Qualitative and Quantitative Evaluation of Antibiotic Residues in Broiler Meat

Aya Salama Kamel[1], Ali Meawad Ahmed[1], Rania Helmy Abdou [2,] and Heba M. Shaheen[1]*

[1]Dept. of Food Hygiene, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

[2] Dept. of Forensic Medicine & Toxicology

[*]Corresponding author: Email: hebashaheen@ymail.com, Mobile No.: 01011433916

Abstract

Antibiotic concentrations in broiler meat are of exceptional health difficulty to human due to its dangers on the purchaser health. This learn about supposed to display the antibiotic residues in 60 frozen broiler samples accumulated from unique hen stores and retail markets in Ismailia Governorate by the use of High-Performance Liquid Chromatography. The outcomes confirmed that 71.7% of the examined samples had been containing at least one antibiotic residue whilst 28.3% had been free from any antibiotic residue. The incidence of tetracycline and sulfonamide in the evaluated samples were 38(88.4%) and 28(65.1%) respectively. The imply awareness values of chlorotetracycline (CTC), doxycycline (DOC), oxytetracycline (OTC) and tetracycline (TTC) in chicken samples were 120.1±30, 156.12±40.86, 115.09 ±10.68 and 139.26±32.21 µg/Kg respectively. While for Sulfonamides they had been 64.16±12.41µg/kg; 75.54±15.64µg/kg; 60.75±12.34 µg/kg and 79.34±24.6 µg/kg for Sulphadiazine, Sulfadimidine, Sulfamethazinee and Sulfaquioxaline residues respectively. Based on the national and global regulations, the received consequences published that the awareness of the detected antibiotics had been exceeding the recommended most residue limit in most broilers is extraordinary hazard on the human fitness if this antibiotic overuse continues.

Keywords: Antibiotic, residues, Tetracycline, Sulfonamide, Broilers

Introduction

Broiler manufacturing is amongst the most unexpectedly upward industries around the globe, where its meat affords a well-balanced source of proteins with imperative

amino acids, vitamins and minerals required by using all a while in addition to its correct style and the most inexpensive price of all meats.

Antibiotics have a wide use as therapeutic, growth promoting and prophylactic agents in chicken production ***(Donoghue, 2003 and Jinap et al., 2010).*** Sometimes they are used illegally as feed supplements to stimulate growth and productivity ***(Laxminarayan et al., 2013).*** The broad use of antibiotics and their incorrect application in hen enterprise incorporates the risk of presence of their residues in fowl tissues main to a massive fitness hazards for the customers including; mutagenicity, carcinogenicity, toxicity and hypersensitivity ***(Nisha, 2008)*** in addition to appearance of resistant pathogenic strains of bacteria ***(Hussein and Khalil, 2013)*** which results in reducing the efficiency of antibiotics used for animal treatment, leading to the treatment failure of livestock ***(Laxminarayan et al., 2013).***

The extend of antibiotic resistance is a crisis of most important concern globally which considered as public health hazards ***(Haller et al., 2002),*** where there is a high risk comes from the transfer of antibiotic-resistant bacterial strains through the food chain ***(Barton, 2007).*** To avoid these hazards, the maximum residues limits (MRLs) of antibiotics in foodstuffs of animal origin, have been recommended by means of the European Commission and listed in the Commission Regulation ***(EU 37/2010)***.

Antibiotics as Tetracycline (TCs) and Sulfonamides (SAs) are delivered in veterinary medicinal drug for greater than 40 years in the past which are wide spectrum antibacterial capsules active against each most sorts of bacteria. They nevertheless the most often used antibiotics in the broiler manufacturing system for therapeutic, prophylactic, or growth-promoting purposes. They are used to deal with many diseases induced by using bacteria and sure different microorganisms as these of digestive and respiratory tracts ***(Wasch et al., 1998).*** Also, they are positive in opposition to some pathogenic agents which often unaffected through other antibiotics e.g. Rickettsia, *Mycoplasma pneumonia*, *Chlamydia spp*. and *Legionella spp* ***(Al-Ghamdi et al., 2000).*** The most frequent TCs used for animal's therapy are tetracycline (TC), Oxytetracycline (OTC), Chlortetracycline (CTC) and Doxycycline (DC). In Egypt, TC compounds are the most well-known antibiotics used in broilers farms due to their highly low cost,

handy accessibility and availability. However, its overuse and inadequate lengths of their withdrawal length lead to the presence of high awareness degree of this antibiotic in broiler meat that may purpose chance to human fitness **(Centikaya *et al.*, 2012)**.

High overall performance liquid chromatography (HPLC) which used in the present study was tasked for measuring antibiotic residues in broiler meat. Antibiotics attention in wholesome safe meals should be below the maximum residual limits (MRL) recommended by the global Codex Alimentarius Commission (CAC) and Egyptian Organization of Standardization (EOS); consequently, this find out aimed to make sure meals protection by doing a reliable screening evaluation to decide the concentration degree of common veterinary antibiotics in broiler meat.

Materials and Methods

1-Collection of samples: sixty frozen broiler samples were collected from poultry shops and retail markets in Ismailia Governorate, Egypt. The collected samples were packed in a sterile impermeable polyethylene bags, stored sealed and cooled in an insulated box contained beaten ice and then transferred to the laboratory of the Faculty of Vet. Medicine, Suez Canal University, beneath a septic condition, barring undue delay.

2-Four plates Method

This is a qualitative method which has the capacity to discover the presence of antibiotics whose ranges are above the endorsed maximum residues level. Broiler samples previously thawed had been positioned in replica on the surface of four plates cast (Test agar CONDA PRONADISA, Spain) in Petri dishes. Then, plates have been inoculated with Bacillus subtilis BGA in specific pH (6, 7.2). Trimethoprim is covered into the medium at pH 7.2 in arrange to extend the compassion of detection of the sulfonamide residues. Chicken samples with following dimension: (8 mm of diameter against two mm of thick) were positioned on the agar surface.

3- Determination of Antibiotics Residues: determination of tetracycline and sulfonamide concentrations in the examined samples was once utilized by way of the use of High-Performance Liquid Chromatography (HPLC) technique.

3.1. Tetracycline detection: -

Extraction: Extraction method related to a method described by ***Şenyuva et al., 2000***

Chromatographic state:

Mobile phase was distal water / acetonitrile (85:15 v/v) Column (C18 – 150 x 4.6 mm –5 µm), Temp. of column 25ºC, Flow rate 1.5 ml/min. Fluorescence finding wave length 360 nm. Detection time (4-6 min), Injected volume: 25µl.

3.2. Sulfonamide detection: -

Extraction: Extraction method related to a method described by **Biswas *et al*., (2007)**.

Chromatographic condition

Analysis of the sulfonamide standards and extracted samples have been carried out the use of a HPLC device geared up with HPLC column oven L- 7300, detector L-7400, auto sampler L-7200, pump L- 7100, vacuum degasser L-76610, interface module D- 7000 and HSM software (Hitachi D-7000 series, Japan).

4- Statistical analysis:

All values are presented as means ± standard error. The antibiotic concentrations were expressed as part per million (ppm). The statistical analysis for this study was performed using **(SPSS 16, 2007)**.

#	Microorganism Test	Medium Ph	Temp. incubation	Antibiotics Families	Antibiotic types
1	Bacillus subtilis	6.0	30	Tetracyclines	Penicillin G
2	Bacillus subtilis	7.2	30	Sulfonamides	Sulfadimerazine and trimethoprim

Results and Discussion

Tetracycline and sulfonamide are common broad-spectrum antibiotics used on a big scale in broiler manufacturing for preventing and treating most bacterial infectious illnesses.

This research was designed to consider the antibiotic attention degree in frozen broiler meat marketed in Ismailia Governorate, Egypt. While assessing the antibiotic residues in the examined samples (Table 1), the consequences showed that 71.7% of samples have been contaminated with at least one residue whilst 28.3% have been free from any antibiotic residue. This result was higher than this obtained by ***Pantha et al., (2019)*** who found that only 30.81% of the examined samples containing antibiotic residues also it was higher than this given by ***Tajick and Shohreh (2006)*** in which antibiotic residues were found in 50% of the samples. Similarly, according to ***Gwachha (2017)*** presence of antimicrobial residues in meat of broiler marketed at Kathmandu valley was 33.03% and ***Abdelmoaty (2015)*** who found that antibiotic residues in fresh samples of broiler meat in Egypt was 34%. This version may

be as a result of the difference in sample size, collection sites, use of feed components and the use of antimicrobials before advertising and marketing of broiler besides emphasizing in their withdrawal period, sensitivity and specificity of the kits used, methodology and different obstacles as nicely as violation in use of antibiotics

\Out of the contaminated samples, Tetracycline (TCs) was detected in 88.4% and not detected in 11.6% of the samples, while sulfonamides (SAs) was detected in 65.1% and not detected in 34.9% of the samples as shown in (Table 2).

These results were greater than those detected by ***Bani-Asadi et al. (2021)*** who found that 28 out of 90 (31.11%) raw chicken meat and by ***Salama et al. (2011)*** who found that only (44%) of the contaminated samples contained TC residues, ***El-Ahwal et al. (1998)*** who found that all examined chicken meat samples were free from these residues and ***Pantha et al. (2019)*** who found that The concentrations of tetracycline and sulfonamide were 33.33% and 41.67% respectively. Also lower result was reported by ***Mehtabuddin et al. (2012)*** who noted that 43% of chicken meat samples contained SAs residues also ***Jammoul and El Darra (2019)*** noted that sulfonamides were the family less detected than the other families. While they were lower than those obtained by ***Aman et al. (2017)*** and those reported by ***Karmy (2001)*** who found that all samples of broiler meat collected from Assuit markets were positive for OTC residues. Nearly similar results were reported by ***Jammoul and El Darra (2019).*** This find out about showed the perfect residue degree used to be for tetracycline followed with the aid of sulfonamide. This high stage of tetracycline might also be due to the universal use of this crew for remedy of diseases and as feed additives.

A. Tetracycline

Tetracyclines are broad-spectrum antibiotics widely used in modern animal husbandry for treatment of microbial diseases, and also improve food quality production.

Results given in Table (3) printed that out of the 38 samples that had been contaminated with tetracycline residues, 22 (57.9%) was contaminated with Chlortetracycline, whilst 35(92.11%), 17(44.74%) and 28(73.68%) was contaminated with Deoxycycline, Oxytetracycline and tetracycline residues respectively. These results were higher than those obtained by ***Aman et al., (2017)***. The mean tetracycline values in the examined samples were 120.1±30, 156.12±40.86,

115.09±10.68 and 139.26±32.21 µg/Kg for Chlortetracycline, Deoxycycline, Oxytetracycline and tetracycline residues respectively while the minimum - maximum values ranged from 92.52-232.96 µg/kg; 145.21-266.78 µg/kg; 92.85-128.73 µg/kg and 131.71-248.63 µg/kg for Chlortetracycline, Deoxycycline, Oxytetracycline and tetracycline residues respectively (Table 4). Lower results were obtained by ***Abou-Raya et al. (2013)*** who recorded The limits of detection (LOD) for chicken samples were averaging 4.4, 5, 13 and 10 ng/g for OTC, TTC, CTC and DOC, respectively and by ***Aman et al. (2017)*** who determined that The residue concentrations of OTC detected in chicken meat samples was ranged from 0.08 to 0.35 µg/g with a mean value of 0.1296 ± 0.0532 µg/g and that of TC have been detected in breast muscle have been ranged from 0.13 to 0.80 µg/g with a mean value of 0.3905 ± 0.2633 µg/g whilst ***Bani-Asadi et al. (2021)*** found that There were no detectable concentrations of tetracyclines in all studied samples.

Based on the countrywide and global regulations, consequences given in table (5) confirmed that eight (36.36%) and seventeen (100%) of samples contaminated with Chlortetracycline and oxytetracycline respectively have been inside the Egyptian permissible limits for tetracycline and EU Council Directive 96/23/EC which is (100 µg/Kg), while 14 (64.4%) and 0% of the samples respectively exceed the maximum residue limit (MRL) for tetracycline as mounted via ***(EOS, 7136, 2010).*** On the other hand all samples contaminated with Doxycycline and tetracycline their concentrations exceed the maximum residue limit (MRL) for tetracycline as established by ***(EOS, 7136, 2010).*** Different result was reported by **Al-*Bahry et al. (2013*)** who observed that the values of the 4 kinds of tetracyclines (TC, CTC, DTC and OTC) residues existing in the examined hen samples had been beneath endorsed most residue limit (MRL) while nearly similar results had been got by ***Nhiem et al. (2006)*** in Vietnam who discovered that 5.5% of examined animal tissue were contaminated with tetracycline residue which exceeded MRL values Tetracyclines may also lead to risky residue in animal-derived food products ***(Pena et al., 2007)***, which consequences in viable fitness risks for shoppers which include; mutagenicity, carcinogenicity, hypersensitive reaction and bone marrow toxicity ***(Nisha, 2008)*** in addition to appearance of resistant strains of pathogenic bacteria.

B. Sulfonamides

Sulfonamides (SAs) are artificial huge spectrum antibiotics which are in many instances used in broiler manufacturing for therapy of many kinds of infectious diseases of digestive and respiratory tracts and halt the boom of bacteria in animal production.

Results given in Table (3) confirmed that out of the 28 samples that have been contaminated with sulfonamides residues, 18 (64.29%) was contaminated with Sulfadiazine, while 28(100%), 5(17.8%) and 28(100%) was once contaminated with Sulfadimidine, Sulfamethazine and Sulfaquioxaline residues respectively. The suggest Sulfonamides values in the examined samples had been 46.16±12.41µg/kg; 75.54±15.64µg/kg; 60.75±12.34 µg/kg and 79.34±24.6 µg/kg for Sulphadiazine, Sulfadimidine, Sulfamethazinee and Sulfaquioxaline residues respectively while the minimum - maximum values were ranged from 17.35-90.11 µg/kg; 18.32-120.16 µg/kg; 14.75-88.18 µg/kg and 19.5-110.32 µg/kg for Sulphadiazine, Sulfadimidine, Sulfamethazinee and Sulfaquioxaline residues respectively (Table 4)

Based on the national and worldwide regulations, outcomes given in table (6) showed that only three (10.7%) and 7(25%) of the samples contaminated with Sulfadimidine and Sulfaquioxaline respectivelty were inside the Egyptian permissible limits for Sufonamides and EU Council Directive 96/23/EC which is (100 µg/Kg), while 25 (89.29%) and 21(75%) of the samples respectively exceed the maximum residue restriction (MRL) for tetracycline as determined by **E.O.S., no. 7136, (2010)**. On the other hand, all samples contaminated with Sulfadiazine and Sulfamethazine their concentrations have been inside the Egyptian permissible limits for Sulfonamides and EU Council Directive 96/23/EC. These bought effects have been increased than these suggested by means of ***Mehtabuddin et al. (2012)*** who discovered that the detectable stages of sulfonamide residues in 23% of rooster samples were exceeding the recommended most residual stage and have been unfit for human consumption also extraordinary outcomes had been stated with the aid of ***Jammoul and El Darra (2019)*** who located that all the mean values for the sulfonamides were acceptable and inside the MRL (100 µg/kg)

according to the ***European Commission EC (2010).***

The huge use of SAs as a end result of their low price in broiler production farms has resulted in springing up of many sulfonamide resistant lines of micro organism in addition to high attention degree of SAs in the marketed broiler meat if insufficient withdrawal instances have now not been found or if these pills have been indecently administered **(Kishida, 2007)**

Conclusions

The results of this study concluded that the examined broiler meat was contaminated with a variety of antibiotics awareness such as (sulfonamides and tetracycline) at distinctive ranges which can produce bad influences on human health which differ and are additionally based upon attention and duration of publicity to the contaminant.

Table (1): Positive and negative samples for presence of antibiotic residues in broiler meat samples

Sample No.	Positive No.	Negative No.
60	43 (71.70%)	17 (28.30%)

Table (2): Number and percentage of the presence of antibiotic families in broiler meat samples (No=43)

	Positive Samples		Negative Samples	
	No.	%	No.	%
Tetracycline	38	88.40	5	11.60
Sulfonamides	28	65.10	15	34.90

Table 3: Number and percentage of the presence of tetracycline family's residues in broiler meat samples

Tetracyclines	Chicken meat samples		Sulfonamides	Chicken meat samples	
	Positive No. (%)	Negative No. (%)		Positive No. (%)	Negative No. (%)
Chlortetracycline	22 (57.90)	16 (42.10)	**Sulfadiazine**	18 (64.29)	10 (35.71)
Doxycycline	35 (92.11)	3 (7.59)	**Sulphadimidine**	28 (100)	0 (0.0)
Oxytetracycline	17 (44.74)	21 (55.26)	**Sulfamethazine**	5 (17.86)	23 (82.14)
Tetracycline	28 (73.68)	10 (26.32)	**Sulfaquinoxaline**	28 (100)	0 (0.0)

Table 4: *Statistical Analytical Results of Tetracyclines and Sulfonamides residues (µg/ kg) in broiler Meat Samples*

Groups	Minimum	Maximum	Mean	± S.E.
Chlortetracycline	92.52	232.96	120.10	30.01
Doxycycline	145.21	266.78	156.12	40.86
Oxytetracycline	92.85	128.73	115.09	10.68
Tetracycline	131.71	248.63	139.26	32.21
Sulfadiazine	17.35	90.11	64.16	12.41
Sulphadimidine	18.32	120.16	75.54	15.64
Sulfamethazine	14.75	88.18	60.75	12.34
Sulfaquinoxaline	19.50	110.32	79.34	24.66

Table 5: *Mean Tetracyclines concentration levels compared to Egyptian standard of broiler meat***.**

	Tetracyclines			
	Chlortetracycline	**Doxycycline**	**Oxytetracycline**	**Tetracycline**
Within the Egyptian Standard	8 (36.36%)	0 (0%)	17 (100%)	0 (0%)
Exceed the Egyptian Standard	14 (64.64%)	35 (100%)	0 (0%)	28 (100%)
Total	22	35	17	28

Table 6: *Mean Sulfonamides concentration levels compared to Egyptian standard of broiler meat***.**

	Sulfonamides			
	Sulfadiazine	**Sulphadimidine**	**Sulfamethazine**	**Sulfaquinoxaline**
Within the Egyptian Standard	18 (100%)	25 (89.29%)	5 (100%)	21 (75%)
Exceed the Egyptian Standard	0 (0%)	3 (10.71)	0 (0%)	7 (25%)
Total	18	28	5	28

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الملخص العربي

التقييم النوعي والكمي لبقايا المضادات الحيوية في لحم الدجاج اللاحم

ايه سلامه كامل- على معوض احمد- رانيا حلمى عبده- هبه محمد شاهين

تشكل متبقيات المضادات الحيوية في لحوم الدجاج خطوره كبيره لما قد تسببه من مشاكل عديده على صحة المشتري. لذلك تم تجميع 60 عينة من الدجاج اللاحم المجمد المتداول فى اسواق ومتاجر محافظه الاسماعيليه وفحصه باستخدام تقنيه الكروماتوجرافيا السائلة عالية الأداء, حيث أكدت النتائج أن 71.7٪ من العينات التي تم فحصها كانت تحتوي على بقايا مضاد حيوي واحد على الأقل بينما 28.3٪ كانت خالية من أي بقايا للمضادات الحيويه. فكانت نسبة وجود التتراسيكلين والسلفوناميد في العينات التى تم فحصها 38 (88.4٪) و 28 (65.1٪) على التوالي, حيث كانت نتائج القيم المتوسطه للكلوروتتراسيكلين, الدوكسيسيكلين, اكسي تتراسيكلين والتتراسيكلين, 120.1 ± 30 ، 156.12 ± 40.86 ، 115.09 ± 10.68 و 139.26 ± 32.21 ميكروغرام / كغ على التوالي. بينما كانت بالنسبة لعائله السلفوناميد 64.16 ± 12.41 ميكروجرام / كجم. 75.54 ± 15.64 ميكروجرام / كجم ؛ 60.75 ± 12.34 ميكروغرام / كغ و 79.34 ± 24.6 ميكروغرام/كغ لبقايا السلفاديازين ، سلفاديمدين ، سلفاميثازيني وسلفاكويوكسالين على التوالي. وتبين من النتائج التي تم الحصول عليها, ان معظم العينات التي تم فحصها تحتوى على نسبه عاليه من متبقيات المضادات الحيويه والتى تتجاوز الحد الاقصى الموصى به من المنظمات الدوليه والمحليه مم يشكل خطر كبير على الصحه العامه للمستهلك إذا استمر هذا الاستخدام المفرط للمضادات الحيوية.