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ORIGINAL ARTICLE

Antibiotics in Poultry Meat and Products' Residual Levels and Implications to Public Health

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Abstract

The great overuse of antibiotics in poultry farms for the treatment of infections or as food additives to improve chicken growth is a major human health concern. Macrolide, tetracycline, quinolones, and beta-lactams are some of the most important antibiotics used in chicken farms and may have residues in their meat. Therefore, the current study was applied to investigate the antibiotic residues in a total of 400 samples of raw chicken breast, liver, nugget, burger, and luncheon (80 each) collected from retail markets and grocery stores in Beni-Suef governorate, Egypt. The results revealed that 72.5% of chicken breast samples had antibiotic residues. The screening of the four antibiotics families in chicken liver samples has shown that danofloxacin (quinolones) and streptomycin recorded the highest percentages (72.5% each), while the lowest one was recorded for penicillin (60%). Similarly, the highest levels of antibiotics in breast samples were for tetracycline, sarafloxacin and danfloxacin (63.75% each), while the lowest one was penicillin (53.75%). On the other hand, poultry products (nuggets, burger, and luncheon) were negative for antibiotic residues. The calculated estimated daily intakes (EDIs) for all examined antibiotics showed higher exposure levels than the fixed values of acceptable daily intakes (ADIs) which are a major health problem. Guidelines for the prudent use of antimicrobial agents for chicken should be adopted to reduce the prevalence of antibiotics resistance in human.

Keywords

Beta-lactams, Chicken, Macrolide, Tetracycline, Quinolones

1. Introduction

Chicken meat is profoundly expended all over the world since they are a cheap source of protein. The poultry meat request is universally expanded by customers causing a dynamic advancement within the poultry industry. Since the request for poultry meat is exceeding, the need for antibiotics in the poultry industry to improve production and control infection in poultry had been increased as well **(Mohammadzadeh et al., 2022).** The overuse of antibiotics and antimicrobials may lead to harmful drug residues in poultry meat and tissues **(Donoghue, 2003; Nisha, 2008).**

Quinolones and fluoroquinolones are critical antimicrobials utilized in human and veterinary medication **(Velissariou, 2006; Andreu et al., 2007; Cañada-Cañada et al., 2009; Cháfer-Pericás et al., 2010).** Quinolones may have a specifically poisonous impact or lead to the rise of drug-resistant microbes predicating a potential hazard to human. Moreover, they may cause hypersensitivity reactions in some individuals **(WHO, 1996).** The most common causes of antibiotic residues in poultry meat are uncontrolled usage, failure to follow label guidelines, or inadequate withdrawal period **(Donoghue, 2003; Cetinkaya et al., 2012)**.

Anti-microbial buildups in poultry meat may have a number of negative impacts on consumers. They can cause, even at low concentrations, allergy, direct toxicity, and the creation of drug-resistant bacteria **(Kirbis et al., 2007).** Moreover, long-term intake of trace amounts, through contaminated food by human may cause normal microflora in the alimentary tract to acquire antibiotic resistance **(Myllyniemi et al., 2000; Javadi, 2011).** Furthermore, antibiotics may cause distinctive issues such as teratogenic deformation of the developing fetus and hypoplasia of developing teeth in newborn children **(Şenyuva et al., 2000).**

Evaluating antibiotics residual levels in poultry meat at regular intervals is very important to guarantee that public health is not jeopardized by them **(Purow et al., 2005),** as well as it is important to ensure that antibiotics residues do not exceed the maximum permissible levels after the withdrawal period which is defined as the time it takes for the residue to reach a safe concentration **(Alm El Dein and Elhearon, 2010).** Moreover, sufficient heating temperature, cooking method, and time as well as the influential role of freezing to reduce the incidence of some antibiotics residues in poultry edible tissues are also crucial factors to ensure a high safety level for consumers **(Hussein and Khalil, 2013).** Therefore, the main goal of this study was to determine the residual levels of different antibiotics commonly used in the poultry industry including tetracycline group (tetracycline), aminoglycosides (streptomycin), β-lactams (penicillin), fluoroquinolone group antibiotics (flumequine, ciprofloxacin, danofloxacine, and sarafloxacin), and macrolide group (erythromycin) in chicken breast, liver and some frozen poultry products (luncheon, burger, and nuggets).

2. Materials and Methods

2.1. Chemicals

Concentrated Hydrochloric acid, Phosphate buffer, pH 8.0 (±0.1), Methanol, Ethylenediamine tetraacetic acid, Trichloroacetic Acid (TCA) LC-grade (99.9% purity), Disodium salt (Na2 EDTA) and Sodium Hydroxide (98%) were obtained from Sigma (St. Louis, MO, USA). Potassium phosphate monobasic and Acetic acid glacial (Analar), purity 98% LC-grade were obtained from fisher scientific (Leicestershire, UK). Antibiotic standards for fluoroquinolones, tetracycline, streptomycin, erythromycin, and penicillin were purchased from Sigma (St. Louis, MO, USA).

2.2. Antibiotic Sensitivity Discs

Penicillin P2 or P10, Tetracycline Te30, Streptomycin S10, Erythromycin E15, and Neomycin N5.

2.3. Collection of Samples

A total of 400 samples of breast muscles, chicken liver, and frozen chicken products (nuggets, burger, and luncheon) (80 each) were collected from poultry shops and grocery stores in the period from October 2021 to January 2022. Each sample was wrapped in a separate labeled polyethylene bag and transported in an insulated ice box at 4°C ± 1°C to the department of food hygiene, Animal Health Research Institute, Dokki, Giza. All samples were analyzed for detection of antibiotics residues according to the **United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) (2011)**

2.4. Samples Preparation and Storage

Ten grams of each sample were stomached with 40±1.0ml of phosphate buffer for 60 sec for muscle and 30 sec for liver tissues. After stomaching, the tissues were allowed to be settled for a minimum of 45 min before use. Previously made wells on 7 agar plates were filled with 200±4µl of the previously prepared buffered sample extract. The antibiotics standard references (SRs) were put in one well as following; Plate 1, Tetracycline; Plates 2 and 3 for Penicillin; Plate 4, Streptomycin; Plates 5 and 6, Erythromycin; Plate 7, Neomycin or Gentamicin. 200±4µl of the SRs concentration were pipetted into the test well. Plate 7 was incubated at 37 ±1ºC for 16-18 hrs, while the plates from 1 to 6 were incubated at 29±1ºC for 16 to 18 hrs.

2.5. Preparation of Spiked Samples

Samples free from antimicrobial residues were collected, prepared, and spiked with different concentrations of the tested antibiotics. The samples extraction and determination of antibiotics were done, and the recovery of the antibiotics from the spiked samples was determined.

2.6. Reading the Bioassay

After incubation of the plates, the diameter of the inhibition zones was recorded for both the tested and the standard reference antibiotic. The standard curve was used for the quantitative determination of the tested antibiotics.

3. Results and Discussion

Tetracycline group (tetracyclines), aminoglycosides (streptomycin), β-lactams (penicillin), fluoroquinolone group antibiotics (flumequine, ciprofloxacin, danofloxacin, and sarafloxacin), and macrolide group (erythromycin) are some of the most commonly used antibiotics in poultry production in Egypt.

The data presented in **Table (1)** showed that high and low quality frozen chicken products (luncheon, burger, and nuggets) had no antibiotic residues. On contrary, fresh chicken breast and liver samples showed positive results with 72.5 and 76.25 %, respectively. The obtained results are in good agreement with **Dipeolu (2004),** who found ciprofloxacin residues in 80% of fresh chicken liver samples. Additionally, **Hussein and Khalil (2013)** found no antibiotics residues in chicken burger and luncheon except ciprofloxacin which detected in 80% of the samples.

On the other hand, **Dipeolu (2004)** found that 21% of the fresh and frozen chicken breast fillets were positive for oxytertracycline and enrofloxacin.

Table 1. Qualitative antibiotics residues in chicken breast, liver and some frozen chicken products.

Samples	Positive samples		Negative samples	
	NO.	%	NO.	%
Raw breast	58	72.5%	22	27.5%
Liver	61	76.25%	19	23.75%
Nuggets	0	0%	80	100%
Burger	0	0%	80	100%
Luncheon	0	0%	80	100%

The absence of antibiotics residues in frozen chicken products may be attributed to the low quantity of chicken meat which added to the product or due to the different processing stages such as grinding and cooking that may lead to the degradation of residues. Temperature, cooking, and freezing time are some of the factors which effect on antibacterial drug residues **(Rose et al., 1996; Heshmati, 2015).** Cooking methods (boiling, roasting, braising, grilling, and frying) and freezing could reduce the concentration of tetracycline residues in poultry product by 94**%** **(Fathy et al., 2015)**. Also, **Khan et al., (2015)** illustrated that ciprofloxacin and oxytetracycline residues could be degraded by heat treatment and freezing. Furthermore, **Yıbar and Soyutemiz (2013)** reported that the level of ciprofloxacin residues in chicken tissues decreased after grilling by 33.34% and boiling by 22.42%.

Table 2. Fluoroquinolones residues (ppm) in chicken breast and liver samples (n= 80).

Compound	Breast			Liver		
	Positive samples			Positive samples		
	No.	%	Mean ± SE	No.	%	Mean ± SE
Danafloxacin	51	63.75%	2.8±0.9	58	72.75%	2.7±0.8
Sarafloxacin	51	63.75%	3±1.2	55	68.75%	5.6±1.7
Flumequine	48	60%	3.1±1.1	50	62.5%	3.2±1.2
Ciprofloxacin	46	57.5%	7.5±1.7	50	62.5%	10.9±2.1

Fluoroquinolones residues are very harmful to human health, even at a low level, it inhibits the facultative anaerobic human intestinal bacteria which leads to the development of drug-resistant bacteria **(Muriuki et al., 2001)**.

Fluoroquinolones were detected in 73% of the examined liver samples and 63% of breast samples, which agreed with **Naeem et al., (2006)** who detected ciprofloxacin residues in 58 and 85% of chicken breast and liver in Pakistan. However**,** **Lemus et al., (2008)** found that 7% of liver and breast samples were positive for Fluoroquinolones residues. On the other hand**,** **Jelena et al., (2006), Kim et al., (2011),** and **Er et al., (2013)** evaluated quinolones and found that the percentage of positive samples was ranging from 1.25% to 32.5%. Ciprofloxacin recorded the highest percentage among all the antibiotics residues tested (32.5%).

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The results of the current study showed that danofloxacin, sarafloxacin, flumequine, and ciprofloxacin were detected in chicken liver by percentages of 72.75, 68.75, 62.5, and 62.5% with mean values of 2.7±0.8, 5.6±1.7, 3.2±1.2, and 10.9±2.1 mg/kg, respectively. As for breast muscle, the percentages of positive samples for danofloxacin, sarafloxacin, flumequine, and ciprofloxacin were 63.75, 63.75, 60, and 57.5% with mean values of 2.8±0.92, 3±1.2, 3.1±1.1, and 7.5±1.7 mg/kg, respectively **(Table, 2).**

The obtained results illustrated that the residual levels of the fluoroquinolones group are higher in liver than breast muscle and these results agreed with **Salehzadeh et al., (2007)** who explained that ciprofloxacin is higher in liver than muscle. Furthermore, **Omotoso and Omojola (2014)** detected ciprofloxacin residues in chicken sample by 354.83 ± 716.43 µg/kg**.** Furthermore, **Mitrowska et al., (2010) and Sattar et al., (2014)** recorded high levels of tetracycline, ciprofloxacin, enrofloxacin, and penicillin in liver, thigh, and breast meat of broilers. These results may be attributed to insufficient guidance of withdrawal periods, absence or ineffective enforcement of restrictive legislation, excessive and prolonged antibiotic use, weak treatment records, failure to identify treated animals, off-label usage of antibiotics, and lack of consumer awareness of the risk they pose to human health **(Muhammad et al., 1997; Cerniglia and Kotarski, 1999)**.

High tetracycline and ciprofloxacin residue levels found in samples may be due to inadequate veterinary supervision. Moreover, antibiotic residues especially fluoroquinolones are very resistant to microbial degradation. Therefore, health problems may occur due to their accumlation in the human body **(Ejobi et al., 1996)**.

Antimicrobials commonly used in the poultry industry are aminoglycosides (streptomycin), β-lactams (penicillin), folate pathway antagonists (sulfonamides-sulfamethazine), quinolones (ciprofloxacin), and tetracycline **(Lemus et al., 2008).** The toxicity of each one of these antibiotics is different, for example, aminoglycosides are hepatotoxic and ototoxic, Folate pathway inhibitors have teratogenic effects, and can cause urinary tract problems **(Jelena et al., 2006)**. On the other hand, β-Lactam causes neurotoxicity in cases where kidney function is altered or there are pre-existing brain lesions. Furthermore, the aforementioned antimicrobial classes can cause disruption of the intestinal flora **(Omotoso and Omojola, 2014)**.

Scientific evidence shows that the classes of antimicrobials commonly used in the poultry industry in the world are aminoglycosides (streptomycin), β-lactams (penicillin), and folate pathway antagonists (sulfonamides-sulfamethazine), quinolones (ciprofloxacin), and tetracycline **(Lemus et al., 2008)**. The toxicity of each one of these classes is different, for example, aminoglycosides are hepatotoxic and ototoxic, Folate pathway inhibitors have teratogenic effects, and can cause urinary tract problems **(Jelena et al., 2006)**. On the other hand, β-Lactam causes neurotoxicity in cases where kidney function is altered or there are pre-existing brain lesions. Additionally, the three antimicrobial classes disrupt the intestinal flora **(Omotoso and Omojola, 2014)**.

Table 3. Residues of antibiotics other than Fluoroquinolones (mg/kg) in chicken breast and liver samples (n=80).

Compound	Breast			Liver		
	Positive samples			Positive samples		
	No.	%	Mean ± SE	No.	%	Mean ± SE
Tetracycline	55	63.75%	7.18±0.85	51	68.75%	9.88±1.05
Penicillin	48	53.75%	4.87±0.64	43	60%	6.09±0.8
Streptomycin	58	56.25%	4.43±0.49	45	72.5%	8.29±0.63
Erythromycin	53	57.5%	7.8±1.022	46	66.25%	13.10±1.6

The mean residual levels of tetracycline, penicillin, streptomycin, and erythromycin in chicken breast were 7.18±0.85, 4.87±0.64, 4.43±0.49, and 7.8±1.022mg/kg with percentages of 63.75, 53.75, 56.25, and 57.5 respectively. As for liver samples, the mean residual levels of tetracycline, penicillin, streptomycin, and erythromycin were 9.88±1.05, 6.09±0.8, 8.29±0.63, and 13.10±1.6mg/kg with percentages of 68.75, 60, 72.5, and 66.25 respectively **(Table, 3).** These results indicated that the residual level of different antibiotic groups is higher in liver than in breast muscle; erythromycin was the highest in liver and breast followed by tetracycline, penicillin, and finally streptomycin. The obtained results were in good agreement with **Gomes and Demoly (2005)** who noted that about 12% of thigh, breast, liver, and kidney samples contained penicillin G with mean values of 0.055, 0.073, 0.133, and 0.198mg/kg respectively. **Salama et al., (2011)** found that 21(42%) of breast and 26(52%) of chicken liver samples contained tetracycline. The contamination ranges were 0.124-5.182 and 103-8148mg/kg for breast and liver, respectively. High levels of penicillin G are worrying because recent studies have shown that *Staphylococcus aureus* recovered from 150 samples of chicken and raw meat has a high resistance to penicillin G (53.8%) **(Salama et al., 2009).** The widespread and inappropriate use of antibiotics in the broiler industry increases antimicrobial resistance (AMR), which reduces the ability of antibiotics to fight previously treatable infections, leading to many adverse effects on human health.

Table 4. Antibiotics residues in chicken breast and liver samples compared with maximum residual limits (MRL).

Antibiotic	MRL (mg/kg)*		Above MRL			
	Liver	Breast	Liver		Breast	
			NO.	%	NO.	%
Fluoroquinolones						
Ciprofloxacin	0.2	100	29	36%	21	26%
Danofloxacin	0.4	200	20	25%	19	24%
Sarafloxacin	0.08	10	24	30%	20	25%
Flumequine	0.5	500	18	23%	16	20%
Tetracycline	0.6	200	28	35%	23	29%
Penicillin	0.05	50	22	28%	18	23%
Streptomycin	0.6	600	24	30%	15	19%
Erythromycin	0.1	100	20	25%	17	21%

MRL= maximum residues limits recommended by **Codex Alimentarius Commission (2018)**

Results in **Table (4)** showed that the percentages of fluoroquinolones antibiotics of liver samples that were above the maximum residue limits (MRL) ranges from 23 to 36%, as for tetracycline, penicillin, streptomycin, and erythromycin, these percentages were 35, 28, 30, and 25 respectively. Regarding breast muscle samples, the percentages of antibiotics above MRL were lower than that of liver samples (ranging from 19-29%). In this respect**,** **Salama et al., (2011)** found that 12(8%) chicken livers and 20(13.33%) chicken breasts contained tetracycline residues above the Codex Maximum Residue limit (MRL). Liver samples had a higher incidence and level than those found in breast or thigh samples. Moreover, **Baazize-Ammi et al., (2019)** found that 28.75, 85, 71 and 80% of chicken meat were above MRL in relation to tetracycline, penicillin, and erythromycin, respectively. **Bilashoboka et al., (2019)** found that 53% of muscle, 65% of liver, and 7.1% of kidney beef samples were above MRL established by **Codex Alimentarius Commission (2018)** in relation to tetracyclines. The high levels of the tetracycline, penicillin, streptomycin, erythromycin, and fluoroquinolone group obtained in the current study may pose a potentially serious health threat to the life of consumer. Moreover, these high levels showed that the contribution of chicken meat to dietary intakes were higher than the acceptable daily intakes (ADIs) which indicates the abuse of antibiotics in chicken farms in addition to wide variety of antibiotics used without attention to the withdrawal time **Myllyniemi et al., (2000).**

4. Conclusion

In conclusion, danofloxacin and streptomycin recorded the highest percentages (72.5% each) in chicken liver samples, while the lowest one was recorded for penicillin (60%). Similarly, the highest levels of antibiotics in breast samples were for tetracycline, sarafloxacin and danfloxacin (63.75% each), while the lowest one was penicillin (53.75%). On the other hand, poultry products (nuggets, burger, and luncheon) were negative for antibiotic residues. Large percentages of the examined liver and breast samples were above the MRL which is a major health problem. Therefore, guidelines on the rational use of antimicrobials in chickens should be adopted to reduce the prevalence of resistant antibiotics in humans.

5. Author Contributions

All authors contributed equally to the study including design of the experiments, methodologies, analysis and interpretation of results and drafting the manuscript.

6. Conflict of Interest

The authors declare no conflict of interest.

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