing and/ or the appropriate use of antibiotic residues in chicken reared in Brazil. Incidentally, poultry consumers may have mutagenic, carcinogenic, and allergic reactions as a result of the drug residues, as well as bone marrow depression, reproductive disorders, toxic effects on intestinal natural flora, and the emergence of antibiotic-resistant bacteria. Antibiotic resistance is projected to kill approximately 7,000,000 people worldwide each year (Muhammad *et al*., 2020).

CONCLUSION

The results of the present study concluded the presence of antimicrobial residues in poultry meat, giblets, and skin samples from domestic broilers chicken at significantly higher values than native breeds followed by imported frozen broiler chicken. High percentages of examined samples contained antibiotic residual values above the MRLs stated by the European Commission, which is a serious matter, therefore, very extensive work in this regard is needed to monitor the antimicrobial residues in poultry. Besides, a campaign must be initiated to educate the farmers about the withdrawal period of drugs as well as the adverse effects of drug residues on human health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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