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Critically important antibiotic-residues assessment in raw meat of various origins marketed in Egyptian markets by mass spectrometer.

Islam Arfa[1], Shimaa Edris[1], Omar Ahmed-Farid[2], Islam Sabeq[1]*

[1]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Toukh, Qalyubia 13736, Egypt;*
[2]*Egyptian Drug Authority (EDA; formerly: NODCAR), Giza, Egypt.*

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ABSTRACT

The current study aimed to assess critical antibiotic-residue levels in raw beef of various origins marketed in Egyptian markets and to judge consumption acceptability about maximum residue limits (MRLs) established by the National Egyptian Food Safety Organization (NFSA). At sales points, 30 samples of raw bovine ribeye and kidney samples (15 of each) were collected and evaluated for the presence of antibiotic residues by Liquid Chromatography-Tandem Mass Spectrometry. Overall, results revealed that amoxicillin, ampicillin, cefotaxime, ciprofloxacin, danofloxacin, enrofloxacin, norfloxacin, oxytetracyclines, tylosin, sulfonamides, gentamycin, and florfenicol verified positive in approximately 67%, 53%, 40%, 37%, 27%, 17%, 33%, 23%, 27%, 46.6%, 50%, and 13% of the samples, respectively. At least two antibiotic residues from one antibiotic class and up to ten antibiotic residues from seven different antibiotic classes were identified in the investigated ribeye and kidney samples. Residues of β-Lactam antibiotics, such as amoxicillin and ampicillin, and cephalosporins like cefotaxime were the most commonly recognized class in 93.33% of the samples, followed by fluoroquinolones at 70%. The MRLs for amoxicillin, cefotaxime, ciprofloxacin, enrofloxacin, norfloxacin, oxytetracycline, Tylosin, and gentamycin residues were violated in 100%, 13.3%, 20.0%, 20.0%, 66.7%, 6.7%, 53.3%, and 33.3% of ribeye samples, respectively. However, the MRLs for amoxicillin (26.16-351.22 µg/kg), cefotaxime (27.17-226.22 µg/kg), ciprofloxacin (30.48-279 µg/kg), and sulfonamides (19.31-266.83 µg/kg) were violated by 53.3%, 80.0%, 13.3%, and 33.3% of kidney samples, respectively, making them dangerous for human consumption. Conclusively, the prevalent multiple-residues index, violation rates, and critically important antibiotic detection in current samples indicate widespread abuse and misuse of antibiotics in the Egyptian veterinary sector.

1. INTRODUCTION

The secondary growth-promoting effects of antibiotics led to their widespread use as feed supplements, along with their primary therapeutic uses to treat or prevent human and animal diseases. Generally, animals receiving antibiotics in their diet gain 4–5% more body weight than those not receiving antibiotics (Bacanlı and Başaran, 2019; Bacanlı, 2024).

On the other hand, antibiotic overuse created two major health risks, both direct and indirect. The administration of antibiotics to animals raised for food directly contributes to the existence of antibiotic residues in animal-derived products (liver, kidney, muscles, fat, milk, and eggs) that are fit for human consumption. These residues can be either the parent chemicals or their metabolites (Baynes et al., 2016). Direct exposure to antibiotic residues in animal-derived foods, such as meat, may relate to a variety of human health issues, including allergic reactions, teratogenicity, carcinogenicity, obesity, reproductive impacts, and teratogenicity. Secondary health risks arising from antibiotic usage to meet the rising animal protein need and intensive farming in developing countries include resistance development, which can impact bacterial populations both directly and indirectly, ultimately leading to the inability to cure diseases (Manyi-Loh et al., 2018).

The most common antibiotics used for prophylaxis, growth promotion, and treatment in food animals are sulfamethoxazole, enrofloxacin, benzylpenicillin, Tylosin, amoxicillin, trimethoprim, oxytetracycline, ampicillin, and streptopenicillin, as well as amikacin, neomycin, enrofloxacin, doxycycline, tetracycline, tilmicosin, and colistin sulfate (Alaboudi et al., 2013; Caneschi et al., 2023; Redwan Haque et al., 2023).

The "One Health" plan requires collaboration across the veterinary, environmental, and human health sectors to be successful (Otu et al., 2021). Surveillance and monitoring of antibiotic usage and resistance are among the national action measures that each country must establish to address direct and indirect antibiotic health concerns (Manyi-Loh et al., 2018; Sabeq et al., 2022). Though it primarily depends on each country's financial resources and the severity of issues, these activities are necessary to be consistent with the primary goals of the World Health Organization's Global Action Plan (GAP) of the "One Health Program" (Manyi-Loh et al., 2018). Successful implementation of residue surveillance and monitoring measures safeguards the country's animal resources, secures the safety of commodities, protects the health and welfare of food animals, prevents disease transmission, and controls them for people's safety and health (Garcia et al., 2020).

Worldwide, the evaluation of the risk and control of veterinary medication residues in food products derived from animals, including meat, dairy, kidney, and muscle, is based on comparable guidelines. Tolerances—the maximum allowed amounts of veterinary medication residues—are established by the Food and Medication Administration (FDA) in the United States of America (USA), while the European Union (EU) maximum residue limits (MRLs) are established by the Committee for Medicinal Products for Veterinary Use (CVMP) and are published by the European

* Correspondence to: islam.sabek@fvtm.bu.edu.eg

Medicines Agency (EMA) (Baynes et al., 2016). The National Egyptian Food Safety Organization, or NFSA, is the equivalent regulatory body in Egypt responsible for establishing MRLs for veterinary medications, including antibiotics. While the EU has adopted MRLs produced by the Codex Alimentarius Commission (CAC) since 2009 (European Union and The Council of the EU, 2009), provided that the EU delegation at the CAC does not object to the MRLs, the NFSA has approved CX/MRL2-2018 (FAO and WHO, 2018) since 2020 without requiring an extra MRL application and evaluation.

Over the last fifty years, methods for identifying antibiotics through analytical methods have focused on stopping the growth of pre-existing bacterial cultures. However, with the introduction of more advanced technology, such as high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS), these approaches are now widely utilized as confirmatory tests at official laboratories, national reference laboratories, and European reference laboratories. In less than eight minutes, LC-MS/MS could identify thirty to forty-six antibiotics (Ghimpețeanu et al., 2022).

In light of maximum residue limits (MRLs) set by the National Egyptian Food Safety Agency (NFSA), the current study aimed to evaluate essential antibiotic residual levels in raw meat of different origins both domestic and imported marketed in Egyptian marketplaces and determine acceptability.

2 .MATERIAL AND METHODS

2.1 . Experiment management and Ethical approval

All methods used in this study were approved by the Benha University, Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee Research Ethics number (BUFVTM 01/06/ 24) .

2.2 . Sample collection

Thirty random samples of fresh beef ribeye loin (*Musculus longissimus thoracis et lumborum, LTL*) cut and kidney (15 of each) were randomly obtained from multiple butcher shops in Cairo, Egypt, and immediately sent to the laboratory for analysis. Each sample represents one animal and/or one butcher shop in order to widen the survey's distinctive samples and area.

2.3 .Sample preparation and distribution.

All samples originated from male cattle of various ages. Each fresh *LTL* was aseptically prepared from both sides of the carcass and weighed approximately 500 g .

Two samples weighing about 100 g (50 g each) were dissected and frozen at -21 ºC to evaluate antibiotic residual levels. The samples were individually packed in airtight plastic bags and immediately transferred on ice gel packs to the laboratory for further investigation.

2.4. Liquid chromatography-tandem mass spectrometry analytical method (LC-MS/MS)

2.4.1 .Sample Preparation and residue extraction

Approximately 10 g of bovine tissue was homogenized with a high-speed homogenizer. Then, 2 g of homogenized sample was transferred to a 50-mL centrifuge tube. Next, 10 mL of extraction solvent (80:20 v/v acetonitrile + 0.1% formic acid) was added. Vortex for 5 minutes, then sonicate for 10 minutes. Centrifuge at 4000 rpm for 10 minutes at 4°C. The supernatant was transferred to a clean tube. 5 mL of hexane was added, vortexed for 2 minutes, and then centrifuged at 4000 rpm for 5 minutes. The overlying hexane layer was then discarded. The aqueous layer was passed through a solid-phase extraction (SPE) cartridge preconditioned with methanol and water. Five milliliters of methanol were used to elute the antibiotics, and the resulting eluates were evaporated to dryness at 40 °C under a nitrogen stream. One milliliter of mobile phase (50:50 v/v acetonitrile with 0.1% formic acid) was used to reconstitute the residue. After that, the reconstituted solution was filtered into an LC vial using a 0.22 µm syringe filter.

2.4.2 .LC-MS/MS Analysis

An LC-MS/MS system with an AB Sciex Triple Quad™ 5500 mass spectrometer and an Agilent 1260 Infinity HPLC system were used. An Agilent ZORBAX Eclipse Plus C18 chromatography column with a 2.1 x 100 mm and 1.8 µm particle size was installed. Two injectable mobile phases were added: acetonitrile with 0.1% formic acid (A) and water with 0.1% formic acid (B). The Gradient Program is set to begin with 90% A and 10% B, then progress linearly to 10% A and 90% B over 10 minutes, hold for 2 minutes, and return to initial conditions over 2 minutes. The flow rate was set to 0.3 mL/min, and the injection volume was 10 µL. The Mass Spectrometry conditions were Electrospray Ionization (ESI) in positive mode, Capillary Voltage of 4500 V, Source Temperature of 350 °C, Desolvation Temperature of 500 °C, and Multiple Reaction Monitoring (MRM) for specific antibiotic transitions. The required actions were taken to guarantee the accuracy and precision of the measurements by the manufacturer during the LC-MS/MS equipment calibration process, which involved comparing a measurement antibiotic standards (Analytical-grade standards of common antibiotics including tetracyclines, sulfonamides, quinolones, macrolides, etc., Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) or instrument of known accuracy with another standard or instrument to eliminate deviations.

2.5 .Statistical analysis

The descriptive data analysis was conducted with SPSS Version 22 (SPSS Inc., Chicago, IL, USA). The antibiotic-residue incidence rate was determined as the ratio of positive samples to total samples multiplied by 100. The antibiotic-residue concentrations in monitored samples were compared to the NFSA and European Union MRLs to determine whether they violated the MRL standard. To calculate the multiple antibiotic-residue index (MARI), the number of antibiotic-residues detected in one sample was divided by the total antibiotic-residues detected in the current study's samples. The total violation rate is obtained by dividing the number of samples that exceeded the MRLs by the total sample analyzed in the present investigation and multiplying by 100.

3. RESULTS

At least two antibiotic residues with a multiple antibiotic residue index (MARI) of 0.17 (2/12) were determined from either one antibiotic (kidney4 and kidney8) or two antibiotic classes (kidney1, kidney13, and kidney15), and up to ten antibiotic-residues with MARI of 0.83 were calculated from seven antibiotic classes, such as the muscle10 sample. Interestingly, muscle samples showed higher levels of MARI than kidney ones (Figure 1)

Figure 1 depicts the multiple antibiotic-residue index (MARI) and classes discovered in native Egyptian raw beef ribeye and kidney samples (n = 30).

Sample	Antibiotic residues		β-lactams			Fluoroquinolones				Tetracyclines	Macrolides	Sulphonamides	Aminoglycosides	Amphenicols
ID	MRI	Classes No.	Penicillin		Cephalosporins									
Muscle 1	0.25	3	Amoxicillin						Norfloxacin			Sulfonamides		
Muscle 2	0.33	3	Amoxicillin		Cefotaxime	Ciprofloxacin			Norfloxacin					
Muscle 3	0.25	3					Danofloxacin			Oxytetracyclin			Gentamycin	
Muscle 4	0.42	4	Amoxicillin	Ampicillin						Oxytetracyclin	Tylosin		Gentamycin	
Muscle 5	0.42	4		Ampicillin			Danofloxacin		Norfloxacin		Tylosin		Gentamycin	
Muscle 6	0.58	4	Amoxicillin	Ampicillin		Ciprofloxacin	Danofloxacin		Norfloxacin			Sulfonamides	Gentamycin	
Muscle 7	0.33	3	Amoxicillin	Ampicillin				Enrofloxacin			Tylosin			
Muscle 8	0.50	5		Ampicillin			Danofloxacin		Norfloxacin		Tylosin	Sulfonamides		Florfenicol
Muscle 9	0.67	6	Amoxicillin				Danofloxacin	Enrofloxacin	Norfloxacin	Oxytetracyclin		Sulfonamides	Gentamycin	Florfenicol
Muscle 10	0.83	7	Amoxicillin	Ampicillin		Ciprofloxacin	Danofloxacin		Norfloxacin	Oxytetracyclin	Tylosin	Sulfonamides	Gentamycin	Florfenicol
Muscle 11	0.58	6	Amoxicillin		Cefotaxime	Ciprofloxacin			Norfloxacin	Oxytetracyclin	Tylosin		Gentamycin	
Muscle 12	0.25	3		Ampicillin				Enrofloxacin			Tylosin			
Muscle 13	0.33	3	Amoxicillin				Danofloxacin		Norfloxacin			Sulfonamides		
Muscle 14	0.75	6	Amoxicillin	Ampicillin			Danofloxacin	Enrofloxacin	Norfloxacin	Oxytetracyclin	Tylosin	Sulfonamides		Florfenicol
Muscle 15	0.25	3						Enrofloxacin		Oxytetracyclin			Gentamycin	
Kidney 1	0.17	2			Cefotaxime	Ciprofloxacin								
Kidney 2	0.33	3	Amoxicillin	Ampicillin								Sulfonamides	Gentamicin	
Kidney 3	0.42	4	Amoxicillin	Ampicillin	Cefotaxime							Sulfonamides	Gentamicin	
Kidney 4	0.17	1	Amoxicillin	Ampicillin										
Kidney 5	0.42	4	Amoxicillin	Ampicillin	Cefotaxime							Sulfonamides	Gentamicin	
Kidney 6	0.42	5		Ampicillin,	Cefotaxime	Ciprofloxacin						Sulfonamides	Gentamicin	
Kidney 7	0.25	3		Ampicillin,								Sulfonamides	Gentamicin	
Kidney 8	0.17	1	Amoxicillin	Ampicillin										
Kidney 9	0.25	3	Amoxicillin		Cefotaxime	Ciprofloxacin								
Kidney 10	0.25	2	Amoxicillin	Ampicillin		Ciprofloxacin								
Kidney 11	0.33	4			Cefotaxime	Ciprofloxacin						Sulfonamides	Gentamicin	
Kidney 12	0.25	3	Amoxicillin		Cefotaxime	Ciprofloxacin								
Kidney 13	0.17	2			Cefotaxime	Ciprofloxacin								
Kidney 14	0.33	4	Amoxicillin		Cefotaxime							Sulfonamides	Gentamicin	
Kidney 15	0.17	2	Amoxicillin		Cefotaxime									
Total	No.		20	16	12	11	8	5	10	7	8	14	15	4
	Frequncy %		67	53	40	36.67	26.67	16.67	33.33	23.33	26.67	46.66	50	13.33

Ampicillin, danofloxacin, sulfonamides, and florfenicol residues in *LTL* samples did not exceed MRLs in comparison to reference Codex/NFSA and EU MRLs and were therefore approved for human consumption. However, with varying frequency, remaining screened antibiotic-residues surpassed MRL levels (Table 1). The Codex/NFSA and/or EU MRLs for amoxicillin, cefotaxime, ciprofloxacin, enrofloxacin, norfloxacin, oxytetracycline, Tylosin, and gentamycin residues were violated in 100%, 13.3%, 20.0%, 20.0%, 66.7%, 6.7%, 53.3%, and 33.3% of *LTL* samples, respectively, and were deemed dangerous for human consumption (Table 1).

Table 1. Range and mean levels of twelve antibiotic-residues in studied meat tissue (n=15) and their acceptability compared to Codex/NFSA and EU MRLs.

Residues	Range (µg/kg)		Mean ± SE		Unaccepted *LTL*		MRLs (µg/kg)	
	Minimum	Maximum			>MRL	*N* (%)	NFSA	EU
Amoxicillin	82.45	362.00	224.88	24.22	15.0	100	50	50
Ampicillin	4.42	18.44	9.75	1.33	<MRL	0	50	50
Cefotaxime	98.17	114.62	106.39	3.00	2.0	13.3	20	
Ciprofloxacin	75.59	179.77	121.81	11.45	3.0	20.0	100	150
Danofloxacin	5.78	40.99	22.60	3.42	<MRL	0	200	
Enrofloxacin	59.51	193.23	130.24	13.85	3.0	20.0		100
Norfloxacin	2.81	25.40	15.55	2.00	10.0	66.7		
Oxytetracyclin	48.22	314.45	169.56	21.05	1.0	6.7	200	100
Tylosin	120.80	403.26	302.16	27.49	8.0	53.3	100	100
Sulfonamides	3.54	30.41	16.87	2.34	<MRL	0	100	100
Gentamycin	56.18	389.49	211.93	34.60	5	33.3	100	50
Florfenicol	7.39	22.15	15.10	1.81	<MRL	0		200

>MRL, higher than maximum residue limit; NFSA, national Egyptian food safety organization; EU, European union; SEM, mean standard error.
It should be mentioned that samples are only deemed to have breached when they surpass the NFSA/MRLs (µg/kg). If NFSA/MRLs are not present, comparisons are made with the EU. Violated *LTL* frequency was calculated by dividing the number of samples that exceeded the MRLs by the total sample analyzed (*n* =15) and multiplying by 100.

Ampicillin (33.25 to 279.81 µg/kg) and gentamycin (14.56 to 323.29 µg/kg) residues in kidney samples did not exceed MRLs compared to reference Codex/NFSA and/or EU MRLs and were thus approved for human consumption (Table 2). While 53.3%, 80.0%, 13.3%, and 33.3% of kidney samples breached Codex/NFSA and/or EU MRLs for amoxicillin (26.16-351.22 µg/kg), cefotaxime (27.17-226.22 µg/kg), ciprofloxacin (30.48-279 µg/kg), and sulfonamides (19.31-266.83 µg/kg), rendering them hazardous for human consumption (Table 2).

Table 2. Range and mean levels of twelve antibiotic-residues in studied Kidney (*n* =15) and their acceptability compared to Codex/NFSA and EU MRLs.

Residues	Range (µg/kg)		Mean ± SEM		Violated *LTL*		MRLs (µg/kg)	
	Minimum	Maximum			>MRL	*N* (%)	NFSA	EU
Amoxicillin	26.16	351.22	149.66	25.97	8.0	53.3	50	50
Ampicillin	33.25	279.81	167.50	23.84	<MRL	0	50	50
Cefotaxime	27.17	226.22	139.74	15.37	12.0	80.0	free	free
Ciprofloxacin	30.48	279.00	172.41	22.31	2.0	13.3	200	150
Sulfonamides	19.31	266.83	136.37	22.23	5	33.3	100	100
Gentamicin	14.56	323.29	144.43	26.93	<MRL	0	5000	750

>MRL, higher than maximum residue limit; NFSA, national Egyptian food safety organization; EU, European union; SEM, mean standard error.
It should be mentioned that samples are only deemed to have breached when they surpass the NFSA/MRLs (µg/kg). If NFSA/MRLs are not present, comparisons are made with the EU. Violated *LTL* frequency was calculated by dividing the number of samples that exceeded the MRLs by the total sample analyzed (*n* =15) and multiplying by 100

4. DISCUSSION

The objective of the current study was to determine consumption acceptability concerning maximum residue limits (MRLs) approved by the National Egyptian Food Safety Organisation (NFSA) and to evaluate critical antibiotic-residue levels in raw meat of different origins, both native and imported, sold in Egyptian markets.

The European Medicines Agency (EMA) categorized antimicrobials into four groups, A through D, in December 2019 (European Medicines Committee, 2020). All four categories are considered critically important in the veterinary sector. Fortunately, none of the twelve antibiotic residues identified in the current study fall under Category A, which prohibits their use as veterinary medications in the EU, especially for animals that produce food. Cefotaxime, third-generation cephalosporins, and quinolones were identified in 40 and 70% of the samples tested, respectively.

These two highly significant (B category) antibiotics are used in human medicine, and their use in animals should be restricted to reduce the risk to the public's health. They should only be used in cases where no clinically useful antibiotics from Categories C or D are available. In human medicine, antibiotics in Category C have alternatives; however, for veterinary purposes, there are no substitutes in Category D, and they should only be considered if no antibiotics in Category D have the potential to be clinically effective. Of the Category C antibiotics detected in recently analyzed beef and kidney samples, aminoglycosides (such as gentamicin, 50%), amphenicols (such as florfenicol, 13%), and macrolides (such as tylosin, 27%). Category C antibiotics have alternatives in human medicine, but should only be utilized for veterinary purposes if none of the antibiotics in Category D are clinically effective. Aminopenicillins, including amoxicillin and ampicillin, tetracyclines such as oxytetracycline, and sulfonamides belong to Category D and should be used prudently as first-line therapies wherever possible in the veterinary sector (European Medicines Committee, 2020).

Ceftiofur is approved for use in a variety of production animals, but cefotaxime is not FDA-approved for use in animals, and administering cephalosporins illegally to food-producing animals in the United States violates FDA guidelines, hence, no withdrawal times have been defined for food-producing animals (Papich, 2016). Furthermore, the MRLs for cefotaxime were not disclosed by the NFSA or Codex standards, despite previous research in Europe claiming MRLs of 20 µg/kg. Consequently, forty percent of the meat and kidneys under study are unfit for human consumption based on cefotaxime limitations.

Quinolones, sometimes known as fluoroquinolones (ciprofloxacin, enrofloxacin, danofloxacin, and norfloxacin), are medically recognized as effective treatments and preventative measures for both humans and animals (Er-Demirhan et al., 2013; Ramatla et al., 2017). Enrofloxacin is a third-generation fluoroquinolone antibacterial drug specifically designed for use in veterinary medicine. According to reports, cattle can partially metabolize enrofloxacin to ciprofloxacin; therefore, the residues of both ciprofloxacin and enrofloxacin are evaluated as a combined amount (European Union and the Council of the EU, 2009). The codex and NFSA standards did not report the MRLs for the sum of ciprofloxacin and enrofloxacin (FAO and WHO, 2018). The EU was the only body to impose MRLs on kidney and muscle tissues, respectively, at 200 µg/kg and 100 µg/kg (European Union and the Council of the EU, 2009). Furthermore, none of the standardization organizations established Norfloxacin MRLs. In Ankara, Turkey, 57.7% (60/104) of beef samples tested positive for quinolones, with an average ELISA concentration of 6.64 ± 1.11 µg/kg (Er-Demirhan et al., 2013).

The sum of enrofloxacin and ciprofloxacin residues was identified in seventeen bovine muscles (17/90) and kidney tissues (17/94), with just two samples falling below the NFSA MRLs (100 and 200 µg/kg) (Myllyniemi et al., 2000). While oxytetracycline was confirmed positive in 26 bovine and porcine samples, two meat and eleven kidney samples did not exceed the MRL (200 and 1200 µg/kg) (Myllyniemi et al., 2000). In 50 chicken, 50 hog, and 50 beef raw meat muscle, liver, or kidney samples from South Africa, HPLC verified the presence of ciprofloxacin, sulphonamide, and tetracycline residues in 8.3, 88.8, and 14.6% of the samples, respectively. Ciprofloxacin and sulphonamide had the lowest and highest occurrences. In depth, ciprofloxacin was not found in either of the raw beef muscle or kidney samples. Tetracycline occurred in only 11.1% of the beef kidney samples (2/18), and sulphonamide was retrieved in 6.6% of the beef muscle (1/15) and 22.2 kidney samples (4/18). Furthermore, HPLC analysis revealed that the concentration ranges of sulphonamide and tetracycline in beef kidney samples were 26.9–82.1 µg/kg and 41.8–320.8 µg/kg, respectively. None of the beef muscle or kidney samples exceeded the relative codex antibiotic MRL (Ramatla et al., 2017). Quinolones (5%), notably enrofloxacin and its metabolite, ciprofloxacin, are the most commonly detected antibiotic residues in bovine samples (Carretero et al., 2008). Additionally, two (2.7%) bovine samples tested positive for sulfonamides (sulfamethoxypiridazine and sulfadiazine), tetracyclines (chlortetracycline), and macrolides (tylosin and tilmicosin). However, none of them exceeded the established MRLs (Carretero et al., 2008).

In Zambia, HPLC results revealed that 34.4% (77/224) and 17.4% (39/224) of beef samples tested positive for oxytetracycline (range: 27.26–481.61 ng/g, mean = 199.6 ± 46 ng/g) and sulfamethazine (range: 11.92–259.98 ng/g, mean = 86.5 ± 8.7 ng/g), respectively. Residues of oxytetracycline and sulphamethazine exceeding the Codex MRLs were estimated in approximately 45.5% and 12.8% of the samples, respectively. Compared to the European Union MRL, 76.6% and 33.3% of the samples had violative oxytetracycline and sulfamethazine residues, respectively (Nchima et al., 2017).

Tetracyclines (81.82%) had the highest detection frequency in cattle muscle samples, followed by fluoroquinolones (63.64%) and sulfonamides (54.55%), all of which were found at values ranging from ND to 424.40 µg/kg. In muscle samples from cattle, oxytetracycline was the antibiotic most often found, with a detection frequency of 77.27% and values ranging from ND to 16.64 µg/kg. Norfloxacin was detected in samples from southern Xinjiang, China, with a detection frequency of 18.18% (4/22) and at concentrations as high as 0.13 µg/kg (Zhang et al., 2021).

Fortunately, florfenicol (13%) was the lowest detected antibiotic class in the currently assessed sample. The absence of chloramphenicol, which was banned for veterinary use due to severe side effects, in any of the currently analyzed samples and the lowest prevalence of florfenicol (13%), may indicate that authorities have successfully managed to restrict the marketing and entry of banned antibiotics. Of the antibiotics in the amphenicol class (thiamphenicol, azidamfenicol, and chloramphenicol), florfenicol is the only one that was developed especially for use in veterinary medicine. This is because it tackles the shortcomings of chloramphenicol, an antibiotic that was banned because of the negative side effects it caused in people who consumed animal-derived food (Picco et al., 2001).

Previously, the HPLC approach revealed the presence of tylosin in 84 beef neck samples, ranging from 36 to 1209 µg/kg (Gürel Yücel et al., 2023). These findings revealed that not only is antibiotic abuse and unlawful usage prevalent, but also vital human antibiotics such as cefotaxime and norfloxacin are illegally utilized in veterinary settings (Sabeq et al., 2022). The current findings revealed the coexistence of up to 10 antibiotic residues in one sample, and all samples contained at least two residues, which was much higher than previous research that identified up to four antibiotics in 34.09% of the samples. Inadequate withdrawal periods before sale or inadequate post-medication standard control were the reasons for the high residue levels of veterinary-approved antibiotics (Zhang et al., 2021). Additional reasons are linked to the regional and national variations in antibiotic usage trends in agriculture. Global antibiotic consumption is anticipated to increase by 67% between 2010 and 2030 (Patel et al., 2020). Antibiotics that are illegal in other nations—including industrialized nations—are still widely utilized in the majority of developing nations (Manyi-Loh et al., 2018). However, because antibiotics can be purchased illegally, through unregulated supply chains, and without a prescription, their widespread abuse and misuse have a significant impact on the consumption profiles of antibiotics in poor nations (Sabeq et al., 2022). Abuse in prophylactic and therapeutic use (overdosing, noncompliance with the withdrawal period), the use of contaminated water, or improper disposal of contaminated animal dung (improper recycling procedures) are all potential major sources of antibiotics in meat. These practices can cause antibiotics to spread throughout the food chain (Ghimpețeanu et al., 2022).

Prolonged exposure to antibiotic residues, particularly in children, immunocompromised individuals, has been associated with immune system impairment, intestinal flora destruction-related digestive issues, kidney problems, carcinogenic consequences, and antibiotic resistance (Ghimpețeanu et al., 2022). Therefore, the World Health Organization (WHO) strongly advises against using any antibiotics, including those used for growth promotion and disease prevention without a diagnosis, in healthy food-producing animals. The overall goal is to encourage the cautious use of antibiotics to slow down antimicrobial resistance and maintain antibiotics' usefulness in medicine (World Health Organization, 2017). This led several developed nations to enact and enforce legislation aimed at reducing the overuse of antibiotics as a preventative measure or as a tool for animal growth (Ghimpețeanu et al., 2022). Animal products must meet MRL requirements while being imported or exported. The lack of global standardization for MRL standards and withdrawal periods, along with MRL violations,

could pose challenges to international food trade between countries (Canton et al., 2021).

5. CONCLUSIONS

The most often identified class in 93.33% of the samples was residues of β-Lactam antibiotics, with fluoroquinolones coming in second at 70%. While only five antibiotic residues were identified in kidney samples, twelve were confirmed in *LTL*. The percentage of breached and hazardous LTL samples varied from 13.3% to 100% with NFSA MRLs. Nevertheless, the range of kidney samples violated was 13.3% to 80.0%, indicating that they were not suitable for human consumption. Multiple-residues index, violation rates, and the finding of very important antibiotics in recent samples all point to widespread abuse and misuse of antibiotics in the Egyptian veterinary sector. The present research suggests that the NFSA should activate stronger punitive measures to ensure food suppliers and business operators fulfil their pledge and apply regulatory action to samples that have been violated. To survey meat samples and stop the spread of high MRL violation rates, it is necessary for NFSA to perform wide-ranging surveys and/or equip governmental meat inspection authority with sophisticated instruments.

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