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Safety evaluation of some retailed chicken meat products with special reference to their antibiotic residue in Egypt

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Abstract

The demand for poultry meat and eggs has expanded in Egypt, which has leading to the intensification of agricultural practices and the usage of antimicrobials, especially tetracycline and sulfonamides. According to the FAO/WHO Codex Alimentarius committee, consumer, health was put at risk when antibacterial residue concentrations increase in foods to the maximum residual level. Therefore, this study has been conducted for determination of tetracycline and sulfadimidine residues in chicken meat and giblets. Therefore, 90 random samples of chicken meat, liver and gizzard (30 of each) were collected from Benha city, Qalubiya governorate, Egypt, , to estimate their contents of such antibacterial residues using high performance liquid chromatography. The obtained results indicated that 48.8% of the examined samples were positive for antibiotic residue. the examined samples 20, 33.3 and 40% of the chicken meat, gizzard and liver samples were positive for tetracycline residues with average concentrations of 116.9, 488.6 and 609.5 ppb; While, at the same time, sulfadimidine was detected in 13.3, 16.7 and 23.3% of the same examined samples mean values of 59.2, 97.6 and 144.9 ppb, respectively. Referring to the obtained results, the liver samples showed higher antibacterial residual rates, followed by gizzard and meat samples, respectively. Furthermore, conducting a strict monitoring measure over poultry rearing farms and retailers is highly recommended to avoid the emergence of public health hazards concerning these antibiotic residues.

Keywords: Poultry meat, Antibiotic residue, sulfadimidine, tetracycline, Egypt.

1. Introduction

As a significant source of animal protein, chicken is in high demand worldwide, leading the poultry sector to rise steadily. poultry farms frequently use growth promoters, such as antimicrobials, to increase chicken output and prevent illnesses when demand exceeds supply **(Mohammadzadeh *et al.*, 2022).**

Geidam et al. (2009) state that antibiotics are naturally occurring, semisynthetic and synthetic antibacterial compounds can be used topically or orally, parentally. For example, sulfonamides, Tetracyclines, and Fluoroquinolone antibiotics have been used for many years in chicken farming to treat bacterial infections, promote growth, and prevent illnesses **(Olatoye and Ehinmowo, 2010).**

Antibiotics have been utilized extensively in animal husbandry for preventative and therapeutic purposes. Accurately 30% of antibiotics used in Europe are for veterinary use, with the bulk of these antibiotics being given to poultry and pig animals for therapeutic purposes **(Van et al., 2020).**

Antibiotics are important for treat and controlling infectious illnesses in chicken productions . They are also used illegally as feed additives to increase animal productivity and development **(Graham *et al.*, 2007).** Antibiotics can be misused and, increases the likelihood that their residues will be found in the chicken's edible tissues and induce toxins and allergies in hypersensitive consumers **(Canton *et al.*, 2021).**

Because of its broad spectrum of antibacterial activity against bacteria in environments, tetracycline is important in veterinary therapy **(Grossman, 2016)**, Additionally ,Sulfonamides are also often used in chicken production as therapy and for preventative measures against a variety of illnesses as coryza, fowl typhoid, pullorum disease, and coccidiosis **(Al-Nazawi and Homeida, 2005; Khalafalla *et al.*, 2022)**.

Sulfonamides were been utilized extensively due to their low cost, which may led to an increase in bacterial species resistant such drug. In addition, sulfonamide residues will found in retail chicken withdrawal times are not allowed on the farm or if these drugs are administered indecently **(Cheong *et al.*, 2010).**

The abuse of antibiotics may result in the emergence of resistant bacterial strains, decreasing the effectiveness of antibiotics used to treat animals, causing treatment failure in animals, and adversely impacting animal welfare **(Manyi-Loh *et al.*, 2018)**. A antibiotic resistance is a global issue that warrants serious attention. As the antibiotic residues had been noticed as potential for public health risks to human population, the risk of human diseases might acquire antibiotic-resistant pathogens from animal flora **(Arsène *et al.*, 2022)**. The spread of germs that are resistant to antibiotics across the food chain poses another concern **(Larsson and Flach, 2022).**

Numerous research investigations have reported finding antibiotic residues in samples of chicken meat. Broiler meat and liver samples sold in Tehran were found to have antibiotic residues, with a high occurrence rate of sulfonamide residues (31.1% and 34.4%, respectively) **(Bani-Asadi *et al.*, 2021).** Further, **Khalafalla *et al.* (2022)** reported finding antibiotic residues in 60% of the chicken meat samples they analyzed in Egypt, where 20, 60, and 50% of domestic grill carcasses they looked at, respectively, surpassed the MRLs for oxytetracycline, enrofloxacin, and sulfadimidine.

In Egypt, the production of broilers contributes significantly to food security. According to figures from the Egyptian Ministry of Agriculture, an estimated 1.4 billion hens generate 1.59 million tons of meat annually.

Exports are little as most of Arab states meet a large amount of their own needs through production, while most of imports come from nations like Brazil and India where production costs are significantly cheaper than in Egypt **(USDA, 2022)**.

Accordingly, the purpose of present study aims is to monitor the incidence of antibacterial residues in chicken meat and some edible offal (liver and gizzard) collected from different poultry slaughter shopes in Benha city, Qalubiya governorate, Egypt.

2. Materials & Methods

2.1. Collection of samples:

Nighty random samples of chicken meat, gizzard and liver (30 of each) were collected from different poultry slaughter shops in Benha city, Qalubiya governorate, Egypt. The collected samples were been transferred to the laboratory for determination of their residues of two of the most used antibacterial in poultry production represented by tetracycline and sulfadimidine.

2.2. Determination of antibacterial residues by HPLC:

- **HPLC conditions**

HPLC used for determination was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150× 4.6 mm) column (Shimadzu, Kyoto, Japan). Data were integrated and recorded using the Chemstation Software program.

2.2.1. Determination of tetracycline residues (Samanidou *et al.*, 2007)

2.2.1.1. Extraction of the drug from the sample

Two grams of each sample were finely chopped and minced using a Sartorius mincer followed by mixing with 0.1 g of citric acid + 1 ml of nitric acid (30%) + 4 ml methanol and 1 ml deionized water in a blender for 2 minutes. The suspension with solid particles has been kept in an ultrasonic bath for 15 minute and centrifuged for 10 minute at 5300 RPM. After filtering through a 0.45 µm nylon filter, 20 µl of solution was injected into HPLC for analysis.

2.2.1.2. Calibration curve

The curve was prepared by using concentrations of 10, 20, 30, 40, 50 and 60 µg/L of tetracycline in eluent. The detection limit for tetracycline was 0.01 ppm and the retention time was 4.4 minutes.

Table (1): Conditions of HPLC for determination of Tetracycline:

Parameter	Tetracycline
Injection volume.	50 µl
Flow rate.	1ml/min
Wavelength.	312 nm
Column temperature.	36ºC
Stop time.	20 min
Post time .	6 min
Mobile phase.	0.05m phosphoric acid: acetonitrile= 50:50 (PH 2.0 V/V)

2.2.1.3. Quantization of the amount of antibacterial residue by detecting peak height or peak area level:

The samples were prepared by adding 500 µl spiking solution to 10 gm (tetracycline) as a control sample to obtain spiking levels equal to 1 µg/ml. Accordingly, the retention time was estimated by which the concentrations of these antibiotics could be measured.

2.2.1.4. The calculation of the exact concentration of antibiotic residue in the examined sample in correlation to the standard curve

A standard calibration curve was applied using 5 known standard solutions of tetracycline : 100 ppb, 200 ppb, 300 ppb, 400 ppb and 500 ppb tetracycline.

2.2.2. Determination of sulfadimidine residues (Patyra *et al.*, 2018)

Sulfadimidine was obtained from Sigma Aldrich (St. Louis, MO, USA). Sulfadimidine residues have been extracted with the using of a methanol and acetonitrile mixture (50:50 v/v).

2.2.2.1. Sample Preparation

5 gm of each sample were transferred to 50 mL polypropylene centrifuge tubes and prepared by adding appropriate sulfonamide working solutions. After vortexing for 30 seconds, the samples have been kept at room temperature for 60 minute. Then, 20 mL of extraction mixture consisting of ethyl acetate/methanol/acetonitrile (50:25:25 v/v/v) was added and the content of the tubes was shaken at room temperature for 30 minutes on a horizontal shaker and centrifuged at 3500 rpm for 10 minutes at 20◦C.

2.2.3. Clean-Up:

The Solid-Phase Extraction (SPE) apparatus and Strata-SCX cartridges (500 mg, 3 mL) were used. Before to sample loading, the cartridges have been represented by 5 mL of 40% acetic acid in acetonitrile. After percolation, washed with 2.5 mL of acetone, 2.5 mL methanol and 2.5 mL of acetonitrile. The analysts were discarded by 2×2.5 mL of mixture of 2% ammonium solution in acetonitrile. The extract was evaporated to dryness under a nitrogen stream at 40◦C±5 ◦C.

2.2.4. Chromatography:

The gradient was applied with 0.08% acetic acid in Milli-Q water (phase A), acetonitrile (phase B), and methanol (phase C). The gradient is shown in Table 2. The flow rate was 0.6 mL/min, and the injection volume was 40 µL. The column temperature was 25 ◦C. All analyzed sulfonamides' excitation and emission wavelengths were 405 and 495 nm, respectively

Table (B) the gradient elution of sulfonamides with HPLC detection.:

Time (minute)	Acetic Acid in Milli-Q Water (A) (%)	Acetonitrile (B) (%)	Methanol (C) (%)
0-10	48	10	42
10-15	41	10	49
15-17	41	10	49
17-20	18	40	42
20-22	48	10	42
22-27	48	10	42

2.6. Statistical Analysis

The data collected were analyzed using Duncan posthoc by SPSS® version 16.0 and the statistical probability (p value) according to **Feldman *et al.* (2003)**.

3. Results

Referring to the recorded data in **Tables (1)**, 48.8% of the examined samples were positive for antibiotic residue, represented by 31.1% and 17.8% for tetracycline and sulfonamide residue, respectively.

Table (1) Incidence of antibiotic residues in the examined samples of chicken meat and offal (n=90)

Antibiotic	Positive samples		Negative samples	
	No.	%	No.	%
Tetracycline	28	31.1	62	68.9
Sulfonamide	16	17.8	74	82.2
Total (90)	44	48.8	46	51.2

Tables (2 and 3) showed that liver samples had the highest residual levels with an incidence of 40 and 23.3%, followed by gizzard and meat samples concerning tetracycline and sulfadimidine residues, respectively.

Table (2) Incidence and fitness of chicken samples based on their tetracycline residues (n=90).

Chicken samples	Tetracycline		MRL (ppb)	Fit samples	
	No.	%*		No.	%*
Meat	6	20	200	28	93.3
Gizzard	10	33.3	600	26	86.7
Liver	12	40	600	23	76.7
Total (90)	28	31.1**		77	85.6**

* percent concerning to the number of samples of each product (30).
** percent concerning to the total number of the examined samples (90).
MRL. Maximum Residual Limit according to NFSA (2020).

Table (3) Incidence and fitness of chicken samples based on the sulfadimidine residues (n=90).

Chicken samples	Tetracycline		MRL (ppb)	Fit samples	
	No.	%*		No.	%*
Meat	4	13.3	100	29	96.7
Gizzard	5	16.7	100	27	90
Liver	7	23.3	100	26	86.7
Total (90)	16	17.8**		82	91.1**

* percent concerning to the number of samples of each product (30).
**- percent concerning to the total number of the examined samples (90).
MRL. Maximum Residual Limit according to NFSA (2020).

Tetracycline and sulfadimidine were been detected in a total of 31.1 and 17.8%, respectively; where tetracycline was detected in significant higher concentrations ($p \geq 0.05$) than sulfadimidine in the same examined samples as it was detected with the mean values (ppb) of 116.9, 488.6 and 609.5 in the examined meat, gizzard and liver samples, respectively; while sulfadimidine was detected in the mean values (ppb) of 59.2, 97.6 and 144.9 in the same samples, respectively **(Fig. 1)**.

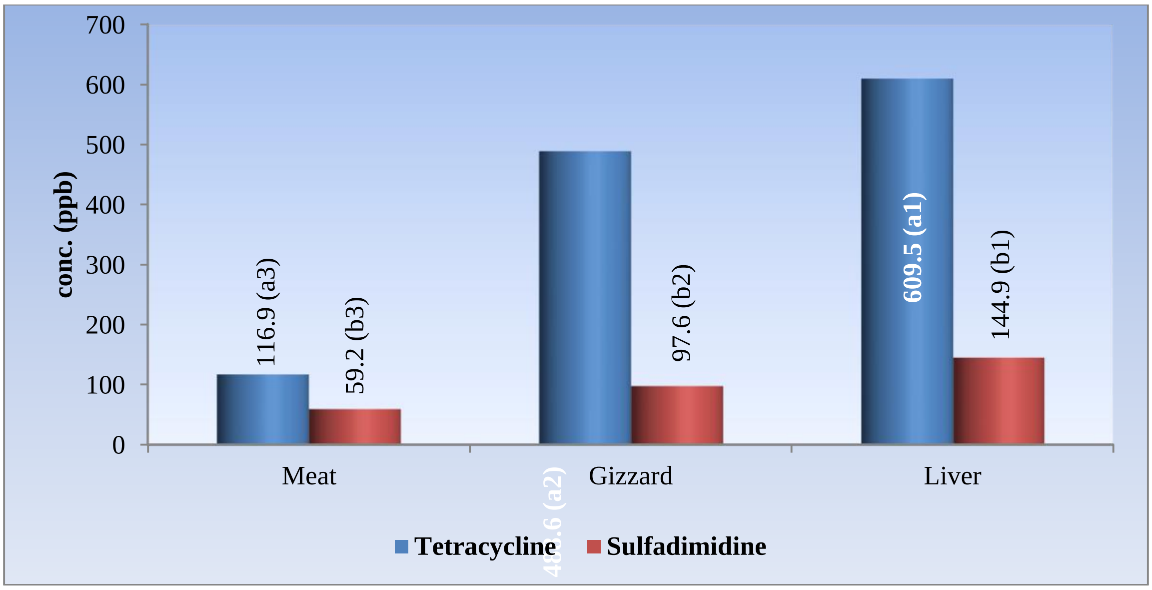

Fig. (1) Mean concentrations (ppb) of tetracycline and sulfadimidine residues in the examined samples (n=30).
a, b: Means with different superscript letters significantly differ within the same product sample (P<0.05).
1, 2: Means with different superscript numbers significantly differ within the same antibiotic (P<0.05).

4. Discussion

The chicken meat and edible giblets are not only delectable, simple to cook, and the least expensive of all meats, but they also offer a special, well-balanced supply of proteins with vital amino acids, minerals and vitamins, which need (**Henry *et al.* (2019),** a high regular percentage of the Egyptian diet's poultry products come from chicken flesh and edible giblets.

In chicken production, antibiotics are mostly used for nutritional, prophylactic and growth-promoting purposes **(Jinap *et al.*, 2010).** There are possible health risks for consumers as a result of this widespread use of antibiotics in the chicken business, including carcinogenicity , mutagenicity, also bone marrow toxicity , in addition to may be allergies **(Nisha, 2008),** as well as the appearance of a resistance strains of pathogenic bacteria **(Hussein and Khalil, 2013).**

While investigating the antibiotic residues in the examined samples, the consequences showed that a total of 48.8% of the examined samples had antibiotic residues. This result was higher those obtained by **Gwachha (2017),** who recorded that the presence of antimicrobial residues in meat of broilers which has been marketed at Kathmandu Valley, Nepal was 33.03% and **Pantha *et al.* (2019),** found that 30.81% of the examined chicken samples contained antibiotic residues; also, it was nearly similar to those recorded by **Tajick and Shohreh (2006)** who found antibiotic residues in 50% of the samples; in add to it has been lower than those been recorded by **Kamel *et al.* (2022)** who was found that 71.7% of their examined samples have been positive for antibiotic residue. Furthermore, The obtained results were lower than those detected by **Salama *et al.* (2011),** found that 44% of the examined meat samples contained tetracycline residue

Tetracycline & sulfonamide are common broad-spectrum antibiotics used on a large scale in broiler production to prevent or treat many bacterial infectious diseases **(Sarkar *et al.*, 2023)**.

Tetracycline is one of the most often recommended antibiotics due to its broad-spectrum bacteriostatic activity due to its low cost and. A naturally occurring tetracycline molecule is synthesized or obtained from the fungus *Streptomyces rimosus*. Due to its high water solubility, it is poorly metabolized by the body and excreted in its original form **(Slana and Dolenc, 2013).** Tetracycline antibiotic over dose or amounts of withdrawal in poultry production led to residues that may harm human health **(Centikaya *et al.*, 2012).**

Out of the examined samples, tetracycline was detected in 20, 33.3 and 40% of the examined chicken meat, gizzard and liver samples, with the mean concentrations (ppb) of 116.9, 488.6 and 609.5, respectively. In regard to the Egyptian standards, 85.6% of the examined samples were fit for human consumption, represented by 93.3, 86.7 and 76.7% acceptance ratio for meat, gizzard and liver samples, respectively **(Table 2 and Fig. 1).**

Sulfadimidine was detected in 13.3, 16.7 and 23.3% of chicken meat samples, gizzard & liver samples, with mean concentrations (ppb) of 59.2, 97.6 and 144.9, respectively. Regarding the Egyptian standards, 91.1% of the examined samples were fit for human consumption, represented by 96.7, 90.0 and 86.7% acceptance ratio for meat, gizzard and liver samples, respectively **(Table, 3 and Fig. 1).**

The obtained results lower than those recorded by **Mehtabuddin *et al.* (2012)** who noted that 43% of chicken meat samples contained sulfadimidine residues , also lower than the results which been recorded by **Aman *et al.* (2017)** who recorded that the mean tetracycline values in the examined meat samples were 139.26±32.21 ppb, and **Pantha *et al.* (2019)** who found that the concentrations of tetracycline and sulfonamide were 33.33% and 41.67% respectively., **El-Ahwal *et al.* (1998)** found that all examined chicken meat samples were free from these residues**, Bani-asadi *et al.* (2021)** did not detect tetracycline in the examined chicken meat and liver samples; while sulfadimidine was detected in 31.1% and 34.4% of the examined meat and liver samples with a mean concentration of 6.05 and 9.26 (ppb), respectively. On the other side, **Ulomi *et al.* (2022)** detected tetracycline and sulfadimidine in all of the examined chicken liver samples (100%) collected from sold in Kinondoni and Ilala Municipalities, Dar es Salaam, Tanzania.

Sulfonamides are synthetic antibiotics that also have a broad spectrum of activity against the majority of Gram-positive and many Gram-negative organisms. Veterinarians frequently provide them to hens for medicinal, preventative, or growth-promoting reasons in order to stop bacterial development in animal production **(Bani-Asadi *et al.*, 2021).**

The variations of the obtained results of antibacterial residues between the examined samples may be attributed to different collection sites, the formula of feed components, the use of antibiotics prior to the marketing and advertising of broilers, as well as sensitivity and specificity of the used kits, methodology, other barriers, and violations in the use of antibiotics.

5. Conclusions

The results allow concluding that the examined broiler meat contained antibiotic residues (tetracycline and sulfonamides) at distinctive ranges constituting a public health hazards and give attention to reducing such antibacterial for long time or high dose in the animal feed or water as therapy. Also, withdrawal time should be respected when using antibiotics and no send the flock for the market before complete the withdrawing period after the last dose according to manufacturing instruction of the producers of this antibiotics.

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